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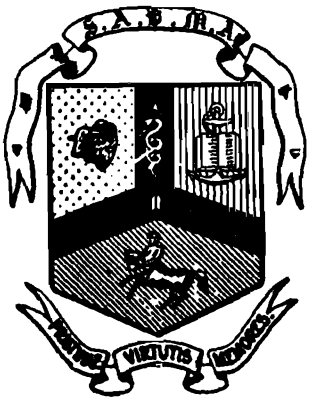

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VAN

DIE SUID-AFRIKAANSE VETERINÊR-MEDIESE VERENIGING

ELECTRICAL ANAESTHESIA

N. L. WULFSOHN[1] AND J. D. H. POOLE.[2]

INTRODUCTION

The advent of chemical anaesthesia in 1845 using nitrous oxide and ether in 1846, opened the way for painless surgery. The work on electricity by Michael Faraday in 1832 led to the possibility of its use as an anaesthetic agent after it was learned that electrical currents could cause unconsciousness. The first experiments in this direction were conducted by Mach in 1875. (1) Since then, many additional experiments have been attempted. (See Table 1).

Several types of current have been tried, ranging from direct currents,(2) pulsating direct currents,(3) alternating currents,(4) to mixed alternating and direct currents.(5) In 1962, Wulfsohn and McBride(6) reported on the use of alternating currents in a group of 36 animals. The best results were found with an alternating current of 1500 cycles per second (cps). This was then applied to a further 53 experimental animals. Since that time, this technique has been applied to a number of domestic animals. The purpose of this paper is to report on experiments on 30 animals in this latter group, consisting mainly of various species of the larger domesticated farm animals, and to review the salient features of electrical anaesthesia research.

MATERIALS AND METHODS

Equipment

The electrical apparatus consisted of a signal generator which produced a 1500 cps alternating current sine wave, which was fed into a high-power, distortion-free amplifier.

Two electrodes were used and were either 0.25-inch silver discs or Luer-Lock needles.

TABLE 1.—DEVELOPMENT OF ELECTRONARCOSIS

Year	*Investigator*	*Type of current*	*Species tested*
1875	Mach	Direct current	Fish, Frog, Dog.
1890	D'Arsonoval	Interrupted direct current	Animals.
1892	Hutchinson	Interrupted direct current	Animals
1901	Pampilon	Interrupted direct current	Animals
1902	Leduc	Interrupted direct current	Animals, himself
1907	Tuffier & Jardrey	Interrupted direct current	Animals, operated on human
1914	Robinovitch	Interrupted direct current	Animals
1934	Van Harreveld & Kok	Alternating current 60cps	Animals
1936	Burge	+v to leg, —v to head	Frog
1944	Frostig *et al.*; Thompson	Alternating current 60cps	Dog, Man
1945	Rose & Rabinov	Interrupted direct current	Animals
1946	Tietz	Alternating current 60cps	Man, 1400 cases
1947	Tietz	Alternating current 60cps	Man, 710 cases
1948	Patterson & Milligan	Alternating current 60cps	Man, 2500 cases
1950	Monro	Alternating current 60cps	Man
1953	Hirschfield	Alternating current 60cps	Man
1954	Blignault	Alternating current 60cps	Man
1955	Kerbikov	Interrupted direct current	Man
1956	Knutson *et al*	Alternating current 700 cps	Animals, Man
1960	Anan-ev	Alternating & direct current	Animals, Man
1961	Hardy, Fabian & Turner	Alternating current 700 cps	Animals, Man
1962	Wulfsohn & McBride	Alternating current 1500 cps	Animals, Man

1. MB., Ch.B., D.A.(Eng), F.F.A.(S.A.).
2. S.A. Cyanamid (Pty.) Ltd., Johannesburg.

The electrodes were applied either in the "transverse position", just anterosuperior to each external auditory meatus, or in the "sagittal position," one electrode at the intersecting point of the lines joining each eye to the opposite ear, the second electrode just inferior to the external occipital protuberance in the midline. Both needle electrodes in the sagittal position point posteriorly.

To lower skin resistance, electrode jelly was applied between the skin and the electrodes, or 0.5 ml. saline solution was injected through each needle. The electrode resistance was measured. From experience we noted that if the resistance was above 600 ohms AC, anaesthesia would be poor. The lower the resistance, the better the anaesthesia.

Procedure

Premedication: Only three animals were given drugs. In these three cases promazine hydrochloride was given intravenously ten minutes before induction of electrical anaesthesia. A sheep was given 12.5 mg., one heifer calf 65 mg., and another heifer calf 125 mg. No drugs were administered to any of the remaining animals.

Induction: The current was brought from zero to the required induction current in 5 seconds. In all cases, the animals fell to their knees and respiration ceased; but, by gradually lowering the current, respiration could be returned to normal within seconds. Investigation showed that as soon as an animal has resumed normal breathing, the current could be slowly raised to the maintenance level. The induction current varies with each species, and was found to be about 10% to 20% above the maintenance level.

Maintenance: At the maintenance level, the analgesia, respiration and muscular relaxation are all satisfactory. If the current is increased or decreased, four planes of anaesthesia can be observed, as described by Wulfsohn and McBride.[6] The maintenance level corresponds to the level at which operations can be performed.

Plane I: In this plane, the animal is asleep, but still can feel pain. Limb movements occur, and on lowering the current stil further, the animal will awaken.

Plane II: (Maintenance level). The animal is asleep and analgesic. Respiration is satisfactory, and operations can be performed at this level.

Plane III: The animal is asleep and analgesic, but increased muscle tone and limb movements appear.

Plane IV: On raising the current above Plane III, gross convulsive movements occur.

During the course of maintenance, the anaesthesia will lighten after 15-20 minutes, and the depth of anaesthesia can be increased by raising the curent level slightly, e.g. 1-2 milliamperes (ma.).

Each species of animal has its own current range for the maintenance level (Plane II). The range of current suitable for Plane II is about 50-60 ma for a donkey; 75-100 ma. for a cow; 25-30 ma. for a horse; 20-30 ma. for a sheep; 20-25 ma. for a goat; and 25-35 ma. for a dog. The current value also varies within each range for each individual animal of a species.

The signs used to detect adequate depth of electrical anaesthesia were analgesia of the flank and perineal skin, using an artery forceps. Respiration and muscle relaxation must also be satisfactory.

Results

See Table 2 for a summary of the results.

Recovery: The most striking part of electrical anaesthesia is the rapid, almost instantaneous recovery. The animal can normally walk away unaided within less than a minute. Those in our experiments would soon proceed to graze.

In all five cases where analgesia was poor, there was either bad electrode contact or too high voltage. In one sheep and two heifer calves, promazine hydrochloride was given intravenously ten minutes pre-operatively, and this helped to produce a smoother anaesthesia.

TABLE 2.—TABULAR SUMMARY OF EXPERIMENTS IN ELECTRICAL ANAESTHESIA IN 30 ANIMALS

Animal Species	No. of experiments	Operations		Duration of operation—(minutes)	Induction (ma.)	Maintenance		Analgesia	
		Skin incision & suture	Other			(ma.)	Volts	Good	Poor
Donkey.....	4	1		5–15	50–100	50–60	1–7	3	1
Bull calf.....	5	—	Castration	5–20	80–100	80–100	0.5–1	4	1
Heifer calf.....	10	5	Rumenotomy Laparatomy	15–40	75–100	75–105	0.75–15.8	9	1
Sheep.....	3	—	Castration Laparotomy	10	35	22–30	0.5–3	3	—
Horse.....	1	—	——	10	100	25	—	1	—
Goat.....	2	—	——	5	30–60	20–25	6–11	2	—
Dog.....	5	3	Hysterectomy	10–44	35–45	28–32–5	3–8–8–1	3	2

If a sudden loud noise is made near an animal under electrical anaesthesia, a reflex movement occurs; but with promazine hydrochloride this is abated.

DISCUSSION

Problems

The main problems in this method are: (1) site of electrode placement, and (2) electrode resistance.

1. *Site of electrodes*: If a transverse position for electrodes is used, the animal appears to be in a deeper sleep. In the sagittal position, the anaesthesia is equivalent to a very high spinal anaesthetic, and sensitivity of the face remains. If the transverse electrodes are too far anterior or if the anterior sagittal electrode is too far anterior, the animal may bellow, bray or bleat, and as in one dog, sneeze repeatedly until the electrodes are moved more posteriorly.

   If the anterior sagittal needle electrode is angulated to point posteriorly-left, the anaesthesia appears to be deeper. However, where it was pointed posteriorly-right in one experiment on a dog, diaphragmatic heaving and vomiting occurred.

2. *Electrode resistance*: Skin and other tissue resistance has to be overcome in the passage of a current into the brain. To reduce this resistance, hair was shaved and electrode jelly was applied between disc electrodes and skin. Alternatively, needle electrodes were used, and saline solution injected through the needles. If the resistance was too high, good anaesthesia was never achieved.

Side Effects

Psychological changes: There were no perceptible mental changes either immediately, or in those animals kept under observation for several months.

Studies on humans who have been anaesthetized electrically also revealed no psychological changes([8 10]).

Electroencephalographic changes: No abnormalities were noted in a group of rabbits that had been tested([8]). This is in agreement with the findings of other workers([9]).

Autonomic nervous system: The small rise in blood pressure and pulse rate is due to sympathetic stimulation([14]).

Hormones: There is a rise of adrenal corticoids, adrenaline, noradrenaline, and pituitary trophic hormones([10]).

Blood chemistry: There is no change in blood cholesterol, serum potassium, sodium, chloride, or calcium. There is a slow rise in blood sugar([11]).

Muscle tone: At 1500 cps., muscle relaxation is good enough to permit performance of surgical procedures.

Brain temperature: Passage of electrical current through tissue produces a rise in brain temperature. In electrical anaesthesia, the highest temperature reached in the brain after 20 minutes was less than 30°C, which is within physiological limits([12]).

Histology: Other investigators have not observed any pathological changes in the brain or spinal cord following electrical anaesthesia([13]) Similarly, our own studies on brains of rabbits revealed no pathological changes after electrical anaesthesia.

Type of anaesthesia: Electrical anaesthesia produces a light plane of anaesthesia, which is sufficiently deep to perform operations. When the sagittal position for electrodes is used, the anaesthesia appears to be akin to a very high spinal anaesthesia.

Advantages

The advantages foreseen for electrical anaesthesia are:—

Rapid reversability which produces almost immediate recovery.

Absence of toxicity, side effects, and after effects, particularly useful for poor-risk patients.

Low maintenance costs.

Portability of apparatus, useful, for example, at the site of an accident.

Possible lack of effect on foetus, which would be an advantage for Caesarean operations.

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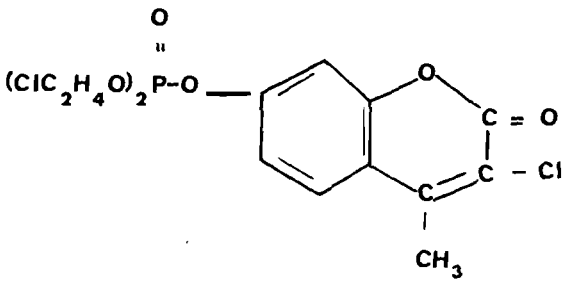

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MANHANDLING THE BABY CHICK: THE EFFECTS OF HUSBANDRY AND VACCINES ON YOUNG CHICKS

VICTOR R. KASCHULA*, V.S.A.I.D. NIGERIA.

INTRODUCTION

Modern artificial methods of poultry raising and husbandry often run counter to natural laws. A clutch of chicks hatched by their mother form a homogeneous unit closely adapted to their environment genetically and largely protected by maternal antibodies against the diseases likely to be encountered. In artificial incubation the chicks are heterogeneous as regards both possible egg borne diseases and antibody protection. It has long been known that antibodies are transmitted through the yolk from the hen to her chick. Brandly *et al*[1] and others have shown that Newcastle disease antibodies are so transmitted via the yolk-sac. Markham[2] has pointed out that this is nature's way of cushioning the severity of infections when the chick is suddenly exposed to them. It is assumed that the same principle of protection applies in most diseases. Antibodies for the viruses of infectious bronchitis, laryngotracheitis, leucosis and avian encephalomyelitis are transmitted from parent to offspring. The chick is probably protected against many other viruses and bacteria in the same way.

Eggs also can be the medium of transmission of such diseases as Newcastle disease[3 4] lymphomatosis[5 6], avian encephalomyelitis[7], pullorum disease[8], fowl typhoid[9], chronic respiratory disease[10] and others.

ARTIFICIAL INCUBATION

Artificial incubation was becoming increasingly popular in Europe and America in the last decade of the nineteenth century and it was at that time that pullorum disease was first recognised as a separate disease entity[11]. There does not seem to be any indication that this disease was a problem before artificial incubation had been practised, and in Africa the disease is still no problem where natural hatching is practised. Artificial incubation has been practised in Egypt since ancient times where a form of custom hatching was done. The eggs were placed in warmed mud ovens. It is not known what the situation with pullorum disease was in ancient days but the system is still practised today in a traditional way in many villages in Egypt and it is known that pullorum disease is now a problem in these "balady" hatcheries[12]. In China a similar system of artificial hatching has also been practised since ancient times.

An interesting point is that pullorum disease does not exist in village poultry of Nigeria and this is probably true of all Africa where the hen still rears her chickens naturally. Another disease that does not exist in these villages is lymphomatosis, but on the other hand several diseases which are no problem in the modern commercial industry are highly prevalent in the villages because of the different ecological situation[13].

In modern intensive practice, the chicks are not only raised under artificial conditions but are often subjected to vaccination and medication. The dangers inherent in such practices will be discussed.

VACCINATIONS

There are two important factors associated with vaccination of the baby chick, namely (i) age and, (ii) parental immunity. Age influences two separate sub-factors, namely the immunizing ability of the young chick and its

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sensitivity to organisms including those of live virus vaccines. Parental immunity affects vaccination in that it prevents proper immunological response to live and probably killed virus immunogens.

Effects Of Age

Twenty years ago the only vaccinations practised in chicks was against fowl pox and this was generally applied after a month of age. Today, vaccination of young chicks with Newcastle disease, infectious bronchitis and fowl pox has become commonplace. In 1962 alone, four billion doses of combined Newcastle disease and infectious bronchitis vaccines were sold in the U.S.A. Much of this vaccine was used on baby chicks. The increase in certain disease problems has coincided with the increased use of some of these vaccines.

Respiratory disease in young chicks as a major problem is of fairly recent date. Bankowski[14] has stated that there has been a sharp increase in respiratory disease in California since 1949. He suggested this was not only due to an increase in the poultry population, but may very well be correlated with the widespread use of live virus vaccines. He associated this with the widespread use of the mild B1 strain which was introduced then and has been the most widely used strain in vaccines in the U.S.A. It should be pointed out, however, that not only has the B1 virus been used increasingly but many other Newcastle disease vaccines strains have also been used and some of these are more pathogenic than the B1 strain. In addition, many vaccines incorporate infectious bronchitis viruses which are much more pathogenic to baby chicks than to older ones. Probably the most important and significant change that has occurred in the last fifteen years is the age at which these vaccines are used and method of administration. Formerly only the wing-web and intramuscular routes were used on chickens over a month of age. Very little respiratory disease was seen during that period. Later milder vaccines such as the B1 and others permitted administration of the viruses to baby chicks through the drinking water, intra-ocularly or by aerosol sprays. It would appear that respiratory disease became a major problem when these vaccines were used. It seems that the administration of these viruses by the respiratory tract, eye or by mouth to baby chicks has been an important factor in the increase in respiratory disease as has been noted in Nigeria.

Kaschula[15] has described his observations on the influence of Newcastle disease vaccination on the increase of respiratory disease in Nigeria and thought that the Komarov "intraocular" strain used on chicks of one to four days old was probably responsible for the marked increase in respiratory disease in that country. When this vaccine was used in chicks older than two weeks of age the precipitation of respiratory disease was insignificant. The age factor in these studies appeared to be a crucial one.

It is generally accepted that young chicks are more susceptible to infections than older ones. The American or pneumotropic type of Newcastle disease is known to be much more pathogenic to baby chicks than it is to older ones[16 17 18 19 20]. In a similar way infectious bronchitis virus is more pathogenic in baby chicks than those even a few weeks old[21 22].

Many other diseases are transmitted through the egg at hatching and some of these may cause serious disease in the baby chick. Such diseases are avian encephalomyelitis and pullorum disease. Pneumonia, aspergillosis and omphalitis are prevalent diseases in the young-chick. The newly hatched chick is subject to many other conditions such as nutritional disturbances, vices, etc. The viruses of lymphoid leukosis complex are apparently contracted through the egg or at hatching, but, because of the long incubation period, manifest themselves only months later. Mycoplasmosis is also transmitted through the egg and may become involved in the syndrome of chronic respiratory disease later on. Probably many other diseases of a less spectacular nature are contracted at this time. Parental immunity largely influences the susceptibility of the young chick to disease.

Fowl pox usually does not attack very young chicks in modern commercial brooder-houses because exposure usually does not occur until

an older age, but it is a severe reaction because young chicks are highly sensitive to the virus. Thus Cunningham[23], Delaplane[24], Beaudette[25] and Lubbehusen *et al*[47] advised against too early vaccination and recommend 4-12 weeks as being the best age, but under mass production methods a month of age may be more convenient when it can be combined with other vaccines. In Nigeria severe reactions have been seen when vaccination was done at one-day old. In one flock of 2,000 20% of the chicks had large granulating ulcers up to 1 cm in diameter at the point of vaccination on the wing-web two to three weeks later and the presence of fowl pox virus was shown by laboratory tests. Mortality was not high, yet above normal, but it is obvious that such reactions are undesirable and should be avoided. Although similar reactions had been seen in other flocks, they did not occur in many others. In another instance in the same country baby chickens were being reared in a battery brooder house where cannibalism (feather-eating) was rife. Some chicks in the brooder house were reacting to the virus of fowl pox contracted naturally. In contact unvaccinated chicks developed extensive lesions at the site of cannibalism and there is no doubt that this was inoculated by infected chickens through the saliva. The fever of the pox no doubt aggravated their miserable condition and increased the cannibalism and mortality rate. Since natural fowl pox is a mild disease, reactions from vaccinations should be negligible. Here again age is an important factor — chickens should not be vaccinated under one month of age. No severe reaction has ever been seen in chicks over that age, although it may affect egg production in laying hens.

Another important consideration as far as age is concerned is the immunizing ability of the baby chick. Experimental evidence strongly indicates that the baby chick is incapable of producing as good an immunity as an older one. Thus Wolfe and Dilks[26] have shown that at hatching, chicks have a poor immunological capacity but this is strengthened substantially as they grow older and at six weeks was equal to that of adult birds.

It has been shown by several other workers that the day-old chick in the absence of immunity from its parent does not show the maximum response to Newcastle disease antigens[27-33]. These findings suggest that, at best, vaccination at one-day old will give poor protection to a natural disease which is particularly severe in the baby chick.

Effect of Parental Immunity

A factor of great importance to vaccination response in baby chicks is the state of parental immunity conferred by antibodies derived from the parent and carried through the yolk. The young chick is thus born with a store of antibodies in its yolk-sac which protects it for a variable period of up to a few weeks depending on the antibody levels of the parents[1,2].

It was at first thought that parental immunity prevented multiplication of live Newcastle disease viruses but it was shown by Levine and Fabricant[34] and others[44,45] that the young chicks could be vaccinated via the respiratory tract or intra-ocularly even though they bore parental immunity. However, it was later shown that this immunity was unsatisfactory. Thus Bankowski and Cortsevet[36] showed that chickens, from immunized parents, vaccinated intranasally with B1 at 5 days of age had no immunity at 32 days of age whereas chicks from susceptible parents vaccinated at the same age had a good immunity at 32 days of age.

Markham *et al*[37] showed that high level H-I antibodies at the time of primary vaccination appear to suppress the response to intraocular vaccination with B1 virus.

D'yankova[38] found that chicks vaccinated at 5 days of age from hens immunized 1-2 months previously with B1 and H vaccines had no increase in antibody titre whereas chicks which did not have a passive immunity acquired a firm active immunity following H vaccination.

Marek and Raszewska[39] found that chicks vaccinated orally with F-107 at 6 weeks and 12 weeks developed good immunity while vaccinated day-old chicks from immune parents had a poor antibody response as only 30% developed an immunity.

Other factors influencing immunity and susceptibility

Apart from the greater sensitivity of baby chicks to pathogens, toxins and other harmful materials, the presence of hereditary susceptibility and resistance may play an important role in the prevalence of certain diseases particularly those of the leukosis complex.

It also appears that initial vaccinations influence response to revaccinations later. Observations in the field in Nigeria have shown that when chicks were vaccinated with an intra-ocular vaccine at one-day old followed by an intramuscular vaccine after one month of age, the response was not as good as when the chicks received no previous vaccination and only had the intramuscular vaccination after one month of age[40].

Kaschula[41] described a case where 4-day old chicks from immune parents were vaccinated with La Sota Newcastle vaccine in the drinking water. They then received several doses of Komarov intramuscular virus in the next few months but finally contracted Newcastle disease of the virulent type when they were seven months old. 98 of these birds died out of a total of 106 from acute Newcastle disease. It appears that the residual immunity produced by the La Sota vaccine prevented a good immunity from developing with the subsequent intramuscular vaccinations. In view of previous experiences and reports with re-vaccination of birds with a residual immunity, it seems that re-vaccination should be done by either the intra-ocular or intranasal routes or by the drinking water method and not by either the wing-web or intramuscular routes[42 43 35].

The above brief discussion indicates many serious complicating factors when immunizing baby chicks, which will be further discussed.

Discussion

Moses[46] has discussed the problems connected with vaccination of young chickens and he has been very critical of vaccination at too early an age and has recognised that harm can be done by reactions to vaccinations. He strongly advises using sanitary measures to prevent disease till the birds are old enough for vaccination. He also stresses the importance of avoiding spread from contact vaccinated flocks. Beaudette[3] has outlined principles of vaccination against Newcastle disease and has stressed the importance of waiting till the chicks were one month old. Although vaccines have been improved since 1948, when that paper was written, it is still considered that most of his views are basically sound and still apply today. The use of different routes of administration, however, are newer developments and must be considered in present day methods.

The present trend in the poultry industry is to give more medicines and vaccines to the baby chick. In the U.S.A. in 1962 four billion doses of Newcastle disease-infectious bronchitis vaccines were sold; much of this was used in the baby chick. There has been a corresponding increase in the use of drugs and medicines. The discussions and arguments given above caution against this tendency.

While the respiratory forms of Newcastle disease are more pathogenic for the young chick they may also attack it even if it has a parental immunity[34 35 36 37]. There does not seem to be sufficient evidence to justify vaccination via the respiratory route at one-day old to protect against natural infection in the first few days of life. Since the vaccination in itself is regarded as being both harmful and also to interfere with subsequent vaccinations, the justification for its practice is questioned. It is considered that isolation of susceptible chicks should be a prime management consideration and brooder houses should be kept strictly isolated from grower and adult houses. No vaccines should be used near the small chicks. The mashes should be special rations free of harmful drugs. It is recommended that the papers of Moses[46] and Beaudette[3] be consulted in formulating vaccination procedures.

In many years of experience with the virulent form of Newcastle disease in Africa, the author has only once seen it attack a flock of chickens on commercial poultry farms under the age of one month and this was in chicks 28 days old. There was the grossest negligence present. Under ordinary conditions existing on

commercial farms there is practically no danger of the disease under one month of age. The respiratory form of the disease is however a different matter, as it will attack chicks from a day old irrespective of whether they have a parental immunity or not. As stated above it is doubtful if vaccination is desirable and strict isolation along lines already mentioned is preferred.

The author has used combined Newcastle disease and live fowl typhoid vaccines by injection for a long period of time. He has found it to be very practical and the two vaccines did not interfere with one another. Many workers have shown that Newcastle disease, infectious bronchitis and fowl pox vaccines can be combined successfully, without interfering with immunities developed. It seems that as mass production of poultry increases, methods that economise in labour and handling of chicks will be needed. When vaccines are given after a month of age there is much less risk of respiratory diseases than when they are vaccinated at day old. Furthermore when they were vaccinated after a month the immunities were always superior and with the Roakin and Komarov Haifa strains this appeared to be lifelong, precluding the necessity of booster vaccinations except an annual one. Water or aerosol vaccines likewise should not be given under 3 or 4 weeks of age since they are especially likely to induce respiratory disease at an early age.

From what has been said above it would appear that vaccines and vaccination practices and routines should be carefully reviewed since it is believed that these may be responsible for much more harm than is generally realised.

(to be continued)

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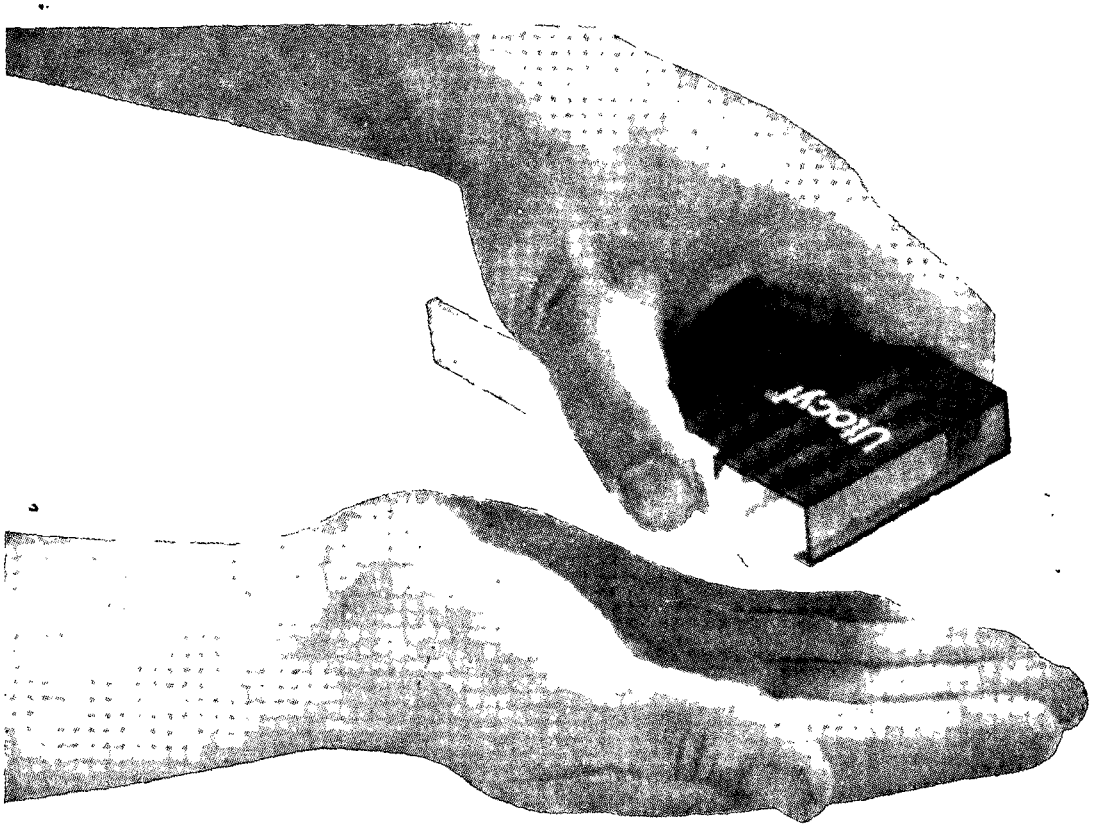

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A COMPARISON OF ANTHELMINTICS ADMINISTERED INTRA-RUMINALLY IN SHEEP

A. J. SNIJDERS AND J. P. LOUW*

SUMMARY

Controlled and critical anthelmintic tests following intra-ruminal administration are described. Anthelmintic efficacy was tested against the nematodes *Haemonchus contortus, Ostertagia circumcincta* and *Ostertagia* spp., *Trichostrongylus colubriformis* and *Trichostrongylus* spp., *Gaigeria pachyscelis, Bunostomum trigonocephalum, Strongyloides papillosus, Oesophagostomum venulosum, O. columbianum* and *Chabertia ovina.*

The anthelmintics tested included methyridine, phenothiazine, rametin and thiabendazole.

A discussion of anthelmintic evaluations in the light of these experiments is presented.

INTRODUCTION

Intra-ruminal administration of anthelmintics ensures that the entire dose enters the rumen. The possibility that the drug may bypass the rumen to the abomasum is excluded.

This method of administration for anthelmintics intended for oral use has been advocated by Boray[1] and other Australian workers[2,3].

The anthelmintics used in the tests described below are intended for oral administration without premedication with copper sulphate.

The tests were conducted in sheep with natural or artificial infestations.

MATERIALS AND METHODS

(1) Various breeds of sheep four to eight months of age were placed in pens with concrete floors and fed a mixture of chopped lucerne hay and pelleted concentrate *ad lib.* On arrival all sheep for controlled tests were treated simultaneously with thiabendazole(1) and Lintex(2) at 88 mg/K.

(2) Methods of infestation and autopsy procedures have been described by Reinecke, Horak and Snijders[4]; Reinecke[5], suggested the larval doses.

(3) The anthelmintics were made up to convenient volumes in water and administered by hypodermic syringe through a cannula inserted in the rumen.

Experiment 1: Controlled anthelmintic test

TABLE 1.—EXPERIMENT 1: EXPERIMENTAL DESIGN

DAY	Number of infective larvae dosed to each sheep		
	O. columbianum	*H. contortus*	*T. colubriformis*
−15	1,000	1,000	1,000
−13	1,000	1,000	2,000
−11	1,000	1,000	2,500
−9	1,000	1,000	2,500
−7	300	1,000	2,000
−5	300	1,000	2,000
−3	500	1,000	2,000
TOTAL	5,100	7,000	14,000
−1	Slaughtered Day −1 Controls Sheep 1 and 2		
0	Divided into groups and Sheep 11–50 treated		
+9	Slaughtered sheep 3–50		

* c/o MSD (Pty.) Ltd., 142 Pritchard Street, Johannesburg.

(1) THIBENZOLE, registered trade mark of Merck & Co., Inc., Rahway, N.J., U.S.A., and contains 2-(4¹thiazolyl)-benzimidazole.

(2) LINTEX, registered trade mark of Farbenfabriken Bayer A. G., Leverkusen, Western Germany, and contains N-(2¹-chlor 4-nitrophenyl)-5-chlorsalicylamid.

Materials and Methods

1. Fifty, four month old Merino wethers were used.
2. The experimental design is summarised in Table 1.
3. Anthelmintics were administered intraruminally as follows:

(a) Sheep 11—18: TBZ A = Thiabendazole 68% w/w water dispersible powder containing 34% particles 44-440μ, dosed at 50 mg/K.

(b) Sheep 19—26: TBZ B = Thiabendazole 68% w/w water dispersible powder of standard particle size, dosed at 50 mg/K.

TABLE 2.— RESULTS OF EXPERIMENT 1: CONTROLLED TEST

No. of animals	Group	Worms recovered *post mortem*										
		H. contortus				*T. colubriformis*				*O. columbianum*		
		(1) 4th	5th	A	Total	4th	5th	A	Total	4th	5th	Total
1	Day—1	3520	1820	—	5340	2300	2550	—	4850	1200	—	1200
2	Controls (2)	5000	3050	—	8050	2500	3120	—	5620	825	—	825
	Average	4260	2435	—	6695	2400	2835	—	5235	1012	—	1012
3	Day +9	120	1920	4380	6420	0(3)	20	11410	11430	655	20	675
4	Controls	660	1220	4135	6015	0	2000	9965	11965	530	0	530
5	"	155	1950	3120	5225	0	240	10870	11110	680	90	770
6		230	2740	950	3920	50	2490	4195	6735	225	20	245
7		940	3500	2635	7075	0	2400	10345	12745	785	0	785
8		1130	1350	1410	3890	0	2400	5615	8015	735	10	745
9		490	2100	2320	4910	0	2840	6965	9805	350	0	350
10		120	1480	1020	2620	0	2330	5150	7480	440	0	440
	Average	481	2032	2496	5009	6	1840	8064	9910	550	17	567
11	TBZ A(4)	0	0	10	10	0	0	10	10	700	0	700
12	50 mg/K	0	0	0	0	0	0	0	0	365	0	365
13		20	10	10	40	0	0	155	155	595	0	595
14		0	0	0	0	0	0	0	0	420	0	420
15		0	5	0	5	0	0	95	95	595	0	595
16		0	0	0	0	0	0	70	70	395	0	395
17		0	0	45	45	0	0	70	70	885	20	905
18		0	0	0	0	0	0	0	0	335	0	335
	Average	2	2	8	12	0	0	50	50	536	2	539
	Average % efficacy	99	99	99	99	—	100	99	99	0	—	0
	% Range	(96–100)	(98–100)	(98–100)			100	(98–100)				
19	TBZ B(4)	0	0	35	35	0	0	45	45	610	0	610
20	50 mg/K	0	0	5	5	0	0	260	260	535	0	535
21		0	5	40	45	0	0	0	0	705	5	710
22		0	45	30	75	0	0	5	5	620	0	620
23		0	0	0	0	0	0	15	15	265	0	265
24		0	0	5	5	0	0	5	5	140	0	140
25		0	0	0	0	0	0	55	55	780	30	810
26		0	25	175	200	0	0	10	10	705	0	705
	Average	0	9	36	45	0	0	49	49	545	4	549
	Average % efficacy	100	99	99	99	—	100	99	99	0	—	0
	% Range	(100)	(99–100)	(93–100)		—	(100)	(97–100)		0	—	

TABLE 2.—(*Continued*). RESULTS OF EXPERIMENT 1: CONTROLLED TEST

No. of animals	Group	Worms recovered *post mortem*										
		H. contortus				*T. colubriformis*				*O. columbianum*		
		(1) 4th	5th	A	Total	4th	5th	A	Total	4th	5th	Total
27	PTZ (4)	0	0	0	0	0	90	820	910	560	10	570
28	660 mg/K	0	0	0	0	0	0	300	300	615	0	615
29		0	5	0	5	0	1175	1570	2745	380	0	380
30		0	5	0	5	0	40	995	1035	560	0	560
31		0	0	0	0	0	1845	4015	5860	340	0	340
32		0	0	70	70	20	130	1225	1375	480	0	480
33		0	0	0	0	0	300	1245	1545	1080	0	1080
34		0	0	5	5	0	520	1080	1600	300	0	300
	Average	0	1	9	10	2	512	1406	1921	539	1	540
	Average % efficacy	100	99	99	99	—	72	83	81	0	—	0
	Range	(100)	(99.8 –100)	(93– 100)		—	(0– 100)	(50– 96)				
35	Ram. (4)	0	0	20	20	0	1880	1580	3460	1165	50	1215
36	50 mg/K	0	20	0	20	0	4180	3925	8105	2075	220	2295
37		0	10	0	10	0	2420	5310	7730	690	5	695
38		0	0	0	0	0	3370	5065	8435	1260	0	1260
39		0	0	5	5	0	425	9095	9520	1700	70	1770
40		0	0	0	0	0	2050	3365	5415	650	50	700
41		0	20	0	20	0	595	5295	5890	1975	85	2060
42		0	115	5	120	0	1980	2475	4455	930	70	1000
	Average	0	21	4	24	0	2112	4514	6626	1306	69	1374
	Average % efficacy	100	99	99	99	100	0	44	33	0	0	0
	Range	(100)	(94– 100)	(99– 100)			(0– 77)	(0– 80)				
43	Meth. (4) 237 mg/K.	0	1280	860	2140	0	1440	1090	2530	525	20	545
44		0	0	25	25	0	2880	1435	4315	615	0	615
45		50	1170	610	1830	0	850	385	1235	540	0	540
46		80	1900	1170	3150	0	170	540	710	570	0	570
47		0	2395	2535	4930	10	2660	1525	4195	1665	370	2035
48		30	1900	1390	3320	0	3090	5000	8090	1070	35	1105
49		5	1635	2240	3880	0	285	2410	2695	525	0	525
50		0	900	4135	5035	0	0	7395	7395	1400	655	2055
	Average	21	1397	1621	3039	1	1422	2472	3895	864	135	999
	Average % efficacy	96	31	35	39	—	23	70	61	0	0	0
	Range	(83– 100)	(0– 100)	(0– 99)		—	(0– 100)	(8– 95)				

(1) 4th, 5th, A = Fourth stage larvae, fifth stage and adult worms

(2) No peptic digestion or examination for third stage larvae

(3) O = <5

(4) See Materials and Methods for explanation of abbreviations.

(c) Sheep 27—34: PTZ = Phenothiazine in a liquid suspension with a particle size 25,000 cm^2/g and purity 49% w/v. Dosed at 660 mg/K.

(d) Sheep 35—42: Ram. = Rametin[3], 80% phthalophos w/w water dispersible powder dosed at 50 mg/K.

(e) Sheep 43—50: METH = Mintic[4] oral form containing 36% base dosed at 237 mg/K.

All dosages were calculated on active ingredient by independent assay.

