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#### SECOND ARNOLD THEILER MEMORIAL LECTURE

#### VETERINARY EDUCATION

WAYNE H. RISER, D.V.M., M.S.\*

(Presented on Thursday, September 2nd 1965 at the University of Pretoria)

May I first express my appreciation for the great honour you have bestowed on me by your invitation to present this lecture in commemoration of Sir Arnold Theiler. This is indeed a privilege for which I am very grateful.

The work of Sir Arnold Theiler and his staff at Onderstepoort was well known when I was a veterinary student at Iowa State College. It was a model for the development of much of the veterinary bacteriology, parasitology, pathology and disease control in America. But one has to come here, talk to his daughter, Dr. Gertrude Theiler, and visit his former students and associates to begin to realize the full impact of the influence that his name and this institution have had on South Africa, the image of veterinary medicine, and even on the world. Without his leadership and the contributions of this institution to animal and human health, the world would have progressed at a much slower pace.

There are many intelligent and well-trained scientists, but few ever reach the heights and contribute as much as Arnold Theiler did. The reasons for this distinction have always intrigued me. What makes a man stand out head and shoulders above his fellow men? It seems that in addition to intelligence and ability there must be dissatisfaction with the situation in which he finds himself and a rebellious determination to do something constructive about it. Those who have made their mark, as Arnold Theiler did, have never spared themselves in their efforts. Furthermore they have inspired their associates and demanded the same dedication of them.

Such leaders are often severe taskmasters, because there is no substitute for intense drive, concentration, discipline and hard work. The people I have talked to these past two weeks who knew and worked with Arnold Theiler have all told affectionate and amusing stories that testify to this. He and his colleagues accomplished what they did, sometimes under stress and against great odds, by sheer determination.

Arnold Theiler founded not only a world renowned research institute but one of the top schools as well. Since veterinary education was of major interest to him, I approach the subject of my lecture this evening very humbly as I set forth my views in the shadow of his vision and accomplishments.

There are many parallels between the development of veterinary medicine here and in the United States. In both countries veterinary medicine is less than one hundred years old. In the early 1920's the profession's future was even in doubt when the draft horse was being replaced by mechanical transportation and it was gloomily predicted that the need for veterinarians might be limited. Contrary to this, veterinary activities have expanded rapidly in all phases and the past 25 years have brought unusual strides.

Beginning with the 1930's, in addition to all the large animal services, an interest in the medical care of pet animals developed and progress in this area has been surprising. In America it now provides half of today's veterinary practice income. In our economy, caring for pet animals has become big business. It is estimated that there are more than 26 million dogs and 26 million cats; the dog food industry alone has a sales volume in excess of \$400 million annually. Since we are a nation with both food

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and labour surpluses, the pet industry does much to help agriculture by providing employment, lowering food inventories, and stimulating retail sales.

A bit later I shall speak about the growth of our government-supported medical research program, but in passing let me say that the calculated value of laboratory animals and facilities to house them is now greater than the value of all livestock in our country. This is accounted for by the accrued costs in care, feed and professional time invested in research animals. Thus it is easy to see that laboratory animal management and husbandry have opened a whole new field for veterinarians.

These changing times, with the increase in both human and animal populations, have brought a new look to veterinary medicine. There are 18 veterinary schools in the United States serving a population of about 200 million and three schools in Canada serving their 20 million. In all, 22,000 veterinarians serve these 220 million people. It is believed that this ratio represents only the minimum needed. As our population grows, our facilities to train more veterinarians must be increased proportionately. Since World War II there has been a recognized shortage of veterinarians in every phase of veterinary activity and most surveys show that today the country could beneficially absorb the services of 25 to 50 per cent more veterinarians than are available.

#### Purpose and Scope of Veterinary Medicine

Veterinary medicine has undergone continuous alteration to meet the challenges of our changing world. In 1887, President Chamberlain of lowa State University said: "The purpose of a veterinarian is to supplant quacks, save life, prevent contagion, and promote health among domestic animals." At this early stage it was hard to imagine that veterinarians would ever be concerned with other than farm animals.

Recently I have asked several educators what they think the purpose of veterinary education is today. The answers have been extremely interesting. One said: "The present aim of veterinary education is to provide a well-rounded knowledge in biological science with the development of particular skills for treating animals." Another

said: "Instruction at a professional level with subjects geared toward practice, research, and continuing study."

Dean Schmiterlöw of Sweden said the chief aims and order of their veterinary education, as set up by his government, are first to pursue scientific research in animal diseases and secondly to transmit the new knowledge to students. It is mandatory that all faculty members be engaged in research in order to teach. The training in veterinary medicine is directed toward:

- 1. Animal production
- 2. Basic medical research
- 3. Control of food products
- 4. Laboratory animal care
- 5. Zoonoses
- 6. Radiology and toxicology
- 7. Aid to underdeveloped countries.

From these remarks it becomes apparent that the philosophy of veterinary education is undergoing sharp revision. It is now called upon to share in the responsibility of the scientific medical community. This brings about a realization that veterinary medicine has to change quickly and radically to keep pace. Our profession, being younger and working alone, had not found its challenges as demanding as those of medicine; consequently the veterinarian was not as well trained as the physician to take par in the widening scope of biomedical science.

In America the main stimulus for advanced training in medicine has been the availability of funds through our federal government in the form of training and research grants. These are available to physicians, veterinarians, dentists and associated biologists. As a result of this effort, now a quarter of a century old, our volume of scientific medical knowledge has doubled every eight years. This has brought pressure on veterinary schools and their personnel to keep abreast with and to become part of this flow of knowledge and advancement. This means that in the curriculum, particularly in the clinical areas, emphasis is placed on depth of knowledge as opposed to mere technical skills.

Liaison with Medicine and the Development of Specialties

A demand has naturally grown for the veterinarian to become part of the medical research team because physicians find themselves poorly trained to handle animals and to cope with the husbandry and disease problems. This has brought about a workable realization of a philosophy advocated years ago by Osler of "one medicine," which means combining human medicine, dentristry, veterinary medicine, nursing and all the supporting skills of the biologist. Consequently the veterinary profession has been pulled somewhat away from agriculture toward medicine. While our application in some countries is still primarily in the field of agriculture, our training and knowledge, particularly graduate training, are becoming more closely allied with medicine.

As a result of this challenge and union, the veterinary schools of America today are rapidly aligning themselves with the medical schools. While this is not so necessary at the undergraduate level, it has proved to be essential for members of the faculty and for graduate students in the development of specialties and research programmes.

The transition of the veterinary school from its affiliation with agriculture to that of medicine deserves note. Fifteen years ago the "one medicine" philosophy did exist on paper but it was practised only to a minor degree in a few of our veterinary schools. Today, of our 18 schools in the United States and three in Canada, all but five are now closely allied with medical schools. Two, namely California and Michigan State, are organizing new medical training centres of which the School of veterinary medicine is a part.

This new philosophy of "one medicine" has encouraged men to become clinical scholars and to prepare themselves at both the clinical and research levels. In such an environment, keen young investigators with a desire to participate both in teaching and in research have a much improved situation. The clinically oriented scholar spends half his time in research and half in clinical teaching. Such a climate generates a spirit of creativity, scholastic adventure and high purpose in the students, as it draws clinical practice nearer to basic science.

Depth in clinical investigation inevitably leads to the development of specialties. This has brought two types of specialists: one of discipline and one of species. One is a generalist, for example, who works with one or more specific species, be it small animals, cattle or horses. The other is the discipline specialist who crosses species lines as he develops skills in cardiology, dermatology, haematology, radiology, ophthalmology, anaesthesiology or orthopaedics. In any clinic, the generalist is the man responsible to the client for the patient. Then, when he needs help in depth, he asks for assistance from the discipline specialist.

Two factors have been discussed briefly that have had a great influence on the image of veterinary medicine in America. These are (1) a broader purpose and scope for the profession and (2) a closer liaison with human medicine. These bring the development of specialties through depth of training.

This point of view is sometimes challenged by those within our profession who are hostile to these changes; and think that it is enough for the young graduate veterinarian to be a highly skilled technician in all aspects of practice. They are sceptical of the "one medicine" philosophy. They prefer that research orientation be left to those who want to take graduate training.

However the tired argument that teaching and research do not flourish together must be rejected. These are unusual times, and veterinary medicine has a special opportunity to serve the welfare of man today when the very existence of man is threatened; survival and progress are keyed to health, and health depends on food of animal origin.

#### Budget, Manpower and Facilities

This broadened purpose of veterinary medicine, with the members of the profession bent on the attainment of ambitious scholarly goals, requires increased budget, manpower and facilities. Each school must have a capacity and a willingness to free its faculty from excessive patient load, teaching duties, and administrative burdens, so as to allow time for research. To produce veterinary graduates who are highly trained and motivated, it is necessary to increase

the size of veterinary facilities so that there will be more individual student attention from clinicians who have the combined qualities of a competent practitioner and a basic scientist. It is also necessary to add more facilities and equipment for each school. To accomplish this, school budgets must be greatly increased. Immediately it becomes obvious that there is a limit to regular tax sources. In our country the expansion of our veterinary schools is being accomplished with supplemental funds. These are sought from foundations, trusts, legacies, donations, fund raising campaigns, federal grants and alumni support. Unfortunate as it may seem, the major effort of our veterinary deans is fund raising. This task is difficult, and it is new to many of our schools. But such endeavour is necessary if expansion of our profession is to continue.

#### Advisability of Developing New Veterinary Schools

In America, as indicated earlier, many veterinary positions are unfilled. At the same time, pressures have mounted at state and national levels for more schools because there are two to three times more eligible students applying for veterinary training than can be accommodated with our present facilities. For example, the University of Pensylvania will admit 75 in its September class but there were 328 qualified applicants. By qualified is meant those who have had at least two years of University training in science, a sufficiently high grade average, and the aptitude and personality acceptable to the admissions committee. The situation is similar in all other American schools.

Since World War II seven new schools have come into being in the United States and one in Canada. In every instance the addition of a new school has benefited the livestock industry and the image of veterinary medicine. To quote Brig. Gen. Wayne Kester: "There is nothing wrong with veterinary medicine that more veterinarians and veterinary schools cannot cure"

The new schools have fostered a friendly spirit of competition for achievement between faculties. New academic positions have been created and these provide younger, qualified men with an opportunity to advance to heads of

departments where they can develop their own fields of interest while they are young and in their most productive years.

The demand for increased numbers of academically trained men stimulated the development of graduate training and there was a general rise in the salary level not only of school personnel, but of all veterinary civil employees.

The establishment of the new schools in California and western Canada were of particular interest. Veterinarians in these two areas felt isolated for the stream of veterinary medicine. Because their environment and disease conditions were different, they sometimes had difficulty interesting investigators and clinicians in their problems.

#### Size of the Veterinary School

The number of students that can be successfully trained in a class is largely determined by the availability of enough case accessions for study and a good clinical teaching staff. It has been found that the optimum class size is 50; in a few instances, where both accessions and staff are available, the number may be expanded to 75. If the demand for students and veterinarians is more than that, a second school should be considered.

#### Methods of Expanding Present Schools

Even in some of our states that have veterinary schools, pressure has been exerted on them both to accept more students and to turn out more graduates; as a result a second school in the state has been proposed. However the legislators and budget directors find that funds cannot be made available for this and they have asked the deans of the existing schools to give consideration to expanding present facilities. So far two solutions have been found that allow them to stay within the 50 to 75 class limit.

Texas A. & M. University: Certain interests in Texas, such as the cattle breeders, have asked for a second school so that the veterinary capacity could be doubled and so that girls could be admitted for training. The school formerly held classes for two semesters with a long break in the summer. Now three terms will be held each year, with only a short break

after each one. A student will have received the same amount of training and graduate at the completion of three calendar years instead of four. This will increase the output of students 25 per cent without increasing the size of the class. The solution necessitates a 25 per cent increase in staff and the installation of air conditioning for class rooms and laboratories so they can be used in the hot summer months.

Michigan State University: Because of their inability to admit all qualified students, they were asked to increase their numbers from 75 to at least 90 in each class. Their answer was to limit each class to 50 but to admit a new class twice each year, in March and September. There are two terms of 24 weeks per year and the course will be completed in three calendar years. Thus there would be two graduations each year. This system will not only increase the output of veterinarians by 25 per cent but will increase the total number of graduates per year from 75 to 100. This also provides the advantage that a student may drop out for a six month term because of finances, illness, scholastic reasons, or to take extra training in a particular subject.

To implement this plan it was necessary to provide two teaching staffs. Each staff will teach for six months and do research for six months. The laboratories were increased in size to accommodate the whole class of 50 instead of 38, or half the class, as formerly.

Medical schools have also had expansion problems. Many experiments have been tried and two are of special interest.

University of Iowa Medical School: This school is located in a city of 40,000 and serves a farming community. The accessions are numerous enough but the variety for a well balanced training programme is lacking. The last two years are devoted to clinical subjects and the student is free to go to any accredited medical centre to take these subjects, as long as he passes a comprehensive examination on his return. For example, the maternity cases are limited and to gain experience the student may go to the maternity clinics of a large city, such as Chicago or Detroit, for his obstetrical course. He is graduated as soon as he completes a successful examination in all clinical subjects,

though he must spend a minimum of two years in the process. This is a method for supplementing the training where clinical accessions are not balanced.

Northwestern University Medical School: At all our medical schools the competition for admittance is so great that at present only students who have completed four years in college are accepted. Thus the total number of years required to complete a medical education makes it an expensive and long process which consumes some of the student's most productive years. Northwestern University is attempting to shorten the course of study by two years be selecting their student from high school graduates of superior calibre and admitting them to the medical course at that time. During the first two years these students spend their time taking undergraduate courses in English, mathematics, physics, chemistry and biology and whatever elective courses in the humanities and arts they choose. Then they are admitted to the four year medical course. As long as their work is satisfactory, they will not have to compete again for entrance to medical school. This should allow students to select a variety of subjects, some of which may be difficult, without having to be concerned with maintaining very high grade averages.

#### School Evaluation

For the past six years I have served on the Council on Education of the American Veterinary Medical Association. The main function of this Council is to inspect veterinary schools and to make recommendations for improvement. In fulfilling this duty I am regularly asked how that school compares with others and what beneficial changes might be made.

In my visit to the faculty of Onderstepoort my impression is that this school, both staff and physical plant, compares very favourably with the American schools. I am particularly impressed with the enthusiasm of the students to learn and with the willingness of the faculty members to extend themselves for the benefit of the students. This is in the finest tradition established by Arnold Theiler and were he alive today he would be proud of the effort extended in this direction.

Because of the close relationship with the Onderstepoort Institute the opportunities to study tropical and subtropical animal diseases are unequalled anywhere else in the world The scope includes microbiology, parasitology, protozoology, toxicology, pathology and other associated environmental problems.

I am not familiar with the procedures here, but in most institutions doing both diagnostic service and research, constant vigilance must be exercised to see that the service load does not overshadow and bury the research projects to the extent that they are not completed and the results are not published. In America, lack of direction has often led to a duplication of work and a waste of time, money and effort.

In the clinical areas of this school the three major disciplines are represented by competent staffs. The impression is gained from visiting these men that their teaching and clinical service load occupies them to the extent that little time is left for specialty training, research and study. This is a common fault of all our American schools. Most of our clinicians are skillful generalists but the staff lacks specialists trained in the fields of radiology, radiobiology, ophthalmology, cardiology, dermatology, anaesthesiology and neurology.

#### Veterinary Extension

In reflecting on improving veterinary education it becomes apparent that the flow of new medical knowledge is ever increasing. When the graduate leaves academic circles to go on his own, whether it be in practice or government service, he loses a means of stimulation to keep abreast with his profession.

Our national and state associations have felt the responsibility for bringing to their members several forms of continuing education. Films, study sets illustrating diseases and lists of qualified speakers have been of assistance.

More effective is the extension program for continuing education. A number of states have employed a veterinarian as director of veterinary extension. His job is to arrange for programmes to be brought directly to small groups of veterinarians in the area in which they practice or are employed. A specialist who conducts a concentrated course in a specific field is secured for instruction. The length of the course is usually from two to four days and it is given to a limited number of veterinarians, ten to twenty at a time.

The enrollees pay fees sufficient to cover the salary of the demonstrator and all other expenses of the course.

#### Conclusion

Finally, veterinary education with all its changes and modifications still honours the high principles layed down by Arnold Theiler and other pioneer educators of his time.

It is indeed a privilege to have been greeted by the men of this great Institution and School which he founded. The warmth and sincerity of the welcome accorded Mrs. Riser and me by the gracious veterinary families throughout the Republic of South Africa will be long and gratefully remembered. Kindly accept our heart-felt thanks for all your hospitality and many courtesies. We will hope for an opportunity to reciprocate in kind when you come to America.



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#### OPENINGSTOESPRAAK BY DIE SESTIGSTE KONGRES VAN DIE SUID-AFRIKAANSE VETERINÈR-MEDIESE VERENIGING

PROF. H. O. MÖNNIG

U uitnodiging aan my om hierdie kongres te open, beskou ek as 'n eer en 'n voorreg waarvoor ek u graag wil bedank. Hoewel ek die laaste 20 jaar min tyd gehad het om aan die werksaamhede van ons Vereniging deel te neem, vertrou ek dat die uitnodiging nie aandui dat ek as 'n buitestaander beskou word nie

Ek verwelkom die geleentheid om 'n paar gedagtes in verband met ons land se wetenskaplike werksaamhede aan u voor te lê en ek doen dit veral graag in hierdie kring waar ek weet dat dit op vrugbare aarde sal val om twee belangrike redes. Eerstens omdat die veearts, deur sy opleiding en werk, in aanraking is met 'n aansienlike deel van ons wetenskaplike kennis, en tweedens omdat veeartsenykundige navorsing in ons land groot suksesse behaal het, as gevolg van 'n heldere en gesonde opvatting van doelstellings en metodes, gepaard met 'n ontdekkingsdrang en 'n diensmotief wat op Onderstepoort steeds duidelik merkbaar was.

U is goed daarvan bewus dat, in die huidige tyd, die vinnig groeiende wetenskaplike kennis en die toepassing daarvan, wat tegnologie genoem word, die belangrikste dryfkragte geword het in ons hele ekonomie en selfs ook in ons maatskaplike lewe. Soos Lord Todd, baie jare voorsitter van die Britse "Advisory Council on Scientific Policy" onlangs gesê het: "Whether we like it or not, science and technology are shaping our future". Wetenskap en tegnologie het dus 'n saak van eersterangse nasionale belang geword en speel gevolglik in toenemende mate 'n rol in die vorming van staatsbeleid in alle vooruitstrewende lande.

Die vinnige toename van die mens se belangstelling in die wetenskap en die gevolge daarvan, gedurende die laaste eeu en veral in ons leeftyd, word self in sommige kringe as 'n natuurverskynsel beskou en dit word bestudeer as 'n "wetenskap van die wetenskap". Ook word ernstige aandag gegee aan die produktiwiteit en lonendheid van navorsing. In die ontwikkelingstadium van ons land kan ons ons aandag gee aan meer praktiese probleme en die lonendheid van byvoorbeeld veeartsenykundige navorsing is sekerlik al voldoende bewys.

As dit dan so is, dat die wetenskap en die tegnologie ons toekoms bepaal, of ten minste sterk rigting daaraan gee, is dit ook noodsaaklik dat ons die regte rigting gee aan ons wetenskaplike aktiwiteite. Om dit te kan doen vereis 'n aansienlike mate van studie, beplanning en koördinering en 'n organisasie wat vir die doel geskep is.

Opnames en studies moet gemaak word van langtermyn- en korttermynprobleme van ons nywerhede, insluitend landbou, van menslike behoeftes, van probleme wat verbonde is aan die gebruikmaking van natuurlike hulpbronne, die voordelige en ook die beperkende faktore wat eie is aan ons land, asook die moontlikhede van ons werkkragte en finansiële middels. Dit dek 'n baie wye veld, maar slegs wanneer ons 'n taamlik volledige beeld van dit alles gekry het, kan ons op 'n gesonde grondslag vir die toekoms beplan en die regte rigting aandui.

Vir hierdie doel is dit nodig dat daar volledige samewerking moet wees tussen die bekwaamste en mees ervare persone wat in wetenskaplike, ekonomiese en selfs sosiale aangeleenthede betrokke is en geen groep kan toegelaat word om eensydig of afsydig te werk te gaan nie.

Dit sal ook duidelik wees dat "wetenskap ter wille van die wetenskap" in 'n groot mate iets van die verlede is, met dié voorbehoud dat die persoon met geniale idees altyd in die geleentheid gestel moet word om sy idees op te volg. Alle wetenskaplike vordering berus op idees, vindingrykheid en visie en daar is slegs 'n klein persentasie van wetenskaplikes wat hierdie eienskappe in 'n aansienlike mate besit. Hulle inisiatief moet te alle tye aangemoedig word. By die beplanning en programmering van navorsing moet hierdie persone 'n belangrike rol speel en daarom moet wetenskaplike beplanning nie van bo af gedoen word nie, maar moet dit deur die navorsers self aangevoor word.

Om effens af te wyk na iets van meer praktiese aard, wil ek graag 'n aangeleentheid noem wat my al baie jare interesseer. Ons huidige beesrasse is geteel van primitiewe rasse wat, nóg as vleis- nóg as melkbeeste, ekonomies was, maar deur teling is daar heelwat van hulle gemaak. En nou sukkel ons met dié beeste, wat hier na Afrika ingevoer is, om hulle te beskerm teen bosluisoorgedraagde en ander siektes van Afrika en om hier met hulle te boer onder die droogte- en ander moeilike toestande van hierdie vasteland. En dit terwyl die natuur hier aan ons 'n dier gegee het, nl. die eland, wat gladnie so ver agter is nie ten opsigte van kwaliteit as 'n vleisras, wat natuurlik bestand is teen die meeste van die genoemde siektes en wat goed aard onder die moeilikste omstandighede. Persone wat ondervinding van die eland het, sê dat mens tweemaal soveel elande as beeste op dieselfde veld kan aanhou, omdat die eland elke soort gras en bossie vreet. Dit is myns insiens beslis en baie duidelik ons plig om 'n intensiewe teelprogram met elande te begin, want dit lê voor die hand dat ons uit die eland die ideale vleisras vir Afrika-toestande sou kan teel.

Planning of scientific activities is not concerned only with research. Education and the application of scientific knowledge, especially in all industries, are equally important. I should, therefore, now like to express a few thoughts on these two aspects of our scientific activities.

The education and training of scientists and engineers are presently receiving serious consideration in all countries. There is a variety of problems in this field and not the least of these is the fact that, in all countries, about one third of the science students at universities fail to complete their studies, while less than one half obtain their first degree in the normal, or minimum period of time. This is not a suitable occasion to consider the various possible reasons for this unsatisfactory state of affairs. But I do

believe that a clear objective is basic to successful study, as the results of our Veterinary Faculty would appear to prove, and that a desire to achieve something worth while is the best stimulant to a purposeful academic career.

The problem of failing students has stimulated interest in the size of universities as a possible factor and opinions on this matter differ widely.

A university has two main functions, viz. education and research and both should be taken into account when the size of universities is discussed. With regard to education, the problems of size of a university and of failing students both, though not necessarily related, are connected with undergraduate students, especially the first two years of study. Problems of research are connected with post-graduate study. Therefore, when a solution to undergraduate educational problems is sought, this should not be done in a way that would be detrimental to research and post-graduate training, which are or should be the real and ultimate objectives of a university.

As far as research is concerned, universities have very special and real advantages over other types of organisations. Due to the fact that their staff includes scientists of all disciplines, as well as economists, sociologists and other specialists, they are able to extend our knowledge in depth and in breadth without limit in either direction. Much of the most valuable research being done at the present time—and this will be even more so in future—is concerned with the border areas between the conventional disciplines such as physics, chemistry and biology and can be successfully pursued only by teams of specialists from different disciplines.

As I have stated before, progress through research depends on ideas and originality, which are not very common qualities.

It is therefore necessary to build up teams around a suitable, gifted leader and for this reason a large university department with one such leader, is far more successful than several small departments, not only in research but also in drawing good staff, which is most important, and in training post-graduate students. It is obvious that this will also be advantageous to undergraduates, but I shall refer to that again later.

Large departments and faculties can better afford and make better use of expensive equipment. It is obvious that one expensive apparatus is much more economical and will be put to better use in a large department than two or more in smaller departments at different universities.

A research problem that is constantly growing is documentation and the study of literature relating to any research project. This can only be solved by the best library facilities and staff. A recent editorial in "Science" states "Among all the methods of storage and retrieval of scientific information, one of the best is still that of storing it in human brains and retrieving what is most pertinent at a particular time by asking intelligent questions". Again it will be obvious that a large university can meet these problems much better than several smaller ones. With regard to research and post-graduate training, we must conclude that the large university has definite advantages.

Coming now to the question of education, let us start with a little arithmetic. If a country has, say 3,000 first year students in chemistry and if it is accepted that classes should not be larger than say 150, these 3,000 students require 20 lecturers. What is the difference whether these 20 lecturers are placed at four or at ten universities? There is very definitely a difference.

As previously mentioned, a large department under a gifted leader will draw the best staff. Moreover, a number of lecturers in one department will be inclined to compare notes on methods of teaching, contents of courses and other aspects which are important as a result of the rapidly changing concepts of chemistry—and other science subjects—in this atomic age.

One must conclude that, if the size of universities has anything to do with the success or failure of students, the odds are decidedly in favour of large universities because they are better able to ensure good quality in teaching, which is really the only thing that matters. Much, of course, depends on proper organisation and, if this condition is satisfied, the optimum size seems to lie around 10-15,000 students.

There is one recent tendency, mainly experienced at large universities, which causes concern and which may stimulate a preference

for smaller institutions. I am referring to political activities of students, such as have occurred in several countries and especially at the University of California in Berkeley with its 28,000 students, where university life and work have now been strained and at times even disrupted for almost a year. The cause of these occurrences is apparently the susceptibility of many students to the ultra-liberal concepts of our day. The liberalistic theory maintains that we are unable to recognise truth, or in other words to be sure that we are right and that, for this reason everyone should have a right to his opinion. As James Burnham states in his book "Suicide of the West", Aristotle already pointed out that this statement refutes itself. If we cannot know what is true, the statement that we cannot know may also be untrue.

A university is essentially a place where one searches for truth and accepts nothing as truth unless it is supported by all available knowledge, and then even with the reservation that further knowledge may alter one's views. This is certainly the first principle students should learn. And, although they should discuss everything, they should be taught not to jump to conclusions and still less to act on them. Especially those who are inclined to subscribe to liberalistic theory should realise that, according to that theory, they cannot know whether they are right.

A commentator on the Berkeley situation states that there are certain to be more Berkeleys "unless ways are found of dealing with them—refraining from obstructing the students' rising concern for civil rights and liberties and perhaps giving them an increased role in formulating the rules governing their universities". This is typical modern liberalism—talk of rights and liberties, without any concern about duties and responsibilities, and letting the children run the house, lest they should feel frustrated and develop complexes. Well, if one wants more Berkeleys, this may be one of the best ways to get them.

There must be limits even to academic freedom and the question should be asked: "Whither should academic freedom lead us?" Surely to knowledge, human happiness and prosperity; certainly not to the complete materialism and atheism of the communist ideology and for this reason there should be discipline even at a university. Dedicated as it should be and guiding its students to study and to the search for truth, in the spirit of humility which characterises the educated, a university need not fear to grow.

En nou ten slotte 'n paar gedagtes oor die toepassing van ons wetenskaplike kennis. Daar lê baie kennis opgesluit in pulikasies in biblioteke. Soms word sulke kennis vergeet en jare later weer ontdek, soos die genetiese werk van Mendel, en dit is sekerlik nie ekonomies of bevorderlik vir die mens se welsyn nie. Maar baie kennis leef ook in die geheue van wetenskaplikes en kom tog nie tot sy reg deur praktiese toepassing nie, omdat daar verskillende soorte klowe bestaan tussen die wetenskaplike en die persoon in die praktyk. As ons in ons land die bes moontlike vordering wil maak, moet ons ernstige aandag aan hierdie vraagstuk gee.

Om meer spesifiek te wees, laat ons net die toestand oorweeg wat die toepassing van veeartsenykundige kennis deur die boere van ons land betref. Ons weet almal hoe moeilik dit is om beter metodes van voorkoming van siektes te laat posvat en toegepas te kry.

Ek het byvoorbeeld gedurende die laaste 40 jaar menige keer gesê en geskryf dat daar in die Karroo geen ernstige wurmparasietprobleem by skape behoort te wees nie, en dat wat daar is grotendeels kunsmatig gemaak word, veral by die drinkplekke van die diere. Nogtans sien mens nog oral by sulke plekke hoe die water oorloop en 'n streep groen gras onderhou word, sodat die parasiete daar weelderig kan teel en die skape wat kom suip kan besmet.

As daar dan boonop nog 'n groot deel van ons boere in die ekstensiewe skaap- en beesboerderystreke sonder veeartsenykundige hulp en advies moet klaarkom, wat word dan van die kennis wat ons besit? Ongelukkig besef baie min boere en ook ander persone hoeveel hulp 'n veearts werklik in staat is om te gee.

Daar word gepraat van 'n komende vleistekort en 'n bottertekort in 'n land wat vleis en botter behoort uit te voer. Ook word gesê dat ons meer moet uitvoer. Ons moet meer wol produseer. Ons moet surplus mielies aan die vee voer. Dit kan alles gedoen word, veral met diere van beter kwaliteit en as ons die lekplekke wat siektes veroorsaak kan toestop. Maar

watter boer kan dit riskeer om meer kapitaal in beter diere te belê sonder veeartsenykundige hulp?

Die probleem van veeartsenykundige hulp in ons land is grotendeels 'n saak van afstande, m.a.w. vervoerkoste en tydverlies op die pad. Nòg die boer op die platteland nòg die veearts kan dit bekostig, maar die twee sal moet bymekaargebring, word. Ek wil graag voorstel dat die Staat 'n deel van die koste moet bydra, op 'n stygende skaal soos die afstand groter word. Byvoorbeeld sal die Staat niks bydra nie vir die eerste vyf myl (of tien myl heen en terug), dan van oor 5-20 myl 50 persent, van oor 20-70 myl 70 persent en oor 70 myl 85 persent van die reiskoste en tyd teen sê 15c per myl. Die koste van ondersoek en behandeling moet die boer self dra, maar die probleem van afstand sal hierdeur baie verlig word. Dit moet ook net geld vir gevalle van groot diere of 'n siekte in 'n kudde, want kleiner diere kan na die veearts gebring word, as daar een is.

Ons spandeer enorme bedrae aan gesondheidsdienste en hospitaalbehandeling van mense. Die wat dit nie kan bekostig nie, word na hospitale vervoer en soms wekelank daar so te sê gratis behandel. Dit word tyd dat daar aan die siek diere, 'n belangrike bron van ons voedsel en ons gesondheid, ook hulp gegee word.

Aanvanklik sal die koste van so 'n skema seker taamlik hoog wees, maar dit sal 'n geleentheid bied vir veeartse om hulle op die platteland te vestig en, soos daar meer van hulle kom, sal die gebiede wat hulle moet bedien steeds kleiner en die koste laer word. Die boer sal altyd 'n deel van die koste moet dra, behalwe nog die koste van ondersoek en behandeling, sodat hy nie maklik van die skema misbruik sal maak nie. Volgens bogenoemde voorstel, sal 'n boer wat 70 myl van die veearts weg is nog R8.25 aan reiskoste moet betaal en die staat R12.75.

Mnr. die President, ek weet dat u die kwessie van veeartsenykundige dienste aan ons boere al lank en ernstig oorweeg en ek hoop dat 'n geskikte oplossing, waarsonder ons eenvoudig nie langer kan voortgaan nie, binnekort gevind sal word.

Dit doen my nou groot genoeë om hiermee u kongres as geopen te verklaar, met my beste wense vir vrugbare en suksesvolle besprekings.





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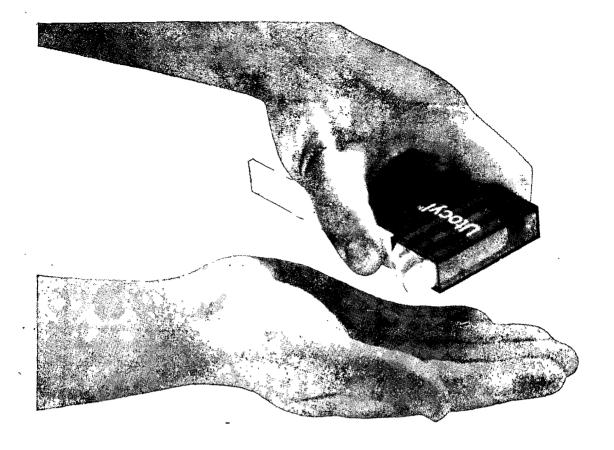
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#### PRESIDENTIAL ADDRESS

### SIXTIETH ANNUAL SCIENTIFIC CONGRESS OF THE SOUTH AFRICAN VETERINARY MEDICAL ASSOCIATION

H. P. STEYN

Ladies and Gentlemen, on occasions such as these we like if possible to report some progress in the affairs of our Association and thus also of the Profession.

There has been a certain amount of progress but not sufficient to warrant that degree of satisfaction which we would like to express.

Some of you may recollect that a few years ago I spoke of the inestimable value of the services rendered to the livestock industry of this country by the veterinary profession. I then expressed the hope that there would be funds made available for the advancement of veterinary education and research, in the form of donations, bequests etc. This, except for a few rather small scholarships and donations, has not happened. One of the reasons for the absence of any significant offers of this kind is probably because there has been no suitable fund raising organization which could collect and administer such funds.

Before dealing more fully with this aspect I wish to announce that we have received an offer of a rather handsome scholarship from the Meat Board viz. R5,000, with the offer of further amounts depending upon results. For this we wish to express our sincere thanks and appreciation to the Meat Board.

The Council of the South African Veterinary Medical Association felt that the time had arrived for the creation of an organization capable of receiving and administering large sums of money which may be made available for the advancement of veterinary science. With this object in view, a draft constitution for a South African Veterinary Foundation has been drawn up and will be submitted to the forthcoming Annual General Meeting of members for their approval and adoption. Should this proposed

draft be approved, the establishment of the Foundation and its administration by a Board of Trustees will take place immediately.

The Board of Trustees will be elected by all members of the Association as soon as possible, but a provisional Board may be appointed forthwith to enable the Foundation to become operative immediately. The provisional Board would hold office until the election of the first Board.

Several promises of donations to this fund have already been received and some prospective donors are anxious to know how soon the fund will become operative, as they are waiting anxiously to be able to make donations to it.

The objects of the proposed Foundation are really quite simple. They are the advancement of veterinary education and research and, if funds permit, to assist in the establishment of veterinary faculties.

During the past year a few statements are said to have been made in regard to veterinary education, which if true, are too vital to be disregarded by us.

It is rumoured that responsible people have said that up to seventy-five students per year could be trained at the existing faculty of veterinary science. Well, of course seventy-five students per year could be trained there, just as forty-five per year are being trained there now, and for that matter a hundred or more could doubtless be trained at this faculty. Our concern is not with the mere mass production of graduates, but rather with the adequate and efficient training of these young men.

There is evidence of over crowding of the faculty by too many under-graduates at present. It has been said that the present faculty is not

ideally situated from the point of view of the available livestock population in the immediate vicinity. There is no doubt about the truth of this allegation.

If it is the intention to equip veterinarians adequately to assist the stock farmer, it is essential that under-graduates should be in a position to see normal stock farming practices frequently in order to familiarize themselves with normal animals and normal animal husbandry.

As an argument against this statement, it is said that certain European and other veterinary faculties are situated within the environs of large cities where veterinary students see very few farm animals. That is true too, but the comparison is incomplete in that conditions are very different from our own in the examples mentioned. There are a number of 'faculties training proportionately very many more veterinarians to cope with very many fewer diseases.

Furthermore, why should we be bound by comparisons. Our object should surely be to train people in such a way that the demands set by our particular conditions can be met most effectively.

There has not yet been any evidence to indicate that our present system of training verterinarians at only one centre is the best for the welfare of the stock breeding industry of the Republic. The contrary would be more correct viz. that the advantages to be gained from having more than one training centre would far outweigh the disadvantages.

I believe it has also been said that it would be cheaper to expand and extend the existing faculty than to establish a second faculty. This is obviously an undeniable fact; but one's immediate reaction is to ask why the stock farmers of the Republic should be satisfied with the cheaper facilities, if the slightly more expensive establishment would result in better service?

We accept the statement by the Hon. the Minister for Agricultural Technical Services, of a few years ago, that it would not be possible or practical to consider the establishment of a second faculty in the "near future", but what we would like to hear from him is that he has accepted the need in principle for another faculty as soon as possible.

For years it has been my earnest endeavour to convince the authorities of the need for additional training facilities for veterinarians, those additional facilities have been granted so far as Onderstepoort is concerned, but we have done our best by quoting facts and figures which have not at any time been contradicted, to indicate the need for a second training centre.

In 1962 the Minister, while opening our Congress at Onderstepoort said: "The Department is busy formulating a scheme which will enable it to engage private practitioners on a part-time basis for the just mentioned (i.e. tuberculosis control) and possibly other future campaigns".

This is now three years ago and the tuberculosis control scheme is apparently still only in the blue-print stage and so too is the parttime employment of veterinarians. Our anxiety about the part-time employment of veterinarians is not caused by the need to find employment for veterinarians but by the urgent need for veterinary services in the rural areas. Various tuberculosis eradication schemes have been in the planning stage for the past 20-25 years, or longer, but they do not seem to progress beyond this, thus another of these protracted gestations. Can we be blamed if we begin to feel that the various schemes are not likely to progress much beyond the planning stage?

We feel that the need for better veterinary services in the rural areas is a most urgent one and if the control of tuberculosis can also improve rural services, you might say that two birds would be killed by one stone, and a very great service would be rendered to the cattle industry, more especially the dairy industry. It seems unnecessary for me to repeat what the estimated financial benefits are following the eradication of tuberculosis. Senior members of the Department of Agricultural Technical Services assert that they are opposed to parttime appointments for various reasons, one of them being that it would be impossible to exercise control over part-time employees. We have found that the Department of Public Health employs a very large number of parttime District Surgeons, that the results are generally quite satisfactory and that work done

by this method of employment would not be done at all if part-time employment was not used, as it would be impossible to obtain full-time District Surgeons wherever they are needed. There are in fact 90 full-time posts of which many are vacant, and 394 part-time District Surgeoncies.

The problem of control of these men is apparently not an obstacle to part-time employment at all.

It would seem therefore that the only objection to the part-time employment of veterinarians arises more from Departmental opposition than from any other valid reason.

It is said too that the creation of part-time veterinary posts would result in a large number of resignations by full-time employees in favour of part-time appointments. This problem could surely be overcome by limiting the number of part-time appointments to a few selected rural districts and gradually increasing these as more graduates become available. By doing this the Department would be increasing its available man power rather than reducing it. The opposition to part-time appointments seems difficult to understand.

A recent development in the registration of dangerous drugs under the Farm Feeds and Stock Remedies Act, i.e. Act No. 36 of 1947 has caused very considerable alarm among veterinarians. This is that all remedies intended for veterinary use should be registered thus enabling unqualified dealers throughout the country to sell these drugs, many of which are dangerous, over the counter like groceries and hardware.

The sale of many of these remedies is prohibited by the Medical, Dental and Pharmacy Act, unless they are prescribed by a doctor, a dentist or a veterinarian. At present a concerted effort is being made by the medical profession and pharmacists, to obtain better control over dangerous and potentially dangerous drugs, yet the Department of Agricultural Technical Services sees fit to register all drugs which may be used for animals and are labelled accordingly.

The remedies used for animals do not differ from those used for the two legged species and therefore the registration of all drugs intended for use on animals simply creates a loophole for the distribution of dangerous remedies, which it is quite certain was not the original intention when Act 36 of 1947 was promulgated.

A host of dangerous remedies have come onto the market since 1947, whereas the Act has not been brought up to date to cope with the new situation, yet a new interpretation of the Act is implemented without due consideration of the consequences.

Many of these new remedies as well as a large number of older drugs are dangerous to animals and humans, and it is unwise to render these freely available through untrained channels to the lay public. This is a matter which is receiving our urgent attention.

We are told that the legal advisers have placed this new interpretation upon the Act. That may be true, but the legal advisers cannot be expected to be aware of the practical implications of their interpretation of a technical Act, and if these are likely to be undesirable, surely the precedent which had been practised for nearly 20 years could remain in force until the Act was suitably amended.

In conclusion I would like to indicate to Dr. Monnig how strongly I support his expression of opinion about the need for a change in the administration and organization of agricultural research and education in the Republic. The opinion expressed by him that this research and education should not be controlled by the Public Service is not new. It has been said by a number of people over a long period. I do not know whether he is going to be successful in bringing about this most desirable change, but I can assure him that by far the greater majority of the members of the Veterinary Profession are convinced of the wisdom of his recommendation.

The present system has been in existence for as long as agricultural research and education has been known in this country and up to a point yeoman service has been rendered, but the limitations of the system are so obvious that it seems strange that it should be necessary to point them out or to endeavour to persuade people of the need for a change.

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	Tax Relief	Tax Relief	Tax Reli <b>ef</b>	Tax Relief		Tax Relief	Tax Relief	Tax Relief	Tax Relief
R 5000 8000 12000	R 37 120 146	R 76 240 292	R 109 360 438	R 143 472 585		R 38 122 149	R 74 245 299	R 108 368 449	R 143 483 599
					*				
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-	Annual Contribution				Annual Contribution				
Taxable	R300	R600	R900	R1200	Taxable	R300	R600	R900	R1200
Income	Tax Relief	Tax Relief	Tax Relief	Tax Relief	Income	Tax Relief	Tax Relief	Tax Relief	Tax Relief
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#### SOME ASPECTS OF RECENT ADVANCES IN ANIMAL GENETICS

#### Jan Hofmeyr

#### Animal Husbandry and Dairy Research Institute Pretoria

Presented to the 60th Scientific Conference of the South African Veterinary Medical Association September 1965.

#### INTRODUCTION

It is a regrettable but inevitable consequence of the tremendous rate of development in most biological sciences that few, if indeed any, scientists can claim to command the whole field of their particular interest. It therefore lies beyond the scope of this paper even to attempt a review of the current, bewildering flood of publications which have a clear bearing on and are relevant to this topic.

Generally speaking, geneticists are still exploring the same basic themes, viz. the nature and mode of action of the gene and chromosomes, theoretical and experimental studies on the inheritance of qualitative and quantitative characters, the response of the latter to artificial selection, mutation and radiation genetics, and so forth. However, in certain laboratories, work has penetrated to a deeply fundamental level in both the purely biological and mathematical fields.

Although not all animal geneticists actually handle domestic animals in their research, they are nevertheless all engaged in manipulating genetic material in one way or another, and at various levels. Some knowledge and understanding of the fundamental mechanisms of heredity is therefore deemed to be essential. This is necessary in view of the succeeding processes of development, growth, reproduction and production.

The main object of this paper is to consider briefly some significant aspects of the wide field of animal genetics.

#### GENETICS OF MICRO-ORGANISMS, BIOCHEMICAL AND MOLECULAR GENETICS

Studies at cellular and molecular level with minute organisms such as viruses and bacteria are making a vast contribution to knowledge of the fundamental facts of life. This contribution is being made mainly through the revelation of a wealth of structural detail within the cell by electron microscopy, information on the structure of DNA and recent advances in the genetics of micro-organisms. The simplest form of life known to science is the virus particle. Here life is reduced to its absolute bare essentials—a large molecule of nucleic acid surrounded by a protective layer of protein. Yet this simple form of life has two fundamental characteristics common to all other forms of life, i.e. a fixed structural form and the ability to replicate that form. One of the more important problems is to bridge the gaps in existing knowledge of exactly how this replication is performed.

It is well established that in the living cell the activities of life proceed under the direction of the genes. The specialised character of a cell is genetically determined and depends on the type and amount of proteins it contains. Cells thus have specific patterns of protein synthesis.

It is also known that the material responsible for this genetic specificity in living organisms is DNA (deoxyribonucleic acid). A great deal is known about the chemical and physical structure and the properties of DNA molecules. A fair amount is known about the way in which specific types of such molecules record and transmit coded genetic information to the

ribosomes in the cytoplasm where protein (enzyme, hormone, etc.) synthesis occurs<sup>1,2</sup>. The genetic information inherited is merely a structural pattern, and differences in the amount and timing of the protein or enzyme provided cannot be coded into the DNA system. This is probably determined by non-genetic factors.

Considerable information has been acquired recently on the biochemical reactions within cells involved in the phenotypic expression of genetic information. Several compounds can interfere at specific points in the sequence of this expression.

In the case of multi-cullular organisms, cells are grouped in tissues where their group function is regulated by primary controllers, among which are various hormones. Hormones effect the activity of genes, and the discovery of this link between hormones and gene action completes a conceptual bridge extending from molecular level to animal ecology and behaviour<sup>3</sup>.

Since little is known about the means whereby organisms acquire new or modified functions, active research has been prompted in the fields of biochemical and molecular genetics. Such research is done for example by identification of individual genes in the viral nucleic acid and then studying the part each plays in the genetic architecture and reproduction of the virus<sup>4</sup>. These studies, concerned with gain and loss of specific biochemical function and with structural change in the active sites, have cast new light on certain biochemical or metabolic disorders and conditions fairly frequently encountered in both humans and animals<sup>5,6</sup>.

Perhaps yet the most striking feature of these studies at molecular level is, that they have given chemical substance to most of the theoretical dictums of classical genetics.

Remarkable evidence on the phenomenon of genetic transduction has emerged from studies on the genetics of *Escherichia coli* and some of the viruses infecting this bacillus. It was demonstrated that when certain pro-viruses turn into infective viruses, thereby killing their hosts, they may carry away with them fragments of genetic material from their hosts. On infection of another host the transduced genes may be combined with genes of the new host. It appears that in transduction, genes of

the host that are linked to genes of the virus can replicate at the unrestricted viral pace when a virus enters the vegetative state. On the other hand, the genes of the virus can become an integral part of the genetic mechanism of the host. In such cases they replicate at the rate of the host's gene complex. These facts, as well as the discovery of episomes (added bodies) and pro-viruses, have not only cast light on a new phenomonon viz. additions to a chromosome of fragments of genetic material arising outside the cell, but have led to new theories on certain aspects of the genetics and transmission of disease resistance.

It is understandable that many fundamental studies on viruses and other micro-organisms are of great interest to the pathologist. The same characteristics which render certain pathogenic organisms exceptional experimental material for genetic studies, also cause concern and pose serious problems to the pathologist. With their tremendous rate of reproduction and recurrent mutation, these organisms harbour a great potential for adaptation to adverse changes, whether induced naturally or otherwise in their environment. Genes or combinations of genes ensuring the greatest adaptive potential are rapidly perpetuated in ensuing generations. This offers the most acceptable explanation for the emergence of such resistant strains of pathogenic organisms as the colon bacterium. Escherichia coli, and Staphylococcus aureus.

Recently acquired knowledge of the genetics of pathogens has renewed and stimulated interest in disease-resistance in man and domestic animals. The possibility of the existence of disease-resistance in indigenous livestock has been suggested, amongst others, by Ross et al<sup>8</sup>. Higher mortality rates among "exotic" or introduced animals have invariably been used to support these statements although other factors (stress, etc.) might also be involved.

That differences exist between breeds of sheep in their resistance to footrot and parasitic infection (viz. Romney in N.Z.) is well known, while the existence of differences among strains of the Merino in their resistance to fleece-rot was indicated by *Dunlop and Hayman*<sup>9</sup>.

Interesting evidence was produced by Hutt<sup>10</sup> and Young et al<sup>11</sup>, suggesting the feasibility of breeding for resistance to certain pathogens and

disease conditions. The successful demonstration of selection for resistance to avian leucosis has led to its general acceptance as part of sound poultry breeding programmes. This disease, according to *Hutt* <sup>10</sup>, costs the U.S.A. approximately 75 million dollars annually through pullet mortality.

In a recent review of the genetic aspects of disease resistance, Fredeen<sup>12</sup> suggests that there is sufficient evidence to justify selection for resistance to scrapie in sheep and atrophic rhinitis in pigs. However, there is reason to believe that selection for resistance to other diseases such as mastitis might not be so successful or as easily accomplished as in the case of avian leucosis.

#### **IMMUNO-GENETICS**

Research in the field or immuno-genetics has shown spectacular advances. Studies in this field have emphasized the importance of serological and biochemical markers as research tools in biological research. The value of bloodgrouping is well known. It is commonly used for parentage tests, for the identification of monozygous twins and free martinism in the field, and for a variety of research purposes. Apart from its great value in taxonomic and evolutionary studies13 it has offered a useful means of studying biochemical polymorphism in livestock. Studies have already been undertaken in order to investigate the possible existence of relationships between serum proteins and economically important characters in animals14,15,16.

Recently Ashton<sup>17</sup> reported an association between serum transferrin type and fertility in cattle. Highly significant differences in breeding efficiency were found in favour of matings between individuals, both of which were homozygous at the specific locus, compared with matings when one or both parents were heterozygous. Breeding efficiency with homozygous bulls was approximately 4 per cent higher than the mean. More recent work<sup>18</sup> showed that the transferrin locus affects fertility both at fertilization and in utero.

These results will probably have to be reassessed in the light of Ashton's 10 most recent paper in which he reports the discovery of a new transferrin D allele. Rausch et al<sup>co</sup> could not succeed in finding an association between transferrin and fertility, but a significant correlation was demonstrated between transferrin type (Tf E) and low milk production.

Several investigations of this nature are being conducted in order to examine pleiotropic effects of blood group genes or the possible existence of linkage of specific blood group loci with genes producing defects, for example,

Blood-typing provides a useful means of studying the genetic structure of breeds and strains, or lines within breeds. Changes in specific gene frequencies may be caused by natural selection or by other forces which usually operate in non-randomly bred populations.

The discovery of a specific marker gene in the blood group or transferrin systems could be a tremendous asset in selection for, say, production characters or disease resistance.

#### POPULATION AND QUANTITATIVE GENETICS

In the field of quantitative or biometrical genetics much has been published during the past few years on theoretical and experimental research. Studies on theoretical problems have been greatly assisted by the use of electronic computers. Much of this work was stimulated by the existence of a wide gap in quantitative genetics between, on the one hand, the mathematician, who often is obliged to work with over-simplified genetic models departing widely from reality, and, on the other hand, the animal breeder or researcher who works with an organism of extreme genetic complexity. The Monte Carlo technique<sup>21</sup> permits a simulation of genetic processes through the use of repetitive sequences involving random numbers generated by a high-speed computer. Modern computers have thereby not only widened the horizon for those geneticists with theoretical and mathematical inclination, but they offer means by which problems can be approached from a new dimension. Various studies are under way in an attempt to expand and to generalise selection theory in order to accommodate several components such as linkage and epistasis<sup>22,23</sup>. Fraser has also examined the effects of reproductive rate and intensity of

selection on genetic structure. This may lead to the development in the foreseeable future of more realistic genetic models, which will replace those at present in use based on over-simplifications and unproved assumptions.

Furthermore, computers facilitate the handling of bulky data and thereby enable the population geneticist to analyse the genetic structure of breeds, herds and strains. Studies examining a wide range of genetic and phenotypic parameters of animal populations have now become a more or less routine undertaking.

Hertability and the assessment of breeding values

The development of the statistical concept of heritability and its introduction by Lush and his scholars have had an important impact on the current approach to animal breeding. It has encouraged studies on the variation of productive characters by directing such studies towards estimates of the additive fraction of genetic variance. Heritability denotes that fraction of the observed or phenotypic variance which is caused by differences in genotype. It expresses the proportion of the total variance that is attributable to the additive or average effects of genes. Recent advances in biometrical genetics<sup>24</sup> have facilitated the rough estimation of contribution of certain non-additive genetic components of variance to the residual genetic variation. Heritability may be defined in statistical terms as the regression of genotype on phenotype or the squared coefficient of the correlation between them. Perhaps the most important function of heritability in practice is its predictive value since it expresses the reliability of phenotypic value or individual merit as a guide to or indication of breeding value. This, incidentally, accounts for the fact that heritability features in most formulas concerned with breeding methods<sup>25,26</sup>. heritability of a character enables a prediction to be made of the genetic progress possible under various systems of selection and it is consequently a most important concept in practical animal breeding.

Generally speaking, where the heritability of a character is high, for instance where the phenotype is a fairly true measure of the genotype, mass selection or selection on individual merit is advocated. Where heritability is low and the

phenotype is therefore not a reliable indication of the genotype, or where expression of a character is sex-limited (milk and egg production, etc.) the emphasis is on progeny testing.

With the application of general principles and concepts associated with population genetics. the emphasis has clearly been placed on the general or average breeding value of an animal. as distinct from its special or specific breeding value. The former refers, in the case of a sire. to the average effect of his genes on his progeny sired from a random sample of the females of the breed. The specific or special breeding value refers on the other hand to his combining ability with a selected group of females. This latter value is of interest only where family or line-breeding is practised as is often the case in small closed herds or stud breeding. This last is in direct contradistinction to the breeding situation found with the use of A.I.

Breeding values are estimated from an individual's own performance or merit or from those of his ancestors, collateral relatives or offspring. In some cases they are estimated from the combined information.

While performance recording is hardly a new idea the systematic employment of such recorded data, as well as the methods of recording, is of more recent origin. These new highly organised methods of performance and progeny testing based on principles of population genetics, are at present very popular in many countries. They range from elaborate testing centres to rather sophisticated statistical methods.

#### Animal breeding with Artificial Insemination

The development of A.I. coincided more or less with the introduction of the newly emerged concepts of population genetics which served the purpose of mutual stimulation. With new ideas on the principles of estimating breeding values and the sudden demand created by A.I. for "proven" and "superior" sires, research was also stimulated in the field of the methodology of progeny testing.

The application of A.I. as a breeding technique has had a tremendous impact on animal breeding. This is particularly true of dairy cattle breeding in most western countries and also of sheep breeding in the U.S.S.R. (Total

number of animals inseminated—59 million cows, 46.6 million sheep, 0.942 million pigs—<sup>27</sup>).

While the remarkable improvements in technique (semen dilutions, deep freezing etc.) were widely acclaimed on the one hand, the application of A.I. met some serious opposition from breeders.

Many of the sponsors of A.I. have unfortunately, although perhaps conveniently, inferred that it offers a means of greatly accelerating genetic improvement under all circumstances.

It is also true that, with a few exceptions<sup>28,29,30,31</sup> very little serious attention was initially given to the full genetic implications of this method of breeding. However, a most interesting booklet was published recently by the M.M.B., England<sup>32</sup>. This booklet constitutes one of the rare attempts to present the views and considered opinions of thirteen internationally renowned scientists on the proposition that two million dairy cattle may be bred annually to two hundred sires of known breeding merit without genetic deterioration.

In terms of animal breeding and breed improvement, A.I. offers obvious advantages when compared with the usual small herd or flock units, for example

- (a) the larger populations per sire provide correspondingly more scope for sire selections;
- (b) it facilitates the application of more advanced breeding systems and genetic manipulations;
- (c) it makes possible earlier and more accurate assessment of breeding values;
- (d) it reduces the generation interval by the earlier use of young sires and allows for greater selection differentials thereby increasing the possible genetic improvement per generation or year.

Robertson and Rendel<sup>30</sup> estimated the maximum rate of progress with A.I. making optimum use of progeny tests in a breeding unit of 10,000 cows, at 2 per cent per year, i.e. double the rate with natural breeding. It is, however, more likely that this figure is nearer 1.5 per cent or less with most A.I. organisations.

During the earlier stages of A.I. the major concern appeared to be the possible increase of undesirable recessive anatomical and physiological defects. Experience so far has indicated that the incidence of these defects is actually lower than with natural mating<sup>33</sup>. A fairly high incidence of progressive posterior paralysis (spastic paresis) in Red Danish cattle was recently reported by Rasbech<sup>34</sup>. It appeared that of 287 bulls examined at A.I. centres, 12.2 per cent were affected and produced offspring with typical symptoms. Genetically speaking, the identification of heterozygous (carrier) sires is a fairly simple process for which one of three standard test matings can be undertaken<sup>35</sup>.

It must be admitted that only a few A.I. populations have reached the stage where this breeding method is effectively canalizing the course of a whole breed. Such a situation exists perhaps only in the Red Danish Dairy Breed (RDB). This is an intriguing genetic set-up and is being followed with great interest by animal breeders and geneticists. The present situation is briefly as follows<sup>35</sup>: Approximately 0.75 million cows (99 per cent) of this breed are being bred annually to 651 bulls (an average of 1,141 cows per bull—in one case as many as 34,106 cows were inseminated from a single sire). The ten most popular bulls provided semen for 259,867 cows-34.5 per cent of the population. The most disturbing indication of population inbreeding is the fact that 497 of the 651 RDM sires at A.I. centres are the offspring of 25 bulls. which were also used or are still in use at the centres.

It is reasonable to assume that A.I. will tend first to decrease the inbreeding in a population for a number of generations. With the initial wide choice of many unrelated sires the relationship between females is kept low and the average inbreeding coefficient calculated for the population is therefore also relatively low. This was confirmed in the case of the RDB by Rottensten<sup>36</sup>. In an earlier investigation by Robertson and Mason<sup>37</sup> the inbreeding was estimated at 11.2 per cent for males and 4.7 per cent for females.

These facts from the more recent developments in the RDB, however, provide a glimpse of the nature of at least some of the genetic problems which might arise with A.I., particularly where advanced planning and breeding systems are not seriously considered or applied.

One of the most thought-provoking papers on this topic is that of *Dunlop and Young*<sup>38</sup> in which they consider the possibilities of A.I. for improving the present genetic status of the Australian Merino in flocks of 1,000, 3,000, 5,000 and 7,000 ewes. While the set of circumstances and some of their assumptions might be criticised, their general conclusions are worthy of note:

- (a) in relatively small flocks, in the given circumstances, A.I. shows no advantage over natural mating and might not even equal it in view of the deleterious effects of inbreeding on fleece weight and reproduction;
- (b) in relatively large flocks, in the given circumstances, the predicted rate of genetic progress, varying with flock size and time, lies between 18 and 26 per cent for fleece weight;
- (c) the absolute rates of progress possible under either A.I. or natural mating are small.

#### Genotype-environment interactions

The relative importance of genotype-environment interactions is still a much-debated topic amongst both scientists and breeders. The real issue is to determine the ranges in genotype and environment over which these interactions are large enough to be of economic importance<sup>39</sup>. A wide range of studies on various organisms, laboratory and farm animals, have been undertaken in order to shed more light on the possible importance of such interactions and their effect on selection and breeding systems.

A theoretical solution to the problem of dealing with selection in different environments has been suggested by Falconer<sup>40</sup>. The principle involved is briefly this: A character measured in two or more different environments is regarded as being not one but two or more as the case may be. This is assumed since the physiological mechanisms are to some extent different and therefore the set of genes involved are also

different. Falconer uses the example of growth rate to explain the situation. On a low plane of nutrition growth rate may be principally a matter of efficiency of food utilization whereas, on a high plane of nutrition, it might be a matter of appetite. From a knowledge of the heritabilities of the different characters in each environment and the genetic correlations between them, the correlated response and expected rate of improvement through selection for growth rate, on any one of the planes of nutrition, can be predicted from given equations. One can furthermore assume that if the genetic correlation is high, the two characters can be regarded as being essentially the same. Provided, then, that no other factors affect the heritability or the intensity of selection applied, it can be accepted that it will matter little in which environment selection is carried out. On the other hand, with a low genetic correlation between the characters and different levels of nutrition, it would be more profitable to exercise selection in the environment in which the population is supposed to perform or is destined to live. This, of course, is subject to heritability and selection intensity not being markedly higher in the alternative environment. Many research workers are engaged in studies designed to check theoretically derived and expected values. Koger<sup>39</sup> recently reported on five projects still proceeding in the U.S.A. and which include two each with beef cattle and poultry and one with sheep. No indicative results are yet available.

Fairly comprehensive statistical analyses conducted on dairy cattle data<sup>41,37</sup> have, however, failed to indicate the presence of important genotype-environment interaction. Experimental work on mice<sup>42</sup> and on poultry<sup>43</sup> on the other hand, suggest that selection between varieties or strains is best carried out in the environment for which the selected varieties or strains are destined.

Perhaps one of the largest experiments of this nature on livestock is the strain trial with sheep in Australia. The results of this investigation are also being closely followed in this country since the ranges in genotype and environment involved agrees very well with those within which local producers operate. The results of the first phases of this experiment<sup>44,45</sup> failed to

show important interactions for wool, body and reproductive characters for the five different Merino strains and the three widely differing localities involved. The work is being continued along different avenues:—

- (a) Ram x environment interactions are studied at three levels—
  - (i) Ram x year (and age) effects. The performance of the offspring of a group of rams of the same strain and locality are being observed in successive years (seasons) i.e. the performance of the lambs at different ages.
  - (ii) Ram x year (and drop) effects. Two successive lamb drops, sired by the same rams (same strain) are being compared at the same station and at the same age but in different seasons.
  - (iii) Ram x station effects. The performance of the rams belonging to the same strain is being compared at two stations where the environment differs widely. Sires are transferred from one station to the other.

#### (b) Selection in two environments

In order to determine whether selection in one environment will be effective in producing progress in animals used in a second environment (the classical situation in practice with stud and commercial units) two flocks of Peppin Merinos are kept at different stations and will eventually be exchanged between stations.

Preliminary results do not indicate the presence of any important interactions.

It would thus appear that, in view of the evidence available (or not available), in general, too much emphasis has possibly been put on the importance of genotype-environment interactions particularly within breeds of livestock. Since management and feeding practices are improving, animals are being subjected less and less to the stresses of environmental extremes, and it can therefore be anticipated that, where interactions do exist, their importance will gradually diminish.

#### Selection experiments

Most genetic laboratories or institutions conduct selection studies and the biological material used ranges from bacteria to large animals. In some cases selection theory is examined, in others mating systems are compared, while yet in others the object is to measure correlated response.

Experimental techniques have been vastly improved in recent years. Many of the earlier selection experiments suffered from serious limitations owing to poor experimental design and lack of proper standardisation techniques.

An interesting feature of many selection experiments is that they show sudden responses after a long period of no response to selection (plateau period). The question of whether or not the different patterns of response to selection are due to differences of the genetic systems under selection, or due to differences in experimental procedure, has not yet been settled<sup>46</sup>.

The series of selection experiments conducted in Australia by Dun of the Department of Agriculture, N.S.W., and by Helen Turner and her team of the C.S.I.R.O., on Merinos, has probably aroused more interest amongst many animal geneticists and breeders than most other work of this nature<sup>47</sup>. These experiments are among the largest of their kind and have already yielded a vast amount of applicable information on genetic parameters and genetic correlations between different wool characters. Although this work is still proceeding the results so far must feature as a major contribution to current knowledge of sheep breeding.

The necessity and relative value of selection experiments with large animals are often questioned by those who regard them as time-consuming and costly. Selection experiments, if well planned and conducted, are however, rewarding in many ways and are still the best way of demonstrating principles and checking theory and predicted response.

#### Conclusions

Recent advances in animal genetics have provided a better understanding of how the basic mechanism of inheritance functions.

The information acquired at molecular level has been transformed to supplement the knowledge collated from theoretical and experimental fields to provide a general and acceptable theory on the inheritance of qualitative and quantitative characters in living organisms.

With the aid of current knowledge and theory on quantitative genetics, the biometrical relationships between related individuals and characters can be calculated. It is also possible to-

- (a) fractionate phenotypic variance and separate genetic and non-genetic sources of variation;
- (b) estimate the size of the additive effect of genes as well as the non-additive fractions:
- (c) estimate the breeding value of animals.
- (d) measure the degree of inbreeding and relationship in a population or between individuals;
- (e) estimate various genetic parameters in a population; and
- (f) predict the expected genetic gain with various selection systems and intensities.

For the purposes of planning practical breeding for livestock improvement the only really important additional information required is the economic value of each trait.

Many experiments have been conducted to examine the effect and possibilities of various mating systems. By accepting reasonable assumptions, predictions can be made on the general results where these systems are applied. Even in the case of non-additive genetic variation, which has hardly been exploited purposefully

in practical breeding, geneticists have suggested the use of special breeding systems. Generally speaking where epistatis might be important. linebreeding is advised, while the development of lines which cross well with others is recommended where over-dominance is involved. In some cases where over-dominance or the presence of heterotic effects are clearly established, rotational cross-breeding systems might be profitable<sup>48</sup>. For more sophisticated situations the system of reciprocal recurrent selection has been suggested49 where maximum use is made of both general and specific combining

Selection studies have underlined the importance of large units and the need to limit to the minimum the number of characteristics for which selection is done. They suggest also that, for all practical purposes, little further genetic progress through conventional breeding systems and methods of selection can be anticipated.

In order to remove any doubts on the fact that much has still to be done, it should be stated that on the experimental side, for example. research is needed to confirm many of the theories and hypotheses which have been developed and postulated. There is still insufficient information for instance on correlated characters and response to selection for single characters. The aid of physiologists and biochemists is required to assist in exploring the basic phenomena associated with inbreeding degeneration, heterosis, and the "upset of genic balance" with prolonged single character selection.

Finally, while increased production in livestock is more easily accomplished by improved nutritional and managerial practices, and the advances in these fields are spectacular, genetic improvement is permanent.

#### RENERENCES

- 1. ALLEN, J. M. (1962). The molecular control of cellular activity. McGraw-Hill Book Co., Inc. New York, Toronto & London.
- BEADLE, G. W. (1964). The new biology and the nature of man. Agric. Science Review, II, (3): 1-13.
   DAVIDSON, E. H. (1965). Hormones and genes. Sci. Amer., 212 (6): 36-45.
   EDGAR, R. S. and EPSTEIN, R. H. (1965). The genetics of a bacterial virus. Sci. Amer., 212, (2): 70-78.
   FALCONER, I. R (1965). Biochemical defect causing congenital goitre in sheep. Nature, 205 (No. 4975): 272 (2020) 978-980.

- MELLMAN ET AL. (1965). A new genetic abnormality. Lancet. (7402): 1395.
   JACOB, F. and WOLLMAN, E. L. (1961). Viruses and genes. Sci. Amer., 204 (6): 93-107.
   ROSS, J. G., ARMOUR, J., and LEE'R. P. (1960). Further observations on the influence of genetic factors in resistance to helminthiasis in Nigerian Zebu cattle. Vet. Res., 72: 119-122.
   DUNLOP, A. A. and HAYMAN, R. H. (1958). Differences among Merino strains in resistance to fleece-rot.
- Aust. J. Agric. Res., 9: 260-266.

- 10. HUTT, F. B. (1958). Genetic Resistance to Disease in Domestic Animals. Cornell Univ. Press, Ithaca, N.Y. 11. YOUNG, C. W., LEGATES, J. E. and LECCE, J. G. (1960). Genetic and phenotype relationships between clinical mastitis, laboratory criteria and udder height. J. Dairy Sci., 43: 54-62.
- FREDEEN, H. T. (1965). Genetic aspects of disease resistance. A.B.A., 33, (1): 17-26. STORMONT, C., MILLER, W. J. and SUZUKI, Y. (1961). Blood groups and the taxonomic status of the 13. American buffalo and domestic cattle. Evolution XV: 196-208.
- OGDEN, A. L. (1961). Biochemical polymorphism in farm animals. A.B.A. 29, (2): 127–138.
   NIEMAN-SφRENSEN, A. and ROBERTSON, A. (1961). The association between blood groups and several production characteristics in three Danish cattle breeds. Acta Agric. Scand., XI (2): 163-196.
- RENDEL, J. (1959). A study on relationship between blood group and production characters in cattle. Rept. Vi intern. Blood-group congress in Munich, 21-23 Aug.: 8-23.
- 17. ASHTON, G. C. (1961). Beta-globulin type and fertility in artificially bred dairy cattle. J. Reprod. Fertil., 2: 117-129
- 18. ASHTON, G. C., and FALLON, G. R. (1962). Beta-globulin type, fertility and embryonic mortality in cattle.

  J. Reprod. Fertil., 3: 93-104.
- ASHTON, G. C. (1965). Serum transferrin D alleles in Australian cattle. Aust. J. Biol. Sci., 18: 665-670. RAUSCH, W. H. ET AL. (1963). The association of bovine transferrin types, as determined by disc electrophoresis, with fertility and production. J. Dairy Sci., 46: 648. 20.
- 21. FRASER, A. S. (1957a). Simmulation of genetic systems by automatic digital computers. I. Introduction. Aust. J. Biol. Sci., 10: 484-491.
- FRASER, A. S. (1957b). Simmulation of genetic systems by automatic digital computers. II. Effects of linkage on rates of advance under selection. *Aust. J. Biol. Sci.*, 10: 492–499.
- FRASER, A. S. (1960a). Simmulation of genetic systems by automatic digital computers. V. Linkage, dominance
- 25. FRASER, A. S. (1960a). Similation of generic systems by automatic digital computers. V. Entrage, dominance and epistasis. (Biometrical Genetics—Oscar Kempthorne, Pergamon Press).
   24. KEMPTHORNE, OSCAR, (1960). Biometrical Genetics. Pergamon Press.
   25. JOHANSSON, I. and ROBERTSON, A. (1952). Progeny testing in the breeding of farm animals. Proc. British Soc. Anim. Prod.: 79-105.
- JOHANSSON, IVAR, (1962). Genetic Aspects of Dairy Cattle Breeding. Oliver & Boyd, Edinburgh & London.
- NISHIKAWA, Y. (1964). Der gegenwartige Stand der künstlichen Besamung lantwirt-schafslicher Nutztiere in der Welt. *Tierzuchter*, 16 (13): 665–666.
- JOHANSSON, IVAR, (1946). Den artificiella inseminationen och nötkreatursaveln. S.R.B. Tidskrift, 3: 33-48. JOHANSSON, IVAR, (1953). Veredlungsarbeit am Milchvieh mit Hilfe der künstlichen Besamung. Züchtungskunde, 24 (3): 97-107.
- KORKMAN, NILS, (1947). Avelsarbetats planläggning vid artificiele insemination. Nord. Jordbr. Forskn., 3 (6): 139-176.
- 31. ROBERTSON, A. and RENDEL, J. M. (1950). The use of progeny testing with artificial insemination in dairy cattle. J. Genetics, 50: 21-31.
- EDWARDS, J. (1959). Genetic considerations in breeding two million cattle to two hundred sires. Univ. Res., Cambridge.
- KORKMAN, NILS, (1961). Seminaveln med nötkreatur. Föredrag vid Kungl. Skogs-och Landbruks-akademiens
- sammankomst—9 November. RASBECH, N. O. (1962). (Investigations on progressive posterior paralysis (spastic paresis) in Red Danish Cattle). A.B.A. 31 (2): 191. 1963.
- AARSBERETNING FOR 1964. De samvirkende Kvaegavlsforeninger med kunstig saedoverfdring. 35.
- ROTTENSTEN, K. (1961). (Inbreeding and consanguinity in the Red Danish breed). A.B.A., 31 (3): 341. 1962. 36.
- ROBERTSON, A. and MASON, I. L. (1956). The progeny testing of dairy bulls: A comparison of special station and field results. J. Agric. Sci., 47 (4): 376-381.
   DUNLOP, A. A. and YOUNG, S. S. Y. (1961). A comparison of genetic progress in wool production under artificial insemination and natural mating. Proceed. of a conference on artificial breeding of sheep in Australia held at the School of Wool Technology, University of N.S.W. 8—10th August, 1961: 189-199.
- KOGER, M. (1965). Genetic-environmental interactions in cattle. Paper presented at Annual Meeting of the 39. S-49 Technical Committee, March 29.
- **4**0
- FALCONER, D. S. (1952). The problem of environment and selection. Amer. Nat., 86: 293-298. HOFMEYR, JAN, (1955). A study of Danish and Swedish progeny testing methods for dairy bulls. Kgl. Landbrukshögskolans Annaler, 22: 425-488.
- FALCONER, D. S. and LATYSZEWSKI, M. (1952). The environment in relation to selection for size in mice. J. Genet., 51: 67-80.
- BOWMAN, J. C. and POWELL, J. C. (1962). The effect of different levels of management on the assessment of differences between varieties of chickens. I. Eight week body weights. Anim. Prod., 4: 319-327.
- DUNLOP, A. A. (1962). Interactions between heredity and environment in the Australian Merino. I. Strain and location interactions in wool traits. Aust. J. Agric. Res., 13: 503-531.
- DUNLOP, A. A. (1963). Interactions between heredity and environmental in the Australian Merino. II. Strain and location interactions in body traits and reproductive performance. Aust. J. Agric. Res., 14: 690-703.
  - FRASER, A. and NASSAR, R. (1965). Selection and polygenes. Nature, 206 (No. 4981): 323-324.
- HOFMEYR, J. H., (1963). Skaapteeltnavorsing in Australië, Nieu-Seeland, die V.S.A. en Edinburgh. Verslag oor studie-besoek. Dept. Landboutegniese Dienste.
- LUSH, JAY L. (1960). Improving dairy cattle by breeding. I. Current status and outlook. J. Dairy Sci., 43: 702~706.
- 49. COMSTOCK, R. E., ROBINSON, H. F., and HARVEY, P. H. (1949). A breeding procedure designed to make maximum use of both general and specific combining ability. Agron. J., 41: 360-367.

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#### LIVE VACCINES IN THE CONTROL OF SALMONELLOSIS

#### H. J. W. Botes

Veterinary Research Institute, Onderstepoort.

Presented to the 60th Scientific Conference of the South African Veterinary Medical Association.

September 1965

#### SUMMARY

A comprehensive review is given of the incidence of Salmonellosis in the fowl and calf with special reference to age incidence. Particular attention has also been paid to the most prevalent Salmonella serotypes with the object of production of standard vaccines.

Reference has also been made to detailed investigations into the different aspects of immunization which gave rise to the development of live attenuated (polyvalent) Salmonella vaccines.

The inefficiency of dead Salmonella vaccines has been confirmed. The potency of formol-killed vaccine is primarily dependent on its freshness. Storage for only 6 weeks result in complete reduction in potency.

The immunizing value of live attenuated cultures on the other hand, is directly related to virulence. Attenuation, to a point of complete avirulency, sacrifices a considerable part of immunizing potency. Calves which have recovered from sublethal doses of virulent S. enteritidis var. dublin were completely immune also against infection by the heterologous serotype, S. enteritidis var. typhimurium. The immunity conferred by avirulent strains was less complete but still far superior to that produced by formalinized vaccines.

#### A. GENERAL INCIDENCE

#### 1. Salmonellosis in the fowl:

The frequency of isolation of abundant numbers of *Salmonella* serotypes from poultry and poultry products <sup>1,2,3,4,5,6,</sup>;,<sup>8,9,10,11,12,13,14,15</sup> renders the domestic fowl the largest single reservoir of *Salmonella* in nature<sup>3,4,5,6,13,16,17,18,19</sup>.

Being the natural host for such a large variety of Salmonella renders the domestic fowl also one of the important, if not the most important avenue of Salmonellosis in the human<sup>1,13,16,17</sup>, 19,20,21,22,23,24,25,26,27,28,29

During recent years a significant increase in the incidence of Salmonellosis in the fowl has been reported <sup>2,21,30,31,32</sup>. These findings were substantiated by Moran<sup>33</sup> who concluded that the incidence of Salmonellosis in the fowl has increased by 7 per cent during the 4 year period preceding 1961.

Salmonella serotypes: Salmonellosis in the fowl could be divided into two main groups viz.

- (1) Infections by the host specific, non-motile serotypes, S. enteritidis var. gallinarum and S. enteritidis var. pullorum the causative agents of fowl typhoid and pullorum disease commonly known as B.W.D. respectively. S. enteritidis var. gallinarum and var. pullorum are closely related in so many aspects that they, for practical purposes, could be regarded as variants of the same serotype. This includes antigenicity as well as immunogenicity.
- (2) Infections by Salmonella serotypes other than Salmonella enteritidis varieties gallinarum and pullorum, generally referred to as the paratyphoid group. Fowl paratyphoid includes a large group of acute and chronic diseased conditions resulting from such infections.

Edwards, Bruwer and Moran<sup>4,5,6</sup>, for example, have isolated 60 heterologous *Salmonella* serotypes from acute outbreaks of Salmonellosis in young and adult fowls as well as from symptomless carriers.

Buxton<sup>2</sup> in an excellent review on the incidence of Salmonellosis described 90 Salmonella serotypes originating from poultry sources. Two years later Williams<sup>34</sup> listed 102 Salmonella serotypes, isolated from poultry and poultry products and maintained that even this huge list is not exhausted.

Although such a large variety of Salmonella serotypes are associated with Avian Salmonellosis it is significant that most of the major epidemics are caused by a few serotypes only. With the exception of S. enteritidis var. gallinarum, var. pullorum and var. typhimurium, other serotypes play insignificant roles<sup>2,3</sup> and could justifiably be disregarded from an economic point of view<sup>30,35</sup>.

In turkeys S. enteritidis var. typhimurium plays an even more prominent role<sup>36,37,38,39,40</sup>, <sup>41,42</sup>. Pomeroy and Fenstermacher<sup>41</sup> isolated 24 Salmonellae from diseased turkeys, 75 per cent of which was S. enteritidis var. typhimurium.

'His finding was in agreement with observations previously made by Pomeroy and Fenstermacher<sup>39</sup>, Hinshaw, McNeil and Taylor<sup>23</sup> as well as by Edwards, Bruwer and Moran<sup>6</sup>, who found up to 48 per cent of all Salmonellosis in turkeys due to S. enteritidis var. typhimurium.

Confirmative evidence as to the high incidence of *S. enteritidis* var. *typhimurium* in turkeys was conclusively, illustrated by Lukas and Bradford<sup>43</sup>. The 1,148 outbreaks investigated by these authors were caused by 30 *Salmonella* serotypes, 46.5 per cent of which was *S. enteritidis* var. *typhimurium*.

Infections by serotypes other than the S. enteritidis var. gallinarum—pullorum group were uncommon in South Africa until fairly recently when infections due to the paratyphoid group were diagnosed with increasing frequency (Abrams<sup>44</sup>).

Henning<sup>45</sup> for example, has isolated 216 Salmonella cultures from diseased poultry, 166 (76.8 per cent) of which were S. enteritidis var. galinarum, 45 (20.8 per cent) var. pullorum while the remaining 5 isolates consisted of S. enteritidis var. typhimurium<sup>1</sup> var. anatum<sup>2</sup>, var. amersfoort<sup>1</sup> and S. typhi<sup>1</sup>.

Routine bacteriological examinations carried out on diseased fowls submitted to Onderste-

poort during 1958/63 revealed: an increase in the incidence of S. enteritidis var. typhimurium from 1 incident in 1939 to 19 (8.8 per cent) in 1963; an increase in the incidence of S. enteritidis var. pullorum from 20.8 per cent to 30.6 while S. enteritidis var. gallinarum still remained the most prevalent serotype (55 per cent).

#### Age Incidence:

Poultry of all ages are susceptible to infections by the various Salmonella serotypes, but certain serotypes are more prevalent in different age groups. Although acute mortalities due to S. enteritidis var. pullorum and the paratyphoid group, especially due to var. typhimurium have been encountered in mature fowls<sup>30,34,42,46</sup>, these serotypes are generally associated with severe losses in fowls under the age of 4 to 5 weeks<sup>30,47</sup>. But adult fowls usually serve as symptomless carriers<sup>13,19,34,47</sup> which constitute the most important source of infection by virtue of infected eggs laid<sup>13,15,47,48,49,50,51</sup>.

Fowl typhoid on the other hand is more commonly known as disease of young and mature fowls but severe losses in chickens have also been reported 52,53,54.

The age incidence of Salmonellosis and the serotypes most frequently associated with these outbreaks was magnificently illustrated at Weybridge by Blaxland, Sojka and Smithers<sup>30</sup>. During routine bacteriological examinations, 1,541 Salmonellae were isolated from 18,422 batches of poultry less than 4 weeks old. Eighty per cent of these belonged to the 3 serotypes S. enteritidis varieties pullorum (54 per cent), typhimurium (20 per cent) and gallinarum (14 per cent). These 3 serotypes were also responsible for 97.8 per cent of the 728 epidermics involving mature fowls. Of these, 364 (50 per cent), 267 (36.6 per cent) and 82 (11.2 per cent), was due to S. enteritidis var. gallinarum, var. pullorum and var. typhimurium, respectively.

#### (2) Incidence of Salmonellosis in the calf:

Salmonella serotypres: As in the fowl, Salmonellosis in the calf is caused by a large variety of Salmonella serotypes with S. enteritidis var. dublin and var. typhimurium by far the most prevalent. Gibson<sup>55</sup> for example, has isolated 14 Salmonella serotypes from diseased calves,

96 to 97 per cent of which was S. enteritidis var. dublin and var. typhimurium, with the incidence of var. typhimurium as high as 39 per cent. Buxton<sup>56</sup> on the other hand, described not less than 28 Salmonella serotypes as the aetiological agents of calf paratyphoid and maintained that this list also is not exhausted.

During recent years an apparent increase in the incidence of Salmonellosis in the calf has also been reported <sup>55,56,57</sup>. The data shown in table 1 support this statement and in addition also indicate the present extent of calf paratyphoid in South Africa. These data also show a marked increase in the incidence of *S. enterididis* var. *typhimurium*.

TABLE 1.

Period	% specimens positive for Salmonellae	Sero var. dublin	Other	
Up to 1939 1958/1963 1964/1965	15 19	95 85.1 65	8.7 19.1	6.2 15.9

Henning<sup>45</sup> for example, found 95 per cent of all calf paratyphoid due to *S. enteritidis* var. dublin. Subsequent surveys, conducted on pathological material submitted to Onderstepoort, indicated a marked increase in the incidence of serotypes other than *S. enteritidis* var. dublin.

In a survey conducted during 1958 to 1963 it has been found<sup>58</sup> that 85.1 per cent of calf paratyphoid outbreaks was due to S. enteritidis var. dublin, 8.7 per cent due to S. enteritidis var. typhimurium while the remaining 6.2 per cent was caused by 10 heterologous serotypes. More recently<sup>59</sup>, the incidence of S. enteritidis var. typhimurium was found to be even higher. S. enteritidis var. typhimurium was isolated from 19 per cent and S. enteritidis var. dublin from 65 per cent of all pathological material examined at Onderstepoort during 1964/1965. During the same survey it also became evident that 19 per cent of all material submitted from diseased calves under the age of 3 months, was positive for Salmonella in contrast to 15 per cent during 1958/1963 period.

Dam<sup>60</sup> reported that in the past S. enteritidis var. dublin was the serotype most predominantly associated with Salmonellosis in calves but during recent years a significant increase in the incidence of var. typhimurium has been observed

also in Denmark. During the 1961/1962 season for example, equal numbers of S. enteritidis var. dublin and var. typhimurium were isolated from outbreaks of paratyphoid while in 1962/1963 this ratio was 3 to 1 in favour of var. typhimurium

As in the fowl, Salmonellosis in the calf presents two epidermilogically different identities viz:

(1) Infections by the host specific serotype S. enteritidis var. dublin, characterized by typical necrosis of the liver with or without tumour splenis pending on the acute nature of the disease. S. enteritidis var. dublin infections, show a tendency to occur in the same herds year after year. Dam<sup>60</sup> who quoted detailed investigations by Müller regarded the high percentage of symptomless carriers amongst adult cattle on such farms a most probable explanation for this phenomenon.

In these instances, Salmonellosis usually occurs at the age of 3 weeks to 12 weeks.

(2) Infections by various other Salmonella serotypes with S. enteritidis var. typhimurium by far the most prevalent. Infections due to var. typhimurium is usually associated with acute septicaemia accompanied by diarrhoea without manifestation of the patho-pneumonic necrotic liver lesions. Furthermore, this type of paratyphoid usually occurs amongst very young calves (5 to 10 days old) although older calves are also effected.

Müller<sup>61</sup> convincingly illustrated that the increase in the incidence of *S. enteritidis* var. *typhimurium* in calves is closely associated with a similar increase in the incidence of this serotype in the fowl. Müller<sup>61</sup> in addition concluded that the fowl constituted the main source of infection in calves as far as *S. enteritidis* var. *typhimurium* is concerned.

Prevention of Salmonellosis in the calf due to S. enteritidis var. typhimurium, therefore, should be based on the control of Salmonella infections in the fowl.

#### Age incidence:

Calves may become infected at any period during the first 12 weeks of life, but it would appear as if calves between the ages of 3 week to 12 weeks are the most susceptible. The age incidence of Salmonellosis in the calf was clearly illustrated by Gibson<sup>55</sup> who found the highest incidence (23 per cent) between 4 weeks to 12 weeks with the incidence in the other age groups as follows: under 3 days, 3 per cent, between 3 days and 7 days, 4 per cent and between 1 week and 4 weeks, 19 per cent.

#### B. IMMUNIZATION

#### I. The inefficiency of dead vaccines:

#### 1. Avian Salmonellosis:

In the past control of avian Salmonellosis by means of vaccination was limited to infections by S. enteritidis var. gallinarum only. The earliest scientific attempt in artificial immunization was made by Bushnell and Patton<sup>62</sup> when repeated injections of large doses of dead S. enteritidis var. gallinarum cultures reduced the mortality rate in fowls, kept in fowl typhoid infected runs, from 30 per cent to 6.5 per cent. Their views as to the immunizing value of dead vaccines were shared by Coles<sup>63</sup> who reported the stimulation of an effective immunity lasting for up to 9 months, by 2 applications of formolkilled fowl typhoid vaccine given at short intervals. This immunity was, however, dependent

on the maintenance of rigorous hygienic conditions, a criterion which in itself indicated an ineffective and incomplete immunity.

In other quarters, doubt existed as to the immunizing value of killed fowl typhoid vaccines as well as the duration of whatever protection is conferred. McNutt<sup>64</sup> and Wilson <sup>65</sup>, for example, found killed fowl typhoid vaccines completely ineffective.

Their findings were substantiated by Hall, MacDonald and Legenhausen<sup>68</sup> who in addition concluded that the incomplete immunity conferred by dead *S. enteritidis* var. gallinarum cultures, was of very short duration and never lasted longer than 4 to 6 weeks.

In view of the variability of findings as to the immunizing value of dead Salmonella vaccines extensive investigations have been conducted at this Institute.

These results showed that the immunizing value of formol-killed Salmonella vaccines is primarily dependent on freshness.

A comparison made between the immunizing value of freshly prepared and 6 week old fowl typhoid vaccines are shown in Table 2.

TABLE 2.—THE IMMUNIZING VALUE OF FORMALANIZED FOWL TYPHOID VACCINES—LOSS IN POTENCY

Vaccine	Vaccination titres (group average)	Exposure mortality	Post exposure titres survivors (group average)	% carriers (survivors)	% Immunity above unaffected controls
1. Fresh—(Vaccinated	1:20	0/50	1:100	0	70
(Controls		12/60	1:250	50	0
2. 6 week old —(Vaccinated	1:10	13/50	1-240	62	3.70
(Controls		14/50	1:180	75	0
3. Fresh + adjuvant — (Vaccinated	1:100	0/50	1:220	44	26
(Controls		12/60	1:250	50	0

When applied in large doses of  $20 \times 10^8$ , a formalinized S. enteritidis var. gallinarum; freshly prepared from the most virulent, recently isolated strains stimulated an effective immunity, which was more complete in the fowls injected twice. But fowls vaccinated with an identical vaccine kept for 6 weeks were as susceptible to fowl typhoid as the unvaccinated controls.

Deriving from these reported results, it became evident that the stimulation and maintenance of a fair degree of immunity, although

incomplete, necessitating administration of large doses of a freshly prepared formol-killed vaccine, at regular intervals not exceeding 4 to 6 weeks.

One other possible solution was the use of adjuvants to improve the immunogenicity of killed Salmonella vaccines. But it has been shown<sup>59</sup> that the incorporation of a mineral oil adjuvant (Arlacel A and Bayol F) stimulates higher serum agglutinin values without improving its immunizing value.

This finding strongly suggests that agglutinating antibodies can not be regarded as a criterion for assessment of immunity but merely serve as an indication of the severity of exposure reaction. This was subsequently confirmed when injection of single doses of rough cultures resulted in complete immunity in the absence of demonstrable agglutination titres. As will be seen later, variation in post-infective serum/agglutinin values constitute reliable means of differentiating between completeness of immunity.

Besides its doubtful immunizing value, large doses of killed fowl typhoid vaccines stimulate the production of serum agglutinins specific to *S. enteritidis* var. gallinarum. The presence of these persisting agglutinating antibodies seriously interfere with the whole blood agglutination test as used for detection of carriers.

#### 2. Calf Paratyphoid:

Henning<sup>67,68</sup> developed formalinized alumabsorped S. enteritidis var. dublin vaccines for routine vaccination of calves as well as pregnant cows. Henning recommended subcutaneous vaccination of the calf, shortly after birth with 2 doses of  $20 \times 10^8$  each, given 10 days apart. The pregnant cow also received 2 applications of  $400 \times 10^8$  each, with the first inocultaion done 2 months to 3 months before calving.

Although excellent results were reported breakdown in immunity frequently occurred. This was at the time attributed to infections by either exceptionally virulent strains of S. enteritidis var. dublin or the occurrence of heterologous serotypes, a criterion which gave rise to the production of special calf paratyphoid vaccines. The success following application of these special vaccines was taken as evidence supporting the virulence hypophesis.

It has since been established<sup>58</sup> that the inability of stock calf paratyphoid vaccine to render satisfactory protection was not due to the occurrence of strains of *S. enteritidis* var. *dublin* of more virulent nature but as the result of reduced immunogenicity caused by storage. Whereas a freshly prepared formol-killed calf paratyphoid vaccine applied in large doses rendered effective protection, storage for only 6 weeks resulted in considerable reduction in antigenicity as well as immune stimulating qualities. This important

observation explained the success obtained with special autogenous vaccines which have always been used fresh.

A criterion of significant importance in the use of a dead calf paratyphoid vaccine is the immunological unresponsiveness in calves vaccinated under the age of 2 to 3 weeks. The immunity response in calves vaccinated at different ages are shown in Table 3.

TABLE 3.

Age vaccinated		titres rage)	Mortalit total e	Immunity		
(weeks)	0	н	ated	Controls	(%)	
2 4	0	1:170 1:380	4/4 3/4	4/4 4/4	0 25 33.3	
6 8 10	0 0 1:30	1:700 1:1200 1:3700	2/3 1/3 1/6	3/3 3/3 3/3	33.3   66.6   83.3	

These results show that vaccination will not achieve active immunity until the age of 3 weeks to 4 weeks. All 4 the calves vaccinated at the age of 2 weeks, followed by a second inoculation 10 days later, were unable to resist fatal infections while only 1 out of 4 (25%) of calves vaccinated when 4 weeks old survived exposure. The immunity response increase with age to attain its maximum of 83.3 per cent in calves vaccinated at the age of 10 weeks.

Maternal vaccination on the other hand is a valuable measure of preventing Salmonellosis in the young calf especially as it renders passive immunity during the period of unresponsiveness to the active stimulus. But colostral transmitted immunity as a reliable method of protection during the whole susceptible age tends to be largely unsatisfactory:

(i) As in the calf, the formol-killed pregnant cow vaccine is also subject to remarked and even complete loss in protency. Storage for only six weeks resulted in up to 1,000-fold reduction in its agglutinin-stimulating qualities (Botes 1964). There have been occasions when vaccination of the pregnant cow resulted in no transmission of passive immunity at all. But vaccination of calves with the vaccine intended for cows, using cow dosages  $(2\times40\times10^9)$  provided some degree of protection although far from satisfactory.

(ii) Maternal vaccination will impart protection to the very young calf only. As is shown in Table 4 colostral transmitted immunity declines rapidly from the second week after birth to be completely ineffective 4 to 6 weeks later, thus rendering the calf fully susceptible again. There are reported cases where calves temporary protected by passive immunity have succumbed of paratyphoid when the immunity has reached a low level i.e. 6 to 8 weeks after been born.

Deriving from this, it has been the custom to vaccinate calves, born from vaccinated cows again shortly after birth. The disadvantage to this program is that the active stimulus may reduce the level of passive immunity by a direct neutralizing effect.

TABLE 4 — DURATION OF COLOSTRAL TRANSMITTED IMMUNITY

-	4 weeks	2 weeks	Age 4 weeks	6 weeks	8 weeks
1. Serum titres (O (H 2. Immunity %		1:60 1:800 100	1:20 1:600 85	1:30 1:340 X28.5	1:20 1:570 0

#### 2. Live Vaccines:

Wilson<sup>65</sup> drew attention to the possible use of live S. enteritidis var. gallinarum vaccine. Fowls vaccinated with live cultures developed a solid and lasting immunity. This line of approach was at the time not persued any further, mainly due to the dangers attached to it.

Based on this finding as well as the fact that fowls, recovered from natural infections, were completely immune<sup>69,70</sup>, Smith<sup>71</sup> developed avirulent strains of *S. enteritidis* var. gallinarum, one smooth (9S) and one rough (9R).

The smooth strain produced a more complete immunity but was virulent for day-old chickens, severely affected egg-production when administered at the laying of age and was in addition also as agglutinogenic as virulent strains. The rough strain on the other hand was completely avirulent, produced immunity

lasting for 12 weeks without the stimulation of agglutinins.

A number of reports have since been published <sup>72</sup>, <sup>73</sup>, <sup>74</sup>, <sup>75</sup> confirming the value of Smith's 9R strain in reducing losses due to fowl typhoid.

Harbourne<sup>72</sup> for example conducted field trials on 43 infected flocks; half the fowls in each flock was vaccinated and the rest left as controls. The mortality rate in the vaccinated fowls was much reduced and varied from 0.9 per cent to 5 per cent whereas the corresponding losses in the controls were 2.4 per cent and 39 per cent respectively. The post vaccinal mortality was presumably due to prevaccinal infection a finding which was in conflict with Smith's interference phenomenon; vaccination shortly after exposure has a beneficial influence on the course of the disease.

Harbourne<sup>72</sup> in addition found a small percentage of vaccinated fowls carriers of the infective virulent strain. This finding as well as the persistent post vaccinal mortality could serve as an indication of the relativeness of immunity produced by the live vaccine.

Although 9R is considered completely avirulent as well as inagglutnogenic, Gordon and Luke<sup>74</sup> found serum agglutinins in up to 6.12 per cent of fowls vaccinated. The majority of these reactors also showed typical pathological ovarian changes some of which yielded rough S. enteritidis var. gallinarum isolates. Gordon, Garside and Tucker<sup>73</sup> attributed the mortality rate in unvaccinated fowls in contact with immunized fowls also to the rough variant. This finding was strongly opposed by Harbourne, Williams, Parker and Fincham<sup>75</sup> who claimed that losses due to the vaccinal strain were insignificant. They based their finding on the fact that although 58 out of 1,570 fowls vaccinated, died of fowl typhoid, the vaccinal strain was isolated in pure culture from 3 of these fowls only; death in the remaining 55 was caused by virulent S. enteritidis var. gallinarum.

Harbourne, Parker, Williams and Fincham<sup>75</sup> isolated the vaccinal strain from a large percentage of unvaccinated fowls which confirmed an observation previously made<sup>74</sup> that the vaccinal strain could be transferred to nonvaccinated fowls in close contact.

# ONDERSTEPOORT LIVE ATTENUATED VACCINES

# 1. Development of Vaccinal Strains:

At first, virulent strains of Salmonella enteritidis var. gallinarum, var. typhimurium and var. dublin were subcultured in a modified minimal medium of Davis and Mingioli<sup>78</sup> containing 1 per cent glucose. Morphological, different colonial isolates, selected at weekly intervals, were tested for rough characteristics by means of agglutination and acriflavin tests.

Subculturing in this minimal medium resulted in the selection of transitional forms<sup>77</sup> as well as unstable rough variants. These unstable autoagglutinating rough forms appeared to be too virulent for vaccinal purposes as was indicated by the mice-mucin test.

When injected into calves unstable rough variants of S. enteritidis var. dublin and var.

typhimurium produced undesirable reactions which included swaying temperatures accompanied by inconsistent diarrhoea.

Stable rough forms eventually used for vaccination were finally developed by serial passages of unstable forms through brothsaline suspensions containing 1.0 per cent to 10 per cent monospecific agglutinins.

Besides being completely avirulent for mice, these stable rough forms were also inagglutinogenic and most important did not show any indication of reversion even after 30 subcultures in serum broth followed by passages through mice, fowls and calves.

As is shown in Table 5, large dosis of stable rough variants of *S. enteritidis* var. gallinarum (5503/19/30) and var. typhimurium (2656/34/50) are completely avirulent also for fowls even when employed as a polyvalent vaccine.

TABLE 5.—AVIRULENCEY EXPOSURE (SUBCUTANEOUSLY) OF SUSCEPTIBLE FOWLS TO HIGH DOSES OF ROUGH (AVIRULENT)

AND SMOOTH (VIRULENT) VARIANTS

Serotype.	Strain and exposure dose	Serum agglutinin respond (group average)	Fatal infections out of total exposed	. No. with lesions	Exposure strain isolated out of total No. exposed
1. S. enteritidis var. gallinarum—					
Smooth	$10 \times 10^6$	1:640	4/10	10/10	10/10
rough	$10 \times 10^{7}$	0	0/20	0/20	15/20
2. S. enteritidis var. typhimurium					
Smooth	$10 \times 10^{8}$	1:180	2/10	6/10	6/10 8/20
rough	$10 \times 10^7$	0	0/20	0/20	8/20
3. S. enteritidis var. gallinarum and var.			,	,	
typhimurium—rough	$10 \times 10^{7}$	0	0/20	0/20	11/20

No fatal infections followed inoculation neither did any of the fowls, slaughtered, show symptoms or pathological lesions.

Besides being completely avirulent these results also emphasised the inability of these inagglutinating (O-antigen) rough cultures to stimulate the production of serum agglutinins. This finding was subsequently confirmed when vaccination of 638 fowls with large doses of S. enteritidis var. gallinarum (5503/19/30) and var. typhimurium (2656/34/50) resulted in the complete absence of vaccination mortality, serum titres or lesions attributable to the vaccinal strains.

2. The Immunizing Value of Freeze-Dried Live Vaccines:

Salmonellosis in the fowl:

# 1. S. enteritidis var. gallinarum:

Table 6 illustrates the immunizing value of live attenuated cultures of S. enteritidis var. gallinarum (5503/19/30).

In contrast to a formol-killed fowl typhoid vaccine (Table 2) only a single inoculum of  $50 \times 10^6$  to  $100 \times 10^6$  live cultures is required to produce a far more complete immunity of long duration (Table 4).

TABLE 6.—LIVE ATTENUATED (FRFEZE-DRIED) CULTURES OF S. ENTERITIDIS VAR. GALLINARUM.

No. Inocu- lation	Vacc./Exp. interval	Mortality out of total exposed	Titre of Survivors (group average)	% Carriers (survivors)	% Immunity above unaffected controls
1 (	a. 3 weeks—Vacc	1/200	1:50	10 (4) 56	78
	-Controlsb. 6 months -Vacc	16/50	1:220	56	0
. [	b. 6 months — Vacc	0/40	1:12.5	] 10	90
-	Controls	20/20	_	_	) 0
2	3 weeks—Vacc	0/50	1:80	44	40
	Controls	8/25	1:230	52	0

- (i) immunizing value of a surgle inoculation.
- (ii) duration of immunity.
- (iii) interference effect of a second inoculation.

First, there was absence of fatal infections following exposure 4 weeks after vaccination. The controls showed an infection rate of 88 per cent; 32 per cent mortality while a further 56 per cent was presented with lesions typical of chronic fowl typhoid.

Second, there was a marked reduction in the post-exposure serum agglutinin values. The average serum titre of the exposure survivals vaccinated with freshly prepared and 6 week old formol-killed vaccines were 1:100 and 1:210 respectively, in comparison to 1:50 in the group immunized with the live vaccine. Although no fatal infection followed exposure of fowls vaccinated with freshly prepared formol-killed vaccine, their much higher post-challenge serum agglutinin values indicate a more severe exposure reaction.

In addition to the question of safety and efficiency it was of practical importance to determine the duration of immunity conferred by live vaccines. To secure this information serologically negative New Hampshire hens were vaccinated with a single dose of  $50 \times 10^6$  at the age of 8 weeks. Routine serological tests were carried out at weekly intervals for the first 2 months and after that at monthly intervals. The results of exposing these fowls to fatal infections 6 months after vaccination are also shown in Table 6.

Where it was found that 2 large doses of the formol-killed vaccine produced an incomplete immunity of short duration<sup>66</sup> a single inoculation of live cultures stimulated a more complete and enduring immunity.

The more complete nature of this immunity 6 months after vaccination was evidenced by:

- (1) Marked reductions in the exposure reactions in the vaccinated fowls. The average serum titre following exposure was 1:15 in comparison to 1:50 in the fowls challenged 4 weeks after vaccination (Table 4).
  - (2) Increased age susceptibility to fowl typhoid as was evidenced by the much higher mortality rate in the controls 6 months after vaccination i.e. 8½ months old fowls. The total fatal infection rate in the controls identically exposed at the ages of 14 weeks (Table 4) and 8½ months (Table 5) was 32 per cent and 100 per cent, respectively.
- (3) Marked reduction in the number of lesions-less carriers. Although 21 (10.5 per cent) of the 200, 14 week old fowls exposed 4 weeks after vaccination remained carriers of the exposure (virulent) strain, 8 (4 per cent) showed lesions typical of chronic fowl typhoid only; the remaining 13 (6.5 per cent) were symptomless carriers.

In contrast to this, no symptomless carriers occurred amongst the 8½ months old fowls exposed 6 months after vaccination; the exposure strain was recovered from the chronic heart lesions of 4 out of 40 fowls only.

This increased protection 6 months after vaccination is most probably due to the persistence of the vaccinal strain. The vaccinal strain was isolated from 75 per cent of 60 fowls 6 weeks after vaccination from 40 per cent of 120

fowls 10 weeks after vaccination and from 5 per cent of 40 fowls 8 months after vaccination. Closely associated with this persistence of the rough strains, is the gradual increase in age susceptibility, already mentioned. It, therefore, seems very likely that the fowl is continuously stimulated by the persisting vaccinal strain as it becomes more responsive.

The practical implication of this important finding is obvious. No antibiotica or drugs with bacterial action should be administered shortly before or for a long time interval after vaccination. Such a step would result in complete inactivation of the stimulus.

The absence of demonstrable vaccinal serum agglutinins and the presence of inconstant post infection agglutinating antibodies is noteworthy. This indicates firstly, that vaccination with live rough Salmonella cultures produces immunizing antibodies without the stimulation of agglutinating antibodies. Second, it serves to establish the fact that exposure of fowls vaccinated with attenuated live vaccine very often result in (the acquisition of agglutinating antibodies. This finding is important as it eliminates the possibility that persisting serum titres foud in vaccinated fowls may have been due to the vaccinal strain.

Another observation of great significance in the use of live Salmonella vaccines is the interference effect caused by a second inoculation given shortly after the first. Where it was found that a reinforcing injection given 10 days to 14 days later enhances the immunizing value of formalinized vaccines, a second injection of a live vaccine has the opposite effect; it causes a reduction in its immunizing value.

This important experimental finding was subsequently confirmed, when two inoculations wrongly administered at short intervals failed to reduce losses in a flock naturally infected. Although mortality ceased within days of the first inoculaction, losses due to fowl typhoid commenced again shortly after application of the second.

Very recently a similar incident has been reported where more than one inoculation given at short intervals failed to reduce losses due to fowl typhoid.

It should, therefore, again be emphasised that administration of two inoculations as was the custom with the formol-killed vaccine, would result in marked reduction in the immunizing value of this live vaccine.

# 2. S. enteritidis var. typhimurium:

It is difficult at this stage, to assess fully the significance of the immunizing value of live cultures of S. enteritidis var. typhimurium against infections by virulent homologous strains. Although 93.4 per cent of vaccinated fowls were solidly immune, 83.4 per cent of the 16 week old controls also resisted fatal infections. This finding, therefore, substantiated observations previously made as far as the age incidence of fowl paratyphoid is concerned. It would appear as if fowls older than 4 to 6 weeks possess a high degree of natural resistance to fatal infections by virulent S. enteritidis var. typhimurium.

Exposure of fowls immunized with live attenuated S. enteritidis var. typhimurium nevertheless displayed resistance to fatal infections due to virulent S. enteritidis var. gallinarum.

This immunity, however, was incomplete as a large percentage of vaccinated fowls remained carriers of S. enteritidis var. gallinarum.

It is, therefore, not inappropriate to postulate that live attenuated cultures of *S. enteritidis* var. *typhimurium* would also render as complete an immunity against a homologous exposure as is the case with *S. enteritidis* var. *gallinarum*.

# Polyvalent S. enteritidis var. gallinarum typhimurium Vaccine:

As was expected the inclusion of live attenuated cultures of *S. enteritidis* var. *typhimurium* did not reduce the immunizing value of *S. enteritidis* var. *gallinarum* against infection by virulent homologous strain. On the contrary, it enhanced its immunogenicity as was evidenced by a further reduction in the exposure reactions in both groups challenged 4 weeks and 6 months after vaccination with this polyvalent vaccine. Only 3 of the 40 fowls challenged 4 weeks after vaccination, yielded exposure strain (virulent *S. enteritidis* var. *gallinarum*) isolates, thus bringing the immunity conferred to 92.5 per cent, in comparison to the 90 per cent

resistance revealed by the fowls immunized with live attenuated cultures of S. enteritidis var. gallinarum only (Table 3).

For reasons already mentioned the present information lacks direct evidence as to the immunizing value of this polyvalent live vaccine against fatal infection due to *S. enteritidis* var. typhimurium or any other heterologous serotype. One can only presume that the immunity should also be as specific as against fowl typhoid infection, which would render this polyvalent vaccine a valuable means of controlling paratyphoid infections especially due to var. typhimurium, also in turkeys.

These experimental findings, together with impressions gained from large scale vaccination in the field, lead to the belief that the use of live attenuated vaccines is attended by little risk and gives rise to an enduring immunity. How long this immunity will last is not known as yet, but its more complete nature 6 months after vaccination suggests that the protection conferred by a single inoculation would most probably last for life.

Immunization of 8 week old fowls with a single dose of live fowl typhoid vaccine, therefore, confers a considerable measure of immunity shortly after vaccination which increases in completeness to render protection at the age when mortality from fowl typhoid is likely to be the highest.

The question often arises what immediate procedure should be adopted in event of an acute outbreak of fowl typhoid? Should the vaccine be used without any delay or should the flock be treated with furazolidone, tested for reactors and then vaccinated? This whole question is debatable.