4. Controls 1 and 2 were slaughtered on Day −1 and sheep 3—50 slaughtered on Day +9. A 100 mesh to the linear inch sieve was used and only the ingesta examined.

Results

These are summarised in Table 2. The abbreviations used for anthelmintics in this table are referred to in materials and methods. *Controls*: The function of the Day −1 Controls was to confirm the viability of the infective larvae administered during the period of infestation. The absence of third stage larvae in these animals is not surprising in view of the fact that a 100 mesh to the linear inch sieve was used which would not trap third stage larvae. Furthermore the gut wall was not digested and therefore *Oesophagostomum columbianum* in the third larval stage in this site were discarded.

The presence of fifth stage *O. columbianum* in some animals slaughtered on Day +9 cannot be accounted for. Worms from the first larval dose of *O. columbianum* (Day −15) would only have been 24 days old at this time and still in the fourth stage[6].

The individual sheep in the control group showed considerable variation in worm counts. The average count of 5,009 for *Haemonchus contortus* compares reasonably with the total number of 7,000 infective larvae administered but ranged from 2,620 to 6,420 i.e. 37 and 92% of the larvae administered respectively.

The variation in *Trichostrongylus colubriformis* count was less and from a total dose of 14,000 infective larvae an average of 9,910 worms became established. Individual burdens varied from 7,480 (53%) to 12,745 (91%).

The diarrhoea observed in the infested animals and the comparatively low number of *O. columbianum* recovered out of a total dose of 5,100 infective larvae suggested that too many infective larvae were administered. This agrees with the observations of Sarles[7] and Reinecke[5]. This diarrhoea causes expulsion of *O. columbianum*[4].

Anthelmintics:

Thiabendazole: No significant difference is apparent between the two formulations which were highly effective (99-100%) against *H. contortus* and *T. colubriformis*. No effect was noted against fourth stage larvae of *O. columbianum*.

Phenothiazine: This was highly effective against *H. contortus*, erratic against *T. colubriformis* and had no effect on the fourth stage larvae of *O. columbianum*.

Rametin: This was highly effective against *H. contortus*.

Methyridine: This was highly effective against fourth stage larvae of *H. contortus*.

No data were available, as to the anthelmintic effect of thiabendazole against *Gaigeria pachyscelis*. Accordingly a critical anthelmintic test on three artificially infested Merino ewes was carried out.

Experiment 2: Critical anthelmintic test

Materials and Methods

Three Merino sheep were infested percutaneously with 200 infective larvae of *G. pachyscelis* on Day −60. On Day O all sheep

(3) RAMETIN, registered trade mark of Farbenfabriken Bayer A.G. active ingredient O, O-diethyl-O-(naphthaloximido) phosphate.

(4) MINTIC, registered trade mark of Imperial Chemical Industries, active ingredient methyridine (2-(β-methoxyethyl) pyridine sulphate 47.9% w/v) or methoxyethyl pyridine base 36% v/v.

were weighed and dosed intra-ruminally with different preparations of thiabendazole at 100 mg/K. Sheep No. 1 received TBZ (1) = pure thiabendazole of standard particle size. Sheep No. 2 received TBZ (2) = 50% standard particles and 50% of particles 44—400μ and Sheep No. 3 TBZ (3) = particles of 44—400μ only.

Faecal collecting bags were attached after treatment and replaced daily until slaughter on Day 3. Total counts were obtained of worms passed in the faeces during this period and those remaining behind in the host.

Results

These are reflected in Table 3. Worms recovered distal to the ileum were regarded as killed[8], although Horak[9] has found that *G. pachyscelis* could occur in the colon 200 days after infestation. The interval between treatment and slaughter was minimal based on the majority of worms found in faecal bags on Day +3.

TABLE 3.—RESULT OF EXPERIMENT 2: CRITICAL ANTHELMINTIC TEST AGAINST 60 DAY OLD *G. pachyscelis*

Sheep Number	Treatment* intra-ruminally	Autopsy	Expelled				Efficacy %
		SI(*a*)	CC(*a*)	Day 1	Day 2	Day 3	
1	TBZ (1) 100 mg/K	0	0	0	25	31	100
2	TBZ (2) 100 mg/K	0	5	0	3	12	100
3	TBZ (3) 100 mg/K	8	1	0	25	17	84

* See Materials and Methods
(*a*) SI = Small Intestine
CC = Caecum and Colon

The first two forms of thiabendazole TBZ (1) and TBZ (2) were completely effective. TBZ (3) was 84% effective.

This experiment indicated the efficacy of thiabendazole against 60 day old *G. pachyscelis*.

Further experiments were carried out to assess the efficacy of this compound against various other stages of *G. pachyscelis*.

Experiment 3: *Controlled Anthelmintic Test*

Materials and Methods

1. Ten Dorper (Dorset Horn x Black Head Persian) lambs 4-6 months old were used.
2. Two hundred infective larvae of *G. pachyscelis* were placed on the skin of each on Day O.
3. Treatment with thiabendazole at 100 mg/K intraruminally and slaughter of these sheep took place as follows:
   Sheep No. 62 treated on Day +7 and slaughtered on Day +45.
   Sheep No. 70 treated on Day +17 and slaughtered on Day +45.
   Sheep No. 61 treated on Day +33 and slaughtered on Day +45.
   Sheep No. 71 treated on Day +49 and slaughtered on Day +72.
   Sheep No. 86 treated on Day +59 and slaughtered on Day +72.
4. Controls: Sheep No. 65, 89, 75 were killed on Day +45 and sheep 72 and 68 on Day +72 respectively.
5. *Post mortem*: Total macroscopic counts of *G. pachyscelis* were carried out.

Results

These are summarised in Table 4. Thiabendazole at 100 mg/K was 64%, 25%, 100%, 100% and 100% effective against 7, 17, 33, 49 and 59 day old *G. pachyscelis* respectively.

According to Ortlepp[10] seven day old worms would be in the lungs. The fourth stage reach the intestine after about 13 days and the

TABLE 4.—RESULTS OF EXPERIMENT 3: CONTROLLED ANTHELMINTIC TEST AGAINST *G. pachyscelis*

Sheep No.	Treatment	Age of worms in days		Autopsy worms recovered	Efficacy %
		at treatment	at slaughter		
65	Control	—	45	62	—
89	Control	—	45	68	—
75	Control	—	45	28	—
Average	worms recovered			53	
62	TBZ 100 mg/K	7	45	14	64
70	"	17	45	40	25
61	"	33	45	0	100
72	Control	—	72	70	—
68	Control	—	72	113	—
Average	worms recovered			91	
77	TBZ 100 mg/K	49	72	0	100
86	"	59	72	0	100

fourth moult occurs during the seventh and eighth week after infestation. (In the summary, however, Ortlepp states that the fourth moult takes place three weeks after infestation which conflicts with the text).

The next experiment tested the efficacy of Thiabendazole at 75 and 100 mg/K and Rametin at 75 mg/K against adult *G. pachyscelis* and *Chabertia ovina.*

Experiment 4: *Controlled Anthelmintic Test*

MATERIALS AND METHODS

1. Twenty Dorper lambs 4-6 months of age were used.
2. Two hundred infective larvae of *G. pachyscelis* were placed on the skin of each lamb on Day −84.
3. Mixed faecal cultures were harvested and infective larvae of *C. ovina* estimated. Larval dosage was based on *C. ovina* larvae and administered orally as follows: Day −52, Day −38 and Day −20; 110, 110 and 50 larvae respectively to each lamb.
4. Anthelmintics were administered intraruminally on Day O as follows:
   (a) Sheep 6—10: Rametin 55% w/w water dispersible powder dosed at 75 mg/K.
   (b) Sheep 11—15: Thiabendazole 68% w/w water dispersible powder dosed at 75 mg/K.
   (c) Sheep 16—20: Thiabendazole 68% w/w water dispersible powder dosed at 100 mg/K.
5. No peptic digestion of the abomasum was carried out *post mortem* on Day +3.

RESULTS

The results are tabulated in Table 5.

The faecal cultures administered on a basis of *C. ovina* larvae resulted in a mixed infestation of *H. contortus, Ostertagia* spp., *Trichostrongylus* spp., *C. ovina* and *O. venulosum.* The population of these species in the control animals were variable but all harboured reasonable worm burdens.

Sheep No. 6 and 7 died within 12 hours of treatment with Rametin and were included with the controls.

Thiabendazole at 75 and 100 mg/K was highly effective against all species present. Rametin at 75 mg/K was highly effective against *H. contortus.*

These experiments had provided additional information regarding the effect of thiabendazole against *G. pachyscelis.* The following two experiments were conducted with *Bunostomum trigonocephalum.*

TABLE 5.—EXPERIMENT 4: CONTROLLED ANTHELMINTIC TEST

Sheep No.	Treatment	*H. c.* (*a*)	*Ost.*	*Trich.*	*G. pach.*	*C. ov.*	*O.v.* 4th	*O.v.* Adult
1	Nil	5,395	130	1,370	65	38	10	74
2	"	4,700	70	1,040	32	14	20	76
3	"	1,220	290	300	55	31	70	76
4	"	3,120	250	430	43	19	0	137
5.	"	6,970	140	720	96	24	40	60
6*	"	680	70	340	85	22	20	83
7*	"	6,500	470	1,500	52	52	10	102
TOTAL		28,585	1,420	5,700	428	200	170	608
Average		4,084	203	814	61	29	24	87
8	Rametin 75 mg/K	0	0	100	34	24	30	80
9	"	0	0	90	36	28	0	0
10	"	680	250	1,090	48	3	10	0
TOTAL		680	250	1,280	118	55	40	80
Average		227	83	427	39	18	13	27
Efficacy %		94	59	47	36	38	—	—
11	Thiabendazole 75 mg/K.	0	0	10	0	0	0	0
12	"	0	0	25	0	0	0	0
13	"	0	0	0	2	0	0	0
14	"	0	0	0	3	0	0	0
15	"	0	0	0	0	0	0	0
TOTAL		0	0	35	5	0	0	0
Average		0	0	7	1	0	0	0
Efficacy %		100	100	99	98	100	100	100
16	Thiabendazole 100 mg/K.	0	0	0	0	0	0	0
17	"	0	0	0	0	0	0	0
18	"	0	0	5	0	0	0	0
19	"	0	0	0	0	0	0	0
20	"	0	0	0	1	0	0	0
TOTAL		0	0	5	1	0	0	0
Average		0	0	1	<1	0	0	0
Efficacy %		100	100	99	99	100	100	100

* Died within 12 hours after treatment with Rametin: included as controls.

(*a*) *H.c.* = *Haemonchus contortus*
Ost. = *Ostertagia spp.*
Trich. = *Trichostrongylus spp.*
G. pach. = *Gaigeria pachyscelis*
C. ov. = *Chabertia ovina*
O. v. = *Oesophagostomum venulosum*

Experiment 5: Critical Anthelmintic Test

MATERIALS AND METHODS

Three Merino wethers naturally infested with *B. trigonocephalum* were treated with thiabendazole at 50, 75 and 100 mg/K respectively. Faecal collecting bags were attached and replaced daily for four days and then the sheep were killed.

Total macroscopic worm counts were carried out on faeces and the ingesta *post mortem*.

RESULTS

The results are tabulated in Table 6.

TABLE 6.—EXPERIMENT 5: RESULTS OF CRITICAL ANTHELMINTIC TEST WITH THIABENDAZOLE.

Dosage	Worm counts of *B. trigonocephalum*						
	Expelled in Faecal Bag						
	Day 1	Day 2	Day 3	Day 4	Total	Autopsy	Efficacy %
50 mg/K	0	28	5	0	33	59	36
75 mg/K	1	22	2	0	24	1	96
100 mg/K	0	44	3	0	47	9	84

Thiabendazole was ineffective at 50 mg/K but highly effective at both 75 and 100 mg/K against *B. trigonocephalum.*

Experiment 6: *Controlled Anthelmintic Test*

MATERIALS AND METHODS

1. Twenty Dorper sheep, six months of age, were used.
2. On Day −63 all sheep were infested percutaneously with 60 infective larvae of *B. trigonocephalum* (*Strongyloides papillosus* larvae were also present), and subsequently *per os* with *O. columbianum, O. circumcincta* and *T. colubriformis.*

The experimental design is set out in Table 7.

TABLE 7.—EXPERIMENT 6: EXPERIMENTAL DESIGN

DAY	No. of infective larvae dosed to each sheep			
	B. trigonocephalum	*O. columbianum*	*O. circumcincta*	*T. colubriformis*
−63	60	—	—	—
−50	—	100	—	—
−46	—	100	—	—
−43	—	100	—	—
−40	—	100	—	—
−34	—	100	—	—
−29	—	100	500	500
−25	—	100	400	500
−22	—	100	460	500
−18	—	100	480	500
−15	—	100	125	500
−11	—	100	170	500
−8	—	100	620	500
−4	—	100	460	500
Total	60	1,300	3,215	4,000

3. Anthelmintics were administered intraruminally on Day O as follows:
   Group A, five sheep: Undosed controls.
   Group B, five sheep: Thiabendazole 68% w/w water dispersible powder at 50 mg/K.
   Group C, five sheep: Rametin 55% w/w water dispersible powder at 75 mg/K.
   Group D, five sheep: Phenothiazine, 90% purity, (9,000 cm^2/g by air permeability) at 606 mg/K.
4. Twenty sheep were slaughtered on Day +7.

Results are summarised in Table 8.

The larval doses and interval before slaughter provided fourth, early fifth and adult stages of *O. circumcincta, T. colubriformis* and *O. columbianum* but only adult *B. trigonocephalum* and *S. papillosus* in the controls. There were, however, only small numbers of fourth stage larvae of *O. circumcincta* and *T. colubriformis* and adult *B. trigonocephalum.*

Thiabendazole at 50 mg/K proved highly effective against all stages of worms present at treatment. The efficacy against adult *B. trigonocephalum* averaged 87%, ranging from 73—100%.

Rametin at 75 mg/K was generally highly effective against fifth stage and adult *O. circumcincta,* fifth stage and adult *T. colubriformis* and adult *B. trigonocephalum.*

Phenothiazine at 606 mg/K was highly effective against adult *O. circumcincta, B. trigonocephalum* and fifth stage and adult *O. columbianum.*

DISCUSSION

Intra-ruminal administration

If the anthelmintic administration *per os* is to be assessed on the assumption that the

TABLE 8.—EXPERIMENT 6: CONTROLLED ANTHELMINTIC TEST

Group and Treatment	Worms recovered *post mortem*													
	O. circumcincta				(a) *S.p.*	*T. colubriformis*				(a) *B. trig.*	*O. columbianum*			
	4th xx	5th	A	Total		4th	5th	A	Total		4th	5th	A	Total
Group A Controls	70	710	1530	2310	290	10	650	2175	2835	12	125	99	181	405
	210	1750	1000	2960	380	0	670	2653	3323	17	225	210	130	565
	90	880	780	1750	250	10	990	2065	3065	17	45	180	170	395
	145	600	1550	2295	400	0	800	2325	3125	19	180	282	97	559
	95	1150	1125	2370	90	30	930	1550	2510	11	70	60	230	360
TOTAL	610	5090	5985	11685	1410	50	4040	10768	14858	76	645	831	808	2284
Mean	122	1018	1197	2337	282	10	808	2154	2972	15	129	166	162	457
Group B Thiabendazole 50 mg/K	0	0	0	0	0	0	0	0	0	1	5	0	0	5
	20	0	0	20	0	0	0	0	0	4	25	0	0	25
	0	0	0	0	0	0	0	0	0	0	10	0	0	10
	0	0	0	0	0	0	0	0	0	1	15	0	0	15
	0	0	0	0	0	0	0	0	0	3	10	0	0	10
TOTAL	20	0	0	20	0	0	0	0	0	9	65	0	0	65
Mean	4	0	0	4	0	0	0	0	0	2	13	0	0	13
Efficacy %	97	100	100	99	100	100	100	100	100	87	90	100	100	97
Group C Rametin 75mg/K	40	125	140	305	30	0	0	160	160	1	135	84	4	223
	0	280	190	470	130	0	0	110	110	0	70	84	153	307
	0	40	80	120	130	0	0	20	20	0	150	200	53	403
	20	0	50	70	70	10	180	60	250	0	230	90	257	577
	70	160	0	230	480	0	90	95	185	0	145	142	45	332
TOTAL	130	605	460	1195	840	10	270	445	725	1	730	600	512	1842
Mean	26	121	92	239	168	2	54	89	145	1	146	120	102	368
Efficacy %	79	88	92	90	40	—	93	96	95*	99	0	28	37	20
Group D Phenothiazine 606 mg./K	125	340	20	485	480	0	90	715	805	0	175	8	0	183
	200	450	60	710	320	0	90	100	190	1	130	6	1	137
	10	400	110	520	160	0	620	1265	1885	1	155	10	10	175
	20	50	10	80	180	50	450	210	710	1	98	0	10	108
	90	370	30	490	390	0	90	140	230	0	220	0	1	221
TOTAL	445	1610	230	2285	1530	50	1340	2430	3820	3	778	24	22	824
Mean	89	322	46	457	306	10	268	486	764	1	156	5	4	165
Efficacy %	27	68	96	81	0	—	67	77	75	96	0	97	97	64

* Excluding fourth stage larvae of T. *colubriformis*

Abbreviations:
(a) S.p. = *Strongyloides papillosus* (a) B. trig. = *B. trigonocephalum*
xx4th = Fourth stage larvae
5th = Fifth stage worms
A = Adult worms

drug enters the rumen, it is essential that initial trials should be carried out by administrating the drug into the rumen either by intra-ruminal puncture or other appropriate method.

It is known that many liquids pass directly into the abomasum after oral administration particularly in lambs. This may in part account for conflicting results obtained by various workers after oral administration.

In addition intra-ruminal administration ensures that the individual sheep receives the complete dose with no possible wastage.

Anthelmintic variations

Gordon[2] quoting earlier workers[11 12 13 14] stated that watery solutions may be swallowed into either the rumen or abomasum in about equal proportions of sheep.

The erratic action of the oesophageal groove reflex is a cause of anthelmintic failure in a proportion of sheep. In addition variations in response occur due to the age of the animal. Erratic results were obtained with Neguvon and Mintic administered intra-ruminally[2].

Stampa[15] also described erratic action of Neguvon plus Asuntol following premedication with copper sulphate. This may be ascribed to either failure of the oesophageal groove reflex, or to the variable metabolism of organic phosphate compounds in individual sheep.

Prestimulation with copper sulphate

Gordon and Whitten[10] found great variations in response to copper sulphate and observed that certain sheep were repeatedly refractory.

Modern anthelmintics e.g. methyridine, Rametin and thiabendazole are advocated for use without prior stimulation of the oesophageal groove reflex with copper sulphate.

Intra-ruminal administration of thiabendazole and two formulations of phenothiazine gave results comparable to accepted efficacies. The results of methyridine intra-ruminally, however, were generally poorer than those obtained by oral or parenteral use[17 2 18].

According to Harrow[19] and Broome[20] the efficacy of methyridine against abomasal parasites is influenced by the pH of the abomasal contents. The results of the present experiments and those of Gordon[2 21] indicate that the efficacy may also be influenced by the method of administration or the route taken by the drug following oral administration.

The results obtained by Federmann[22] with Rametin *per os* were better than those observed in these tests following intra-ruminal administration, and the efficacy of this drug may also depend to some degree on its site of lodgement in the animal.

Anthelmintic tests

The critical test[23] and the controlled test[24 8 25] have been used in the experiments described above.

The critical test proved satisfactory for fifth and adult stages of *G. pachyscelis*, as very few worms expelled with the faeces after treatment were digested. However, in Experiment 6 (Table 8) higher efficacy of thiabendazole at 50 mg/K against *B. trigonocephalum* was recorded in this controlled test than in one critical test (Experiment 5; Table 5). The controlled test described by Gibson[25] should be used for the third and fourth stages of *G. pachyscelis*, since sheep cannot be repeatedly infested[5].

The deficiencies of critical tests for anthelmintic evaluation for all stages of *H. contortus* and *O. circumcincta*, as well as all immature stages have been demonstrated[8 4 18]. Controlled tests are the method of choice for abomasal and immature nematodes.

Larval dosages

Larval dosages must be adapted to the virulence and nature of the parasite in question e.g. *G. pachyscelis* and *O. columbianum*. Small numbers of worms, however, are difficult to count and necessitate total counts.

Other considerations which should be borne in mind regarding the size of larval dose and resultant worm population, are the observations by Douglas[20]. This author states that in the case of relatively insoluble drugs the surface area of the worms exposed to the drug has a bearing on the efficacy.

The large numbers of larvae can cause mortality or severe morbidity before completion of an experiment. In Experiment 1 the larval dosage of *O. columbianum* had to be decreased as a result of severe scouring. In addition the large larval doses of *O. columbianum* resulted in a poor establishment of worms (4,540 out of 40,800) confirming the observation of Sarles[7] that the clinical symptoms of

oesophagostomiasis is in direct relation to the number of larvae administered while the worm count is an inverse ratio. The increased peristalsis associated with scouring causes a loss of worms as recorded by Reinecke *et al*[4].

In this same experiment it should be noted that in some instances treated animals harboured more worms than the controls. This may indicate either variation in establishment of worms or probably that treatment removed certain stages only and allowed a higher proportion of young forms to develop naturally. Horak[9] has observed that selective treatment may lead to an improved establishment of worm populations.

In Experiment 6 only 1,300 infective larvae of *O. columbianum* were given to each sheep yet a comparatively constant number of worms was recovered. The total recovery from the controls was 35% of the larvae administered.

In the case of *T. colubriformis*, however, a total of 112,000 larvae to the control sheep resulted in variable worm burdens in Experiment 1 but 71% recovery against 74% out of only 20,000 in Experiment 6. This is even more striking in view of the fact that the Dorper is more susceptible to infection with *T. colubriformis* than the Merino[9]. With this species larval dose is not so critical as with *O. columbianum*.

Infestation with pure strains of a single species is essential if uniform worm burdens are desired. This is well illustrated in Experiment 4 where mixed cultures were used and mixed infestations with marked variations occured. In addition identifications of larval stages and adult worms *post mortem* is facilitated and the possibility of cross reactions between various genera or species as observed by Reinecke *et al*[8], Reinecke[5] and other workers is controlled. This interaction is not only confined to worms occurring in the same organ but extends to other organs as well. Stewart[27] observed that the "self cure" phenomenon of *H. contortus* also effected expulsion of concurrent infestations of *T. colubriformis*.

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THE VALUE OF UNIFORM WORM BURDENS IN THE LARVAL ANTHELMINTIC TEST

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SUMMARY

Simultaneous infestations of worm-free lambs with different species of worms resulted in uniform burdens of *T. colubriformis* and *G. pachyscelis*, regardless of the presence of other species. *Haemonchus contortus* could be established in uniform numbers in the presence of *T. colubriformis*, *G. pachyscelis* and *O. columbianum*, but was adversely affected by *O. circumcincta*. Although *O. columbianum* was recovered in large numbers, worm burdens varied markedly; *N. spathiger* was either absent or present in small numbers, in mixed infestations.

Reinfestation with *G. pachyscelis* of previously infested hosts was unsuccessful within 6 days of initial infestation. Infested adult sheep were resistant to challenge with *T. colubriformis*.

The most effective anthelmintic was phenothiazine with a particle size of 32,200 cm^2/g dosed at 600 mg/K; followed by Rametin at 75 mg/K and Tetrachloroethylene at 0.3 ml/K. Phenothiazine with a particle size of 10,500 cm^2/g or less, at 500 mg/K was only effective against *H. contortus* and *O. columbianum*.

INTRODUCTION

Anthelmintics effective against a wide range of parasites in all stages of development, have been synthesised. Since standard anthelmintic tests have proved inadequate for evaluating the efficacy of anthelmintics against immature worms, the larval anthelmintic test was developed (Reinecke[1]).

As Reinecke[1] has pointed out, in the larval anthelmintic test uniform worm burdens are essential. In this paper further experiments to test the feasibility of establishing concurrent infestations with different species in susceptible hosts are carried out.

Experiment 1: Attempts to establish six species concurrently in uniform numbers in susceptible hosts

Materials and Methods

1. Eighteen weaned Dorper (Dorset Horn X Black Head Persian) lambs, born, reared and maintained worm-free, were used. These lambs were infested percutaneously with infective larvae of *Gaigeria pachyscelis*, and orally with *Oesophagostomum columbianum*, *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Ostertagia circumcincta* and *Nematodirus spathiger*, using the methods of Reinecke, Horak and Snijders[2].

2. The experimental design is summarized in Table 1.

3. The anthelmintics dosed were:

Tetrachloroethylene 99 per cent with 1 per cent Triton X100 as emulsifying agent, mixed with water in the proportion of one part tetrachloroethylene to two parts water.

PTZ A, a phenothiazine powder with particle size of 32,200 cm^2/g and purity which varies from 94.7 to 95.4 per cent w/w.

Rametin (also known as Maretin) an organic phosphate O, O-diethyl-O-napthal-oximido-phosphate, 60.92 per cent w/w.

PTZ B, a mixture of phenothiazine, particle size 10,500 cm^2/g and purity between 37.5 to 38.8 w/v, and hexachlorophene 1.03 per cent, w/v.

4. *Post-mortem* procedures used are described[1,2].

5. Larval stages were identified microscopically according to: Veglia,[3,4]; Ortlepp[5]; Kates & Turner[6]; Douvres[7,8], but classified in their various stages according to Reinecke[9].

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TABLE 1.—EXPERIMENTAL DESIGN.

Day	No. of infective larvae dosed					
	G. pachyscelis	*O. columbianum*	*H. contortus*	*T. colubriformis*	*O. circumcincta*	*N. spathiger*
—48	100					
—42	100	190				
—38		200				
—35		200				
—31		216				
—28		177				
—24		203	506	454	570	484
—21		203	506	454	327	484
—17		171	474	500	447	316
—14		171	474	500	447	316
—12	216					
—11		228	570	532	475	418
— 9		194	570	532	475	418
— 7		200	400	456	530	475
— 5		200	514	522	456	456
— 1		200	514	522	456	456
TOTAL	416	2,953	4,928	4,862	4,683	4,298
0	Day 0 Control. Sheep 1 killed. Sheep 5—18 dosed as follows: Tetrachloroethylene 0.66 ml/K intra-abomasal. Sheep 5 Tetrachloroethylene 0.33 ml/K intra-abomasal. Sheep 6 Tetrachloroethylene 0.33 ml/K *per os*, preceded by 10 ml, 10% CuSO solution. Sheep 7 & 8 PTZ A phenothiazine 600 mg/K *per os*. Sheep 9, 10 & 11 Rametin 50 mg/K intra-rumenal. Sheep 12, 13 & 14 PTZ B 300 mg/K phenothiazine and 10.3 mg/K hexachlorophene intra-rumenal. Sheep 15 & 16 PTZ B 300 mg/K phenothiazine and 10.3 mg/K hexachlorophene *per os*. Sheep 17 & 18					
+7	Day + 7 Controls. Sheep 2, 3 & 4 killed. Treated on Day 0. Sheep 5 to 18 inclusive killed.					

RESULTS

These are summarized in Tables 2 and 3. Unfortunately the caecal and colonic ingesta of 11 of the sheep were discarded accidentally before they were examined. Hence the *Oesophagostomum columbianum* results summarized in Table 2 do not include worms that may have been present in the lumen of the caecum and colon. In seven of the sheep where the ingesta of these organs was available, the number of *O. columbianum* recovered from each sheep is summarized in Table 3.

Worm burdens

Trichostrongylus colubriformis

Uniform worm burdens were achieved in the Day +7 Controls.

Haemonchus contortus and *Ostertagia circumcincta*

The number of worms recovered from Sheep 4 was considerably less than either Sheep 2 or 3 (Day +7 Controls). This deficit was most marked in the fifth and adult stages particularly of *H. contortus*.

Gaigeria pachyscelis

Uniform worm burdens were noted in the controls (Sheep 1 to 4) and in those sheep where the anthelmintics were not effective (Sheep 10, 15, 16 & 18). The worms were all of similar size in the fifth stage.

Oesophagostomum columbianum

Marked variations were noted in the number of worms recovered all in the fourth stage except for some third and fifth stage in the Day O Control, and a few fifth stage in Sheep 16.

Nematodirus spathiger

Few worms in the fourth, fifth and adult stage, i.e. one to 40 were noted in eleven sheep, and none were recovered from the other seven sheep (not tabulated).

TABLE 2.—WORMS RECOVERED *Post-mortem*. WORMS IN CAECAL AND COLONIC INGESTA NOT INCLUDED (SEE TEXT).

Species		*H. contortus*				*O. circumcincta*				*T. colubriformis*				*G. pachysce lis*	*O. columbianum*
Group	Sheep No.	Stage of development			Total	Stage of development			Total	Stage of development			Total	5	Stage of development
		@4	@5	@A		4	5	A		4	5	A			4
Control Day 0.....	1	(60)737	218	703	1,718	(28)892	322	837	2,079	(39)506	189	222	956	31	(132) 1,409
Contro. Day +7.....	2	556	951	1,600	3,107	573	935	1,779	3,287	0	943	1,053	1,996	39	719
Control Day +7.....	3	215	751	2,251	3,217	307	641	1,411	2,359	0	1,188	901	2,089	28	627
Control Day +7.....	4	506	96	24	626	581	439	772	1,792	0	1,269	809	2,078	27	554
C_2Cl_4 0.66ml/K.....	5	0	0	5	5	45	125	55	225	0	255	255	510	22	195
C_2Cl_4 0.33 ml/K.....	6	75	30	5	110	275	565	305	1,145	0	225	90	315	0	650
C_2Cl_4 0.33ml/K.....	7	25	10	0	35	145	340	375	860	0	290	245	535	11	1,425
C_2Cl_4 0.33 ml/K.....	8	5	0	0	5	65	245	165	475	0	420	215	635	9	555
Average Reduction...		93.9%	98.3%	99.8%	99.3%	72.9%	52.5%	83.0%	72.7%	—	73.7%	78.2%	75.7%	67.7%	0.0%
P.T.Z. A 600 mg/K..	9	52	0	0	52	263	49	23	335	0	60	40	100	0	455
P.T.Z. A 600 mg/K..	10	570	50	5	625	215	245	95	555	0	5	25	30	40	580
P.T.Z. A 600 mg/K..	11	85	0	0	85	170	30	10	210	0	135	125	260	8	595
Average Reduction...		68.3%	97.2%	99.9%	89.0%	55.6%	83.9%	96.7%	85.2%	—	94.1%	93.2%	93.7%	48.4%	14.2%
Rametin 50 mg/K....	12	133	3	0	136	160	115	185	460	0	368	233	601	17	264
Rametin 50 mg/K....	13	48	0	0	48	497	135	125	757	0	410	159	569	20	440
Rametin 50 mg/K....	14	157	0	0	157	528	110	91	729	0	363	1,240	1,603	13	375
Average Reduction...		73.5%	99.8%	100.0%	95.1%	18.9%	82.1%	89.9%	73.8%	—	66.5%	40.9%	55.0%	45.2%	43.1%
P.T.Z. B 300 mg/K...	15	625	86	0	711	312	473	683	1,468	0	760	697	1,457	26	645
P.T.Z. B 300 mg/K...	16	542	239	308	1,089	381	850	507	1,738	0	300	653	953	28	635
P.T.Z. B 300 mg/K...	17	277	185	55	517	79	535	683	1,297	0	665	782	1,447	15	660
P.T.Z. B 300 mg/K...	18	305	30	0	335	122	457	534	1,113	0	567	915	1,482	31	940
Average Reduction...		0.0%	77.7%	92.9%	71.4%	54.2%	13.7%	54.4%	43.4%	—	49.4%	17.3%	35.0%	19.4%	0.0%

( )-third stage larvae @ 4 — fourth stage larvae 5 —fifth stage A — adults

TABLE 3.—*Oesophagostomum columbianum*-RECOVERED *Post mortem*.
WORMS IN CAECAL AND COLONIC INGESTA INCLUDED (SEE TEXT).

Group	Sheep No.	Stage of Development				Total
		3	4	5	A	
Control Day 0.....	1	132	1,486	138	167	1,923
Control Day +7.....	2	0	1,432	788	436	2,656
Control Day +7.....	4	0	686	34	112	832
C_2 Cl_4 0.33 ml/K.....	6	0	993	470	167	1,630
Reduction.....		—	6.2%	0.0%	30.1%	6.5%
P.T.Z. A 600 mg/K.....	9	0	635	102	5	742
P.T.Z. A 600 mg/K.....	10	0	840	113	4	957
Average Reduction.....		—	30.3%	73.7%	98.5%	51.3%
Rametin 50 mg/K.....	13	0	457	4	15	476
Reduction.....		—	56.8%	99.0%	94.5%	72.7%
PTZ B 300 mg/K.....	Caecal and colonic ingesta not examined.					

Strongyloides papillosus

One sheep (2) had 23 adult worms.

Anthelmintics

Tetrachloroethylene is the most effective drug against *H. contortus* and *G. pachyscelis*; PTZ A against *O circumcincta* and *T. colubriformis*; and Rametin against *O. columbianum*.

TABLE 4.—RESULTS OF CRITICAL ANTHELMINTIC TESTS IN TWO SHEEP INFESTED WITH *O. columbianum*, TREATED WITH PTZ B AT 300 mg/K, AND KILLED 68 HOURS AFTER TREATMENT

Sheep No.		Worms recovered	
		Stage of development	
		4	A
19	Autopsy	380	313
	From faecal bags	0	12
	Reduction	0.0%	3.7%
20	Autopsy	127	117
	From faecal bags	0	1
	Reduction	0.0%	0.8%

No data were available as to the anthelmintic efficacy of PTZ B at 300 mg/K against adult *O. columbianum*. Accordingly critical anthelmintic tests were performed on two sheep, which indicated that few adult worms (0.8—3.4%) were expelled as a result of the treatment (Table 4).

Experiment 2

As attempts to produce uniform worm burdens with six species simultaneously were unsuccessful, possibly due to the inclusion of too many species, a further experiment with only four species was carried out.

Materials and Methods

1. Sixteen weaned Dorper lambs, born, reared and maintained worm-free, were infested percutaneously with infective larvae of *G. pachyscelis* and orally with *O. columbianum*, *H. contortus* and *T. colubriformis*.

2. The experimental design is summarized in Table 5.

3. The anthelmintics dosed were:

PTZ B, a mixture of phenothiazine, particle

TABLE 5.—EXPERIMENTAL DESIGN.

Day	No. of infective larvae dosed.			
	G. pachyscelis	*O. columbianum*	*H. contortus*	*T. colubriformis*
—41	510	381		
—35		152		
—32		200		
—29		182		
—25		183		
—21		224	1,000	975
—18		200	500	500
—14		192	500	500
—11		180	512	525
— 8		182	544	485
— 6		182	544	485
— 4		205	492	500
— 2		205	492	500
— 1		220	429	500
TOTAL	510	2,888	5,013	4,970
0	Day 0 Control. Sheep 21 killed. Sheep 25 to 36 dosed as follows: PTZ B 500 mg/K phenothiazine and 17.6 mg/K hexachlorophene *per os*, Sheep 25, 26 & 27. PTZ C 500 mg/K phenothiazine *per os*. Sheep 28, 29 & 30. RD 3412 100 mg/K *per os*. Sheep 31, 32 & 33. Rametin 75 mg/K *per os*. Sheep 34, 35 & 36.			
+7	Day +7 Controls. Sheep 22, 23, & 24 killed. Treated on Day 0. Sheep 25 to 36 inclusive killed.			

size 10,500 cm^2/g and purity between 37.5 to 38.8 w/v and hexachlorophene 1.03%, w/v.

PTZ C. Phenothiazine in a liquid suspension, particle size 7,600 cm^2/g and purity which is constant at 36.5%w/v.

RD 3412 an experimental compound.

Rametin an organic phosphate O, O-dimethyl-O-napthal-oximido-phosphate 60.92 per cent w/w.

Results

The results are summarized in Table 6.

Worm burdens

These were remarkably uniform for *H. contortus* in the Day +7 controls. Furthermore the number of *T. colubriformis* and *G. pachyscelis* was uniform, not only in the controls, but also in those groups unaffected by the two anthelmintics PTZ B and RD 3412.

The worm burdens of *O. columbianum*, however, varied markedly.

Anthelmintics

Rametin at 75 mg/K was the most effective anthelmintic against *H. contortus*, *T. colubriformis* and *G. pachyscelis*. Both brands of phenothiazine at 500 mg/K were highly effective against fifth stage and adult *O. columbianum* and less effective against *H. contortus*. RD 3412 was highly effective against fifth stage and adult *H. contortus*.

Comment

This experiment showed that the worm burdens of *H. contortus* unlike Experiment 1, were remarkably uniform in the Eontrols. This favourable result may be due to the absence of *O. circumcincta*. Conversely, *T. colubriformis* was established in uniform numbers, regardless of the presence of other species.

Experiment 3

This experiment was set up, infesting sheep with *H. contortus*, *O. circumcincta* and *T. colubriformis*, to ascertain whether there were any cross reactions between these three species.

TABLE 6.—WORMS RECOVERED *Post-mortem*.