There is general agreement that furazolidone medication causes almost immediate cessation of losses due to Salmonellosis 78,79,80,81,32,42,70,82,83. But administration of furazolidone which at present is the drug of choice has certain disadvantages:—

(1) It reduces losses without complete elimination of the infection<sup>81</sup> with the creation of carriers<sup>37,42,82</sup>. Thes ecarriers form the main source of re-infection after treatment has been discontinued<sup>42,83</sup>.

(2) It has an inhibitory effect on production of agglutinins, resulting in the reduction of the validity of the whole blood agglutination test for detection of carriers<sup>32</sup>.

As vaccination with live vaccines is not recommended within 10 to 14 days of discontinuation of antibiotics or any antibacterial medication for obvious reasons, it stands to reason that losses could continue after cessation of treatment with furazolidone or carriers could easily be overlooked.

Unconfirmed reports<sup>84</sup> indicated that application of Onderstepoort live attenuated vaccine caused almost immediate cessation of further losses. In the light of these findings, as well as observations by Smith<sup>71</sup> and later confirmed by Harbourne, Williams, Parker and Fincham<sup>75</sup> where administration of 9R to fowls already infected had a beneficial influence on the course of the disease, vaccination as the primary control measure should be more practical.

The use of furazolidone or any other drug can obscure the early recognition and elimination of carriers and this disadvantage is no longer outweighed by the inefficiency of vaccination. Veterinarians and breeders should now consider very carefully whether vaccination as a primary measure followed by whole blood agglutination tests, 6 weeks later would not be a more profitable approach.

# (b) Calf Paratyphoid:

# 1. Virulence and immunity:

Botes<sup>58</sup> observed that calves which survived artificial exposure to virulent *S. enteritidis* var. *dublin*, by the subcutaneous route, developed a solid immunity also against infection by heterologous *Salmonella* serotypes.

Completeness of immunity was based on survival rates, temperature reactions, diarrhoea and the differences in pre- and post-exposure serum agglutinin values following intraduodenal infection as compared to these reactions in the controls concurrently exposed.

Calves injected at the age of 4 weeks with  $10 \times 10^8$  virulent S. enteritidis var. dublin reacted severely but recovered spontaneously. They showed temperatures of up to  $106^{\circ}$ F lasting for 6 to 8 days, accompanied by typical calf

paratyphoid diarrhoea. But when exposed to virulent S. enteritidis var. dublin 2 weeks later they were solidly immune as was evidenced by insignificant temperature reaction and gastro-intestinal disturbances. Identical reactions were also obtained in calves injected with virulent S. enteritidis var. dublin and then exposed to S. enteritidis var. typhimurium and vice versa. But the most important criterion indicating the completeness of immunity was the absence of any increase in the post exposure serum agglutinin values irrespective of whether exposed to the homologous or heterologous serotype. The controls on the other hand died within 8 days of infection after revealing typical symptoms.

Of interest is the exposure reactions in 4 weeks old calves vaccinated with 2 large doses of  $100 \times 10^9$  each of formalinized vaccine. Here, exposure was followed by 75 per cent fatal infections after all 4 calves showed severe temperatures reactions and gastro-intestinal disturbances with a 5-fold increase in the post exposure serum titre of the only survivor.

Besides revealing the incompleteness of immunity stimulated by the formol-killed vaccine, these results also clearly indicate the state of unresponsiveness already illustrated (Table 3).

In comparing the immunity response in calves injected with virulent *Salmonella* strains and formol-killed vaccines, also prepared from the most virulent strains, the following deductions are obvious:

- (1) Live virulent cultures stimulate a complete immunity also against infections by heterologous strains.
- (2) Killing and preservation destroys a large percentage of the immunizing properties.
- (3) As will be confirmed later this initial trial indicates that immunological unresponsiveness does not effect the immunizing value of live vaccines.

The close correlation which exists between virulence and completeness of immunity was further illustrated in subsequent experiments where both unstable rough variants with reduced virulence and completely avirulent rough forms were used.

Injection of unstable rough variants with reduced virulence, caused less severe reactions but these were such as to render it unsafe for vaccination purposes. The outstanding symptoms produced were swaying, temperatures accompanied by dehydration in spite of the fact that not all the calves showed diarrhoea. All the calves survived exposure with homologous as well as heterologous serotypes but the resultant temperature and diarrhoeal reactions were taken as an indication of less complete immunity.

Stable rough inagglutinogenic variants of S. enteritidis var. dublin (2267/50/31) and var. typhimurium (2656/301/51) on the other hand was completely avirulent for calves under the age of 4 weeks. When applied to older calves some developed slight reactions which tended to be more severe in older calves. These reactions which occurred 2 to 4 days after vaccination and never lasted longer than 2 to 4 days included temperatures of 102°F and 104°F., with or without diarrhoea.

However, such temperature reactions were very common in calves vaccinated with formolkilled vaccines and these reactions, therefore, are unreliable as evidence of virulence.

It was, however, observed that these reactions and in particular the gastro-intestinal disturbances were more marked in calves heavily infested with internal parasites especially coccidiosis,

Inoculation experiments conducted at Kaalplaas confirmed the avirulent nature of these vaccinal strains where single doses of  $20 \times 10^8$ given simultaneously with heartwater vaccine at the age of 1 week did not produce any clinical symptoms in the 120 calves vaccinated.

(2) The immunizing value of live attenuated calf paratyphoid vaccines.

In comparison to the more virulent strains, the immunity conferred by the completely avirulent strains is less complete but still far in excess of the protection rendered by formol-killed vaccines. It is, therefore, concluded that a strain which is attenuated to a point where it is completely avirulent often sacrifices a considerable part of its immunizing potency.

As is shown in Table 7, 75 per cent of the 12 calves vaccinated at the age of 1 week, 80 per cent of the 5 calves vaccinated at the age of 2

weeks and 83.3 per cent of the 6 calves vaccinated at the age of 4 weeks resisted fatal infections. The corresponding immunity responses in calves vaccinated with the formol-killed vaccine at the ages of 2 and 4 weeks were 0 and 25 per cent, respectively (see Table 3).

TABLE 7.

Age Vaccinated (weeks)			total e	y out of xposed Controls	% Immunity	
1 2 4	0	0 0 1:140	3/12 1/5 1/6	4/4 3/3 3/3	75 80 83.3	( <del>_</del> ) (0) (25)

B Figures in parenthesis represent immunity response inlves ca vaccinated with a formol-killed vaccine.

Acquisition of the much higher degree of immunity in 1 to 4 week old calves vaccinated with the live vaccine does not imply that the state of unresponsiveness is a criterion associated with killed vaccines only. It does, however, prove that live vaccines produce a far more complete immunity. The immunity produced by one inoculation of this live vaccine in 1 to 2 week old calves is as complete as the protection conferred by two inoculations of a formol-killed vaccine in 10 week old calves. The higher degree of immunity stimulated by the live vaccine must to a large extent also be ascribed to the persistence of the vaccinal strains. The vaccinal strain was recovered from the gallbladder of 2 calves 5 weeks after vaccination. It would appear as if the persisting rough variants cause a continuous stimulation as the calf:becomes more and more responsive.

The immunity stimulated by live attenuated S. enteritidis var. typhimurium is equally effective against infection by homologous strains as is the case with S. enteritidis var. dublin.

As was the case with virulent cultures, attenuated strains of both serotypes render protection also against infection by the heterologous serotype, but this immunity is far less complete. The cross immunity conferred by S. enteritidis var. typhimurium appear to be far superior to that of S. enteritidis var. dublin.

A polyvalent vaccine composed of *S. enteitidis* var. *dublin* and var. *typhimurium* not only enhances the immunogenicity of the individual serotypes but in addition renders protection against the *Salmonella* serotypes responsible for up to 97 per cent of all calf paratyphoid.

The freeze-dried, live attenuated polyvalent calf paratyphoid vaccine, as issued at present, therefore, replaces the formol-killed vaccines previously used for vaccination of calves, pregnant cows as well as the large number of vaccines issued on special request.

The trials summarised here illustrate that a single inoculation of live calf paratyphoid vaccine are capable of inducing a solid, lasting immunity which withstands doses of virulent exposure strains far in excess of the infection likely to be encountered in the field. This live polyvalent calf paratyphoid vaccine may be expected to induce an immunity in one and two week old calves comparable to that which follows immunization of 10 week old calves with 2 large doses of freshly prepared formol-killed vaccines.

In making tentative recommendations for the use of the live calf paratyphoid vaccine various circumstances must be considered. Because of the undesirable vaccination reactions sometimes encountered in 6 weeks to week old calves and because of the effective immunity produced in younger calves, routine vaccination under the age of 3 weeks is recommended. The age of vaccination is, however, largely dependent on local conditions. Where calf paratyphoid occurs at a predictable age say in calves 3 to 12 weeks old, vaccination should be done at least 7 days before the expected period of risk even if this implies immunization shortly after birth.

Vaccination at a too early age should be avoided especially where colibacillosis or any other diarrheal condition is a constant factor. This is of great importance as no antibiotic or any drug with antibacterial action should be given for at least 7 days before or 10 to 14 days after vaccination.

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# REFERENCES

- BUXTON, A. (1957a). Public Health Aspects of Salmonellosis in Animals. Vet. Rec. 69: 105.
- BUXTON, A. (1957b). Salmonellosis in animals: A Review. Commonwealth Agricultural Bureaux Farnham Royal, Bucks. England
- BUXTON, A. (1958). Salmonellosis in Animals. Vet. Rec. 70: 1044.
- EDWARDS, P. R., BRUWER, D. W. and MORAN, A. B. (1948 a). The genus Salmonella: Its occurrence and
- distribution in the United States. Kentucky Agricultural Exp. Stat. EDWARDS, P. R., BRUWER, D. W. and MORAN, A. B. (1948b). Further studies on the occurrence and distribution of Salmonella types in the United States. J. Inf. Dis. 83: 220.
- EDWARDS, P. R., BRUWER, D. W. and MORAN, A. B. (1948c). Salmonella Infections in Fowls. Cornell Vet. 38: 247.
- ERIKSSON, B. (1963). Studies on the presence of Salmonellae in freshly slaughtered chickens. Nord. Hyg. T. 44: 99.
- GALTON, M. M., MACKEL, D. C., LEWIS, A. L., HARDY, W. C. and HARDY, A. V. (1955). Salmonellosis in poultry and poultry processing plants in Florida. *Am. J. Vet. Res.* 16: 132. GANDON, Y. (1963). *Salmonella* from French and foreign eggs and egg products. *Ann. Past.* (Paris) 104: 584. GORDON, R. F. and BUXTON, A. (1946). A survey of Avian Salmonellosis in Great Britain. *Brit. Vet. J.*

- KRESSMANN, H. and ALBERT, O. H. (1961). Vier jahre Salmonella-Verordunung und ihr Auswirkung in der Praxis. Berl. Münch Tierärztl. Wschr. 74: 359.
- MOREHOUSE, L. G. and WEDMAN, E. E. (1961). Salmonella and other disease producing organisms in animal byproducts. A Survey. J. Am. Vet. Med. Ass. 139: 989.

  QUIST, K. D. (1963). Salmonellosis in Poultry. Public Health Reports 78: 1071.

  SADLER, W. W., YAMAMOTOTO, R. ADLER, H. E. and STEWART, G. F. (1961). Survey of market poultry
- for Salmonella infection. Appl. Microb. 9: 72.
- VAN OYE, E. (1964). The world problem of Salmonellosis. Dr. W. Junk Publishers. The Hague. EDWARDS, P. R. (1958). Salmonellosis: Observations on incidence and control Ann. N.Y. Acad. Sci. 70: 598. 16
- 17.
- GALTON, M. M. (1963). Salmonellosis in Livestock. Public Health Reports 78: 1066.
  GORDON, R. F. (1959). Broiler Disease. Vet. Rec. 71: 994.
  HOBBS, B. C. (1961). Public Health significance of Salmonella carriers in Livestock and Birds. J. Appl. Bact. 19. **24**: 340.
- ESSEX-CATER, A. J. (1963). An outbreak of human infection due to Salmonella typhimurium phage type 4 associated with the use of unpasteurized liquid egg. J. Hygiene 61: 323.
- GALBRAITH, N. S. (1961). Salmonellosis in man and animals. II. Studies on human Salmonellosis in relation to infection in animals. Vet. Rec. 73: 1296. HARVEY, R. W. S., PRICE, T. H., DAVIS, A. R. and MORTLEY-DAVIES, R. B. (1961). An outbreak of
- Salmonella food poisoning attributed to bakers confining. J. Hyg. (Comb.) 59: 105.
- HINSHAW, W. R., McNEIL, E. and TAYLOR, T. J. (1944). Avian Salmonellosis: Types of Salmonella isolated and their relation to public health. Am. J. Hyg. 40: 264. McCROAN, J. E., McKINLEY, T. W., BRIM, A. and RAMSAY, C. A. (1963). Five Salmonella outbreaks relat-

- NEWELL, K. W. (1963). Value of International Reporting of Salmonellae. Public Health Rep.
  PERRIERA, M. S. and BLAXLAND, J. D. (1955). Salmonella typhimurium infection in man contracted from turkeys. Monthly Bull. Munn. Health Lab. Services 14:52.
  POLAK, M. F. (1960). Kippe- eiprodukten in bakterigen als mogelijke bron von Salmonellose bij de mens. Ned.
- T. Geneesk. 104: 2660.

- SANDERS, E., FRIEDMAN, E. A., McCALL, C. E. and BRAHMAN, P. S. (1963). Surveillance of Human Salmonellosis. *Public Health Rep.* 78: 1080. STEELE, J. H. (1963). Epidemilogy of Salmonellosis: *Salmonella* 1885-1962. *Public Health Reports* 78: 1065. BLAXLAND, J. D., SOJKA, W. J. and SMITHERS, A. M. (1958). Avian Salmonellosis in England and Wales 1948-56 with comment on its prevention and control. *Vet. Rec.* 70 (18): 374.
- 1948-56 with comment on its prevention and control. Vet. Rec. 70 (18): 374.
  BURR, W. E., TOURTELLOTTE, M., LUGINBUHL, R. E. and JUNGHEUR, E. L. (1957). Salmonella heidelberg infection as a problem in pullorum disease control Avian Dis. 1: 298.
- SIEBURTH, J. M. (1957). The effect of furozolidone on the cultural and serological response to S. typhimurium
- infected chickens. Avian Dis. 1: 80.

  MORAN, A. B. (1961). Occurrence and distribution of Salmonella in animals in the United States. Proc. 65th
- Annual Meeting U.S. Livestock Sanitary Association, Minneapolis, Minnesota 441.

  34. WILLIAMS, J. E. (1959). Paratyphoid and Paracolon Infections. Biester and Schwarte—4th Edition Chapter 9.

  35. BLAXLAND, J. D., SOJKA, W. J. and SMITHERS, A. M. (1956). A study of Salmonella pullorum and Salmonella gallinarum from field outbreaks of the disease. J. Comp. Path. 66: 270.
- ABELSETH, M. K. and ROBERTSON, H. E. (1953). Salmonella typhimurium infection in 1952 turkey flocks. A public hazard. Canad. J. Public Health 44: 263.
- MAGWOOD, S. E. and BIGLAND, C. H. (1962). Salmonellosis in turkeys: Evaluation of bacteriological and serological incidence of infection. Canad .J. Comp. Med. 26:151.
- POMEROY, B. S. and FENSTER MACHER, R. (1939). Paratyphoid Infection in Turkeys. J. Am. Vet. Med. Ass. 94: 90.
- 39. POMEROYS, B. S. and FENSTERMACHER, R. (1941). Paratyphoid Infections in Turkeys. Am. J. Vet. Res. 2: 285.
- 40. POMEROY, B. S. and FENSTERMACHER, R. (1943). Salmonella Infections in breeding turkeys. Am. J. Vet. Res. 4: 199.

- 41. POMEROY, B. S. and FENSTERMACHER, R. (1944). Salmonella infections in turkeys. Am. J. Vet. Res. 5: 282
- 42. SMITH, N. W. (1956b). The treatment of experimental Salmonella typhimurium infection in turkey poults and chicks. Vet. Rec. 67: 749
- LUKAS, G. N. and BRADFORD, D. R. (1954). Salmonellosis in turkey poults as observed in routine necropsy of 1148 cases. J. Am. Vet. Med. Ass. 125: 215.
   ABRAMS, L. (1964). Personal communication.
- 45. HENNING, M. W. (1939). The antigenic structure of Salmonella obtained from domestic animals and birds in South Africa. Ondersteplort J. Vet. Sci. and Animal Ind. 13: 79.
- VAN ROEKEL, H. (1959). Pullorum Disease. Biester and Schwarte Editors: Diseases of Poultry, 4th Edition Iowa State University Press, Ames.
- 47. BIESTER, H. E. and SCHWARTE, L. H. Editors (1959). Diseases of Poultry—4th Edition. Iowa State University Press Ames.
- CANTOR, A. and McFARLANE, V. H. (1948). Salmonella organisms on and in chicken eggs. Poultr. Sci. 27: 48.
- 49. JORDAN, F. T. W. (1956a). The occurrence of Salmonella gallinarum in the faeces of fowl typhoid. Poultr. Sci. 35: 1026.

- JORDAN, F. T. W. (1956b). Transmission of Salmonella gallinarum through the egg. Poultr. Sci. 35: 1019.
   WILSON, J. E. (1948). Avian Salmonellosis. Vet. Rec. 60: 615.
   HALL, W. J., LEGENHAUSEN, D. H. and MACDONALD, A. D. (1949). Studies on Fowl Typhoid. I. Nature and dissemination. Poultr. Sci. 28: 344.
   JALL, W. (1956). Fowl Typhoid Riesty, and Schwarte Diseases of poultry. 4th Edition. Journ State Unit
- HALL, W. J. (1959). Fowl Typhoid. Biester and Schwarte Diseases of poultry. 4th Edition. Iowa State University Press, Ames.
- KOMANNOV, A. (1932). Fowl typhoid in baby chickens. Vet. Rec. 12: 1455. GIBSON, E. A. (1961). Salmonellosis in Calves. Vet. Rec. 73: 1284.

- 56. BUXTON, A. (1960). Salmonellosis in Calves. Vet. Rec. 72 (11): 640.
  57. INGRAM, P. C. and LOVELL, R. (1960). Infection by Escherichia coli and Salmonella. Vet. Rec. 72: 1183.
  58. BOTES, H. J. W. (1964). Calf Paratyphoid: Evaluation of formol-killed and live attenuated vaccines. Bull. Off.
- Intern. Epiz. 62: 581.

  BOTES, H. J. W. (1965). Unpublished data.

  DAM, A. (1964). Infectious Diseases among Calves in Denmark with particular reference to Septicaemia with 60.

- Escherichia coli Bull. Off. int. Epiz. 62: 495.
  MÜLLER, J. (1957). De problème des Salmonelloses au Danemark. Bull. Off. int. Epiz. 48: 323.
  BUSHNELL, L. D. and PATTON, J. W. (1924). The use of Vaccines in Poultry Diseases. Poultr. Sci. 4: 64.
  COLES, J. D. W. A. (1946). Fowl typhoid. S.A. Poultry, Pigeon and Bird Magazine 52: 199.
  McNUTT, S. H. (1962). Vaccination of Poultry. J. Am. V. M. Ass. 69: 472.
  WILSON, J. E. (1946). Fowl Typhoid. Certain aspects of the experimentally produced disease. Vet. Rec. 58: 269.
  HALL, W. J., MACDONALD, A. D. and LEGENHAUSEN, D. H. (1949). Studies on Fowl Typhoid. II. Control of the Disease. Poultr. Sci. 28: 789.
  HENNING, M. W. (1953a). Calf paratyphoid. II. Artificial Immunization. Onderstepoort J. Vet. Res. 26 (1): 24
- 68. HENNING, M. W. (1953b). Calf Paratyphoid. III. The transmission of antibodies to newly-born calves. Onder-
- stepoort J. Vet. Res. 26 (1): 45.
  69. SMITH, H. W. (1955a). Observations on experimental fowl typhoid. J. Comp. Path. 65: 37.
  70. SMITH, H. W. (1955c). The chemotherapy of experimental fowl typhoid in fowls. (Gallus domesticus). J.
- Comp. Path. Ther. 67: 55.

  SMITH, H. W. (1956). The use of live vaccines in experimental S. gallinarum infection in chickens with observations on their interference effect. J. Hyg. 54: 419.
- 72. HARBOURNE, J. F. (1957). The control of fowl typhoid in the field by the use of live vaccines. Vet. Rec. 69 (48): 1102
- GORDON, R. F., GARSIDE, J. S. and TUCKER, J. F. (1959). The use of living attenuated vaccines in the control of fowl typhoid. *Vet. Rec.* 71 (15): 300.
  GORDON, R. F. and LUKE, D. (1959). A note on the use of the 9R fowl typhoid vaccine in poultry breeding
- flocks. Vet. Rec. 71:926. HARBOURNE, J. F., WILLIAMS, B. M., PARKER, W. H. and FINCHAM, I. H. (1963). The prevention
- of fowl typhoid in the field using a freeze-dried 9R vaccine. Vet. Rec. 75 (34): 858.

  76. MACKIE and McCARTNEY (1962). Handbook of Bacteriology. Robert Cruickshank—Tenth Edition.

  77. BOTES, H. J. W. (1963). Environmentally induced changes in Salmonella cultures. Onderstepoort J. Vet. Res.
- BELDING, R. D. and MAYER, M. L. (1958). Furazolidone in the treatment of Salmonella infections in turkeys 2. Effect on acute paratyphoid infection in poults. Poultr. Sci. 37: 463. FRANCIS, D. W. (1960). Treatment of natural infection of Salmonella pullorum in day-old chicks with Furazoli-
- done. Avian Dis. 4: 63.

  80. GRUMBLES, L. C., WILLS, F. K. and BONEY, W. A. (1954). Furazolidone in the treatment of fowl typhoid in
- turkeys. J. Am. Vet. Med. Ass. 124: 217.

  HARWOOD, P. D.: Clinical applications of Nitrofurans—Past and Present. Proc. 1st Nat. Symposium on
- WILSON, J. E. (1956). The use of furazolidone in the treatment of infection of day old chicks with S. pullorum, S. gallinarum, S. typhimurium and S. thompson. Vet. Rec. 67: 849.
  WILSON, J. E. (1956). The treatment of carriers of S. pullorum and S. gallinarum with furazolidone. 82.
- Vet. Rec. 68: 748.
- 84. COETZEE, L. (1965). Personal communication.

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# RECENT ADVANCES IN FOOT AND MOUTH DISEASE RESEARCH

P. G. HOWELL

Veterinary Research Institute, Onderstepoort.

(Summary of paper presented to the 60th Scientific Congress of the South African Veterinary Medical Association. September 1965).

It is now almost sixty years since Loeffler and Frosch demonstrated the filterability of the causal agent of foot-and-mouth disease. You will recall that this was in fact the first recognition of an animal pathogen in the group which for many years retained the title of the filter passing organisms. A further claim which this virus has for a special place in history is the fact that it was also the first virus in which antigenic differences between strains were recognised. I think it is true to say that in spite of all these qualifications and the fact that it has received so much attention, it can still claim the distinction of possessing many fundamental biological properties which still remain to intrigue the curious investigator.

The virus has been classified within the group of Picorna viruses which includes the entero viruses of human origin e.g. poliomyelitis, Coxackie and ECHO as well as many viruses of non-human origin but which are pathogenic for animals. Suspensions prepared from infected tissues contain two specific complement fixing components. The infectivity of the virus is confined to the larger of the two particles which measures 25 mu in diameter. By heating this particle to 56°C, or lowering the pH of the suspension to below 6, the infectivity is lost and we are left with a suspension of components indentical with the smaller of the two particles which measures only 7 mu in diameter and which is non-infective. The relationship between these two components has been studied and it is believed that the infective particle is composed of a central ribonucleic acid core, surrounded by a granular structure consisting of these 7 mu protein subunits.

The existance of these two distinct components can be demonstrated by various tech-

niques including agar gel diffusion, ultracentrifugation, chromatography or treatment by fluorocarbons. By the application of these techniques it has been possible to follow the multiplication cycle of the virus within the cytoplasm of the animal cell.

Current opinion accepts that the antigenicity of the foot-and-mouth virus whether live or inactivated is associated with the larger 25 mu particle. Of the remaining physical properties which are of interest to us, perhaps the most important is the resistance to ether, chloroform, bile salts, detergents and heat (85°C/4 hrs) when in the presence of protective colloids. On the otherhand the virus is highly susceptible to changes in pH and at values below 6 it is almost instantaneously inactivated.

At this point I must remind you of the fundamental importance of the living cell in the study of viruses. To the laboratory worker who is continually seeking to develop more sensitive methods and assay techniques the discovery of a new host system invariably heralds another period of discovery and advancement. This is certainly very true of foot-and-mouth disease. Prior to 1950 the guinea-pig was almost exclusively used, but this host exhibited serious limitations since strains had to be adapted before they could be studied and as an assay system for detecting small amounts of natural virus they were of very doubtful value. In 1951 suckling mice were found to be highly susceptible and up to the present they have played an ever increasing role in various studies. Early attempts to propagate foot-and-mouth disease virus in fertile hens' eggs were disappointing and it was not until a lower temperature of incubation and the intravenous route of inoculation was used, that success was eventually claimed. By

this technique virus from cattle could be adapted and thus another very valuable host system. became available. Furthering these investigations the day-old chick was soon added to the list of useful host systems. By far the most spectacular advances however, have been those associated with the introduction of tissue culture. Here again the virus of foot-and-mouth disease was among the first to be propagated in tissue culture. As early as 1931 fragments of embryonic guinea-pig tissue were used to propagate the virus in vitro. From 1955 onwards, the development of monolayer cultures gave considerable impetus and provided the most sensitive assay technique yet available, as well as paving the way for new methods of vaccine development and production.

It is now opportune to turn our attention more specifically to the various problems and see how these host systems in particular, have contributed to recent progress. We would probably not be very far wrong if we suggested that the most important problem is how to bring about effective immunisation.

At present there are 7 recognised serological types of foot-and-mouth disease virus namely O. A, C, SAT, 1, 2, 3, and AS1A 1. Each of these types possess completely distinct immunological characteristics, particularly when evaluated by cross-immunity experiments. By serological methods there is evidence of a group antigenicity, which may vary greatly, depending upon the technique used.

Within each of these main types, evidence accumulated over the past 15 years has revealed the existance of strains of virus having distinct antigenic differences between themselves. They are referred to as subtypes and the demonstration of antigenic differences by either serological methods or cross-immunity experiments are made at a lower level of immunity than in the case of type determination.

We find the real significance of these antigenic differences is therefore related to immunisation, where it has been found that vaccinated cattle are only fully protected when they are exposed or their immunity is challenged, by the homologous subtype strain. Challenge or natural infection by a heterologous subtype may reveal an almost complete lack of immunity. It is some-

times even doubtful if two subtype strains at the extremes of the spectrum of variation can justifiably be classified within the same type. This is usually accomplished by establishing a common relationship to an intermediate strain within the same type.

The problem of effective immunisation thus resolves itself around the question of how to develop a vaccine of sufficient potency and immunogenicity. If for example we wish to build up a strategic reserve in order to have vaccine available in anticipation of an outbreak, it must be of such broad antigenicity as to cover any potential variation in the antigenic composition of the newly emerged field strains.

Alternatively, we must have the technical skill, so that we may be in a position to produce a vaccine homologous to the field strain within the shortest possible time after the presence of the disease has been confirmed.

This need for vaccines of broad antigenicity brings us then to a review of the advances made in the development of live attenuated vaccines. It is generally believed that a live vaccine contains virus in a form which will multiply within the recipient and as far as possible simulate natural infection, without producing clinical evidence of disease. As a result the immunity is expected to be stronger, to last longer and to have a broader spectrum of antigenic effectivity.

Here then we have a good example to illustrate the profound influence which the recently introduced host systems have had on the course of research on this disease. Up to the present time, many subtype strains have been attenuated by the emperical method of serial passage through consecutive groups of mice or fertile hens eggs, with varied success. Sufficient attenuation to render a strain inocuous by passage in either mice or chick embrios may take from 6 months to 2 years to accomplish. Thereafter a further period of potency and safety testing must be undertaken which will take another 3 months to complete. This process is thus time consuming and the chances of success cannot always be assured. In the same way tissue cultures have been used to modify the virus by serial passage. What was at one stage considered to be a major step toward attenuating strains while retaining a broad spectrum of antigenicity, has

in the light of more searching tests shown that this system holds no advantages over the previously mentioned laboratory animals.

One of the disappointments which have been revealed by work with these strains is the fact that in many cases a high dosage level is still required, suggesting that in all probability the modified virus, although live, is nevertheless unable to multiply, thus discounting one of the main advantages of this type of vaccine. Other strains again require very accurate inoculation, to ensure that all the virus is deposited within the substance of the muscle, while others have become so modified that they will only produce a good immune response if they are administered intradermolingually which you will all agree is a highly impractical technique under field conditions. Probably one of the most distressing features of all, has been the variable susceptibility exhibited by different species, or even between breeds within the species, to a modified strain vaccine. This is particularly true where in order to retain antigenicity, vaccines have been prepared from relatively low passage material. To quote an extreme yet pertinant example, many of the modified vaccines which by standard techniques are fully attenuated for cattle, may still produce a virulent clinical and contagious infection when administered to pigs.

As a counter to this element of uncertainty and in an endeavour to place this emperical process of modification on a more scientific foundation, studies have been conducted on the genetics of virus strains. Here the practical application is directed to an attempt to develop and define "genetic markers" that is, finite physical or biological properties which will make it possible to accurately characterise a particular strain of virus at any stage of attenuation or alternatively to provide data which will enable a worker to predict the probable behaviour of a strain under specific conditions.

Some of the properties which have been considered in this regard are the resistance of the virus to heat, pH changes, reproductive ability at elevated temperatures and pathogenicity for various hosts or cell types. The general feeling at this stage is again one of disappointment, since there appears to be few if any markers which can be used to identify levels of attenuation amongst the numerous strains studied.

At this stage it is appropriate to reconsider the role of tissue culture in the development of attenuated vaccines. Apart from their role of providing a host system for serial passage the living cell cultivated on glass has had some refined applications. Notweorthy amongst these is the plaque technique, whereby individual infective units can be assayed, clones selected and highly reproducible, and accurate serological tests can be undertaken.

In an attempt to circumvent the prolonged process of host to host passage, the plaque technique has been used to select from a heterogenous virus population e.g. at a low passage level, those infective particles which exhibit the inherent properties of an attenuated strain. Similarly it is being used as a method of purifying seed viruses, thus ensuring as far as possible a homogenous attenuated vaccine with stable properties.

While it is certainly an achievement to be able to modify strains for attenuated vaccines, it is still another problem to be able to produce them in sufficient quantities and it is in this regard that we once again appreciate the immense value of tissue culture. In this regard one should bear in mind the limitations of small animal colonies, the production of suckling mice is a demanding occupation and inevitably the greatest difficulties are encountered when some unexpected demand is made.

Before proceeding it is opportune at this stage to consider another type of vaccine which is also intimately dependant upon the survival or growth of animal cells in vitro. This was in fact the first vaccine ever used in the control of foot-and-mouth disease, namely the inactivated Here again most attention has been directed to the improvement of the potency of the product. This has been achieved by the consideration of alternate methods of inactivation, involving chiefly chemicals which will act specifically upon the RNA and modify as little as possible the protein coat. As an adjuvant, saponin has found favour particularly in vaccines intended for use in pigs. Fundamental to all these investigations however is the fact that the ultimate potency of a vaccine of this type is dependant upon the mass of antigenic material (i.e. 25 mu particles) which is administered.

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Thus it will be seen that common to both types of vaccine is the basic technological problem of how to produce sufficient virus or "antigen" to meet the ever increasing demands of production.

In Europe some laboratories have prefered to continue with the well established technique of cultivating the virus in fragments of surviving bovine tongue epithelium. Virus yields have been increased by stimulating more active metabolism of the cells under accurately controlled environmental conditions and through the supply of essential nutrients in the media. More recent has been the use of trypsinized cells which may be divided into two categories, either primary cultures or established line cells.

The successful cultivation of these cells in large quantities has been achieved in one European laboratory by the scaling up of the rolling bottle technique. In this particular laboratory a combination of ingenuity and engineering skill has evolved a system in which 10,000 plasma bottle cultures of calf kidney cells are produced each week.

An alternative system of bulk propagation has been developed from the experience gained by the culture of bacteria in large fermentor type tanks. Technical developments have made it possible to create an optimal environment where temperature, gas exchange, pH and the supply of nutrients can be accurately controlled. At present the one disadvantage of this system is the fact that only cells with potential carcinogenic properties can be grown under these conditions. Considerable impetus was given to this technique by the development of a cloned line of cells originating from baby hampster kidney tissue. This cell not only multiplies very prolifically but is highly susceptible to foot-and-mouth disease virus and has as a result played an important role in the tissue culture studies previously mentioned.

Having successfully produced our vaccine we are still faced with the problem of potency and safety testing. There is no doubt that the potency testing and definition of standards of efficacy is still one of the most contentious issues amongst workers in this field and standards might vary considerably e.g. between a vaccine prepared for routine prophylactic immunisation as against

a vaccine prepared at short notice to meet a state of emergency. Apart from the various theoretical and technical difficulties the problem of effective testing is closely linked with economic considerations—the problem of bulk storage over long periods particularly in smaller laboratories and the cost of experimental animals. Earlier workers established very accurate and reproducible methods using adult cattle but as their use became more impractical, attention was once again given to the smaller laboratory animals.

Experience over a number of years has revealed a good correlation between the concentration of neutralising antibodies developing after the administration of vaccine and the protection which the animal will have to subsequent exposure. This assay of neutralising antibodies can also be used as an indication of the potency of a vaccine. As a result recent investigations have shown that day-old chicks will show an immune response in relation to the potency of a vaccine or alternatively use can be made of the colostrol transfer of immunity where pregnant female mice are vaccinated and their young, which you will recall are highly susceptible to field virus, are challenged at a set period after These techniques are all nevertheless still subject to the variations which might be encountered in any biological system and current research is attempting to develop in vitro techniques e.g. complement fixation as a means of measuring the amount of effective antigen in a given dose of vaccine.

Under the heading of epizootology or the consideration of all those factors which determine the occurrence of disease, there are many facets which are as much the concern of the worker in the laboratory as the officer in the field. It should be remembered that no complete survey work can be undertaken without efficient and reliable serological techniques. A notable contribution in this direction has been the development of the colour test. By observing the changes in the colour of a tissue culture system in the presence or absence of the destructive effect of virus, it is possible to measure the neutralising antibody content of a serum sample added to the system. As a result it is now possible to determine the immune status of large groups of animals e.g. game or domestic stock, to a

variety of sub-types without much difficulty. This data will have very useful application in the effect of immune populations on the development of subtypes.

The creation of these immune populations by immunisation has led to the use of vaccine in species which were hitherto neglected in the epizootological cycle. One of the most distressing features of this work has been the difficulties encountered in bringing about an effective immune status in pigs. This particular idiosynchracy of the pig, together with the important role which it plays in agriculture in Europe, presents what must be one of the most perplexing problems in the effective control of the disease today. In general the post vaccinal humerol immunity in pigs is low, irregular and of short duration. Some workers believe that there is no correlation between the neutralising antibody titre and the protective value of a vaccine when determined in pigs.

Irrespective of whether a live or inactivated vaccine is considered, the problem is similar and, unless massive doses of antigen are given e.g. twice the normal cattle dose, no immunity can be demonstrated. Present research is aimed at determining the effect of the repeated administration of vaccine at short intervals which will have the inevitable result of placing even greater demands on the supply of vaccine.

The frequent reference to the effects of stress on the course of disease has prompted a consideration of the influence of these factors on foot-and-mouth disease, particularly in regard to the response of animals to vaccination by the live attenuated vaccines. While it is probably impossible to replicate in the laboratory the conditions under which domestic stock may be subjected, they can to some extent be applied to small laboratory hosts. Here we note a return to the usefulness of the guinea pig, in which host it was observed that the type of floor or even the type of food, may be the determining factor in the severity of the clinical disease and to an extent, the immunological response.

In conclusion I would like to recapitulate briefly what I hope you will have grasped as the underlying theme of this paper, namely that although much has been achieved in this particular field of research, a great wealth of material awaits the interested worker. To what degree success can be claimed will depend upon the criteria that have been set. We should bear in mind that within the past few years the disease has continued to spread to new continents while at the same time within the enzootic areas new variant subtypes have emerged. Our efforts to halt these natural phenomena have perhaps been no more than temporary delaying tactics. Certainly the ultimate remains to be achieved.

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# THE EARLY DIAGNOSIS OF PREGNANCY, THE DEVELOPMENT OF THE FOETAL MEMBRANES AND NIDATION IN THE MARE.

# C. H. VAN NIEKERK

Faculty of Veterinary Science, Onderstepoort

Paper delivered at the Sixtieth Annual Congress of the S.A.V.M.A. at Onderstepoort

# SUMMARY

A method is discussed whereby a diagnosis of pregnancy in the mare can be made as early as 14 days after ovulation. The development and growth of the foetal membranes was studied and recorded. Placentation was found to begin as early as 25 days after ovulation in the form of a band which develops on the allantochorion adjacent to the yolk-sac.

# Introduction

The low foaling percentage in the Republic of South Africa, as well as in many other countries, continues to present a problem. For the investigation of one possible factor responsible for low foal crops, namely early embryonic death and resorption, it is essential that a definite diagnosis of pregnancy be made as early as possible after fertilization.

Research workers have done much to probe this problem and developed tests such as the mucin test<sup>1</sup>. Most of these proved unreliable in the accurate determination of early pregnancy. The Ascheim Zondek test is only of value from 40 days after ovulation up to about 120 days.

The only practical method for a definite diagnosis of pregnancy before 40 days, is by rectal palpation of the uterine horns, uterine body and cervix as described by van Niekerk<sup>2</sup>. Since this publication, further investigations have been carried out.

In the paper presented to the conference thirty six transparencies of the ventral aspect of the pregnant and non-pregnant uteri of mares slaughtered from 2 hours to 34 days after ovulation, were projected to demonstrate the difference in size and tone and the thickness of the wall of the uterine horns between the pregnant and non-pregnant uterus at various stages of pregnancy. These differences were also illustrated graphically by van Niekerk<sup>3</sup>. Transparencies showing the variations in size of the embryonic mass from 16 to 34 days after ovulation were also shown.

Berliner<sup>4</sup> stated, "... the structure and development of the foetal membranes and placenta of the horse at consecutive stages of pregnancy still awaits an exhaustive examination". Development of the allantois with special reference to implantation will be described.

# MATERIAL AND METHODS

Mares of the light farm type were used. All mares were teased daily by active healthy stallions. Data from daily rectal examinations, including the size, thickness and tone of the uterus, cervix and embryonic mass and its position in the uterus were recorded. The day of ovulation was determined by rectal examination of the ovaries.

Eighty five mares were slaughtered at different stages of the oestrous cycle and pregnancy from 2 hours up to 40 days after ovulation. Measurements, photos and transparencies of the uterus, cervix and foetal bulge were taken. The size of the blastocyst and the embryonic mass were also recorded and photographed from 9 days to 40 days after ovulation. The sizes of the foetal sacs were measured separately. Bouins fixative was used to fix the foetal membranes for histological examination.

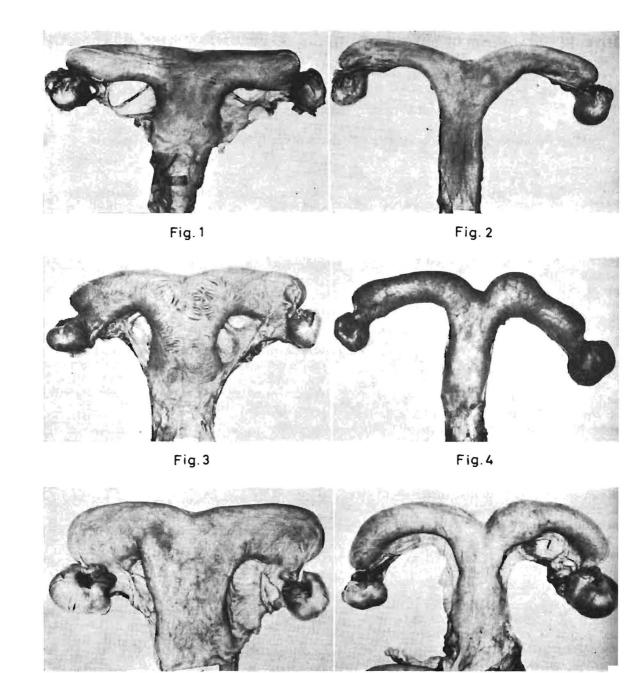


Plate 1.

Fig. 5

Fig.6

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# RESULTS AND DISCUSSION

Up to the 10th day of pregnancy very little difference in thickness and tone between the pregnant and non-pregnant uterine horns was observed.

As the corpus luteum degenerates after the 10th day, in non-pregnant mares, a marked decrease in tone and to a lesser degree in thickness of the uterine wall, sets in.

Where conception occurred, a marked increase in uterine wall thickness and tone were observed from the 11th to the 18th day after ovulation with a further gradual increase up to the 25th day. These increases in tone and thickness of the uterine wall are caused by hypertrophy and hyperplasia of the endometrial glands. The myometrium also undergoes hypertrophy as do the blood vessels.

Plate 1 demonstrates this marked difference in tone and thickness between the pregnant and non-pregnant uteri.

Figs. 1 and 2 show the ventral view of the uteri of a non-pregnant and pregnant mare 14 days after ovulation. At this early stage a marked difference in uterine tone and thickness between the non-pregnant and pregnant mare is noticeable and palpable rectally. In the non-

# PLATE 1.

Ventral aspect of the non-pregnant and pregnant uteri of mares.

# Fig. 1.

Non-pregnant uterus, 14 days after ovulation.

# Fig. 2.

Pregnant uterus, 14 days after ovulation with ventral bulge in left horn.

### Fig. 3.

Non-pregnant uterus, 17 days after ovulation-Age of mare 4 years.

# Fig. 4.

Pregnant uterus, 17 days after ovulation. Embryonic bulge in left horn 2.6 cm, dorso ventral. Age of mare 10 years.

# Fig. 5.

Non-pregnant uterus 20 days after ovulation. Age of mare 4 years.

# Fig. 6.

Pregnant uterus, 20 days after ovulation. Embryonic bulge in left horn 2.8 cm. dorso ventral. Age of mare 4 years. pregnant mare (Fig. 1) it is difficult to palpate the cervix rectally as most of the cervical tone is lost but in the pregnant mare (Fig. 2) the cervix could be palpated as a round, hard and fleshy structure

In Fig. 2 a round ventral bulge, caused by the blastocyst, can be observed in the left horn a few centimetres to the left of the bifurcation. This bulge is not palpable rectally. The marked difference in tone and thickness of the walls of the uterine horns, uterine body and cervix make a diagnosis of pregnancy by rectal palpation fairly easy at this stage. The blastocyst itself measures  $2\frac{1}{2}$  by  $1\frac{1}{2}$  cm.

At 16 days the blastocyst is 5 cm in diameter and the embryonic mass can be palpated in most mares as a ventral bulge the size of a pigeon's egg, 2.4 cm in diameter dorsoventrally.

Figs. 3 and 4 show the ventral aspect of the uteri of a non-pregnant and pregnant mare 17 days after ovulation.

The wall of the uterus of the non-pregnant mare is relatively thin, soft, flabby and without tone and can be moved between thumb and forefinger when palpated rectally. The cervix is relaxed and difficult to pick up rectally. The horns and body of the pregnant uterus (Fig. 4) are round and tubular, firm, resembling a roll of sponge rubber and cannot be compressed completely. In comparison with the non-pregnant uterus it is very easy to palpate the pregnant uterus and cervix 17 days after ovulation. The ventral bulge of 2.6 cm. diameter dorso-ventrally is readily palpable at this stage.

The blastocyst (yolk-sac) measures 6.1 cm. in diameter and remains more or less the same size up to 21 days after ovulation when the allantois commences to develop.

Figs. 5 and 6 are of the uteri of a non-pregnant and pregnant mare 20 days after ovulation. Both mares were of the same type, age and weight. Again, the differences in tone and thickness of the walls of the uterus between the two, are marked. The embryonic bulge in the horn of the pregnant uterus (Fig. 6) measures 2.8 cm. in diameter dorsoventral and the blastocyst 6.1 cm. in diameter.

Both mares had large follicles and both were on heat when slaughtered.

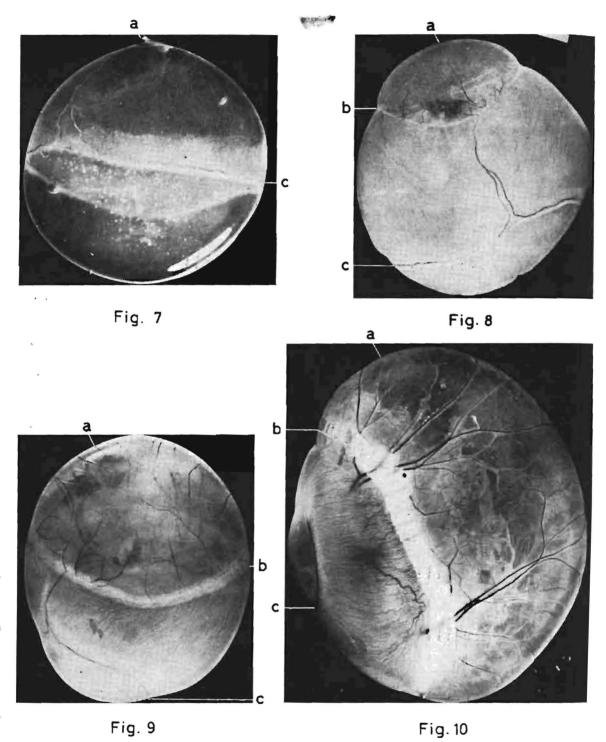


Plate 2.

Plate 2: Figs. 7 to 10 show embryos, within their foetal membranes, of mares slaughtered 21, 25, 27 and 34 days respectively, after ovulation. The size of the yolk-sac of a 21 day old embryo (Fig. 7) is 6.7 by 6.4 cm. The distance from the embryo to the sinus terminalis (c) measures 3.3 cm. and from the sinus terminalis to the opposite pole 3.4 cm.

The allantois was first noticed on the 21st day as a small bulge from the posterior gut of the embryo (Fig. 7 point a). The crown-rump size of the embryo is 7.5 mm. Twenty five days after ovulation the allantois (Fig. 8.a) measures 2.0 cm. from its pole to the yolk-sac junction and the yolk-sac 4.8 cm. The size of the embryonal vesicle (allantois and yolk-sac) is  $6.8 \times 6.6$  cm.

The allantois grows rapidly over the yolk-sac. At 27 days after ovulation the diameter of the allantois (Fig. 9.a) is 4.1 cm. and that of the yolk-sac 5.7 cm. At 34 days the allantoic-sac (Fig. 10.a) measures 6 cm. and the yolk-sac 3 cm. in diameter. The allantois has enveloped the yolk-sac at 40 days after ovulation.

Harvey<sup>5</sup> reported that implantation does not occur in the mare for a period of about 8 weeks, at which time chorionic villi begin to penetrate into the uterine sulci. Prior to this time the chorion is held in apposition to the uterine mucosa by pressure of the foetal fluids.

Zietzschmann and Krölling<sup>6</sup>, state that up to 5 weeks of age the embryonal mass remains unattached in the caudal third of the uterine horn

PLATE 2 Embryos.

Fig. 7

Embryo (a) 21 days old, 7.5 mm. long, on the dorsal aspect of yolk-sac. Allantois just beginning to develop.

FIG. 8.

Embryo 25 days after ovulation.

Fig. 9.

Embryo 27 days after ovulation.

Fig. 10

Embryo 34 days after ovulation.

a = Allantois.

White band on chorion which presents area of proliferation and invagination of trophoblasts.
 Sinus terminalis.

and only at approximately the 14th week does the chorionic sac become intimately attached to the endometrium.

After fixing the foetal membranes in Bouins fluid a white band (Plate 2.b) became visible in the chorionic surface at the allanto-chorionic and yolk-sac junction. The chorion in this area was always attached to the endometrium. Histological sections through this band revealed proliferation and invagination of the trophoblastic wall of the allanto-chorionic membrane. The epithelium of the chorion in this area changes from flat to cuboidal to columnar (See Fig. 11).



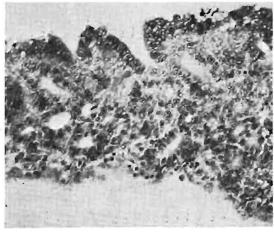


Fig. II: Section through the area of proliferation of the chorion.

Above: Under low power. Below: Under high power.

Twenty five days after ovulation the first signs of this white band, 1 mm. wide, was noticeable (Fig. 8.b). At 27 days it was 3 mm. wide (Fig.

9.b), and 7 mm. wide 34 days after ovulation, (Fig. 10.b). The proliferation, with villi formation, spread from this band over the chorionicsac mainly along the bloodvessels (Plate 2, Fig. 10). At approximately 40 days after ovulation, villi formation has spread over the whole chorionic surface except for the areas in the lumen of the uterine horn pointing towards the fallopian tube and the area bulging into the body of the uterus pointing towards the cervix. Areas over the endometrial cups were also devoid of villi formation.

As the allantois grows over the yolk-sac this band reaches the yolk-sac placenta 40 days after ovulation.

This work indicates that nidation in the mare occurs as early as 25 days after ovulation. This is considered an important factor in the explanation of embryonic resorption which, according to van Niekerk<sup>3</sup>, occurs between 25 and 30 days after ovulation in mares on poor nutrition. During this period placentation changes from a yolk-sac placenta to chorio-allantoic placentation. It would appear that when the embryo reaches an age of approximately 30 days there is sufficient attachment between the allanto-chorion and endometrium to ensure adequate foetal nutritional requirements. Van Niekerk<sup>3</sup> found that malnutrition did not cause embryonic resorption after 30 days of gestation.

### Conclusions

- 1. With some experience it is possible to make a diagnosis of pregnancy at 14 days and a definite diagnosis of pregnancy 16 to 19 days after ovulation by rectal palpation of the uterine horns, uterine body and cervix, and by feeling the embryonic mass which forms a ventral bulge in the uterine horn a few centimetres to the left or right of the uterine bifurcation.
- 2. The first signs of allantoic development was noticed 21 days after ovulation.
- 3. Allanto-chorionic attachment to the endometrium begins in the form of a band on the allanto-chorion and yolk-sac junction 25 days after ovulation. This is given as a reason why resorption due to malnutrition does not occur beyond 30 days of gestation.
- 4. Between the 25th and the 30th days of pregnancy placentation changes from a yolk-sac placenta to a chorio-allantoic placentation and this is probably the reason why embryonal resorption, due to poor nutrition, only occurs between 25 and 30 days after ovulation.

# REFERENCES

- DAY, F. T. and MULLER, W. M. C., 1940. A Comparison of the efficiency of methods of diagnosing equine pregnancy with special reference to the mucin test. Vet. Rec. 52, p. 711-716.
   VAN NIEKERK, C. H., 1965. Early Clinical Diagnosis of Pregnancy in Mares. J. S. Afr. vet. med. Ass. 36, 53.
   VAN NIEKERK, C. H., 1965. Early Embryonic Resorption in Mares. J. S. Afr. vet. med. Ass. 36, 61.
- 4. BERLINER, V. R., 1963. Equine Medicine and Surgery, 1st ed., Chapter 23, p. 611. American Veterinary Publications.
- HARVEY, E. B., 1959. Reproduction in Domestic Animals. Ed. H. H. Cole and P. T. Cupps. p. 435. Academic Press New York.
- ZIETZSCHMANN, O. and KRÖLLING, O., 1955. Lehrbuch der Entwicklungsgeschichte der Haustiere. p. 134. Paul Parey, Berlin.



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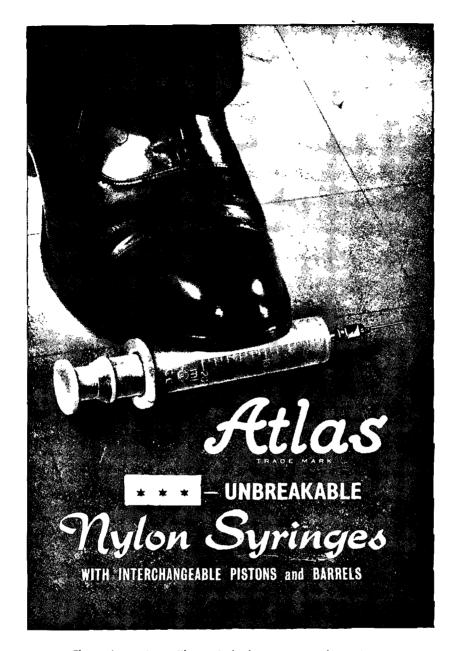
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# ADRENAL FUNCTION AND FERTILITY

# S. J. VAN RENSBURG

Veterinary Research Institute, Onderstepoort

Read in part at the 60th Annual Conference of the S.A.V.M.A. September 1965.

# SUMMARY

Specific involvement of the adrenal in the biochemical, physiological and pathological aspects of reproduction in animals and man are discussed, and syndromes particularly referred to include ovarian inactivity, cystic ovaries, seminal changes in males, prolonged gestation and gestational failure.

Experimental studies demonstrate a profound increase in hypophyseal follicle stimulating hormone when hydrocortisone acetate is administered to ovariectomized sheep. In intact goats this steroid potentiated the growth of luteal phase follicles and reduced the size and progesterone concentration of corpora lutea. Habitually aborting Angora goats had significantly higher blood cortisol levels after adrenocorticotrophin injection; and in aborted foetusses adrenal hyperplasia in conjunction with thymic involution was regularly present.

The level of adrenal function may alter pituitary gonadotrophin in some species by virtue of a direct stimulatory action of glucocorticosteroids on FSH, and indirectly by altering gonad steroid production. androgens may inhibit hypophyseal luteinising hormone particularly in species where the secretion is normally relatively high. Evidence that adrenal secretions, probably androgens, are responsible for the formation of cystic corpora lutea in hyperadrenocorticism, is presented. Available experimental data on the effects of adrenal hormones on the uterus is discussed in relation to the clinical evidence of potent lethal effects on the foetus; the hypothesis that placentfunction is impaired by antagonism of progesterone action is favoured. Attention is drawn to the possible participation of the foetal pituitary-adrenal axis in pregnancy termination.

# Introduction

In view of the common embryological origin of both the gonads and adrenals from the urogenital ridge, the existence of some interplay between these organs is not surprising. The clinical recognition of virilizing and feminizing syndromes following hyperplasia and adrenal cortical tumours has exemplified this association.

Early clinical experience with Addison's disease and experiments involving bilateral adrenalectomy of females have clearly demonstrated the dependence of normal reproductive function on the presence of adrenocortical hormones. Such subjects cease to cycle and the ovaries and uterus become small, but reproductive function may be restored by administration of adrenal steroids, and a quantitative response has been demonstrated1. From their work on ovarian compensatory hypertrophy following unilateral castration, Edgren and Peterson<sup>2</sup> concluded that the absence of adrenocortical hormones disrupts cellular metabolism probably at the level of the ovary, so that the ovary is not capable of a growth response to increased gonadotrophin.

Elucidation of the main pathways of steroid biosynthesis in recent times has to a large extent clarified adrenal-gonad interrelationships. Briefly, acetate is converted to cholesterol, and from this complex steroid all physiologically active steriod hormones are formed by a sequence of degradations and modifications controlled by genetically determined enzyme systems. In the ovary, cholesterol will be converted to progesterone, androgens are formed from progesterone and the androgens are converted to oestrogens. Luteal tissue does not possess enzyme systems for the further conversion of progesterone, and it is therefore secreted as such, but follicle wall

tissue rapidly converts progesterone to oestrogens.

In adrenocortical tissue the same pathway of cholesterol-progestins- androgens-oestrogens exists; however there are additional enzymes which convert most of the progestins and androgens to adrenocortical hormones such as cortisone and cortisol (hydrocortisone). The well known congenital adrenal hyperplasia in man is caused simply by defects in these systems (11 and/or 21 hydroxylases), resulting in the formation of an inactive intermediate. This inactive metabolite cannot exert the usual negative feedback control on pituitary ACTH, which results in excessive stimulation of the adrenals and hence the secretion of enormous amounts of sex hormones.

Defects of enzyme systems are now relatively frequently identified in the human, and though they probably occur in domestic animals, none have been precisely clarified. The very nature of animal selection and elimination, natural and otherwise, would tend to eliminate such individuals. Modern animal breeding practices however embrace the intensive exploitation of production characteristics, in which the variety of hormones influencing growth, lactation, and coat must inevitably be involved. The adrenal gland is intimately concerned in all these processes, as it is in reproduction. The variety of physiological and pathological factors which may influence adrenal function are not within the scope of this discussion, but it is well to note that within a species the genotype may markedly influence the level and nature of adrenocorticosteroid metabolism3,4,5.

# REPRODUCTION SYNDROMES INVOLVING ADRENAL FUNCTION

# Ovarian Inactivity

Döcke and Worch<sup>6</sup> assessed adrenal function in heifers by determining the reduction of circulating eosinophiles 6-8 hours after intramuscular injection of 350 i.u. ACTH, and considered a reduction of 50 per cent or more as a positive response. In normal animals the test was positive, as also in animals on a deficient diet which had retained normal ovarian function. Heifers on the deficient diet with ovarian atrophy and anoestrus responded negatively to this test. Experiments on laboratory animals

have failed to reval any reduction of pituitary gonadotrophin as a consequence of severe protein restriction. Anovulatory cycles in the human are treated with considerable success with adrenal corticosteroids, generally administered during the follicular phase of the cycle.

Of considerable interest are the recent observations by Australian workers<sup>8,9</sup> that transportation stress induces follicular growth and ovulation in anoestrus ewes. After travelling considerable distances, up to 75 per cent of induced ovulations were found when examined five days later. No psychic exhibition of oestrus occurs, which is to be expected in the absence of a corpus luteum preceding the induced ovulation. Braden and Moule<sup>8</sup> failed to induce this phenomenon by injections of ACTH, but observed that severe stress seemed to induce cystic corpora lutea.

Hunter<sup>10</sup> studied ovarian activity in sheep from three different regions, in which differences in the nutritional value of the natural vegetation are recognised. Well defined seasonal trends in the incidence of ovulation were found in the two areas in close proximity to the abattoir, but in the third and most distant region, ovulations were frequent and constant throughout the year.

# Cystic ovaries

In cattle, the marked adrenal hyperplasia associated with ovarian cysts was established by Garm<sup>11</sup>. The increase is mainly due to hypertrophy of the adrenal cortex, and in particular the zona glomerulosa. On these grounds he concluded an increased production of mineralocorticoids. He postulates that the primary defect is the increased production of pituitary FSH, and a failure to produce sufficient LH for ovulation. The prolonged secretion of oestrogen would then result in adrenal cortical stimulation by increasing ACTH release. He also claimed evidence of increased adrenal androgen secretion. The primary defect suggested in this hypothesis does not bear a plausable association with the fact that it is primarily the high producing cow which is afflicted.

Cupps, Laben and Mead<sup>12</sup> confirmed the adrenal hyperplasia in cows with cystic ovaries, but emphasised the involvement of the fasicular zone. The ability of adrenal tissue to convert

acetate to adrenal steroids has been studied in vitro<sup>13</sup>. Homogenates from cows with cysts converted acetate at much slower rates than those from normal animals.

The variety of clinical syndromes and variable histopathology associated with ovarian cysts in cattle suggest the possibility of multiple causes, and this should be considered in future studies. Numerous studies have failed to identify a convincing primary defect. Undoubtedly there are significant disturbances in gonadotrophin secretion, but they must frequently be transient, as evidenced by the high recovery rate both spontaneously and following therapy. Maximal lactational stress is also important in the occurrence of cysts<sup>11</sup>. Involvement of the adrenal, is however, much complicated by the stimulatory effect of prolonged oestrogen exposure. Nevertheless, the marked influence of adrenal corticoid hormones on gonadotrophins (see below), warrants careful consideration of the role played by this gland.

In man, polycystic ovaries frequently accompanied by hirsuitism, (Stein-Leventhal syndrome) is relatively common. In the follicular cysts, steroid biosynthesis proceeds to the androgen stage and conversion to oestrogens appears to be impaired. This does not appear to be a specific enzyme defect, as stimulation with gonadotrophins result in the excretion of large amounts of oestrogens<sup>14</sup>. This effect is also obtained with dexamethasone treatment15. Follicular cysts, and Stein-Leventhal cases do appear to improve after prolonged Prednisone treatment16. On the other hand, the Stein-Leventhal syndrome has also been reported to be a manifestation of hyperadrenocorticism<sup>17,18</sup>. From these results it would appear possible, that adrenal androgen suppression of pituitary gonadotrophins could be implicated; treatment with synthetic glucocorticoid drugs would suppress the adrenals and remove the inhibition.

# Adrenal hypertrophy in bulls

The semen of bulls with fascicular adrenal tumours, and hypertrophy of the fascicular-reticular zones, characteristically have increased sperm concentration, and total sperm per ejaculate, while the fructose content is reduced. Administration of the acetates of cortisone and cortisol induced identical changes in the semen

of normal bulls<sup>19,20</sup>. Furthermore, these workers have presented indirect evidence that cortisol and estrogen may be secreted in abnormally high amounts in the same animal. Adrenal cortical tissue containing hyperplastic fascicular nodules from these bulls has been subjected to incubation studies with added progesterone, which was found to be converted to cortisol at a very much greater rate than normal bull adrenal tissue<sup>21</sup>. These results strongly suggest primary adrenal involvement, but interaction with a possibly deranged steroid metabolism in the testes and actual blood levels of glucocorticosteroids require further study.

Conversely, degenerative changes of the testes seminiferous epithelium have been associated with glomerular zone hypertrophy of the adrenal cortex in a large number of bulls<sup>22</sup>. Renal degenerative changes were found to be extensive in those animals examined histologically and this suggests that impaired kidney function was the major lesion. After adrenalectomy, however, the semen of one of three bulls improved, indicating primary involvement of the adrenal in this animal.

# Prolonged gestation

In the U.S.A., two syndromes in cattle, conditioned by genetic factors, are recognized. In the Guernsey breed, post mature foetusses always exhibit adenohypophyseal aplasia, and physically the foetus is premature in development. In contrast, Holstein-Friesian postmature calves are unusually large at delivery. The adenohypophysis is present, but smaller than normal, and the calves die in a hypoglycemic coma within eight hours of delivery.

Adrenal function has been investigated in these post mature Holstein-Friesian calves and all found to exhibit hypoadrenocorticism<sup>23</sup>; replacement therapy with adrenal corticosteroids is essential for survival.

In the cases of pregnancy in Addisonian patients reviewed by Osler<sup>24</sup> the infants were small and gestation was a mean of 13 days longer than usual.

# Gestational failure

Pregnancy in the classical Cushing's syndrome, (hyperadrenalism) is said to be uncommon and rarely does the infant progress to full term<sup>25</sup>. As

the foetal wastage is so high, and severe adverse maternal effects common, the interruption of early pregnancy is even considered. However in Addison's disease there is also an increased incidence of stillborns, prematures, and abortions. Term infants born to mothers with Addison's disease do as well as those born to normal mothers.

While studying the excretion of 17-hydroxy-corticosteroids in human pregnancy, Appelby and Norymberski<sup>26</sup> encountered two cases of foetal death associated with excessive excretion of these hormones. High values were encountered a month before intrauterine death, and excretion was found to fall sharply after the death of the foetus, even though pregnanediol continued to rise and remained higher than normal.

No direct hormonal studies involving corticosteroids and infertility in domestic animals have previously been reported. In cattle lower blood sodium and higher potassium levels have been associated with repeat-breeder cows, and a possible relationship of adrenocortical insufficiency to sterility discussed<sup>27</sup>. Following a similar study on sheep, Turner and Koch<sup>28</sup> suggested that Merino ewes with blood potassium values near the mode for the flock may have a better reproductive performance than those outside the model class.

In Angora goats, an exceptionally severe problem of spontaneous and usually habitually recurring abortion exists. After an extensive investigation, van Heerden<sup>29</sup> concluded that gestation failed due to failure of endocrine mechanisms maintaining gestation. Studies of the morphology of the endocrine organs of anoestrus normal and aborter goats revealed consistent adrenal hyperplasia in the aborter does<sup>30,31</sup>. Furthermore, blood leucocytes of pregnant aborters exhibited a significant "shift to the left" and the oesinophiles were reduced. Corpora lutea were retarded in growth and markedly cystic in cycling animals. These luteal abberations were reproduced in normal animals in which adrenal hyperplasia had been induced to a similar extent by exogenous ACTH. "Pregnanediol" as measured by the method of Klopper, Michie and Brown<sup>32</sup> was consistently lower but found to be a poor index of luteal function because of the large adrenal contribution. Injections of ACTH markedly reduced pregnanediol excretion. Mohair production and fibre diameter were reduced, and on the basis of known actions of corticosteroids on hair growth, it was postulated that selection for fleece quality favoured animals with adrenal hyperplasia.

# EXPERIMENTAL STUDIES ON ADRENAL HYPER-FUNCTION

1. Blood cortisol in habitually aborting Angora goats

Procedure. Normal and aborter does were injected with 40 i.u. ACTH ("Acton", Frederiksberg Chem. Labs.) intravenously over 10 seconds, and 80 ml. blood withdrawn from the jugular vein one hour later. After centrifugation for 20 minutes, the heparinized plasma was stored at -20°C until assay.

The thawed plasma, adjusted to pH 8.5, was extracted twice with two volumes ethyl acetate. and the extracts washed twice with 1/20th volume water. After drying in a rotary evaporator, the residue was redissolved in 30 ml. 70 per cent methanol and extracted three times with equal volumes hexane. The alcohol was then evaporated to the aqueous layer, made up to 20 mls. with water, and extracted twice with 50 mls. methylene chloride. The dried methylene chloride residue was transfered to small tubes, evaporated under nitrogen, and spotted on methanol washed 1.5 cm, strips of Whatman No. 20 chromatography paper. After descending chromatography in the system benzene: methanol: water 5:2:3 for seven hours, the hormone spots were located by ultraviolet reflex photography, and eluted with 5 mls. methanol in a Zander-Simmer type apparatus. The dried eluate was subjected to a micro-modification of the Porter-Silber reaction (1 ml. total volume) and measured at 380, 410 and 440 mµ, applying Allen's correction.

Results. The capacity of the adrenal cortex to respond to adrenocorticotrophin was examined in a total of 14 does, 7 of which had aborted. Concentration of cortisol in the blood of normal animals averaged 3.67  $\mu$ g/100 ml. plasma (S.E. 0.26, range 2.6–4.7) and in aborters 6.30  $\mu$ g/100 ml. plasma (S.E. 0.64, range 4.0–9.2). This difference is significant (P<0.05); aborter does were much more variable in their response, but only one of the seven fell within the range of

levels found in normal animals. Further current studies indicate that does with values less than 4  $\mu$ g/100 ml. plasma will not abort, and those above 5  $\mu$ g/100 ml. plasma certainly will.

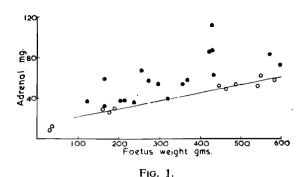
The results presented here do not prove that resting levels are actually any different in the two groups, but exclude the possibility of enzymatic defects in cortisol biosynthesis. The concentration of cortisone was extremely low in a few samples measured, and no obvious differences were apparent. The observed morphological hyperplasia in aborters therefore appears to be due to an increased functional status of the adrenal glands.

# 2. Foetal adrenals and abortion

Procedure. The anterior and posterior pituitary, thyroids, adrenals, thymus, spleen, gonads, kidneys and liver were dissected from freshly aborted Angora foetuses and weighed to the nearest 0.1 mg. Occasional aborted foetuses are grossly oedematous, others are fresh and have a normal appearance, but many are aborted only several weeks after foetal death, as shown by skeletal size and degree of autolysis. The percentage of the bodyweight that each organ constitutes was also calculated, and this data demonstrated the absence of dehydration in aborted foetusses.

Results. Consistent and marked differences between the nine normal and eighteen aborted foetuses were only found in the adrenal and thymus weights. Adrenals were hyperplastic (Fig. 1) and thymus glands atrophic. Thymic atrophy is classically induced by exposure to excessive adrenal corticosteroids, and its presence indicates hyperfunction of the foetal adrenal cortex.

Maternal corticosteroids and ACTH have been found to be at least partially permeable through the placenta in those species studied, though the foetal pituitary also clearly participates in regulating foetal adrenal function. Assuming these findings on laboratory animals are applicable to goats, and that aborter does do



Adrenal hyperplasia in aborted foetusses.

O Normal; • aborted.

have excessive circulating cortisol, an inhibitory effect on foetal ACTH may be expected. Conversely, maternal ACTH diffusing through the placenta will increase adrenal size. The genotype of the foetus may be responsible for the hyperplasia but this seems unlikely as utilising male bucks of different breeds in which no endocrine abortion problem is known does not improve the course of gestation in aborter does29. That a dominant gene causing hyperplasia is present seems unlikely because of the maternal and environmental factors associated with abortion. To conclude, it seems that the regularity of concominant maternal and foetal adrenocortical hyperplasia is possibly due to excessive maternal ACTH. The foetal corticosteroids thus produced may however play an important role in precipitating abortion (see below).

# 3. Influence of cortisol acetate on ovine pituitary FSH

Procedure. Eight mature Merino ewes were randomized into two groups after having been bilaterally ovariectomized two months previously. One group was injected intramuscularly with 200 mg. cortisol acetate in saline suspension for four days while the controls received saline only. The anterior pituitaries were harvested on the morning of the fourth day and kept in each of three changes of cold acetone for 24 hours, after which the glands were stored in a dessicator at 4°C.

The Steelman-Pohley HCG-augmentation assay for FSH was performed on 25 day old Wistar rats using 50 i.u. HCG per rat. A single dose level of 3-5 mg. anterior pituitary powder per rat was administered in 6 injections over 3 days, and the animal autopsied on the morning of the fourth day. Two levels of NIH-FSH-S2, which fell well within the linear response range as previously established for these animals by

assay of five levels of the standard, were run with each assay, and groups of five rats were used for each unknown and standard.

Results. The bodyweights of the sheep at the commencement of the injections and immediately prior to slaughter, their adrenal weights and the concentration and total amount of pituitary FSH are presented in table 1.

TABLE 1.—INFLUENCE OF CORTISOL ACETATE ON PITUITARY FSH IN OVARIECTOMIZED SHEEP.

	D a da	alika (lba )	Adrenal -	ug NIH-FSH-S1 equivalent		
Treatment	Bodyweight (lbs.) Initial Final		weight (gms)	ug/mg. pituitary	mg/total pituitary	
Control	86 86 86 86	86 85 84 85	2.5 3.0 3.4 2.5	15.3 (1.9) 12.7 (2.5) 22.5 (1.5) 12.4 (1.2)	1.9 2.7 3.1 1.8	
	86	85	2.9	15.7	2.4	
Cortisol Acetate	88 90 92 81	88 91 92 81	2.5 2.3 2.3 3.0	36.2 (2.5) 34.4 (1.6) 34.6 (2.9) 30.9 (2.2)	6.7 6.9 7.1 5.6	
	88	88	2.5	34.0	6.6	

Cortisol acetate in saline suspension 200 mg/day for 4 days, pituitaries harvested on morning of fifth day. Figures in parenthesis denote standard error of the mean.

The dosage of cortisol acetate used did not appear to be unphysiological as daily bodyweights were not affected, nor were any differences detectable on inspection of the carcases for fat distribution and muscular development. The treatment however greatly increased the pituitary FSH content, and in the absence of any ovarian steroid feedback these concentrations of FSH probably reflect actual secretion rates. Even within the control group, large adrenals appeared to be associated with increased FSH (table 1). The NIH-FSH-S2 equivalent value for each of the 20 rats used for each group of sheep was expressed in terms of NIH-FSH-S1, the difference between the two groups was highly significant (P < 0.01).

This potentiation of pituitary FSH by cortisol acetate was only demonstrated in ovariectomized ewes, but such an effect in intact anoestrus ewes would explain the stimulation of ovarian activity in transport stressed sheep<sup>8,9</sup> and also

possibly the occurrence of marked follicular growth and oestrus noted at abortion in Angora goats with adrenal hyperplasia. Alternatively this may not be a direct action; it is possible that the exogenous cortisol inhibits the secretion of hormones from the adrenals which may normally exert a suppressive influence on pituitary gonadotrophins.

# 4. Influence of cortisol acetate on ovarian response to gonadotrophin in goats

Procedure. Five 48-hourly intramuscular injections of progesterone (0.25 mg/lb.) were administered to eight anoestrus Angora does and this was followed by a single subcutaneous injection of pregnant mares' serum (12 i.u./lb.) 24 hours after the last progesterone injection. Commencing with the second last progesterone injection, four does received 100 mg cortisol acetate suspension intramuscularly daily until sacrificed nine days after oestrus onset. The does were teased with bucks twice daily through-

out the experiment, and weighed every 48 hours. Progesterone in frozen corpora lutea was determined by the method of Rowlands and Short<sup>33</sup>, using 90 per cent aqueous methanol as the stationary phase and running the chromatograms for 3 hours.

Results. There were no differences in bodyweight, PMS injection — oestrus onset intervals, or duration of oestrus between the two groups. The weight of the uteri of treated animals was also unchanged, the differences found in the ovaries are recorded in table 2.

TABLE 2.—INFLUENCE OF CORTISOL ACETATE ON THE OVARIAN RESPONSE TO GONADOTROPHIN IN ANGORA EWES.

Treatment	Corprora lutea				Fol	Follicle diameters (mm)		
		Mean weight		Progester- one	Politicie diameters (mm)			Mean
	140.	(mg)	(mm)	(ug/g)	1	2	3	1
Control	4 9 3 2	494 459 647 756	2.5 2.2 4.3 7.5	5.0 5.2 4.6 6.7	7.8 9.3 9.0	6.1 7.6 8.0	3.4 6.6 7.7	5.8 7.8 8.2
	4.5	589	4.1	5.4	6.5	5.4	4.4	5.5
Cortisol Acetate	8 2 11 2	455 641 341 619	0.4 2.1 0.1 0	3.0 3.0 4.3 3.0	11.8 16.0 10.1	10.4 13.7 9.0	9.0 13.4 8.2	10.4 14.4 9.1
	5.8	514	0.7	3.3	9.5	8.3	7.7	8.5

Cortisol acetate 100 mg/day commencing on seventh day of progesterone treatment and continuing to autopsy nine days after oestrus onset.

As in our earlier experiments with ACTH administration, growth of the corpora lutea tended to be retarded, but in marked contrast the size of the central cavity was reduced by cortisol acetate. An unexpected finding was a rather consistent reduction in the progesterone concentration in the corpora lutea.

No follicles larger than 3 mm. were present in one animal from each group; in the remainder follicles were consistently larger in the treated group (table 2). Potentiated follicular growth was also found after ACTH administration in both sheep and goats<sup>31</sup>.

# DISCUSSION

Influence of adrenal function on gonadotrophins. Experimentally, the administration of glucocorticosteroids clearly may increase hypophyseal FSH biosynthesis and release. As early as 1951, Soval and Soffer<sup>34</sup> demonstrated that the administration of corticosteroids or ACTH can cause an increase in urinary gonadotrophin excretion in the human; FSH was found to be increased relative to LH<sup>35</sup> and the response is immediate and maintained only during treatment<sup>36</sup>. In this work a marked increase in the

FSH content of ovariectomized ewes resulted from short-term treatment with cortisol acetate.

Experiments such as these would tend to inhibit adrenal steroid secretion, including the sex steroids, by virtue of the suppressive influence on ACTH secretion exerted by the exogenous corticosteroid. In conditions of hyperadrenocorticism excessive oestrogens and particularly androgens may exert effects on pituitary gonadotrophins. Species such as man and rodents which are known to secrete relatively large amounts of adrenal androgens, may then show signs of inhibition of pituitary gonadotrophins<sup>37</sup>; however cattle and sheep appear to secrete only relatively small amounts of adrenal androgens<sup>38</sup> and adrenal virilization is rare. Certainly transport stressed ewes and Angora goats with hyperplastic adrenals show signs of considerable FSH action, while anoestrus cattle appear to have a low level of adrenal function<sup>6</sup>, and anovulatory cycles in man are frequently rectified by exogenous corticosteroids. Though direct studies are lacking, there is no clear evidence that glucocorticosteroids appreciably influence LH secretion, if anything there is possibly some inhibition but this could be due

to androgenic metabolites of the administered steroid.

To conclude, it seems plausable that under pathological circumstances the level of adrenal function may cause profound disturbances in the secretion and ratios of FSH and LH by virtue of the stimulatory and inhibitory actions of its secretions. On these grounds the basic defect in conditions like cystic ovarian disease could be a manifestation of disturbed adrenal function.

Gonadal function. In normal human subjects, the administration of dexamethasome, ACTH and dehydroepiandrosterone increases urinary oestrogen secretion during the follicular phase of the cycle but not during the luteal phase<sup>39</sup>. The Stein-Leventhal syndrome is associated with polycystic ovaries and hyperadrenocorticism<sup>17</sup>; steroidogenesis in the ovarian cysts is blocked at the androgen stage but the administration of FSH appears to overcome the defect resulting in oestrogen secretion. This same effect is obtained with dexamethasone treatment<sup>15</sup>, and it seems most likely that the rise in oestrogen secretion results from increased FSH release.

Oestrogen excretion may be increased, normal or reduced in patients with Cushings syndrome<sup>40</sup>. The ovarian sclerosis and other pathology which is known to accompany androgenic or Cushing oid adrenocortical hyperfunction is stated to bear a superficial resemblence to polycystic ovaries<sup>41</sup>. Understandably, there is a dearth of information on the long term effects of hyperadrenocorticism on pituitary gonadotrophins. In the fourth experimental study reported in this paper, the size of the follicles was considerably increased by cortisol acetate nine days after oestrus onset, while the weight, size of the central cavity and progesterone concentration The luteal inhibition is not was reduced. necessarily due to direct interference with pituitary luteotrophins, but may be a consequence of oestrogen predominance. results are in accord with the shorter oestrous cycles and retarded luteinization we have found in aborter ewes, but preliminary results indicate that the progesterone content of pregnant aborter corpora lutea is normal. Morphologically the corpora lutea of aborter and ACTH treated normal ewes are grossly cystic, yet the cavity is reduced to less than normal with

cortisol acetate treatment. This suggests that another adrenal product capable of inducing cystic corpora lutea, as has been demonstrated with dehydroepiandrosterone in cows<sup>42</sup>, is operative.

Gestation. In rats, ACTH exerts a powerful inhibitory influence on the uterine response to oestrogen, which is abolished by removal of the adrenals<sup>43</sup>. This depression is also produced by purified glucocorticosteroids<sup>44</sup> but the effect appears to be largely limited to water inhibition as the rate of protein, RNA and phospholipid synthesis is hardly affected<sup>45</sup>. Of greater significance is the inhibition of progesterone action on decidual development demonstrated in various experimental designs<sup>46,47</sup>. Unpublished observations in our laboratory clearly demonstrate a selective inhibition of actual placental growth in pregnant rats.

The literature already discussed in this paper emphasises the lethal effects on the foetus of maternal hyper-adrenocorticism in a number of species; the same applies to the rabbit 48,49. Angora goats are remarkable in this respect, in that the degree of elevation of blood cortisol following ACTH reliably detects animals which will abort when bred. Most commonly foetal death occurs several weeks before abortion though this is not always so, and a pathogenesis involving multiple factors which accounts for most of the characteristics of the syndrome has been suggested<sup>31</sup>.

In pregnant sheep, a substantial decrease in the blood cortisol level occurs prior to normal parturition<sup>50</sup>, and our observations indicate that the same applies to goats. This mechanism is obscure, but the involvement of the foetal adrenal is of interest. Adrenal hyperplasia is a consistent feature of aborted Angora foetuses, and in prolonged gestation of cattle the overdue foetuses suffer from adrenal insufficiency23. The drop in maternal corticosteroids prior to parturition could directly induce increased adrenal activity in the foetus. Reduction in placental weight during late pregnancy is exhibited by a number of species<sup>51</sup> and is accompanied by a drop in sodium transfer before parturition. It is therefore tempting to postulate that the foetal pituitary-adrenal axis plays a role in maturation of the placenta and hence the termination of pregnancy.

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# REFERENCES

- 1. CUPPS, P. T., 1955. The ability of certain adrenal steroids to restore reproduction in adrenalectomized female
- COFFS, F. 1., 1933. The ability of certain adrenal steroids to restore reproduction in adrenalectomized female rats. Endocrinology, 57, 1-4.
   EDGREN, R. A. and PETERSON, DE A. L., 1964. The influence of adrenalectomy and corticoid replacement on ovarian compensatory hypertrophy in rats. Acta. Endocr. (Kbh), 47, 485-490.
   SPICKETT, S. G., BADR, F. M., SHIRE, J. G. M., and STEWART, J., 1965. Genetic variability in adrenal structure and function. J. Endocrin., 31. xxvii—xxviii.
   MEDDER, R. J. and COLLINS, R. L., 1965. Histology and weight of the mouse adrenal: a diallel genetic study. J. Endocrin., 31, 95-105.

- study. J. Endocrin., 31, 95-105.

  5. BURSTEIN, S., BHAVNANI, B. R. and KIMBALL, H. L., 1965. Genetic aspects of cortisol hydroxylation in guinea pigs: Urinary excretion and production rates of cortisol and 2 a—hydroxycortisol. Endocrinol gy, 76, 753–716.
- 6. DÖCKE, F. and WORCH, H., 1962. Beziehungen zwischen der Eierstocksfunktion beim weiblichen Rind.
- Zuchthug. Fortpfl Störung. u. Besamung, 6, 28-37.

  SREBNIK, H. H., NELSON, M. M. and SIMPSON, M. E., 1961. Follicle-stimmulating hormone and interstitial-cell-stimulating hormone in pituitary and plasma of intact and ovariectomized protein-deficient rats. Endo-
- ctinology, 68, 317—326. BRADEN, A. W. H. and MOULE, G. R., 1964. Effects of stress on ovarian morphology and oestrous cycles in ewes. Aust. J. Agric. Res., 15, 937-49.
- LANG, D. R., 1964. Ovulations in Merino ewes moved by road or rail in Queensland. Proc. Aust. Soc. Anim. Prod., 5, 53-57.
   HUNTER, G. L., 1959. A contribution to the study of the problem of low fertility among Merino ewes in South Africa. J. Agric Sci., 52, 282-295.
- GARM, O., 1949. A study on bovine nymphomania. Acta Endocr., 2, Suppl. 3. CUPPS, P. T., LABEN, R. C. and MEAD, S. W., 1959. Histology of pituitary, adrenal, and reproductive organs
- CUPPS, P. I., LABEN, R. C. and MEAD, S. W., 1959. Histology of pituitary, adrenal, and reproductive organs in normal cattle and cattle with lowered reproductive efficiency. Hilgardia, 29, 383-410.
   CUPPS, P. T., GARM, O. and LUNAAS, T., 1963. Metabolism of progesterone and sodium acetate by adrenal gland homogenates from normal cows and cows with ovarian cysts. J. Dairy Sci., 46, 550-552.
   CROOKE, A. C., BUTT, W. R., PALMER, R., MORRIS, R., EDWARDS, R. L., TAYLOR, C. W. and SHORT, R. V., 1963. Effect of human pituitary-follicle-stimulating hormone and chorionic gonadotrophin in Stein-Leventhal syndrome. Brit. Med. J., 1, 1119-1123.
   BUTT, W. R., CROOKE, A. C., CUNNINGHAM, F. J. and PALMER, R., 1963. The effect of dexamethasone on the excretion of oestriol and follicle-stimulating hormone in patients with Stein-Leventhal Syndrome. J. Endocrin. 26, 303-304.
- Endocrin., 26, 303-304.

  16. PERLOFF, W. H., SMITH, K. D. and STEINBERGER, E., 1965. Effect of Prednisone on female infertility.

  Int. J. Fert., 10, 31-40.
- ABU-HAYDAR, N., LAIDLAW, J. C., NUSIMOVICH, B. and STURGIS, S. H., 1954. Hyperadrenocorticism and the Stein-Leventhal syndrome. J. Clin. Endocrin. Metab., 14, 766.
   PERLOFF, W. H., CHANNICH, B. J., HADD, H. E. and NODINE, J., 1958. The Stein-Leventhal ovary: A manifestation of hyperadrenocorticism. J. Fert. Steril., 9, 247, 1958.
   CUPPS, P. T., LABEN, R. C., RAHLMAN, D. F. and REDDON, A. R., 1960. Effects of adrenal glucocorticoids

- and testosterone on the semen. J. Anim. Sci., 19, 509-514.

  CUPPS, P. T., 1964. The effects of steroid hormones on the semen from bulls. Proc. V. Internat. Congr. Anim. Reprod. and A.I., 4, 206-210.

  CUPPS, P. T., BRIGGS, J. R., GARM, O. and ONSTAD, O., 1964. Metabolism of progesterone by adrenal gland homogenates from bulls. J. Dairy Sci., 47, 803,806.

  CUPPS, P. T., LABEN, R. C., FOWLER, M. E. and BRIGGS, J. R., 1964. Testicular degeneration associated with kidney degrees in the bull. J. Dairy Sci. 47, 432, 436.
- with kidney damage in the bull. J. Dairy Sci., 47, 433-436.
  HOLM, L. W., PARKER, H. R. and GALLIGAN, S. J., 1961. Adrenal insufficiency in postmature Holstein calves. Am. J. Obstet. Gynec., 81, 1000-1008.
  OSLER, M., 1962. Addisons disease and pregnancy. Acta Endocr. (KbH), 41, 67-78.
  FRANKLIN, R. R. and ALEXANDER, J. A., 1963. Endocrine disorders complicating pregnancy. Am. J. Med.
- Scis., 245, 718-725.

  APPELBY, J. I. and NORYMBERSKI, J. K., 1957. The urinary excretion of 17-hydroxycorticosteroids in
- 26.
- human pregnancy. J. Endocrin., 15, 310-319.

  McWADE, D. H., WILLIAMS, J. A. and DUNCAN, C. W., 1958. Ovarian function, blood chemistry, and reproductive performance of repeat-breeder cows. Proc. iii Symposium on Reprod. and Fert. Ed. Gassner, F. X., 97–106, Pergamon press.
- TURNER, H. N. and KOCH, J. H., 1961. Studies on the sodium-potassium balance in erythrocytes of Australian Merino sheep. II. Obesrvation on 3 Merino strains. Aust.J. Biol. Sci., 14, 260-273.
- VAN HEERDEN, K. M., 1963. Investigations into the cause of abortions in Angora goats in South Africa. Onderstepoort J. Vet. Res., 30, 23-84.

- 30. VAN RENSBURG, S. J., 1963. Endocrinological aspects of habitually aborting Angora-goat ewes. S. Afr. Med.,
- J., 37, 1114-1115. VAN RENSBURG, S. J. 1964. Possible endocrine mechanisms in gestational failure. Proc. V. Internat, Congr.
- Anim. Reprod. and A.I., 3, 375-378.

  32. KLOPPER, A., MICHIE, E. A. and BROWN, J. B., 1955. A method for the determination of urinary pregnane-
- diol. J. Endocrin., 12, 209-219.

  33. ROWLANDS, I. W. and SHORT, R. V., 1959. Progesterone content of the guinea-pig corpus luteum during the
- reproductive cycle and after hysterectomy. J. Endocrin., 19, 81-86.

  34. SOHVAL, A. R., and SOFFER, L. J., 1951. The influence of cortisone and adrenocorticotrophin on urinary gonadotrophin excretion J. Clin. Endocrin., 11, 677-687.
- 35. BROWN, P. S., (1956. Follicle Emberim, 11, 677-657.

  36. BROWN, P. S., THORBURN, AR. and CROOKS, J., 1963. Urinary gonadotrophin excretion during treat-
- ment with corticotrophin. J. Endocrin., 28, 125-126.

  37. VARON, H. H. and CHRISTIAN, J. J., 1963. Effects of Adrenal Androgens on immature female mice. Endo-
- crinology, 72, 210-222. SHORT, R. V., 1960. The secretion of sex hormones by the adrenal gland. Biochem. Soc. Symposium No. 18,
- 39. BARLOW, J. J., 1964. Adrenocortical influences on estrogen metabolism in normal females. J. Clin. Endocrin. 24, 586-596.
- WEST, C. D. DAMAST, B. and PEARSON, O. H., 1958. Urinary estrogen excretion in Cushing's Syndrome. I. Clin. Endocrin. Metab., 18, 15–27.
- INNACONE, A., GABRILOVE, J., SOHVAL, A. and SOFFER, L., 1959. The ovaries in Cushing's syndrome. New Eng. J. Med., 261, 775.
- RAHLMANN, D. F. and CUPPS, P. T., 1962. Effects of estradiol and dehydroepiandrosterone on the reproductive cycle of the cow. J. Dairy Sci., 45, 1011–1014. SZEGO, C. M. and ROBERTS, S., 1948. Pituitary-adrenal cortical antagonism to oestrogenic stimmulation of
- the uterus of the ovariectomized rat. Am. J. Physiol., 152, 131-140.

  VELARDO, J. T. and STURGIS, S. H., 1956. Supression of uterine growth by purified hydrocortisone acetate, 9d flurohydrocortisone acetate and corticotrophin. J. Clin. Endocrin., 16, 496.
- 45. NICOLETTE, J. A. and GORSKI, J., 1964. Cortisol effects on the uterine reponse to estrogen. Endocrinology,
- 64, 955—959.

  46. HISAW, F. L. and VELARDO, J. T., 1951. Inhibition of progesterone in deciudal development by steroid compounds. Endocrinology, 49, 1951.
- VELARDO, J. T., 1957. Influence of purified adrenal hormones and adrenocorticotrophin on progesterone in decidual tissue formation. Am. J. Physiol., 190, 408-412.
- RAKOFF, A. E., PASCHKIS, K. E. and CANTAROW, A., 1942. Atypical response of the rabbit to deoxy-
- Corticosterone acetate. Science, 96, 322.

  MASSON, G. M. C., LEWIS, L. A., CORCORAN, A. C. and PAGE, J. H., 1953. Deoxycorticosterone in rabbits: Stimmulation of rabbit "Toxaemia of pregnancy" J. Clin. Endocrin. Metab., 13, 300-315.

  SABA, N., 1965. Plasma cortisol levels in pregnant ewes. J. Agric. Sci., 64, 11-14.
- 51. McLAREN, A., 1965. Placental weight loss in late pregnancy J. Reprod. Fert., 9, 343-346.

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#### THE RELIABILITY OF THE CALIFORNIA MASTITIS TEST AS A FIELD TEST FOR LEUCO-CYTES IN MILK

G. CREWE, Div. Field Veterinary Services, Dept. Agric. T.S. Pretoria.

#### SUMMARY

California Mastitis Test (C.M.T.) reactions and leucocyte counts of 1,000 milk samples were compared. It was found that a definite gel was indicative of >400,000 leucocytes per ml. and that the C.M.T. could be evaluated as simply positive or negative without any intermediate gradings and still give a reliable result. The method of leucocyte count as carried out in this laboratory was shown to be sufficiently accurate for routine work. A \*commercial C.M.T. solution gave almost identical results to those obtained with a standard C.M.T. solution.

#### Introduction

Mastitis causes the South African milk producer annual losses of millions of Rand. With the ultimate aim being a mastitis control programme it was decided to investigate the accuracy of the C.M.T. as a rapid, easy and reliable test for mastitis.

Experiments were carried out to determine the accuracy of the leucocyte count, as any error would have a direct bearing on the interpretion of the results obtained when leucocyte counts and C.M.T. values are compared.

Experiments which show the relationship between leucocyte count and C.M.T. values were conducted, in an attempt to determine how a standard C.M.T. reagent should be interpreted in order to diagnose a leucocyte count>400,000 per ml.

As a commercial C.M.T. product would be used in the control programme, experiments

were carried out to determine whether a commercial product would give comparable results to those of a standard solution.

#### MATERIALS AND METHODS

California Mastitis Test. The principle of the test is based on the fact that the reagent ruptures the cells releasing nuclear protein which then unites with the reagent to form a gel.<sup>1</sup>

The standard C.M.T. solution was made up as follows; 2 per cent sodium lauryl sulphate (w/v), 0.05 per cent methyl violet (w/v) and pH 7.2, whilst the commercial C.M.T. product had the following composition; 3 per cent sodium lauryl sulphate, bromo-cresol purple and pH 5.4.

All milk samples were examined within four hours of collection.

The C.M.T. was performed by mixing 4 ml. C.M.T. reagent with 6 ml. of milk for 25 seconds, in a shallow container. Figures were allocated according to the degree of gel formation as follows:

0-no gel.

1+-slight sliminess.

2+-definite gel formation.

3+-strong, thick tenacious gel.

Leucocyte Count. Smears were prepared by the method of Prescott and Breed<sup>2</sup> and stained with Newman's stain<sup>3</sup>. Ten fields on each cm<sup>2</sup> were examined and the number of leucocytes per ml calculated.

For the experiments to determine the accuracy of the leucocyte count, smears were made from ten milk samples. Ten fields in each smear were examined and the number of leucocytes per ml. calculated. This was repeated ten times at intervals during the day.

<sup>\*</sup>Rufus Mastest Solution.

In the experiments to determine the relationship between leucocyte count and C.M.T. values, leucocyte counts of >1,000,000 were recorded as millions.

#### RESULTS

1. The determination of the accuracy of leucocyte count.

The results in table 1 shows that although the standard deviation of the samples is rather high, the greatest coefficient of variation is 21 per cent.

2. The relationship between the leucocyte count and C.M.T. values are demonstrated in tables 2, 3, & 4.

TABLE 1. DETERMINATION ON THE ACCURACY OF THE LEUCOCYTE COUNT.

Smear No.		Leuco	cyte Co	unt XI	). repeti	tion (te	n fields	each)			Aver- age	9	V
Sincar No.	a	ь	С	d	e	f	g	h	i	j	Count	X103	%
1	450	900	600	650	600	350	500	450	450	400	535	51.0	9.5
2	50	50	50	50	50	50	150	50	50	50	60	10.0	16.7
3	1,250	800	300	2,600	1,850	1,000	950	750	1,250	850	1,160	140.4	12.1
4	1,350	1,050	950	1,150	1,000	1,250	1,350	1,150	1,200	1,000	1,145	46.5	4.0
5	1,000	1,750	950	2,000	1,100	1,050	1,350	1,050	1,250	1,950	1,355	125.5	9.3
6	3,200	4,550	2,850	3,500	5,100	4,000	4,000	2,800	3,600	5,100	3,870	266.0	6.9
7	2,600	2,500	950	2,500	1,500	1,100	2,050	1,500	1,550	1,300	1,755	193.4	11.1
8	10,000	5,300	7,550	4,900	4,600	2,700	3,450	5,000	8,200	5,750	5,745	704.8	12.3
9	150	50	250	300	150	200	200	300	100	250	195	26.3	13.5
10	50	100	200	300	50	250	150	150	50	50	135	28.9	21.4

S is the standard deviation.

V is the coefficient of variation.

TABLE 2. CORRELATION OF C.M.T. VALUES AND LEUCOCYTE COUNT.

				C.M.T.	Values			
Leucocyte		0		3		2		3
Count X10 <sup>5</sup>	No.	%	No.	%	No.	%	No.	%
50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900	312 57 61 36 21 15 3 4 0 0 3 2 0 0 0 0	61 11 12 7 4 3 0.5 0.6 0 0.5 0.4 0 0 0 0	11 8 19 23 27 16 9 8 4 2 2 3 0 4 1 1	7 5 13 16 18 18 11 6 5 3 1 2 0 3 1 1 0	0 0 3 4 11 5 3 12 9 14 12 18 8 10 7 14 9	0 0 1 2 4 2 1 6 4 7 6 9 4 5 3 7 4 3 3	0 0 0 1 2 2 0 0 1 0 4 2 2 4 5 1 8	0 0 0 0 1 2 2 2 0 0 1 0 3 2 2 3 4 1 1 6
1000	. 0	0	6	4	61	29	79	63
Total	514	100	148	100	213	100	123	100

TABLE 3. PERCENTAGE DISTRIBUTION OF DIFFERENT C.M.T. VALUES WITHIN DIFFERENT CELL RANGES.

Lougaanta	C.M.T. Values						
Leucocyte Count X10 <sup>3</sup>	0	1	2	3			
50—150 200—400 450—650 700—950 >1,000	84 15.1 0.9 0	25 56 7 8 4	1 15 30 25 29	0 5 6 26 63			
Total	100	100	100	100			

TABLE 4. PERCENTAGE C.M.T. VALUES ABOVE AND BELOW 400,000 LEUCOCYTES PER ml.

Leucocyte	C.M.T. values as percentages.						
Count X10 <sup>3</sup>	0	1	2	3			
400 >400	99 1	81 19	16 84	5 95			
Total	100	100	100	100			

Table 4 shows that 99 and 81 per cent of samples having a C.M.T. value of 0 and 1 respectively contain <400,000 leucocytes/ml, whilst 84 and 95 per cent of those with 3 readings have >400,000 leucocytes/ml. This means that there is an error of approximately 20 per cent in the evaluation of C.M.T. readings 1 and 2.

3. Comparison between a standard C.M.T. solution and a commercial product.

Table 5. A comparison between a standard C.M.T. solution and a commercial product.

No. of samples/category							
C.M.T. Values	Standard	Commer- cial	Difference				
0	514	511	3				
1	148 213	126 205	22 8				
3	125	125	Ö				
Total	1,000	1,000	33				

As indicated in table 5 the difference in the readings obtained with the standard and commercial product was never more than one unit and the overall discrepancy was only 3.3 per cent.

#### DISCUSSION

Although the results of the investigation—to show the accuracy of the leucocyte count revealed a high standard deviation for the samples, the coefficient of variation, which compares the relative variability of the samples was only 21 per cent.

Schalm<sup>4</sup>, who did the original work on the C.M.T. classified the reactions as, negative, trace, weak positive, positive and strong positive with the corresponding cell counts of 0-200x10<sup>3</sup>, 150-500x10<sup>3</sup>, 400-1,500x10<sup>3</sup>, 800-1,500x10<sup>3</sup> and 800-1,500x10<sup>3</sup>. It appears that our grading of C.M.T. 1 is comparable to Schalm's grading of trace, whilst his C.M.T. values of 1, 2 and 3 correspond to our C.M.T. 2 and 3 in as much as all contain >400,000 leucocytes/ml.

In this work a leucocyte count of >400,000/ml was regarded as positive for mastitis. If C.M.T. 0 and 1 are both regarded as negative and C.M.T. 2 and 3 as positive, then there will be an error of 20 per cent, negatives being taken as positive and vice versa. However, if the limitations of the leucocyte count are remembered (21 per cent coefficient of variation) then the overall error becomes less and it is justified to classify the C.M.T. reaction for field use as simply positive or negative.

#### CONCLUSION

- 1. Examination of 10 fields per smear is a sufficiently accurate method for determining the number of cells present in milk.
- 2. On comparing the leucocyte content with C.M.T. readings it was found that a definite gel was a clear indication of abnormal leucocyte content. Grading of the positive C.M.T. reactions is unnecessary and leads to confusion on the part of the tester. The C.M.T. should therefore be judged as positive (gel formation) or negative (no gel formation). A simple and reliable test such as this is ideal as an expedient in a national mastitis control programme.
- 3. Comparative tests using the standard and commercial C.M.T. reagents showed the commercial product to be quite satisfactory for use in a control programme.

#### ACKNOWLEDGEMENTS

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#### REFERENCES

- SCHALM, O. W., and NOORDLANDER, D. O. (1957). Experiments and observations leading to the development of the California Mastitis Test. J. Amer. Vet. Med. Ass. 30, 5.
   PRESCOTT, S. C. and BREED, R. S. The determination of the number of body cells in milk by a direct method. Journal of Infectious Diseases 7, 632-640.
   LITTLE, R. B. and PLASTRIDGE, W. N. Bovine Mastitis, Pub. McGraw-Hill Book Company, Inc. New York
- 1964.
- 4. SCHALM, O. W. A syllabus on the bovine mammary gland in health and disease. University of California, Davis, California, U.S.A.

#### VETERINARY NON-PROPRIETARY NAMES

List No. 4 October 1965.

The British Veterinary Codex Revision Committee has adopted the following non-proprietary names for the veterinary substances indicated.

Non-	nron	rietarv	илто
INOII-	טטזט	rietarv	name

#### Other names

OXYCLOZANIDE	3, 5, 6, 3', 5'-pentachloro - 2, 2'-dihydroxybenzanilide;
	I.C.I. 46, 633.
• .	

2-dimethylamino-6-methylpyrimid-4-yl diethyl phosphorothionate; I.C.I. 29, 661.

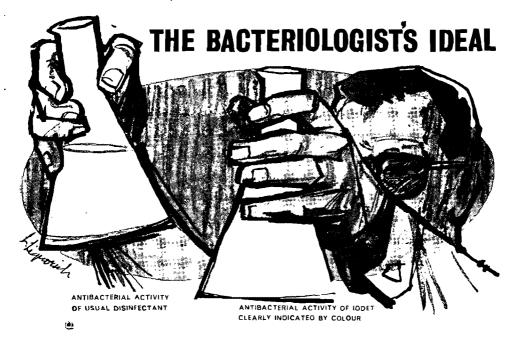
TETRAMISOLE..... 2, 3, 5, 6- tetrahydro-6 phenylimidazo- [2, 1-b] thiazole; I.C.I. 50,627.

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#### THE INCIDENCE AND TYPES OF MASTITIS IN PRETORIA HERDS, BASED ON THE CALIFORNIA MASTITIS TEST AND THE ISOLATION OF BACTERIA.

\*Georgina Crewe

Veterinary Field Services, Onderstepoort.

Presented to the 60th Scientific Conference of the South African Veterinary Medical Association September 1965.

#### SUMMARY

The results of a survey covering 44 herds (1783 cows) representing 10 per cent of those supplying milk to Pretoria are presented. Mastitis was shown to be widespread as evinced by both the California Mastitis Test and bacterial isolation. The correlation between the results of these two techniques is discussed. A very high proportion of staphylococcal to streptococcal infection was found. The implications of the findings are discussed.

#### INTRODUCTION

Little is known of the incidence and types of mastitis present in dairy herds in South Africa. The survey to be reported was therefore instituted.

The milk supply to the City of Pretoria is derived from a wide surrounding area. For the purposes of the survey, 10 per cent of the herds supplying milk to the city were selected at random. These totalled 43 herds representing, all the districts concerned and lying from 10 to 150 miles from the city. In addition, the herd

of the National Dairy Research Institute, Irene, was included as a control. The total number of cows involved was 1783 and the survey was carried out from July to September, 1964.

The following information was recorded in connection with each herd included in the survey.

Of the 43 herds, 30 consisted of Frieslands, 9 of Frieslands and Jerseys, 2 of Jerseys only and 2 of Ayreshires.

Hand milking was used on 34 herds and machine milking on the remaining 9.

In 29 herds disinfectant was added to the udder washing water and in 26 herds udder cloths were used.

The owners were also asked whether they used any tests for the detection of mastitis. Twenty four did not, thirteen used the strip cup and seven used other methods such as the CMT and pH papers.

In spite of the above, all the farmers used remedies for the treatment of mastitis. Penicillin preparations were used by 32 owners, staphylococcal vaccine by 3 and various other remedies by the remainder.

When asked whether they considered mastitis to be a problem in their herd, only seven answered in the affirmative.

Each farm was given a rating for hygiene from 1—3, 3 being the best.

The control herd consisted of Friesland, Jersey and Brown Swiss cows and was machine milked. The hygiene was of a very high standard.

Assisted by:

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Mr. A. Smith, Animal Husbandry and Dairy Research Institute, Pretoria.

Mr. J. H. Grey, Animal Husbandry and Dairy Re-search Institute, Pretoria.

#### **METHODS**

The cows were prepared for milking in the usual manner as used in the particular dairy. Composite samples were collected from each cow for bacterial isolation, consisting of approximately 25 ml. of midstream milk from each quarter collected into sterilized jars.

The California Mastitis Test (C.M.T.) was carried out on the spot on separate samples from each quarter. Approximately equal parts (about 3 ml.) of milk and "Rufus Mastitis Solution" were mixed in a shallow paddle, agitated for some 30 seconds and the reaction evaluated as negative or positive depending on the absence or presence of gel formation. No intermediate ratings were used.

At the laboratory the milk samples were centrifuged, the sediment plated onto blood agar and the cultures incubated at 37°C for 24 to 48 hours. Samples showing gram-negative organisms were discarded. Those showing gram-positive organisms were classified as staphylococci and streptococci and the former grouped according to Baird-Parker's classification.<sup>1</sup>

#### RESULTS AND DISCUSSION

#### California Mastitis Test (C.M.T.)

A positive reaction to this test depends on the number of leucocytes present in the milk. Assuming that the presence of over 400,000 leucocytes per ml. of milk indicates mastitis, previous work<sup>2</sup> has shown the C.M.T., read as positive or negative on gel formation, to be sufficiently accurate for survey purposes.

On this basis no herd was found free of C.M.T. reactors. The distribution of reactors is shown in Table 1.

TABLE 1.—HERD DISTRIBUTION OF C.M.T. REACTORS.

% C.M.T. pos.	% of herds
4-20	14
21-40	25
41-60	34
61-80	25
81-88	2

In table 2 the correlation between positive C.M.T. results and infection in the milk can be

seen. Of the C.M.T. positive cows, 84 per cent were excreting bacteria. Of these, 47 per cent were staphylococcal infections. Ewbank<sup>3</sup> found no association between cell count and the presence of bacteria.

In the present survey 38 per cent of the animals showed positive results to both the C.M.T. and bacterial isolation. According to Barnum<sup>4</sup>, the corresponding figure in Canada was 16.2 per cent.

The control herd (No. 43) showed a relatively high proportion of C.M.T. positive tests (34%) but a relatively low figure (22%) for the presence of bacteria. This can be ascribed to the fact that at the time of the survey difficulty was being experienced with the regulation of the vacuum of the milking machine.

#### Bacterial Isolation.

No attempt was made to divide the staphylococci isolated into pathogenic and nonpathogenic groups as it is maintained that the presence or absence of "bacterial mastitis" depends on the reaction of the udder to the bacteria present.