Species		*H. contortus*				*T. colubriformis*				*G. pachycelis*	*O. columbianum*			
Group	Sheep No.	Stage of development			Total	Stage of development			Total	Stage	Stage of development			Total
		4	5	A		4	5	A		5	4	5	A	
Control Day 0.....	21	693	559	674	1,926	(24)359	314	389	1,086	131	(15) 282	130	162	589
Control Day +7....	22	889	675	1,134	2,698	0	669	856	1,525	130	322	20	182	524
Control Day +7....	23	849	630	1,261	2,740	0	836	834	1,770	154	250	55	175	480
Control Day +7....	24	380	1,175	1,187	2,742	0	698	736	1,434	212	240	315	377	932
PTZ B 500 mg/K...	25	600	35	10	645	0	504	696	1,200	173	320	0	0	320
PTZ B 500 mg/K...	26	433	327	0	760	0	576	927	1,503	109	470	0	1	471
PTZ B 500 mg/K...	27	482	166	146	794	0	1,530	980	2,510	223	560	0	0	560
Average reduction..		28.5%	78.7%	95.6%	73.1%	—	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%	99.5%	30.2%
PTZ C 500 mg/K...	28	866	505	119	1,490	0	362	823	1,185	24	750	0	2	752
PTZ C 500 mg/K...	29	65	0	5	70	0	244	592	836	104	327	11	23	361
PTZ C 500 mg/K...	30	659	177	9	845	0	573	862	1,435	171	700	0	2	702
Average reduction..		24.9%	72.5%	96.3%	70.6%	—	46.5%	6.2%	25.3%	39.4%	0.0%	96.9%	96.3%	6.2%
RD 3412 100 mg/K.	31	305	0	0	305	0	762	1,258	2,020	140	323	17	88	428
RD 3412 100 mg/K.	32	650	40	0	690	0	783	840	1,623	136	570	80	150	800
RD 3412 100 mg/K.	33	670	10	0	680	0	851	1,324	2,175	144	265	26	156	447
Average reduction..		23.3%	97.9%	100.0%	79.5%	—	0.0%	0.0%	0.0%	15.2%	0.0%	68.5%	46.5%	13.5%
Rametin 75 mg/K..	34	60	0	0	60	0	228	0	228	56	382	22	45	449
Rametin 75 mg/K..	35	100	20	0	120	0	611	185	796	140	488	130	212	830
Rametin 75 mg/K..	36	130	20	0	150	0	455	15	470	77	283	2	0	285
Average reduction..		86.5%	98.4%	100.0%	96.3%	—	41.3%	91.7%	67.7%	44.8%	0.0%	60.8%	64.9%	19.2%

( ) Third Stage larvae.

Materials and Methods

1. Ten weaned Dorper lambs born, reared and maintained worm-free were infested orally with infective larvae of *H. contortus*, *O. circumcincta* amd *T. colubriformis*.

2. The experimental design is summarized in

3. Anthelmintics.

SKF 90,590, an experimental compound.

TABLE 7.—EXPERIMENTAL DESIGN.

Day	No. of infective larvae dosed to each sheep		
	H. contortus	*O. circumcincta*	*T. colubriformis*
—25	500	300	1,000
—20	200	300	1,000
—17	218	500	1,000
—13	200	470	1,044
—10	198	1,000	1,000
— 7	207	1,000	1,000
— 5	205	795	1,000
— 3	201	1,000	1,000
— 2	200	1,080	1,120
TOTAL	2,129	6,445	9,164
0	Day 0 Control. Sheep 37 killed. Sheep 41—49 dosed as follows: S.K.F. 90,590 200 mg/K *per os.* Sheep 41, 42 & 43 Methyridine (Mintic) 200 mg/K *per os.* Sheep 44, 45 & 46 CCl_4 50% in peanut oil 0.2 ml/K sub. cut. Sheep 47, 48 & 49		
+3	Faecal worm-egg counts. Sheep — Eggs per gram of faeces 38 — 5,600 39 — 4,600 40 — 2,400 41 — 7,000 42 — 10,600 43 — 6,200 44 — 3,400 45 — 0 46 — 2,200 47 — 2,400 48 — 1,600 49 — 3,600		
+4	Day +4 Control Sheep 38 killed Treated on Day 0. Day +3, 0 e.p.g. Sheep 45 killed		
+7	Day +7 Controls Sheep 39 & 40 killed Treated on Day 0. Sheep 41—44 & 46—49 inclusive killed.		

Methyridine (Mintic) i.e. 2-(beta-methoxyethyl) pyridine sulphate 47.9 per cent w/v in liquid solution.

Carbon tetrachloride 50 per cent in peanut oil.

4. On Day +3 faecal worm egg counts revealed that Sheep 45 was negative. Accordingly this sheep and one control (Sheep 38) were killed on Day +4 and the remainder were slaughtered on Day +7 (Table 7).

RESULTS

Worm burdens

These were uniform for all species, as shown by the Day 0 and Day +4 Controls. Marked variations particularly of *H. contortus*, and to a lesser extent of *O. circumcincta* were noted in the Day +7 Controls.

Anthelmintics

Only in one sheep (45) was methyridine highly effective against all species; in the other two sheep it was highly effective against *T. colubriformis*, had little effect against *O. circumcincta* and no effect on *H. contortus*.

The group treated with S.K.F. 90,590 showed a 42.9 per cent and 10.8 per cent reduction in fifth stage *H. contortus* and adult *T. colubriformis* respectively. Sheep treated with carbon tetrachloride showed a reduction of 36.9 per cent and 13.0 per cent in fifth stage and adult *T. colubriformis* respectively. In view of the marked variations in the worm burdens of the Day +7

TABLE 8.—WORMS RECOVERED *Post-mortem*

Group	Sheep No.	*H. contortus*				*O. circumcincta*				*T. colubriformis*			
		Stage of development			Total	Stage of development			Total	Stage of development			Total
		4	5	A		4	5	A		4	5	A	
Day 0 Control.....	37	(204)244	200	532	1,180	(204)1,406	404	683	2,697	(127)678	1,823	1,612	4,240
Day +4 Control.....	38	461	184	339	984	615	645	762	2,022	288	1,294	3,305	4,487
Methyridine 200 mg/K	45	0	11	0	11	66	55	235	356	0	0	0	0
Reduction.....		100.0%	94.0%	100.0%	98.9%	89.3%	91.5%	69.2%	82.4%	100.0%	100.0%	100.0%	100.0%
Day +7 Control.	39	237	603	958	1,798	1,048	1,087	2,024	4,159	0	1,062	4,111	5,173
Day +7 Control. ...	40	13	0	0	13	719	62	434	1,215	0	1,324	2,958	4,282
S.K.F. 90590 200 mg/K	41	168	45	759	972	1,857	312	669	2,838	0	1,251	3,224	4,475
S.K.F. 90590 200 mg/K	42	153	307	571	1,031	1,278	1,026	2,053	4,357	0	1,884	2,685	4,569
S.K.F. 90590 200 mg/K	43	329	164	953	1,446	987	493	1,233	2,713	0	1,333	3,553	4,886
Average Reduction....		0.0%	42.9%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	—	0.0%	10.8%	1.8%
Methyridine 200 mg/K	44	86	436	873	1,395	147	552	820	1,519	0	403	140	543
Methyridine 200 mg/K	46	234	491	229	954	360	949	992	2,301	0	45	33	78
Average Reduction....		0.0%	0.0%	0.0%	0.0%	71.3%	0.0%	26.3%	28.9%	—	81.2%	97.5%	93.4%
CCl_4 50% 0.2 ml/K...	47	266	190	445	901	1,322	858	1,839	4,019	0	415	3,740	4,155
CCl_4 50% 0.2 ml/K...	48	324	313	660	1,297	796	676	1,032	2,504	0	830	2,827	3,657
CCl_4 50% 0.2 ml/K...	49	90	116	1,318	1,524	833	634	2,635	4,102	0	1,014	2,661	3,675
Average Reduction....		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	—	36.9%	13.0%	19.0%

( ) — third stage larvae

controls, and the fact that individual sheep treated either with SKF 90,590 or carbon tetrachloride had worm burdens that fell within the range of the controls, these results should be treated with reserve.

Comment

It appears that in attempts to establish mixed infestations of *H. contortus* and *O. circumcincta*, one of the Day +7 Controls (Sheep 40) showed marked incompatability between these species, *H. contortus* particularly, being markedly reduced. The other Day +7 Control (Sheep 39) seemed highly susceptible to both species, the total worm burdens of both being higher than either the Day 0 (Sheep 37) or Day +4 Control (Sheep 38). This tendency was reflected in *T. colubriformis* with one Day +7 Control only (Sheep 39) which had 5,173 worms, while the other controls (Sheep 37, 38 & 40) were uniform varying only from 4,240 to 4,487 worms.

Experiment 4

It has been claimed that susceptible worm-free hosts are essential to establish uniform worm burdens experimentally[2]. Experiment 4 is set up to test this claim.

Materials and Methods

1. Ten infested adult Merinos, were each dosed with 16,675 and 37,300 infective larvae of *T. colubriformis* on Day —28 and Day —22 respectively.

2. Anthelmintics dosed on Day 0 were:

Three sheep (54, 55 & 56) were dosed with PTZ D at 500 mg/K *per os*. This phenothiazine powder has a particle size 9,300 cm^2/g and purity which varies from 90.0 to 90.1 per cent w/w.

Three sheep (57, 58 & 59) were dosed with PTZ E at 500 mg/K *per os*. This phenothiazine suspension's particle size is unknown but the purity is 46.0% w/v.

3. All sheep were slaughtered on Day +6 and only the ingesta of the abomasum and first seven metres of small intestine examined for worms (Reinecke, Snijders & Horak,[10]).

RESULTS

These are summarized in Table 9. The marked variations in worm burdens indicated that infested hosts were unsuitable experimental animals. Both brands of phenothiaiznie were ineffective. The reduction of fifth stage worms may lead to false conclusions as to the efficacy of phenothiazine — if due consideration is not taken of the fact that the burdens of four sheep (54, 55, 57 & 58) fell within the lower range of the control sheep.

TABLE 9.—T. *colubriformis* RECOVERED *post-mortem.*

Group	Sheep No.	Stage of Development		Total
		5	A	
Control Day +6.....	50	915	4,930	5,845
Control Day +6.....	51	488	4,956	5,444
Control Day +6.....	52	132	2,067	2,199
Control Day +6.....	53	565	8,903	9,468
P.T.Z. D 500 mg/K.....	54	148	14,716	14,864
P.T.Z. D 500 mg/K.....	55	171	8,478	8,649
P.T.Z. D 500 mg/K.....	56	52	1,472	1,524
Average reduction.....		76.4%	0.0%	0.0%
P.T.Z. E 500 mg/K.....	57	133	2,995	3,128
P.T.Z. E 500 mg/K.....	58	164	5,637	5,801
P.T.Z. E 500 mg/K.....	59	105	10,687	10,792
Average reduction.....		74.5%	0.0%	0.0%

DISCUSSION

The attainment of uniform worm burdens

Attempts to infest worm-free lambs, with different species to achieve uniform mixed worm burdens varied with the species.

Trichostrongylus colubriformis

Concurrent infestations of other species did not affect the establishment of *T. colubriformis* in susceptible lambs. This species was recovered consistently in uniform numbers from the controls and from treated sheep unaffected by the anthelmintics, regardless of the presence of other species.

It must be emphasized that fully susceptible worm-free hosts were essential to achieve this objective. Experiment 4 clearly showed that a massive challenge of infested sheep was unsuccessful. Not only did the worm burdens vary enormously, but some of the worms were retarded in the fifth stage beyond the prepatent period, and many adult females had only one or two eggs in their uteri. These effects on the worms are accepted as evidence of resistance (Stewart & Gordon,[11]; Urquhart, Jarrett & Mulligan,[12]).

Gaigeria pachyscelis

Ortlepp[5] described the life cycle in which the infective stage enters the host percutaneously. The third moult takes place in the lungs at 7 days, and fourth stage larvae enter the intestine 14 days after infestation. The fourth moult is completed by the seventh or eighth week.

In Experiment 1 infective larvae were placed on the skin on Day —48, Day —42 and Day —12 respectively. Only worms in the fifth stage were recovered, (Table 2). It appeared that only the first larval dose had been responsible for the resultant worm burden.

Fourth stage larvae should have been present in the small intestine in the controls. Thus on Day 0, worms 42 days old, and on Day +7, worms 19 days old, if they took would still be in the fourth stage in the small intestine. Their absence indicates that the hosts were resistant to the second and third larval dose.

As a result of the failure to reinfest the same host even within six days of initial infestation, only a single dose of infective larvae was subsequently used (Experiment 2). This resulted in uniform worm burdens in the same stage of development, confirming results previously obtained[1].

Haemonchus contortus and *Ostertagia circumcincta*

Attempts to infest lambs uniformly with both species simultaneously were unsuccessful. The numbers of *H. contortus*, and to a lesser extent *O. circumcincta* in the same host were adversely affected, e.g. Sheep 4 and 40 (Day +7 Controls, Experiment 1 & 3.) Remarkably uniform worm burdens of *H. contortus*, however, were obtained in the absence of *O. circumcincta*, in Experiment 2 in sheep simultaneously infested with *T. colubriformis*, *O. columbianum* and *G. pachyscelis*. These three species, therefore, did

not adversely affect *H. contortus*, as has been shown by Reinecke[1].

The interaction of *H. contortus* with *O. circumcincta* to the detriment of both species after single doses of infective larvae was demonstrated by Turner, Kates & Wilson[13]. This is confirmed even with multiple doses of infective larvae.

Stewart[14,15] showed that dosage with infective larvae of *H. contortus*, will expel adults of this species as well as of *O. circumcincta* and *T. colubriformis*. This is known as "self-cure". This phenomenon is probably not responsible for the variable worm burdens in our experiment as *T. colubriformis* was not affected. The numbers of *T. colubriformis* recovered *post-mortem*, were uniform and not reduced, even in those animals with reduced *H. contortus* and *O. circumcincta* worm burdens (Experiment 1 & 3).

Nematodirus spathiger

No conclusions can be drawn, as this species was only used in the first experiment. The strain was unfortunately lost hence experiments with this species could not be repeated.

Oesophagostomum columbianum

In a previous experiment worm-free lambs were infested simultaneously with *G. pachyscelis*, *T. colubriformis*, *H. contortus* and *O. columbianum*[1]. A total of 947 infective larvae of *O. columbianum* were dosed and resulted in uniform worm burdens. In the present Experiment 2, the same four species were used but the *O. columbianum* dosage was stepped up to 2,888 infective larvae; the resultant worm burdens were not uniform (*vide supra*).

Sarles[16] dosed three groups of sheep with infective larvae of *O. columbianum* every day for 28 days. Each sheep in the first group was dosed with 10; the second with 100 and the third with 1,000 infective larvae a day, respectively. The percentage of infective larvae that developed to adults, as well as the uniform numbers of worms in sheep dosed with a total of 280, was superior to groups dosed either with 2,800 or 28,000 infective larvae. He concluded that there was an inverse ratio between the number of infective larvae dosed and the resultant worm burdens.

The results of the present experiments together with those of the previous experiment[1], confirms Sarles'[16] observations, that moderate numbers of infective larvae, i.e. less than 1,000, produce more uniform worm burdens than do excessive numbers.

Anthelmintics

The anthelmintic with the highest efficacy was P.T.Z. A which consisted of finely ground phenothiazine (32,200 cm^2/g). There was a surprisingly low efficacy in the other coarser phenothiazine compounds. They were not as finely ground (less than 10,500 cm^2/g), and at 500 mg/K although highly effective against fifth stage and adult *H. contortus* and *O. columbianum*, were either only partially effective or not effective, against larval stages and adult worms of other species, particularly *T. colubriformis*.

An unexpected observation was the high efficacy of tetrachloroethylene against all stages of *H. contortus* and to a lesser extent against *O. circumcincta*, *T. colubriformis* and *G. pachyscelis*.

Rametin was highly effective against both immature and adult *H. contortus* and to a lesser degree against other species.

Methyridine was erratic and disappointing when dosed orally against abomasal parasites; it was, however, highly effective against *T. colubriformis*.

The compounds SKF 90,590, RD 3,412 and carbon tetrachloride were generally not effective.

Those anthelmintics which were generally ineffective acted as additional controls in checking the attainment of uniform worm burdens.

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A COMPARISON OF SHEEP WORM DOSING ROUTINES, AS ADJUDGED BY WOOL PRODUCTION, AND BODYWEIGHT CHANGES

C. J. BOSMAN

Maurice Hall[1](a) 1917 not only uttered the penetrating observation, "Permanent Pastures Perpetuate Parasites!" but was also the originator([1]b) (1921) of the "critical tests" for the evaluation of anthelmintics. Taylor([2]) (1964) the philosophic parasitologist has recently emphasized that the animal industry will have to learn to live with some parasites and try to control, rather than eradicate them because eradication of worm parasites is a fools' dream which may never come true under the present intensive farming practices which become more intensive at the beck and call of "progress".

Phenothiazine, introduced in 1938, was the first anthelmintic to gain wordwide acceptance for the control of nematode parasites in a variety of hosts and for more than 20 years it has served the animal industry well, particularly the sheepowner. Of recent years other anthelmintics, with a wider spectrum of activity and greater efficiency against both mature and immature worms, have come to the fore.

Gordon([3]) (1965) records some most interesting facts, judged by increased wool yields, showing the benefits of dosing sheep alternately with different types of anthelmintics. Nunns & Rawes([4]) (1965) showed that treatment of in-lamb ewes allowed the rearing of fat lambs, without the need to dose them.

The true economics of the treatment of sheep for internal parasites have seldom been considered on a comparative basis. Nowadays, so much emphasis is placed on the immediate anthelmintic efficiency of a drug that the subsequent history of the treated flock may be overlooked. In the trials here reported, dosing routines commonly used by farmers were compared. The value of the treatments was assessed by examining the productivity and profitability of the animals over a long period rather than by estimating reduction of infestation after dosing.

THE 1962-63 TRIALS

Three lots each of 75 weaners were selected and suitably marked for identification. Out of each group, twentysix were individually marked, weighed and matched to give three very similar groups of sheep for detailed study. The twentysix individually marked animals were also weighed, and faecal samples were subsequently taken before every dosing from all groups to study worm burden changes through assessing the e.p.g.

GROUP I

THIABENDAZOLE DOSED GROUP

These were dosed on seven occasions with thiabendazole, receiving 7½ ml. of the label recommended suspension (50-60 mg/kg).

GROUP II

PHENOTHIAZINE (PTZ) DOSED GROUP

Each animal in this group received seven doses of a liquid suspension of phenothiazine containing 15 g. of active ingredient (400-500 mg/kg).

GROUP III

PHENOTHIAZINE alternated with NICOTINE-COPPER-ARSENIC (NCA) DOSED GROUP

This group followed the farmer's own routine, and was dosed four times with phenothiazine suspension, and three times with a proprietary nicotine-copper-arsenic drench, which at the label recommended rate of 1½ fl.oz. gave copper sulphate 20 mg/kg, nicotine 10 mg/kg, and an insignificant amount of arsenic (0.4 As_2O_3 mg/kg).

RESULTS

The observations were continued over 306 days. It will be noted from Table I that there is little

Veterinary Research Team Cooper & Nephews S. Af. (Pty.) Ltd., P.O. Box 108, East London.

TABLE 1

E.P.G. Counts during Trial period								
Date	4/1/63	18/2/63	15/3/63	11/4/63	8/5/63	20/6/63	1/8/63	16/9/63
I Thiabendazole	5,900	1,969	1,653	1,450	217	48	38	232
II PTZ.....	6,460	1,848	1,461	1,431	374	54	39	1,182
III PTZ and NCA.	7,196 PTZ	2,025 NCA	2,523 PTZ	1,625 NCA	1,089 PTZ	158 NCA	86 PTZ	1,092

to choose, as regards the number of worm eggs found in the faeces, between the phenothiazine and the thiabendazole treated groups. However, the group which were treated alternately with phenothiazine and NCA passed significantly greater numbers of eggs.

Table II reveals that the thiabendazole dosed sheep gave the heaviest wool clip and the best net return. However, it is interesting to note that the phenothiazine dosed group yielded slightly less wool than the third group which had received much less phenothiazine and had passed more worm eggs. This picture is again reflected in the weights after shearing, that is, the animals regularly dosed with phenothiazine again came off worst.

TABLE II

	I Thiabendazole	II PTZ	III PTZ & NCA
Average gross weight gain before shearing.....	9.0 lb.	4.5 lb.	9.0 lb.
Average wool yield per sheep.....	8.6 „	7.4 „	7.7 „
Net weight gain or loss up until shearing per sheep.....	+0.4 „	—2.9 „	+1.3 „
Gross return on wool* per sheep.....	R4.30	R3.70	R3.85
Cost of remedy per sheep in cents.....	29.5c.	11c.	8c.
Net return per sheep after deducting remedy costs.....	R4.00	R3.59	R3.77

* based on 50 c/lb.

FIRST 1963-64 TRIALS

The weaners used in the second year trials, which lasted 344 days, originated from the same flocks as those incorporated in the previous trial. Four groups, each of 75, and one "control group" of 20 were formed. E.p.g. counts were made on twenty specially marked individuals from each group at the start of the trial, and thereafter at every dosing.

The unexpected good results obtained from the "phenothiazine alternated with NCA" group dosing during the 1962-63 trials led one to suspect that perhaps it was the copper present in the dose which accounted for the better results as the total amount of the efficient anthelmintic, phenothiazine, dosed was less. In the following year therefore another group was inserted which received as much phenothiazine as the phenothiazine alone group, but in addition copper and cobalt.

GROUP I

PHENOTHIAZINE DOSED GROUP

The 75 sheep received 1½ fl. oz. (400-500 mg/kg) each of a prepared drench at every dosing.

GROUP II

THIABENDAZOLE DOSED GROUP

These received 7½ ml. each at every dosing (50-60 mg/kg.)

GROUP III

PHENOTHIAZINE plus COPPER and COBALT DOSED GROUP

These received 400 to 500 mg/kg of phenothiazine and 13 to 17 mg/kg and 5 to 6 mg/kg of copper and cobalt sulphates respectively.

GROUP IV

PHENOTHIAZINE alternated with NICOTINE-COPPER-ARSENIC DOSED GROUP

These received phenothiazine at the rate of 400 to 500 mg/kg and copper sulphate 20 mg/kg, nicotine 10 mg/kg and an insignificant amount of arsenic at each dosing, again following the owner's programme.

GROUP V

UNTREATED CONTROL GROUP

The twenty weaners in this group were treated only when they were in such bad condition that they would have died. They received one dose of NCA and one of phenothiazine during the whole period of observation (344 days).

Results

The results were similar to those of the previous year's trials, as is shown in Tables III and IV:

Once again the wool yields of the phenothiazine alone dosed group (8.5 lbs.) and phenothiazine plus trace copper and cobalt (8.4 lb.) group were lowest, suggesting that this was the result of the full course of phenothiazine. The "alternate dosed" group received less phenothiazine than the PTZ and PTZ-PLUS and the yield of wool was the same as that of the thiabendazole dosed group. This suggests that the depression in wool yield was caused by the full course of phenothiazine and not attributable to lack of copper and/or cobalt.

TABLE III

E.P.G. Counts during trial period.

Date	14/11/63	11/12/63	10/1/64	21/2/64	23/3/64	27/4/64	23/7/64	21/8/64
I PTZ.....	7,340	2,790	3,835	3,900	4,570	2,453	140	160
II Thiabendazole...	7,575	1,420	4,525	3,352	5,410	2,047	35	30
III PTZ-plus.....	7,960	2,515	3,880	4,010	6,830	3,210	230	410
IV NCA/PTZ.....	11,870	12,250	3,861	4,335	6,820	3,005	540	450
V Untreated Control.....	8,736 NCA	10,670	16,420	16,115	10,070	10,131	1,870	1,530

TABLE IV

	I PTZ	II Thiabenda-zole	III PTZ-plus	IV PTZ+NCA	V Untreated control
Average gross weight gain before shearing...	8.2 lb.	11.8 lb.	8.0 lb.	8.1 lb.	3.8 lb.
Average wool yield per sheep.....	8.5 lb.	9.2 lb.	8.4 lb.	9.2 lb.	6.4 lb.
Net weight gain or loss up until shearing per sheep.....	—0.3 lb.	+2.6 lb.	—0.4 lb.	—1.1 lb.	—2.6 lb.
Gross return on wool per* sheep.....	R4.20	R4.60	R4.20	R4.60	R3.15
Cost of remedy per sheep in cents.....	13c.	32c.	15c.	8.5c.	2.56c.
Net return per sheep after deducting remedy costs.....	R4.12	R4.28	R4.05	R4.51	R3.12

* based on 50c./lb.

SECOND 1963-64 TRIALS

There is marketed in Australia and also elsewhere an anthelmintic mixture based on 67.5% w/w technical phenothiazine, and 22.5% w/w -2 phenyl benzimidazole (P.b.). This compounded worm remedy was compared in the field with thiabendazole, following the criteria already mentioned.

Weaners from the same farm and very similar to those used in previous years were divided into three groups.

GROUP I

THIABENDAZOLE DOSED GROUP

This group consisted of 65 lambs and was dosed regularly with thiabendazole at 50/60 mg/kg.

GROUP II

PHENOTHIAZINE plus -2 PHENYL BENZIMIDAZOLE DOSED GROUP

Group II consisted of 65 weaners, and was dosed with the mixed remedy at a rate of 259-337 mg/kg of phenothiazine and 141/163 mg/kg of -2 phenyl benzimidazole (P.b.).

GROUP III

CONTROL GROUP

This group consisted of 20 controls, which received one dose of NCA and one dose of phenothiazine as in previous trials to avoid deaths, but in spite of this two sheep died from worm infestation.

RESULTS

These trials ran for 314 days and the data of e.p.g. counts and productivity is given in the two tables that follow:

TABLE V

E.P.G. Counts during trial period

Dosed with	14/11/64	12/12/64	17/1/65	28/2/65	2/5/65	11/6/65	28/7/65	1/9/65
I Thiabendazole...	4,000	800	4,400	5,500	3,200	3,500	250	375
II PTZ+P.b.....	4,200	400	3,400	4,300	2,500	3,000	50	200
III Untreated Control.....	5,400 NCA	2,500	9,800	8,000	9,100	8,600 PTZ	1,500	2,250

TABLE VI

	I Thiabendazole	II PTZ+P.b.	III Surviving controls
Average gross weight gain before shearing.....	6.2 lb.	3.2 lb.	0.2 lb.
Average wool yield per sheep.....	9.4 „	8.6 „	6.7 „
Net weight gain or loss up until shearing per sheep.....	—3.2 „	—5.4 „	—6.5 „
Gross return on wool* per sheep.....	R4.70	R4.30	R3.35

No comparative costs of the remedies is feasible as the phenothiazine/phenyl benzimidazole mixture is not on sale in South Africa.

* based on 50 c./lb.

These results are of interest because although there is little difference in the e.p.g. counts of thiabendazole and phenothiazine/phenyl benzimidazole dosed groups there is a difference not only in wool yield but also in the body weight losses. Although production of the group which received PTZ and P.b. is lower than the thiabendazole dosed sheep both were far superior to the undosed controls.

DISCUSSION

During the last decade occasional complaints have been heard in South Africa that PTZ did not improve the condition of sheep dosed in spring or mid-summer. Some complaints even went further to suggest poor wool yield and weight losses when animals were regularly dosed with it.

Apart from the above it was realised that too little information was available about the strategic use of worm remedies in South Africa. Meldal-Johnsen[5] (1961) has studied the incidence of sheep worms in the King William's Town area; Muller[6] (1962) did the same around Stellenbosch Both worked in the Cape Province. Reinecke[7] (1964) correlated and added to the information which is essential when cognizance has to be given to the "helminth-life-cycle-season" factors in the

choice of remedy for strategic dosing. Further, it was also appreciated that it was undesirable to use any anthelmintic without discretion and knowledge of the specific problem involved. In other spheres of life specific tools are used for specific purposes, but up to now in South Africa it has been an ardent chase after new remedies to control all worms without any effort to use all the available remedies with discretion so as to obtain good results and, at the same time, to reap better profits.

The emphasis has been more on efficacies of remedies rather than effectiveness and profit together. For instance it is uneconomic to dose with the most costly wide spectrum remedy when only a low infestation of *Haemonchus* is present, which could be dealt with by a cheap remedy which is specific against it.

The time lapse between dosings is also important, because if dosings are spaced too far apart this could do more harm than good.

Climatic conditions also play a vital role in worm infestations and should be reckoned with during the course of the dosing routine.

The life cycle of the parasite to be dealt with also has a bearing on the choice of remedy for inclusion in the dosing programme.

The above problems and factors of internal parasite control prompted a series of field trials in an effort to obtain a better understanding of the problems and needs of the average sheep farmer.

If the e.p.g. tables I, III and V are studied it is evident that the use of different dosing materials did not differ all that much in controlling the persistent parasite challenge, which consisted of *Haemonchus contortus, Trichostrongylus spp.* and *Oesophagostomum columbianum*. When the weight gain and wool yields are correlated with the egg counts, a marked difference is found between the PTZ, PTZ-Plus and PTZ + P.b. groups on the one side, in comparison with the thiabendazole and NCA/PTZ dosed groups on the other side. In spite of comparable egg count levels, the wool yield and weight gains were lower than that of the latter groups. Considering the higher egg count level in the NCA/PTZ dosed groups, one would expect a lower wool and weight production, but this "cheaper" dosing programme compared favourably with the highly "effective" thiabendazole dosed groups.

The question arises — why were the PTZ dosed groups so disappointing in spite of a relatively good worm control?

With the knowledge on the efficacy of phenothiazine gained over the years, manufacturers took endless trouble to produce a purer and finer ground product to ensure the best efficacy. In this effort they overlooked the fact that the finer and purer the product becomes, the absorption of this material into the bloodstream increases accordingly, and it may be this factor that plays a retarding role in the performance of the sheep.

Toxicity trials could not demonstrate beyond any doubt that finer and purer material of PTZ was more detrimental to the health of the animal than the coarser material, but numerous instances have come to the notice of field workers that pointed to phenothiazine toxicity, especially when this product followed a dose of chlorinated hydrocarbons. Is it not possible that we are today dealing with a subclinical toxicity?

In a letter Hebden and Setchell[8] (1962) quoted twelve cases of suspected PTZ poisoning. They came to the conclusion that micronised PTZ may be safe under ordinary conditions, but there are circumstances under which it is dangerous to use, such as dosing PTZ within 14 days after a CCl_4 dose in which case the latter may reduce the kidney excretion of PTZ. This may then possibly enhance toxicity of fine particles more than coarse particle PTZ, due to the different paths of their elimination. Other factors, according to the above authors, which also impair the functions of the kidneys, such as the presence of oxalates or restriction of water intake, may also enhance toxicity. However, in a number of the cases mentioned in their letter, neither of the above factors was involved, and they are of the opinion that more evidence is required before it could be accepted that toxicity was produced by impurities rather than the greater absorption due to the finer particles.

Williams, Humphries and Mills[9] (1963) carried out experiments on the influence of PTZ administration on the cytochrome oxidase activity in lamb brain tissue, and they came to the conclusion that the reduction in cytochrome oxidase activity following administration of PTZ results from some interference other than copper metabolism. The possibility exists that in copper deficient animals the effects of giving PTZ may lower an already depressed cytochrome oxidase. In view of this they suggest that the use of PTZ and its derivatives, during pregnancy and also in young lambs, be attended with caution in parts where copper deficiency exists, and particularly in places where previous outbreaks of swayback have occurred.

According to Radeleff[10] (1964) cyanogenetic plants, which contain cyanogenetic glucosides from which glucosides hydrocyanic acid is produced by

the action of appropriate enzymes, inhibit the action of such enzymes as cytochrome oxidase, depriving the tissues of the necessary oxygen.

Animals grazing on grasses containing a fair amount of cyanogenetic glucosides, may also suffer when they are dosed with PTZ if this drug also has an inhibitory action of the cytochrome oxidase as suggested by Williams et al. (1963).

Oxalates in plants where a deficiency of calcium is present may also interfere with the excretion of the absorbed PTZ because oxalates interfere with the normal function of the kidneys.

Other poisonous plants like ragwort taken at a low level may also upset the proper metabolic functions in the animal, and when dosed with PTZ may also inhibit growth and wool production.

Grazing in the Eastern Cape coastal belt is known to be deficient in copper and has plants poisonous to ruminant, and this may account for the poor results obtained by the PTZ products in this series of trials.

In the PTZ/NCA group the copper in the NCA may have had the desired effect in helping the animals by supplementing the low copper in the grazing and hence produced more wool, in spite of the fact that its general anthelmintic efficacy was mediocre. However, the spacing of the PTZ doses were so far apart that any side effects were masked. Furthermore, this group only received four doses of PTZ.

The reason why the PTZ-plus dosed group did not perform beter may be due to the PTZ having been given on eight occasions.

When considering the economical aspects of the different dosing programmes employed in these trials, it is evident that, in spite of the high returns obtained in the thiabendazole groups, it is not always more profitable than that of the NCA-PTZ routines.

The spacing of doses in these trials was also too long to effect a good control of the parasite incidence. This indicates that the longest period between dosings should be 21 days in the spring to autumn season, especially in a normal or rainy season. Dosing 14 days after every rain may have the desired effect in South Africa.

CONCLUSIONS

These trials disclosed a large number of problems that are worthy of further investigation. They also stressed the necessity for more work on the rotation of anthelmintics in a dosing routine to obtain the programme of remedies which will give the best returns.

The observations were on ranched sheep and the results could differ from those of animals grazing on pastures with higher nutritional levels.

This work also stresses the need for a well planned strategic and tactical dosing programme rather than relying on rule of thumb routine dosings, as the farmers used in their trials.

The fact that most of the factors which may be involved in the depression of weight gains and wool production appear to be subclinical, weight gain and wool production may be accepted as a measure of their influence on the animals' well-being.

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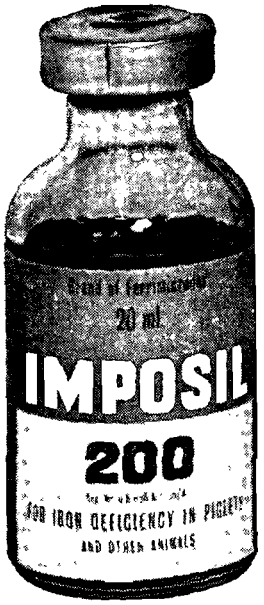

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MELAMINE FOR SHEEP

H. I. MACKENZIE

SUMMARY

A toxicity trial, a feeding trial and nitrogen balance studies were carried out using melamine mixed with maize as a supplement to roughage diets for sheep. The results indicated that melamine can be used as a source of non-protein nitrogen (NPN) by sheep but the efficiency of utilization is governed by the type of roughage in the basal ration.

The most marked effect of roughage quality on melamine utilization was observed when the basal ration was changed from hay containing 9.1% C.P. to hay with a C.P. content of 3.4%. Feed intake dropped drastically and a number of the sheep died.

In general the results suggest that melamine cannot be regarded as a reliable source of N.P.N. for sheep fed roughage diets which are deficient in protein.

INTRODUCTION

Recent research has shown that cyanuric acid is a safe and effective source of N.P.N. for sheep[1][2][3]. This compound, which can be derived from urea, has a relatively low nitrogen content of 32%. Melamine, which is similar to cyanuric acid in structure, contains 66% nitrogen. Investigations were carried out to determine whether melamine could be used as a concentrated source of N.P.N. for sheep on roughage diets which were deficient in protein.

COMPOSITION OF MELAMINE

The material used in all the investigations was of the following composition:—

Assay (by Kjeldahl	Not less than 97.5%
Free acid	Not more than 5 ml. N. per 1%
Sulphated ash	Not more than 0.1%
Average nitrogen content	66.53%

1. TOXICITY TEST

An acute toxicity test was carried out on 4 maiden ewes which were receiving a diet of low quality hay and buiret. Melamine was mixed with water and the slurry was administered as a drench as indicated in Table I.

TABLE I.—LIVEWEIGHT OF EWES AND DOSAGE RATES OF MELAMINE IN WATER.

Sheep No.	Liveweight (kg)	Dosages		Amount ingested	
		Melamine (g)	Water (ml)	Melamine (g)	g Melamine/ kg bodyweight
58	37.5	10	60	6	0.16
54	35.6	20	120	16	0.45
74	33.7	30	180	28	0.83
6	33.3	40	240	39	1.17

The drenches had no apparent adverse effect on the sheep. The only signs of abnormal behaviour were shown by sheep numbers 74 and 6 which tended to salivate profusely immediately after drenching due, presumably, to the large volume of slurry they were forced to ingest.

These findings are in agreement with those of Cronje[4] who showed that sheep could tolerate single doses of melamine of up to 50g.

2. NITROGEN BALANCE STUDIES

Two nitrogen balance studies were carried out using cottonseed hulls and maize meal in the basal rations.

Research Department, African Explosives and Chemical Industries, Limited P.O. Northrand, Transvaal.