TABLE 3.—PERCENTAGE OF STAPHYLOCOCCUS SUB-GROUPING FOR STAPHYLOCOCCUS INFECTED COWS.

Staphylococcus subgroup	No. of cows	% of total Staphs.
I (aureus) II III IV V VI	210 304 138 9 69 35	27 40 18 1 9 5

On this basis there appeared to be a high incidence of bacterial infection (75%) of which 43 per cent were staphylococcal, 5 per cent streptococcal and 27 per cent due to gramnegative bacteria.

Streptococcus agalactiae was found in 41 per cent of the streptococcal infections.

It is of interest to compare these results with those of other workers. Howell et al<sup>5</sup> found a total infection of 46 per cent of which 23.4 per cent were S. aureus and 28.5 per cent streptococcal. Barnum<sup>4</sup> reported 47.7 per cent of infections

TABLE 2.—PERCENTAGE INFECTION AND C.M.T. POSITIVE COWS PER HERD.

No. of Herd	No. of Cows	% CMT pos. Cows	% Total infection	% Stapl	n % Strep infection	% Other
1 2	50 33	70 39	78 93	68 69	2 0	8 24
2 3	32	43	56	25	0 ,	31
. 5	39 50	41 66	87 74	50 30	0 4	37 40
6 7	49	32	64	24	4 4	36
8	50 50	50 12	82 72	32 42	0	46 30
9 10*	50 50	64 18	86 80	48 60	18 12	20 8
11*	50	88	82	48	0	34
12 13	18 50	50 12	83 64	72 48	0 4	11 12
14	50	36	66	30	18	18
15 16	50 10	14 50	78 70	50 70	2 0	26 0
17 18	45 50	50 38	78 92	37 <b>60</b>	20 14	21 18
19	50	38	90	54	26	10
20 21*	50 20	34 70	62 65	26 25	6	30 40
22	16	69	63	63	0	. 0
23* 24*	33 50	69 4	69 66	30 38	3 6	36
25*	50	44	82	40	0	42
26 27	19 28 8	74 75 25	90 82	27 54	0	22 42 63 28 0
28 29	8 48	25 35	100 81	88 22	12 2 0 0	0 57
30	49	62	90	56	0	34
31 32	9 19	44 74	100 79	100 68	0	0
33 34	23 50	65 48	91 72	52 46	0	39 26
35	50	80	96	52	1 6	38
36 37	50 49	60 42	80 68	56 56	4 4	20 8
38	49 50	48	82 82	64	0	1 18
39 40*	50	40 28	80	58 18	0	22 62
41 42	26 50	58 44	81 <b>70</b>	38 34	0	43 36
43* 44*	50 50	34 20	22 42	4 8	0	18 34
'anis 4 - Tur Ave	TRACE C M T		es machine milk		SIFIED ACCRODING	TO LIVEIENE
Hygiene	Average CMT p	% Avera	ge % Av	erage % ylococcal	Average % Streptococcal infection	Average % Gram neg. infection
1 and 2 3	50	80 64		50 34	4 .	31 28
Control	34	22		4 .	ō	18
fet. ourn.—5		<u> </u>	511	<u> </u>		
Vet. 'ourn.—5			511		·	

Hygiene	Average % CMT ps. cows	Average % Total Infection	Average % Staphylococcal infection	Average % Streptococcal infection	Average % Gram neg. infection
l and 2	50	80	50	4 2	31
3	- 35	64	34		28
Control	34	22	4		18

due to haemolytic staphylococci and 35.9 per cent due to Sc. agalactiae. In New York State<sup>6</sup> the incidence of pathogenic staphylococci and streptococci was 13 per cent and 36 per cent respectively. In comparison with these figures the proportion of streptococcal infection found in Pretoria would appear to be extremely low.

Table 4 shows that the incidence of positive C.M.T. reactors and infection could be correlated to the standard of hygiene.

#### **Conclusions**

Mastitis wound appear to be very widespread in dairy herds supplying the Pretoria area. Although largely unappreciated by milk producers, this condition must cause considerable financial loss as will be shown by Mr. Langley later in this symposium. Further research and extension work is urgently required in order to promote economical milk production and ensure a safe and clean supply to the consumer.

#### REFERENCES

- BAIRD-PARKER, A. G., 1963. A classification of Micrococci and Staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. 30: 409.
- CREWE, G. The reliability of the California Mastitis Test as an indication of the Leucocytes in milk under field conditions (in press).
- 3. EWBANK, R. 1962. An evaluation of the C.M.T. and the Negretti Field Test as indicators of subclinical bovine mastitis. Vet. Rec. 74: 38.
- BARNUM, D. A. 1962. A method for the control of staphylococcus and streptococcus mastitis in Ontario. Canadian Vet. J. 3: 162.
- 5. HOWELL, D. et al. 1964. A survey of the incidence of mastitis in Dairy cows in the Reading area. Vet. Rec. 76:
- 6. EDITORS OF MOD. VET. PRACTICE., 1964. What's new in mastitis control? Mod. Vet. Practice. 45: 13.

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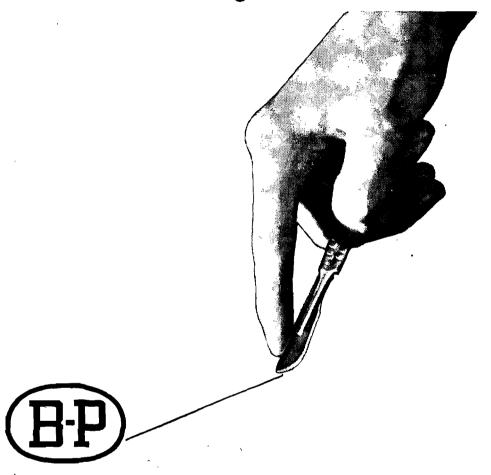
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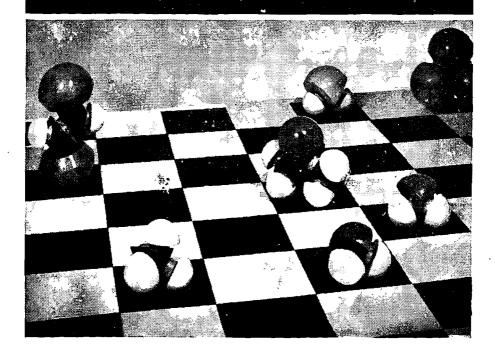


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#### THE EFFECT OF MASTITIS ON HERD MILK PRODUCT ON AND COMPOSITION

(Delivered at the 60th Annual Congress of the S.A.V.M.A. September 1965)

#### J. S. A. LANDREY

National Animal Husbandry and Dairy Research Institute, Irene.

#### SUMMARY

The Irene herd, under veterinary and technical supervision and with adequate resources has managed to control mastitis to reasonable proportions and has reached a stage where the prevalence of infection in the herd is lower than in most herds in the surrounding area.

Despite this fact, it is estimated that mastitis was responsible for a loss of 10.53% in milk production representing an annual financial loss of approximately R1,033 in a herd averaging approximately 80 cows in milk. In addition mastitis was directlyresponsible for a wastage of 6.4% of the cows from the milking herd, a deterioration in the chemical quality of the milk produced and considerable expense in the treatment of infected cows.

The dairy farmer is being urged from all sides to improve the efficiency of production. If this is our object, a loss of 10% on production due to udder troubles can hardly be ignored. We might have to learn to live with the problem but it cannot be allowed to eat us out of house and home.

#### Introduction

A study of recent literature on mastitis leaves one with a number of impressions. One is the fact that the problem is obviously universal in all dairying countries and that the prevalence of udder disease in dairy cattle is much the same today as it was thirty years ago. One must also be impressed by the increased attention that is being given to the problem in surveys, research and eradication programmes. This is possibly due to the success achieved over the past few decades in eradicating or controlling the more spectacular diseases among dairy cattle, thus

leaving the dairy industry in a position to appreciate more fully the depressing influence of the less spectacular erosive diseases on the production and quality of milk. Finally, one is left with the realization that the solution to the problem is, to say the least, elusive. In a paper published at the end of last year<sup>1</sup>, criticism was levelled at the conventional view that the prevalence of the disease is due to poor husbandry and that most farmers could overcome the difficulty by applying what is currently accepted as good management. It is pointed out that there is little or no clarity on what comprises good management in this respect and that mastitis exists and can be a serious problem even in herds where management is good by conventional standards.

In South Africa, this attitude towards the control of mastitis prevails to a large extent. The importance of management of the milking herd and hygiene during the milking operation is generally appreciated but one gains the impression that the attitude is "We know the answers—the onus is on the farmer to see that the management and hygiene in his herd in improved". The need for research on the subject, whether in the field or at research institutions, is often questioned and with a single exception<sup>2,3</sup> very little has been published on the subject in South Africa.

Management on dairy farms in South Africa varies considerably between farms, between areas and between producers of milk for industrial purposes and for urban consumption. Management in many South African dairy herds and in a number of dairying areas is unconventional, to say the least, by recognised dairying standards. Studies in the field on the incidence of mastitis would at least indicate among what

groups of farmers and in what areas mastitis is most prevalent. In addition, such surveys might indicate those managerial practices which are associated with either a high or low degree of infection.

Another attitude towards mastitis in the Republic is illustrated by the opinion expressed recently by the senior technologist of one of our major dairying concerns. He believes that, like the poor, mastitis will always be with us and that we must learn to live with it, In his view, the significance of the problem, to the dairy industry as a whole, is being exaggerated.

In an attempt to present the significance of the problem in perspective—for the Pretoria area at least—two years records relevant to mastitis in the routine herd of the Production Unit at the Animal Husbandry and Dairy Research Institute at Irene have been analysed.

#### IDENTIFICATION OF MASTITIS IN THE IRENE HERD.

During the period August 1963 to August 1965, 156 lactations were commenced and completed in the herd. During this period, identification of udder damage and infection was based on three tests viz:

- Strip-cup tests on each quarter at every milking.
- 2. California Mastitis Tests; on composite samples of each cow's milk once weekly.
- 3. Bacteriological examination of composite samples of each cow's milk at the N.I.V.R., Onderstepoort, approximately once every three months.

Samples reacting possitively to any one of these three tests were regarded as mastitic and the cows were immediately isolated.

Quarter samples from these cows were further examined at Onderstepoort and if positive for pathogenic bacteria, the quarter was treated according to the recommendation of the veterinarian in attendance. Thereafter, samples from infected quarters were subjected to bacteriological examinaiton at regular intervals and if both

clinical symptoms and bacterial infection disappeared, the cow was returned to the routine herd.

Cows showing clinical symptoms and/or reacting positively to the California Mastitis Test but showing no sign of bacterial infection were also regarded as mastitic and were isolated until such time as they reacted negatively to all tests.

#### INCIDENCE OF MASTITIS IN THE IRENE HERD.

In Fig. 1. the proportion of the milking herd isolated because of mastitis over the two year period is illustrated. It is apparent that there has been an improvement. The proportion of affected cows would seem to have stabilized at around 5—6% of the milking herd. There does not appear to be any marked seasonal trend.

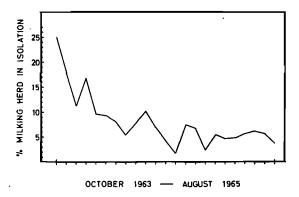


Fig. I

Division on x axis represent months over period indicated.

Of the 156 lactations, 76 or 48.7% were affected by mastitis at least once. Eleven cows were isolated twice during the course of their lactations, one three times and two four times.

Of the 156 lactations, ten were terminated shortly after calving. Three animals were culled because of acute mastitis ascribed to S. aureus. Of the remaining seven, three died and four were culled soon after calving for reasons not associated with mastitis. The remaining 146 lactations commenced normally.

TABLE 1.—THE INFLUENCE OF AGE ON THE INCIDENCE OF MASTITIS.

	Total	Mastit	is Positive	Infected with Pathogenic Bacteria		
Lactation Number	Number of Lactations	No.	As % of Total	No.	As % of Total	As % of Mastitis Cases
1st. Lactation	45 50 61	12 32 32	26.6 64.0 52.5	3 14 16	6.6 28.0 26.2	25.0 43.8 50.0
TOTAL	156	76	48.7	33	21.2	43.4

Infections by pathogenic bacteria were the cause of mastitis affecting 33 lactations 21.2% of all lactations or 43.4% of the affected lactations, staphylococci were responsible for all cases with one exception which was due to streptococci.

Age had a marked influence on infection as is illustrated in Table 1.

In the spring of 1964 the herd was included in the sample of 44 herds comprising the survey in the Pretoria area already described in the previous paper<sup>2,5</sup>.

Based on the results of California Mastitis Tests, the incidence of mastitis in 8 of the 44 herds was lower than that in the Irene herd. As far as bacterial infection was concerned, however, this herd had the lowest incidence of all.

The control of infection to reasonable limits can be ascribed to attention to hygiene, as advised by our Dairy Technologists and to the guidance of Veterinarians of the Division of Veterinary Field Services.

Here then, we are dealing with one of the cleanest herds in the Pretoria area as far as the incidence of mastitis is concerned, with the best technical knowledge at our disposal and with adequate facilities and adequate funds for the treatment of infected animals.

# THE INFLUENCE OF MASTITIS ON MILK PRODUCTION AND COMPOSITION

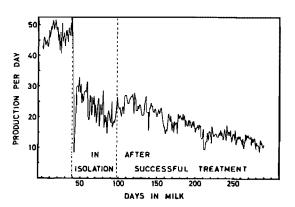
The significance of mastitis, as it affects the efficiency of milk production, is determined mainly by four factors i.e.:

1. The direct loss in milk as a result of the inflammation of the udder.

- 2. The reduction in the lactational life of cows i.e. as a cause of herd wastage.
- The detrimental effect of mastitis on the composition and quality of the milk.
- 4. The cost of treatment.

#### 1. The Direct loss in milk production.

The influence of mastitis on production is illustrated in Fig. II. This cow, a Friesland in her 3rd. lactation, showed symptoms of infection on the 49th. day of lactation and *S. aureus* was identified in the milk from two quarters. It is apparent that her production was adversely affected even after successful treatment.



The influence of mastitis on the total milk production of a quarter varies considerably with the severity of the infection and the stage of lactation at which the quarter is infected. Further, among mastitis cases at Irene, it was found that with infection of one quarter of an udder, the production of the other uninfected

quarters dropped considerably. This could be partially explained by the change of environment and particularly the feeding regime during isolation. However, many cows passed through isolation without suffering any setback to their production whatsoever.

An additional observation was that in quarters obviously affected by mastitis but showing no signs of bacterial infection, production rapidly improved with treatment although the initial drop might have been quite spectacular. This was seldom the case when the inflammation was due to bacterial infection.

If the influence of mastitis on production is to be brought into perspective one must determine the loss of milk that can be ascribed to this cause. In America, the value of this loss is estimated to lie between \$250 million and \$500 million per year. The loss in South Africa is estimated at R8 million annually<sup>4</sup>.

The estimate of the loss of milk production which can be ascribed to mastitis in the Irene herd has been based on applying the average persistency of unaffected cows in any group to the pre-infection performance of cows affected by mastitis in the same age groups.

The "Persistency Index" is the production over the first 30 days of lactation expressed as a percentage of the production over the whole lactation.

The production of cow F26, illustrated in Fig. II, may be used as an illustration of such a calculation.

Production of Cow F26 over first 30 days = 1442.5 lbs.

Estimated laction potential assuming a "Persistency Index" of 15.56 = 9290.5 lbs.

Actual production = 6615.5 lbs.

Estimated loss due to mastitis = 2665 lbs.

On this basis, the loss on the 146 lactations was estimated at 8,265 gallons or 10.53% of the total potential production of the cows.

With the incidence of mastitis higher in older cows, it is hardly surprising that the loss should be higher in the older group as is illustrated in Table II.

TABLE II.—THE INFLUENCE OF AGE ON LOSS IN PRODUCTION DUE TO MASTITIS.

	No. of Lacta- tions	Percentage of Loss in Lactational Production
1st. Lactation	42	2,73
2nd. Lactation	49	9.28
3rd. Lactation	55	17.45
TOTAL	146	10.53

The loss was also appreciably higher in lactations affected by bacterial infection than in those reacting positively to the California Mastitis Test or the Strip cup test but where no bacterial infection could be established. This is illustrated in Table III.

TABLE III.—THE EFFECT OF BACTERIAL INFECTION ON LOSS OF MILK PRODUCTION.

	No. of Lacta- tions	Percentage Loss in Lactational Production		
Bacterial Infection	32	25.0		
Aseptic Mastitis	41	17.4		
Unaffected by Mastitis	73	0.0		
All Lactations	146	10.5		

#### 2. Wastage

During the two year period covered, 17 of the original 156 cows left the herd. Total wastage from the milking herd was therefore 10.9%.

Of these 17 cows, 10 were lost as a direct result of mastitis. One died, one was destroyed and the remaining eight were culled after it had been decided that they were a source of infection to the rest of the herd.

Wastage due to mastitis was therefore 6.4% of the milking herd or 58.9% of the total wastage from the herd.

#### 3. Influence on the composition of Milk.

The influence of mastitis on the composition of milk is simply but effectively described as follows<sup>2</sup>:

"The result of inflamatory reaction is a decrease in those constituents which are elaborated by the epithelial cells and an increase in those derived from the blood".

A complete chemical analysis which included the macro-chemical analysis and a mineral analysis of milk from cows with infected udders at the Research Institute. Irene was conducted and Dr. J. H. Labuschagne reported as follows:

The total solids, fat, solids-not-fat, casein and lactose decreased while the non-casein protein, chlorides and ash constituents increased. The most obvious changes occurred in the non-casein-proteins, lactose and casein contents as well as in the stability of the caseinate complex, which dropped to a very low level. The increase in the non-caseinproteins can, to a great extent, be ascribed to udder cells secreted in the milk. These udder cells are mainly responsible for the rise in the total-protein percentage.

It was found that a complete inbalance existed between the soluble cations and soluble anions. which is the principle factor causing instability of the caseinate complex in mastitis milk. In comparison with normal milk the calcium content increases and the phosphorus content decreases. The changes in these two constituents are largely attributed to the soluble fractions. The soluble inorganic phosphorus, which is closely associated with the mineral balance in milk, drops to a low level whilst the ionizable calcium fraction, which is concerned in the actual coagulation mechanism of the casein practically trebles in comparison to the level found in normal milk. Conflicting results were obtained in the case of the colloidal inorganic fractions of calcium and phosphorus in our investigations, but overseas literature reports high values for these constituents in sub-clinical mastitis milk. No clear behaviour of the magnesium and citrate contents could be noticed as in some cases an increase was noticed whereas in other samples a decrease occurred. White and Davies<sup>6</sup>, reported a decrease in the citrate content of sub-clinical mastitis milk.

#### 4. The cost of Treatment

The treatment of infected cows was under close Veterinary supervision. No attempt has been made to estimate the cost of this treatment -it must have been considerably more than the average dairy farmer would have been prepared to provide.

Of the 76 lactations which were affected by mastitis, 47 or 62.1 % were successfully treated before the lactations were completed. Thirteen of the 33 cases of bacterial infection were successfully treated.

#### REFERENCES

- DODD, F. H., NEAVE, F. K. and KINGWELL R. G., 1964. J. of Dairy Sci. 47: 1109.
   VAN RENSBURG, S. W. J., 1939. Farming in South Africa—Reprint 71.
   VAN RENSBURG S. W. J., 1947. Onderstepoort Journal 22: 91.

- CREWE G., 1964. Bladskrif No. 11, Departement van Landbou-tegniese Dienste.
- CREWE G., 1965.

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#### DIE VOORKOMS VAN MASTITIS IN VARSMELK- EN KAASMELKKUDDES IN SUID-AFRIKA

M. N. HERMANN, Navorsingsintituut vir Veeteelt en Suiwel, Irene.

(Voordrag aan die 60ste Kongres van die S.A.V.M.V., Sept. 1965).

#### SUMMARY

The C.M.T. and leucocyte count was applied to milk from 700 herds producing milk for the fresh milk trade and for production of cheese. From this it appears that the incidence of mastitis is considerably higher in fresh milk herds of the central and eastern Transvaal than n the cheese milk herds of the western Transvaal and northern Cape. 40% of the milk from fresh milk herds react positively the the C.M.T. whereas only 2.5% of cheese milk herds are positive. On the other hand, about 25% of fresh milk and 80% of cheese milk herds produce C.M.T.-negative milk respectively.

No definite conclusions can as yet be drawn from these differences, and further investigation appears essential.

Mastitis word allerweë as een van die belangrikste siektes in melkkuddes beskou en groot aantalle koeie is volgens berigte uit verskillende lande met een of ander vorm daarvan besmet. In Australië is die persentasie besmetting bv. ongeveer 30 persent, in Noorweë 17.7 persent, in die Reading-omgewing van Engeland 46 persent, in Indië 28.6 persent, in Pakistan 25 persent, en in Joegoslawië 44 persent, (Francis, 1962; Fodstad, 1963; Howel, Wilson en Vessey, 1964; Indian Fmg., 1962; Maqboolur, 1964; Milojovic en Milenkovic, 1962). Tydens 'n opname van die higiëniese kwaliteit van melk in Suid-Afrika is onder andere toetse uitgevoer met die doel om 'n algemene indruk te verkry van die voorkoms van mastitis. Die gegewens wat volg is net as 'n voorlopige aanduiding bedoel en heelwat aanvullende werk is nodig, voordat 'n volledige prentjie van die toestand in Suid-Afrika beskikbaar sal wees.

#### I. PROSEDURE EN ONTLEDINGSMETODES

Monsters is vanaf November 1964 tot September 1965 by drie varsmelk- en drie kaasfabrieke geneem van die melk van 240 varsmelk- en 460 kaasmelkkuddes. Van die varsmelkkuddes is ongeveer 60 in die omgewing van Pretoria, terwyl die res oor die Oos-Transvaalse Hoëveld versprei is. Die kaasmelkkuddes is dié van produsente in Wes-Transvaal en Noord-Kaap.

Die melkmonsters is by die fabrieke uit die skaalbak geneem en direk getoets. Die toetse wat toegepas is, is die California Mastitis toets (C.M.T.) en die mikroskopiese telling van leukosiete.

#### DIE C.M.T.-TOETS

Een ml. van 'n 3 persent oplossing van natrium-laurielsulfaat met 'n pH van 6.6—6.8 en 0.01 persent bromkresolpers word by 1 ml. melk gevoeg en in 'n bakkie met 'n wit agtergrond vermeng. Die mate van slymerigheid wat ontwikkel, en die kleurverandering van die indikator is 'n aanduiding van die aantal leukosiete in die melk en die pH, en daarom 'n indirekte aanduiding van uierontsteking.

#### MIKROSKOPIESE TELLING VAN LEUKOSIETE

'n Hoeveelheid van 0.01 ml. melk word op 'n 1 cm.² oppervlakte van 'n voorwerpglasie gesmeer, laat lugdroog, onvet met benseen, fikseer met alkohol en gekleur met 'n 0.7 persent alkoholiese oplossing van metileenblou. Ten minste 10 velde per monster word getel en die gemiddeld daarvan bereken.

#### 11. RESULTATE EN BESPREKING

Die resultate van C.M.T.-toetse en leukosiettellings word in Tabelle 1 en 2 aangegee.

TABEL 1.—MASTITIS-VOORKOMS IN MELKKUDDES, SOOS AANGEDUI DEUR MIDDEL VAN C.M.T.-TOETSE.

C.M.T.	Varsmelkkuddes (% van 240 monsters)	Kaasmelkkuddes by			
		Vaalharts (% van 72 monsters)	Lichtenburg %( van 179 monsters)	Reivilo (% van 184 monsters)	
Negatief. positief (1+) sterk positief (2+ en 3+).	23.8 32.9 43.4	64 30.5 5.5	82.2 12.8 5.0	87 10.8 2.2	

TABEL 2.—MASTITIS-VOORKOMS IN MELKKUDDES, SOOS AANGEDUI DEUR MIDDEL VAN LEUKOSIETE TELLINGS.

Aantal leukosiete	Varsmelkkuddes (% van 282 monsters)	Kaasmelkkuddes by			
		Vaalharts (% van 106 monsters	Lichtenburg (% van 77 monsters)	Reivilo (% van 130 monsters)	
500,000 en minder	28.0 37.2 34.8	83.0 14.2 3.0	72.7 22.0 5.2	85.4 13.8 0.8	

In beide tabelle is 'n duidelike verskil tussen varsmelk- en kaasmelkkuddes waarneembaar. Die C.M.T.-resultate en die leukosiet-telling is ongelukkig nie heeltemal betroubaar vir die aanduiding van mastitis nie. Volgens Aynsley en Buol (1965) is 9 persent van 538 monsters wat C.M.T.-negatief was, positief bevind met behulp van bakteriologiese toetse, terwyl 43.6 persent van die positiewe gevalle geen mastitispatogene opgelewer het nie. Soortgelyke resultate is deur Rude (1963) gerapporteer. resultate in Tabelle 1 en 2 kan dus net 'n benaderde aanduiding van die mate van besmetting gee. Gray en Schalm (1962) het 12,438 monsters van 1243 koeie getoets en gevind dat die gemiddelde afname in melkproduksie 6.0, 10.0, 16.0 en 24.5 persent is, by C.M.T.-resultate van  $\pm$ , 1+, 2+ en 3+. As hulle werk as grondslag gebruik word en aangeneem word dat daar dieselfde afname in kuddemelk as by indiwiduele koeie is, beteken dit dat by 43 persent van die varsmelkkuddes en 2 persent van die kuddes in die Reivilo-area 'n produksieverlies van ongeveer 20 persent as gevolg van mastitis plaasvind.

Die vraag ontstaan nou waarom daar so 'n groot verskil tussen die verskillende gebiede is. Hierdie vraag kan tans nie beantwoord word nie; alhoewel met behulp van Tabel 3 op enkele verskille tussen boerdery-praktyke in die gebiede gewys sal word.

Die varsmelkboerdery is intensief van aard. Die plase is, veral by die stedelike gebiede, veel kleiner as in die kaasmelkgebiede. Die koeie

TABEL 3. VERSKILLE IN MELKBOERDERYPRAKTYKE TUS-SEN VARSMELK- EN KAASMELKPLASE

	Varsmelk (Sentraal- Tvl.)	Vaalharts (Kaasmelk)	Reivilo (kaasmelk)
Aantal koeie in melk Produksie/koei/dag. Produksie/kudde/dag. Melking in 'n stal Melking onder 'n afdak Melking in die opelug Aantal melkings/dag. Kalwers by die koeie Kragvoer per koei (lb.). Handvoeding T.V.V./koei Ure veldweiding/koei/dag	±50 20-25 lb. 100-150 gel. 100% — — 2 2 9.5 lb. 13.9 lb. 7.5	8 24 lb 19 gel. 20.8 % 45.9 % 33.3 % meesal 2 min 3'4 lb. 14.75 lb.	53 7 lb. (i) 60 gcl. 22.2% 14.8% 63.0% meesal 1 96% 0.84 lb. 2.02 lb. 16.8
Ure op aangeplante wei- dings/dag	1.8	3.7	0.5

(i) 11.4 lb. as die hoeveelheid wat deur die kalf gesuip word, in aanmerking geneem word. Toestande in die Lichtenburg-area kom min of meer ooreen met die by Reivilo, alhoewel die kuddes kleiner is en minder kalwers nog aan die koeie suip.

kry heelwat kragvoer. By Vaalharts is die plasies klein en die boerdery ook relatief intensief. By Reivilo, daarenteen is die boerdery baie ekstensief van aard en die koeie word gewoonlik eenmaal per dag vanaf die weidings gemelk.

Mastitis word met verskillende faktore geassosieer soos voedingskondisies, klimaat, higiëniese praktyke, seisoen van die jaar, stadium van laktasie, melkopbrengs, ens. Daar is egter heelwat teenstrydige getuienis in hierdie verband. Sommige werkers het bv. 'n verwantskap gevind tussen hoë opbrengs en mastitis, terwyl andere hierdie bevindinge nie kan bevestig nie (Sien Fell, 1964). Met die eerste oogopslag wil dit voorkom asof melkopbrengs per koei ook hier 'n rol speel. Die hoë opbrengs van die koeie in Vaalharts spreek egter daarteen, want daar is wesenlik minder mastitis as in die varsmelkgebiede. Dit wil wel voorkom asof daar meer mastitis in Vaalharts as in Reivilo is. Die verskil is egter nie groot nie en daar moet in gedagte gehou word dat die produksie per kudde kleiner is. Die kuddemelk kan dus positief reageer as net een koei aangetas is, terwyl 'n groter mate van besmetting by groter kuddes vir 'n positiewe toets nodig is.

Ander faktore wat 'n rol mag speel, is die feit dat die koeie in die ekstensiewe areas binne 'n veel groter oppervlakte beweeg wat besmettingsoordraging verminder. 'n Verdere verskilpunt is die melking in stalle waar daar moontlik, veral by ontoereikende higiëne, meer geleentheid vir versameling van ongewensde organismes bestaan-soortgelyk aan die sogenaamde hospitaalsiekte in kraaminrigtings.

Die rol van voeding in mastitis is heelwaarskynlik ook belangrik. Sommige ondersoekers meen dat goed gevoerde en gesonde koeie minder vatbaar is, terwyl andere weer die teenoorgestelde vind. Daar kan, op die oog af beoordeel, nie beweer word dat die koeie in die varsmelkkuddes swakker gevoer word nie. Verskil ten opsigte van die ander gebiede is die groter hoeveelheid kragvoer. In hoeverre dit 'n rol kan speel, is nie duidelik nie.

Noorlander (1962) beweer dat mastitis hoofsaaklik, of byna uitsluitlik, by koeie voorkom wat vir baie jare op 'n hoogs ontoereikende dieët was of wat uitgeput is deur lang periodes van geforseerde hoë laktasie. Hierdie bewering mag miskien in ons varsmelkgebiede van toepassing wees.

Daar moet egter ook nie uit die oog verloor word nie dat die Wes-Transvaal en Noord-Kaaparea taamlik verskillend is van Sentraal- en

Oos-Transvaal. Daar is onder andere verskille ten opsigte van reënval, temperatuur, plantegroei en bodemgesteldheid. Volgens die klimatologiese indeling van gronde word die grond in die Noord-Kaap-area as woestyngronde beskou, terwyl die Oos-Transvaal grond van 'n humide klimaat het, waar 'n neiging is tot 'n tekort in spoorelemente (met uitsondering van die swart turfgronde). Daar sal dus verskille wees in die chemiese samestelling van die voer, veral met betrekking tot spoorelemente.

Uit die gegewens, tans tot ons beskikking, is dit ongelukkig nie moontlik om enige definitiewe afleidings te maak nie. Daar sal nog veel meer navorsing gedoen moet word. Die verskillende toestande in Suid-Afrika bied egter interessante moontlikhede om die benadering tot mastitis, wat miskien ook 'n "beskawingsiekte" is, op 'n beter grondslag te plaas.

#### III. OPSOMMING

Melk van 700 kuddes wat varsmelk en kaasmelk produseer, is by fabrieke met behulp van die C.M.T.-toets en leukosiet-telling vir die teenwoordigheid van mastitis getoets. voorkoms van mastitis skyn in die varsmelkkuddes van Sentraal- en Oos-Transvaal veel hoër te wees as in die kaasmelkkuddes van Wes-Transvaal en Noord-Kaap. Van die varsmelkkuddes reageer ongeveer 40 persent van die kuddes se melk sterk positief met die C.M.T.toets, teenoor 2-5 persent by die kaasmelkkuddes, terwyl net ongeveer 25 persent varsmelkkuddes negatief reageer teenoor ongeveer 80 persent kaasmelkkuddes.

Geen definitiewe gevolgtrekkings kan nog gemaak word oor die oorsake van hierdie verskille nie en verdere ondersoekwerk skyn noodsaaklik te wees.

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#### **VERWYSINGS**

- AYNSLEY, L. H. en BUOL, J. M. (1965). The use of a direct test as an indication of sub-clinical mastitis in dairy cattle. Vet. Rec. 77, 379.82. Dairy Sci. Abstr. 27, 306.
   FELL, L. R. (1964). Machine milking and mastitis—A Review Dairy Sci. Abstr. 26, 551-69.
- 3. FODSTAD, F. H. (1963). Statistical investigation on the difference in type of mastitis by different methods of milking. Dairy Sci. Abstr. 25, 333.

FRANCIS, J. (1962). Austr. J. Dairy Techn., 17, 144. Aangehaal deur Fell, 1964.
 GRAY, D. M. en SCHALM, O. W. (1962). Amer. J. Vet. Res. 94, 541-43. Dairy Sci. Abstr. 24, 400.
 HOWEL, DEBORAH, WILSON, C. D. en VESSEY, M.P (1964). A survey of the incidence of mastitis in dairy cows in the Reading area. Vet. Rec. 76, 1107-12. Dairy Sci. Abstr. 27, 27.
 INDIAN FMG. (1962). Why lose your dairy cows to mastitis? Indian Fmg. 11, 33-34. Dairy Sci. Abstr. 24, 260.

- 8. MAQBOOLUR RAHMAN, M. D. (1964). Bovine mastitis caused by micro-organisms in Pakistan. Dairy Sc
- Abstr. 26, 597.
  MILOJEVIG, Z. en MILENKOVIC, D. (1962). The incidence of abnormal milk in bulk supplies. Dairy Sci. Abstr. 25, 161.
- 10. NOORLANDER, D. O. (1962). "Milking machines + Mastitis" Madison, Wisconsin. Aangehaal deur Fell, 1964
- RUDE, R. A. Jr. (1963). Comparison of California Mastitis Test readings and bacteriological culture results. Vet. Med. 58, 332 and 327. Dairy Sci. Abstr. 25, 480.

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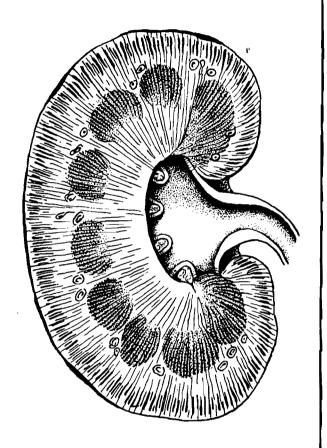
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#### DIAGNOSIS AND CONTROL OF HERD MASTITIS BY EXAMINATION OF CAN SAMPLES

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#### INTRODUCTION

For the purpose of the veterinary control of consumer milk supplies as well as for surveys of udder health prior to the introduction of, or during herd mastitis control schemes, a reliable and accurate yet practical method of assessment of the incidence and type or cause of mastitis in the herds becomes essential. When problem herds have thus been identified, detailed investigation of herd conditions and the examination of each cow is justified.

Direct and indirect tests for the presence of an excessive number of leucocytes alone do not indicate the aetiology of mastitis and may even indicate nothing more than prolonged lactation. In can samples of commercial milk a variable degree of bacterial contamination may complicate bacteriological procedures designed to establish the presence of particular or potentially mastitogenic organisms. The use of selective media has limited application and may reduce the sensitivity of methods of isolation. Dilution of pathological secretion with normal milk, particularly where a low incidence of mastitis is present in a large herd, is a further factor to be considered. Herds in South Africa are probably considerably larger on an average than in Europe, and production per capita lower.

A random sample of 10 per cent of the herds supplying Pretoria with fresh milk was used for this work, and can milks, obtained from 44 herds, each consisting of as many as 50 cows, were examined. The results were compared with independent assessments undertaken on the same day by Crewe et al<sup>1</sup> and based on the examination of milk from every cow in each herd.

#### MATERIALS AND METHODS.

Using standard procedures for thorough agitation and aseptic sampling, three-ounce samples were obtained from marked 10 gallon cans immediately after production and cooling on the farm and again on delivery at the reception depot on the next day. Samples were stored at room temperature until delivery to the laboratory, usually some 16–18 hours after production. The survey took place during the cooler part of the year, i.e. May to August.

After 20 inversions of the sample container through 180°, aliquots of the can samples from each herd were examined individually or combined to form various composite farm and depot samples respectively. In this manner a series of paired single and combined samples was obtained.

Each sample was subjected to:

- 1. A laboratory version of Schalm and Noorlander's<sup>2</sup> California Mastitis Test (C.M.T.), consisting of mixing equal portions of milk and reagent (commercial) in a hollow tile by vigorous stirring with a platinum wire and noting the consistency and reaction. (pH);
- direct microscopic counting of the number of leucocytes/ml according to the method of Prescott and Breed, as quoted by Little and Plastridge<sup>3</sup> and counting ten fields per cm<sup>2</sup>. Newmans modified stain was employed
- 3. The T.T.C. test for inhibitory substances according to the method advocated by the British Milk Marketing Board<sup>4</sup> but with the samples first heated to 80°C for 5 seconds and then cooled to 37°C;

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- 4. Bacteriological examination for Staphylococcus aureus and mastitis streptococci using sediment from 10 ml. of centrifuged milk for surface inoculation on three different media:
- (a) Blood Tryptose Agar (B.T.A.).
- (b) Blood Tryptose Agar with 20 i.u. of Polymyxin B sulphate per ml. added (B.T.P.A.).
- (c) Chapman's Mannitol Salt Agar (6.5% NaCl) (C.M.S.A.).

Plates were incubated at 37°C for 24 hours and then for a further 24 hours when no initial growth was observed. Likely colonies were classified as Staphylococcus aureus on the basis of morphology, fermentation of mannitol and production of pigment, coagulase and alpha-, beta-, or alpha-beta toxins. Streptococcus agalactiae, Sc. dysgalactiae and Sc. uberis were identified on the basis of the CAMP reaction, splitting of aesculin, and sugar fermentations, and grouped as mastitis streptococci for the purpose of this work in order to differentiate them from other streptococci found in commercial milk.

#### RESULTS

Survey data show that the position regarding the health of the udders of the cows in 44 of the herds supplying Pretoria with fresh milk is far from satisfactory. This conclusion is reached when considering the following facts:

- (a) Single can samples:
  - Of 199 single-can farm and depot samples examined bacteriologically, 169 or 84.4 per cent yielded Staphylococcus aureus and 19 or 9.5 per cent yielded Mastitis streptococci.
  - Single-can samples showed up to 9.5 million leucocytes per ml. and only 8 (4.2%) contained what might be considered a normal number of leucocytes i.e. 300,000/ml. (See table 1).
- (b) Combined-can (herd) samples:
  - 1. All of the 44 herds were found to be supplying milk from which S. aureus could be isolated, and mastitis streptococci were found in 5 (11.3%) of the bulk milk supplies.

TABLE 1. FREQUENCY DISTRIBUTION OF LEUCOCYTE COUNTS/ML. IN SINGLE AND COMBINED (HERD) CAN MILK.

Leucocytes/ml.	No. of samples Single-Can Combined-Can		Herd Mastitis assessment.
>300 × 10 <sup>3</sup> 300 × 10 <sup>3</sup> —	8 (4.2%)	0	M-Free
500 × 10 <sup>3</sup> 600 × 10 <sup>3</sup> —	40 (20.1%)	8 (9.1%)	M-Low
1.5 × 10 <sup>6</sup> 1550 × 10 <sup>3</sup> —	95 (47.2%)	22 (50%)	M-prevalent
5 × 10 <sup>6</sup> >5 × 10 <sup>6</sup>	51 (27%) 5 (2.5%)	14 (30.9)%	M-severe

- Herd milk samples contained as many as 4 million leucocytes/ml and no herds were producing milk free of pathological secretion if the Danish standard of 300,000 leucocytes/ml is accepted<sup>5</sup> while 80 per cent of herds gave evidence of moderate to severe udder irritation (See table 1).
- A. Comparison of results obtained by the examination of 120 paired samples obtained from the same cans on the farm and at the receiving depot respectively:
  - 1. Leucocyte content/ml. of milk: In some instances the counts obtained were identical or nearly so, on others they were widely divergent. However, the mean count for the 120 farm samples amounted to 1,406,800 and that for the depot samples was 1,286,300, and the difference is not considered to be significant when taking into account the various errors which may occur, e.g. in the actual counting procedure. It is therefore concluded that a relatively satisfactory agreement exists between the leucocyte counts of farm and depot samples.
  - 2. California Mastitis Test. Complete agreement was obtained in 71 (59.1%) out of 120 sample pairs. In 17 (14.5%) instances, however, one of the samples in a set gave a negative reaction and the other a positive one. Positive reactions were 1-4+, and of those positive samples not in complete agreement with each other, 20 (16.5%) varied 1+, 6 (5%) varied 2+ and 23 (21.2%) varied 3+ or 4+.
  - 3. Inhibitory Substances in Milk. In 101 (84.2%) pairs, the can and depot samples were in complete agreement on the presence or absence of inhibitory substances. In the 19 pairs in which agreement could not be shown, inhibitory substances were

- detected in 16 depot samples and in 3 farm samples. No attempts were made to identify these inhibitory substances.
- 4. Bacteriological results. In 10 pairs (8.3%) the bacteriological results failed to agree. In 9 instances S. aureus was isolated from only one of the pairs of samples, and in 3 instances mastitis streptococci could be found in only one of a set. Assuming that the absence of growth in one sample from a positive pair constitutes a false negative, 3 farm and 6 depot samples were falsely negative for S. aureus whilst 2 farm and 1 depot sample were falsely negative for mastitis streptococci.
- B. Comparison of the results obtained using different media for the isolation of specific organisms from can samples of milk.
  - (a) S. aureus was isolated from 250 (90.6%) of the 276 single and combined cansamples examined. The frequency of successful isolation on the different media used may be summarisee as follows:—

On B.T.A. 216 or 86.4% of isolations were positive, on B.T.P.A. 234 or 93.4% of isolations were positive, and on C.M.S.A. 238 or 96.5% of isolations were positive. In 204 (81.6%) of the 250 samples positive for S. aureus the organism was isolated on all three media. In the remaining 46 samples, positive isolations were obtained on C.M.S.A., B.T.P.A. and B.T.A. in 34, 30 and 12 instances respectively.

(b) Mastitis Streptococci were isolated from 31 (10.8%) of 288 single or combined can-samples examined; in all but one instance the B.T.A. and B.T.P.A. plates were in agreement. (C.M.S.A. inhibits the growth of mastitis streptococci and was therefore not used for this purpose).

From these results it is concluded that C.M.S.A. is the media of choice for isolation of S. aureus, but as streptococci do not grow therein and staphylococcus toxin production cannot be assessed without the presence of erythrocytes in the medium, the use of B.T.P.A. alone or in conjunction with C.M.S.A. is

considered advisable when the two most common causes of infectious mastitis are considered.

C. Analysis of survey data to determine the extent to which combined can (herd) milk samples may be relied upon to provide a true reflection of the mastitis situation on the herds.

For this purpose the results of Crewe's<sup>1</sup> mastitis assessment (I) (based on examination of each cow in the herd) were compared with an assessment based on results obtained from herd can-milk examination (II):

- (a) The assessments I and II agreed on the presence or absence of mastitis strepticocci in 27 of the 44 herds (61.3%). In 19 herds II yielded 15 false negatives and 2 false positives.
- (b) Methods I and II agreed on the presence of S. aureus infection in 39 (86.4%) of the 44 herds. In 3 instances method II gave a false negative and in 2 instances a false positive.
- (c) Assuming leucocytes in excess of 300,000/ml. to be an indication of pathological secretion<sup>5</sup>, all 44 herds were shown by method II to be producing milk with a variable degree of admixture of such abnormal milk. This agreed with Crewe's assessment (I).
- (d) The number of leucocytes/ml. was taken as a measure of the degree of admixture of pathological secretion in the bulked herd sample, and this was compared with the percentage of C.M.T.-positive cows in the herds examined.

The mastitis assessment (II) was graded on a basis similar to that used by Schalm<sup>6</sup> to grade his C.M.T.-reactions, and the results summarised in Table 2.

TABLE 2. INCIDENCE OF C.M.T.-POSITIVE COWS IN HERDS GRADED ACCORDING TO LEUCOCYTE CONTENT OF HERD MILK.

Method II	Mastitis	No. of	Method I		
Leucocytes/ml in Herd can Sample.	Assess- ment Herds		Mean percentage of C.M.T. cows/ herd, and range		
>5 × 10 <sup>6</sup> 1550 × 10 <sup>3</sup> —5 × 10 <sup>6</sup> 600 × 10 <sup>3</sup> —1.5 × 10 <sup>6</sup> 250 × 10 <sup>3</sup> —550 × 10 <sup>3</sup> >200 × 10 <sup>3</sup>	3+ 2+ 1+ Slight	0 13 26 5	0 69.4 (42.88) 42.6 (4—75) 31.6 (1.—41)		

D. The accuracy of the herd milk leucocyte count as compared with that of the constituent can samples.

In order to assess the accuracy of the actual leucocyte counts in combined can samples they were compared with the expected counts obtained by calculating the mean counts of the can milk samples which were used to constitute the combined can sample. The degree of variation was expressed as a percentage of the mean leucocyte count of the constituent can samples. The results are summarised as follows:

TABLE 3. PERCENTAGE VARIATION OF THE ACTUAL FROM THE EXPECTED LEUCOCYTE COUNT IN COMBINED CAN (HERD) SAMPLES OF VARIOUS COMPOSITION.

No. of herd samples	No. of single cans per herd sample (gallons)	Mean and range of variation
11	2×10	±30.4% (7-64)
19	3×10	±34.1% (0-109)
6	4×10	±38.3% (5-100)
3	5×10	±53.3% (43-69)

Although the numbers are statistically inadequate, there is an obvious tendency for accuracy to decrease as the number of cans per combined sample increases. In order to limit such loss of accuracy to  $\pm 50\%$  it would appear desirable to limit the number of cans per combined sample to 5, but this hypothesis requires further investigation.

The leucocyte count forms the basis for evaluating the C.M.T. test and for establishing the presence of abnormal secretions in milk. It is therefore necessary that the counts be seen in correct perspective. Prescott and Breed<sup>13</sup> developed a convenient technique for counting the leucocytes in milk by microscopic examination of a fixed and suitably stained smear of milk spread over a square cm. Various modifications have been advocated. After a critical study, Strinadka15 reported on the factors contributing to inaccuracies. It is generally accepted that the number of fields which should be examined is determined by the leucocyte content, although Schneider and Jasper<sup>16</sup> contend that the method only becomes reliable when making 4 smears from each sample and examining 200

fields altogether, using a very low multiplication factor for final calculation. This method would obviously be too cumbersome for routine application.

Crewe<sup>17</sup> found that examining only 10 fields per single smear was accurate enough for practical purposes and gave good correlation with C.M.T. readings. In our own limited investigations, using 4 smears per sample and examining 80 fields for each sample to obtain an eventual count, we found that the counts obtained after examining only 10 fields (initial count) gave responsible results in as much as the standard deviation (S) was 10.07 and the coefficient of variation amounted to 19.18 per cent (See table 4).

# E. The value of the C.M.T. as an indirect measure of the actual leucocyte content of bulked samples.

A total of 97 (34.6%) out of 280 single or combined can samples gave negative reactions to a laboratory version of the C.M.T. already described. Based on Schalm's grading of the C.M.T. reaction, these survey results may be summarised in the following table:

TABLE 4. COMPARISON OF INITIAL AND EVENTUAL COUNTS OF LEUCOCYTES IN MILK USING 10 FIELDS IN ONE BREED SMEAR AND 80 FIELDS IN 4 BREED SMEARS OF THE SAME SAMPLE RESPECTIVELY, EXPRESSED IN THOUSANDS/ML.

Initial	Eventual	Mean(x)	Deviation from x
250	250	250	0
600	725	662	62
300	350	325	25
800	712	756	44
600	712	656	56
250	275	263	13
100	] 187	143	43
1450	762	1,100	350
150	125	138	12
200	225	213	13
200	210	205	1 5
100	135	118	18
600	900	750	150
1650	1,737	1,694	44
500	487	493	7
700	550	625	75
		8,391	

From this it is calculated that S (Standard deviation) = 10.07 and V (Coefficient of variation) = 19.18%

Discussion: This laboratory method of performing the C.M.T. ensures accuracy of proportions but precludes ready recognition of the so called "precipitate" or slimy sediment which is readily observed in using the flat bottomed plastic paddles in the stable. It may therefore be assumed that trace or even 1+ reactions might

be missed in the laboratory method. 2+ and 3+ reactions however, representing leucocyte counts of >800,000/ml. should give clearly recognised reactions.

TABLE 5.—Frequency distribution of positive and negative C.M.T.-reactions interms of leucocyte content of can milk samples.

Schalm's C.M.T Leucocytes/ml		C.M.Treactions Negative Positive				Total C.M.T	
grade			1+	2+	3+	Total	readings
Negative Trace 1+ 2+ 3+	<200,000	4 47 72 (40%) 29 (17%) 0	0 16 104 129 6	0 0 1 9	0 0 0 2 0	16 105 140 9	4 63 177 169 9
		152	155	13	2	270	422

From these results it would appear that 72 (20.3%) and 29 (8.2%) of the 355 milks which had more than 400,000 and 800,000 leucocytes/ml. respectively gave false negative C.M.T. reactions. Even taking into account the inherent errors present in our method of establishing the actual leucocyte content of milk, this still represents a high percentage (28.5%) of false negative results.

However, none of the samples with less than 200,000 leucocytes/ml gave a false positive reaction to the C.M.T. There appeared to be no correlation between the degree of reaction and the leucocyte count.

It is concluded that the laboratory version of the California Mastitis Test is not sensitive enough to distinguish between gross and slight admixture of pathological secretion in bulk milk samples, and that a considerable number of milks with a high leucocyte content may be falsely classed as negative. These findings do not agree with those of others 8,9,10,16. The basis of the C.M.T. reaction<sup>11,12,16,20</sup> has been well established, and the only problem should be the variation in subjective personal appreciation of the degree of reaction. From this one must conclude that the laboratory version we employed was not satisfactory. Apart from apparent greater sensitivity there seems to be considerable advantage in the application of the Brabantsche mastitis test using apparatus which objectively measures the viscosity of resultant gels19.

#### DISCUSSION AND CONCLUSIONS

Mastitis may be defined as an inflammation of the mammary gland characterised by tissue changes which lead to temporary or permanent changes in the nature of the secretion and caused by infectious, toxic, chemical, thermal or traumatic agents. Changes in the nature of the secretion may also be seen at the end of prolonged lactation without any mastitis being present, and this applies particularly to cell Conversely, organisms capable of content. producing mastitis e.g. staphylococci, streptococci, etc. may be present without eliciting any tissue reaction. These facts should be borne in mind when interpreting the results of examination of milk samples. Crewe<sup>1</sup> has for example shown that only 16 per cent of herd owners considered that they had a mastitis problem although none of the 44 herds were considered to be producing normal milk when judged by the presence of leucocytes and S. aureus. Danish rules for the control of mastitis<sup>5</sup> require that isolation of Group B, C, G or L streptococci from milk be notified regardless of the leucocyte content, whereas S. aureus is only notifiable where the leucocyte count exceeds 300,000/ml. The presence of "carrier" cows or those harbouring temporary bacterial infections should therefore be considered.

Hauke<sup>18</sup> has reviewed the literature and concludes that an increase in leucocyte content is the most reliable indication of udder irritation, regardless of cause. Most authorities consider

100,000/ml. as the normal leucocyte content of cows in an average lactation, but some feel inclined to move this figure up to 500,000. Bulk milk should therefore, at most, contain this number of leucocytes, and dilution of pathological secretion with normal milk is therefore capable of masking instances where only a few affected cows are present in a herd. South African dairy herds are usually quite large, and herds containing 75 or more cows are not unusual, whils; average production is usually in the vicinity of 2 galls, or less per day. This is in sharp contrast with European countries, where herds are smaller and production per capita higher, and the dilution factor in our milk supplies may prevent us from accepting overseas standards without further investigation. Where chronic mastitis is well established in a herd, however, many cows become involved due to the infectious nature of some forms of mastitis or to a common non-bacterial cause to which all cows are exposed.

. The results of this survey show that can milk samples obtained from the farm or the depot are satisfactory for establishing, with a fair amount of accuracy, the presence of a mastitis problem in herds where the condition is not grossly apparent to the owner, and that up to 5 ten-gallon can samples may be combined into a single sample without great loss of accuracy when compared with an assessment based on individual cow examination. This appears to justify the institution of a system of survey and control which will utilise leucocyte content and the presence of S. aureus and mastitis streptococci as a basis for diagnosis. It should however be stressed that at least three consecutive tests over a period of several months are necessary for the results to be conclusively negative. The use of the leucocyte content alone as a diagnostic method for mastitis is considered inadequate in as much as it does not indicate the aetiology and may well result in the diaryman treating his cows with large quantities of antibiotics when a managemental fault is the cause of his problem. Not only would such a procedure be costly and wasteful and fail to bring about an improvement, but it would certainly add to the contamination of our milk supplies with residues of antibiotics and other chemotherapeutic substances.

The use of sensitive and selective media for the isolation of mastitogenic organisms is necessary,

as well as concentration of the sample in order to ensure positive isolations where organisms are present. Danish rules require at least  $3 \times 0.01$  quantities of whole milk to be inoculated, but concentration and the use of sediment is considered better though somewhat time consuming. The presence of large numbers of leucocytes may well result in the destruction of so many bacteria that even grossly abnormal secretions may not yield the causative organism (Jasper<sup>17</sup>) unless special procedures are adopted e.g. enrichment of aseptically drawn cow or quarter samples by incubation, or concentration of commercial milk samples by centrifugation.