Trial 1. *Methods*

Three sheep aged two years were fed the basal ration which consisted of cottonseed hulls (5.8% C.P.) *ad lib.* and 100g. maize meal, 7g. bonemeal and 3g. salt per sheep per day for 24 days. Nitrogen balance was measured in standard metabolism crates during the last 5 days of this period.

An amount of 10g. melamine per sheep was added daily to the maize meal, bonemeal and salt mixture for a continuous period of 42 days and nitrogen retention was measured three times viz. during the last 5 days of each fortnight.

The sheep were group fed in a stall when they were not in the metabolism crates.

Results

Because of the variability between sheep in response to treatments, the results of the balance studies are presented in full.

Table 2.—Nitrogen balance data (g. per sheep per 5 days).

Sheep No. & Wt. (kg)	Av. N Content of roughage (%)	Period 1 Basal only 19*+5** days	Period 2 Basal + 10g. melamine 9*+5** days	Period 3 Basal + 10g. melamine 9*+5** days	Period 4 Basal + 10g. melamine 9*+5** days
		0.93	0.96	0.97	0.88
D12	Roughage intake	2129.5	4,246.5	4,436.8	5,383.5
	N intake	34.01	70.04	86.27	88.58
47.0	N loss	40.76	62.39	69.36	78.26
	N balance	—6.75	+7.65	+16.91	+10.32
D23	Roughage intake	2,195.0	3,025.0	3,654.5	4,772.5
	N intake	28.78	73.97	80.54	83.98
42.5	N loss	55.94	62.95	71.04	83.75
	N balance	—27.16	+11.02	+9.50	+0.23
D28	Roughage intake	2,819.0	1,764.0	2,342.0	1,671.5
	N intake	28.21	64.77	72.01	61.82
36.4	N loss	34.21	97.43	67.21	64.35
	N balance	—6.00	—32.66	+4.80	—2.53

* Housed in a stall and group fed.
**Housed in metabolism crates.

The results indicated the following trends:

(a) The voluntary roughage intake of 2 of the 3 sheep was increased by the addition of melamine to the diet. The intake of the third animal fluctuated widely.

(b) Melamine was utilised by the sheep as an additional source of nitrogen within 2 weeks after the commencement of feeding.

(c) The amount of nitrogen retained by all of the sheep dropped during the sixth week of feeding due to an increase in nitrogen losses relative to the nitrogen intake. A sharp rise in faecal nitrogen accounted for the major portion of the increase. There was a slight decrease in urine nitrogen.

Trial 2. *Methods*

Four sheep aged 2 years were used in this trial.

The basal ration consisted of cottonseed hulls (7.4% C.P.) *ad lib* plus 100g. maize meal, 10g. bonemeal, 10g. salt and 1000 I.U. Vitamin A per sheep per day. The sheep were fed the basal ration only, for 29 days and nitrogen balance was determined during the last 5 days of this period. Melamine was then added to the ration at a rate of 10g. per sheep per day for a further 28 days and balance studies were carried out twice viz. during the last 5 days of each fortnight. The sheep were individually fed in stalls when they were out of the metabolism crates.

Discussion

The results showed the following trends:

(a) Melamine tended to depress voluntary roughage intake.

(b) The feeding of melamine restored nitrogen equilibrium in the sheep within a fortnight.

(c) Nitrogen retention decreased two weeks after a positive nitrogen balance had been achieved.

TABLE 3.—NITROGEN BALANCE DATA (G PER SHEEP PER 5 DAYS)

Sheep No. & Wt. (kg)	Av. N content of roughage %	Period 1 Basal only 24*+5** days	Period 2 Basal + 10g. melamine 9*+5** days	Period 3 Basal + 10g. melamine 9*+5** days
		1.05	1.26	1.26
D9	Roughage intake	3,880.5	2,602.0	2,300.0
	N intake	48.73	76.34	64.33
34.6	N loss	49.00	55.50	56.69
	N balance	—0.27	+20.84	+7.64
E23A	Roughage intake	4,296.0	4,193.0	4,523.0
	N intake	52.88	96.36	99.26
40.9	N loss	63.44	69.96	75.01
	N balance	—10.56	+26.40	+24.25
D24	Roughage intake	2,863.5	2,687.5	2,751.0
	N intake	38.56	83.11	83.67
37.7	N loss	45.03	45.92	71.96
	N balance	—6.47	+37.19	+11.71
N.N.	Roughage intake	1,922.5	2,073.5	861.5
	N intake	29.15	77.71	67.04
35.0	N loss	36.55	50.54	46.67
	N balance	—7.40	+27.17	+20.37

* Housed in stalls and individually fed.
** Housed in metabolism crates.

As in the previous trial, this resulted mainly from a decline in the apparent digestibility of nitrogen in the diet. Nitrogen losses in the urine rose slightly during the last fortnight of the test.

3. FEEDING TRIAL

A conventional feeding trial with four groups of 9, three year old sheep was carried out to compare the value of melamine with urea as a source of supplementary nitrogen for sheep on hay diets.

Methods

Before the trial started, the sheep were dosed with an anthelmintic and a vitamin A supplement and then conditioned to pen feeding for a period of 43 days on a diet of good quality hay *ad lib* plus a supplement of 100g. maize meal and 18.6g. biuret per sheep per day.

During the trial, the sheep were weighed fortnightly after 16 hours starvation. Hay was offered in weighed amounts which exceeded the daily consumption by at least 30%. Daily intake was determined by weighing the residues. Lick consumption was measured weekly.

The trial was divided into two phases and the basal rations were as follows:

Phase 1. (42 days) *Eragrostis curvula* hay (9.1% C.P.) plus a lick of equal parts by weight of dicalcium phosphate and salt.

Phase 2. (28 days) *Eragrostis curvula* hay (3.4% C.P.) and the lick.

The supplements fed once daily throughout both phases were as follows:

Group 1. 100g. maize meal per head per day.

Group 2. 100g. maize meal + 9.8g. melamine per head per day.

Group 3. 100g. maize meal + 19.6g. melamine per head per day.

Group 4. 100g. maize meal + 14.0g. urea per head per day.

Note: The nitrogen contents of 9.8g. melamine and 14.0g. urea were equal (6.5g N).

RESULTS

It will be noted from Table 4 and Figure 1 that Phase 2 was terminated after 28 days. This was necessary because the sheep in Groups 2 and 3 had lost excessive amounts of weight and several were in a critical condition. The treatments were stopped and all the sheep were put back on to the original adaption diet to regain weight.

TABLE 4.—LIVEWEIGHT CHANGES AND LICK AND HAY CONSUMPTION OF SHEEP WITH AN AVERAGE INITIAL LIVEWEIGHT OF 38.7 KG.

	Phase 1 Hay 9.1% C.P. Period 42 days			Phase 2 Hay 3.4% C.P. Period 28 days		
	Weight Change (kg)	Lick Consumption (g/h/d)	Hay Consumption (g/h/d)	Weight Change (kg)	Lick Consumption (g/h/d)	Hay Consumption (g/h/d)
Group 1 (Control)	+3.73	11.91	1,102.25	—4.54	17.58	539.78
Group 2 (Melamine 9.8g)	+2.67	22.96	1,052.35	—6.35	18.71	390.10
Group 3 (Melamine 19.6g)	+4.44	22.68	988.85	—7.61	19.56	390.10
Group 4 (Urea 14.0g)	+3.58	18.14	1,111.32	—0.76	10.49	644.11

Note: The changeover from Phase 1 to Phase 2 was made without an adaptation period.

The differences in liveweight changes were not significant in Phase 1. In Phase 2, Group 4 lost significantly ($P = 0.01$) less weight than the other three groups and the weight loss in Group 1 was significantly ($P = 0.05$) less than that in Groups 2 and 3.

It should be noted that 10 days after the commencement of Phase 2, the sheep in Group 3 started refusing to consume the supplement. Group 2 displayed a similar tendency towards the end of the comparison. The actual amounts of the supplement eaten by these two groups during Phase 2 were:

	Maize meal (g/h/d)	*Melamine* (g/h/d)
Group 2	89.4	8.8
Group 3	68.4	9.7

When Phase 2 was terminated and the sheep received the adaptation diet, Groups 1 and 4 gained weight but Groups 2 and 3 continued to lose. Over a period of 31 days, five sheep in Group 2 died.

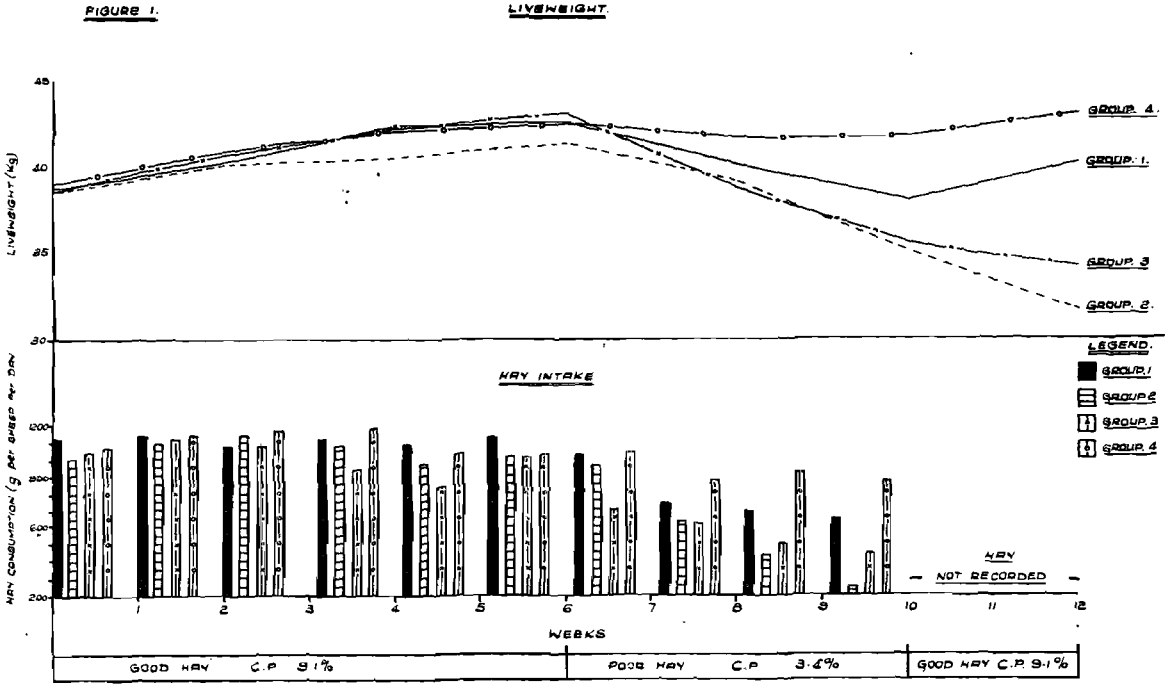

Figure 1

Liverweight and hay consumption of sheep on a diet of hay and a supplement of maize meal and N.P.N.

Discussion

In Phase 1 the addition of melamine or urea to high quality hay did not exert any influence on liveweight. This finding indicates that the utilization of melamine, as is the case with other sources of N.P.N., is negligible in the presence of roughages containing adequate supplies of natural proteins[5][6]. However there was an indication that the sheep receiving melamine consumed less hay than those which were fed urea or the basal ration only.

In Phase 2 the voluntary hay intakes of all groups dropped. The amounts consumed by the control group and the group which received urea (Group 4) was considered to be "normal" for diets of this type but the intake of the sheep receiving melamine was exceptionally low. The effect of this pattern of consumption was clearly reflected in the weight losses.

It was not possible to determine the precise reason for the refusal of the sheep to consume all of the melamine-containing supplements during Phase 2. It would appear that it resulted from a general lack of appetite rather than from a decrease in palatability with time, because during the recovery period, these sheep showed a marked reluctance to eat supplements which contained no melamine.

The pattern of lick consumption in Phase 1 was similar to that shown by sheep which were fed cyanuric acid in earlier trials. The addition of N.P.N. to the diet increased the voluntary intake of a mixture of dicalcium phosphate and salt[3]. This tendency did not, however, persist during Phase 2.

There was no obvious reason why sheep died in Group 2 only. Throughout the test period, the only symptoms of a physiological disturbance that were evident were slight scouring and, in the latter stages of Phase 2, emaciation and a severe reduction in feed intake. No detailed post-mortem examinations were carried out.

The only published record of melamine toxicity appears to be the observations made by van der Merwe on 2 sheep. He showed that melamine administered at the rates of 50g. and 70g. per sheep per day caused death in 6 days. Post-mortem lesions included degeneration of the liver, kidneys, bladder and lungs[7].

Conclusion

The value of melamine as a source of N.P.N. for sheep appeared to be largely influenced by the type of roughage with which it was fed. In common with other sources of N.P.N., melamine did not exert any measurable effect on liveweight when used as a supplement to high quality hay which was fed *ad lib*. However, when the crude protein content of the roughage was between 5.5 and 7.8% the addition of melamine to the diet restored nitrogen equilibrium within two weeks, but the efficiency of utilization tended to decline with time because of a decrease in the apparent digestibility of the nitrogen.

The most significant finding however, was that sheep which received supplements containing melamine showed a marked reduction in feed intake when the bulk of the basal ration consisted of extremely low quality hay. This tendency persisted after the sheep were offered a melamine-free diet of high quality and a number of the animals died. The precise cause of death was not established.

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AN EVALUATION OF SOME WINTER FEED SUPPLEMENTS FOR SOUR VELD AREAS

K. M. BUCHANAN* AND D. K. SHONE

INTRODUCTION

The true sour veld regions of South Africa correspond to those areas where the veld grasses have the lowest protein values in winter. Generally these grasses are coarse and unpalatable and they frequently form the sole diet of ranging animals. The low quality of the grazing and the severity of the winter, impose on maintenance feeding the most exacting conditions to be found in this country.

Supplements containing urea are commercially available for feeding to ruminants for the maintenance of bodyweights during autumn and winter. The currently available supplements contain urea in combination with other materials.

urea and salt
urea and molasses
urea, maize and molasses distillers dried solubles (m.d.d.s.)
urea, maize and natural proteins.

The usefulness of these supplements is dependent upon the extent to which they meet the nutrient requirements of the animal for maintenance and whether the advantages gained in achieving this object justify the cost of the supplementary feeding.

Beneficial results from the use of all four forms of urea supplements have been reported. In South Africa the feeding of urea and salt alone has been found to reduce the loss in bodyweight occurring in winter in sour veld areas[1,2]. The feeding of a carbohydrate, readily available to the rumen micro organisms, together with the urea, has been found to improve the utilization of the urea[3,4]. Maize has been demonstrated to be superior to molasses in improving the utilization of urea[5,6]. Improved utilization of urea when fed with a source of natural protein, has also been reported[7,8].

The purpose of this trial was to compare the suitability of commercial supplements for maintenance feeding under sour veld conditions as measured by their ability to maintain the bodyweight of sheep.

In terms of texture, palatability and feed value, the hay used in this trial was considered to be representative of sour veld grazing in winter, and the trial was conducted in the latter half of the winter.

MATERIALS

Feeds

The basal diet fed to all groups was dry veld hay cut in winter in the Bapsfontein area of the Transvaal and obtained as a single consignment.

The analysis of the hay was:

crude protein	5.0 per cent
fat	1.2 per cent
fibre	40.7 per cent
nitrogen free extract:	42.1 per cent

The composition of the supplementary feeds is given in table 1. All feedstuffs used in the manufacture of the supplements were obtained from single batches. The urea, maize and salt block (R) is commercially available and was bought as a single consignment. This block had a declared urea content providing a protein equivalent of 28.6% and also contained 8% of natural protein derived from maize and molasses distillers dried solubles.

The urea, molasses, maize and natural protein block (V) was developed for possible commercial use and contained urea which provided a protein equivalent of 23%. It contained 10% of natural protein from various plant sources.

Molasses meal contains 70% molasses absorbed onto dried cane pith.

Animals

Ninety-six German Merino ewes approximately 18 months old with an average liveweight of 80 lbs.

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TABLE 1.—THEORETICAL COMPOSITION OF FEED SUPPLEMENTS PER CENT.

	Urea Maize Salt Block. (R)*	Urea Maize Protein Block. (V)	Molasses Concentrate Plus Salt.	Molasses Meal Concentrate No Salt.	Urea Molasses Lick	Urea/ Salt Block	Phosphorous Lick
Urea	10.0	8.0	7.5	7.5	7.0	30.0	—
Protein Plus Protein Equivalent	37.0	33.0	22.0	22.0	20.0	—	—
Natural Protein	8.0	8.0	not significant	not significant	—	—	—
Molasses	—	3.0	52.0	37.1	65.0	11.0	—
Molasses Distillers Solubles	unknown but present	14.0	8.0	8.0	—	—	—
Maize	do	—	—	—	—	—	—
Salt	do	22.0	22.0	22.0	8.0	36.0	50.0
Phosphorus	1.7	1.8	1.2	1.2	0.78	3.8	6.0

* Values for Urea Maize Salt Block (R) are estimated with the exception of urea and phosphorus which are declared by the manufacturer.

were obtained for the trial. After arrival the sheep were drenched with thiabendazole and then adapted to the basal diet of veld hay on which they remained for nine weeks — during this period the sheep were shorn and their liveweights dropped to 66.8 lbs.

METHODS

The trial was arranged as two randomised block experiments. Period 1 of experiment I was run concurrently with experiment II; period 2 of experiment I followed directly afterwards.

Experiment I

Thirty-six sheep were randomly distributed into six pens of six sheep each. The three replicated treatments were randomly allocated to the pens.

The treatments were as follows:

Period 1 — 52 *days*

Group A — urea, maize & m.d.ds block (R) ad lib

Group B — urea, maize and natural protein block (V) ad lib

Group G — Control group. Salt/phosphate lick available ad lib

Period 2 — 52 *days*

Group A — Block R fed at a fixed daily quantity

Group B — Block V fed a fixed daily quantity

Group K — Control group. Salt/phosphate lick available ad lib.
This group was previously group D of experiment II.

The sheep had free access to hay and water at all times.

At the end of the first 52 day period, the control sheep (G) were replaced because of severe emaciation.

Experiment II

Sixty sheep were randomly distributed into 10 pens of 6 sheep each. The replicated treatments were randomly assigned to pens. Hay and water were available ad lib to all sheep. The experiment ran for 52 days.

The treatments were as follows:

Group C — Control group — fed no supplements

Group D — Molasses meal concentrate (no salt) fed a fixed daily quantity.

Group E — Urea, salt block — available ad lib.

Group F — Molasses meal concentrate (22% salt) available ad lib.

Group H — Urea and molasses solution — available ad lib.

Liveweights

All sheep were deprived of food and water for 14 hours before weighing. All sheep were individually weighed each week. The weight at the commencement and at the termination of the trial are the mean of three weights taken at 48 hour intervals. The final liveweights were recorded after all the experimental groups had been on the control diet for one week.

Feeding

Feed and water consumption were recorded daily. Hay was fed long in racks. Blocks were placed on the floor with all wrapping removed.

EXPERIMENT I

TABLE 2.—MEAN DAILY CONSUMPTION OF WATER, HAY AND SUPPLEMENTS, AND THE MEAN LIVEWEIGHTS AT THE START AND THE END OF THE TRIAL

Period 1.	Group A	Group B.	Group G.
	Urea Maize Salt Block (R)	Urea Protein Maize Salt Block (V)	Salt Phosphorus Lick
Mean consumption of water lbs.	3.03	5.21	2.73
Mean consumption of hay lbs.	1.70	1.50	1.59
Mean consumption of supplement lbs.	0.208	0.420	0.045
Mean liveweight start lbs.	66.77	64.88	67.33
Mean liveweight end lbs.	65.70	65.25	62.35
Gain or Loss lbs.	—1.07	+0.37	—4.98
Period 2	Group A Urea Maize Salt Block (R)	Group B Urea Protein Maize Salt Block (V)	Group D. Salt Phosphorus Lick
Mean consumption of water lbs.	3.80	5.02	2.73
Mean consumption of hay lbs.	1.72	1.59	1.52
Mean consumption of supplement lbs.	0.286	0.286	0.044
Mean liveweight start lbs.	66.45	65.25	67.24
Mean liveweight end lbs.	65.45	66.60	60.49
Gain or Loss.	—1.00	+1.45	—6.75

EXPERIMENT II

	Group C. Control Group.	Group D. Molasses Meal. No Salt.	Group E. Urea Salt Block.	Group F. Molasses Meal Plus Salt.	Group H. Urea and Molasses Solution
Mean consumption of water lbs.	2.21	2.83	2.36	6.06	3.55
Mean consumption of hay lbs.	1.56	1.60	1.48	1.56	1.55
Mean consumption of supplement lbs.	0.02	0.50	0.04	0.02	0.44
Mean liveweight Start lbs.	68.66	65.66	66.50	68.66	67.16
Mean liveweight End lbs.	64.65	66.11	63.69	64.65	65.48
Gain or Loss lbs.	—4.01	+0.45	—2.81	—4.01	—1.68

When a fixed daily quantity of a block was fed, the block was crumbled and fed in tins. The molasses meal concentrate when fed ad lib, was stamped down tightly in tins. The urea molasses solution was fed in open drums fitted with a perforated wooden float to limit intake.

RESULTS

The mean daily consumption of hay, supplements and water and the mean liveweights at the commencement and termination of the experiments, are presented in Table 1.

Experiment I

Two deaths occurred in period 2 of this experiment. One sheep in Group A, fed the urea, maize, m.d.ds. block (R) died as result of severe haemonchus infestation during the experiment, while the second sheep from Group B (Block V) died two days after the termination of the experiment. The latter sheep lost 10 lbs. during the last two weeks of the experiment, while the remaining 5 sheep in this pen either maintained or gained weight over this period. The sheep in Group A has been omitted in the statistical treatment of the data.

There was no significant difference in the hay consumption between the groups. The sheep on the urea, molasses, maize and natural protein block (V) drank significantly more water ($P = < .05$) than the sheep in the other two groups.

The weight lost by the control sheep in both periods of this experiment was statistically highly

significant ($P = < 0.01$) when compared to the weights of the sheep fed the urea, maize and m.d.d.s. block (R) and those fed the urea, maize, molasses and natural protein block (V).

Over the two periods the sheep (group B) fed the urea, maize, molasses and natural protein block (V) gained weight while those fed the urea, maize, m.d.d.s. block (R) lost weight, but no conclusion can be drawn as the calculated protein equivalent intake of the sheep fed block V was 18 per cent greater than that of the sheep fed block R.

In the second half of the experiment the groups were fed on equal quantities of block R and V with the result that in this period the sheep fed on block R had an increased intake of calculated protein equivalent of 11% over the sheep fed on block V. In spite of this difference in calculated protein equivalent there was no statistically significant difference in bodyweight between the two groups over this period. The sheep on block V has a mean liveweight gain of 1.3 lbs. while those fed block R lost 1.0 lbs. One of the sheep fed on block V died two days after the termination of the experiment from haemonchosis and it is probable that this sheep affected the gains which may have resulted in significant differences emerging between the sheep fed on R and V blocks.

Experiment II

Two sheep fed the urea salt block (group E) died. It was established by post mortem examinations that both deaths were due to poverty. Further deaths due to poverty were expected in this group as well as in the control group (group C) and it was decided to terminate the experiment at 52 days. Had the experiment continued, it is probable that all the sheep fed the urea, salt block (group E) and those fed no supplement (group C) would have died of poverty. The dead sheep have been omitted from the liveweight results which are thereby improved.

The control sheep fed no supplement, lost significantly more weight ($P = < 0.05$) than the sheep fed on the molasses meal concentrate (no salt), the molasses meal concentrate (22% salt) and the urea molasses solution.

The sheep fed on the molasses meal concentrate (no salt) were the only ones to gain weight. The difference of 1.76 lbs. per head between the sheep fed on the molasses meal concentrate with and without salt is thought to be too great to be attributed to the slightly greater intake of 0.04 lbs. per day of the molasses meal concentrate (no salt). This aspect is being investigated further.

The sheep fed the molasses meal concentrate (22% salt) (group F) consumed significantly ($P = < 0.05$) more water than any of the other groups. The intake of water was directly related to the amount of salt consumed. The consumption of the concentrate by this group increased to a level thought to be greater than was needed for maintenance purposes and intakes were to a degree restricted by not replenishing the concentrate promptly.

There were no significant differences in hay consumption between any of the groups.

Discussion

In considering the results of this trial, the pre-experimental treatment of the sheep must be taken into account. The sheep were on a sub-maintenance diet for 2 months prior to the trial and during this period, shortly after being shorn, the weather was extremely cold with snow and driving winds. The sheep, as a result, had already lost an average of 13.2 lbs. bodyweight before the commencement of the trial.

While statistically significant differences in bodyweights were obtained between groups, extention of the trial may have altered the criteria of measurement from one of differences in bodyweights to simply one of death or survival. It is reasonable to assume that, had the sheep been heavier at the start of the trial, wider differences would have appeared and further differences may have emerged.

Under the conditions of this trial, all sheep which did not receive some readily available carbohydrate, lost weight and would have died if the trial had been extended. Some South African workers[1 2 9] have shown that urea fed without a readily available carbohydrate can reduce the loss of liveweight experienced by animals grazing winter veld, but results in the field are often variable[10] and in this trial, urea fed alone, failed to arrest weight loss. The generally variable nature of the protein, cellulose and lignin fraction of roughage feeds is well known[11] and the variation in results obtained by different workers can probably be explained by differences in the energy availability and/or the nitrogen content of the roughages used[12 13 14].

The significant differences obtained between the urea, maize, molasses and natural protein block (V) and the urea, maize and m.d.d.s. block (R) suggests that the form of natural protein may be of significance. The protein in block V was derived from maize and cotton seed cake, while

that from the urea, maize and m.d.d.s. block (R) is derived from maize and molasses distillers dried solubles.

The sheep receiving the molasses meal concentrate (22% salt), lost weight, while the sheep receiving molasses meal concentrate (no salt), gained weight. The loss of weight of the former group cannot be explained satisfactorily on the basis of a lower intake, the only difference in treatment between the two groups being the inclusion of 22% salt in the supplementary feed of the sheep that lost weight. Depression in digestibility of crude fibre and protein on high salt levels (8%) has been reported[15], and this may be an explanation of the apparent depression of weight gain.

Summary

The efficacy of six forms of supplementary feeds designed for grazing animals during winter in sour veld areas were compared using German Merino sheep. All the supplements which combined urea with some form of carbohydrate were effective in reducing losses in bodyweight.

The control sheep on the basal diet of poor quality sour veld grass hay, as well as the group which received the urea and salt alone, lost significantly more weight than the other groups and would have died if the trial had continued.

This trial indicates that urea fed with a readily available carbohydrate plus a natural protein, is superior to urea and a readily available carbohydrate fed alone. It is thought that the type of natural protein included may be of some importance.

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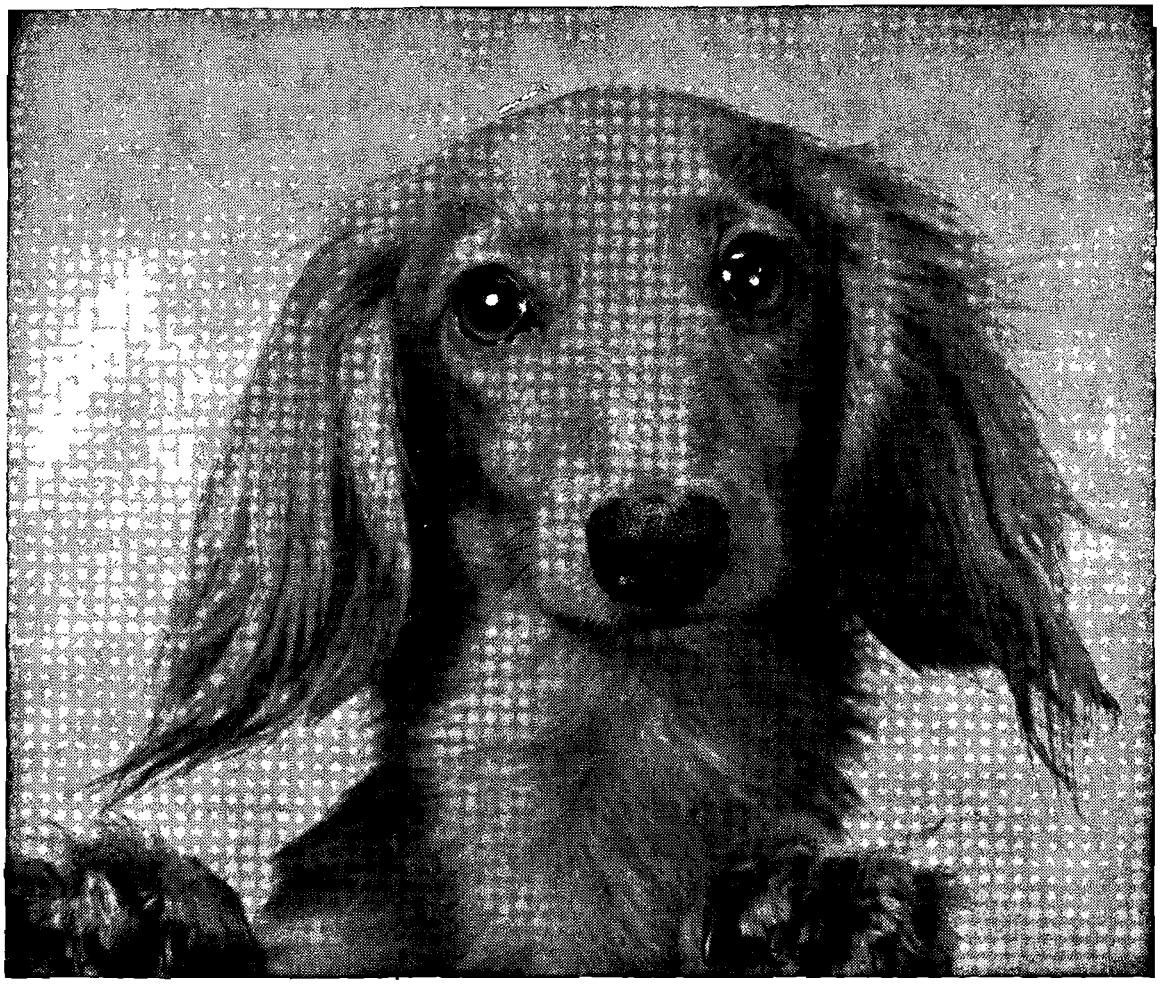

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THE HAEMAGGLUTINATION TEST AND IMMUNITY TO *PASTEURELLA HAEMOLYTICA*

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SUMMARY

A low grade immunity against *Pasteurella haemolytica* injection was produced in mice with whole cell vaccine. The apparent improvement in the antigenicity of such a vaccine when an adjuvant was added to it was not confirmed by statistical analysis of the results.

The haemagglutination test can be used to get some idea of the immunity of a group of sheep but is not reliable where individual animals are concerned. Polysaccharide, which is responsible for the formation of haemagglutinating antibodies, has some immunizing properties. A protein fraction of the cells is, however, also capable of stimulating the production of protective antibodies, and sera with low haemagglutination titres may be capable of conferring immunity to mice. Immunity to *P. haemolytica* is therefore not dependent on a single antibody.

INTRODUCTION

The incidence of mortality in sheep, caused by *Pasteurella* infection has increased greatly in South Africa during the past three years. *Pasteurella haemolytica* has been recovered alone or in association with *Pasteurella multocida* from at least 60% of the cases while no viruses or pathogenic pleuropneumonia-like organisms could be isolated[1]. Further, *P. haemolytica* has been isolated frequently from sheep suffering from diseases such as mastitis, abscessation, encephalitis and arthritis and according to a number of workers[2 3 4] this microbe is an important pathogen of sheep.

The immunization of animals against haemorrhagic septicaemia and other forms of pasteurellosis has given variable results[5 6 7 8]. One of the major obstacles in the evaluation of immunity tests has been the lack of a suitable serological test; thus the value of vaccine has been tested either on a small number of cattle, buffalo or pigs or by field trials. In the experiments reported here, the antigenicity of various *P. haemolytica* vaccines was found by actively immunizing mice and testing the immunity by the injection of living culture; in sheep immunity was assessed by determining the protective value of their sera for mice.

P. haemolytica, however, produces haemagglutinating antibodies which may be used to identify the different serotypes[9 10 11 12]. As this test is simpler to perform than the tedious mouse protection tests, an attempt was made to find if it would be a satisfactory means of determining the immunity status of immunized sheep.

MATERIALS AND METHODS

Media and vaccine production.

The following media were used:- Bain & Jones broth (B.J.)[13], Sterne & Hutchinson broth (S.H.)[14], Gallo & Valeri broth (G.V.)[15] and Y.P.C. broth[16].

After preparation, the media were dispensed in 200 ml or 500 ml amounts in small or large Roux flasks and sterilized by autoclaving at 120°C for 30 minutes.

For vaccine production, each Roux flask was inoculated with the overnight growth obtained from a blood tryptose agar slant. The flasks were agitated for 24 hours at 37°C; formalin (0.5% final concentration) was added to the cultures which were allowed to stand at 37°C for a further 48 hours.

When adjuvants were added to killed cell vaccine, 50.0 ml aliquots of culture were centrifuged at 4,000 g for 60 minutes and sufficient supernatant fluid drawn off with a syringe to compensate for the volume of adjuvant to be added. The adjuvants used were prepared and added to the cultures as indicated in Table 1.

In the experiment designed to find which antigen stimulated the formation of protective antibody, the extracts were prepared from dry cells. Polysaccharide was extracted by the phenol-water method of Mergenhagen[25] and protein was precipitated from the water phase by the method of Tauber & Garson[26].

TABLE 1.—PREPARATION OF ADJUVANT CELL VACCINES.

Adjuvant	Composition of Adjuvant	Volume of adjuvant added to culture ml.	Volume of concentrated
Oil[17,18]	White oil A*1 —11.4 ml Lubrol*2 — 0.6 ml	6.0	54.0
Lanolin[19]	White oil A —40 ml Lanolin*3 (white) — 4 ml Lubrol —0.25 ml	17.0	34.0
Aluminium[20] hydroxide	Al $(OH)_3$ gel*4 —1 ml Dist. water —3 ml	2.0	48.0
Tricalcium[21] phosphate gel	$CaCl_2$ $6H_2O$ (132 g/l), —15 ml. Na_3PO_2, $12H_2O$ (152 g/l)—15 ml Water —145 ml[22]	25.0	25.0
Saponin[23]	5% solution (white)	0.5	49.5
Calcium[15] chloride	Prepared according to Gallo & Valeri[15]	7.13	53.0
Aluminium[24] phosphate gel	Na_3PO_4. $12H_2O$, (136.84 g/l)—100 ml $AlCl_3$. $6H_2O$, (84.2 g/l)—100 ml	25.0	25.0

*1 Caltex *2 I.C.I. *3 Baird & Tadlock *4 B.D.H.

Immunity experiments.

The pathogenicity of five strains of *P. haemolytica* for mice was compared. Of these, strain 4367, isolated from the pneumonic lung of a goat, gave the most reproduceable results; 0.5 ml of a 1:4 dilution of a suspension equivalent to Brown's opacity tube No. 10, administered intraperitoneally, consistently killed 75% to 85% of the mice. This challenge dose was used in all the subsequent immunity experiments. The pathogenicity of the strain, which was also used for the preparation of vaccine, was maintained by regular passage through mice.

Groups of fifty mice were used to compare the antigenicity of different vaccines. Each mouse was given two subcutaneous injections, each of 0.2 ml, with an interval of three weeks between them, and was challenged ten days after the second injection.

Eight animals per group were used to study the antibody response in sheep. Each animal was given two intramuscular injections, each of 5 ml, with an interval of three weeks between them. The sheep were bled just before the first injection and then every ten days for periods of up to three months depending on the requirements of the experiment. The sera were stored at −20°C. The mouse protective value of each serum sample was tested in the following way. Three mice were each given 0.5 ml serum intraperitoneally and three were given 0.3 ml. Due to the large number of sera which had to be tested, not all could be titrated on the same day. The tests were therefore spread over three days and two mice treated with 0.3 and 0.5 ml of every serum, respectively on each of the three days. This had the added advantage that each serum was actually tested against three slightly different challenge doses which made the final result more reliable and significant.

This method was experimentally shown to give the clearest distinction between normal and immune sera. Increasing the dose accentuated the protective property of the normal serum, whereas if the dose was reduced, little difference could be shown between normal and immune serum.

The protective value of immune serum for mice was expressed as the percentage protection it afforded compared with the control mice which received pre-immunization serum.

The technique used for the haemagglutination tests was a slight modification of that described by Carter[27,28]. Guinea-pig red cells were used instead of human 0 cells and it was found that clearer agglutination patterns were obtained if the red cells, after treatment with antigen, were suspended in 1:200 normal rabbit plasma. Prior treatment of the cells with formalin or tannic acid had no advantage[29,30,31,32], nor did cells from different guinea-pigs influence the test. As slightly different titres were obtained with different anti-

gen preparations, one batch of antigen was used for all comparative tests.