The results of this pilot survey of herds around Pretoria are alarming, and justify further surveys in other parts of the country where milk production for both the industrial and fresh milk market is undertaken. For the purpose of comparison and proper evaluation, adoption of a standard procedure is recommended. pending on the results of such a survey a positive approach to the mastitis problem is suggested. This will include some form of unification of the present widely diversified interest in the problem. Today municipal authorities, the Milk Board, the Dairy Board, the various Divisions of the Department of Agricultural Technical Services and the milk co-operatives each have their own individual system of control (testing, advisory and statutory). Dairy farmers have, by and large, not realised the losses they incur from mastitis and how these losses may be reduced. Veterinary practitioners are rarely called upon to deal with mastitis as a herd problem. Dairy farmers obtain information from various other persons and organisations who advise, recommend and often sell to the dairyman, mostly without any investigation into the cause or extent of the condition. This is brought about by the ready availability of indirect mastitis diagnostic aids as well as disinfectants and drugs for mastitis, and encourages an ad hoc approach to the problem which can clearly not succeed. Use of the leucocyte content of milk for survey or diagnostic purposes must inevitably lead to unnecessary treatment of cows and contamination of milk supplies with antibiotics. irritant properties of most therapeutic substances on intramammary infusion is not commonly appreciated, and increased leucocyte content of milk after such therapy is inevitable regardless of anti-bacterial activity. For the improvement of udder health in our herds, the following steps are suggested:

- 1. A wide survey of the incidence and type of mastitis in the herds in the important milk production areas which should include both fresh milk and factory milk supplies. To this end the results of this investigation may be used for the establishment of a standard survey procedure.
- 2. Determination of the areas where the problem is of sufficient magnitude to warrant more intensive investigation.
- 3. Identification of problem herds and investigation into conditions on the farm concerned with the view to correcting faulty milking techniques and general management.
- 4. Procuring the active and long term cooperation of the dairyman by instituting of a financial penalty for delivery of abnormal milk or a premium for mastitisfree milk. The Milk and Dairy Boards have statutory powers to do this as part of marketing control.

5. The formation of a specific mastitis control or udder health control organisation by the collaborative efforts of veterinary services, local authorities, dairy cooperatives, animal husbandry services, the milk and dairy marketing boards, and farmer's organisations. This organisation could be staffed by seconded personnel or be subsidised by levy and other funds, but the farmer with a mastitis problem should himself be responsible for certain costs in connection with his own herd. The organisation would undertake sampling and laboratory services, field investigation, diagnostic and advisory services, and conduct the scheme on a national basis.

Mastitis control is basically a veterinary responsibility, but full use should be made of the abilities and services of agriculturalists, animal husbandrymen, engineers, microbiologists. dairy technologists and the numerous other disciplines which can contribute to an improvement of the situation. The mastitis problem affects not only our national income but also the nutrition and public health of our people.

#### ACKNOWLEDGEMENTS.

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#### REFERENCES

- 1. CREWE, G. (1965). The incidence and type of mastitis in Pretoria herds based on C.M.T. and bacterial isolation— In press.
- SCHALM, O. W. and NOORLANDER, D. O. (1957). Experiments and observations leading to the development of the California Mastitis Test J.A.V.M.A. 30: 5.
   LITTLE, R. B. and PLASTRIDGE, W. N. (1946). Bovine Mastitis. McGraw Hill Book Co. Inc., N.Y. and
- BRITISH MILK MARKETING BOARD. Standard methods for the detection of antibiotics and other inhibitory substances in milk, I. (1964). Thames Ditton, England.
   MINISTRY OF AGRICULTURE, DENMARK: Instructions of the Veterinary Directorate of 1.7.64 to labora-
- tories in the control of bovine mastitis.
- SCHALM, O. W. (1962). A Syllabus on the bovine mammary gland in health and disease. Univ. of California, Davis, California, U.S.A. CREWE, G. (1965). The reliability of the California Mastitis Test as an indication of leucocytes in milk under
- field conditions-In press.
- GRAY, D. M. and SCHALM, O. W. (1960). Interpretation of the California Mastitis Test on milk from individual mammary quarters, bucket milk and bulked herd milk. J.A.V.M.A. 136: p.195-198.

  9. JENSEN, P. T. (1957). Investigations into the Whiteside and C.M.T. tests for the detection of pathological secretion in herd milk samples. Nord. Vet. Med. 9, 560-608.

  10. LEIDE, W. and SCHALM, O. W. (1961). Die Anwendung des Schalm Test (C.M.T.) bei Sammelmilch zur heine mild deutsch bekan reallegabet. Tienerter J. Impedieur 16: 219-229.
- bestimmung minderwertige milch durch hohen zellgehalt. Tierartztl. Umschau 16: 219-229.

- PAAPE, M. J., HAFS, H. D. and TUCKER, H. A. (1963). The relationship of Feulgen D.N.A. in milk to the number of somatic cells. J. Dai. Sci. 46: 625.
   CARROL, E. J. and SCHALM, O. W. (1962). The effect of Desoxyribonuclease on the California Mastitis Test.
- Dai. Sci. 45: 1094-1097
- PRESCOTT, S. C. and BREED, R. S. (1911). The determination of the number of body cells in milk by a direct method. J. Infect. Dis. 7: 632-640.
   SCHALM, O. W. (1962). Bovine Mastitis: A program for its control in California. Can. Vet. J. 3: 90-92.
   STRYNADKA, N. J. AND THORTON, N. R. (1937). The accuracy of the direct microscopic (Breed) count of bacteria and leucocytes in milk. J. Dai. Sci., 20, 1937, 685-692.
   SCHNEIDER, R. and JASPER, D. E. (1964). Standardisation of the California Mastitis Test. Am. J. Vet. Res.

- 25: 109, p1636.

  17. JASPER, D. E. (1963). Laboratory Notes Calif. Vet. 17: 5 p.37-38.

  18. HAUKE, N. (1961). Cell content in normal and abnormal milk—a review. D. Tierartzl. Wschrft. 68: 666-724.

  19. JAARTSVELD, F. H. J. (1962). De uitvoering en waarde van de B.M.R. bij een georganiseerde mastitisbestrijding.
- JAARTSVELD, F. H. J. (1962). De uitvoering en waarde van de B.M.R. bij een georganiseerde mastitisbestrijding, Tydschr. v. Dgkde. 87: 16, p.1069.
   JAARTSVELD, F. H. J. (1961). Bydrage tot de diagnostiek van Mastitis bij Runderen in het hader van een georganiseerde bestrijding. Thesis Utrecht.
   MCKAY, K. G. (1960). The California Mastitis testing program. J. Dai. Sci. 43: 896.

#### LETTERS TO THE EDITOR

Earlier this year a report was published on the occurrence of Listeriosis in Chinchillas. Subsequent to this, two further outbreaks of the disease have been bacteriologically confirmed. The initial investigations were carried out at the Veterinary Investigation Centres at Stellenbosch and Allerton and confirmed at Onderstepoort.

Cultures of the strains isolated from these three outbreaks were sent to Dr. E. H. Kampelmacher, Head of the Laboratory for Zoonoses, Rijks Instituut voor de Volksgeszondheid, Utrecht, Netherlands, for serotyping. findings showed that they all belonged to serotype I which has a world wide distribution and is also pathogenic for man and ruminants.

It is clear that Listeriosis in Chinchillas is widespread in the Republic and the possibility that domestic animals may become infected is great. This again could endanger the health of stockmen, purveyors of animal products and consumers.

Veterinarians are therefore urged to acquaint themselves with the symptomatology and pathology of the condition in order to assure early diagnosis and immediate implementation of control measures.

C. M. Cameron.

Department of Bacteriology, Onderstepoort.

<sup>1.</sup> DU PLESSIS, J. L. and CAMERON, C. M., 1965. LISTERIOSIS IN CHINCHILLAS. J.S. Afro. vet. med. Ass. 36(1), 107.

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CHAP 44

#### SOME ASPECTS OF THE SHEEP AND WOOL INDUSTRY IN AUSTRALIA<sup>3</sup>

#### K. VAN DER WALT\*.

Presented to the 60th Scientific Conference of the South African Veterinary Medical Association.

September 1965

#### ECOLOGY AND GENERAL FARMING PRACTICE.

The 170 million sheep in Australia are distributed mainly over the eastern and western poles of the continent below the tropic of Capricorn. Relatively small numbers exist in the central desert while the northern tropical area (Queensland) has eight million sheep.

Sheep farming is practised in three major agro-climatic zones:—

(1) A high rainfall area with an annual precipitation of over 20 inches. This littoral zone which girdles the east and west coasts and parts of the south coast, has a Mediterranean climate with dry summers and wet winters. On the tablelands of New South Wales, however, the rainfall is distributed evenly throughout the year.

Farms are relatively small and free-hold with cleared and improved land fetching high prices. This area lends itself to improved and artificial pastures and carries Merinos, and cross-bred sheep for fat lamb production.

- (2) Inland from the coastal area is the wheatsheep zone where sheep raising for both wool and fat lamb production is combined with wheat farming. In N.S.W. this area lies on the western slopes, i.e. west of the Great Dividing range.
- (3) The largest area is a semi-arid pastoral zone with a mean annual rainfall below 20 inches and high evaporation. Merino sheep predominate here. In the north-eastern part of this area the size of farms is measured in square miles and ranges from 30,000 acres to 1 million acres. These huge properties are leased from the State government for periods of 28 years with an

Sheep farming on these stations is on a massive and extensive scale. The sheep are dependent on natural vegetation which varies from open forest grazing, Acacia scrub, to savannah-shaded or open Mitchell grass plains. Drinking is from open surface water, in river channels, stored in earth tanks, or reticulated through surface drains fed from artesian bores that deliver up to a million gallons per day. This type of farming obviously brings with it the problems of adequate fencing, grazing and predator control, mustering, dosing etc., and the small number of farm workers, with their sheep dogs, controlling these operations, remain a source of admiration to the South African.

The general topography and climate of Australia is very similar to South Africa. The tableland rising from the coast is not as high as that in South Africa and receives a more evenly distributed rainfall but severe frosts occur during the winter months.

About 75 per cent of the sheep in Australia are Merinos. The sheep industry is the basis of the whole Australian economy, with wheat and beef being the other main agricultural exports. During the last decade a start has been made with the development of the vast natural resources of this wealthy continent and industrialization is progressing rapidly. A lack of population numbers seems to be the only restrictive factor in the bright future of this fast developing country.

option of renewal, provided that the tenant is worthy and improvements are undertaken. These permanent improvements become the property of the lessee.

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The problems of the sheep industry.

In the absence of major epizootic diseases, severe intermittent droughts are undoubtedly the major problem affecting sheep farming in Australia. During my visit in June the worst-drought in living memory raged in some of the eastern parts of the country. Where food and water gave out altogether, farmers had to kill thousands of stock, while in other instances sheep in poor condition, were sent by rail two thousand miles to the west. Drought feeding consisted mainly of wheat which is cheap and available, but fodder was at a premium. The price of a ton of lucern increased from A£18 to over A£50.\*

At that time it was estimated that, if the drought did not worsen, between 25 and 30 million sheep would be lost through deaths and decreased reproduction. There have been seven serious Australia-wide droughts since about 1880. To give some indication of the ravages of previous droughts, it has been estimated that with the prolonged drought of 1895–1903 the national sheep figures dropped from 103 million in 1893 to 53 million in 1903, and that 30 years passed before the numbers increased to the 106 million of 1891. By 1942 the numbers were 125 million but drought cut these back to 90 million by the end of 1946. Only in 1953 was the predrought level reached again.

Low lambing percentages and high lamb losses are other major problems of the industry. Infertility caused by oestrogen-containing clovers and *medicago* spp. especially in Western Australia, adds to reproductive failure. Other toxicological aspects receiving attention are the poisonous properties of *Phalaris tuberosa* and lupinosis.

Internal parasitism and blowflies constitute the major disease problems. Toxoplamsosis, enzootic icterus, footrot, urolithiasis and posthitis cause losses but are relatively unimportant. Used to a host of major epizootic diseases as we are, it is difficult for the South African veterinarian to see any disease problem at all in Australia. The effects of predators on sheep farming are important. The two most important predators are dingoes and feral pigs, with foxes, eagles and crows playing a minor role. Dingoes actually still limit the distribution of sheep and are particularly troublesome in Western Australia. In Queensland most properties are protected by state erected dog-proof barrier fences costing A£600—A£1,000 per mile. Feral pigs, introduced by mariners during the early to middle years of the last century, infest large parts of the inland drainage systems of Queensland, the Darling Range system and northern New South Wales, and can cause particular havoc amongst young lambs.

#### Organisation and Financing of Research.

Research is undertaken by the C.S.I.R.O. (Commonwealth Scientific and Industrial Research Organization), the Universities, and the Departments of Agriculture of the different States.

The C.S.I.R.O. is a Commonwealth organiestablished by the Commonwealth Government to undertake industrial research related to the national problems of the primary and secondary industries and to develop Australia. It is financed by the Commonwealth Treasury together with additional funds from such sources as the Wool Research Trust Account, the Dairy Produce Research Trust Account and the Australian Cattle and Beef Research Trust Account. These funds are raised by a levy of two shillings per bale from the woolgrowers and the Commonwealth Government Contributes A£2 for every A£1 of the producers. In this way A£1.5 million becomes available The funds are administered by every year. appropriate bodies, such as the Australian Wool Board, appointed by the Commonwealth Government.

The C.S.I.R.O. is organised into a number of divisions responsible for independent research, or at times co-operating with Universities and State Departments of Agriculture. The Divisions of Animal Health, Animal Physiology, Animal Genetics and Plant Industry are the most important contributors to sheep and wool research.

The Faculties of Veterinary Science of the Universities of Sydney, Queensland and Mel-

<sup>\*</sup> By the middle of September it had still not rained. The drought was further complicated by two spells of very cold weather resulting in the first snow for 128 years at Hornsby near Sydney. Mackay (Queensland) at 21°S.L. had the first snow in recorded history.

bourne, the Faculties of Agriculture in the latter Universities and in the Universities of Western Australia, Tasmania, and Adelaide, together with the Faculty of Rural Science at New England University, and the Faculty of Wool Technology, University of N.S.W. are responsible for teaching and also undertake research and post-graduate training.

Financial support for the Universities is derived from Commonwealth funds and is allocated to the different Universities by the Australian Universities Commission.

The Departments of Agriculture of the respective States are responsible for diagnostic, regulatory, and regional research and extension services. Each State maintains its own laboratory services and undertakes investigational and research work of local interest. The main financial responsibility for all these services rests with the individual States, assisted by funds from the industry.

Research projects on sheep and wool problems are pursued at all the above institutions. The projects are financed by grants from the Wool Research Trust Fund of the Australian Woolboard amounting to A£0.25 million and A£0.2 million per annum for the Universities and State departments respectively. Allocation of monies from the Research Fund is suggested by the Wool Production Research Advisory Committee to the Wool Board, which decides. This Committee has a veterinarian as its executive officer and membership is composed of a nominee of the Minister of Primary Production, a representative of C.S.I.R.O. with the remainder from Universities, Departments of Agriculture and producers. These members are all nominated by the Wool Board and the producers have the controlling vote.

Research aims and trends.

The sheep industry is Australia's greatest asset, and periodic droughts, low carrying capacity of indigenous pastures, and a low reproduction rate her greatest problems. It is, therefore, not surprising to find that these factors influence the general trend of research.

All aspects of pasture improvement, increased reproduction, decreased lamb mortality, and increased production per acre receive attention. These central themes run through most projects. Bringing the fruits of research to the farmers and ensuring wide application is as difficult as in South Africa, and every effort is being made to support and strengthen the extension services.

To undertake this research task Australia requires in the next five years three hundred people with post-graduate training in plant and animal biochemistry, ecology, genetics, nutrition, physiology, entomology, mycology, toxicology, various aspects of animal health, extension education and field experimentation. At present an average of about twenty persons are trained in these disciplines annually.

To overcome this basic obstacle, study grants for post-graduate training receive high priority. This support has the added advantage of research being done at the same time.

In the past seven years Australia has spent about A£18 million on all research, i.e. production and textile, relating to the sheep and wool industry. By 1960 it became evident that the greatest disadvantage in this programme was a lack of definition of the problems facing the industry. This led to the formation of committees to define problems in reproduction, lamb survival, body and wool growth, pasture utilization, nutrition, drought flock and property management, genetics etc., Available information was documented and promising ideas were classified. This information was circulated to research and extension officers.

By 1963 the idea was conceived to call a conference for national stocktaking to see what had been achieved and to promote some reappraisal by workers in various fields of the sheep industry. In December of that year the first sheep and wool technical conference was held at Luera. Here available knowledge, whether applied by farmers or not, was discussed, areas requiring elucidation defined, and a priority list of projects drawn up. It was decided to evaluate the aims and trends emanating from this conference in five years and to hold similar conferences every subsequent five years. This particular conference also set the pattern for similar conferences in other directions, e.g. cattle, pasture, etc.

Apart from post-graduate training the highest priority is given to the following projects:

- (a) Increasing production through regional research;
- (b) conducting field surveys to define problems;
- (c) providing answers to problems which have already been recognised and defined;
- (d) providing information of both biological and economic value which can be incorporated into extension programmes.

Priority is given to extension programmes which are likely to be successful in:

- (a) Increasing production in a way that is economically satisfying to the industry;
- (b) providing practical, economically sound answers to problems which have been organised and defined;
- · (c) affecting production in extensive regions or large areas extending from State to State.

From the above priorities it should not be deduced that insufficient attention is being paid to basic research which cannot be directly translated into financial gain. The opposite is true but the Australians strive for a balanced laboratory-field programme and attempt to gain the maximum benefit from practical application of research results.

#### Current research

It is not possible to do justice to the standard and scope of the Australian research in a brief discussion. General progress in fields of particular importance to the South African sheep industry is the following:

Pasture improvement: For a comparatively small research investment on pasture improvement, the results have so far contributed an extra 30 million sheep to the total population, i.e. more than fifty per cent of the overall increase. In addition, it is estimated that the nitrogen fixed by legumes annually amounts to A£1,600 million worth of fertilizer, and an average increase of five bushels of wheat per acre has also resulted from increased soil fertility.

Research is aimed at introducing exotic species of legumes, improving established strains and creating, by selective breeding, new strains which will be useful in tropical and semi-arid areas. One of the main breakthroughs for temperate Australia in this field came with the realization that the *Rhizobia* required for vigourous growth of legumes are far more specific than previously realised and that inoculation with the correct *Rhizobium* is the first requirement.

By selective breeding C.S.I.R.O. evolved a new legume *Siratro* which appears particularly promising in the tropical areas where Townswille lucerne and certain *Glycine* strains are already used for pasture improvement.

Lucerne strains are selected for the temperate areas and particular attention is being paid to the adaptation of strains to the arid and semi-arid zones of South Australia. Lotononis, a legume introduced from the Worcester district in the Cape has proved particularly frost and drought resistant.

The basis of general pasture improvement in temperate Australia, however, remains subterranean clover, medicago spp., Phalaris and Rye grass and in Western Australia lupins as well.

The legumes are introduced directly into native pastures after suitable inoculation and pelleting of the seed, and preparation of the pasture through fertilizing with lime, phosphate, sulphur, and the required trace elements. Fertilizer and seed are dropped from aircraft or by introduction with a chisel plough.

The tremendous impact on carrying capacity can be judged from areas on the east coast of N.S.W. where 10–14 sheep per acre can be grazed under experimental conditions, while five sheep per acre are common on some farms. Improved pastures are already able to support 200 million sheep; a figure which can only be attained by 1980 because of low reproduction. The lack of sheep numbers becomes even more significant when it is realised that at present an additional two million acres are improved annually.

Increased reproduction by sheep: Studies conducted on a broad front include attempts to increase fertility in the ram and ewe, improvement of management and feeding, and basic research in endocrinology and physiology.

Oestrogen containing clovers cause sterility in the ewe, resulting in drastic reduction of lambing percentages. This problem varies with soil types and climate, but fortunately, this property of clovers can be overcome relatively easily by selection for low oestrogen containing strains.

Seminal degeneration as a result of extremely hot climates, and similar climatic effects on pregnancy, and on birth weight of lambs are being studied. In one of these projects canulization of the spermatic artery and the study of testicular blood flow with radio-isotopes belong to the most sophisticated techniques observed in the study of male reproductive physiology.

A study of the effects of undernutrition on ram fertility has revealed the important role of high energy rations in maintaining spermatogenesis. The close correlation between bodyweight (and therefore nutrition) and the ability of the ewe to reproduce or bear twins is most interesting. Ewes weighing less than 60 lb have a low conception rate while the possibility of bearing twins increases significantly when body weight exceeds 120 lb.

Autumn joining (May), when the Merino reaches a peak in reproductive activity, results in constant and significant increases in the lambing rate. The use of teaser rams and flushing 16 days before joining again increases lambing percentages significantly.

As spring mating holds definite advantages in certain circumstances, the use of one per cent teaser rams for alternate three week periods from the end of July to mating in the middle of October yielded 92 per cent oestrus periods as against 11 per cent where no teasers were used. These results have, however, been very variable and need further elucidation.

An important observation affecting the pregnant ewe is that a high plane of nutrition should not only be maintained before mating but continued for the first month after conception. On a falling plane of nutrition during the latter period, foetal loss may take place, resulting in a 30 per cent or more reduction in lambs born.

Approximately 80,000 sheep are inseminated artificially in Australia every year. Half of the total number is done by one private practitioner who has perfected this technique to the extent where, with trained helpers, he can inseminate up to 90 animals in an hour.

Lamb losses: Over 90 per cent of deaths in lambs occur within the first three days of life. The incidence of loss in Australia varies from about two to 29 per cent, according to climate and rainfall. Losses in the temperate zone with rainfall over 20 inches per annum, averages about ten per cent as opposed to twenty per cent in semi-aric tropical Australia. The majority of deaths is attributed to starvation, exposure to heat or cold, or a combination of these factors.

Amongst well nourished cross-bred ewes dystokia is the primary cause of lamb mortality. Infectious abortion has been attributed to Listeria monocytogenes, Vibrio fetus, Brucella ovis, and Toxoplasma gondii, but the overall loss caused by these organisms is low. In certain areas predation is a problem but, as in South Africa, these losses include attacks on abandoned or moribund animals or scavenging.

As losses in young sheep retard the increase in numbers, Australian workers have embarked on a series of studies to define the problem more accurately. These include field surveys of losses, nutrition of the pregnant ewe, effects of climate and of birth weight, and physiology of the new born lamb.

In field surveys a number of farmers cooperate by sending all, or a representative sample of dead lambs to laboratories for necropsy. The carcasses are gathered early in the morning, put into double polythene fertilizer bags, these being finally packed into grain bags. Specimens are sent by express train and arrive at the laboratories in excellent condition. Necropsy findings are not aimed so much at defining the exact cause of death but rather at establishing the time and circumstances of death. Results are classified into prenatal death, postnatal death where the lamb has never walked, death some time after birth where the fat stores have been metabolized etc. A number of other observations are recorded, e.g. presence and location of food in the intestinal tracts. type and time of predator attacks and congenital abnormalities.

The exact subdivisions of the above catagories and the specific observations required for each post-mortem examination are standardised on special forms and punch cards. The adoption of a standard procedure enables workers in different areas to compare their results.

Supplementing the ration of ewes during the last quarter of gestation with 8 oz. of grain daily, yielded a significantly higher survival rate in lambs. Diminished milk secretion due to malnutrition, absent or defective teats etc., caused a high lamb mortality and a low birth weight also decreased the survival rate.

Studies on neonatal physiology and lamb and mothering behaviour have elucidated some causes of death during the first three days of life. In hot humid climates lambs may suffer from heat prostration which is aggravated when the lamb is forced to move about. Resistance to cold stress is influenced by temperature, humidity, rate of air movement, the occurrence of rain or snow and the amount of milk suckled. Dry newborn lambs are able to maintain their body temperatures in ambient temperatures as low as -5°C where as cold wind and driving rain soon causes death from exposure.

A significant inverse relationship exists between birth weight and cooling after birth. Smaller lambs are less able to survive cold stress. Undernutrition of the pregnant ewe not only produces a lighter lamb but the total energy reserves in lambs from well fed and poorly fed ewes were approximately 1,000 and 400 K. cal respectively.

Mismothering in ewes and the suckling behaviour in lambs are being studied. In respect of the latter it is interesting to find that the suckling reflex of lambs decreases remarkably in twelve hours. If a lamb is prevented from suckling for a number of hours the chance of survival decreases proportionately.

In one experiment the improved management of the lambing flock reduced lamb losses from 24 to 11 per cent. This reduction was accomplished by a drift system where the whole flock was managed through a series of ten, 16 acre paddocks. Each morning the dry ewes were moved to the adjacent paddock while the wet ewes were left behind. The lambs were weighed, tagged and mothered daily and were docked, castrated and the Mules operation performed between the third and fifth day of life. With

their mothers they joined the main flock on the ninth day when the remaining dry ewes arrive in that particular paddock again.

Supplementary and drought feeding: Research in nutritional and allied fields is so wide that a comprehensive discussion is impossible.

Of particular interest to the South African is the emphasis on improved pasture and grazing management to carry the sheep throughout the year rather than supplementary feeding in winter as advocated here. Introduction of legumes with adequate fertilisation of pasture, conservation of grazing for winter, plus ensilage and hay making comprise the basic plan for feeding. If used, grain supplementation is in the form of wheat.

During the present drought, feeding blocks containing urea were being used extensively but the urea approach to overcome nitrogen deficiency in winter feeding, is much less evident in Australia than in South Africa. Perhaps the lack of cheap and readily available molasses in distant areas accounts for the reluctance to use urea molasses mixtures. The Australians, however, correctly maintain that little evidence exists under field conditions to warrant preference of urea over other forms of feeding sheep. The very efficient conservation of nitrogen by the undernourished sheep through recycling of urea to the rumen mainly through the saliva, has somewhat reduced the importance of a protein deficiency in winter grazing. A lack of energy in winter grazing seems, however, to be of great importance in computing adequate winter rations.

In conclusion it can be restated that through investigations into numerous aspects of rumen physiology, tracing the rôle of individual microand macro food components, and by supporting laboratory findings with field trials, the Australians are advancing on a broad front of sheep nutrition of great importance for South Africa.

Selective breeding: Some extensive and important breeding experiments are in progress but the contribution of this work to the economics of sheep industry as a whole is kept in proper perspective. The Australians realise that, to increase the production of wool and mutton per acre, the aim must be to produce sheep of high fertility and viability carrying

adequtae wool, rather than a high wool yielding, small, subfertile animal with a lowered resistance because of wool packing. It has been estimated that in stud flocks with optimal nutrition, increase in wool production through selective breeding will not amount to more than one ounce of greasy wool per head per year. This is four to six ounces per sheep generation of six years but it will take many years to transmit this progress from the studs to the national flocks. The contribution of one extra lamb every generation is evident.

A number of flocks are being selected for single characteristics but one of the most interesting results comes from the differences in flocks selected for fold score. In the fold plus flock, i.e. bred for well developed skin folds, it was found that there was an increased wool yield, but a lowered fertility and a higher lamb mortality. The lamb mortality did not only increase due to dystokia which is more prevalent in the fold plus lamb, but deaths continued also nearer to weaning age.

Fold plus rams appear more susceptible to summer heat with resultant semen deterioration, but this was not the only reason for lowered fertility since the trend persisted in fold plus ewes mated to fold minus rams.

In the fold minus flock the wool yield was not as high, but fertility as well as viability of the off-spring was significantly higher. Another interesting observation was that fold minus rams were easily trained with an artificial vagina while this was more difficult in the fold plus rams.

#### DISCUSSION

Wool is the main agricultural export product of both Australia and South Africa and the two countries have very similar production problems. Australians are straining every nerve to increase production and they believe that they are still able to increase their sheep population to 450 millions. In South Africa, however, the sheep population has not increased in the last decade or more and it is authoritatively stated that as we have reached the ceiling of our carrying capacity, we should concentrate on increased production per sheep.

One of the most serious threats to the wool industry is a diminished supply of the raw product resulting in a temporary unrealistic increase in price. This may permanently push the manufacturers into the arms of the artificial fibre trade with disastrous results for the whole sheep industry. The fate of the South African wool industry is thus intimately bound to that of the other wool producing countries and they in turn can expect us to do our share in production. In these circumstances it is not unfair to compare the trends in Australia and South Africa and to ask what we can learn from each other.

Until a very short while ago the veterinary profession in South Africa has had to contend with major epizootic diseases which have fortunately not existed in Australia. These diseases have seriously hampered our effort to inincrease production but we are now at the stage where we can pay attention to the erosion and reproductive diseases.

The contention that South Africa's carrying capacity will not allow bigger sheep numbers is, in the light of Australian experience, unrealistic and, for the progress of the industry, this idea must be abandoned. The highveld and a large area of Natal is not so different from the tableland and coastal belt of New South Wales that some of the spectacular increase in carrying capacity cannot be translated to South Africa. Similarly much of the winter rainfall area can be improved for sheep and cattle.

Up to the present the South African veterinary profession can hardly be blamed for the lack of definitive field studies on lamb losses, disease patterns, reproductive failure etc. A lack of veterinarians and more pressing stock diseases has precluded this, but the time seems to be at hand to initiate some of the more important of these projects.

The results of Australian research and experience with similar projects can be of great help in South Africa. Much has been done in that country to improve natural pasture and in the selection of legumes for tropical and semi-arid areas. They have also worked out procedures for field studies of lamb mortality, reproductive failures, and nutrition which can

immediately be applied to our own projects. Some of these projects are either under way or in the process of being undertaken and reference to Australian work can only be profitable.

Some suggestions with an Australian background are the following.

Compilation of a comprehensive bibliography of all research papers which have so far been published in South Africa on the sheep industry, supplemented by important publications from overseas which have a bearing on our research. The relatively small pig industry in South Africa has recently, through the Pig Research Committee, produced a South African bibliography for the pig industry!

To compile information on techniques which can be used in field investigations with sheep and to distribute this to all interested workers. A conference similar to the Sheep and Wool Technical Conference of Australia will bring together research workers of various disciplines working in sheep and wool research. Such a gathering will be able to define the most urgent problems and issue a priority list of research projects to be undertaken.

In conclusion the importance of post-graduate training and refresher courses cannot be overstressed. The Veterinary Faculty should do everything in its power to promote this. An exchange of post-graduate students between Australia and South Africa should be encouraged for the mutual benefit of both countries. In this respect an interesting example is the annual exchange of a prominent research worker between Australia and New Zealand for a period of six weeks, These visits are financed by the respective Wool Boards of the two countries.

#### **ACKNOWLEDGEMENTS**

I wish to record my sincere appreciation for the hospitality with which I was received everywhere in Australia by veterinary and other colleagues, during my recent visit. They all gave unstintingly of their time and experience and much of the information in this presentation was gained through personal communication.

My particular thanks go to Dr. G. R. Moule of the Australian Wool Board for checking the factual statements about Australian research.

#### REFERENCES

The author will be pleased to supply references or sources of information about statements in this article to any interested person.



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#### THE PATHOGENIC MYCOPLASMA OF DOMESTIC ANIMALS

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Veterinary Research Institute Onderstepoort.

Presented to the 60th Scientific Conference of the South African Veterinary Medical Association.

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#### SUMMARY

A description of the *Mycoplasmataceae* together with the historical development of our knowledge of these organisms and a brief review of the diseases and conditions attributed to them is presented.

#### Introduction

#### 1. Description.

Since the discovery of the causal agent of Contagious Bovine Pleuropneumonia in 1898<sup>1</sup>, the *Mycoplasmataceae* have become increasingly important to the veterinarians. The causal organism of Contagious Bovine Pleuropneumonia was at first classified as a virus due to its filterable properties, but after cultivation on artificial media and intensive studies on its unique morphology it maintained an isolated position in the microbiological world for many years.

Mycoplasmataceae are the smallest organisms that can be cultured on cell free media. The organisms possess no cell wall and are extremely pleomorphic. This pleomorphism is attributed to the plasticity of the organism, which some workers claim can be distorted into any shape by external physical forces<sup>2</sup>. Other workers claim that filaments and various morphological elements are part of the life cycle<sup>3</sup>.

Mycoplasmataceae can be cultured on liquid and solid media. On solid media they produce typical colonies barely visible to the naked eye. The colonies often have a dark central portion well embedded in the agar surrounded by a lighter peripheral zone growing on the surface.

Mycoplasmataceae have proved to be very exacting in their nutritive requirements. The pathogenic types are especially difficult to isolate and culture, and this explains why they have

evaded detection for so long. The requirement of cholesterol<sup>4</sup> as a growth factor in the media is a property shared by no other bacteria. Most media are enriched with serum or ascitic fluid to supply the cholesterol requirements. The organisms are resistant to penicillin but are sensitive to most of the broad spectrum antibiotics especially the tetracyclines.

#### 2. Historical Outline.

Contagious Bovine Pleuropneumonia was prevalent in Europe for centuries. Its infectivity was recognised by Willems, who in 1852 used virulent lymph injected into the tail tip to produce probably the earliest known veterinary vaccine<sup>5</sup>.

In 1898 Nocard and Roux cultured the causal organism by inoculating virulent pleuritic fluid into collodion sacs containing broth and inserting it into the peritoneal cavity of rabbits. Later Martins broth enriched with serum was used successfully as a culture medium. In 1900 Dujardin-Beaumetz<sup>6</sup> described the growth of the organism on solid media and in 1910 Bordet<sup>7</sup>, Borrel and Dujardin-Beaumetz<sup>8</sup>, the morphology and staining techniques.

The "virus" of Pleuropneumonia maintained an isolated position in the microbiological world until Bridre<sup>9</sup> in 1923 cultivated the causal organism of Infectious Agalactiae which resembled it morphologically and culturally. In 1934 Shoetensack<sup>10</sup> discovered another of this group from nasal secretions of dogs suffering with distemper. In 1936 the first saprophytes were

discovered by Laidlaw<sup>11</sup> from sewage filtrates and Seiffert<sup>12</sup> from soil in 1937. Dienes<sup>13</sup> isolated the first of this group from man in 1937. Longley<sup>14</sup> in 1940 described another of this group connected with Caprine Pleuropneumonia. Many more isolates followed and this prompted workers to refer to them as the Pleuropneumonia-like organisms (P.P.L.O.)

Of interest here is the discovery in 1936 of the cocobacilliform bodies, causing a chronic coryza of slow onset and long duration, by Nelson<sup>15</sup>. It was only in 1952 that the organism was cultured by Markham and Wong<sup>16</sup> in Edwards media and recognised as a *Mycoplasma*.

#### 3. The Pathogenic Mycoplasma.

Mycoplasmataceae have been isolated from the muccous membranes of many animals and man. Many of these, isolated from apparently normal individuals, have aroused much speculation as to their true nature. Edwards isolated Mycoplasmataceae from the genital tracts of cattle<sup>17</sup> and concluded that they were responsible for inflammatory processes causing poor fertility<sup>18</sup>. Albertsen<sup>19</sup> however, isolated the organism from normal healthy A.I. bulls and considered them non-pathogenic. Edwards20 also isolated three Mycoplasma strains from the vagina and throat of dogs. The a strain was derived mainly from the vagina but the  $\beta$  and  $\gamma$  strains were isolated from the throat as well as the vagina. It was also suggested that these may be of significance in inflammatory conditions of the genital tract<sup>21</sup>. In poultry Nelson's coccobacilliform bodies (M. gallisepticum), the aetiological agent of chronic respiratory disease, was differentiated from various non-pathogenic strains (M. gallinarum). Yoder and Hofstad<sup>22</sup> have since divided Mycoplasma of avian origin into 12 serological groups. Six of these have proved to be pathogenic.

Many attempts have been made to differentiate between pathogenic and non-pathogenic Mycoplasmataceae but no real conclusion has been arrived at. Edward<sup>4</sup> has shown that the pathogenic Mycoplasmataceae require cholesterol for growth. The saprophytic strains can be cultured without this factor<sup>23</sup>. Many recognised non-pathogens however, are difficult to culture and their cholesterol requirements are of the same order as the pathogenic types.

An interesting parallel in the pathogenic mycoplasma has emerged from the fact that most can produce serositis and arthritis on subcutaneous or I.P. injection. Many Mycoplamas organisms that are considered as commensals could however play a rôle in producing pathological conditions in combination with other agents or as a secondary infection. These must be considered as potential pathogens and may yet prove to be of great economic importance in animal husbandry.

#### Contagious Bovine Pleuropneumonia.

South Africa has been free of this disease for many years. The last outbreak was reported in the Waterberg district in 1921. The disease has however been diagnosed clinically, serologically and bacteriologically in Ovamboland, South West Africa.

M. mycoides, the aetiological agent, was the first orgainsm of this group to be isolated in 1898 by Nocard and Roux. It serves also as the type species for this group.

Transmission is by droplet infection, with direct contact of infected and susceptible animals<sup>5</sup>. Diseased animals in the acute stage are the most active spreaders of the disease but the clinically "recovered" animal with sequestra in the lungs are the most dangerous transmittors. Very often outbreaks can be traced back to the introduction of a "clinically" normal animal which was later found to harbour the organism in sequestrae in the lungs.<sup>5</sup>

The incubation period of this disease varies. Under natural conditions it is between 3-6 weeks but a period of 94 days<sup>24</sup> can elapse before symptoms appear.

In acute cases the temperature rises and the animal becomes listless. The coat is staring, anorexia and respiratory distress are observed. A dry husky and painful cough can be provoked on moving the animal or on percussing the chest<sup>5</sup>. The animal stands with its elbows turned out and each inspiration is followed by a contraction of the abdominal wall. Percussion of the chest cavity is dull depending on the extent of the lesions. Sometimes the whole pleural cavity can be filled up with a fibrinous mass.<sup>25</sup> Oedema of lower areas is sometimes present.

Subacute and chronic cases are not easy to diagnose. The lesions in the lungs are localised and the only symptom may be a rare cough. Acute cases may "recover" and usually go over to the chronic stage.

Lesions of the acute cases are very typical. The chest cavity shows a fibrinous pleurisy. The affected lungs are enlarged and in early cases small, dense, sharply circumscribed nodules may be found throughout the organ. Later extensive areas become consolidated with large amounts of straw coloured fluid excuding from cut surfaces. The interlobular septa is thickened and saturated with fluid giving the typical "marbling" effect. In chronic cases the interlobular septa are prominent containing large amounts of connective tissue. Sequestra of varying sizes can be found.

Diagnosis is by clinical symptoms. Fresh exudate and effected lungs on ice can be submitted for bacteriological confirmation and sera for the complement fixation test.

#### Pleuropneumonia of goats

This disease has not been reported in South Africa. Sheep are also susceptible<sup>14</sup>. Mortality is much higher than in Pleuropneumonia of cattle. Transmission is by direct contact. Incubation is 8–28 days. With the onset of symptoms the course is rapid and death occurs usually within a week. Temperature is normal but in many cases a rise in temperature occurs a day or 2 before death. Copious nasal discharge is a common feature. Coughing is not common. There is a loss of condition, listlessness and dyspnoea is marked. Appetite is not impaired.

At post mortem there is a pleuritis of varying degrees with a serous or sero-fibrinous effusion (never purulent). In the lungs a diffuse lesion often involving the apical and cardiac lobes is of an oedematous nature in both the alveolar and interlobular septa. Marbling is not as marked as in Bovine Pleuropneumonia. On incision a straw coloured fluid exudes from the affected areas. Less common is the focal lesion which may be single or multiple. These are hepatised 1 cm areas yellowish-grey in colour and slightly raised. A narrow grey zone surrounds the

lesion. Diagnosis is from the clinical symptoms, confirmed bacteriologically.

Chronic Respiratory disease.

In 1936 Nelson<sup>15,26</sup> subdivided coryza in fowls according to the onset, type and duration of symptoms. He differentiated between a coryza of slow onset and a chronic nature caused by a coccobacilliform body and an acute coryza of rapid onset and short duration caused by *Haemophilus gallinarum*. In 1943 Delaplane<sup>27</sup> described a chronic respiratory disease in poultry caused by an agent that could be propagated in embrionated eggs. In 1952 Markham and Wong<sup>16</sup> cultured this agent in artificial media and recognised it as belonging to the *Mycoplasma* group. They also recognised it as the coccobacilliform bodies of Nelson.

The disease is transmitted by contact. It can be brought onto a clean farm by carrier birds or infective material. The disease is also transmitted through the egg. Van Roekel<sup>28</sup> observed lesions in embrios and chicks obtained from commercial flocks affected with the disease. These findings were supported by Fahey and Crawley.<sup>29</sup>

The incubation period varies between 11 and 18 days<sup>30</sup>. With experimental infections this can be reduced. Young chickens are more susceptible than adults. Highly bred birds such as broilers have proved to be very susceptible. Mortality is low in adult birds but amongst broilers can be as high as 30 per cent<sup>31</sup>.

In adult birds the symptoms are nasal discharge, which in early cases can be unilateral, tracheal râles, gaping and coughing. In badly infected flocks swollen sinusses and eye lesions, common in turkeys, are also observed. The disease affects the feed intake, egg production declines, and the birds loose weight. Among younger birds and broilers the disease is more pronounced. Symptoms can vary according to the nature of synergistic agents. Fahey and Crawley<sup>32</sup> isolated a viral agent during their studies. Adler<sup>33</sup> describes Infectious Bronchitis and Fabricant<sup>34</sup> Infectious Bronchitis and pathogenic *E. coli* as complicating agents.

Diagnosis is made from the symptoms, history and confirmed with the plate agglutination, tube agglutination or haemagglutination inhibition tests. Isolation and identification of the causal organism is the absolute confirmation of the presence of the disease.

Infectious Synovitis.

Olson<sup>35</sup> and Wills<sup>36</sup> independently described a disease of chickens involving the synovial structures. The disease often occurred together with respiratory diseases and a haemorrhagic condition. The infectious agent could be propagated in embrionated eggs and filtration experiments<sup>37</sup> showed that the agent was either a large virus or small bacterium. Lecce<sup>38</sup> using the satellite phenomenon of *Micrococcus sp.* on *Mycoplasma* colonies cultured the agent and proved it to be a *Mycoplasma*.

The disease is observed primarily in young birds 4-1235 weeks of age in broiler flocks. Mortality is low but morbidity may be as high as 75 per cent. The infection spreads slowly and the incubation period varies between 24-80 days<sup>39</sup>. Transmission can be affected by inoculation of infective material into the foot pad or by I.V., I.M., intrasinous, I.P. and conjunctival routes. Egg transmission has also been reported<sup>40</sup>. Direct contact is necessary for the spread of infection.

The first symptoms are pale combs, lameness and retarded growth. As the disease progresses the combs shrink and becomes bluish-red in some cases. Swellings of the joints and breast blisters are observed, the hock joint is most commonly affected and contains a viscous creamy to grey exudate which becomes caseous. Some birds exhibit no joint lesions but a more generalised form of the disease. They are listless, emaciated and have a greenish diarrhoea.

The causal organism is present in the blood<sup>41</sup> in infectious levels after 8 hours following an I.V. injection, and 48 hours after I.M. injection. This infectious level can persist until the 15th day. The agent can be recovered for all tissues except the bile and intestinal contents suggesting a true viraemia. The organism disappears from body tissues after 52 days. The spleen is enlarged with the liver also enlarged and having occasionally a mottled greenish or dark red appearance. The kidneys are swollen and mottled to pale.

The organism is susceptible to the broad spectrum antibiotics especially the chlorte-tracyclines<sup>42</sup>.

Infectious Agalactiae of Sheep and Goats.

When the agent of Infectious Agalactiae was cultured in 1923 by Birdré and Donatien<sup>9</sup> it became the second organism of this group known to microbiologists.

The organism, M. agalactiae, can remain in the dung for considerable periods<sup>2</sup>. Infection occurs per os and may remain latent until stress conditions allow the manifestation of disease symptoms. It is during the lambing season that the disease is most prevalent. It produced a catarrhal or parenchymal mastitis with a viscous purulent discharge or a watery milk that divides into 2 layers, an upper bluish grey and a lower sediment of yellow green granules. The mammary tissue becomes flabby, with hard nodules. Infected quarters are permanently damaged.

In severe outbreaks lambs, females and males develop a parenchymatous keratitis or panophthalmia with an eventual rupture of the cornea. Joints of the legs are also infected. They become oedematous and purulent. Rupture may occur. The monocytes show a considerable increase in the blood stream. The disease is most prevalent in the mediterranian countries and the near east. It has not yet been found in South Africa.

Bovine Mastitis.

In 1963 Stuart et al<sup>43</sup> published a detailed report on an outbreak of mastitis in Friesian cows caused by a *Mycoplasma* resembling *M. bovigenitalium*.

Twenty six out of 28 animals in their first lactation and 6 out of 29 in their second lactation were affected. The affected quarters became enlarged and tense without evidence of pain. The animals otherwise showed no effects. Milk from infected quarters was slightly yellow when first drawn and when left to stand for a few minutes separated to form a deposit or an upper layer of fine flakes from a whey like fluid.

Cultures of the *Mycoplasma* infused into the teat canals set up a mastitis indistinguishable from natural cases. Infected quarters usually returned to normal the following lactation.

#### ENZOOTIC PNEUMONIA.

Synonyms: Virus pneumonia of Pigs.

This is the most common respiratory disease in pigs<sup>44</sup> and though mortality may be very low, morbidity is high. The disease has been transmitted by bacterial free filtrates and the infective agent can be grown in tissue culture<sup>45,46</sup>. Pleomorphic organisms in tissue cultures and in smears made from infective lungs are always present.

The disease is characterised by a chronic non-productive cough. The incubation period varies between 7-30 days. The onset of the disease is gradual and the cough may persist for a long time. Recovery is very gradual and may be complete but some animals remain infective although they have apparently recovered. These animals are unthrifty and there is reduced efficiency of food conversion. The disease is more severe under crowded conditions.

Transmission is by droplet infection during coughing and sneezing. The organism remains infective outside the body for a very short time. After 24 hours infected houses that had been vacated are no longer infective for susceptible pigs. Pigs out of doors are rarely affected by the disease.

Lesions are usually confined to the anterior and inferior lobes and there is consolidation of these areas. The lesions are pale pink to grey and in early cases not well demarcated. The cut surface is oedematous and there is an accumulation of exudate in the bronchioles. Later the lesions become darker in colour and partially collapsed.

Due to the fact that the tetracyclines can prevent the establishment of an infection and also reduce the severity of the disease, and also the presence of pleomorphic organisms in tissue cultures and infected lungs this organism is very likely one of the *Mycoplasma* group.

Apart from the diseases mentioned above *Mycoplasmataceae* have been isolated from many other conditions. Many of them have proved to be non-pathogenic. Some however, have proved to be virulent when inoculated into animals. The rôle these organisms may play in animal health is not completely clear at the moment.

#### Swine

In 1945 McNutt et al.<sup>47</sup> studied an outbreak of arthritis in weaned pigs. *Erysipelas* was recovered from some of the infected pigs but many purulent lesions yielded no bacteria when cultured. The agent was however cultured in embrionated eggs, and proved infective when inoculated S.C. and I.P., producing arthritis, pericarditis, pleuritis and peritonitis.

Switzer<sup>48</sup> in 1953 isolated a filterable agent from the nasal mucosa of swine with atrophic rhinitis. This agent was propagated in embrionated eggs and could on I.P. injection produce polyserositis and arthritis. The agent had no connection with atrophic rhinitis, and was later recognised as a *Mycoplasma*, and named *M. hyorrhinus*.

Carter<sup>49</sup> in 1954 also isolated *Mycoplasma* from pneumonic lungs of swine. These organisms could also produce polyserositis and arthritis in pigs although pneumonia could not be produced.

Lecce<sup>38</sup> in 1960 described two agents in a fatal disease of colostrum free piglets reared in isolation. As soon as these piglets were moved to farms and exposed to conditions where pigs had been reared naturally they developed a polyserositis, arthritis, haemorrhagic gastroenteritis and died. He proved that the polyserostis and arthritis was caused by a *Mycoplasma* and gastroenteritis by *Haemophilus suis*. If colostrum free piglets were reared in isolation and infected with *Mycoplasma* they developed a very mild polyserositis. Naturally raised piglets proved to be immune when infected with *Mycoplasma*.

Sheep and Goats.

Cordy<sup>50</sup> et al. in 1955 described a highly fatal disease of sheep and goats characterised by septicaemia and arthritis. The aetiological agent proved to by a *Mycoplasma*. The disease according to them differed from Infectious agalactiae and Caprine pleuropneumonia. Culturally the organism resembled that of Caprine pleuropneumonia. It is of interest to note that this organism is also pathogenic for swine.<sup>51</sup>

Boiden<sup>52</sup> et al. in 1958 recovered from ovine pneumonia a *Mycoplasma* and a virus. The *Mycoplasma* did not produce pneumonia by

intratrachial inoculation but did produce arthritis. The virus was responsible for the pneumonic lesions.

#### Calves.

Meyer<sup>5</sup> in 1910 described in South Africa a poly-arthritis that resulted in calves injected subcutaneously with M. mycoides. Moulton<sup>53</sup> et al. 1956, described a Mycoplasma from arthritis in calves. The Mycoplasma was not related to M. mycoides. The lesions were of a fibrinous nature.

Simmons 1963<sup>54</sup> described a fibro-purulent arthritis in calves from which a Mycoplasma was recovered. Infected animals were negative to serological tests with M. mycoides.

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#### REFERENCES

- 1. NOCARD, ROUX, BORREL, SALIMBENI and DUJARDIN-BEAUMETZ. (1898). Le microbe de la péripneumonie. Ann. Inst. Past., 12, 240-262.
- 2: KLEINEBERGER-NOBEL, E. (1962). Pleuropneumonia-like organisms (P.P.L.O.). Mycoplasmataceae. Academic press. London and New York.
- FREUNDT, E. A. (1960). Morphology and classification of the P.P.L.O. Ann. N.Y. Acad. Sci. 79, 312.
   EDWARD, D. G., f.f., and FITZGERALD, W.A. (1951). Cholesterol in the growth of organisms of the Pleuro-
- pneumonia group. J. gen. Microbiol. 5, 576. HENNING, M. W. (1949). Animal diseases in South Africa. Second edition. Central News Agency Ltd. South Africa
- 6. DUJARDIN-BEAUMETZ, R. (1936). Le microbe de la péripneumonie et sa culture. These de Paris, as quoted by Turner.

- BORDET, J. (1910). La morphologie du microbe de la pérepneumonie des bovides. Ann. Inst. Past., 24: 161.
   BORREL, DUJARDIN-BEAUMETZ, JEANTET and JOUAN. (1910). Le microbe de la péripneumonie. Ann. Inst. Past., 24: 168.
   BRIDRÉ, J., DONATIEN, A., and HILBERT. (1923). Le microbe de l'agalaxie contagieuse mouton et de la chévre. Ann. Inst. Pasteur, 39: 925.
   SHOETENSACK, H. M. (1936). Pure cultivation of filterable virus isolated from canine distemper (Part 11). Morphological and cultural features of Asterococcus canis, Type 1, n.sp. and Asterococcus canis, Type 2, n.sp. Vicerte Appl. 13: 175.

- LAIDLAW, P. P., and ELFORD, W. J. (1936). A new group of filterable organisms. Proc. roy. Soc., B. 120: 292.
   SEIFFERT, G. Filtrable Mikroorganismen in den freien Natur. Zbl. Bakt. Abt. 1. Orig. Beiheft. 140: 168.
   DIENES, L., and EDSALL, J. (1937). Observations on L-organisms of Kleineberger. Proc. Soc. exp. Biol., N.Y. 82: 36.

- N.Y. 82: 36.
   LONGLEY, F. O. (1940). Contagious Pleuropneumonia of Goats. Indian J. Vet. Sci. 16: 127.
   NELSON, J. B. (1936). Coccobacilliform bodies in birds infected with coryza of slow onset. J. Exp. Med. 63: 515.
   MARKHAM, F. S. and WONG, S. C. (1952). Pleuropneumonia-like organisms in the aetiology of Turkey sinusitis and Chronic respiratory disease of chickens. Poulry sci. 31: 902.
   EDWARD, D. G. f.f., HANCOCK, J. L. and HIGNETT, S. L. (1947). Isolation of Pleuropneumonia-like organisms from the Bovine genital tract. Vet. Record. 59: 329.
   EDWARD, D. G., f.f. (1952). The Pleuropneumonia group of organisms and their significance in genital infection Brit. J. Venereal Diseases 27: 29.
   AI BERTSEN B. F. (1955). P.P.L.O. in the semen of Danish artificial insemination bulls. Nord. Veterinärmed.

- 19. ALBERTSEN, B. E. (1955). P.P.L.O. in the semen of Danish artificial insemination bulls. Nord. Veterinärmed. 7: 169.
- 20. EDWARD, D. G., f.f., and FITZGERALD, W. A. (1951). The isolation of organisms of the pleuropneumonia group from dogs. J. gen. Microbiol. 5.: 566.
- CHÚ, H. P. and BEVERIDGE, W. I. B. (1954). Chronic balano-posthitis in dogs associated with Pleuropneumonia-like organisms. Symposium sur les uretritis non gonococcignes, Monaco. YODER and HOFSTAD, M. S. (1964). Characterisation of Avian Mycoplasma. Av. Dis. 8: 481.
- 23. RAZIN and KNIGHT, B. C. J. G. (1960). A partially defined medium for the growth of mycoplasma. J. gen.
- microbiol. 22: 1492. 24. PRIESTLEY, F. W. (1961). Report to the Govt. of the Somalia on Contagious Bovine Pleuropneumonia. F.A.O. Rome 1961.
- Own observations.
- 26. NELSON. J. B. (1936). A coryza of slow onset. J. Exp. Med. 63, 509.
- DELAPLANE, J. P. and STUART, H. O. (1943). The propagation of a virus in Embrionated Chicken eggs causing a chronic respiratory disease in chickens. Am. J. Vet. Res. 4: 325.
   VAN ROEKEL, H., OLESUIK, O. M. and PECK, H. A. (1952). Chronic respiratory disease of chickens.
- Am. J. Vet. Res. 13: 252.