The histograms were compiled from the average titres obtained from five serum samples collected from each group of sheep at ten day intervals over a period of fifty days.

Results

There was little difference in the densities of the growths obtained in B.J., S.H., G.V. and Y.P.C. broths inoculated with *P. haemolytica* and incubated for 24 hours at 37°C, but as the growth in B.J. broth was slightly better it was used for all further experiments. The average density of cells obtained fell between the opacity of Brown's opacity tube No. 10 to twice the opacity of tube No. 8.

Vaccines prepared from cells grown in the different broths showed no difference in antigenicity, nor could any difference in antigenicity be found in cells from 8, 12, 16 and 24 hour-old cultures.

The results of an experiment designed to find if adjuvants would improve the immunizing properties of cell vaccine for mice are shown in Table 2.

The immunity produced by each of the vaccines was significantly better than that possessed by the untreated mice but none of the adjuvants improved the antigenicity of the 'cells only' vaccine in a statistically significant way.

Some adjuvant-vaccines were also tested in sheep, but as shown in Table 3, none of them improved the antibody response. On the contrary, the highest protection and haemagglutination titres were obtained in the sheep which received vaccine without any adjuvant.

TABLE 2.—COMPARISON OF PROTECTION OBTAINED IN MICE WITH DIFFERENT ADJUVANT VACCINES.

Vaccine (Adjuvant)	Total Mice challenged	Survivors	Protection %
Cells only.....	30	19	63.3
Oil.....	41	33	80.5
Lanolin.....	43	34	79.1
$Al(OH)_3$.....	40	34	85.0
Ca_3PO_4.....	45	31	68.9
$AlPO_4$.....	46	37	80.4
Saponin.....	41	25	61.0
$CaCl_2$.....	44	28	63.6
None (Controls)..	50	14	28.0

TABLE 3.—ANTIBODY RESPONSE IN SHEEP TO ADJUDVANT VACCINES

Vaccine	Haemagglutination Average titres over 2 months Reciprocals	Passive mouse protection value of sera
		Average % increase in protection over 2 months
Cells only.....	50.0	27.8
Cells plus Oil.....	33.3	15.4
Cells plus Aluminium phosphate.....	46.0	9.7
Cells plus Aluminium hydroxide.....	28.18	14.9

The antibody response of sheep to oil adjuvant vaccine was examined and, as can be seen in Figure 1, there is a marked parallelism between the haemagglutination and the mouse protection titres.

However, four of twenty-four individual sera with high haemagglutination titres had poor protective properties while others which protected mice well had low haemagglutination titres.

This discrepancy between haemagglutination titres and protection titres was brought out more clearly by the following experiment. The haemagglutination titres and mouse protection titres of the sera of eighteen normal sheep were determined. The results were plotted and as shown in Figure 2, there is no correlation between haemagglutination and mouse protection titres.

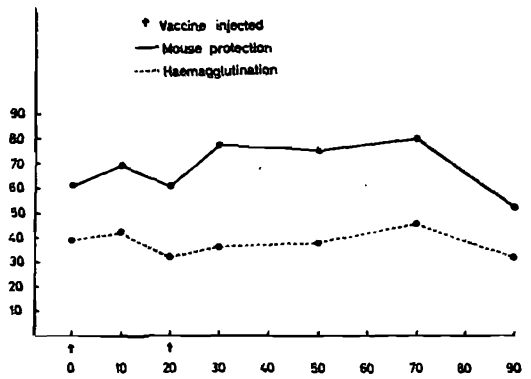

Figure 1.

Antibody response of sheep immunized with oil adjuvant vaccine.

It is clear that although the haemagglutinating antibody is closely associated with protection, the protective value of serum is not solely dependent on it.

To determine which antigen was in fact responsible for stimulating the formation of haemagglutinating and protective antibodies, polysaccharide and protein fractions and whole cells were administered to groups of sheep. The results of the serum titrations are shown in Figures 3 and 4. Polysaccharide is responsible for the formation of haemagglutinating and mouse protective antibodies. Protein on the other hand stimulated the formation of protective antibody but not of haemagglutinating antibody. The fact that immunity to *P. haemolytica* is not due to a single antibody is further confirmed by the finding that the best results were obtained with whole cell vaccine.

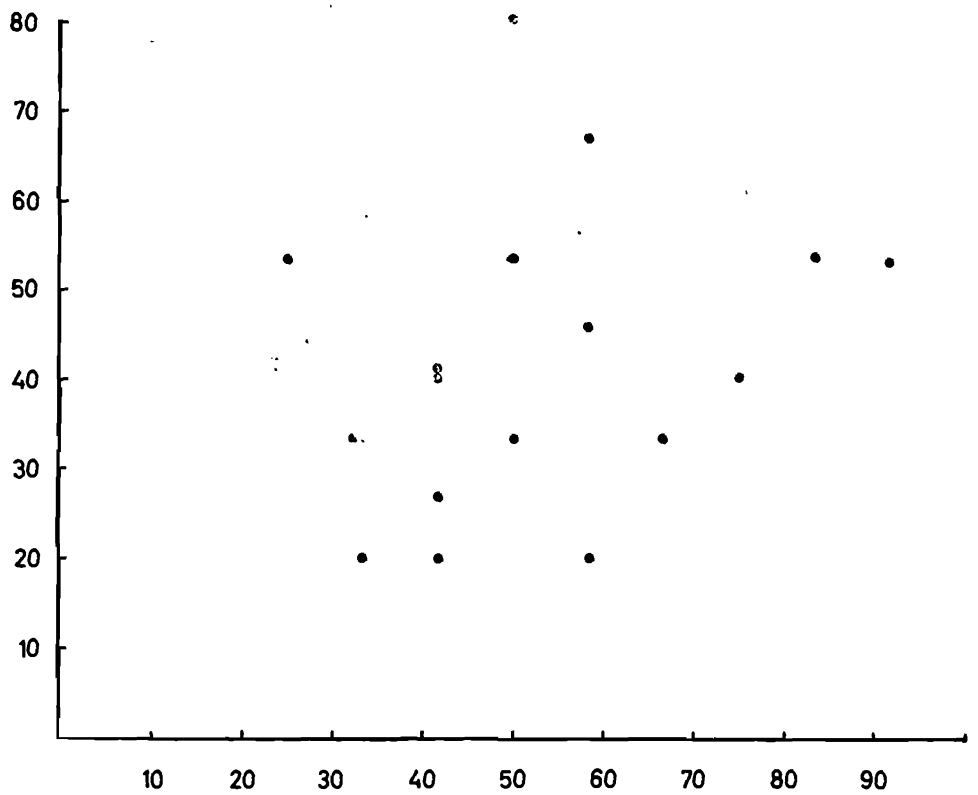

Figure 2

Haemagglutination and Mouse Protection Titres of Normal Sheep.

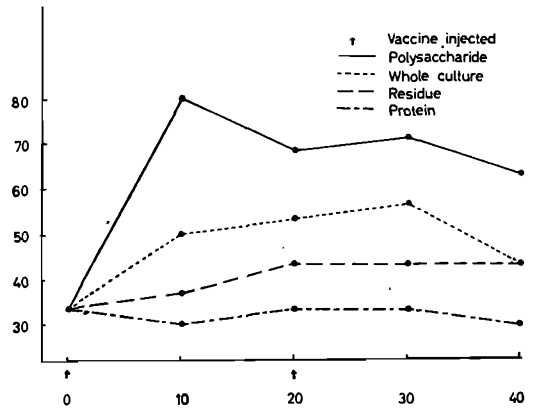

Figure 3.

Haemagglutination titres obtained with different cell fractions.

Discussion

Rebers and Heddleston[33] have shown that the surface polysaccharide of *Pasteurella multocida* is able to evoke a solid immunity to fowl plague in chickens and Carter[34] demonstrated the significance of the protective property of polysaccharide of this organism. He was able to show a close correlation between haemagglutinating antibody and mouse protection in pasteurella antisera. The experiments carried out in this study show that the polysaccharide of *P. haemolytica* has some immunizing value and that the haemagglutination test gives some measure of the immunity of a group of sheep but is not reliable where individual animals are concerned.

In 1965 Rebers and his co-workers[35], however, isolated a toxic, particulate, protective antigen, not a polysaccharide, from *P. multocida.* The results reported here have shown that cell fractions other than polysaccharide also play a role in producing immunity to *P. haemolytica.*

Immunity to *P. haemolytica* is therefore not dependent on one single antigen or antibody and for this reason other serological[36] and biological tests in addition to the haemagglutination test should be used to assess the value of *Pasteurella* vaccines.

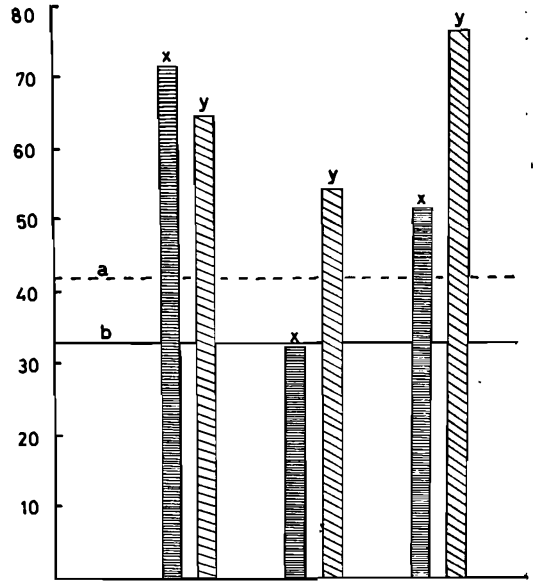

Figure 4.

Comparison of haemagglutination and mouse protection titres, obtained with different cell fractions.

a = *Haemagglutination titres of normal sheep serum.*

b = *Level of protection afforded to mice by normal sheep serum.*

x = *Haemagglutination.*

y = *Mouse protection.*

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BILTONG-INDUCED, *S. ENTERITIDIS* VAR. *TYPHIMURIUM* FOOD POISONING — A CASE REPORT

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SUMMARY

A short report on an outbreak of *S. enteritidis* var. *typhimurium* food poisoning caused by consumption of biltong is given.

The possible source of infection is briefly discussed.

INTRODUCTION

Galbraith[1] quoting the Monthly Bulletin of the Ministry of Health and Laboratory Services (Reports 1950-1960) reported an increase in the incidence of human Salmonellosis from 1369 cases in 1949 to 7846 in 1959. Of these var. *typhimurium* was responsible for 75.2 per cent with the highest incidence of 90 per cent reported during 1954. Ninety-eight per cent of *S. enteritidis* var. *typhimurium* positive cases reported in 1959 was traced to meat (47 per cent) and eggs (42 per cent).

The important rôle played by meat as a source of human Salmonellosis has been emphasized repeatedly[2 3]. Anderson, Galbraith and Taylor[4] reported 90 incidents of human food poisoning caused by var. *typhimurium* all of which were traced to calves' meat.

S. enteritidis var. *dublin,* on the other hand, plays a less important rôle in human Salmonellosis. Gibson[5] quoted only 3 reported cases where var. *dublin* was involved. This finding is rather surprising as the host-adapted var. *dublin* is the most predominant serotype associated with bovine Salmonellosis[5 6 7].

During recent years Salmonellosis amongst beef and dairy cattle has become a serious problem[8]; Moore, Rothenbacher, Bennett and Barnes[9] found 78 apparently healthy cattle, slaughtered for human consumption, carriers of *Salmonella.*

Seen in the light of the high incidence of Salmonellosis in the bovine and the large amount of biltong (dried meat) annually consumed, it is surprising that only a few cases of food poisoning due to this delicacy have been reported. Even more surprising is, that in not one of these cases, var. *dublin* or var. *typhimurium* were incriminated. The two cases reported were caused by the rather uncommon serotypes, var. *lomita*[10] and var. *newport*[11].

Case History

An ox, specially conditioned, was slaughtered during June, 1965. Preparation of biltong was done according to the customary method described by Van den Heever[12]. As the biltong was not completely dry, only a small portion was sampled by members of the family during early August. The same afternoon all persons who participated in this sampling, developed headache, nausea and diarrhoea.

The biltong was suspected as being the source of infection and 4, six inch pieces were forwarded to Onderstepoort for bacteriological examination. A small portion (1x1 cm) of each piece was homogenized, suspended in sterile physiological saline and plated directly onto S.S. agar. After 12 hour incubation pure cultures of *S. enteritidis* var. *typhimurium* were obtained from all four plates.

DISCUSSION

As far as the author is aware, this is the first reported case of biltong-induced food poisoning caused by *S. enteritidis* var. *typhimurium.*

The important question arising from this case is whether infection took place during processing (salting) or whether the animal was suffering from *Salmonella*-septicaemia at the time of slaughter.

The available data strongly suggest that the animal was the source of infection and, most probably would have died shortly of acute Salmonellosis, had it not been slaughtered.

In support of this conclusion Van den Heever[12], found it extremely difficult to contaminate biltong artificially during the salting process but dry biltong prepared from the meat of a calf which had died of Salmonellosis (var. *dublin*) was infected even 6 months later[13].

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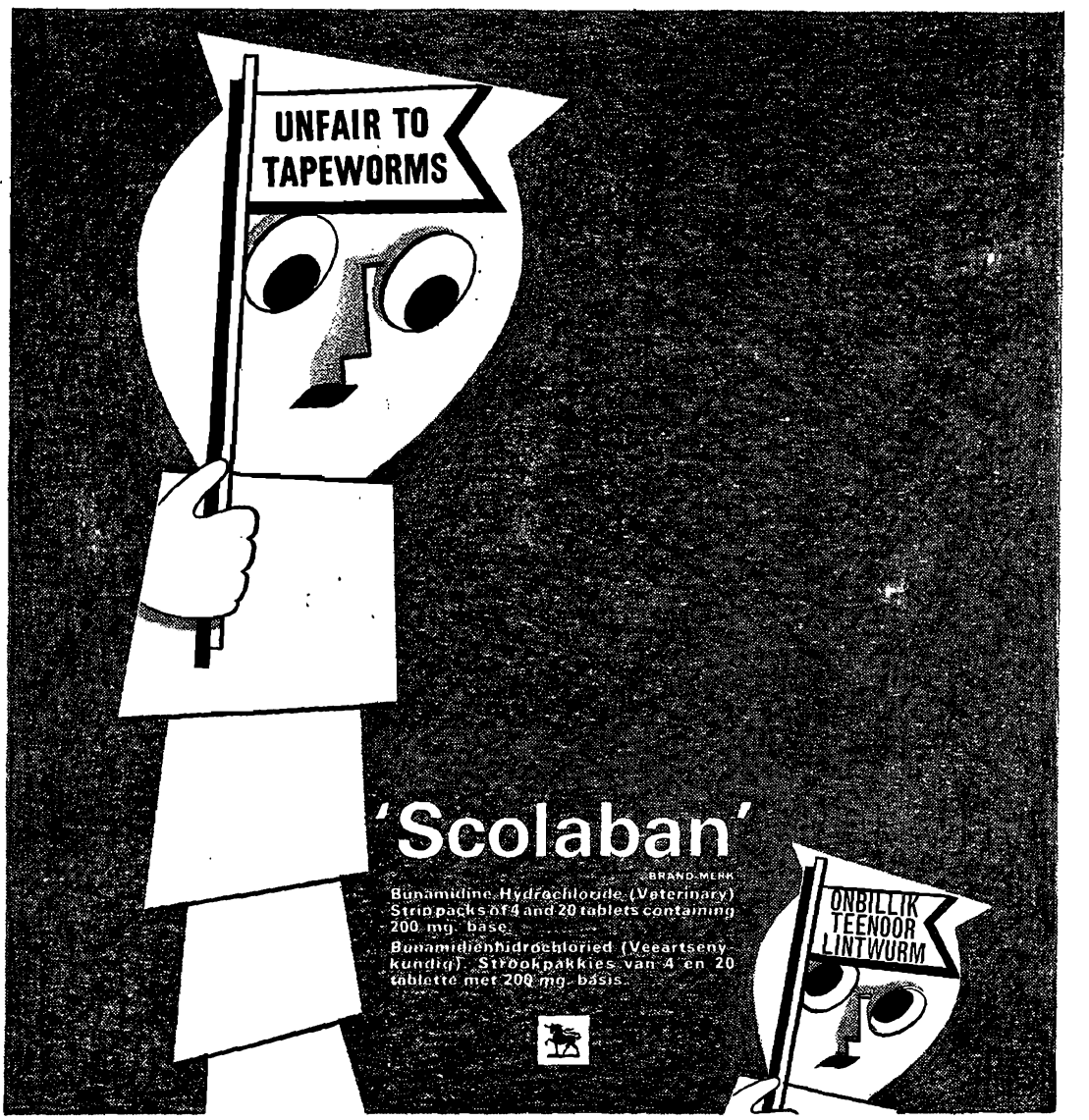

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THE AVIAN AND BOVINE COMPARATIVE TUBERCULIN TEST USING ONDERSTEPOORT PPD TUBERCULINS

R. W. WORTHINGTON* AND H. H. KLEEBERG*.

SUMMARY

Investigations were undertaken in tuberculous and non-tuberculous cattle using Onderstepoort PPD tuberculins. The results were analysed statistically and the most suitable interpretation standards for the comparative test determined. It was shown that the comparative test was able to differentiate more accurately between tuberculous and non-specificially sensitized cattle, than a test using bovine tuberculin alone.

INTRODUCTION

The difficulties encountered with the non-comparative tuberculin test (bovine PPD alone) and methods of overcoming them were discussed in a previous paper[1]. The comparative test using mammalian and avian tuberculins simultaneously in the same animal has been used with success in many countries[2 3 4 5 6 7 8] but this test has not been popular with South African veterinarians. Reasons generally given are that there are no logical grounds for using the test as avian tuberculosis is rare in South African poultry and Johnes disease almost non-existent. The avian tuberculin prepared at Onderstepoort from the strain D_4 was also blamed for causing too large reactions in tuberculous cattle and for not covering a sufficiently wide range of non-specific sensitizations.

Recent investigations into the cause of non-specific reactions have shown that the most common cause of sensitization in non-tuberculous herds in South Africa is probably *M. avium* or closely related avian-like strains([9]). These findings indicated that the avian-bovine comparative test could probably be used to advantage in this country.

The locally isolated strain, 20485, is now being used instead of D_4 for the production of avian tuberculin. Our bovine tuberculin is produced from the strain AN[5] which is also the strain used in Holland. Onderstepoort avian tuberculin is issued in the same strength (25,000 TU per ml) as avian tuberculin used in other countries but our bovine tuberculin is issued at a strength of 70,000 TU per ml while the European common market countries use mammalian tuberculin at a strength of 50,000 TU per ml and England, the United States, Australia and New Zealand use tuberculin containing 100,000 TU per ml. Our interpretation standards for tuberculin testing have been derived arbitrarily, similar to standards used overseas. In view of our different tuberculins, our different animals, husbandry methods and climatic conditions, it was necessary to investigate thoroughly the comparative test using Onderstepoort tuberculins under local conditions. Suitable interpretation standards could then be derived using the data from this study.

MATERIALS AND METHODS

All cattle were tested with Onderstepoort PPD tuberculins. The bovine tuberculin containing 70,000 TU per ml and avian tuberculin 25,000 TU per ml. Amounts of 0.1 ml. of each tuberculin were injected in two sites about six inches apart on the same side of the neck, the avian tuberculin was injected in the anterior site. Skinfold thickness was measured with spring operated semi-automatic calliper and recorded at the time of injection and 72 hours later. Reactions were recorded as the increase in skinfold thickness measured in millimeters.

Tuberculous cattle: The cattle used were from eight heavily infected tuberculous herds containing a total of 1,700 cattle which are being used in experiments on the chemotherapy of bovine tuberculosis with the drug Isoniazid. All cattle were

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tested before treatment started, 2 months after the start of treatment and 6-8 months after the start of treatment. When tuberculous cattle are treated with Isoniazid they gradually lose their sensitivity to tuberculin as they become cured([10 11]). The records of these tests were nevertheless useful because by carefully studying the reactions of each cow over at least two consecutive comparative tests it was possible to judge with a high degree of accuracy which animals were in fact tuberculous. Only reactions in 443 cattle believed to be tuberculous and tested before treatment were included in the investigation. The herds investigated were all within 100 miles of Onderstepoort, in the highveld region. All herds, except one, were commercial dairy herds, 93% of the cattle were Friesians, 6% Jersey and 1% Brown Swiss.

Non-Tuberculous cattle: In order to obtain a large sample of non-specific reactors, herds were selected in which this type of reaction occurred more frequently than usual. Only cattle from herds considered to be free from tuberculosis were used. Herds were considered to be free of tuberculosis when a consideration of the herds' history indicated that there was no infection, when reactions were generally small, hard and circumscribed, when reacting animals showed a tendency to convert to negative on retesting, and when reactions to avian tuberculin were generally larger than to bovine tuberculin.

The animals used came mainly from three sources:

(1) Herds in which Isoniazid treatment had been used. In this case only herds in which the spread of the disease had been completely arrested were used. Animals which were previously positive reactors were not included. Records of six monthly tests of each animal for a period of 3-4 years were available. Only animals from the clean part of the herd in which no new cases of tuberculosis had been detected during this time were included.

(2) To boost the number in the sample we included cattle which had been tested by colleagues of the Veterinary Field Services. Only herds judged to be free of tuberculosis by the veterinarian performing the test, and where a study of the available records confirmed this opinion were included.

(3) Herds which were specifically investigated by the authors because of their history of containing non-specific reactors.

The reactions in 940 non-tuberculous animals from some 3,500 cattle in 22 herds were included in this study. The sample again included mainly Friesian cattle, mainly from the Transvaal Highveld region. Some herds widely distributed over the whole country and of varying breeds were, however, also included.

The reactions (skinfold increase) to bovine tuberculin, to avian tuberculin and the difference bovine reaction — avian reaction were recorded. Frequency distribution tables of reactions to the two tuberculins and the differences between the reactions to the two tuberculins were constructed for tuberculous cattle and non-specific reactor cattle. According to the range of the reactions class intervals of 1 or 3 mm were chosen and histograms drawn. The mean reactions were calculated for each distribution from the grouped data by standard statistical methods([12]). Less-than-cumulative frequencies were calculated for the distributions of the differences bovine reaction — avian reaction for the tuberculous and the non-tuberculous cattle. Cumulative frequencies were then expressed as percentages and plotted on a large scale on graph paper as less than cumulative frequency curves, using class intervals of 1 mm. Similarly less than cumulative frequency curves were constructed for all the reactions to bovine tuberculin in the tuberculous cattle and for the reactions of 1 mm or more to bovine tuberculin in the non-tuberculous cattle.

Results

In the 1,700 cattle tested in tuberculous herds, 443 or 26.1 per cent were considered to be tuberculous. The mean reaction to the bovine tuberculin in these animals was 14.0 mm and the mean reaction to the avian tuberculin was 4.5 mm. The distribution of the reactions to the two tuberculins are shown in histogram form in Fig. 1. Both distributions show a marked positive skewness which precludes the use of normal distribution theory for further analysis of the data. It is very obvious that the reactions to bovine tuberculin were generally much greater than the reactions to avian tuberculin.

In the 3,500 cattle tested in non-infected herds, 940 or 26.9 per cent reacted to one or both of the tuberculins. The mean reaction to bovine tuberculin in the reactor cattle was 2.3 mm and the mean reaction to avian tuberculin was 3.7 mm. In contrast to the position in the tuberculous herds it can be seen in Fig. 2 that the reactions to bovine tuberculin are generally smaller than the reactions to avian tuberculin. Both distributions again show a definite positive skewness. The range of the reactions to both avian and bovine tuber-

culin was much smaller in the non-tuberculous cattle than in the tuberculous animals.

The frequency distributions of differences between the reactions to bovine and avian tuberculin (bovine reaction — avian reaction) in the tuberculous cattle and in the non-tuberculous reactor cattle are shown in histogram form in Fig. 3. The reactions to bovine tuberculin were greater than the reactions to avian tuberculin in almost all the tuberculous animals. Alternatively, most of the non-tuberculous animals were more sensitive to avian tuberculin than to bovine tuberculin. The mean of the difference of the reactions was 9.66 mm in the tuberculous cattle and -1.22 mm in the non-tuberculous cattle. The distribution in the case of the non-specific reactors appeared to be approximately normal, but in the tuberculous animals there was a marked positive skewness. The less than cumulative frequency curves for the two distributions are shown in Fig. 4. From the curves one can read off the percentage of tuberculous cattle in which the difference between the reactions was less than any given limit. In other words the percentage of tuberculous cattle which would have been incorrectly diagnosed as negative if this limit was regarded as the definitive limit for distinguishing tuberculous from non-tuberculous cattle. Similarly it is possible to read off from the graph the percentage of non-specific reactor cattle in the sample that fell below any given limit and, therefore, by subtraction the percentage of non-specific reactors with reactions above this level, i.e. the number of non-tuberculous cattle that would be classed as positive. These results for various differences in the two reactions are given in Table 1.

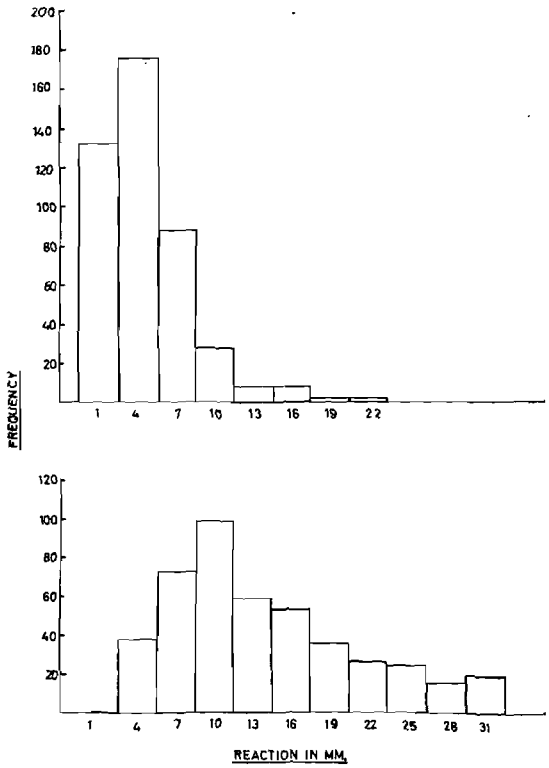

FIG. 1.

Frequency distributions of reactions to avian (above) and bovine (below) PPD in 443 *tuberculous* cattle.

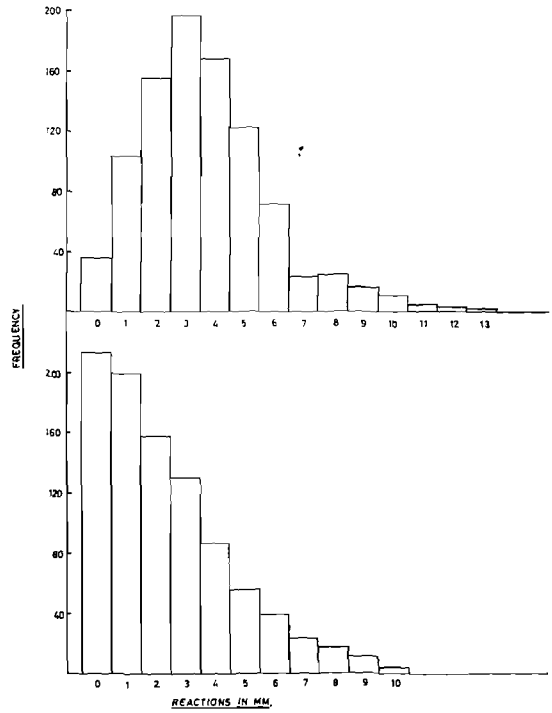

FIG. 2.

Frequency distributions of reactions to avian (above) and bovine (below) PPD in 940 *non-specifically sensitized cattle.*

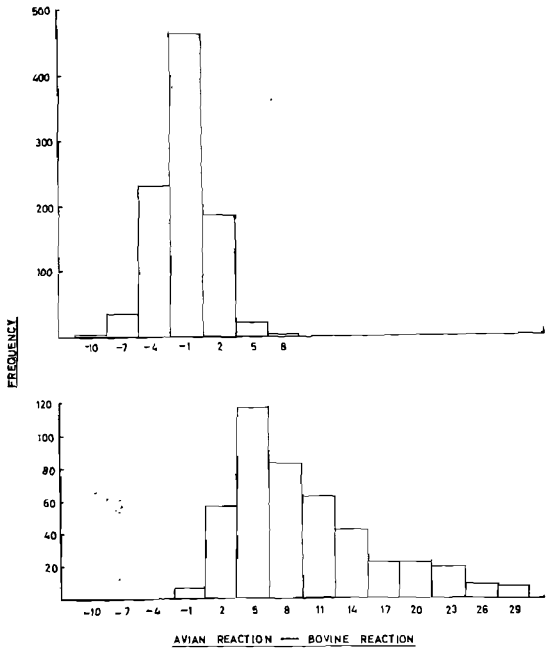

FIG. 3.

Frequency distributions of the difference bovine reaction — avian reaction in 940 *non-specific (above) and* 443 *tuberculous cattle (below).*

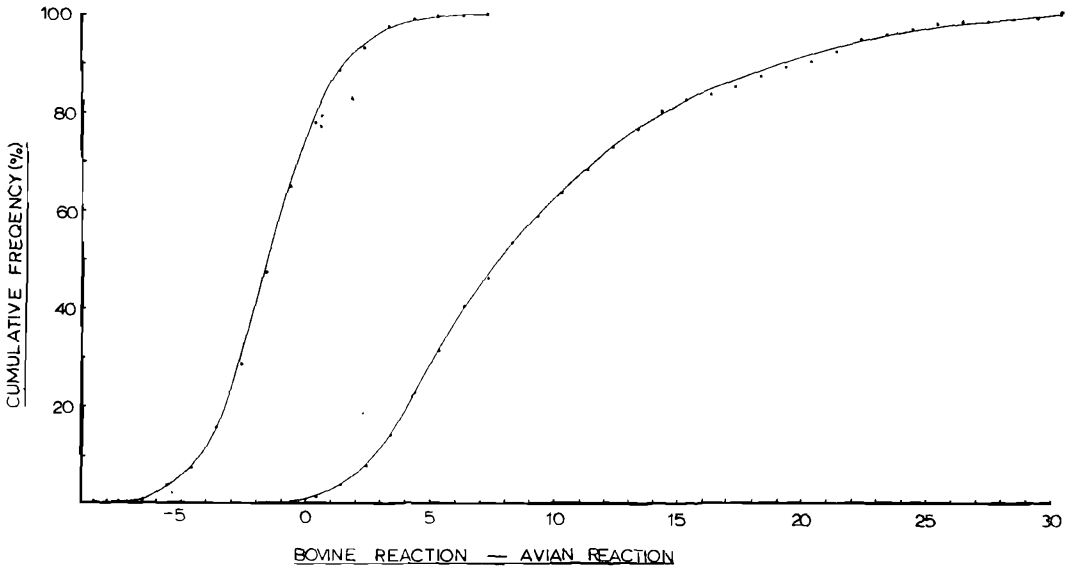

FIG. 4.

Cumulative frequency curves of the difference bovine reaction — avian reaction in non-specifically sensitized (left) and tuberculous (right) cattle.

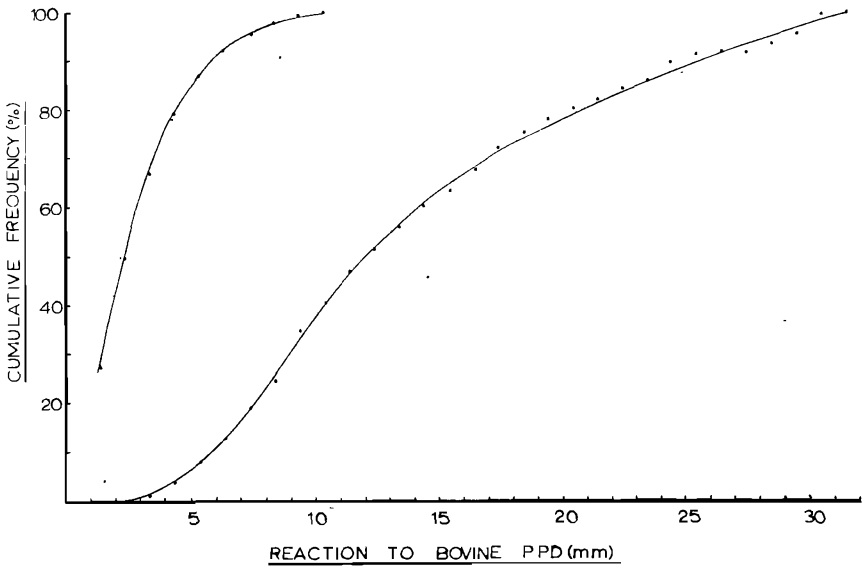

FIG. 5.

Cumulative frequency curves of reactions to bovine PPD in non-specifically sensitized (left) and tuberculous (right) cattle.

TABLE 1.—DIAGNOSTIC ACCURACY OF THE COMPARATIVE TEST.

Difference avian reaction—bovine reaction	% Tuberculous animals in which the difference was less than the limit given in column 1	% Non-specific reactors in which the difference was greater than the limit given in colum 1
—1.0 mm.....	0.0%	41.5%
0 mm.....	0.7%	27.0%
1 mm.....	2.5%	14.0%
2 mm.....	6.0%	8.0%
3 mm.....	12.0%	4.0%
4 mm.....	19.0%	1.5%
5 mm.....	28.0%	0.5%

Less than cumulative frequency curves of all the reactions to bovine tuberculin in the tuberculous and the non-specific reactor cattle are shown in Fig. 5. From these curves the percentage of tuberculous cattle that would be classed as negative, and the percentage of non-specific reactors that would be classed as positive at any level can also be determined. These figures are given in Table 2.

TABLE 2.—DIAGNOSTIC ACCURACY OF THE TUBERCULIN TEST USING BOVINE TUBERCULIN ALONE.

Reaction to bovine tuberculin	% Tuberculous animals with reactions lower than limit given in column 1	% Non-specific reactors with reactions greater than limit given in column 1
2 mm.....	0%	57.5%
3 mm.....	1.0%	39.5%
4 mm.....	2.5%	25.0%
5 mm.....	6.0%	16.0%
6 mm.....	11.0%	10.0%
7 mm.....	17.5%	5.5%
8 mm.....	22.5%	3.0%

Discussion

A number of criticisms may be levelled at the method of investigation especially the fact that the procedure used to classify animals as tuberculous or non-tuberculous was the tuberculin test. The tuberculin test was, however, used on a herd basis and only animals from pathologically proven tuberculous herds were included in the sample of tuberculous reactors, furthermore animals were judged as tuberculous on the results of two or three consecutive comparative tests which is a far more accurate method of diagnosis than a single test. The samples were large and a few wrongly diagnosed cases should not effect the final results to any extent. Control slaughter was not possible because of economic reasons. To check the interpretation formulas derived from this work post-mortem examination of a large number of reactors should be done at the beginning of an eradication campaign. The samples were not representative of all breeds and of all geographic areas of the country. The main tuberculous problem, however, occurs in our commercial dairy herds which consist predominantly of Friesian cattle and this group was well represented.

A big percentage of non-specific reactors in South Africa are more sensitive to avian tuberculin than to other mycobacterial sensitins[9]. The frequency distributions of the reactions are, therefore, similar to what was anticipated with the tuberculous cattle being markedly more sensitive to bovine PPD and the non-tuberculous cattle more sensitive to avian PPD. What is, however, of great importance is to derive optimal interpretation standards for the comparative test with Onderstepoort PPD[5] in naturally sensitized South African cattle. An ideal test would detect 100% of tuberculous cattle, and class 100% of non-specific reactors as negative. This ideal cannot be achieved at the present time. For epidemiological reasons it is preferable to have an interpretation formula which errs slightly to the strict side. In practice a herd is never declared negative as a result of a single test, three negative tests being required in the herd before it is granted accreditation. Theoretically, if the first test is 95% accurate 5% of tuberculous animals will escape the first test, 95% of these should be detected at the next test so that only 0.75% will be undetected after 2 tests and 0.01% after three tests. A triple test which detects 95% of infected animals each is, therefore, a very accurate test. A method which still further increases the accuracy of the tuberculin test is to classify herds as tuberculous and non-tuberculous and to interpret very strictly in tuberculous herds and more leniently in non-tuberculous herds. This method is used for interpretation of comparative tests in England[13] and for the interpretation of the non-comparative test in South Africa[14]. This procedure should be adopted when laying down interpretation standards for the comparative test.

After careful scrutiny of the data given in Table 1 the following formula was considered to be the most satisfactory.

Tuberculous Herds:

Avian reaction equal to or larger than bovine reaction — negative for TB.

Bovine reaction up to 2 mm larger than avian reaction — suspicious for TB.

Bovine reaction more than 2 mm larger than avian reaction — positive for TB.

The reaction to bovine tuberculin must, however, be 4 mm or greater before an animal can be declared positive.

Non-tuberculous Herds:

Bovine reaction up to 2 mm larger than avian reaction — negative for T.B.

Bovine reaction 2-4 mm larger than avian reaction — suspicious for TB.

Bovine reaction more than 4 mm larger than avian reaction — positive for TB.

It can be seen from Table 1 that, if this key had been used 94% of tuberculous animals in our sample would have been classed as positive and 99% as positive and suspicious. If the second formula had been used in the non-tuberculous cattle only 1.5% of non-specific reactors would be classed as positive. Furthermore the operator should be allowed to use his discretion to class positive reactors as suspicious or vice-versa, so that, when the whole herd history and other relevant factors are considered, the accuracy should be still further increased. In this respect the type of reaction i.e. whether oedematous, painful, hot and diffuse or whether hard and circumscribed should be particularly considered. A problem in using the comparative test is the classification of animals highly sensitive to both avian and bovine tuberculin. These cattle might have been sensitized by *M. avium* or another species of *Mycobacterium* and later infected with bovine tuberculosis. These cases should, therefore, be regarded as highly suspicious in tuberculous herds.

The principal finding of this study is that the comparative test is a more accurate diagnostic method than a test using bovine tuberculin alone. Another study by R. Worthington has shown that there was no significant difference in the distribut-

ion of reactions to bovine tuberculin in tuberculous cattle tested with bovine tuberculin alone or by the comparative test. Whether there is a significant difference in non-specific reactors is not known. It can, however, be seen from the results given in Tables 1 and 2 that the comparative test is able to differentiate more accurately between tuberculous cattle and non-specific reactors than a test using bovine tuberculin alone. In the comparative test the most definitive limit for interpretation is a 2 mm difference between reactions to bovine and to avian tuberculins. In this case 6% of tuberculous cattle are missed and 8% of non-tuberculous cattle are declared positive. With bovine tuberculin alone the most definitive limit is a reaction of 6 mm. In this case 11% of the tuberculous cattle are missed and 10% of the non-tuberculous cattle are declared positive. At the moment a limit of 6 mm is used in non-tuberculous herds and 4 mm in tuberculous herds[14]. The 4 mm limit would have missed only 2.5% of tuberculous cattle in this sample but would have made 25% of non-specific reactors positive.

In South Africa the bulk of tuberculin testing is done with bovine tuberculin only. This method has given satisfactory results, but when large numbers of non-specific reactors are encountered, confusion arises. In known problem herds and when retesting suspicious reactors the comparative test should always be used. The interpretation of tests as described in this article is based to a large extent on a consideration of the herd history and test results. In small herds and individual animals the operator will have less information available; in this case the comparative test is again superior. In general, when the comparative test is used, non-specific reactors can be more readily recognized and, therefore, retesting, test slaughter and unnecessary slaughter can often be avoided. Great economic benefit could, therefore, be derived from a more general use of the comparative test.

ACKNOWLEDGEMENTS

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FURTHER STUDIES OF THE TOXICOLOGY OF *LASIOSIPHON BURCHELLII* MEISN (HARPUIS BOS)

M. TERBLANCHE*, M. J. PIETERSE†, T. F. ADELAAR* AND J. D. SMIT*.

SUMMARY

An acetone extract of the plant has been prepared and its toxicology studied on sheep, rabbits, guinea-pigs, rats, dogs and pigs. Considerable species variations in susceptibility were found. The guinea-pig was found to be a very suitable laboratory test animal. The symptoms and pathological lesions caused by the extract are described.

INTRODUCTION

Lasiosiphon (*Gnidia*) *burchellii* Meisn. (Afrikaans Harpuisbos) was first proved toxic to sheep by Steyn(1) in 1932. In 1939 Van der Walt(2) reported negative tests for prussic acid on this plant. A chemical investigation on this plant with the purpose of isolating the toxic principle has now been undertaken. For the purpose of determining the toxicity of plant extracts a small laboratory animal was required. A batch of dried plant material which was toxic to a Merino sheep at a rate of 3.3 g/kg, producing the typical symptoms described by Steyn(1)(3), was therefore dosed through a stomach tube to two adult female rabbits. The first received 2.2 g/kg/day for 5 days and the second 3.3 g/kg/day for 6 days. No symptoms or deaths occurred. Due to the bulk of the material the dosage could not be increased. The rabbit, therefore, seemed to be insusceptible. No other small laboratory animal was available to which plant material could be dosed in large amounts. Large amounts of extracts, therefore, had to be prepared so as to determine its toxicity to sheep. A toxic extract was prepared and dosed to guinea-pigs. These proved highly susceptible and from then on were used as test animals. The symptoms in this species differed somewhat from those in the sheep in that they did not show the typical dyspnoea with loud groaning. After further work an improved method of preparation of the extract, which is described here, was developed. This was first dosed to 9 sheep which developed the typical symptoms. It was then used on 5 rabbits, 22 guinea-pigs, 7 rats, 2 pigs and 3 dogs. The results of the findings are reported here.

PREPARATION OF THE TOXIC EXTRACT

A batch of the plant collected in the Kuruman district was first proved toxic by dosing it per stomach tube to a Merino ewe at the rate of 3.3 g/kg. The animal showed typical symptoms as described by Steyn(1) and died after 19 hours.

The ground leaves were extracted continuously for five days with acetone in a soxhlet apparatus. On cooling the solvent a precipitate formed which was filtered off and recrystallised from ethyl acetate. This product proved non-toxic to sheep and guinea-pigs. The acetone was evaporated *in vacuo* yielding a black tarry toxic substance. This was extracted with petroleum ether (B.P.40-60°C) giving a black oily extract which proved to be non-toxic. The petroleum ether insoluble residue was shaken repeatedly with cold water and the extract so obtained proved non-toxic. The residue, an amorphous dark green substance, was highly toxic and formed the extract used in the toxicological studies. The yield was approximately 1 g from 45 g of plant material.

SPECIES SUSCEPTIBILITY

Details of the various toxicity trials are given in note form in Appendix 1. The findings may be summarised as follows:

The M.L.D. of the extract for sheep was found to be approximately 40 mg/kg which is equivalent to 1.8 g/kg of the dried plant. Nine sheep were used to establish this figure.

This plant is known amongst the Zulus in Dundee as Usinga Lwe Salukasi; which means that which will make the old woman's back strong.

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† Soils Research Institute, Pretoria.

The guinea-pig was found to be highly susceptible with an M.L.D. (extract) of approximately 22.4 mg/kg or 0.9 g/kg of the dried plant. This animal is, therefore, suitable for use in testing the toxicity of plant extracts.

The rabbit proved to be very refractory, the M.L.D. of the extract lying between 1-1.5 g/kg which is equivalent to 45-67.5 g/kg of the dried plant. This finding explained previous failures to produce poisoning in rabbits by dosing the dried plant as the very large doses required could not be administered.

The M.L.D. of the extract for the rat was found to be between 200 and 300 mg/kg or 10 to 15 times higher than that for the guinea-pig.

The extract was also tested on three dogs but they invariably vomited after being dosed and showed no further effects.

Of two pigs used, one showed vomition and diarrhoea after being dosed at the level of 100 mg/kg while the other died after receiving four daily doses at the same level.

CLINICAL SYMPTOMS

Sheep.

The acute cases died within 24-48 hours after dosing. In some no symptoms were observed prior to death. A typical symptom was accelerated respiration characterised by a short sharp inspiration and a prolonged forced expiration accompanied by a groaning sound which may start within hours after dosing and can be heard from a dozen paces off. On auscultation sharp, dry alveolar sounds were heard. The animals were reluctant or unable to stand. One animal when forced to stand collapsed in spasms. A clear or haemorrhagic nasal discharge often occurred. The animals often pushed against objects with their heads and refused all food although the ruminal movements might persist. At death the heart continued to beat for 30-60 seconds after respiration had ceased.

In subacute cases, i.e. those that showed symptoms for a week or more with subsequent recovery or death, the dyspnoea and nasal catarrh described above was also present accompanied by ruminal atony, anorexia and a foetid diarrhoea. In some cases the dyspnoea disappeared but the diarrhoea persisted even for weeks with ultimate recovery or death. Tachycardia (140 (88-190) beats/minute) appeared on the first day after dosing and persisted for the course of the symptoms. The temperature may be immediately elevated by up to 3°C until death or return to normal after a week in long standing cases. It might then again show a rise just prior to death.

One animal showed slight but constant muscular tremors with nodding of the head which started 3 days after dosing and persisted for 2 days while another showed complete paralysis of the hindquarters.

The mortality was some 80% of cases which developed symptoms.

Guinea-pig.

Most of the fatal cases died within 5 to 20 hours or up to 3 days after dosing without any symptoms having been observed. The most common symptom seen was paresis which set in within 10 minutes after dosing. The animals showed reluctance to move and when forced to do so exhibited incoordination which progressed to total paralysis.

In the long standing cases a diarrhoea lasting 1-2 days, listlessness and staring coat, which lasted up to 6 days, were seen.

The Rat.

Symptoms in the rat resembled those seen in the guinea-pig.

The Rabbit.

Three of the four fatal cases died without any symptoms having been seen. The fourth showed loss of condition and dehydration probably due to a lack of intake of food and water. One rabbit showed an increased amount of mucous in the faeces.

The Pig.

The pig that recovered showed a yellow diarrhoea as did the one that died but in this case it later became haemorrhagic.

The Dog.

The only symptom seen was vomition.

CHEMICAL PATHOLOGY

Blood samples were collected from the more chronic cases in sheep daily for the first week and twice weekly thereafter. The following were determined:- Red cell sedimentation rate, packed cell volume, haemoglobin concentration, leukocyte count, blood glucose, urea nitrogen, creatinine, cholesterol, calcium, inorganic phosphate, magnesium, chloride, bicarbonate, serum proteins, bilirubins, glutamic oxalo-acetic transaminase, alkaline phosphatase, zinc sulphate turbidity test. The methods were as previously recorded[4].

Despite this fairly comprehensive battery of tests the only positive findings in the sheep were a

tendency to hyperglycaemia and a rise in blood urea nitrogen between the 2nd and 4th days. There was also a more persistent tendency for an increase in the bicarbonate level. The pigs showed a moderate rise in blood urea nitrogen and creatinine.

AUTOPSY FINDINGS

Sheep.

The most significant finding in sheep which had died of acute poisoning was a marked emphysema and moderate congestion and oedema of the lungs accompanied by a generalised congestion of the carcass. Focal hyperaemia was found in any or all of the following:- Rumen, omasum, abomasum and caecum. Histological examination of four spleens revealed caryorrhexis of the lymph follicles in one. Focal congestion was found in one heart.

In the subacute cases the congestion and oedema of the lungs was more pronounced and the emphysema less marked. Generalised congestion, especially of the liver, was common. Three cases showed nephrosis but unfortunately blood analyses had not been carried out in two while the third had not shown a rise in blood urea nitrogen. Atrophy of the lymphoid tissue of the spleen was seen in two cases. Other very common findings were atony of the fore-stomachs, hyperaemia of the alimentary canal, sometimes with focal haemorrhages, and signs of a diarrhoea. The canal was mainly affected cranially. Other lesions encountered were frequently subepi-and subendocardial haemorrhages, marked chronic myocarditis with round cell infiltration and connective tissue formation in one case, myocardial focal necrosis in another, and myocardial oedema in a third.

Rabbit.

Lesions encountered in the rabbits included: generalised congestion, hyperaemia and oedema of the lungs, moderate hyperaemia and petechiae in the gastro-intestinal canal especially caudally, soft faeces in the rectum. Two cases showed multiple focal ulcerative purulent caecitis. Although no diarrhoea had been seen clinically, rectal content was soft. Liver lesions seen varied from congestion only, slight central fatty changes with necrobiosis, focal neutrophil infiltration, to light diffuse fatty changes with focal purulent hepatitis. Focal myocarditis, hydropericard, nephrosis, splenic haemorrhages and focal haemorrhages in lymph nodes were seen in individual cases.

Guinea-pig.

The most common lesions were in the gastro-intestinal canal. In the stomach and small intestine these varied from hyperaemia to acute haemorrhagic inflammation with blood in the lumen. Hyperaemia and petechiae in the caecum were also common. The lungs showed a mild emphysema often occompanied by hyperaemia and oedema. In many cases the liver was unaffected but congestion, fatty changes, diffuse necrobiosis, hyaline droplet degeneration, neutrophil infiltration, embolism and severe central necrosis in individual cases were encountered. Other lesions found were congestion and haemorrhages in the myocard and adrenals; congestion of the kidneys, brain pancreas and lymph nodes. One case showed reactive hyperplasia in a lymph node.

The Rat.

The one case examined showed generalised congestion and slight haemorrhages in the lungs, congestion of the liver, marked haemorrhages in the spleen and haemorrhagic enteritis of the small intestine with blood in the lumen.

Pig.

The one case showed emphysema of the lungs, congestion of the liver, severe haemorrhagic gastritis especially of the pyloric region.

DISCUSSION

To date the toxicity of *Easiosiphon burchellii* Meisn. has been proved experimentally only in sheep(1). An acetone extract has been prepared from the dried plant material which when dosed to sheep produced the identical symptoms to those caused by the plant. It was, therefore, assumed that the extract contained the active principle. This extract was then dosed to different species of animals. The guinea-pig proved to be highly susceptible (1.5 to 2.0 times as susceptible as sheep). This is an extremely useful finding for the purpose of testing the toxicity of further purified extractions of the plant. The rabbit was proved to be susceptible to the toxic substance, though between 45 to 68 times less so than the guinea-pig, despite the fact that toxicity trials with plant material had failed to produce symptoms. For practical purposes it can be regarded as insusceptible. The rat and pig also proved susceptible. They were, however, inferior to the guinea-pig as experimental animals for our purposes as the rat was 10 to 15 times less susceptible than the guinea-pig, while the pig frequently vomited the dosed material and also showed a lower susceptibility. The dog showed vomition only and was, therefore, entirely unsuitable.

The symptomatology and pathology of each species was also studied. The symptoms and lesions

described by Steyn[1][3] and mostly based on one experimental sheep, were reproduced and some elaborations made. During an outbreak of this poisoning in 1961[5] where 50 sheep had died, marked emphysema of the subcutaneous tissues was seen. The emphysema of the lungs was also much more pronounced than was found during the present investigation. In addition emphysema in the mediastinum was also seen. The symptoms and lesions found in the sheep, closely resembles that described for *Lasiosiphon anthylloides* Meisn. by Alexander[6]. It, therefore, seems very likely that they might contain the same toxic principle(s).

It is difficult to postulate the pathogenesis of the disease at this stage, because of the surprisingly relatively mild postmortem findings found after the violent symptoms of dyspnoea, ruminal stasis and diarrhoea seen in most cases. However, it does seem that the toxic principle(s) primarily affects the heart and lungs and causes irritation of the gastro-intestinal canal. Steyn[2] and Alexander[6], on the other hand, were of the opinion that these plants contain only a gastro-intestinal irritant. Alexander especially thought the acute gastric pain directly or indirectly responsible for all the symptoms. In some of our cases liver-, kidney-, and spleen lymphoid tissue damage was also seen. Only the sheep appears to develop the very marked dyspnoea. The other animals studied showed primarily gastric irritation and in some instances (e.g. the guinea-pig and the rat) paralysis or otherwise sudden death.

Appendix I

TOXICITY TRIALS

Sheep.

The extract was administered per ruminal fistula to 9 sheep varying in body weight between 23.7 and 40 g/kg with the following results.

1 sheep dosed at 200 mg/kg died in 2 days.

1 sheep dosed at 100 mg/kg died in 8 days.

Of 3 sheep each dosed at 50 mg/kg, 1 died in 2 days, 1 in 8 days and the third showed no effects.

1 sheep dosed at 40 mg/kg died after 8 days.

One sheep initially dosed at 30 mg/kg showed no effects and was given 40 mg/kg 14 days later. It subsequently showed symptoms.

1 sheep dosed at 30 mg/kg showed symptoms.

1 sheep dosed at 20 mg/kg showed no effects.

The acute M.L.D. of the extract for sheep appeared to be approximately 40 mg/kg which is equivalent to 1.8 g/kg of the dried plant material.

Rabbits. (Dosed per stomach tube).

1 rabbit dosed 0.5 g/kg showed no effects. On the 5th day it received 1 g/kg again with no result. On the 13th day it was given 2 g/kg and died 2 days later.

1 rabbit given 1 g/kg showed only mucous in the faeces.

Of 3 rabbits given 1.5 g/kg one died within 24 hours and 2 on the 4th day.

The acute M.L.D. of the extract for rabbits would appear to lie between 1 and 1.5 g/kg or 45 g to 67.5 g/kg of the dried plant. The rabbit is, therefore, highly resistant.

Guinea-pigs.

No. of Animals	*Dosage rate mg/kg.*	*Effect*
1	500	Died on day 2..
6	100	All died on day 2
6	58.5—30	All died on day 2
2	27.3 & 24.8	No effects
2	22.4 & 23.4	Died on day 2
2	23 & 22	Symptoms only
2	21 & 20	No effects

In addition one animal was dosed at 10, 20 and 40 mg/kg on successive days with no results. On day 18 it was given 60 mg/kg and died 2 days later.

The M.L.D. of the extract would appear to be in the neighbourhood of 22.4 mg/kg (or 0.9 g/kg of the dried plant).

Rats.

2 rats dosed at 200 mg/kg showed symptoms for from 3 to 5 days and recovered.

1 rat dosed at 300 mg/kg died within 24 hours. M.L.D. of extract 200-300 mg/kg.

Pig.

Barrow, 2½ months old, body-weight 19.4 kg dosed extract at 50 mg/kg. Showed yellow watery diarrhoea on days 3 and 4, recovered. On day 22 given 60 mg/kg, diarrhoea following 2 days. Day 28 given 100 mg/kg, vomited, diarrhoea, recovered.

Boar, 2 months old, body-weight 10 kg, dosed at 100 mg/kg/day for 4 consecutive days. Haemorrhagic diarrhoea day 4, died day 5.

Dog.

Non-descript bitch (weight 4.75 kg) dosed per capsules at following levels and intervals: Day 1, 50 mg/kg; days 7 to 10, 40 mg/kg/day; days 27 and 28, 30 mg/kg and day 29, 20 mg/kg. On all occasions the only effect was vomition.

Fox terrier bitch (7.5 kg) was dosed at 50 mg/kg on an empty stomach and then fed. No symptoms or vomition occurred that day but copious vomition took place overnight. No further effects.

Fox terrier bitch (7.7 kg) dosed at 50 mg/kg in divided portions in meat over 4 hours. Vomition 15 minutes after last dose but vomitus re-eaten. Copious vomition that night, no further effects.

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A TOXICOLOGICAL STUDY OF THE PLANT *SESBANIA PUNICEA* BENTH

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SUMMARY

Cases of *Sesbania punicea* Benth. poisoning have been encountered and the toxicology of the plant studied for the first time in South Africa.

Most domestic animals and poultry appear to be susceptible. The whole plant is toxic but the seed is most toxic followed by the flowers and leaves.

The toxicity, symptoms, chemical pathology and pathology are described and discussed in conjunction with a review of the literature. The main effect appears to be irritation of the gastro-intestinal canal and cardiac failure with terminal kidney dysfunction in some cases.

INTRODUCTION

Sesbania punicea Benth. which grows as a small tree or bush, has been introduced into South Africa and proved very popular in gardens because of its bright orange coloured flowers. (The botanical description is given in Appendix 2). It seeds prolifically and often spreads into uncultivated areas. The plant has been proved toxic to sheep, barrows, fowls, ducks, pigeons, rabbits, guinea pigs and even to frogs and toads. (See references 1 to 7). In view of these facts and as several cases of suspected *S. punicea* poisoning, where the seeds were fed to pigeons, fowls and peacocks by well meaning owners, had been encountered, it was decided to investigate the toxicity of the plant under local conditions.

TOXICOLOGICAL TRIALS

The details of some of the trials are summarized in Appendix 1. The main findings will be indicated here.

Sheep: (Adult Merino sheep of both sexes were used). The acute M.L.D. of the ground seed appeared to be between 0.5 and 1.0 g/kg body weight, which is in accord with Marsh's[1] findings of 1.1 g/kg (49.89 g/100 lb.) Chronic toxic dose: As little as 100 mg/kg/day caused death after 6 doses. This is even less than the 440 mg/kg/day reported by Marsh[1]. While the fresh leaves failed to cause death in one sheep, they did so in two others at a rate of 10 g/kg/day after 4 and 8 days respectively. The fresh flowers were also toxic and the smallest amount to kill a sheep was 5 g/kg/day after 3 doses. The variation in the toxic dose can be ascribed to the fact that the animals were not dosed at the same time and, therefore, received the fresh material in different stages of plant growth and of freshness.

FIG. 1.
Sesbania punicea Benth.

The clinical symptoms observed included depression, listlessness, progressive weakness, anorexia,

ruminal stasis, diarrhoea, tachycardia, marked hyperaemia of the conjunctiva, an elevated temperature and occasionally dyspnoea. Animals may be found dead without any symptoms having been observed. The diarrhoea was more prominent in sheep receiving the fresh flowers. It was first watery and greenish, later becoming putrid.

The chemico-pathological findings were a tendency to hyperglycaemia and terminal rises in blood urea nitrogen and creatinine especially in the sheep poisoned with the flowers. (See table 1 and 2). The methods used were the same as described in a previous paper[8]. There were also rises in serum glutamic oxaloacetic acid transaminase in some of these sheep.

TABLE 1.—CHEMICO-PATHOLOGICAL CHANGES IN SHEEP NO. 2 DOSED WITH THE FRESH FLOWERS.

Day	1	13	15	20	24
B.U.N. ..... (mg %)	28	28	61	120	240
Creatinine ..... („)	1.9	1.7	1.1	3.9	8.7
Glucose ..... („)	55	58	70	85	76

B.U.N. = Blood urea nitrogen.

TABLE 2.—CHEMICO-PATHOLOGICAL CHANGES IN SHEEP NO. 4 DOSED WITH FRESH FLOWERS.

Day	1	9	10	13	14
B.U.N. ..... (mg %)	24	26	20	98	138
Creat. ..... („)	0.9	0.6	0.3	5.5	9.2
Glucose ..... („)	70	61	60	47	84

Autopsy findings included generalised congestion, subepi-, endo- and myocardial haemorrhages, focal hyperaemia of the ruminal wall, diffuse hyperaemia of the intestines with focal haemorrhages in the rumen, abomasum and intestines (one case showed focal necrosis in the rumen-wall), hyperaemia and oedema (occasionally haemorrhages) of the lungs. Different combinations of these lesions were seen in individual cases but the most common appeared to be the haemorrhages in the heart, the hyperaemia and oedema of the lungs and the varying degree of hyperaemia to haemorrhages in the gastro-intestinal-canal. In spite of biochemical kidney dysfunction observed, only one sheep (sheep No. 2 in table 1), showed histological kidney damage. It showed diffuse hyaline degeneration of the tubular epithelium in the cortex. Some sheep showed slight degenerative liver changes and in one case a focal lymphocytic infiltration was seen in the adrenal. The degenerative changes found in the liver and kidney are in accord with the findings of Marsh[1], while Emmel[7] found necrosis of individual kidney cells and in some cases necrosis of entire tubules in the pigeon.

Rat: The rat proved susceptible, fatal poisoning being caused by a single dose at 3.0 g/kg and by repeated doses at 1.0 g/kg. Symptoms noted were hyperaemia and exudation of the conjunctiva, slight oedema of the eyelids and posterior paresis before death. Autopsy findings were a generalised congestion, mild to marked congestion of the lungs often with emphysema and hyperaemia of the gastro-intestinal canal, especially of the stomach and small intestines. No histological examinations were performed. In cases of acute poisoning symptoms were frequently not observed before death. Paralysis was the symptom seen. In subacute cases the symptoms were listlessness, anorexia, watery eyes, slight oedema of the eyelids with polypnoea in one case and diarrhoea in another. Autopsy findings were similar to those in the sheep except that occasional oedema of the lungs accompanied the hyperaemia. In many cases lesions in the gastro-intestinal tract were not present.

Rabbit: The susceptibility of the rabbit was found to be similar to that of the other laboratory animals tested, a single dose of 0.5 g/kg and repeated doses of 0.1 g/kg/day of the seed proving fatal, while 5.0 g/kg/day of both fresh flowers and dried leaves caused mortality within 2 to 5 days. The symptoms were similar to those in the other animals but in addition the more chronic cases showed a very marked hyperaemia around the edges of the eylids appearing after 2-9 days and

giving the animals a bespectacled appearance. In some cases this was followed by alopecia round the eyes and purulent conjunctivitis. These lesions were not associated with photosensitivity as their severity was unaffected by the colour of the animal and exposure to sunlight.

The most common autopsy findings were hyperaemia and oedema of the lungs, hyperaemia and occasional haemorrhages in the stomach, petechiae in the caecum and congestion of the liver. In three animals the spleen showed congestion with atrophy and caryorrhexis of the lymphoid tissue histologically.

Fowl: The seed whether ground or whole proved toxic to the fowl. Adult White Rock, White Leghorn and Austra White hens were used. As little as 2.0 g/kg of the dried ground seed and 2.5 g/kg of the whole seed (70 seeds) caused death. In 7 and 6 week-old Leghorn chickens dosed with the whole seed there was a tendency towards a lower susceptibility, 5.0 and 6.0 g/kg (35 and 30 seeds) being required to kill them. Emmel[7] reported that 150 seeds were required to kill an "adult chicken" while Shealy[2] killed fowls with 9 seeds.

The symptoms observed were depression, ruffled feathers and drooping wings, ataxia, progressive debility and diarrhoea. Symptoms appeared from 24 to 48 hours after a single dose and deaths occurred from 2 to 8 days after dosing. Hyperaemia and haemorrhages in the proventriculus were constant and characteristic post mortem lesions. The haemorrhages were found on the crest of the proventricular papillae. Petechiae were noted on the mucous membrane of the gizzard after removal of the keratinized layer. These lesions were seen especially in the region adjacent to the proventriculus. The kidneys were pale in colour and the lungs showed marked congestion and oedema. In a few cases focal necrotic lesions, round and 1 cm in diameter, were found in the crop mucous membrane. In some, only hyperaemia of the crop mucous membrane was seen. On histological examination hyperaemia and haemorrhages were seen in the lamina propria of the proventriculus. Cross sections of the papillae showed haemorrhages in the lamina propria of the plicae, especially those nearest to the gland opening. Cloudy swelling and congestion were observed in the liver and kidneys. The lungs showed marked congestion and oedema.

Pigeon: Fifteen racing pigeons were dosed with the whole seed at levels ranging from 1.0 to 9.0 g/kg. Of these 12 died, including the bird receiving the lowest dose. Individual resistance is indicated by the fact that three birds which received 1.5, 3.5, and 8.0 g/kg respectively showed no effects. However, the bird receiving 8.0 g/kg was seen to have vomited soon after dosing, which may also have occurred in the others. This may explain the variable results in this species. In one bird a total dose of only 6 seeds proved lethal. Symptoms appeared 9 hours after dosing and death occurred 10 to 40 hours thereafter. The symptoms and post mortem findings were, except for vomition, similar to those seen in the fowl. Hyperaemia of the proximal part of the small intestine was intense and the kidneys and liver showed advanced cloudy swelling and degeneration histologically.

Turkey: The susceptibility and reactions of the Beltsville Small White and American Bronze Turkey hens were similar to those in the fowl. As few as 20 (2.5 g/kg) seeds caused death in a 7 weeks old American Bronze poult.

The *Duck*: No symptoms were produced in Pekin ducklings nor in adult muscovies with doses as high as 6.5 g/kg and 5.0 g/kg respectively of the whole seed. This resistance may be related to the speed at which the seeds passed whole through the tract of the duck. In a subsequent test it was found that in the fowl when 40 seeds were dosed, an average of only 7 could be recovered in the droppings over the following 48 hours. In the duck given the same dose 19 seeds were recovered over the first 30 hours. Furthermore, the greater the number of seeds dosed, the larger percentage were excreted undigested e.g. in 5 adult ducks dosed with 50, 40, 30, 25 and 20 seeds, 44, 19, 13, 8 and 7 seeds respectively were recovered within 30 hours.

The Horse: One horse (body weight 470 kg) was dosed with ground seed at a level of 0.1 g/kg on two successive days. The only effect noted over the next two days was softening of the faeces. The dosing was then repeated over a further two days (days 5 and 6). It died on the 8th day of the experiment. Hyperaemia of the conjunctiva and a diarrhoea were noted from the 5th day and icterus and excessive lachrimation appeared on the 6th day.

Chemico-pathological tests on blood samples revealed a rise in unconjugated bilirubin (from 1.4 to 6.5 mg %) with no change in the conjugated fraction, a slight rise in blood urea nitrogen (from 24 to 31 mg %) and creatinine (from 0.9 to 2.2 mg %) and a slight drop in haemoglobin concentration (from 17 to 13.5 mg %) which was not, however, accompanied by a similar drop in haematocrit. No changes occurred in serum glutamic oxaloacetic or pyruvic transaminases, serum alkaline phosphatases, total plasma protein or the zinc sulphate turbidity test.

Autopsy findings were:- Generalised congestion, liver slightly swollen and yellowish with multiple red specks on cut surface, no significant changes histologically. Kidney dark in colour but normal histologically. Slight hydropericard with multiple subepicardial petechiae especially along the coronary vessels and in the coronary fat and subendocardial haemorrhages in the left ventricle, especially over the papillary muscles. Myocard congested. Lungs congested and oedematous with focal emphysema along edges. Spleen marked congestion. Hyperaemia of the fundic region of the stomach. Diffuse hyperaemia and focal submucosal haemorrhages in intestines.

Bovine: A yearling Afrikaner heifer (wt. 234 kg) was dosed ground seed at the rate of 1 g/kg. Apart from slight hyperaemia of the conjunctiva, no symptoms developed over the following week. Doses at 0.25 g/kg were then administered on three consecutive days and the animal died the day after the last dose.

A yearling ox (wt. 157 kg) was given 0.1 g/kg daily (week-ends excepted) on 25 occasions over 36 days. It showed marked symptoms over the last 14 days and died on the 39th day.

The symptoms were as described for the sheep with ruminal atony and dehydration very pronounced. The second animal showed weakness of the hindquarters over the last 8 days.

Chemico-pathological examinations were carried out on the second animal and the more significant results are shown in table 3.

TABLE 3.—CHEMICO-PATHOLOGICAL CHANGES IN BOVINE NO. 2 DOSED WITH GROUND SEED.

Day	1	7	9	16	19	26	33	36	37
B.U.N..... (mg %)	18	18	20	18	20	20	42	33	61
Creat..... („)	1.3	0.3	0.9	0.9	0.6	0.9	1.9	1.7	4.1
Glucose..... („)	82	79	85	70	65	72	79	64	93
SGOT ..... K.U.	109	86	50	74	366	132	206	139	203
SGPT ..... K.U.	36	73	67	92	151	92	151	144	112

Intermediary readings showing no significant changes have been omitted.

As will be seen there were rises in blood urea nitrogen, creatinine, glucose and serum glutamic oxalo-acetate and pyruvate transaminases.

Autopsy findings were as in the sheep. Again no histological lesions were found in the kidneys.

Pig: Three pigs were used in experiments in which the ground seeds were mixed with the feed.

Pig No. 1 was a two months old gilt (wt. 14.5 kg) given ground seed at 0.5 g/kg on 5 consecutive days. It vomited on the 2nd day and did not consume all its ration on the 5th day. It was not dosed on days 6 and 7 but again on day 8 (0.75 g/kg) and was found dead on day 9.

Pigs 2 and 3 were dosed as indicated in table 4.

TABLE 4.—DOSAGE REGIME PIGS 2 AND 3

	Days of Exp.	Dosage .. Rate g/kg	No. of doses given
Pig 2.....	0–13	0.25	10
	14–37	0.50	19
	38–99	1.0	62
Pig 3.....	0–13	0.25	10
	14–37	0.50	19
	38–61	1.0	19
	62–100	2.0	29

Both animals showed vomition and very poor appetites during the experiments, so that the amount of toxic material ingested was considerably less than that administered. Pig No. 2 died on the 100th day, while under anaesthesia with trichlorethylene. The experiment on Pig No. 3 was abandoned as the animal was practically starving. It subsequently recovered very rapidly.

Pig No. 1 showed lethargy, anorexia, vomition, paresis of the hindquarters and terminal diarrhoea. The other animals showed similar symptoms except that No. 3 did not develop diarrhoea.

No significant chemico-pathological changes were found in any of the pigs.

Autopsy findings were:-

Pig No. 1. Marked haemorrhagic gastritis, hyperaemia and focal haemorrhages of the intestines, no haemorrhages in the heart.

Pig No. 2. Diffuse hyperaemia of the gastro-intestinal canal and purulent pneumonia probably due to aspiration of vomitus.

Dog: Two dogs were each dosed twice with ground seed in gelatine capsules at 1.1 and 2.2 g/kg respectively. On all occasions the animals vomited. After its second dose one dog appeared unwell for a week but recovered.

Discussion

The results confirm and augment the findings of other workers to date. The seeds, flowers and leaves were found to be toxic in descending order. However, Marsh[1] who dosed sheep with up to 15 g pods/kg (716 g/100 lb) with negative results, killed guinea-pigs with extracts made from the pod and concluded that it does contain some toxin. Repeated dosing with small amounts has demonstrated a cumulative effect. As a large variety of animals and birds have been proved susceptible, the plant, which is becoming very widespread especially in gardens, may prove a danger to livestock and even humans.

Six species of Sesbania, (*S. hamata* Phill., and Hutch., *S. macrantha* Welw., *S. aculeata* Pers., *S. aegyptiaca* Poir., *S. mossambicensis* Klotz., and *S. cinerascens* Klotz) occur naturally in South Africa[13]. Nothing is known about their toxicity at the moment, except that the last named two have given positive tests for alkaloids[13]. Guisti[5] reported finding cyanogenetic glucosides in the seed but we could not confirm this finding. Robey[9] isolated a sapotoxin from the seed but conceded the probability of other toxic principles being present.

Appendix 1
Toxicity Trials

Species	Dosage (g/kg)	Days given	No. of Animals	Result
Sheep	1.0	1	1	Symptoms day 4, died day 5.
(ground seed)	0.5	1	1	Slight symptoms, recovered.
	0.25	Daily	3	All died day 6–7 after 4–5 doses.
	0.1	1–5	1	Symptoms days 3–6, died day 7.
(fresh leaves)	5.0	1–3, 5–7	1	6 doses, no effect.
	10.0	8–10, 12–13		6 doses, no effect.
	10.0	1, 7, 9–10 20–23	1	Symptoms days 2–23, died day 24. Marked ruminal effect.
	10.0	1–4	1	4 doses. Died day 5.
(Fresh flowers)	1.0	1–3	(No. 1)	3 doses in 5 days, no effect.
	5.0	6–10, 13–14,		9 doses in 11 days, no effect.
	8.0	15–18, 20–21		6 doses in 8 days symptoms days 19–23.
	8.0	29		No effect.
	10.0	30		Symptoms day 31, died 32.
	2.0	1–2	(No. 2)	No effect.
	4.0	3		No effect.
	8.0	6–7		No effect.
	10.0	8–11, 13–14		Marked symptoms died day 26.
	10.0	1–2	(No. 3)	Symptoms day 2, died 3.
	10.0	1, 3, 7, 9, 10	(No. 4)	Slight symptoms days 2–12 Marked symptoms 13–14, died 15.
	5.0	1–3	(No. 5)	Symptoms days 2–3, died 4.
Rat	3.0	1	1	Symptoms days 2–7, recovered.
(ground seed)	2.0	1	1	Dead at 48 hrs.
	1.0	Daily	4	All died days 3–9, after 2–6 doses.
	0.5	1–3, 6–11	1	Symptoms day 4 onwards. Dosing discontinued day 11, recovered.
Guinea-Pig	3.0	1	1	Dead 24 hrs.
(ground seed)	2.5	1	1	Paralysis, died 26 hrs.
	2.0	1	2	(*a*) died 26 hrs. (*b*) recovered.
	1.0	Daily	4	Died days 9–13 after 6–8 doses.
Rabbit	5.0–1.0	1	4	All died, 48 hrs.
(ground seed)	0.5	1	1	Died 72 hrs.
	0.25	1	1	No effect.
	0.5	Daily	1	4 doses, died day 5.
	0.25	Daily	1	16 doses, died day 26.
	0.1	Daily	8	All died, day 3 to 14.
(dried leaves)	5.0	Daily	1	4 doses, died day 5.
(dried flowers)	5.0	Daily	2	(*a*) 1 dose, died day 2. (*b*) 3 doses, died day 5.