- 29. FAHLEY, J. E. and CRAWLEY, J. F. (1954). Studies on Chronic Respiratory disease of chickens. III. Egg transmission of a Pleuropneumonia-like organism. Canad. J. Comp. Med. & Vet. Sci. 18: 67.
  HOFSTAD, M. S. (1952). Chronic Respiratory disease. Progress report of Vet. Med. Res. Inst. Iowa State
- College, Ames, Iowa.
- 31. HOSTAD, M. S. (1959). Diseases of Poultry. Edited by Biester and Swart. 4th Ed. The Iowa State University Press, Ames, Iowa.
- FAHLEY, J. E. and CRAWLEY, J. F. (1954). Studies on chronic Respiratory disease of chickens. 11. Isolation of a virus. Canad. J. Comp. and. Vet. Sc. 18: 13.
   ADLER, H. E. and YAMAMOTO, R. (1957). Pathological and non pathological Pleuropneumonia-like or-
- ganisms in Infectious sinusitis of turkeys. Am. J. Vet. Res. 18: 655.

  EABRICANT, J. and LEVINE, P. P. (1962). Experimental production of complicated Chronic respiratory disease infection ("air sac" disease). Av. Dis. 6: 13.
- OLSEN, N. O., BLETNER, J. K., MUNRO, D. A. and ANDERSON, G. C. (1954). Enlarged joint condition in poultry caused by an infectious agent. Poultry Sci. 33: 1075.
- WILLS, F. K. (1954). Preliminary report on transmission of an agent producing arthritis in chickens. Texas Agr. Exper. Sta. Prog. report 1674.

  37. COVER, M. S., GELETA, J. N. and WALLER, E. F. (1956). The aetiology of an arthritic disease of chickens.
- Am. J. Vet. Res. 17: 12.
- LECCE, J. C. (1960). Porcine polyserositis with arthritis: Isolation of a fastidious Pleuropneumonia-like organism and Haemophilus suis. Ann. N.Y. Acad. Sci. 79: 670.
- OLSON, N. O. (1959). Diseases of Poultry Edited by Biester and Swart. 4th ed. The Iowa State University Press, Ames Iowa.
- SNOEYENBOS, G. H. and BOSCH, H. I. (1958). A further indication of egg transmission of Infectious synovitis.
- Av. Dis. 2: 494.

  BENTON, W. J. and COVER, M. S. (1959). The infectivity of blood and tissues from chickens with Infectious synovitis. Av. Dis. 3: 361.
- 42. MUNRO, D. A., OLSON, N. O., SHELTON, D. C. and WEAKLY, C. E. (1956). Synovitis control. 5. Intramuscular streptomycin and a comparison of continuous and intermittent feeding of Aureomycin and Furazolidone
- (NF-180). Poultry Sci. 35: 1161. STUART, P., DAVIDSON, I., SLAVIN, G., EDGSON, F. A. and HOWELL, D. (1963). Bovine mastitis caused by Mycoplasma. Vet. Rec. 75: 59.
- WHITTLESTONE, P. (1957). Some respiratory diseases of pigs. Vet. Rec. 69: 1357.

  GOODWIN, R. F. W. and WHITTLESTONE, P. (1962). Production of Enzootic pneumonia in pigs with an agent grown in tissue culture from the natural disease. Br. J. Exp. Path. 44: 291.
- BETTS, A. O. and WHITTLESTONE, P. (1963). Enzootic or virus pneumonia of Pigs: The production of Pneumonia with tissue culture fluids. Res. Vet. Sci. 4: 471.

  McNUTT, S. H., LEITH, T. S. and UNDERBJERG, G. K. (1945). An active agent isolated from hogs affected
- with arthritis. (Preliminary report). Am. J. Vet. Res. 6: 247. SWITZER, W. P. (1953). Studies on atrophic rhinitis of swine II. Intraperitoneal and intranasal inoculation of
- young pigs with a filterable agent isolated from nasal mucosa of swine. J. Am. Vet. Med. Assoc. 48: 392. CARTER, G. R. and SCHRODER, J. D. (1956). Virus Pneumonia of Pigs in Canada with special reference to the rôle of Pleuropreumonia-like organisms. Cornell Vet. 46: 344. CORDY, D. R., ADLER, H. E. and YAMAMOTO, R. (1955). A pathogenic Pleuropneumonia-like organism from goats. Cornell Vet. 45: 50. CORDY, D. R., ADLER, H. E. and BERG, J. (1958). The pathogenicity for swine of a pleuropneumonia-like 49.
- organism from goats. Conrnell Vet. 48: 25
- BOIDIN, A. G., CORDY, D. R. and ADLER, H. E. (1958). A Pleuropneumonia-like organism and a virus in ovine pneumonia in California. *Cornell Vet.* 48: 410.
- MOULTON, J. E., BOIDIN, A. G. and RHODE, E. A. (1956). A pathogenic Pleuropneumonia-like organism from a calf. J. Am. Vet. Med. Assoc. 129: 364. SIMMONS, G. C. and JOHNSON, L. A. Y. (1963). Arthritis in calves caused by Mycoplasma sp. Austr. Vet. J.
- 39: 11.



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#### A NOTE ON THE TOXICITY OF COTYLEDON ORBICULATA L.

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#### SUMMARY

Cotyledon orbiculata L. has for long been suspected of being a hazardous plant to livestock in South Africa. Final proof of its toxicity has now been accumulated. A description of the plant and its toxicity to sheep, goats and rabbits is presented together with the symptoms, biochemical changes and pathological lesions observed. This plant is another Cotyledon species causing the disease "Krimpsiekte".

#### INTRODUCTION

Cotyledon orbiculata was found toxic and to produce the disease "Krimpsiekte" by Davy1, Kehoe<sup>2</sup> and Kamerman<sup>3,4</sup>. According to later workers4,5,6,7,8,9 this species was apparently confused with Cotyledon leucophylla C.A.Sm (a Transvaal species) upon which the previous work was actually done. Henning10 and Sapeika<sup>11</sup>, who both collected material from the Karoo, probably used the correct species in their studies. The former<sup>10</sup> found his material to be non-toxic. Sapeika<sup>11</sup> on the other hand, feeding extracts of the plant to guinea-pigs found one specimen to be negative but a second specimen appeared to have the same pharmacological action on the heart as did Cotyledon walichii Harv. He concluded that the plant contained significant amounts of the active principle.

During October 1963 a farmer at Maltahöhe in South West Africa lost a number of sheep, showing symptoms closely resembling those of Krimpsiekte. He suspected plant poisoning and submitted a bagful of the suspected material for examination. The plants received were identified by the Chief of the Botanical Research

Institute in Pretoria as Cotyledon orbiculata L. The results of the biological tests performed with this material are presented here in an attempt to dispel the doubts that exists regarding the toxicity of this plant.



Fig. 1 Cotyledon orbiculataL.

#### Description of the plant:

Cotyledon orbiculata L. (family Crassulaceae) commonly called Pig's Ear, Honde-oor or Plakkie occurs as entirely glabrous succulent shrublets of up to 80 cms. in height, throughout the Cape Province (with the exception of the north eastern areas) Basutoland, Southern Natal, the eastern Orange Free State, and Southern and Central South West Africa. The plant, which flowers during the spring and summer months, carries stout erect branches with the leaves not clustered at the end of these. The main branches are themselves much branched; the leaves are opposite, 5-15 cm. long, 2-5 cm. wide, obovate, usually mucronate and dark green to glaucous red margins. Peduncles are 20-40 (-70) cm. long, glabrous, sometimes branched, each bearing a terminal cyme of 10-30 flowers. flowers are 2.5-5 cm. long, pendulous when flowering and erect when fruiting. Calyces are short and fleshy with free lobes up to 6 mm. long; Corolla tubes are oblong-campanulate, slightly inflated above the insertion of the filaments, longer than the corolla lobes and red or sometimes yellow or orange in colour. Ten stamens are borne in two series, with a tuft of hairs around the insertion into the corolla tube and the outer stamens are the shorter. There are five scale-like nectaries at the base of the ovary, alternate with the carpels. The ovary has 5 free or partly fused carpels and the syles are free.

This species is very similar to Cotyledon leucophylla R. A. Dyer ex C.A. Sm., which occurs only in the Transvaal. According to Dyer<sup>12</sup>, C. orbiculata may be distinguished from the latter by the corolla tube being inflated and longer than the free corolla lobes; it being cylindrical and shorter than these lobes in C. leucophylla.

#### TOXICITY TRIALS

(i) Rabbits: The fresh plant material, i.e. flowers, stems and leaves, was minced separately and each dosed to a rabbit at a rate of 5 g/kg. on the first day and subsequently at a rate of 7 g/kg./day. The animal receiving the flowers died 48 hours after the commencement of the experiment and the other two after 72 hours.

(ii) Sheep: Sheep No. 1, a young Merino ewe of 26 kg. was dosed with 7 g/kg. bodyweight of the mixture of semi-dried stems and leaves. Death followed within 24 hours.

Sheep No. 2, a 4-tooth Merino ewe of 31.7 kg, was dosed once with 1 g/kg, of the same and died 72 hours later.

Sheep No. 3, an adult Merino ewe of 32.7 kg. was dosed once with 250 mgm./kg. and kept under observation for 14 days. No symptoms occurred and it was assumed that this dosage level was a sub-toxic one. To determine whether there was a cumulative effect the same sheep was dosed with 250 mgm./kg./day on the 15th and 16th days after the commencement of the experiment. On the following day (17th) the animal showed symptoms of tachycardia, ruminal stasis and lethargy. The symptoms became progressively worse up to the 19th day; the sheep then improving until it appeared completely normal by the 25th day. The animal was rested up to the 29th day and the whole procedure then repeated. Dosing commenced with 250 mgm/ kg. on the 30th day; no symptoms occurred during the following week and the sheep was then dosed with 250 mgm/kg, of material daily on the 38th and 39th days. Symptoms were seen once more, lasting for eleven days instead of the six days observed earlier and they were more severe. The animal was again left without further dosing until the 57th day when the dosage was slightly increased to one dose of 300 mgm./kg. No symptoms occurred during the following week. Administration of material was continued with a dosage level of 300 mgm./ kg./day on the 64th and 65th days. occurred within 24 hours after the last dose.

Sheep No. 4, an adult Merino ewe weighing 26.8 kg. was given six doses of 100 mgm./kg./day spread over 8 days (Saturdays and Sundays were excluded). The animal was not dosed on the 9th and 10th days, since a ruminal stasis had developed. From the 11th to the 15th day the animal received 100 mgm./kg. of material daily, when symptoms occurred lasting 5 days. It, therefore, seemed that a daily dosage level of 100 mgm./kg. had a cumulative effect, similar to that of 250 and 300 mgm./kg./day used with sheep No. 3. Dosing was resumed from the twenty-fourth to the thirty-sixth day of the

experiment using a dosage level of 50 mgm./kg. daily (i.e. 9 dosages over 13 days). Severe symptoms occurred which lasted for seven days after the last administration. The animal was once again rested and then given 25 mgm./kg. of material daily from the 50th to the 132nd day (i.e. 69 dosages spread over eighty-two days). No symptoms occurred. By accident the animal was dosed with 0.9 g./kg. on the 134th day. She became very ill and died 3 days later. It is

interesting to note that if this animal is compared with sheep No. 2 (which died 3 days after one dose of 1 g./kg.), it showed no increased susceptibility due to the prolonged dosing at that level.

Sheep No. 5. Up to this stage the well known symptom of torticollis in Krimpsiekte had not been seen, therefore the dosing pattern shown in Table 1 was followed:

TABLE 1.—THE DOSING PATTERN FOLLOWED IN SHEEP NO. 5 WITH Cotyledon Orbiculatal L.

Days	1	8	15	30	50	65	78	86	99	106	113	128	134	141
Dosage mg/kg	250	300	325	350	400	425	450	475	500	525	550	600	650	700

The sheep became ill following all doses of 300 mg/kg, and over but recovered completely before the succeeding one in the regimen shown in Table 1. The sheep was discharged from the experiment on the 150th day. Apparently the time between each dose was sufficient for the animal to recover from all effects of the previous dose.

(iii) Goats: As goats are known to be very susceptible to the toxic effects of the plant, two were dosed with the above material. The first received a single dose of 500 mgm/kg. and the second two such dosage levels on two successive days. The first animal became and stayed ill for eight days and then recovered, while the latter one died within 24 hours following the last dose.

#### SYMPTOMATOLOGY

In all instances where the animals became acutely ill and died, tachycardia (e.g. 160-200/min), polypnoea (e.g. 60/min), associated with a grunt on expiration, and ruminal stasis (e.g. 0/5 minutes) were seen. A slight rise in body temperature of 1-2°F occurred in some cases twenty-four hours prior to death, which followed after 1-3 days of dosing.

Animals which did not die, presented tachycardia (e.g. 114-120/mins) lasting for two to three days; no polypnoea, a progressive ruminal atony (e.g. 5, 4, 3, in 5 minuts) until a complete stasis was reached after one to four days, and then took anything up to thirteen days to recover. Such cases also showed lethargy, anorexia and a muco-nasal-catarrh, lasting for six to eight days. These animals tired quickly when chased, were very unsteady on their feet and often stumbled. Muscular tremors were sometimes observed. A soft stool was only occasionally seen. In the goats, muscular tremors and shivering were much more pronounced and diarrhoea was marked.

Sheep No. 4, which received the chronic dosage level of 50 mgm/kg/day, exhibited anorexia, a hollow appearance of the flanks and a slightly arched back in the initial stages of intoxication. Sluggishness of the ruminal movements was observed later. These symptoms became worse until complete runimal stasis supervened and dosing had to be discontinued. No tachycardia or polypnoea was seen in this nstance.

In the case of sheep No. I sudden death followed bloat. Torticollis was not observed in any of the experimental animals.

#### CHEMICAL PATHOLOGY

Haematological examinations were performed using standard techniques. Chemical estimations of the various blood constituents studied were done according to the methods indicated in parentheses; Urea nitrogen (B.U.N.)<sup>(13)</sup>, creatinine <sup>(14)</sup>, glutamic-oxalacetic transaminase <sup>(15)</sup>, bilirubin <sup>(16)</sup>, total plasma protein <sup>(17)</sup>, cholesterol <sup>(17)</sup>, glucose <sup>(14)</sup>, calcium <sup>(18)</sup>, magnesium <sup>(19)</sup>, total inorganic phosphate <sup>(17)</sup>, chloride <sup>(14)</sup> and bicarbonate <sup>(20)</sup>. The zinc sulphate turbidity test was used as set out by Kunkel <sup>(21)</sup>.

A tendency towards elevation of the blood urea nitrogen (B.U.N.) and to a lesser extent of blood creatinine levels was noticed soon after the animals were dosed, these values returning to normal as the symptoms disappeared and the animals improved. This is illustrated by the figures in Tables 2 and 3.

TABLE 2.—THE CHEMICO-PATHOLOGICAL CHANGES IN THE BLOOD OF SHEEP NO. 2.

Day	1	2	
B.U.N. mg.% Creatinine mg.%	23.9 0.6	23.9 0.9	31.3 2.0
Dosage g/kg	1.0	0	0

TABLE 3.—THE CHEMICO-PATHOLOGICAL CHANGES IN THE BLOOD OF SHEEP NO. 5.

Day	86	87	89	92	106	107	109	113	114	120
B.U.N. mg.%	22.0 0.6	64:4 1.7	27.6 0.6	17.6 0.9	_	46.0 2.2	25.8 1.4	_	40.5 1.7	18.4 0.6
Dosage mg/kg	475	0	0	0	525	0	0	550	0	0

Sheep No. 4 and one of the goats showed more or less the same pattern. Sheep No. 1 and the other goat died before blood could be collected. Sheep No. 3 did not show any rise in B.U.N. or creatinine.

Seventy-eight blood samples from the various animals used were examined and no abnormalities in the red blood cells as shown by the sedimentation rate, haematocrit and haemoglobin concentration were observed. No liver damage as evidenced by assay of serum glutamicoxalacetic transaminase activity, conjugated and unconjugated bilirubin concentrations and performance of the zinc sulphate turbidity test was observed. Values for total plasma proteins, serum cholesterol, blood sugar, calcium, magnesium, phosphates, chlorides and plasma bicarbonate appeared unaffected in all the experimental animals.

#### **PATHOLOGY**

Congestion, oedema and emphysema of the lungs were the only lesions found in the rabbits used. No histological examinations were performed on the specimens from these animals.

Generalised congestion of the carcass was seen in the sheep and goats. Mild hyperaemia and oedema of the lungs, with marked sub-epiand subendocardial haemorrhages were present together with focal hyperaemia of the abomasum and small intestines in many instances. On

histological examination of necropsy material fatty infiltration of the liver and the presence of groups of large pigment bearing cells in the lymph nodes were sometimes encountered, but no kidney damage was discernible.

#### DISCUSSION

This plant has evoked symptoms or caused death in all of the ten experimental animals dosed with it. Except for torticollis, all the symptoms of the disease, Krimpsiekte, have been reproduced and Cotyledon orbiculata L. should now be added to the short list of Cotyledons which have been proved beyond doubt to cause this disease; namely:

C. decussata Sims<sup>3</sup>; C. Leucophylla C.A. Sm.<sup>2</sup>; C. ventricosa Burm.<sup>4</sup>; C. wallichii Harv.<sup>10</sup>; and C. panniculata L.f.<sup>3</sup>.

The single minimum lethal dose of this particular batch of plant material in a nearly dried state, seemed to lie between 0.7 and 1.0 g/kg. body-weight. Definite indications that the toxic principle has a cumulative effect were found. For example, while 700 mgm/kg. was found to be a toxic but sub-lethal dose, two doses of 300 mgm/kg. on successive days proved lethal, and dosage levels of as little as 50 mgm/kg/day produced symptoms after some time. (13 days in this instance).

Evidence has also been found that the animal recovers completely after a rest of approximate-

ly 14 days following the last dose and no increased susceptibility remains. This is not in accord with statement by Henning<sup>10</sup>, namely, that the condition may not appear until months or even years after exposure to toxic material. There is also evidence that animals can completely detoxicate small amounts of toxin administered daily. In this regard sheep No. 4, which showed signs of the disease three times in succession, was rested and then dosed with 25 mgm/kg/day over 82 days without showing any ill effects. This animal was subsequently accidentally dosed with 0.9 g/kg and reacted exactly like a completely susceptible sheep (e.g. No. 2.).

Indications of a mild kidney dysfunction have been found as seen by the blood levels of urea and creatinine. No histological lesions were, however, found in these organs.

Other symptoms which have not been recorded previously but were seen in our animals were a marked ruminal atony, tachycardia and polypnoea.

#### **ACKNOWLEDGEMENTS**

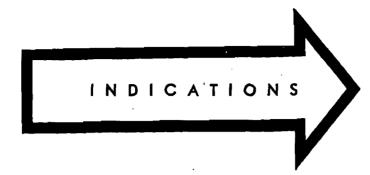
The Chiefs of the Veterinary Research Institute, Onderstepoort, and the Botanical Research Institute, Pretoria, are thanked for permission to publish this paper. The valuable technical asistance of Miss A. de Villiers, Messrs. B. P. Maartens and A. M. van Straten is acknowledged. The Department of Pathology is thanked for the histological examinations. Mr. H. Tolken of the Botanical Research Institute, Pretoria, supplied the botanical description and the photograph of the plant.

#### REFERENCES

- BURTT DAVY, J., 1908-1909. Notes from the Economic Herbarium. Transvaal Agric. J. 7: 652-657.
   KEHOE, D., 1912. Preliminary note on the poisonous properties of Cotyledon orbiculata. 2nd Rep. Dir. Vet. Res. Union of S. Afr. 387-397.
   KAMERMAN, P., 1926. The toxicity of the genus Cotyledon, the cause of the disease Krimpsiekte. S. Afr. J. Sci. 23, 185 197.
- Sci. 23: 185-187.
- 4. KAMERMAN, P., 1932. The toxic principle of Cotyledon orbiculata. D.Sc. Thesis. Univ. Pretoria (Unpublished).
- STEYN, D. G., 1932. A study of the factors concerned in the determination of the toxicity of Cotyledon orbiculata L. 18th Rep. Dir. Vet. Serv. & Ani. Ind. U. of S. Afr. Pt. 2: 899-938.
- STEYN, D. G., 1932. Known poisonous plants in the Union of South Africa. J.S. Afr. vet. med. Ass. 3: 178.

- STEYN, D. G., 1932. Ridwin poisonous plants in the Union of South Africa. J.S. Apr. Vet. Med. Ass. St. 176.
   STEYN, D. G., 1934. The toxicology of plants in South Africa. Central News Agency. South Africa.
   STEYN, D. G., 1949. Vergiftiging van Mens en Dier. Van Schaiks. Pretoria.
   WATT, J. M. and DREYER-BRANDWYK, M. G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd Ed. E. & S. Livingstone Ltd., Edinburgh & London, p. 311.
   HENNING, M. W., 1926. Krimpsiekte. 11th & 12th Rep. Dir. Vet. Educ. & Res. U. of S.A. p. 331-364.
- 11. SAPEIKA, N., 1936. The pharmacological actions of plants of the genera Cotyledon and Crassula N.O. Cras-
- sulaceae. Arch. Internationales de Pharmacodynamie et de Therapie. 54: 307-328.

  12. DYER, R. A., 1937. Flowering Plants of South Africa. 1st Ed. L. Reeve & Co., Ashford, LKent. Vol. 17, plate
- 13. BROWN, J. M. M., 1957. Some simple laboratory procedures for the practitioner. J.S. Afr. vet. med. Ass. 28:
- HAWK, P. B., OSER, B. L. & SUMMERSON, W. H., 1954. Practical Physiological Chemistry. 13th Ed. McGraw-Hill Book Co., Inc., New York, Toronto & London. p. 899.
   KING, J., 1958. Routine methods for the estimation of serum transaminase. J. Med. Lab. Technol. 15: 17-22.
- 16. MALLOY, H. T. and EVELYN, K. A., 1937. The determination of bilirubin with the photo-electric colorimeter. J. Biol. Chem. 119: 481-490.
- KING, E. J. and WOOTTON, I. D. P., 1956. Micro-analysis in Medical Biochemistry. 3rd Ed. J. & A. Churchill Ltd., 104 Gloucester Place W.1., London.
- FERRO P. V. and HAM, A. B., 1957. A simple spectrophotometric method for the determination of calcium. Amer. J. Clin. Pathol. 28: 689-693.
- 19. NEILL, D. W. and NEELY, R. A., 1956. The Estimation of Magnesium in serum using Titan Yellow. J. Clin. Pathol. 9: 162-163.
- VAN SLYKE, D. D., STILLMAN, E. and CULLEN, G. E., 1919. Studies of acidosis. XIII. A method for titrating the bicarbonate content of the plasma. J. Biol. Chem. 38-167-178.
   KUNKEL, H. G., 1947. Estimation of alteration of serum gamma globulin by a turbidimetric technique. Proc.
- Soc. Exp. Biol. Med. 66: 217-224.

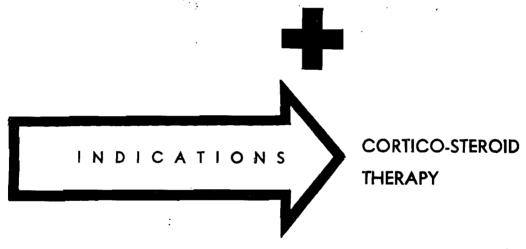


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## THE ANTHELMINTIC EFFICACY OF BITHIONOL AGAINST PARAMPHISTOMUM MICROBOTHRIUM, FASCIOLA SPP. AND SCHISTOSOMA MATTHEEI.

#### I. G. HORAK

Veterinary Research Institute, Onderstepoort.

#### SUMMARY

- 1. Bithionol at 25 to 100 mg/Kg. was 92.7 to 100% effective against adult and immature *P. microbothrium* in sheep and 99.9 to 100% effective against immature worms in cattle at dosage rates of 25 and 35 mg/Kg. respectively.
- At 75 mg/Kg. it was 98.7 to 100% effective against mature Fasciola gigantica and F. hepatica in sheep, but at 100 mg/Kg. it had little or no effect on 14 and 28 day old F. gigantica.
- 3. At a dosage rate of 100 mg/Kg. Bithionol had no effect on S. mattheei in sheep.
- 4. The above dosage rates produced no symptoms of toxicity in sheep, but in cattle at both 25 and 35 mg/Kg ataxia and abdominal pain were present.

#### Introduction

The efficacy of Bithionol\* (2, 2 thiobis (4, 6-dichlorophenol)) against adult *Paramphistomum* spp. and *F. gigantica* has been reported by Guilhon and Graber<sup>1</sup>, while Ueno, Watanabe and Fujita demonstrated its efficacy against *F. hepatica*<sup>2</sup>. Lämmler showed that it had no effect on *S. mansoni* in mice and golden hamsters<sup>3</sup>.

In previous papers Horak described the efficacy of Lintex\* against immature P. micro-bothrium, and Freon\*\* against adult Param-

phistomum spp. in sheep<sup>4,5</sup>. Lintex, however, had no effect on mature stages in sheep or cattle and only a variable effect against immature worms in cattle.

The present controlled anthelmintic tests were conducted to assess the efficacy of Bithionol primarily against *P. microbothrium* in sheep and cattle, but *Fasciola* spp. and *S. mattheei* were included in two of the experiments.

#### MATERIALS AND METHODS

#### 1. Anthelmintic.

The dose of Bithionol for each animal was weighed and a few drops of a wetting agent added, before adding water and dosing.

#### 2. Controlled Anthelmintic Tests

#### A. Infestation.

#### P. microbothrium.

Sheep and cattle were infested per os with metacercariae encysted and counted on cellulose strips as described by Swart and Reinecke<sup>6</sup>. Adult infestation was confirmed by faecal worm egg counts.

#### F. gigantica and F. hepatica.

Metacercariae, encysted on cellulose strips, were counted and dosed orally to sheep.

#### S. mattheei.

Cercariae, suspended in water, were counted by using the method employed by Reinecke, Horak and Snijders for the estimation of infective larvae of *Oesophagostomum columbianum*?. These cercariae were divided into four approximately equal doses, and four sheep were each infested percutaneously.

<sup>\*</sup>Actamer, Monsanto Chemical Company, Missouri.

<sup>\*</sup> Farbenfabriken Bayer A.G. Leverkusen.

<sup>\*\*</sup> Freon B U: E. I. Du Pont de Nemours and Co., Delaware.

#### B. Experimental design.

Four separate experiments were planned and the experimental design of each of these is given in Table 1.

#### C. Recovery and counting of worms.

#### P. microbothrium.

Worms were recovered and counted as described by Horak<sup>5</sup>.

#### F. gigantica and F. hepatica.

The liver was removed in Experiment 2 and the major bile ducts opened and all visible fluke removed. The liver was then sliced into approximately \(\frac{1}{4}\)" thick strips and the remaining fluke expressed from these strips by manual pressure. The fluke thus obtained were pooled and counted.

In Experiment 3 both F. gigantica and S. mattheei were present in the liver.

The liver was removed and sliced into strips approximately ½" thick. These strips were washed in normal saline to remove all exposed worms. The strips were then incubated in normal saline at 40°C to stimulate the remaining worms to crawl out<sup>8</sup>, thereafter the worms that still remained in the liver were expressed. The worms thus obtained were pooled and counted.

In addition the lungs were examined for liver fluke and these were removed and counted.

#### S. mattheei.

The schistosomes were obtained from the liver as described in Experiment 3 above. The worms present in the blood vessels on the walls of the alimentary canal (from the rumen to the rectum) and in the mesenteric veins were counted in situ.

#### D. Assessment of anthelmintic efficacy.

The difference between the numbers of worms recovered from the controls and from the treated animals represents the anthelmintic efficacy of the drug.

#### 3. Worm Distribution.

The distribution of F. gigantica and S. mattheei, in the various organs of the sheep slaughtered, was noted.

#### RESULTS

#### Experiment 1.

Immature and adult P. microbothrium in sheep.

At a dosage rate of 75 mg/Kg. live weight Bithionol was 92.7% effective against 3 day old worms; at 25 and 50 mg/Kg. it was 99.6 and 100% effective respectively against 16 day old worms. A dosage rate of 75 mg/Kg was 100% effective against adult paramphistomes.

#### Experiment 2.

Immature P. microbothrium and mature F. gigantica and F. hepatica in sheep.

At 75 mg/Kg Bithionol was 100% effective against immature paramphistomes and mature *F. hepatica* and 98.7 and 100% effective against *F. gigantica*.

All the *F. hepatica* were recovered from the bile ducts while many of the *F. gigantica* were still present in the liver parenchyma.

#### Experiment 3.

Immature P. microbothrium, F. gigantica and S. mattheei.

At a dosage rate of 100 mg/Kg. Bithionol was 100% effective against 21 day old paramphistomes but had very little and no effect on 14 and 28 day old *F. gigantica* and no effect on 47 day old *S. mattheei*.

Very few F. gigantica were recovered from the major bile ducts, the majority were found in the liver parenchyma.

#### Experiment 4.

Immature P. microbothrium in cattle.

Bithionol was 99.9 and 100% effective against 9 day old worms at dosage rates of 25 and 35 mg/Kg. respectively.

#### Distribution of F. gigantica.

The majority of worms were found in the liver but small numbers were constantly found in the lungs and one worm was found in the ruminal wall.

#### Distribution of S. mattheei.

The majority of schistosomes were found in the mesenteric veins and their branches on the walls of the alimentary tract but large numbers were constantly present in the liver.

TABLE 1.—EXPERIMENTAL DESIGN OF EXPERIMENTS 1 TO 4.

periment 1.							
Sheep No.	Day 0	Day 3	Day 10	Day 16	Day 21	<b>Day</b> 979	Day 984
1	P.M/C	T	S		_		
2	P.M/C	С	S				
3	P.M/C	_		T	S	_	
4	P.M/C	_		С	S		
5	P.M/C	_		T	S		
6	P.M/C					T	S
7	P.M/C	_				С	· Š
8	P.M/C				_	$ar{\mathbf{T}}$	Š

Sheep No.	Day 0	Day 53	Day 69	Day 74–76		
9	F.g.M/C	P.M/C	T	S		
10	F.g.M/C	P.M/C	С	S		
11	F.g.M/C	P.M/C	T	S		
12	F.g.M/C	P.M/C	C	S		
13	F.h.M/C	P.M/C	Ť	S .	•	
14	F.h.M/C	P.M/C	Ĉ	S		

Sheep No.	Day 0	Day 19	Day 26	Day 33	Day 47	Day 105~109	Day 112–116
15	S.m.C.		P.M/C	F.g.M/C	T	_	S
16	S.m.C		P.M/C	F.g.M/C	С		l s
17	S.m.C	F.g.M/C	P.M/C	1 , 1	Т	S	ļ
18	S.m.C	F.g.M/C	P.M/C	1 [	C	S	

Experiment 4.				
Bovine No.	Day 0	Day 9	Day 20–23	Day 28-52
1 2	P.M/C P.M/C	TC	S S	
3	P.M/C P.M/C	T C	<u> </u>	S S

P.M/C = Paramphistome metacercariae.
F.g.M/C = F. gigantica metacercariae.
F.h.M/C = F. hepatica metacercariae.
S.m.C. = S. mattheei cercariae.
T = Treated.
C = Control.
S = Slaughtered.

TABLE 2.—CONTROLLED TESTS ON IMMATURE AND ADULT P. microbothrium in sheep.

Sheep No.	No. of M/c	Bithionol	Age of We	orms on Day	Param- phistomes	0/
	Dosed	mg/Kg	Treated	Slaughtered	Recovered	Efficacy
Immature Paran	phistomes					
1 2	115,000 107,000	75 Cont	trol ,	10 10	5,705 78,090	92.7
3 4	20,000 20,000	25 Cont	16 trol	21	38 10,784	99.6
5	20,000	50	16	21	o	100.0
Adult Parar	nphistomes					
6	5,000	75	979	984	0	100.0
7	10,000	Cont		984	1,610	
8	10,000	75	979	984	´ 0	100.0

M/c = Metacercariae.

TABLE 3.—THE EFFECT OF BITHIONOL AT 75 MG/KG ON P. microbothrium and Fasciola SPP. In sheep.

Chan No	No of Mio	A == = 6 M/s	Age of W	orms on Day	Fore in	Worms	0/
Sheep No.	No. of M/c Dosed	Age of M/c in Days	Treated	Slaughtered	Eggs in Gallbladder	Recovered	Efficacy
Immature P. mic	robothrium						
9	20,000	6	16	21	_	0 .	100.0
10	20,000	6	Control	21	_	13,590	100.0
11	20,000	34	16	23	_	0	100.0
. 12	20,000	34	Control	23	_	5,806	100.0
13	20,000	34	16	22	_	0	100.00
14	20,000	34	Control	22	_	15,940	100.00
F. gigantica							
9	1,188	293	69	74	0	5 Dead	100.0
10	551	293	Control	74	0	40	100.0
						65 Dead	
11	968	8 8	69	76	0	2 alive	98.7
12	542	8	Control	76	0	149	70.7
F. hepatica		•					
13	. 919	293	69	. 75	Many	0	100.0
, 14	515	293	Control	75	Few	′ 10	100,0

M/c = Metacercariae.

TABLE 4.—THE EFFECT OF BITHIONOL AT 100MG/KG ON P. microbothrium, F. gigantica and S. mattheei in sheep.

. Sheep No.	No. of M/C or Cercariae	Age of W	orms on Day	Worms Recovered	Percentage Efficacy
. Sheep No.	Administered	Treated	Slaughtered	Recovered	Lineacy
P. microbothrium 15 16	30,000 30,000	21 Control	90 86	0 1,049	100.0
17 18	30,000 30,000	21 Control	83 79	0 2,176	100.0
F. gigantica 15 16	400 400	14 Control	83 79	53 86	38.4
17 18	400 400	28 Control	90 86	197 159	0.0
S. mattheei 15 16	1,140 1,140	47 Control	116 112	170 138	0.0
17 18	1,370 1,370	47 Control	109 105	270 85	0.0

M/C = Metacercariae.

TABLE 5.—CONTROLLED ANTHELMINTIC TESTS ON IMMATURE P. microbothrium in Cattle.

Bovine No.	No. of M/c Dosed	Bithionol mg/Kg	Age of W	orms on Day	Param- phistomes	% Efficacy
Bovine 140.	Dosed	mg/Kg	Treated	Slaughtered	Recovered	Enicacy
1 2	117,000 110,000	25 Con	g trol	23 20	30 55,608	99.9
3 4	116,000 115,000	35 Con	9 trol	52 28	0 68,743	100.0

M/c = Metacercariae.

TABLE 6.—THE DISTRIBUTION OF F. gigantica RECOVERED FROM SHEEP.

Shoon No.	. No. o	f Liver Fluke Recover	red from	Total No. of Liver Fluke
Sheep No.	Liver	Lungs	Ruminal wall	Recovered
15 16 17 18	49 84 187 156	3 2 10 3	0 0 0	53 86 197 159
Average	119	4.50	0.25	123.75
%	96.2	3.6	0.2	100.0

TABLE 7.—THE DISTRIBUTION OF S. mattheei IN SHEEP.

Sheep No.	No. of Schistosomes Recovered from			Total No. of Schistosomes
	Walls of the Alimentary Canal	Mesentery	Liver	Recovered
15 16 17 18	56 52 84 17	71 75 107 38	43 11 79 30	170 138 270 85
Average	52	73	41	166
%	31.3	44.0	24.7	100.0

#### DISCUSSION

#### P. microbothrium.

At a dosage rates of 25 to 100 mg/Kg. live weight Bithionol exhibited remarkable efficacy against both adult and immature worms in sheep and immature worms in cattle. The activity against adult paramphistomes in sheep confirms the findings of Guilhon and Graber in cattle<sup>1</sup>. It is, therefore, possible to remove both adult and immature paramphistomes with a single drenching whereas previously anthelmintic activity had been confined to either the adult or immature stages<sup>4,5</sup>.

In an outbreak of acute paramphistomiasis in cattle, Malan treated 10 animals with Bithionol at an estimated dosage rate of 20 mg/Kg. as the exact weights of the animals were not known. All the animals died after treatment from the effects of the infestation with no apparent dimunition of worm burdens<sup>9</sup>. The discrepancy between these results and those obtained in the present experiments might be due to the reduced dosage rate as Guilhon and Graber only recorded high efficacies at dosage rates exceeding 25 mg/Kg.<sup>1</sup>.

#### F. gigantica and F. hepatica.

Bithionol was highly effective against 69 day old worms of either species but even at an increased dosage rate had little or no effect on immature worms.

The sheep in Experiment 2 were originally infested with F. gigantica and F. hepatica metacercariae for life cycle studies but were then placed in this experiment and infested with P. microbothrium. This explains the variation in age of the liver fluke metacercariae and the difference between the numbers of metacercariae dosed to, and worms recovered from the treated and control sheep. The treated sheep being selected on the larger number of metacercariae that they had received.

The dead liver fluke recovered from the livers of sheep 9 and 11 were in an advanced stage of decomposition. These worms could thus not be taken as an indication of the total number of worms present at the time of treatment as some may have disintegrated entirely.

The recovery of eggs from the gall-bladders of the two sheep infested with *F. hepatica*, while eggs were absent in all the sheep infested with *F.* 

gigantica, would indicate the earlier maturity of F. hepatica. The length of the prepatent periods of these two worms has been given by Kendall and Parfitt10, and by Alicata11.

The liver fluke recovered from the lungs were usually found in pairs in circumscribed foci characterised by haemorrhage and necrotic lung tissue. There was no difference in size between these fluke and those recovered from the liver.

#### S. mattheei.

Bithionol at 100 mg/Kg. live weight had no effect on this schistosome thus confirming the findings of Lämmler in mice and golden hamsters infested with S. mansoni3.

Khayyal reported on the distribution of S. mansoni in infested mice and recovered very few worms from their livers<sup>12,13</sup>. The present results. however, seem to indicate that large numbers of S. mattheei may be found in the livers of infested sheep.

Toxicity.

No symptoms of toxicity were seen in any of the sheep, but one sheep which was given 200 mg/Kg. exhibited diarrhoea of approximately 24 hours duration on the day after dosing. The cattle seemed more susceptible to the toxic effects of this remedy and even at the low dosage rates of 25 and 35 mg/Kg. developed ataxia and colic of approximately 24 hours duration on the day following drenching.

#### **ACKNOWLEDGEMENTS**

The Chief, Veterinary Research Institute, is thanked for facilities to carry out these experiments and permission to publish the results; Messrs. J. H. D. Maré, L. P. Heitmann, J. V. Badenhorst and J. A. Muller for their technical assistance and the Section of Bacteriology, Onderstepoort, for supplying and housing the cattle.

#### REFERENCES

- 1. GUILHON, J. and GRABER, M., 1962. Action du bithionol sur les amphistomes et sur Fasciola gigantica.
- Bull. Acad. vét. Fr., 35: 275-278.

  UENO, H., WATANABE, S. and FUJITA, J., 1960. (Studies on anthelmintics against the common liver fluke. II. Action of Bithionol in cattle.) J. Jap. vet. med. Ass., 13: 151-155. (In Vet. Bull., 31: No. 149).
- LÄMMLER, G., 1964. Die experimentelle Chemotherapie der Trematoden-Infektionen und ihre Problematik. III. Chemotherapeutische Untersuchungen an experimentell mit Schistosoma mansoni infizierten Tieren. Z.
- Tropenmed. Parasit., 15: 337-368.
  HORAK, I. G., 1962. Studies on paramphistomiasis. IV. Modified critical and controlled anthelmintic tests on
- HORAK, I. G., 1962. Studies on paramphistomiasis. IV. Modified critical and controlled anthelimitic tests on the conical fluke Paramphistomiam microbothrium. J. S. Afr. vet. med. Ass., 33: 203-208.

  HORAK, I. G., 1964. Studies on paramphistomiasis. VI. The anthelimintic efficacy of Lintex and Freon against Paramphistomium spp. in sheep and cattle. J. S. Afr. vet. med. Ass., 35: 161-166.

  SWART, P. J. and REINECKE, R. K., 1962. Studies on paramphistomiasis. II. The mass production of metacercariae of Paramphistomium microbothrium Fischoeder 1901. Onderstepoort J. Vet. Res., 29: 189-195.

  REINECKE, R. K., HORAK, I. G. and SNIJDERS, A. J., 1963. Techniques for testing anthelimintics against importure Occupancy and controlled anthelimintic and the production of the parameters.
- immature Oesophagostomum columbianum. The Evaluation of Anthelmintics: In Proc. 1st Internat. Conf. World Ass. Adv. vet. Parasit., August 22-23, 1963: Hanover, Germany: 167-180.

  8. SARASIN, G., 1965. Personal communication.

  9. MALAN, W. du T., 1965. Unpublished data.

  10. KENDALL, S. B. and PARFITT, J. W., 1962. The chemotherapy of fascioliasis. Brit. vet. J., 118: 1-10.
- ALICATA, J. E., 1938. Observations on the life history of Fasciola gigantica, the common liver fluke of cattle in Hawaii, and the intermediate host, Fossaria allula. Hawaii Agric. exp. Sta., Bull. 80, 22pp. KHAYYAL, M. T., 1964. The effects of antimony uptake on the location and pairing of Schistosoma mansoni.
- Brit. J. Pharmacol., 22: 342-348.
- KHAYYAL, M. T., 1965. Significance of worm shifts in experimental Schistosoma mansoni, with emphasis on the action of anaesthetics. Nature, 205: 1331-1332.





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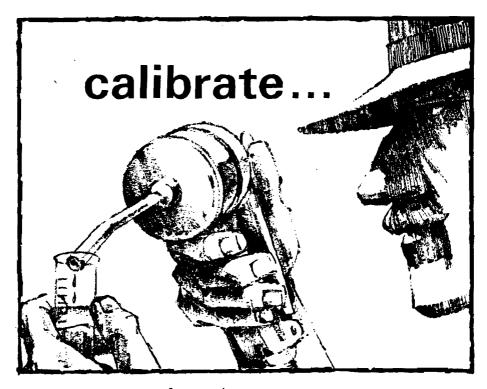
"In the toxicity trials, doses of 600 mg./Kg. and below did not produce untoward clinical effects."

#### References:

(1) Drudge, J. H., Szanto, J., Wyant, Z. H., and Elam, G.: Critical tests on thiabendazole against parasites of the horse, J. Parasitol. 48 (Suppl.): 28, April 1962 (In Soc. proc.) (2) Drudge, J. H.: A new Grug for Parasite Control, Blood Horse, September 15, 1962.

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## CLINICO-PATHOLOGICAL STUDIES OF BABESIA CANIS INFECTION IN DOGS, IV. THE EFFECT ON BILIRUBIN METABOLISM

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#### SUMMARY

The appearance of bilirubin in the plasma of dogs suffering from babesiosis has been studied in some 111 cases which for convenience were divided into three more or less arbitrary categories, Cat. I representing early acute cases with erythrocyte counts of more than three million per cu. mm., Cat. II with extreme mucosal pallor but no icterus, and Cat. III showing any degree of icterus.

It was found in the majority of the early acute cases that such bilirubin as was present was in unconjugated form indicating extensive haemolysis. The well-developed but non-icteric ones were intermediate in position in that both predominantly haemolytic and predominantly hepatic types were represented, while the icteric category all showed liver damage of varying degrees, in some cases so extensive as to suggest intrahepatic cholestasis at the cellular level.

#### Introduction

The most obvious change in the composition of blood plasma in conditions involving jaundice is the presence of bile pigments. This has been remarked on previously, in connection with babesiosis in the dog, where the popular names tend to stress the observation that jaundice is frequently seen in severe or neglected cases.

The most important bile pigment, bilirubin, is formed, briefly, as follows: At the end of the life-span of an erythrocyte it is engulfed by a reticulo-endothelial cell in which it disintegrates. The porphyrin ring of haemoglobin is opened and a bilirubin-iron-globin complex formed. Iron is released from the complex and combines with  $\beta$ -globulin for storage as ferritin or goes into the labile iron pool for reuse in the synthesis of haemoglobin. Excessive haemolysis

and thus excessive release of iron gives rise to haemosiderosis which is an accumulation of insoluble particulate matter in the cells appearing as golden brown granules in routinely stained sections. Haemosiderin, which is a ferric hydroxide and mucopolysaccharide complex, is similar in structure to ferritin. The globin set free goes into the protein pool.

In babesiosis the parasites cause haemolysis which is frequently in this country so massive that the plasma becomes a deep portwine red and the kidney threshold (130 mg. per 100 ml. of plasma in man) exceeded very considerably, with resultant haemoglobinuria. Haemosiderosis is a frequent histological finding in advanced cases of the disease.

The bilirubin resulting from normal breakdown of haemoglobin is taken up by the liver, conjugated and excreted into the bile.

In human medicine the main interest in the van den Bergh diazo test<sup>2</sup> has for the greater part of half a century been centred on its use in the differentiation between "medical" or hepatocellular, and "surgical" or obstructive forms of jaundice. Chapman and coworkers3 have recently reviewed the present status of this subject in the light of the newer knowledge4-9 of the identity of the "direct" and "indirect" reacting pigments gained during the period 1953-1958. It had been found that the "indirect" reaction was caused by bilirubin as formed in the reticulo-endothelial system and liberated into the plasma in bound form with plasma albumin<sup>10</sup>. This bilirubin was practically insoluble in water but soluble in alcohol. The "direct" reacting fractions were shown 6,7 to consist of the mono- and diglucuronides of bilirubin which were derived from enzymatic (transferase) action by the microsomes of hepatic polygonal cells. These enzymes catalysed<sup>11,12</sup> the transfer of the glucuronide radical from uridine diphosphate glucuronic acid to various receptors, in this case bilirubin. These glucuronides were shown to be readily soluble in water and were the forms as secreted in the bile. It was thus suggested that the basis of "direct" and "indirect" reactions was simply a matter of the solubilities of the ester glucuronides and of bilirubin itself in water and alcohol respectively.

It became clear that the limitation of the value of the test arose from the fact that in unrelieved extrahepatic obstruction very soon the hepatic cells and bile ducts became involved and that shortly after the early phase a "mixed" picture would be presented.

Moreover, in severe cases of hepatocellular damage, the excretory function of hepatic cells may be interfered with to such an extent that the picture of cholestasis may well be produced. The lesion would be a biochemical one entirely.

Without adequate clinical evidence then the test would lose much of its value.

Other workers including Watson<sup>13</sup> working on a basis of the ratio between the glucuronides and total serum bilirubin suggested that "low bilirubin ratios in cases of elevated total serum bilirubin are indicative of retention jaundice of various degrees. The lowest ratios (less than 20%) are characteristic of haemolytic disease or constitutional hepatic dysfunction of the Gilbert While the majority of patients with jaundice due to parenchymal liver disease have bilirubin ratios in the same general range as found in cases of biliary obstruction (45% to 80%) there is a significant segment of cases with diffuse liver disease in which ratios below 40% are encountered. In the range of 35% to 40%there is small likelihood of confusion with biliary obstruction and below 35% almost none". Other views had however been expressed on this matter14-16. These workers discounted the diagnostic value of the diazo test except when suspecting haemolytic anaemia.

For the purpose of the present study it was evident that the differentiation between bilirubin and its glucuronide conjugate could provide information of considerable interest, for the reason that the disease starts with haemolysis, frequently on a massive scale, progressing to

anoxic hepatocellular damage. The picture presented would not be confused by any extrahepatic obstructive element. It could be anticipated that the test would reveal the extent and time of development of cellular damage and that the trends would be clarified by the figures obtained.

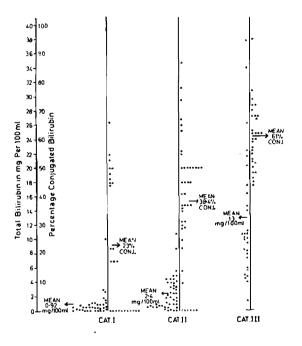
#### MATERIALS AND METHODS

The case material for this study was as before' derived from clinical cases of *Babesia canis* infection presented at the Small Animal Clinic at Onderstepoort for diagnosis and treatment. These were divided into three more or less arbitrary categories:—

Category I: Early acute cases with pale pink mucous membranes.

Category II: Cases showing great mucosal pallor but no icterus.

Category III: Cases showing any degree at all of clinical icterus.



FREQUENCY OF TOTAL BILIRUBIN VALUES IN BABESIOSIS WITH PERCENTAGES CONJUGATED

The samples were collected as previously described and both total and conjugated bilirubin estimated by a modification of the classical diazo method of Malloy and Evelyn<sup>17</sup>.

Unconjugated bilirubin was calculated by diffference between total and the direct reacting (one minute) bilirubin glucuronides.

#### RESULTS

The findings are plotted graphically as a scatter diagram representing on the left side of the vertical lines the total bilirubin figures obtained, and on the other the percentages of these consisting of conjugated bilirubin. These are as before separated into categories I to III. See the Figure.

Of the 35 cases in Cat. I, eight gave zero values for total bilirubin and for these, naturally no percentage glucuronide was indicated. The great majority had less than 1 mg, per 100 ml. of bilirubin. The exceptions containing 2.7, 3.0 and 10.2 mg, per 100 ml, would probably represent borderline cases, with appreciable liver damage already present. By the criterion of their erythrocyte count they were however placed into Category I. Even with these higher figures included the overall mean value was 0.92 mg. per 100 ml. Only five cases had 50% or more of their bilirubin present as the glucuronides. Almost one third had all the bilirubin present in the free or unconjugated form. The mean percentage of glucuronides was calculated at 23%.

The Cat. II cases, 46 in number, gave an average figure of 2.4 mg. of bilirubin per 100 ml. The highest values were 10.8 and 8.7 mg. per 100 ml. with the rest scattered between 0 and 5.6, and the mean was 2.4 mg. per 100 ml. The percentage of conjugation showed very great variation from six at 0 (quite unconjugated) up to 87%, with a mean of 38.4%.

In the third category where all the thirty patients were icteric to some degree the total bilirubin averaged out at 13 mg. per 100 ml. with a range of 1.4 (exceptionally low) to 37.8 (exceptionally high) and a mean percentage conjugated of 61%.

#### DISCUSSION

The pattern of development of babesiosis is again shown up by these figures. In the first category cases nearly all the bilirubin present resulted from haemolysis above physiological levels. Comparativley little bilirubin was in the

conjugated form but there was some evidence of early liver damage.

Cat. II showed a mixture of haemolytic and hepatitic types with a distinct upward trend in bilirubin figures, which illustrated a progressively greater involvement of the liver as the erythrocyte count fell below 3,000,000 per cu. mm. and the mucous membranes tended to become ivory white.

The levels of total bilirubin found in the Cat. III (icteric) cases were very high by accepted<sup>18</sup> standards for dogs with their comparatively low threshold for this pigment. The higher ones certainly tended to suggest a biochemical cholestatic lesion. This was supported by the always predominantly conjugated form of pigment.

From this series and from experience of this test over many years it has become clear that in *B. canis* infection clinical jaundice is never seen unless the liver cells are fairly heavily involved in the disease syndrome. No matter how peracute the disease no such thing as jaundice from pure haemolysis has ever been found. In the more or less pure haemolytic stage (category I) the total bilirubin values are practically invariably of a low order as compared with those in which liver damage is evident.

In no case of this disease has "free" bilirubin alone ever been found to rise to anything like the levels found in cases of human neonatal jaundice with kernicterus, regardless of the peracuteness of the disease or the degree of haemolysis. In these severe cases there is often a severe "spilling" of haemoglobin into the urine, but never icterus unless the liver is already extensively involved.

Maegraith<sup>19</sup> has discussed the question of bile pigments in plasma and urine at length in a study of pathological processes in malaria and blackwater fever. He stated that liver function could be disturbed during all forms of malaria in man, whether naturally or artificially acquired and that the degree was dependent to some extent on the plasmodial species concerned and on the severity of the infection. The involvement of the liver was sometimes quite transitory and slight but could be so extensive, particularly in *P. falciparum* infection, as completely to dominate the clinical picture.

On the question of whether a pure haemolytic jaundice could exist without any involvement of the liver, he quoted conflicting views expressed by different authors. Some held that iaundice in malaria, unaccompanied by bilirubinuria, was nearly always haemolytic in type whereas other workers stated categorically that there was an hepatic element in all forms of jaundice. All the quoted work however dated from before the modern understanding4-9 of bilirubin metabolism gained during the sixth decade of the century, and also before enzyme determinations<sup>20</sup> introduced during the same period were available for detection of liver cell degeneration or necrosis.