Species	Dosage (g/kg)	Days given	No. of Animals	Result
Adult Fowl..... (ground seeds)	4.5–2.0	1	4	Symptoms in 12–48 hrs. All died between hr. 26 and day 5.
(whole seeds).....	5.0–2.5 (156–70 seeds)	1	9	Symptoms 24–48 hrs. Died days 2–8.
7-week chicken..... .. (whole seed)	6, 5, 2.5 (46, 45, 21 (seeds)	1	3	2.5 g/kg—no effect. Other 2—symptoms 24 hrs. died 48 hrs.
6-week chicken..... (whole seed)	7.0–4.5 40–25 seeds)	1	4	4.5 & 6.5 g/kg—no effect. 6.0 & 7.0 g/kg—symptoms 24 & 18 hrs. resp. Died day 4 & 2 resp.
Pigeon..... (whole seed)	9.0–1.0	1	15	12 showed symptoms at 9 hrs., died 20–48 hrs. Individuals at 1.5, 3.5 & 8.0 g/kg—no effect.
Adult Turkey..... (whole seed)	4.5, 4.0, 3.5 (314–138 seeds)	1	3	Symptoms 24 hrs. Death 48 hrs. Except at 4.0 g/kg.—recovered after 7 days.
7-week Poult..... (whole seed)	3.0, 2.5 (22, 20 seeds)	1	2	Symptoms 24 hrs., died 48 hrs.
7-week Duckling..... (whole seed)	1.0–6.5 (15–85 seeds)	1	8	No effect.
Adult Muscovy.....	4.0, 5.0 (178, 210, seeds)	1	2	No effect.

Appendix 2

DESCRIPTION OF THE PLANT*

Family: Leguminosae Synonyms: *Daubentonia punicea* D.C. *Piscidia punicea*, *Aeschynomene miniata*, *Daubentonia longifolia* D.C.(7), *Sesbania cavanillesii* Wats(10) *Sesbania drummondii* (Rydb) Cory(11).

Popular names: Coffee Bean(1) (4), Rattle Box(4), Siene Bean(4) and False Poinciana(12) in the U.S.A. It is a laxly branched glabrous bush or small tree, with slender terete branches, drooping leaves, and racemes of large orange-red flowers. Leaves six to eight inches long; short petiole and long rachis very slender; leaflets, eight to fifteen pairs with an odd one, opposite, subsessile, oblong or obovate-oblong, tip rounded, apiculate, nerves spreading; stipules setaceous, caducous. Flowers large, in a drolping, shortly peduncled raceme six to ten inches long, scarlet in bud, but paling as they open to orange; bracteoles setaceous; pedicels one-fourth to half an inch long. Calyx turbinate, truncate, very shortly lobed. Standard orbicular, nearly an inch broad, recurved. Wings two-thirds of an inch long, oblong, obtuse. Keel petals as long as the wings, strongly falcate, claws very long; limb oblong, obtuse. Ovary slender, strongly incurved, style long, stigma terminal. Legume stipitate, two to four inches long, acuminate, 4-angled; angles with coriaceous wings. Seeds 4 to 10, globosely reniform.

This plant is a native of South Brazil, Argentine and Mexico.

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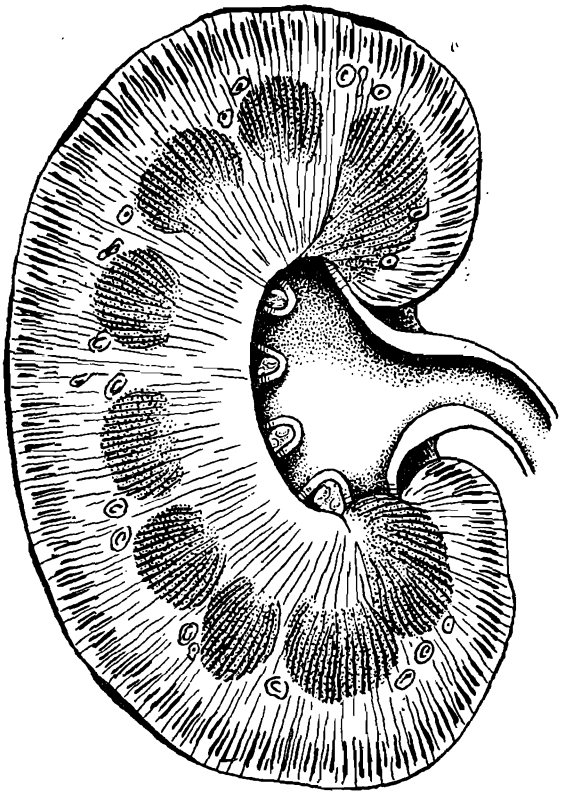

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A REPORT ON NEGATIVE EXPERIMENTS WITH FERRIC CHLORIDE AS A PROPHYLACTIC AGENT AGAINST GOUSIEKTE

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SUMMARY

In 1964, Visser(1) reported experiments indicating that ferric chloride, when given in the drinking water, acted as a prophylactic agent against poisoning by the plant *Pachystigma pygmaeum* (Schltr.) Robyns. (Gousiekte).

Experiments on these lines have been conducted not only with *P. pygmaeum*, but also with *Pavetta harborii* S. Moore and *Fadogia monticola* Robyns. The ferric chloride was administered either as a dose with the plant material or added to the drinking water in varying amounts. In all cases the findings were negative. These results are not in accord with those of Visser.

INTRODUCTION

Gousiekte* is a chronic disease of ruminants characterised by sudden death due to cardiac failure following on a chronic interstitial myocarditis after a latent period of 1-8 weeks after ingestion of the plant. The plant *Pachystigma pygmaeum* (Schltr.) Robyns was incriminated as a cause of the condition by Theiler in 1923(2). Later, however, the plants *Pavetta harborii* S. Moore(3) and *Fadogia monticola* Robyns(4) were also proved to cause the identical syndrome. *P. pygmaeum* poisoning has long been a serious economic problem especially in the Western and North Western Transvaal.

In 1964, Visser(1) reported success in preventing deaths from *P. pygmaeum* poisoning by the addition of 0.5 g ferric chloride per gallon to the drinking water. These experiments were carried out on the experimental farm "Swartrand" in the Western Transvaal.

For the purposes of our experiments, a diagnosis of gousiekte was made on the following criteria.

1. Clinical symptoms, where observed. These usually follow exertion on the part of the affected animal and consist of dyspnoea, tachycardia and anxious bleating soon followed by collapse and death. Examination of the resting animal often reveals a tachycardia and systolic murmur.

2. Macroscopic post mortem lesions. These are characterised by a thinning of the myocard with cardiac dilation, marked oedema and congestion of the lungs and often hydropericard, hydrothorax and ascites.

3. The presence of the very characteristic focal chronic myocarditis as described by Theiler(2) and Smit(5).

EXPERIMENTAL

Pachystigma pygmaeum (Schltr.) Robyns.

Experiment 1: Four two toothed Merino sheep, three wethers and one ewe, were used. Each received 200 g of a known toxic batch of the dried plant as a watery suspension per stomach tube daily except at week ends. Three of the sheep also received ferric chloride, which was added to the suspension as a watery solution, while the fourth received the plant material only. Details of dosages and survival periods are shown in Table 1. All died of typical Gousiekte as confirmed histologically.

Experiment 2: This experiment was conducted on the farm on which Visser(1) had carried out his work. Twenty four Merino wethers, all of approximately 12 months of age and 30 kg. body weight, were divided into two groups and each was penned individually. Each animal was fed 1 lb. fresh *P. pygmaeum* each morning. In the afternoon they were each given 3 lbs. of chaffed lucerne hay provided all or most of the test plant had been

* "Gousiekte" can be translated as quick or sudden disease.

TABLE 1.—PACHYSTIGMA PYGMAEUM (SCHTR.) ROBYNS.

Sheep	Weight in kg.	No. of dosages	Total Plant dosage		Daily dosage $FeCl_3$ in g.	Exper. day of Death
			Actual kg	As g/kg.		
1	28.1	54 in 65 days.....	10.8	384	0.5	66
2	20.9	76 in 91 days.....	15.2	727	1.0	95
3	27.2	26 in 31 days.....	5.2	191	2.0	32
4	22.7	85 in 103 days.....	17.0	749	None	104

eaten. Records of plant and hay consumption were kept. All animals had free access to a bonemeal and salt lick and were given 5 litres of water each per day. In the case of Group 1, 8.5 g $FeCl_3.6H_2O$ (= 5.1 g $FeCl_3$) was added to the drinking water. Of this they consumed an average of 1.5 litres each per day at the beginning of the experiment but this rose to some 3 litres per day later. The consumption of ferric chloride was, therefore, 1.5 to 3.0 g per day. The survival times in the two groups are shown in Table 2.

TABLE 2.—SURVIVAL TIMES OF SHEEP IN EXPERIMENT 2.

Day of Experiment	No. of Deaths	
	Gr. 1	Gr. 2
43–45	1	3
46–50	2	4
51–55	2	2
56–60	2	2
61–65	3	1
71	1	0
158	1	0
Totals.....	12	12

Ten of the 12 sheep in group 1 died showing the typical macroscopic post mortem lesions of gousiekte and of these only one did not show the histological lesions. One was slaughtered in extremis on day 63 and gousiekte confirmed histologically. Feeding of the plant was stopped on the 92nd day of the experiment owing to lack of material. At this stage one sheep in group 1 was still alive. It had shown polypnoea, tachycardia and a systolic murmur from the 76th to 112th day but these symptoms had disappeared by the 126th day. It was slaughtered on day 158 and gousiekte lesions were demonstrated both macro- and microscopically. All twelve sheep in this group, therefore, contracted gousiekte. Similarly all twelve sheep in the control group died of gousiekte as determined by macroscopic post mortem findings and all but one showed the diagnostic histological changes to some degree.

Pavetta harborii S. Moore:

Experiment 3: The dried, ground plant material used in this experiment had been tested previously and it had been found that when it was dosed to goats at the rate of 200 g per day until a total intake of 88 g/kg body weight had been reached, the majority of animals died from 1 to 8 weeks later.

In the experiment 8 goats were dosed 200 g of plant material daily. This was given per stomach tube as a suspension to which varying amounts of ferric chloride were added. In the cases of the first 3 goats, dosing was continued until death after the last day indicated in Table 3.

TABLE 3.—PAVETTA HARBORII S. MOORE—GOATS.

Goat No.	Weight in kg.	No. of Dosages	Total Plant Dosage		Daily Dosage $FeCl_3$ g	Expr. day of Death
			Actual kg.	As g/kg.		
1	36.3	38 in 44 days.....	7.6	209	1.0	46
2	33.6	25 in 30 days.....	5.0	149	1.0	30
3	29.1	19 in 23 days.....	3.8	131	None	23
4	40	29 in 35 days.....	5.8	145	0.5	43
5	46.8	34 in 41 days.....	6.8	147	2.0	51
6	19.5	14 in 18 days.....	2.8	143	3.0	23
7	40.5	25 in 32 days.....	5.0	123	4.1	55
8	27.2	18 in 22 days.....	3.6	132	10.2	40

All the animals died of typical gousiekte as judged by clinical symptoms, when seen, and macroscopic post mortem lesions. The typical histological change was seen in 5 of the animals but could not be detected in Nos. 1, 4 and 6.

Six sheep of mixed gender were then each fed with 200 g dried plant material every morning. In the afternoons they received chaffed lucerne hay ad. lib, provided all the test plant had been eaten. They were each given 4 litres of water daily, to which varying amounts of a ferric chloride solution were added for five sheep as set out in Table 4. The 6th sheep served as a control. Of this they consumed 1 to 2 litres daily. The actual amount of $FeCl_3$ taken in was, therefore, 0.25 to 0.5 times that of the amounts given in Table 4. They all died after 37 to 53 days, showing typical symptoms and lesions.

TABLE 4.—PAVETTA HARBORII S. MOORE—SHEEP.

Sheep No.	Weight in kg.	No. of dosages	Total Plant eaten		Daily Dosage Fe Cl_3 g Offered	Expr. day of Death
			Actual kg	As g/kg		
29	32.6	39 in 43 days.....	7.8	239	1.0	50
30	26.8	42 in 46 days.....	8.4	313	2.0	47
31	27.7	41 in 46 days.....	82.	296	3.1	47
32	26.7	36 in 36 days.....	7.2	278	4.1	37
33	33.6	49 in 51 days.....	9.8	292	5.1	53
34	40.0	39 in 44 days.....	7.8	195	None	45

Fadogia monticola Robyns:

An experiment similar to those described was conducted on two Merino sheep using this plant. The details are shown in Table 5.

Both animals showed the typical macro- and microscopic changes of gousiekte on post mortem examination.

CONCLUSION

Ferric chloride had no prophylactic or therapeutic value in Gousiekte, whether caused by *Pachystigma pygmaeum* (Schtr.) Robyns, *Pavetta harborii* S. Moore or *Fadogia monticola* Robyns.

This is not in accordance with the findings of Visser.

TABLE 5.—FADOGIA MONTICOLA ROBYNS

Sheep No.	Weight in kg.	No. of dosages	Total plant dosage		Daily Dosage $FeCl_3$ in g	Expr. day of Death
			Actual kg.	As g/kg.		
35	30	57 in 68 days.....	10.4	346.7	2.0	69
36	30.1	55 in 63 days.....	11.0	365.5	None	66

ACKNOWLEDGEMENTS

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ADVANCES IN "GEELDIKKOP" (*TRIBULOSIS OVIS*) RESEARCH.
8. A CRITICAL ANALYSIS OF THE SYMPTOMATOLOGY OF GEELDIKKOP.

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SUMMARY

The symptomatology of geeldikkop is reviewed and interpreted in the light of present day knowledge. Some symptoms previously not recorded are described and an attempt is made to show that an infectious agent is more than likely one of the main factors responsible for precipitating the acute syndrome. The similarity of many symptoms seen in the early stages of the disease are compared with those of bluetongue. Indications are given as to probable causes of death during the various stages of the condition.

INTRODUCTION

Although geeldikkop has almost certainly been known from the earliest times of farming in the Cape Province, the first official record of the disease is that of Hutcheon[1], which appeared in 1887. The only comprehensive descriptions of the symptomatology of the syndrome by authors who have actually been concerned with work on the disease in their various capacities are those of Theiler[2], Steyn[3 4 5] and Quin and Rimington[6]. General details of the condition are to be found in the volume on Animal Diseases in South Africa by Henning[7] (now out of print) and in the monographs of Blum[8], Clare[9] and Watt and Breyer-Brandwijk[10]. These authors have drawn largely on the earlier work cited for their material. Brief accounts of the syndrome, again depending on the above mentioned sources, are to be found in many contemporary textbooks on Veterinary Toxicology. All published illustrations of the disease in South African literature are similarly reproduced from those in the original papers mentioned.

Recent work on the aetiology, epizootology, pathology, chemical pathology and biochemistry of the disease[11-18] makes it imperative that the symptomatology of geeldikkop is reviewed most critically in the light of the findings which have emerged. Geeldikkop and the closely related haemolytic disease, enzootic icterus, are thought to be two extreme manifestations of a single disease entity[13-18]. This in turn is possibly associated with a subclinical, lowgrade and chronic selenium intoxication[13-15 17]. The acute manifestations, geeldikkop and enzootic icterus, are known to be precipitated by certain severe non-specific stressors the most potent of which, in the case of geeldikkop, is thought to be a relatively mild disease of probable virus origin[14 19].

In this paper the geeldikkop syndrome alone will be reviewed with these recent findings as background material. Enzootic icterus and further proof of it's relationship to geeldikkop will form the subject of subsequent papers in this series.

General discussion of the geeldikkop syndrome.

In it's classical form the disease is one of small ruminant animals characterised by severe photosensitization, a mild to severe icterus which is best classified as an intrahepatic cholestasis, mild to severe renal lesions, blood electrolyte imbalances, certain blood cell dyscrasias and numerous other biochemical features of great interest[13-17]. Affected animals may present either a mild symptom complex in which complete recovery is the rule or an extremely severe one which almost invariably has a fatal termination. Between these two extremes numerous variations of the disease pattern are encountered, the dominant symptoms being determined by the biochemical disturbance which has assumed the greatest prominence. This in turn depends largely on nutritional, management and environmental factors, intercurrent infections or internal parasitism and the nature of the precipitating stressor factor. Of these, good nutrition and management play an undoubted rôle in reducing the severity and incidence of the syndrome[14 17] while adverse environmental conditions and the other latter factors acting singly or in concert

undoubtedly introduce numerous detrimental complicating conditions.

The typical syndrome offers it's own particular horrors; the possible sequelae are however infinitely more terrible. In animals which are fortunate (or unfortunate) enough to recover, these include permanent blindness, mutilation or at the best disfigurement, lameness, infertility or chronic nephritis. Fatal terminations include death from suffocation, septicaemia, general toxaemia, nephrosis, Addisonian types of electrolyte imbalances, ketosis, dehydration, starvation and heat stroke, each of which are attended by their own peculiar set of symptoms.

Apart from these symptoms which are either germane to the syndrome or arise from secondary complications we must consider many which belong properly to the precipitating condition, be it an infectious agent or otherwise. These include pyrexia and severe gastro-intestinal stasis early in the syndrome, serous nasal discharges and erosions on the nostrils before the animal is truly photosensitive, haemorrhages on the coronary bands of the feet and pododermatitis, torticollis and numerous symptoms of probable nervous origin.

1. *The severe acute syndrome*:

While Theiler's original description of the disease is characteristically meticulous([2]), that of Steyn([4]) is more systematic and easier to follow, in that it presents a clearer pen-sketch of the course of the disease. I have therefore based my discussion of this part of the disease complex largely on Steyn's presentation. According to this author the course of the typical syndrome may be roughly divided into five stages viz:-

(a) Stage 1: Affected animals show marked pruritus, erythema, hyperaesthesia and some hyperthermia in all unpigmented and exposed areas. The typical "flinching" attitude of an hyperaesthetic sheep on exposure to sunlight is shown in Plate 1, fig. 1. Pain is intense (exposure time was 5 minutes).

(b) Stage 2: This generally follows within one to two days of the appearance of the first symptoms and includes marked oedema of the affected areas extending often down the limbs, conjunctivitis, rhinitis, labiitis and general apathy. Consequent to the buccal and nasal lesions are reluctance to move the mandibles, difficulty in feeding and drinking and dyspnoea. In some cases a slight febrile reaction may be observed. These first two stages are regarded by Steyn (*op. cit.*) as representing the primary symptomatology of the disease. The following stages include secondary symptoms or sequelae. A typical case of stage 2 is shown in Plate 1, Fig. 2.

(c) Stage 3: This corresponds roughly to the period covered by the fourth to eighth day of the disease. Oedema of the affected areas is severe and attended by seeping of much of the oedamatous fluid through the skin with crust formation. Such effusions are at first serous and become purulent later. Copious purulent nasal and occular discharges are evident which result in sealing of the eyelids and nostrils with tenacious crusts. Icterus has become apparent and in some cases there is a febrile reaction. Many cases show a peculiar stretching of the neck, in which the head may be bent over backwards. (See Plate 1, Fig. 6). This has been interpreted *inter alia* as an attempt to overcome obstruction of the nasal passages. Keratitis is a prominent symptom. Many sheep succumb during this stage from suffocation or toxaemia. Rapid loss of condition is general.

(d) Stage 4: The oedematous swellings, which by now have commenced to subside are replaced by extensive mummification of the skin of the affected areas. This is attended with loss of sensation and in many instances a complete inability to open the jaws. Forcible opening of these structures is often impossible without considerable damage to the necrosed tissues. Icterus is intense, marked cachexia is the rule and secondary infection of the gangrenous areas is most marked. Fairly high fevers are common and constipation is frequent. A case typical of this stage of the disease is shown in Plate 1, Fig. 4.

PLATE 1

Development of the lesions of photosensitization in typical cases of the acute syndrome:- fig. 1: "flinching" attitude adopted by shorn early cases of the disease (stage 1) when exposed to solar radiation. Hyperaesthesia and pain are intense. Exposure time in this case, 5 minutes; fig. 2: Stage 2, showing oedema of the face, lips, nose, ears and eyelids and serious discharges from the eyes and nose; fig. 3: torticollis in an early case of the disease; fig. 4; severe sloughing lesions and purulent panopthalmia in an advanced case of the disease; fig. 5: severe facial gangrene and sloughing of the ears and nostrils and eyelids in the terminal stages of the disease; fig. 6: the sheep on the left shows the peculiar upward jerking of the head seen frequently in early cases; the group of five sheep behind him are highly photosensitive and take advantage of the small amount of shade offered by the former animal.

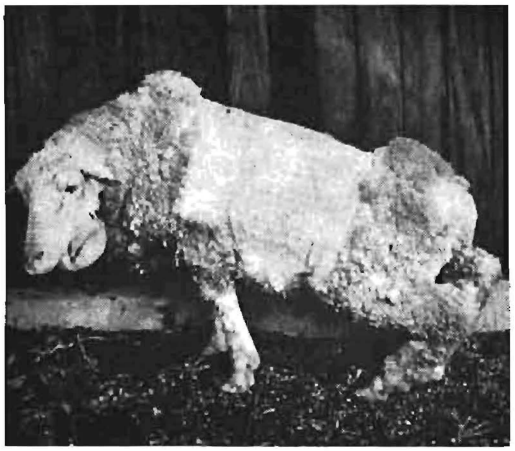

FIG. 1

FIG. 4

FIG. 2

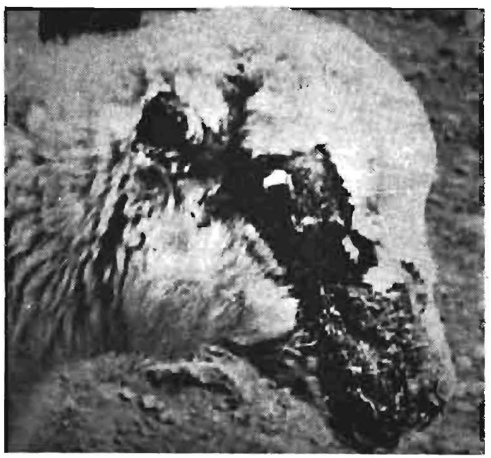

FIG. 5

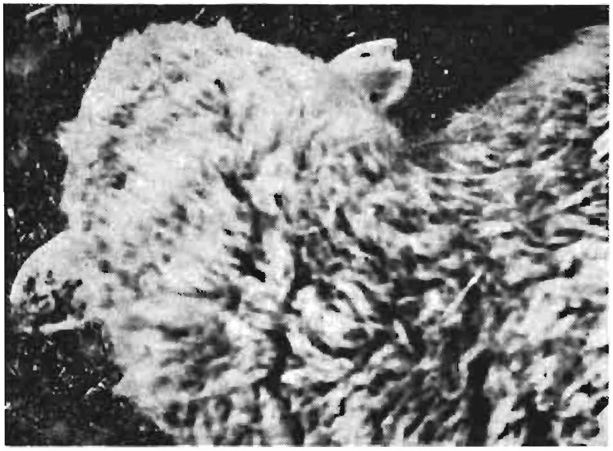

FIG. 3

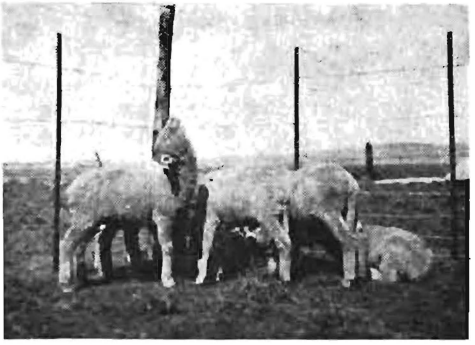

FIG. 6

(e) Stage 5: There is considerable sloughing of the hard dry necrosed skin, especially on the face, lips and ears. The latter two structures may slough away entirely. The newly developing skin under the facial sloughs is exposed as a thin red hairless structure which frequently becomes severely sunburnt. A secondary acute purulent dermatitis may appear once more. Purulent panophthalmia with rupture of the cornea is a frequent finding. In more fortunate cases the acute keratitis proceeds no further than total opacity of both corneae. Dyspnoea is generally still severe owing to damage to the nasal respiratory passages. In many animals laminitis and coronitis lead to permanent lameness and hoof malformations. Shedding of the fleece towards the end of this stage is frequent. The duration of the syndrome up to this point has been about three to five weeks. A case showing extensive gangrene of the face is presented in Plate 1, figure 5.

Theiler (*op. cit.*) mentions the following points of interest which can be added to the above outline of the disease:

(i) "Geeldikkop was characterised by the occurrence of a febrile reaction in the course of it's evolution. This was sometimes the first symptom, sometimes it appeared only after the onset of the swellings in the head; and only in very light cases, that went over in an early recovery, a distinct disturbance was absent"; and again in a later passage — "As already stated the fever may appear before the onset of other clinical symptoms, with them or after them. In the first instance we can speak of a prodromal fever; it lasts about one to two days. This occurrence was however not the rule, neither the simultaneous appearance of fever and swellings. In most instances it appeared after the onset and the curve drawn followed a fairly typical type." These temperature reactions will be discussed at greater length later in this paper.

(ii) "The initial external symptoms were of two different kinds, both affecting the psyche of the animal: depressive and excitative ones. The former were most frequently met with and had a sudden onset". Such symptoms included apathy, dejection, anorexia, seeking of shade, unwillingness to move and lagging behind the flock. Of the excitatory symptoms Theiler says: "When the illness started with a stadium excitationis, the sheep were found to be very restless, not feeding at all, lying down and rising again. When in the standing position the head was raised and suddenly jerked vertically and this motion was repeated time after time. Later the sheep often took up a sternal position and repeated the same movement". The reader is referred back to Plate 1, Fig. 6 showing a sheep which has been photographed during such a movement. It is interesting to note that Theiler and Steyn differ in the time of the appearance of this symptom and in their interpretation of these signs. (*In the case of Theiler's report I have inferred an interpretation differing from that of Steyn, since none is actually given. These symptoms are stated to precede obstruction of the external nares*). To these early symptoms Theiler adds frequent shaking of the head and grinding of the teeth. These are manifestations of pain and probably different in origin to the peculiar movements of the head mentioned above.

(iii) "The second important lesion to be met with was the icterus. It appeared in the latter portion of the disease" — i.e. *from the fourth to the tenth day* after the onset of the first symptoms in the various cases which he studied. (The italics are mine). Theiler found that in most cases the appearance of the icterus coincided with the "acme of the fever" or appeared shortly afterwards, increasing in severity while "the temperature descended in a lytic manner to a collapse in cases of death". The "acme of the fever" mentioned corresponds to Steyn's stage 3 of the disease.

Quin and Rimington[6] state that in severe cases "the coronet of the hoof and the base of the horns assume a dark purple red colour". These symptoms appear concomittantly with the severe facial oedema. Such cases are described as "running a high temperature and showing a certain measure of shock". Constipation and rumen atony are mentioned as prominent signs but no indication of when they are first detected is given.

During the course of the last ten years the author and his coworkers have had the opportunity of examining and working on a few hundred cases of all stages of the disease. The investigations concerned have covered almost the entire area in which the disease is enzootic and have been conducted under a wide variety of climatic and seasonal conditions. These studies have included detailed work on the epizootology, chemical pathology, histopathology and biochemistry of the disease[11-19] and the results obtained have been carefully correlated with the symptomatology of each case. Many of these animals have been maintained under carefully controlled conditions[20] and

the disease has been allowed to run it's full course in the absence of secondary complicating factors[14].

The results of these studies have been most illuminating particularly with regard to the aetiology of the condition. By and large, Steyn's description of the acute syndrome with regard to the sequence of events is a fairly accurate portrayal of the course of the disease. There are however a number of extremely important additions and corrections which must be made to this description. Many of the symptoms can now be interpreted more clearly in the light of our present knowledge. In this paper I will present the results of a carefully controlled study of the symptomatology of 89 cases of the typical acute syndrome. These animals were maintained and nursed as described previously[14 20]. The studies were controlled with collateral work on 30 sheep obtained from Karoo farms on which no geeldikkop was present at the time of the investigations concerned. The control animals were free from any clinical and *biochemical* signs of either geeldikkop or enzootic icterus. Both affected and control animals were virtually free from internal parasites. In many instances old calcified lesions of oesophagostomiasis were evident on autopsy, but few mature worms were to be seen. The affected animals were sheep of both sexes and all ages ranging from lambs of two months old to one ewe aged about twelve years. The majority were however about 2-3 years old. In most instances the animals had not been inoculated against bluetongue. In some cases such inoculations were done only a few days before geeldikkop broke out on the farms concerned or else the adult members of the flock had been inoculated during one of the preceding years. The majority of the farmers in the semidesert areas where geeldikkop is enzootic are notoriously lax with regard to regular inoculation programmes and the information which can be obtained from them on this matter is often disappointingly unreliable.

A. *The prodromal stage of the disease.*

In previous papers I have indicated that profound biochemical disturbances and blood cell dyscrasias may be present in apparently normal sheep in flocks where the syndrome is rampant[13-16]. The prominent symptomatology of 9 such cases is presented in Table 1. These data are restricted to haematological and chemical pathology findings since apart from a fairly high fever in many of these animals, they all appeared to be clinically normal. The appetite appeared to be normal in all cases and no disturbances of habitus were observed. The animals were selected at random from two flocks with an extremely high incidence of active cases of the disease. The haematology of these animals has been fully described in a previous publication[16].

In all instances the leukopaenia was extremely severe and in many cases it was difficult to carry out a satisfactory differential white cell count. The leukopaenia appeared in most instances to be due to a lymphocytopaenia. These findings have been noted by Clark[21] in his studies on ovine pregnancy toxaemia and are best explained as being a result of severe or prolonged stress. Similarly the

TABLE 1.—ANALYSIS OF SOME PROMINENT SYMPTOMS OBSERVED DURING THE PRODROMAL STAGE OF THE DISEASE. (PRECLINICAL CASES.)

Total number of cases studied = 9. All sheep were maintained under controlled sheltered conditions.

Symptom.	Number of cases
Severe leukopaenia, due in most instances to severe lymphocytopaenia	9
Eosinopaenia	7
Thrombocytopaenia	3
Anaemic changes. (Mild to severe and including anisocytosis, presence of macrocytes, normoblasts and Jolly Bodies in blood films.)	9
Hypoglycaemia	6
Low total plasma protein values indicative possibly of water retention	5
Blood Electrolyte disturbances (including hyponatraemia, hypochloraemia, hyper- or hypokalaemia)	9
Slight elevation of blood urea nitrogen	9

eosinopaenia may be attributed to a stress reaction. The thrombocytopaenia is transient and often associated with bone marrow hypoplasia[12 16 17]. A neutropaenia is often evident and is associated with a marked "shift to the left"[16]. The anaemia seen is undoubtedly the result of a low grade haemolytic crisis [14 15 22] and is also often associated with bone marrow hypoplasia[12 16 17].

In a previous paper[14] dealing with biochemical changes in 28 cases of the acute syndrome, I have stressed the fact that from the time the earliest clinical symptoms of the disease are observable there is a markedly elevated γ-globulin level in the plasma proteins of affected animals. This cannot be attributed to disturbances of liver function[14]. To these cases must be added a further five in which the same phenomenon has been studied. These animals are included amongst those whose symptoms are described in the following stage of the disease.

The presence of a febrile reaction in most of the animals studied in this stage of the disease, coupled with an obvious stress reaction and high γ-globulin levels in animals innocent of vaccine immunities must surely indicate that an infectious agent is operative in precipitating the acute syndrome, as has been argued previously[14]. Temperatures recorded varied between 103° and 105.5°, in febrile reactions. If such is the case then the infection must also be clinically mild, but none the less severe enough to induce profound disturbances in animals predisposed to acute attacks of geeldikkop[13 19]. In clinically normal sheep drawn from the pool of animals available for experimental purposes, Clark[22] has noted the appearance of a mild hyperbilirubinaemia[15] when these sheep have been subjected to mild stress reactions. In most cases these sheep have been purchased from farms in areas where geeldikkop or enzootic icterus are prevalent. I shall, in a following publication in this series, endeavour to show that even the mildest form of stress can precipitate enzootic icterus in sheep prone to this syndrome whereas in geeldikkop, the less violent of the two manifestations, a much more powerful stimulus is probably required.

The hypoglycaemia observed in these sheep is interesting. Two possibilities have to be considered in any attempt to explain these symptoms, viz. (a) severe disturbances in carbohydrate metabolism are a prominent feature in acute geeldikkop. These have been associated with marked suppression of the activity of enzymes like triose-phosphate dehydrogenase[14] and (b) a severe and complete gastro-intestinal stasis is a prominent symptom in at least half the cases which have been studied at the time of appearance of the first symptoms. This will be described under the following stage of the disease. It's very severity, however, points to it's existence long before symptoms are first noted.

Water retention and blood electrolyte disturbances which have been observed in these cases[15] are in all likelihood a further manifestation of a reaction to stress.

B. *Early cases of the disease — The stage of Photosensitivity.*

For the purposes of this discussion I have combined Steyn's stages one and two since in a highly photosensitive sheep the duration of the first stage may be as short as thirty minutes after exposure to sunlight and from the practical point of view there seems to be little justification for separating symptoms of the acute photosensitivity syndrome. They are induced by a peculiar set of circumstances in diseases of this nature; the *sequence* of events up to the appearance of the sequelae is always the same. The only variables are the photodynamic agents concerned and the severity of the reaction. In the case of geeldikkop tradition accords the role of main photodynamic agent to phylloerythrin[11]. We know today that coproporphyrin III may be just as, or even more, important in this respect[14]. The severity of the reaction depends primarily upon the duration of irradiation. Geeldikkop is no exception to this rule, but in this disease a number of *internal* factors are operative in determining the severity of the symptom pattern. We will consider these in a moment.

A prerequisite for the genesis of phylloerythrin sensitivity is an abundance of green plant food in the diet; the amount of phylloerythrin which is formed in the forestomachs being proportional to the amount of chlorophyll which is present in the diet[11]. In geeldikkop these conditions are often not fulfilled since in the first instance the disease makes it's appearance amongst a flock when severe drought conditions prevail on the farms concerned and secondly the presence of a severe gastro-intestinal atony seen in at least half the cases studied so far, will drastically reduce the amount of phylloerythrin absorbed from the gastro-intestinal tract. I have had numerous spectacular failures in attempts to produce phylloerythrin photosensitivy for student demonstration purposes, by ligation of the common bile duct in sheep which have been given green lucerne *ad libitum* for at least one month beforehand. These failures have in all instances been due to severe gastro-intestinal stasis consequent to the operation. Although phylloerythrin is readily detected in the contents of the gastro-intestinal tract of these animals, it does not appear to be absorbed from the static digestive tract.

In geeldikkop the primary biochemical lesion is a failure in the transport of bile pigments, bile acids, porphyrins, copper ions and compounds like the sulphonephthalein dyes across the hepatic cell membranes([13 14]). In the absence of phylloerythrin it is the coproporphyrin III which is not excreted via it's normal biliary route that becomes the prime photodynamic agent([14]). Geeldikkop becomes in this respect a true cutaneous hepatic porphyria([14]) and must be regarded as such for the purposes of this discussion.

The symptoms which are seen in this stage of the acute syndrome fall into two groups which must be clearly separated from one another, viz: (a) those due to the primary biochemical disturbance of biliary excretion, notably the symptoms of photosensitivity and the icterus, and (b) those which are unrelated to the photosensitivity syndrome and which I think are undoubtedly due to the precipitating condition, be it an infection or otherwise. These include the febrile reaction, serous nasal and occular discharges, rhinitis, pulmonary oedema and dyspnoea, coronary band haemorrhages and complete gastro-intestinal stasis. An analysis of some of the prominent symptoms seen in 36 cases of this stage of the disease are presented in Table 2.

TABLE 2.—ANALYSIS OF SOME PROMINENT SYMPTOMS SEEN IN EARLY CASES OF GEELDIKKOP.

Total number of early cases studied = 36. Duration of illness in all instances = 1–3 days. All sheep were maintained under controlled sheltered conditions.

Symptom.	Number of cases	Approx. % of Total
Lesions of photosensitivity: Mild to severe oedema and erythema of ears, eyelids, nose, face and dewlap	36	100
Icterus: Severe	9	25 } 92
Mild to fair	24	67 }
Not present	3	8
Serous occular and nasal discharges not associated with frank rhinitis or conjunctivitis	8	22
Rhinitis	10	28
Early erosions or necrotic lesions on the external nasal septum	6	17
Conjunctivitis and Keratitis	4	11
Dyspnoea varying from mild to severe. (In one instance only was this possibly due to caking of nasal discharges.)	9	25
Pulmonary oedema. (Mild to severe and confirmed in most cases on autopsy)	5	14
Coronary band haemorrhages, with or without oedema of the extremities and coronitis. (In four cases the base of the horns showed similar lesions as well.)	13	36
Complete gastro-intestinal stasis (confirmed in most cases on autopsy)	17	47

The symptoms of photosensitivity and the photosensitization syndrome need no further description. They are accurately portrayed in the papers by Theiler([2]) and Quin and Rimington([6]), or in the monographs by Steyn([3]), Henning([7]), Blum([8]) and Clare([9]). In geeldikkop the severity of these symptoms is due in part to the conditions mentioned above and in part to the severity of the biochemical lesions in both liver and kidneys([13 15]). The hepatic block to porphyrin excretion may be partial or complete thus leading to more or less photosensitivity. The early appearance of severe renal lesions is a potent complicating factor since these constitute a distinct block to the secondary or "escape route" for porphyrin excretion([13-15]). Once such lesions are established elimination of the photodynamic agents is effectively blocked and photosensitization must be extremely severe in animals exposed to intense solar radiation. In most cases of geeldikkop photosensitivity precedes the appearance of clinical icterus. This, I think, is due to the very low levels of

either phylloerythrin or coproporphyrin which need be in the circulating blood to evoke photosensitivity. There is no evidence to indicate that a biochemical block of hepatic porphyrin excretion precedes that of bilirubin conjugate excretion.