Gilles and coworkers<sup>21,22</sup> have reported on a similar study of Babesia canis infection (experimentally produced). Measurements of plasma bilirubin were made in 17 animals. In ten which were severely anaemic and clinically jaundiced bilirubin values were between 2.0 and 6.0 mg. per 100 ml. Seven died, and three were killed in the early stages of recovery. Pathological changes were found histologically in the livers of all seven that died and of one of the killed group. The remaining seven animals which had bilirubin "within normal limits", were all anaemic but one, and none showed jaundice. Three of these died from the disease and the remainder were killed at various stages of recovery. Structural changes were seen in the livers of four.

In summary then it is evident that determination of bilirubin, while not capable of detecting very early liver damage, is nevertheless a useful means of throwing light on the progress from the initial haemolysis due to the activity of B. canis through to the stage when liver damage is so extensive as to present a biochemical picture of obstruction at the cellular level.

#### **ACKNOWLEDGEMENT**

Permission by the Chief, Veterinary Research Institute, Onderstepoort to publish this paper is acknowledged with thanks.

#### REFERENCES

- MALHERBE, W. D., 1965. Clinico-pathological studies of Babesia canis infection in dogs. I. The influence of the infection on bromsulphalein retention in the blood. J.S. Afr. vet. med. Ass. 36: 1, 25-30.
   VAN DEN BERGH, A. A. H. and MULLER, P., 1916. Über eine direkte und eine indirekte Diazoreaktion auf Bilirubin. Biochem. Z. 77, 90.
- 3. CHAPMAN, J. A. et al 1960. An analysis of the clinical application of the van den Bergh reaction in jaundice. Amer. J. Med. Sci. 239, 11-16.
- 4. COLE, P. G. and LATHE, G. H., 1953. The separation of serum pigments giving the direct and indirect van den
- COLE, P. G. and LATHE, G. H., 1953. The separation of serum pigments giving the direct and indirect van den Bergh reaction. J. Clin. Path. 6, 99.
   COLE, P. G. et al 1954. Separation of the bile pigments of serum, bile and urine. Biochem. J. 57, 514.
   BILLING, B. H. and LATHE, G. H., 1956. The secretion of bilirubin as an ester glucuronide giving a direct van den Bergh reaction. Biochem. J. 63, 6P.
   SCHMID, R., 1956. Direct-reaching bilirubin, bilirubin glucuronide, in serum, bile and urine. Science 124, 76.
   SCHACHTER, D., 1957. Nature of glucuronide in direct-acting bilirubin. Science 126, 507.
   BILLING, B. H. and LATHE, G. H., 1958. Bilirubin metabolism in jaundice. Amer. J. Med. 24, 111.
   GREENSPAN, E. M. and DREILING, D. A., 1957. Introglobulin fractional analysis as an aid in the differentiation of medical from surgical jaundice. Constructure of medical from surgical jaundice. Constructure of programment of the constructure of medical from surgical jaundice. Constructure of programment of the constructure of the construction of medical from surgical jaundice. Constructure of the construction of medical from surgical jaundice. Constructure of the construction of medical from surgical jaundice. Construction of the construction of medical from surgical jaundice. Construction of the construction of
- tiation of medical from surgical jaundice. Gastroenterology 32, 500.

  11. SCHMID, R. et al 1957. The enzymic formation of bilirubin glucuronide. Arch. Biochem. Biophys. 70, 285.

  12. ARIAS, I. M. and LONDON, I. M., 1957. Bilirubin glucuronide in vitro: demonstration of a defect in Gilbert's
- disease. Science 126, 563.

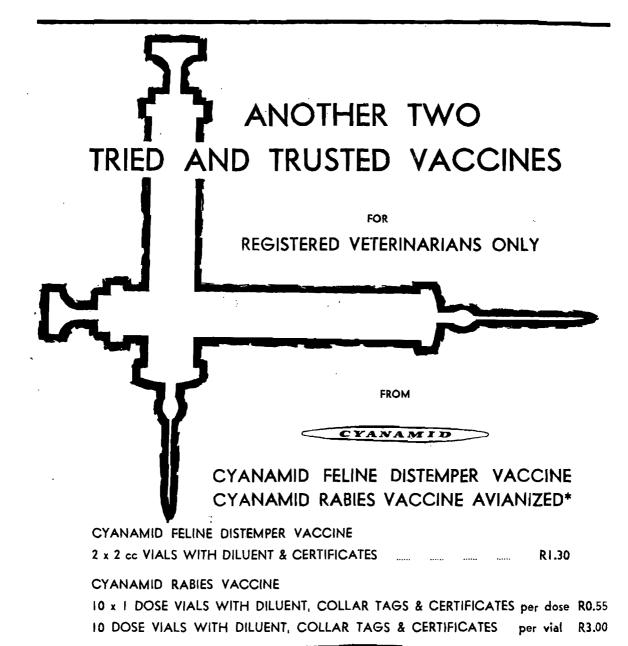
  WATSON, C. J., 1956. The importance of the fractional bilirubin determination in clinical medicine. Ann. Int. Med. 45, 351.
- 14. HOFFBAUER, F. W. et al 1949. Limitations and merits of a single serum sample analysis in the differential diagnosis of jaundice. J. Lab. Clin. Med. 34, 1259.
   15. KLATSKIN, G. and DRILL, V. A., 1950. The significance of the "one-minute" prompt direct reaching bilirubin
- in serum. J. Clin. Invest. 29, 660.
- 16. ZIEVE, L. et al 1951. Normal and abnormal variations and clinical significance of the one-minute and total serum bilirubin determinations. J. Lab. Clin. Med. 38, 446.

- 17. MALLOY, H. T. and EVELYN, K. A., 1937. The determination of bilirubin with the photoelectric colorimeter.
- J. Biol. Chem. 119, 481.
  CORNELIUS, C. E. and KANEKO, J. J., 1963. Clinical Biochemistry of Domestic Animals. Academic Press, New York and London.
- MAEGRAITH, B. G., 1948. Pathological Processes in Malaria and Blackwater Fever. Blackwell Scientific Publications Oxford.
- 20. MALHERBE, W. D., 1963. Enzyme chemistry: an aid to veterinary diagnostics. J. S. Afr. vet. med. Ass. 34: 2, 257-262.
- 21. GILLES, H. M. et al 1953. The liver in Babesia canis infection. Ann. Trop. Med. Parasit. 47: 4, 426-431.
- 22. MAEGRAITH, B., et al 1957. Pathological processes in Babesia canis infections. Z. Tropen. med. Parasit. 8:

#### THANKS AND AN APPEAL

We wish to thank all those firms who have supported us in the past year by making use of our advertising columns.

Further, we appeal to all our members and readers to support these firms and the many others who will be advertising in this journal during the coming year.





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# REPORT ON THE OCCURRENCE OF SPELEOGNATHUS STRIATUS IN THE DOMESTIC PIGEON

### S. M. SHANE

Veterinary Research Institute, Onderstepoort.

### SUMMARY

The incidental finding of an apparently non-pathogenic respiratory mite of pigeons, Speleognathus striatus is recorded.

### Introduction

Speleognathus striatus was described by Crossley<sup>1</sup> in Texas, following a survey of respiratory mites in Columbiform birds. Fain<sup>2</sup> recorded the parasite from the red-eye turtle dove (Streptopelia semitorquata) at Astrida, in Ruanda-Urundi. No record can be found of its occurrence in the Republic of South Africa.

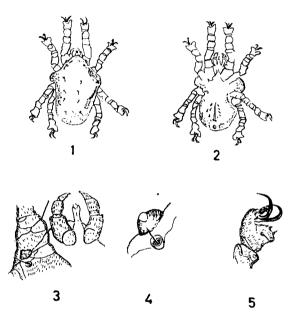
The mites were observed during routine examination of pigeons submitted to the Poultry Section, Onderstepoort Veterinary Research Institute. Five cases were observed; three birds from Pretoria, and two from Welkom.

### **OBSERVATION**

None of the birds displayed upper respiratory tract symptoms during a two day observation period. Anti-mortem examination of the Pretoria case revealed hyperaemia of the palate, and in two individual birds, excessive lachrymation was noted. The Welkom birds were completely asymptomatic.

Post-mortem examination of the nasal region, revealed the presence of white 0.5 mm. diameter, mites moving rapidly over the nasal mucosa. The parasites were concentrated in the aboral portion of the turbinate structures. One mite was observed moving rostrad adjacent to the palatine cleft. Approximately twelve parasites were located in each nasal cavity, but rapid motility, and the convolutions of the turbinates made accurate counting impossible. The situation of the parasites corresponded in each of the infested birds.

### Speleognathus striatus



- 1. Adult female. Dorsal view x 150
- 2. Adult female. Ventral view x 150
- 3. Mouth parts and position of eye x 400
  - 4. Eye x 1500
  - 5. Tarsus x 400

### DISCUSSION

Wormersley quoted by Crossley¹ has indicated that the hydrophobic cuticle facilitates the transmission of the mite in drinking water. The communication of the oral and nasal cavities via the palatine cleft, together with the ability of the Columbiformes to suck liquids may be contributory factors.

The pathogenicity of these mites is assumed to be mild. The turbinate mucosa was only slightly hyperaemic and the mucus secretion was not visible enhanced. Accordingly speci-

mens were not submitted for histopathological examination. The possibility of lesions resulting from heavy infestation must be considered. Irritation of mucosae would conceivably lead to nasal congestion or in extreme cases secondary bacterial infection.

It is not possible to comment on the distribution of the parasite in the Republic. The demonstration of the mite from unrelated lofts in widely separated areas may indicate a fairly disseminated infestation of South African pigeons.

### **ACKNOWLEDGEMENTS**

The author is sincerely grateful to Prof. R. du Toit for identification of the mite, and to his department for technical assistance. The Chief, Veterinary Research Institute, Onderstepoort is thanked for permission to publish this article.

### REFERENCES

- 1. CROSSLEY, D. A. Jnr., 1952. "Two New Nasal Mites from Columbiform Birds". J. Parasitol. 38, 385.
- 2. FAIN, A., 1956. Rev. Zool. Bot. afr. 53, 18.

### A NEW SALMONELLA SEROTYPE

Salmonella enteritidis var. louwbester, 16:Z:e.n.x.

H. J. W. Botes.

Veterinary Research Institute, Onderstepoort.

This microbe, a typical Salmonella, was isolated from the liver of a calf which died from enteritis.

It fermented, with acid and gas production, glucose, dulcitol, mannitol, maltose, arabinose, rhamnose, inositol, sorbitol and trehalose but did not produce acid or gas from lactose or sucrose. It was motile, indol and urea negative and M.R. and H<sub>2</sub>S (TSI) positive.

On serological examination, it was agglutinated by diagnostic single factor somatic (O) antiserum 16 and possessed the flagellar (H) antigens Z and e.n.x. It completely absorbed the antibodies against factors 16: e.n.x. from a

monospecific S. enteritidis var. hvittingfoss (16: b: e.n.x.) antiserum but did not remove the antibody against the flagellar phase (b). It removed both flagellar phase antibodies (Z and e.n.x.) from a monospecific S. enteritidis var. greenside antiserum (50: Z: e.n.x.).

A freeze-dried subculture was identified by Dr. Joan Taylor of the Salmonella Reference Laboratory, Colindale, London as a new Salmonella serotype, possessing antigens 16: Z: e.n.x.

The name, S. enteritidis var. louwbester, has been proposed and accepted.

### **ACKNOWLEDGEMENTS**

I have pleasure in thanking the Chief, Veterinary Research Institute, Onderstepoort for permission to publish this report, Dr. Joan Taylor for typing the strain and Mr. L. R. Bester for his careful technical assistance.

# BESNOITIOSIS IN SOUTH AFRICAN ANTELOPES: A PRELIMINARY NOTE ON THE OCCURRENCE OF BESNOITIA CYSTS IN THE CARDIOVASCULAR SYSTEM.

- P. A. BASSON-Dept. of Pathology, Onderstepoort.
- J. W. VAN NIEKERK-Senior State Veterinarian, Skukuza.
- R. M. McCully—Staff member of Geographic Pathology Division, Armed Forces Institute of Pathology, U.S.A., temporary assignment Dept. of Pathology, Onderstepoort.
- R. D. BIGALKE—Section of Protozoology, Onderstepoort.

During the course of a survey of blue wildebees (Connochaetes taurinus (Burchell, 1823)) in the Kruger National Park, for schistosomiasis and gedoelstial myiasis, numerous round white objects measuring slightly less than half a millimeter in diameter were found attached to the endocardium and the intima of various blood vessels. Upon subsequent examination of microscopic sections, these objects proved to be cysts of a Besnoitia sp.

A foot and mouth disease survey within the Park subsequently afforded an opportunity of checking additional blue wildebeest and other antelopes for similar cysts. These were found in 19 out of a total of 21 blue wildebees, 33 out of 74 impala (Aepyceros melampus (Lichtenstein, 1812)) and one out of six kudu (Tragelaphus strepsiceros (Pallas, 1766)) examined.

The significance of these findings, other than the discovery of the parasite, is the fact that the cysts were numerous in the cardiovascular system, and distinctly rare in other tissues. The peripheral veins, especially those of the limbs, and the jugular veins were the more heavily parasitized. The cysts were frequently concentrated on the valves. Cysts were also observed within lymphatic channels in the subcutis of some animals.

With the exception of a few cysts in the conjunctival sclera of several eyes which were closely examined, none of the clinical manifestations usually associated with besnoitiosis of cattle were detected.

An investigation, initiated in August 1964, to determine the significance of the presence of *Besnoitia* in these wild ruminants is still in progress. The results of this investigation, to date, show promise of having a far-reaching effect on our knowledge of besnoitiosis. The first of a series of articles on these findings will be reported later in the Onderstepoort Journal of Veterinary Research.

### APONOMMA EXORNATUM (KOCH) AS A CAUSE OF MORTALITY AMONG MONITORS

E. Young\*

National Zoological Gardens of South Africa, Pretoria.



Aponomma exornatum in the nostrils of a Land monitor (Varanus albigularis). Photo: Dick Wolff.

During 1964 our Land monitors (Varanus albiqularis) and Water monitors (Varanus niloticus) suddenly started to die. They all showed

dyspnoea and usually died from suffocation soon after the onset of the first symptoms. The nostrils of affected monitors were distended and closer examination revealed the presence of numerous ticks in their nasal passages. These ticks, which had occluded the nasal passages completely, were identified by Professor R. du Toit, of Onderstepoort, as adult Aponomma exornatum (Koch).

As many as 56 ticks were removed from the nostrils of one adult Water monitor which showed severe dyspnoea. After the removal of ticks, the monitors recovered immediately.

A. exornatum is commonly found on monitors in South Africa. These ticks may be responsible for the mortality of many monitors in nature.

\*Present Address:

Kruger National Park, P.O. SKUKUZA.

# A SUSPECTED CASE OF TETANUS IN A WATERBUCK (KOBUS ELLIPSIPRYMNUS)

### E. Young.

National Zoological Gardens of South Africa, Pretoria\*

Symptoms, typical to that of the bacterial dissease, Tetanus, which is caused by the anaerobic *Clostridium tetani*, were observed in a young Waterbuck heifer, which had a large wound on her inner thigh. The wound was contaminated with horse manure.

The onset of symptoms was sudden and included spasms of the masseter muscles (Lock jaw), starring eyes, prolapse of the third eyelids, an extended head and neck, stiffness of the limbs, a rigid tail and general muscular spasms and hyperaesthesia. The animal remained conscious and sometimes relaxed for a few moments but the spasmodic convulsions could then easily be brought about by any external stimulus.

Other possible causes of such a symptomcomplex, like Heartwater, strychnine poisoning etc. were excluded and a diagnosis of Tetanus was made on the typical symptoms and epizootology.

\*Present address:

Kruger National Park, P.O. Skukuza.

### TAENIA SOLIUM Lin., (1758) IN THE CHAC-MA BABOON, PAPIO URSINUS (Kerr, 1792)

### Anna Verster

Viljoen<sup>1</sup> fed large number of viable *Cysticercus* cellulosae to six domestic dogs, a black backed jackal (Canis mesomelas Schreber, 1775) and an unspecified baboon. None of these animals, however, became infested.

Recently attempts were made to infest three vervet monkeys, Cercopithecus aethiops (Lin., 1758), and a chacma baboon, Papio ursinus (Kerr, 1792), with C. cellulosae. Each animal was fed five cysticerci daily for three days, i.e. each animal was given a total of 15 cysticerci. The viability of the cysticerci was tested in vitro before each experimental feeding. The animals were killed seven days after the last exposure and examined for helminths.

No helminths were recovered from any of the three monkeys, but two *Taenia solium*, approximately 4 mm. long, were recovered from the baboon.

### REFERENCES

1. VILJOEN, N. F., 1937. Cysticercosis in Swine and Bovines with special reference to South African conditions. Onderstepoort J. vet. Sci., 9: 337-570.

### THE BUSINESS MEETING

MINUTES OF THE SIXTIETH ANNUAL GENERAL MEETING HELD IN THE AUDITORIUM OF THE FACULTY BUILDING, ONDERSTEPOORT, ON WEDNESDAY 15TH SEPTEMBER 1965

### PRESENT:

Dr. H. P. Steyn (President). Prof. R. Clark (Vice-President). Dr. S. W. J. van Rensburg (Acting Secretary), and the following members:

Drs. Abrams, L., Adelaar, T. F. Albertyn, A. A. L., Anderson, A. A. L., Bauling, E. C. B. Bekker, P. M., Belonje, P. C., Bezuidenhout, R. J., Bishop, G. P., Bisschop, G. H. R., Bisschop, J. H. R., Bisschop, P. J. N., Bosman, P. P., Botes, H. J. W., Boyazoglu, P. A., Brown, J. M. M., Brownlie, J. F., Bunton, P. H., Cable, H. R., Cameron, C., Canham, I. S., Cavanagh, F. E., Clark, R., Cotton, C. G., Combrink, A., Coubrough, R., de la Harpe, P., de Villiers, W. J. B., de Lange, M., Doré, J. L., Dorrington, J. E., Ducasse, F. W., .m du Plessis, J. L., du Preez, J. H., du Toit, R., Ehret, W., Erasmus, J., Flanagan, H. O., Frean, J. R., Greathead, M. M., Grosskopf, J. F. W., Gurnell, T. O., Holtz, H., Horak, I., Horton, B. G. W., Hugo, P. P., Howell, R. J., Jansen, B. C., Jenkins, W., Kluge, E. B., Lambrechts, H. B., le Riche, E. O., le Roux, D. J., Lombard, S., Louw, T. A. T., Malherbe, W. D., Matson, B. A., Masters, P. M. S., McFarlane, I. S., McHardy, W. M., Meeser, M. J. N., Morgenthal, J. C., Neethling, D. A., O'Grady, J. M., Oosthuizen, J. J., Osbourn, D. E., Osrin, R. B., Paine, B. T., Petrick, S. W., Poole, J. D. H., Pols, J. W., Potgieter, L. N. D., Reinecke, R., Rippon, C. B., Robinson, E. M., Roos, C. J., Ryksen, W. J., Schmidt-Dumont, A. M. A., Schutte, J. A., Snijders, A. J., Stampa, S., Stevens, G. J. H., Steyn, D. G., Sutton, G. D., Steenekamp, N. C. F., Smit, J. O., Tarr, A. F., Terblanche, H. J. J., Thorburn, J., Thornton, D. J., Thorold, P. W., Tustin, R. C., Turner,

S. C., Trichard, C. J. V., Truter, D. E., Uys, P. L., Van Aardt, W. P., van Blerk, N. S., van Drimmelen, G. C., van Heerden, A., van Heerden, K. M., van Heerden, S., van Niekerk, J. W., van Niekerk, C. H., van Rensburg, S. J., van Schalkwyk, I., van den Heever, L. W., van der Merwe, C. P., van der Merwe, C. F., van der Walt, K., van der Watt, J. J., Warnes, W. E. J., Wessels, C. C., Wessels, T. C., Wheeler, W. J., Williams, J. G., Wilson, R. A.

### APOLOGIES FOR ABSENCE:

Drs. Campbell-Dickson, C. H. Flight, G. Martinaglia, H. G. J. Coetzee, H. Nelson, P. Snyman.

### DEATHS:

The President referred to the loss the Association had sustained through the death of the following members: R. Alexander, W. H. Chase, E. T. Clemow, L. L. Daly, A. M. Diesel, R. S. Garraway, C. Jackson and I. P. Marais.

He asked members to stand in silence for a minute as a mark of respect.

1. CONFIRMATION OF THE MINUTES OF THE 59TH. ANNUAL GENERAL MEETING

Notice of meeting was taken as read, and minutes of the 59th. Annual General Meeting were confirmed.

- MATTERS ARISING FROM THE MINUTES OF THE 59TH. ANNUAL GENERAL MEETING
  - (a) The Benevolent Fund

The Secretary reported that Council had considered this proposal very carefully and was not in favour of increasing the membership fees in order to make a bigger allocation to the Fund. Donations to this Fund should be made on a

voluntary basis, and members were urged on their own to make use of every opportunity for collecting monies and making donations to the Benevolent Fund.

### (b) Congress Fund

After proper investigation, Council had concluded that it was not desirable to establish a separate Congress Fund. Any profit made at the Onderstepoort Congresses is usually absorbed by the losses on Congresses at other centres.

### 3. Presidents Report.

The Presidents report on the activities of the Association during the year is printed elsewhere in this issue.

During the discussion on this report the question was raised as to whether it was proper for a practitioner to appoint an assistant to conduct a branch practice for him. Dr. Jansen stated there were two points to consider: (a) When a person is practising in a certain area and there is another area in the vicinity that needs service, the practitioner in the former is entitled to open a branch practice in the latter or to appoint an assistant on a proper contract to run it. (b) As professional people we have an obligation to the public whereby a practice started in an area must be so conducted that it supplies reasonable service.

Dr. Tarr pointed out that the opening of branches is a common practice in all professions.

### 4. Membership

- (a) Resignations: V. Caeiro—Agreed.
- (b) New Members: The following were proposed and accepted:

Drs. J. A. Badenhorst, H. J. Bertschinger, F. M. Bristol, H. R. Cable, A. Combrink, R. C. Cook, B. Easton, I. L. Firth, J. M. P. Fleming, T. Foulkes, G. J. Futter, T. O. Gurnell, C. Hibl, Mrs. J. M. Hofmeyr, R. I. C. Hyam, W. B. Hyman, E. A. Jaques, A. Kossuth, D. T. Longland, C. T. Nilsen, L. N. D. Potgieter, A. J. Pyle, B. M. Shaw, A. M. Simon, M. J. O. Taylor, S. L. Trevor, L. B. V. Valkanyi, H. A. Van de Pypekamp, P. J. van der Lingen, C. J. van Wyk, J. A. van Wyk, J. K. G. van Zyl, I. S. Watt.

### (c) Associate Membership

Dr. Jansen proposed and Dr. Clark seconded Dr. O. G. H. Fiedler. Unanimously accepted.

### REPORTS.

### Finance:

Since reports of the other Committees were dealt with by the President, the Secretary submitted only the Auditor's Report and Financial Statement. He explained that the excess of R80-20 of Expenditure over Income was mainly due to a loss of R179-71 on the Cape Town Congress and increased cost of production of the Journal. The Report was adopted.

On proposal by Dr. K. van der Walt it was unanimously agreed that the President be reimbursed for all phone calls made by him.

### 6. WORLD VETERINARY ASSOCIATION.

The meeting was informed that the next International Veterinary Congress will be held in Paris on 17—22 July 1967.

### 7. Guide to Professional Conduct.

Dr. Jansen indicated that in its deliberations the Veterinary Board is guided by the "Guide to Professional Etiquette". This was drawn up years ago and many changes have taken place since. The time has therefore arrived to revise the guide, and he appealed to the profession to scrutinize it very carefully and to suggest changes and make recommendations.

As some members complained that they had not received the relevant Circular, it was decided that another be sent out to members.

During this discussion it became evident that there was much uncertainty amongst practitioners on the association of Veterinarians names with veterinary hospitals on name plates, letterheads and in telephone directories. Both the President and the Chairman of the Veterinary Board (Dr. Jansen) pointed out that this was contrary to the Ethical Code.

### 8. SITING OF VETERINARY HOSPITALS AND CON-SULTING ROOMS.

The President indicated that this question has caused trouble in some cities. Since the relevant by-laws are not uniform throughout the Republic, the branches of the Association were

asked to submit the names of legal persons with a thorough knowledge of the local ordinances who could be consulted. The response was poor and he again appealed to the branches to assist in this matter.

### 9. Administration of the Association.

The President expressed the hope that a Secretary would soon be appointed to fill the vacancy resulting from the death of Dr. Diesel. In the meantime Dr. S. W. J. van Rensburg would act.

# 10. CONSTITUTION OF THE S.A. VETERINARY FOUNDATION.

The President submitted a copy of the Constitution and stated that this had been drafted by the legal adviser of the Association. It has been closely scrutinized by Council and he now asked the meeting to approve of it.

- Dr. K. van der Walt expressed the fear that the Foundation may harm the existing Faculty Fund. He therefore proposed that it be merged with the latter Fund. This was seconded by Dr. W. Malherbe.
- Dr. C. Orsin proposed and Dr. J. Thornburn seconded that the meeting accepts the Constitution, and this proposal was carried, only three members voting against it.

### 11. ELECTION OF OFFICE BEARERS.

The voting to fill the four vacancies on Council resulted in the election of Drs. Clark, Hofmeyr, Jansen and van den Heever. Further, Dr. M. de Lange was elected to fill the vacancy created by the unopposed election of Dr. A. F. Tarr as Vice-President.

The new Council therefore consists of:

President:

Dr. H. P. Steyn.

Vice-President:

Dr. A. F. Tarr.

Elected Members:

Dr. R. Clark.

ĭ

Dr. D. Coles.

Dr. M. de Lange.

Dr. J. Doré.

Dr. C. F. B. Hofmeyr.

Dr. B. C. Jansen.

Dr. M. C. Lambrechts

Dr. L. W. v.d. Heever.

### 12. GENERAL.

### (a) Coat of Arms.

The newly designed Coat of Arms was passed round for members to see. It had been suggested that, provided there was a demand for it, lapel badges be made.

A list was accordingly passed round, on which members could indicate whether they want such a badge or not.

### (b) Registration of Stock Remedies.

In response to a request by the President, Mr. P. M. Bekker explained the implications of the Fertilizers, Farm Feeds, Sterilizing Plants and Stock Remedies Act (No. 36 of 1947).

Resulting from a decision of the Department that the application of the Act be entrusted to a Chemist, he had been appointed and had to give effect to decisions which had already been taken by the law advisers. It amounted to this, that no one is permitted to sell a Stock remedy unless it has been registered.

The President expressed the fear that laymen could obtain drugs that are highly dangerous.

Dr. Jansen stated that registration was necessary, but indicated the desirability of including a separate register for dangerous drugs.

After further discussion the President pointed out that registration is no guarantee of quality and that the Act affords no protection to the Veterinarian. He informed members that Council had appointed a Committee to investigate, and this was approved by the meeting.

### 13. VENUE OF 1966 CONGRESS.

It was decided that this be held at Onderstepoort.

### 14. RESOLUTIONS.

None were submitted.

### 15. ADJOURNMENT.

Dr. Osrin proposed a hearty vote of thanks to the President, after which the meeting adjourned at 12.30 p.m.

### VOORSITTERS VERSLAG

Die afgelope jaar het redelik bevredigend verloop mits ons onthou dat alle werk wat deur Raadslede verrig word gedurende hulle vry tyd gedaan moet word.

Die Raad het soos gewoonlik sewe keer vergader. Die twee mees belangrike werksaamhede van die afgelope jaar was die voortsetting van die ondersoek na Nasionale Veterinêre dienste en die formulering van die S.A. Veterinêre Stigting.

· U het alreeds al die voorlopige vraelyste wat deur die komitee opgestel is ontvang. Die antwoorde wat tot dusver van lede ontvang is toon 'n bevredigende belanghebbing aan professionele sake asook die belange van die veenywerheid.

Die formulering van die antwoorde wat tot dusver ontvang is word nou onderneem en dit gaan 'n reuse taak wees om al die antwoorde te ontleed en gevolgtrekkings te formuleer. Die komitee sal na hierdie eerste stap besluit hoe daar verder opgetree moet word want ons wil graag weet hoe georganiseerde landbou en andere instansies ageer op sommige van ons vrae.

Die eintlike doelwit van die ondersoek is vanselfsprekend om te bepaal of huidige algemene veeartsenydienste bevredigend is en indien nie, hoe verbeterings aangebring moet word.

Die werk is egter van so 'n reuse omvang dat dit werklik die aandag van 'n voltydse kommissie van ondersoek behoort te geniet.

Die tweede belangrike ondersoek was om te bepaal of dit moontlik sou wees om 'n Suid-Afrikaanse Veterinêre Stigting te stig. U Raad het gevind dat dit wel moontlik is, en 'n konsep konstitusie vir so 'n stigting sal later vandag aan u voorgelê word vir u goedkeuring. Of die Stigting die algemene openbare byval en ondersteuning gaan geniet wat 'n noodsaaklikheid is om daar 'n sukses van te maak sal die tyd alleen ons kan leer. Graag wil ek u aandag vestig op ons balansstaat en wins en verlies rekening. U sal sien dat ons bruto bates gedurende die jaar vermeerder het met byna R1,000 na 'n totaal van R29,499. Hierdie totale bates is egter onderverdeel aan die volgende fondse:—

- Die algemene fonds wat ongeveer R12,271 beloop.
- (ii) Die liefdadigheidsfonds wat op R8,908 staan.
- (iii) Die Major Brown prysfonds wat R5,770 bedra.

Dan is daar allerlei skulde van ons Vereniging van R2,549.

As u na ons bates kyk sal u sien dat daar 'n bedrag van ongeveer R2,000 is wat aan die Vereniging verskuldig was deur lede d.w.s. agterstallige subskripsies. U moet egter onthou dat die balansstaat afsluit op 31 Maart elke jaar. Van die agterstallige subskripsies word die meerderheid gedurende die jaar opbetaal; daar is egter 'n baie aansienlike bedrag agterstallige subskripsiegelde wat elke jaar blykbaar groter word.

Daar is lede wat soveel as R90 agterstallig is met hul subskripsies en dan is daar 'n aantal wat R30 na R40 en meer skuld. Ons subskripsie is R15.00 en as iemand R90 agterstallig is beteken dit dus dat hy ses jaar agterstallig is. Dit is duidelik dat die toedrag van sake nie stilswyend deur ons toegelaat kan word nie. U Raad stel dus voor om in die toekoms niemand toe te laat om meer as 'n jaar agterstallig te laat raak tensy daar 'n bevredigende rede is nie. U moet onthou dat ons subskripsie eintlik vooruit betaalbaar is.

Die laaste punt van belang onder finansies wat ek onder u aandag moet bring is dat ons oor die afgelope jaar 'n verlies van R80.21 gely het. Die sal u in die wins en verlies staat sien. U sal sien dat ons grootste uitgawes aan ons tydskrif en aan salarisse toe te skryf is. Ons het ook 'n verlies van R179 aan ons kongres in Kaapstad te danke.

Nietemin sou ons nie op 'n verlies uitgekom het as daar nie so veel lede met hul subskripsies agterstallig was nie. Derhalwe doen ek 'n ernstige beroep aan alle lede om tog asseblief hul subskripsies te betaal, en veral die lede wat lank agterstallig is.

Aangaande die plek waar ons vergader sal u later in die vergadering gevra word om te besluit waar ons volgende kongres gehou gaan word. Ten spyte van die verlies wat in Kaapstad gely is, ondersteun ek nog hartelik die beginsel om kongresse al om die ander jaar weg van Pretoria te hou. Die voordele hieraan is so groot dat ten spyte van die ongerief aan die Departement van Landbou-tegniese Dienste, ek daarvan oortuig is dat ons nie moet afsien van die gebruik nie. Met so 'n reëling sou Onderstepoort reeds twee keer soveel kongresse hê as die res van die land.

Gedurende die jaar is besluit om die S.A.V.M.V. wapen te registreer. Daar is toe gevind dat daar sekere heraldiese foute in die samestelling van die wapen was. Die verbeterde wapen is nou geregistreer en ek stuur 'n eksemplaar van die ou en die verbeterde wapens rond sodat u hulle kan vergelyk.

U sou opgemerk het dat u Raad besluit het om opgesomde notule van Raadsvergaderings aan alle lede te stuur. Ek vertrou dat dit u goedkeuring wegdra, maar ek moet u daarop wys dat die prosedure 'n groot uitgawe veroorsaak. Die omsending van die notule is egter 'n noodsaaklikheid en u Raad sou graag van u hoor of u tevrede is met die manier waarop die notule uitgestuur word. Dit mag nodig wees om die notule 'n weinig meer te beknop, maar verkorting kan oordrewe word en die notule nutteloos maak.

Gedurende die afgelope jaar is daar een nuwe tak naamlik Wes-Transvaal en twee nuwe groepe, naamlik die Klein Diere Praktisyns in die Kaap en die Kommersiële Groep in Johannesburg, gestig.

Die Wes-Transvaal Tak het sy eerste vergadering met volslae sukses op Potchefstroom gehou en die Voorsitter, Komitee en lede van die Tak word geluk gewens met hulle onderneming.

Ook word die nuwe groepe alle geluk toegewens en vertrou ons dat kollegas hul doelwit met die nuwe organisasies sal verwesenlik sien. U Raad het heelwat aandag aan die nuwere ontwikkelings geskenk en het sy goedkeuring daaraan geskenk. Daar is egter 'n gevoel dat ons moet waak teen oormatige versplintering onder ons betreklike klein totale ledetal.

Nou, vriende, is dit my onaangename plig om sekere ongewenste en onetiese gedragsverskynsels van enkele lede onder u aandag te bring. Daar was gedurende die afgelope jaar 'n paar oortredings waaromtrent daar in party gevalle klagtes by die Veeartsraad ingedien is. Hieromtrent gaan ek nie veel sê nie want sommige hiervan is nog onder oorweging, maar ek wil 'n waarskuwing uitspreek. U weet dat daar 'n verskeidenheid van maniere is waarop 'n man homself kan adverteer. Nou ja, die Veeartsraad het nou deur jare van ondervinding bekend geraak met al die (verskoon die woord) slinkse wyses van advertensie. Ook kan die Veeartsraad alreeds met 'n groot mate van korrektheid voorspel watter soort van verskonings geopper gaan word as mense gevra word na 'n verduideliking.

Die slotsom is dat die Veeartsraad nou geduld met die soort van verskynsel verloor en kollegas sou gerade wees om hierop ag te slaan, want iets sal gedoen moet word om die enkele veeartse wat op enige ongewenste metode 'n voorsprong oor hulle kollegas probeer kry, te straf.

Een van die verskynsels wat onder ons aandag gebring is is die gebruik van die "M.R.C.V.S." wat op subskripsie verkry is, asof dit 'n tweede graad of kwalifikasie is. Die besluit van 'n vorige jaarvergadering hieromtrent is baie duidelik gestel en alom bekend gemaak. Persone wat die publiek probeer bedrieg deur misbruik te maak van enige soortgelyke titel moet dus min simpatie verwag indien hulle by die Veeartsraad aangekla word.

Ten slotte wil ek graag alle Raadslede van die S.A.V.M.V. en alle kollegas hartelik bedank vir hulle getroue medewerking en ondersteuning oor die jare.

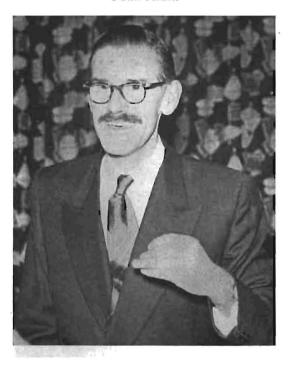
Aan ons Raadslede, en veral aan ons Onder-President wie, tot my innige spyt, besluit het om homself nie herkiesbaar te stel nie, wil ek namens u en myself my waardering vir alles wat hulle gedoen het uitspreek. Die manne moet heelwat van hulle vry tyd op offer om toe te sien dat die werksaamhede van ons organisasie vorder. Daar word elke jaar baie verrig en as u gekose Raadslede wie met die toegeweidheid wat hulle toon gewerk het nie sou daar geen vordering wees nie.

Sommige van u mag voel dat daar nie bevredigende vordering is nie. Ek persoonlik sorteer onder die groep, maar ons moet onthou dat ons Raadslede almal deeltyds in hul vry tyd die werk moet doen. Ons kan nie verwag dat daar meer as een Raadsvergadering ongeveer elke twee maande gehou moet word nie, en daartussen word nog telke komitee vergaderings gehou. Daarbenewens is daar nou agt takke

en vier groepe. Gewoonlik is daar meer as een Raadslid op elke tak se jaarvergadering. U sien dus dat ons nie met reg kan verwag dat veel meer gedoen kan word nie.

Daar is nog twee van u ampsdraers wat 'n spesiale woord van waardering verdien naamlik ons oorledene Sekretaris en sy opvolger agerende dr. Schalk van Rensburg (Sen.), en dr. Nobby Clark. Twee laasgenoemde het vrywilliglik in die bres gespring as agerende Sekretaris en Redakteur respektiewelik, en ons het dit hoofsaaklik aan hulle, met hulle ondersteuners, te danke dat ons vandag hier met so 'n mate van sukses ons normale verrigtinge kan voortsit.

### **OBITUARY**



It is with great regret that we have to report the sudden death of Campbell Dickson of Johannesburg who died suddenly on the 22nd October 1965 while on holiday on the Wild Coast.

He was born in Edinburgh in 1913 and obtained his M.R.C.V.S. at the Royal "Dick" in 1934 after which he practiced in England. In 1945 he came out to South Africa and joined the firm of which he later became a partner and with which he remained until his death.

He was a great clinician and true gentleman who gained for himself the admiration and respect of his clients and colleagues. In its obituary the Johannesburg "Star" said that he was probably the best loved man in the northern suburbs of the city. He was well known to many Onderstepoort graduates as a fair and friendly examiner. He is also well known to the profession as the editor of that remarkable private veterinary digest the "Pennent".

He was the first secretary of the Rosebank Rotary Club and that body has now decided to name its music scholarship after him.

Campbell Dickson was cremated in East London and a memorial service was held at St. Columbia's Church, Parkview.

We extend our deepest and most profound sympathy to his widow and to his brother in Edinburgh.



### VICE PRESIDENT

The new Vice President of the Association, Ashton Forrester Tarr was born at Senekal O.F.S., in 1911 and matriculated at Dale College, King William's Town. He graduated from Onderstepoort in 1935. While at University he captained both the 1st cricket and 1st hockey teams and was awarded colours.

He joined the state service in 1936 and was stationed in various parts of Natal and at Umtata. From 1944 he has practised in Pietermaritzburg. He has been a member of Council

since 1957 and has been a very regular attendant at meetings despite the inconvenience of the travelling involved.

He is a man of wide interests being a steward of the City Sporting Club, an active Rotarian and a member of various other societies.

He has maintained his interest in sport being a Past Chairman and Life Member of the Pietermaritzburg Wanderers Club.

His election is a fitting tribute to one of our senior colleagues.

### HONORARY ASSOCIATE MEMBER



Dr. O. G. H. Fiedler was unanimously elected an Honorary Associate Member of the association at the 60th annual general meeting held in September 1965.

Otto George Hans Fiedler was born in 1912 at Berlin, Germany. From 1931 until 1933 he studied Zoology, Botany, Physiology, Chemistry, Physics and Philosophy at the Berlin University. Thereafter he continued his studies with Entomology, Parasitology, Agricultural Zoology and Plant Pathology at the Faculty of Agriculture, also at the University of Berlin. He was promoted to Dr. rer. nat. in March 1937 with the distinction of "Magna cum laude".

From January 1937 onwards he was Scientific Assistant and later on became Custos and Head of a Section at the Zoological Museum of

the University of Berlin. In 1938 he won a research fellowship of the "Reichsforschungsrat" (German Research Council) and proceeded to East Africa for the study of insect pests in tropical agriculture.

In 1948 he was employed by the Division of Veterinary Services. First he was connected with the Tsetse Fly Campaign in Zululand and later on transferred to Onderstepoort where he worked on ectoparasites and vectors of diseases of livestock as well as the means of their control. In 1952 he won the internationally contested senior research felloship of the Combined S.A. Control Board (Wool, Meat and Dairy) for the study of parasites and transmitters of diseases of the sheep. In 1956 he joined the scientific staff of Agricura Laboratoria, Ltd. where he is responsible for research and development of insecticides and dipping materials. On invitation he attended the First International Congress of Parasitology (21st-26th September, 1964) in Rome, Italy and read two papers on the problem of blowflies of woolled sheep and their control.

He has published 59 scientific papers so far, 35 of which deal with subjects of veterinary interest, such as tsetse flies and the Campaign in Zululand, the blowfly problem in South Africa, parasitic mites, lice and ticks and their control by means of the new synthetic insecticides.

He made a special study of the organophosphorus compounds and found that some of them were of particular value for the protection of sheep against blowfly strike, as e.g. Diazinon, and that other compounds of this group are very potent killers of ticks and can be used as dipping material for cattle. He also found the itch mite of sheep in South Africa as well as different methods to control this pest.

### **BOOK REVIEWS**

### ANIMAL ANAESTHESIA.

Volume Two: General Anaesthesia.

BY: MELCHIOR WESTHNES AND RUDOLF FRITSCH.

Translated by: A. D. Weaver.

Edinburgh and London. Oliver and Boyd. 1st English Edition. (1965), pp XXI, 458. Price 95s.

The first English edition of this book is a translation of "Die Narkose der Tiere", published in German in 1961 by Paul Parey, Berlin. The translator, A. D. Weaver, is to be complimented on his work. The English translation of volume I was published in 1964 and dealt with local anaesthesia. Volume II is devoted entirely to general anaesthesia. This outstanding book is probably the most comprehensive work on the subject available today and will be of particular value to veterinarians and students who are not au fait with German.

The book is divided into four sections. Section I defines anaesthesia and then continues with a chapter on the theories of anaesthesia. The physiological principles are considered particularly with regard to respiratory and circulatory aspects. The care of the patient and the choice of the anaesthetic agent, two factors of major importance, are also discussed in this section.

Section II described the drugs employed in anaesthesia and the indications for various volatile, gaseous and injectable agents. Analeptic and antidotes, muscle relaxants, sedatives and drugs depressing the autonomic nervous system are discussed in respect of their pharmacological and practical uses.

Section III covers the various techniques used in anaesthesia, Intravenous, intraperitoneal, intrathoracic and intramedullary injections of anaesthetic agents in the different species are discussed followed by a description of the technique for inhalation anaesthesia. New apparatus such as anaesthetic machines are deemed essential for modern operating conditions and valuable information on the use and handling of these machines is included. Anaesthetic complications are inevitably encountered in many anaesthetic procedures and accidents usually arise when least expected. The precautions and steps to be taken in these emergencies are discussed at length.

The final section is devoted to a description of anaesthesia in the different species including the domestic animals, laboratory animals, wild and zoo animals and birds. It also includes a chapter on legislation in Great Britain relating to anaesthesia and a list of drug synonyms. Unfortunately many of these drugs are unobtainable or are registered under different names in the Republic of South Africa. This, however, detracts very little from the value of the available information.

It is extremely difficult to criticise a book which has to cover such a wide field. With the tremendous advances made in veterinary anaesthesia and the rate at which new information becomes available it is understandable that any book of this nature will not contain the latest information in respect of every new drug. As stated by the translator, no drugs introduced since 1961 have been discussed. The chapter on electrical anaesthesia deals with the history of the technique but it is felt that a short description of the practical application and possible uses would not be out of place.

This is a comprehensive, detailed work and forms a most welcome addition to the library of the clinician, general practitioner or student as well as to the research worker handling laboratory animals.

D.G.S.

### CANINE AND FELINE NUTRITIONAL REQUIREMENTS

Proceedings of a Symposium organized by the British Small Animal Veterinary Association. Editor: Oliver Graham-Jones. Pergamon Press. London. 1965. pp. 160. Price 63/-.

This is a welcome contribution to the limited available literature, on fundamental research in canine and feline nutrition, and its related areas of nutritional diseases.

The sixteen papers have been classified under "Some Physiological and Biochemical Aspects of Nutrition" and "Practical Dietetics of the Canine and Feline". The papers are generally of a high standard, presenting interesting results and opinions as well as giving some background of previous literature.

In the first section mineral and vitamin requirements are evaluated, and the protein-

calorie ratios as influenced by growth. A commendable paper is also included on the effects of deficiencies on bone and central nervous system development, as well as one on the effects of worm infestations.

The second section of the book deals with certain aspects of applied nutrition, however several of the papers are inclined to be more of academic interest and would have better fitted in the first section. From the general practitioner's viewpoint it would have been more advantageous, if in the latter half of the book, more emphasis were given to the ingredients, methods of compilation and handling of rations.

The contributions to the symposium have brought forward a publication which will be of substantial benefit to persons involved in small animal nutrition studies.

P.A.B.

# COMPARATIVE PHYSIOLOGY AND PATHOLOGY OF THE SKIN

Edited by ARTHUR J. ROOK and G. S. WALTON, Blackwell Scientific Publication, Oxford. pp. 795. Published Price £7-15-0.

The book contains 55 papers delivered at a symposium at Cambridge by 46 authorities drawn from the medical and veterinary field.

The papers could be classified under the following headings:

### COMPARATIVE DERMATOLOGY.

Under this heading the present state of our knowledge of human and veterinary dermatology is compared and the tasks, possibilities and aims of comparative dermatology are discussed.

### HAIR.

The genetics of hair growth, replacement of hair, normal and abnormal hair in man and animals are some of the most important aspects included under this heading. This is a very valuable contribution to the veterinary literature as very little is known in this field of veterinary dermatology.

### NUTRITIONAL INFLUENCES ON THE SKIN.

Nutritional influences on the skin in man and in animals constitute the next group of papers. Emphasis is placed on the pathological lesions produced by deficiencies.

### PORPHYRIA AND LIGHT SENSITISATION.

This interesting subject is very well covered by authorities from the human and veterinary fields. The various types of porphyrias are discussed in great detail.

### THE MAST CELL.

This forms a very valuable section of the symposium. The history, pharmacology and the pathology of the mast cell in man and animals receive attention.

### IMMUNOLOGY.

In a very comprehensive series of lectures all the various aspects of skin sensitivity are discussed. This includes papers on allergy, anaphylaxes, contact dermatitis, urticaria, atopy and reactions to arthropods and drugs.

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TUMOURS OF THE SKIN.

Various aspects of skin tumours received attention. Normal skin, the distribution and incidence of skin tumours are some of the important aspects under discussion. The aetiology, with special reference to virus and chemical agents as skin carcinogens, forms another major part of the lectures.

The symposium certainly covers a very wide field. It is a valuable contribution to our knowl-

edge of skin pathology. The application to the veterinary field of the knowledge available in human dermatology and the adoption of a uniform scientific terminology offer immense prospects for immediate advances in diagnosis and therapy and for planned research.

This book is a most welcome addition to the library of the pathologist, clinician and general practitioner.

J. D. S.

Canine Neurology—Diagnosis and Treatment—B. F. HOERLEIN, W. B. SAUNDERS Company, Philadelphia and London, 1965. Pages: (x)+303. Numerous illustrations. Price \$13.50.

Clinical neurology is a much neglected subject in veterinary literature. Besides a relative dearth of articles dealing with clinical aspects of brain conditions, only a couple of books have appeared in English and German on the subject. Spinal compression, particularly disc herniation, has received more attention, led originally by Swedish veterinary surgeons.

The publication of a new book on neurology is thus a welcome event, even more because the book under review could, with almost equal justification, have been entitled "Canine Neurosurgery".

The scope of the book is as follows: After a general introduction follows a chapter on basic neurologic diagnostics by Carl H. Clark. Then radiographic examination is described as well as laboratory examinations. The only other author who contributed, is R. W. Redding on canine electro-encephalography. Disorders and surgery of peripheral nerves occupy 20 pages, those of the spine 123 pages and of the brain 78 pages.

Although reference is made from time to time in the text to anatomy and physiology of the nervous system, the reader would be well advised to brush up on neuro-anatomy and neurophysiology in order to derive full benefit from the book.

Many of the operations described will not be attempted by the general practitioner unless he is strongly orientated surgically and exceptionally well equipped, but there are many pages of recent information, much of it not readily available to most veterinarians. In this respect spinal trauma and cranial injuries come to mind as examples. Although a full range of surgical conditions of the nervous system is described, non-surgical conditions are also included. Hoerlein has succeeded in being relatively brief without sacrificing essentials.

This book should therefore have a thorough appeal to all veterinary clinicians.

The many illustrations are clearly executed and the printing is in double column on fine quality gloss paper.

C.F.B.H.

### CAPE EASTERN AND KAROO BRANCH.

Account of the 12th A.G.M. and conference held at Weaver's Hotel, East London, on the 24th July, 1965.

### PRESENT:

Drs. R. A. Painter, J. Fick, W. van Aardt, C. P. v.d. Merwe, K. Mason, W. Galpin, L. W. Rossiter, C. Marlow, J. Snyman, B. Rippon, C. P. Harte, G. C. Dent, L. A. Simpson, R. Buchalter, E. M. van Tonder, H. Lucouw, J. H. Viljoen, C. Wilkens, H. E. William Jones, K. M. van Heerden, C. Osrin, J. M. de Wet, G. Thompson, C. W. A. Belonje, H. Botha, E. Leeb-du-Toit.

### VISITORS:

Dr. & Mrs. C. Bennett, Dr. & Mrs. Whitehead, Dr. B. J. Erasmus, Prof., G. van Drimmelen.

### THE BUSINESS MEETING

### Apologies:

Drs. Schutte, McIntyre, Flight, Jonker, Dovey, Immelman, Bunton, Kropwinicki.

### New Members:

Drs. C. P. van der Merwe, J. H. D. Snyman, C. P. Harte, H. J. Viljoen, L. A. Simpson, B. Rippon.

MINUTES OF 11TH A.G.M. AND FINANCIAL STATE-MENT:

These, having been circularized were taken as read.

### MATTERS ARISING:

The schedule of fees, which was circularized, was accepted after a long discussion as to mileage fee to be charged particularly in country practice.

A separate Book Fund had been started to enable the committee to put into circulation the quarterly Bulletin as proposed.

### CHAIRMAN'S ADDRESS.

In his address the chairman, Dr. Belonje, explained the need for a revision of our constitution. He also told the meeting of how the Book Fund for the quarterly Bulletin had come into existence.

### CONSTITUTION:

The proposed revision of the constitution as circularized was accepted with the exception that the meeting decided that the Asst. Chief Veterinarian, East London, should ex officio, be a member of the committee.

### ELECTION OF NEW OFFICE BEARERS.

Chairman: Dr. C. W. A. Belonje.
Secretary: Dr. E. Leeb-du-Toit.
Ex Officio: Dr. J. M. de Wet.
Additional: Dr. R. Buchalter,
Dr. J. H. D. Snyman.

### SCIENTIFIC CONFERENCE

This was opened by Mr. Bennett, M.P. for Albany. Mr. Bennett said that there was a definite need for a second Veterinary Faculty and explained why he felt that this faculty should be situated at Rhodes University.

The following papers were delivered:—

Dr. G. B. Whitehead,—"Insecticide Resistance".

Dr. B. J. Erasmus,—"Modern Trends in Virology".

Dr. C. H. B. Marlow—"Some Practical Aspects of A.I. as applied to Sheep and Goats".

Dr. H. J. Viljoen—"Internal Parasites of Sheep".

Dr. W. P. van Aardt-"Knoopderm".

A Social Gathering at the home of Dr. & Mrs. C. W. A. Belonje in the evening rounded off a very enjoyable day.

### **BOOK NEWS**

When the first British Veterinary Codex appeared in 1953 it was hailed as "A milestone in veterinary literature". It was the only book providing authoritative standards for drugs used in the prevention and treatment of animal disease, recognised formulary of veterinary preparations and systematically presented information on the actions, uses and dosage of This work has now been veterinary drugs. completely revised. Monographs on new drugs and preparations to keep abreast with the latest developments in prophylaxis and treatment have been added and the result of this complete revision is the appearance of the 2ND BRITISH VETERINARY CODEX; 880 pages; R12.10.

The name of Dr. N. T. M. Yeates, Professor of Livestock Husbandry at the University of New England, Armidale, N.S.W., Australia, should be familiar to all animal scientists in view of all his scientific publications. They will therefore be interested to know that a book by him has just been published. This is MODERN ASPECTS OF ANIMAL PRODUCTION. The four main topics of this are: all aspects of reproduction, the influence of climate, meat, and wool. 371 pages; numerous illustrations and references. R9.00

Telephone: 2-1289. Postal Address: P.O. Box 15, Pretoria. The important role played by the veterinarian in wild life preservation is being increasingly recognised. We have therefore no hesitation in recommending THE MANAGEMENT OF WILD MAMMALS IN CAPTIVITY by Lee S. Crandall. This book is packed with detailed information, based on over 50 years' experience. It covers 761 pages and has a large number of references at the end of every chapter. R10.18.

VETERINARY MEDICINE AND HUMAN HEALTH by C. W. Schwabe which was published recently is a book which should occupy a prominent place on the desk of every veterinary and medical officer concerned with public health, and of those interested in the Zoonoses. It was reviewed in the June 1965 issue of this Journal. 516 pages; many illustrations, tables and references. R12.75.

Diseases of metabolism in both large and small animals frequently prove puzzling to the veterinarian and are a source of great concern to their owners. They are fully dealt with in **METABOLIC DISORDERS OF DOMESTIC ANIMALS**, by D. E. Stevenson and A. A. Wilson; 198 pages; many references and tables. R3.75.

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