The severity of clinical icterus depends similarly on the severity of the simultaneous hepatic and renal cell blocks([13-15]). In spite of the large amounts of conjugated bilirubin found in the plasma of affected animals([13-15]) bilirubinuria is seldom seen in any stage of geeldikkop. This is one of the most potent arguments in force for the existence of collateral and possible identical biochemical lesions in both liver and kidneys, since the comparatively mild histopathology seen in either organ cannot be reconciled with the severe biochemical disturbances of pigment excretion([13-16]).

In the case of icterus however there is an added complication which markedly affects the severity of this symptom and the time of it's appearance. This is the degree of intravascular haemolysis which may be present. As will be seen from the figures in table 2 an extremely severe icterus has been observed in twenty-five per cent of cases within the first three days of illness. If this was to be due to a disturbance in pigment excretion alone then one would expect that the plasma of these animals would contain mainly bilirubin glucuronides. That this is not so, is shown by the figures given in Table 3, taken from the blood studies of the animals concerned.

TABLE 3.—PLASMA BILIRUBIN LEVELS AND SOME PROMINENT SYMPTOMS OF ACUTELY ILL EARLY CASES OF GEELDIKKOP.

Sheep No.	Duration of illness.	Main symptomatology	Total bilirubin	bilirubin glucuronides	unconjugated bilirubin
Vos. H.	1–2 days	Mild oedema, erythema and icterus. Extremely ill.....	11.26	6.25	5.01
Vos. I	2–1 days	Mild oedema, erythema and icterus. Extremely ill.....	12.50	6.88	5.62
V.W. 20	1–2 days	Mild oedema, erythema and icterus.....	6.00	2.70	3.30
V.W. 24	1–2 days	Mild oedema, erythema and icterus, Dyspnoea.....	6.90	3.80	3.10
V.W. 25	2 days	Mild oedema and erythema. Icterus, Dyspnoea, severe coronitis, in extremis....	14.70	8.40	6.30
V.W. 26	2 days	Mild oedema and erythema. Icterus, Dyspnoea, in extremis.....	12.30	6.50	5.80
Vos. A	2–3 days	Severe oedema, erythema and icterus....	18.75	10.63	8.12
Vos. B	2–3 days	Severe oedema, erythema and icterus....	37.51	24.38	13.13
Vos. C	2–3 days	Severe oedema, erythema and icterus.....	31.25	20.00	11.25
Vos. E.	2–3 days	Severe oedema, erythema and icterus....	20.63	12.50	8.13
Vos. F	2–3 days	Severe oedema, erythema and icterus....	25.00	15.63	9.37

Note: Figures for bilirubin and it's conjugates are expressed in mg. %.

Subsequent work has shown that there is little or no interference with bilirubin conjugation in geeldikkop([14]). I have discussed at length the mechanism of intravascular haemolysis in geeldikkop elsewhere([13-15 23]). It is sufficient for the purposes of this discussion to note that the markedly increased red cell fragility which is a prominent feature of geeldikkop is due possibly to inhibition of glyceraldehyde-phosphate dehydrogenase. Since the activity of methaemoglobin reductase in the erythrocyte depends largely upon the efficiency of this enzyme([13-15 24]), methaemoglobincythaemia is often seen in the early cases of the syndrome. "Gun-metal blue" or blackish brown pigmentation of the kidneys and haemochromatosis are by no means uncommon autopsy findings in geeldikkop([16]) and one is forced to conclude on the basis of the evidence available that an explosive

haemolytic crisis occurred in at least a quarter of the animals which we have studied. This is the only possible explanation I can find for the severity of the icterus seen in the animals noted in Table 2 in this stage of the disease. It is an important one for it places us squarely within the confines of the symptomatology of enzootic icterus.

Depending as it does, upon all the factors noted thus far, the symptomatology of this stage of the geeldikkop syndrome can be a very variable one. Outbreaks are encountered in which the symptoms of photosensitivity are dominant, icterus is mild and transient and if the animals are properly nursed the course of the entire syndrome is mild and lasts no longer than a week. In other outbreaks photosensitivity may yield first place to icterus as the main symptom. In such instances the incidence of renal pathology is considerable, haemolytic anaemia severe and the morbidity and mortality rate are extraordinarily high in the following stage of the disease. One is often confronted in these cases with chemical pathology, autopsy and histopathological findings which can best be described as typical of a mixed geeldikkop — enzootic icterus syndrome([16]). In other flocks both extremes of the acute syndrome may co-exist and numerous variations of the disease pattern are seen in animals falling between these extremes.

From the point of view of the farmer and the veterinarian concerned with the welfare of his animals this is the critical stage of the disease. Immediate and vigorous action is required to prevent further exposure to solar radiation and to subdue the existing acute dermatitis for it is here only that one has any hope of arresting the relentless progress of the lesions of photosensitization towards the devastating sequelae mentioned earlier.

Many of the animals which were studied during this stage of the disease showed a febrile reaction. It was usual to take the temperatures of the animals concerned at 8 a.m. and again at 10 p.m. This febrile reaction, present from the onset of obvious symptoms, varied from 103.5° — 105.5° and lasted in most instances for no longer than three days. Marked fluctuations between the limits mentioned were seen in the individual animals, either on the same day or during the following two days. On the whole the temperature curve seen was similar to that observed by Theiler (*op. cit*) in his experimental animals. One point must however be made quite clear with regard to the febrile reactions in geeldikkop. The temperature reactions which I have described and which Theiler noticed at the commencement of the disease in his animals were present before any secondary infection of the lesions of photosensitization could occur. The following stage of the disease is one which is marked by extensive necrosis of the affected areas with secondary infection contributing largely to the symptomatology, and thus is one in which a severe febrile reaction is not an unexpected phenomenon. It is necessary to draw a clear distinction between these two separate febrile reactions. In the instance of these early cases the febrile reaction noted has probably continued over from the prodromal stage. It is by no means a constant finding in patients at this stage, but it's presence is significant. These findings are again in accordance with those of Theiler. It is very interesting to note that he was struck by the resemblance of his animals' temperature curves to those seen by him in cases of bluetongue([2]).

We have now to consider the symptoms which are not properly those of photosensitization or those to which a different explanation can be attached with equal force. The reader is referred back to Table 2. In a large percentage of cases the presence of a clear serous discharge from the eyes and nose was noted. In many this was associated with the febrile reaction mentioned. In some severely icteric animals these serous effusions were distinctly yellow in colour, but in none were they associated as yet with clinically obvious rhinitis or conjunctivitis. On the other hand we found in many cases frank rhinitis from the outset accompanied by much sneezing and snorting and a thick muco-purulent nasal discharge. *Oestrus ovis* infection is very prevalent in the areas where geeldikkop is prevalent, but in all the animals we have examined showing these symptoms it could be dismissed as not being one of the causes. Many of these animals presented an acute conjunctivitis as well as rhinitis and in some keratitis was obvious as well. Both symptoms are features of photosensitization in all animals; they are also symptoms of many infectious diseases. In some of the cases we have studied where, judging by the mildness of oedema and erythema on the exposed areas, photosensitivity was only just becoming apparent, rhinitis, conjunctivitis and, in some, keratitis were established symptoms.

In 17 per cent of our early cases the presence of circumscribed superficial erosions on the external nasal septum was noted. The same symptom has been observed in phylloerythrin photosensitization induced by oral administration of icterogenin (unpublished work) and in cases of bluetongue exposed to solar radiation at the height of their reaction. (Clark and Brown — unpublished observations).

Dyspnoea was seen in twenty five per cent of the cases studied and varied from mild to severe. In only one instance was this associated with

obstruction of the nasal passages by a muco-purulent discharge. In fourteen per cent of these cases it was undoubtedly due to pulmonary oedema, mild in some and severe in others. In the remainder we could find no obvious clinical reason for the dyspnoea. The presence of myocardial lesions has been noted earlier[16] in an histopathological study of geeldikkop material. It is likely that such lesions escaped detection in these cases.

In approximately 36 per cent of our cases hoof lesions were prominent signs. These varied from mild to severe and included haemorrhages in the coronary band and the hoof substance just below it, clinically identical to those seen in bluetongue, coronitis, pododermatitis and oedema of the extremities just above the coronary band. In four of these animals similar lesions were present at the base of the horns. As stated earlier in this paper lesions of this nature were first recorded by Quin and Rimington (*op. cit*) and were found to be associated with a high fever in the animals concerned. In this context I would like to quote translated passages taken from letters from two of my co-workers in the field, van Heerden[24] and van Tonder[25]:- (a) dealing with an extensive outbreak in January of 1964 in the Rietbron and Beaufort West areas — "In all the animals which had geeldikkop we found an extremely severe coronitis which was absolutely identical to that of bluetongue. The coronitis was also found in a large number of animals which showed no signs of geeldikkop and which had not had the disease. Further, cyanosis was encountered in affected sheep in which icterus was mild and also in those with coronitis in the absence of geeldikkop. In some cases the coronitis had subsided leaving a break in the hoof substance with "slipper" formation. The above symptoms were so obvious that one was forced to conclude that bluetongue was also present among the flock", and (b) dealing with various other outbreaks in the same season — "By far the majority of cases were seen amongst young merino lambs. A few cases were also seen in Angora goats. Many of the affected lambs showed a definite coronitis and pododermatitis. These cases were noticed particularly from February onwards and seeing that the lambs were susceptible to bluetongue, the strong possibility exists that this disease played a complicating rôle, leading to the higher incidence of geeldikkop amongst the lambs."

These letters are interesting not only in that there is a strong suspicion that bluetongue is operative as a precipitating stressor in geeldikkop outbreaks, but also in indicating why the incidence of the syndrome can be so high in lambs.

In a previous study[14] and earlier in this paper the presence of high gamma globulin levels in the blood of these early cases has been noted. At this stage of the disease this hypergamma-globulinaemia cannot be attributed to hepatic pathology, nor can it for the reasons given earlier be explained away as a post-innoculation reaction.

A further symptom which we have seen rather infrequently during outbreaks of geeldikkop is that of torticollis. It was seen in none of the animals in our controlled studies but has been observed in isolated instances during the course of our work. A case of this nature is shown in Plate 1, fig. 3. (I am grateful to my co-worker Wilkins for this photograph and the relevant case report[26].)

The peculiar raising and lowering of the head first noted by Theiler and Steyn (*op. cit*) and shown in Plate 1, fig. 6 has often been seen by us in the course of recent field investigations. It occurs during this and the following stage of the disease. In early cases it does not appear to be due to an attempt to expel nasal discharges or to gulp air through the month. It is often associated with amaurosis and the "pushing" symptoms seen in matricaria poisoning[27], together with the same aimless walking backwards and forwards until some solid object is encountered, against which to push. This peculiar head movement cannot be associated with the intense pain of photosensitization lesions, since the natural reaction of a photosensitive sheep is to place the head in whatever shade is offered. This tendency is clearly demonstrated by two of the sheep in Plate 1, fig. 6. I believe this particular set of symptoms to be of purely nervous origin, although as yet our histopathological studies have not indicated that this may be the case.

Many animals have been encountered in this stage of the disease in a comatose or semi-comatose condition. These have often been pregnant ewes or fat wethers and in the majority of cases ketosis has been shown to be the complicating factor[15]. The field veterinarian should be constantly on the alert for this posibility since it requires immediate specific treatment.

Complete gastro-intestinal stasis as indicated in Table 2, was seen in at least half the cases in the controlled studies. In most instances considerable atrophy of the gastro-intestinal tract was evident, the contents of which were invariably in a state of advanced desiccation[16]. Considering that symptoms in this stage of the disease had only been present for three days at the most, the severity of the stasis indicates that it must have preceded the onset of the clinical symptoms by quite a few days.

C. *Advanced cases of the disease — the ictero-nephrotic and Addisonian syndromes.*

Cases of this nature belong to Steyn's stages

three and four noted earlier and exhibit the characteristic sequence of symptoms described there (q.v.) Table 4 presents an analysis of the prominent symptoms seen in 28 cases of stage three of the disease. Photosensitivity has largely subsided, but the lesions of photosensitization are prominent and are passing over into gangrenous sloughs. Secondary infection of these lesions has commenced, giving rise to a marked fever in most cases and a considerable degree of toxaemia. The eye lesions

TABLE 4.—ANALYSIS OF SOME PROMINENT SYMPTOMS SEEN IN EARLY CASES OF GEELDIKKOP.

Total number of advanced cases studied = 28. Duration of illness in all instances = 4–7 days. All sheep were maintained under controlled sheltered conditions.

Symptom.	Number of cases	Approx. % of Total
Lesions of photosensitivity: Mild to severe oedema of head, face, limbs etc.....	6	22
: Mild to severe sloughing lesions.....	20	93
: Mild to severe gangrenous lesions.....	2	9
Icterus : Severe.....	9	32 } 68
: Mild to fair.....	10	36 }
Not present.....	9	32
Rhinitis.....	4	14
Dyspnoea not associated with rhinitis.....	2	9
Conjunctivitis, Keratitis or panophthalmia.....	7	25
Complete gastro-intestinal stasis (confirmed at autopsy).....	9	32
Severe emaciation.....	9	32
Cases *in extremis* when first examined.....	8	29

which progress rapidly towards purulent panophthalmia are largely due to mechanical injury, solar irradiation and desiccation (in the absence of the blinking reflex) with superimposed secondary infection. This stage is dominated by renal pathology([13-15]) and clinical icterus is consequently severe. Nitrogen retention is invariably severe. There is often a marked uraemia and creatininaemia associated with albuminuria([13-15]) Malaise, extreme apathy and emaciation are prominent symptoms and many animals are found *in extremis* in this stage. The cause of death may be attributed to renal failure, toxaemia or septicaemia. In most of our cases kidney collapse seemed the most important in this respect. Gastro-intestinal stasis is once more a prominent symptom and ketosis a frequent complication.

An analysis of the prominent symptoms seen in eight cases of the fourth stage of the syndrome is presented in Table 5. This stage of the disease represents the second and third weeks of illness. Gangrene and secondary infection of all the areas affected by the ravages of photosensitization dominate the symptom picture. Unless drastic treatment is applied at this stage, the mortality rate is very high. Many cases are encountered *in extremis*. Nephrosis is severe in many cases but adrenal exhaustion is by now quantitatively more important. Dehydration, hyponatraemia, hypochloraemia, hypoglycaemia and hyperkalaemia are often present([15 16]) and there is a marked lymphocytopaenia and eosinopaenia([16]). A large number of sheep succumb at this stage to these Addisonian electrolyte imbalances and dehydration.

In many cases a large number of sheep which have passed through the third stage and appear to be making a successful recovery following careful treatment and nursing, suddenly collapse and die within 1-3 days. In all cases of this nature which we have been able to examine, collapse and death have been attributed to adrenal failure. This is another condition requiring immediate specific treatment for which the field veterinarian should be on the *qui vive*.

Febrile reactions are common and their severity depends on the severity of secondary infection. Gastro-intestinal stasis is invariably present in a most severe form as can be expected in cases ill as long as this and requires immediate and forceful treatment if the sheep are to recover.

TABLE 5.—ANALYSIS OF SOME PROMINENT SYMPTOMS SEEN IN ADVANCED CASES OF GEELDIKKOP

Total number of advanced cases studied = 8. All cases were in the second to third week of illness and all were maintained under controlled sheltered conditions.

Symptom.	Number of cases
Mild to severe gangrenous lesions of photosensitization on head, face and limbs	3
Mild to severe healing sloughs on head, face and limbs	4
Icterus : Severe	4
Mild to fair	2
Not present	2
Panophthalmia	1
Dyspnoea and cyanosis	1
Coronary band haemorrhages, with or without oedema of the extremities and coronitis	3
Severe emaciation	4
Complete gastro-intestinal stasis (confirmed at autopsy)	7
Severe Dehydration (confirmed by chemical pathology and autopsy)	2
Cases in extremis when first examined	5

In those cases where the renal lesions have subsided, the degree of icterus has declined simultaneously. *Bilirubinuria* and *coproporphyrinuria* are seen rather infrequently, but the hepatic excretion of bile pigments and porphyrins seems to have become reconstituted to varying degrees. In cases where icterus is still severe, uraemia and marked failure of hepatic bromsulphalein clearance are still very much in evidence.

Depending entirely on the vigour of treatment and nursing care up to this stage many animals may recover albeit with permanent mutilations but unfortunately many more succumb to the causes mentioned. Many of these deaths can be prevented if the cause is recognised and treated in time.

The symptoms which I have attributed to the precipitating causes have either largely passed over or are entirely obscured by the sequelae of the lesions of photosensitization and the various biochemical disturbances which now come to the fore. Coronitis and coronary band or hoof substance haemorrhages are persistent symptoms as can be seen by inspection of Table 5.

D. *Recovering cases of the disease — Steyns Stage 5.*

Although we have had the opportunity of examining large numbers of these cases, only eight have been studied under the controlled conditions mentioned. These studies contribute little to the descriptions already extant and there is no need to dwell further on the devastations of the sequelae. Convalescence is protracted and it may be many months before severely affected animals cease to be an economic burden to their owners. Fecundity is severely impaired by the poor physical condition of these animals. Rams are often rendered permanently sterile following gangrene and mechanical injury of the exposed scrotum. Libido is nonexistant for many months. Balano-posthitis is sometimes seen as a secondary complication.

Serious breaks in the wool and shedding of the entire fleece are common sequelae. Many farmers bearing this in mind attempt to finish shearing before the onset of the first summer rains and the appearance of geeldikkop. This, although it has it's advantages, can have fatal consequences. Summer thunderstorms in the Karoo are often accompanied by hail and bitterly cold south-easterly winds. Atmospheric temperatures may fall within a matter of hours from 100-115°F to near freezing point. We have often witnessed the tragic spectacle of thousands of newly shorn animals in all stages of geeldikkop succumbing to exposure. Many of those that survive the exposure often perish from acute broncho-pneumonia. It is imperative that recovering animals are not subjected to further stress for at least six weeks after subsidence of the acute

symptoms. We do not know how long the kidneys or the adrenals may remain *loci minores resistentiae*. The red cell lesions are ever present as also are permanent damage to numerous enzyme systems[22 24 28].

E. *Symptoms seen in the Angora goat, the Karakul, Blackheaded Persian sheep and wild antelope.*

All breeds of sheep and goats are susceptible to geeldikkop. Those with black pigmented skins and thick wool cover of the face, ears and extremities suffer far less from photosensitization than do the white breeds with woolless faces, ears and limbs. Ocular lesions, icterus and all the biochemical disturbances mentioned earlier are nonethe-less present in the black breeds and mortality, consequent to the factors mentioned, may be high. The Blackheaded Persian sheep develops lesions of photosensitization mainly on the limbs (especially on the plantar aspect of these), axillae, scrotum or vulva and mammae if present. Eye lesions are always present.

The symptom pattern in the Angora goat is identical to that in other white breeds of sheep and goats.

There exists no known reason why the disease should not occur in cattle or wild antelope which have been raised in the Karoo. The former occur in limited numbers in the Karoo and are more often than not maintained on artificial pastures. I have received numerous reports of geeldikkop in cattle from farmers but have never been able to confirm them. Similarly I have been told of severe outbreaks amongst springbuck (*Antidorcas marsupialis marsupialis*), blesbuck (*Damaliscus albifrons*) and steenbuck (*Raphicerus campestris campestris*) in which white areas of the skin, the muzzle and the eyes were primarily affected and in which icterus and cachexia were most obvious to the farmers concerned.

F. *General discussion.*

The purpose of this paper is not only an attempt to explain the symptomatology of geeldikkop in the light of present day knowledge and place on record some observations which do not appear in older accounts of the syndrome, but also to attempt to focus more attention on the possible rôle of an infectious agent in precipitating the syndrome. Our views on the basic aetiology remain unaltered[13-17]. It must not be inferred from the data presented here that an infectious agent is the only "trigger" factor. This is certainly not the case[13-17]. Similarly the emphasis which has been placed on bluetongue must not be construed as proof that this disease is in anyway concerned with geeldikkop. Although many of the lesions I have described are highly reminiscent of bluetongue, the presence of this disease amongst flocks in which geeldikkop is rampant still has to be confirmed by conventional means. One of the most important reasons for the failure of transmission experiments conducted by the earliest workers on the disease[11], Theiler[12], and later workers like Adelaar[11] may be that by the time the symptoms of geeldikkop have appeared the infectious stage of the precipitating disease has passed over, possibly some while before. The presence of a hypergamma-globulinaemia from the very onset of the symptoms of geeldikkop lends some considerable weight to this view.

I have, in a previous paper on the intrahepatic cholestases, indicated the close similarity between certain aspects of the symptomatology and chemical pathology of geeldikkop in sheep and chlorpromazine induced icterus in man[19]. A careful study of published case reports of the latter condition indicates that in many instances the appearance of icterus in patients receiving the drug has been preceded some while before by a mild febrile reaction, influenza-like symptoms and digestive disturbances. Why should the same state of affairs not pertain in the case of geeldikkop? The infectious agent must, as I have stated earlier, be mild enough to evoke a response which will pass unnoticed in the flock unless it is specifically searched for, yet be such that it will precipitate an acute episode, geeldikkop, in sheep where severe biochemical lesions have been in existence for some time, possibly from birth. Similarly in another paper[14] I have compared geeldikkop with human cutaneous hepatic porphyria. Porphyrias of this nature may be the result of many different aetological factors, all have in common the fact that acute attacks are precipitated in *asymptomatic* individuals by a variety of conditions which may be regarded as stress factors. Severe icterus is often but not always associated with the acute episodes and the liver pathology is reminiscent in some cases of geeldikkop and in others of enzootic icterus.

In conclusion I would like to draw the attention of readers to the important and well-known work of Neitz and Riemerschmid on the influence of solar radiation on the course of bluetongue in sheep[29 30]. The severest and most fatal reactions were observed when the solar radiation was very intense, i.e. in February, when geeldikkop is often rife. One of their main conclusions is that sheep in the field may react very severely to bluetongue if they become photosensitized. Their observations

on the alarming results of innoculation of sheep with the bluetongue vaccine then in use are worthy of further study with regard to the context of the present paper.

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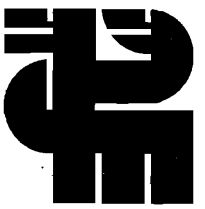

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EVALUATION OF ISONIAZID IN THE FIELD CONTROL OF BOVINE TUBERCULOSIS

H. H. KLEEBERG*, R. C. NIXON** AND R. W. WORTHINGTON*

SUMMARY

The use of isoniazid therapy and prophylaxis together with controlled slaughter in 30 tuberculous herds is reported. A dosage rate of 20 mg/kg proved superior to 10 mg/kg. Treatment periods of 9-11 months were not superior to seven months. Determination of drug levels in blood and tissue proved the slow absorption but high diffusibility of isoniazid in ruminants. With intravenous administration bacteriocidal blood levels are reached. Toxic symptoms were seen at a dosage rate of 30 mg/kg, marked ataxia at 60 to 70 mg/kg and death at 100 mg/kg. Cure was established by disappearance of tuberculin sensitivity, post mortem examination and bacteriological examination of lesions. Of positive reactors remaining in the herds 3-4 years after start of treatment, 73% became tuberculin negative. Bacteriological cure was attained in 78% of the 213 cattle examined at post mortem. The epizootology in the herds is discussed with special reference to the spread of the disease after treatment was completed. Only three major breakdowns were encountered. Advantages and disadvantages of the method are discussed.

INTRODUCTION

In the eradication of tuberculosis, the accent has been placed on those measures which are most efficient in preventing the spread of infection to clean animals. During this century, a step-by-step development has taken place in the efforts to control bovine tuberculosis. Ostertag thought that the elimination of clinical cases would suffice. Bang then introduced the segregation of tuberculin reactors from healthy stock, and later the slaughter-out policy of all reactors was used successfully in the U.S.A. BCG vaccination was given a fair trial in bovines, but it is out of favour in developed countries because it causes sensitivity to tuberculin. Recent trials in Uganda and Malawi, and work by Doyle et al[1] and Chodnik[2], have shown, however, that freeze-dried BCG can be of value under certain conditions. Extensive trials of chemotherapy and chemoprophylaxis of bovine tuberculosis have been done in South Africa in the last eight years. Since 1959 a number of papers have been published on this subject in this Journal[3-6], and in other publications[7-9]. The present paper reports the findings on 30 herds treated in the Transvaal and Natal under field conditions, our experiences with higher dosages of isoniazid (INH) and its toxicity, and new data on the pathology, bacteriology and epizootology over a period of years.

MATERIALS AND METHODS

For the estimation of INH blood, milk and tissue levels, a microbiological method by vertical diffusion, as described previously[5], was used. Cattle were dosed orally with 10, 20, 30 and 40 mg/kg, and 10 and 20 mg/kg intravenously, and blood samples collected every 2 hours for 24 hours. Milk was collected after 15 hours. To evaluate the diffusability of isoniazid into body tissues, 12 cattle were killed three to four hours after oral administration of 20 mg/kg, the tissue homogenised and the amount of isoniazid per gram determined in spleen, liver, lung, lymph nodes and abscess material.

To establish the toxic and lethal doses of isoniazid for cattle, goats and sheep, five animals of each species were dosed daily with increasing amounts until 100 mg/kg was reached, and this dose was continued for three days. Another six head of cattle were given isoniazid intravenously.

Altogether 213 isoniazid treated cattle were autopsied at Onderstepoort. The site of the tuberculous lesions was lung tissue in 80%, lymph nodes of the lungs and mediastinum 32%, mesenteric lymph nodes in 54%, pleura and peritoneum in 30%, lymph nodes of the head in 12%, the udder in 7%, and liver or peri-portal lymph nodes in 5% of cases. In one third of the cases only the lungs were involved, in one third only the lungs

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and the bowel, and in 21% generalised tuberculosis was found. Another 50 cases were seen in abattoirs during routine meat inspection, but visible lesions could not be found in the majority of cases within the time allotted for inspection.

Bacteriological studies were done on all pathological material or representative samples of it. In all, 380 organs were found to be tuberculous. Where possible all purulent, caseous and calcified material was removed and used for inoculation of cultures and injection of guinea pigs. Altogether 1218 guinea pigs, 2150 solid egg media and 2306 fluid media were used to test the viability and virulence of surviving *M. bovis*.

In the field, 1323 tuberculous cattle were dosed with 10 mg/kg INH daily for 7 to 10 months, 209 head with 15 mg/kg INH, 464 with 20 mg/kg INH and 254 with 30 mg/kg INH (see Table 1).

TABL. 1.—GENERAL INFORMATION ON TREATED HERDS.

Identity of herd	Breed	Size of herd	Incidence of TB at start		Isoniazid treatment			Follow-up	
			No.	%	Dose mg/kg	Method of Administration	Duration of treatment months	No. of tests	Duration of Experiment years
De Wildt	Jersey	25	10	40%	10	Solution by bottle	7	10	1¾
Elsburg	Friesian	129	79	60%	10	Solution by bottle	8	6	2
Residencia	Friesian	357	44	11%	10	Powder in feed	8½	10	4
Brakpan	Friesian	138	68	50%	10	Powder in feed	8	13	5
Pinetown	Friesian	69	66	95%	10	Powder in feed	9	7	2
Walkerville	Friesian	317	157	50%	10	Powder in feed	8	10	3
Naboomspruit	Afrikander	1000	153	16%	10	Dosing syringe	8	11	5
Heidelberg	Friesian	239	87	40%	10	Powder in feed	9	7	3½
Lion's River	Friesian	270	60	22%	10	Powder in feed	8	5	3
Mount Ashley	Friesian	311	58	19%	10	Powder dosed	8	5	3
Boksburg F	Friesian	267	136	50%	10	Powder in feed	8	9	4
Henley-on-klip	Ayreshire	198	24	12%	10	Solution by bottle	10	11	4½
College	Friesian	160	56	35%	10	Powder in feed	8	4	3
Ashburton	Friesian	150	67	45%	10	Powder in feed	8	5	3
Merrivale	Friesian	504	153	30%	10	Powder in feed	8	5	3
Klipriver	Friesian	147	69	50%	10	Powder in feed	8½	8	3
Camperdown	Friesian	68	31	46%	10	Powder in feed	8	3	2½
Paulpietersburg	Guernsey	54	26	50%	15	Dosing syringe	9	10	2½
Boksburg J	Jersey	170	61	35%	15	Powder in feed	11	10	4¾
Grasmere	Friesian	269	113	32%	15	Powder in feed	7	5	1¾
Garsfontein B	Friesian	54	18	33%	*(10) 20	Powder in feed	7	6	2¾
Garsfontein W	Friesian	476	171	29%	**20(30)	Powder in feed	7	7	2¾
Delmas	Jersey	165	66	33%	20	Powder in feed	6	4	1½
Springs	Friesian	368	74	26%	20	Powder in feed	6	6	2
Roodepoort	Friesian	290	84	25%	*20(10)	Powder in feed	8	4	1½
Boksburg W	Friesian	58	25	50%	20	Powder in feed	6	3	1
Stainbank	Ayreshire	81	44	54%	20	Powder dosed	6	3	2
Olifantsfontein	Friesian	312	65	22%	**30(10)	Powder in feed	9	4	1½
Rustenburg	Jersey	43	19	45%	*30(10)	Powder in feed	6	4	1½
Holmdene	Friesian	415	170	42%	**25(10)	Powder in feed	7	2	¾

* = 10 mg/kg for 2 months, 20 mg/kg for 5 months.
** = 20 mg/kg for 4 months, 30 mg/kg for 3 months (twice weekly).
* = 20 mg/kg for 4 months, 10 mg/kg for 4 months.
** = 30 mg/kg for 3 months, 10 mg/kg for 6 months.
* = 30 mg/kg for 3 months, 10 mg/kg for 3 months.
** = 25 mg/kg for 4 months, 10 mg/kg for 3 months.

The selection of herds for field experiments was determined by the incidence of tuberculosis, incapability of the owner to slaughter-out, and the facilities on the farm for accurate dosing and identification of animals. Identification was done by eartag numbers, but this was not always satisfactory, and leniency in this respect sometimes caused delays at tests and uncertainty about the identity of animals. Records were kept by the State Veterinarian, the Chief — Veterinary Field Services, and Onderstepoort. Some farmers were also requested to keep records, but only a few were sufficiently accurate.

The diagnosis was based on the results of tuberculin testing which were confirmed by post-mortem after slaughter of selected cases. Interpretation of the test was done rather strictly with the Interpretation standards suggested by Kleeberg[10] and Worthington and Kleeberg[11]. Non-specific sensitisation appeared in many herds. In the more recent experiments the comparative bovine/avian test was therefore generally used. All herds were retested 2 months after the initial test, and from then on at intervals of 3, 6 or 9 months. During the later years, half-yearly testing was the rule, but sometimes delays in testing were unavoidable and intervals of 9 to 12 months occurred. Elimination of clinical cases by slaughter was exercised in some herds. Separation of negative and positive animals was done in very few herds.

Chemoprophylaxis was used in 17 herds. Non-reactor cattle were always regularly tested. Farmers were instructed to measure the drug with special measuring spoons. Administration was effected by daily dosing or feeding of the drug in the ration. The control of dosing was done in most herds by checking the amount of drug used within a certain period. At each test the tuberculosis position of the herd was discussed with the farmer and he was advised to remove certain animals.

Judgement of apparent cure was based on the results of consecutive tuberculin tests. When the tuberculin sensitivity decreased steadily or disappeared completely after therapy, the animal was regarded as harmless to negative stock and kept on the farm. Removal was advised when an animal maintained its tuberculin reactivity or when an upward trend was seen after a previous decrease. Selection for slaughter commenced 2 years after the start of the control measures, and continued until the last reactor was removed.

Renewed spread of the disease was established when 2 to 3 definite new positive cases were found. As the farmers could not be forced to remove known treated cases many retained permanent reactors for years. Owners were also warned continually not to introduce untested stock, but nevertheless some did. Many farmers wished to retreat cattle which continued to react as well as newly discovered cases. This was allowed in eight herds as farmers often had supplies of drugs left over, and it was thought too dangerous to leave reacting animals untreated when a farmer would not remove valuable cattle.

BLOOD AND TISSUE LEVELS OF ISONIAZID IN CATTLE AND OTHER RUMINANTS

It has been suggested in previous papers([5, 6]) that dosages of 20 mg/kg were not much superior to doses of 10 mg/kg, and that a dose of 15 mg/kg failed to produce a clearly better therapeutic result in one field trial. Newer experimental evidence with higher dosages in other animals([12–15]) and man however indicate that higher dosages can bring about quicker improvement. Considering the 4 hour serum values in Table 2, it is evident that doubling and tripling of the dose increased the

TABLE 2.—BLOOD, TISSUE AND MILK LEVELS OF ISONIAZID

Dosage and Administration	Interval between dosing and bleeding in hours						
	2	4	4	4	8	15	15
	Serum	Serum	Liver, Spleen	Lung	Serum	Serum	Milk
10 mg/kg per os.....	1.2	1.3	—	—	0.9	0.4	0.24
20 mg/kg per os.....	1.3	2.0	1.6	1.0	1.6	0.7	0.4
30 mg/kg per os.....	5.3	3.5	—	—	2.1	1.0	0.7
40 mg/kg per os.....	6.2	5.5	—	—	3.4	—	—
10 mg/kg i.v.....	6.0	3.0	—	—	1.5	—	—
20 mg/kg i.v.....	16.0	8.7	—	—	5.7	—	—

Figures represent mcg/ml microbiologically assayed.
Figures give the mean value of 4 to 20 cattle.

concentration in the serum accordingly, and it is probable that the two hour values after 30 and 40 mg will have a bacteriocidal effect on *M. bovis*. The two hour INH serum level was also determined in goats, sheep and calves. After a loading dose of 10 mg/kg it was shown that these animals had a higher two hour value than cows with the same dose. The concentration for calves and sheep was doubled and that of goats tripled. This indicates that the early blood levels are mainly determined by the rate of absorption, which is quicker in young and small animals than in the rumen of a fully grown cow. The constancy of blood levels produced by a single dose once a day can possibly be explained by the fact that great amounts of food and fluid are kept in the stomachs of ruminants for long periods, and absorption of fluid is slow. Intravenous administration at 10 mg/kg and 20 mg/kg caused zero hour blood level of 100 mcg/ml and 200 mcg/ml respectively. These very high levels were not toxic. Side effects were observed about 5 hours after doses of 70 mg/kg i.v. when breakdown of the drug by the liver was more advanced. The drug levels in tissue

give proof of the high penetrating ability of INH into the tissues and they confirm the results of work on other mammalian species([16] [17]). The milk levels were about 65% of the blood levels, and in cows dosed with 20 mg/kg INH, one gallon of the 12-15 hour milk contained only 1.8 mg isoniazid.

INTRAVENOUS ADMINISTRATION OF ISONIAZID

To study the effect of very high peak levels as produced by intravenous administration, two small trials were undertaken. As the only predictable artificially producible form is miliary tuberculosis of the lungs caused by intravenous infection, 6 calves and 5 goats were injected with 1 mg and 0.5 mg respectively of the virulent *M. bovis* strain 9473. Three weeks later, when clinical symptoms were apparent, the 5 controls were sacrificed and viability counts were done on typically diseased lung tissue. The six remaining animals were treated intravenously once daily for 5 or 10 consecutive days with 10 or 20 mg/kg INH. The following day they were sacrificed and viability counts were done in the same way from a representative piece of lung tissue. When treatment started the bacilli had increased about hundredfold, shown by an average colony count of 240,000 viable units per 50 mg of lung tissue. After a total dose of 50 to 100 mg/kg INH this figure diminished to 1/35th and after 200 mg/kg INH to 1/80th of the original number of organisms. It therefore appears that very high tissue levels are in fact bacteriocidal.

Six untreated tuberculous heifers and cows were bought and two each were treated with 20 mg/kg INH intravenously for 8, 16 and 24 days respectively. They were then slaughtered, and all presented chronic tuberculous lesions in the lungs and various lymph nodes. Bacteriological examination of those lesions revealed that the two animals treated for eight days still contained virulent *M. bovis*, but of the others two were completely sterilized and two contained only a few attenuated organisms. The experiments indicate the more theoretical than practical value of starting chemotherapy with two weeks of intravenous INH administration.

TOXICITY OF ISONIAZID IN RUMINANTS

It was shown that the tolerance of the drug in cattle at therapeutically effective dosages is very good. Over 1300 head of cattle of all breeds tolerated 10 mg/kg INH daily for periods of 7-11 months. Another 673 cattle received 15 or 20 mg/kg INH daily for periods of 4-11 months without showing any adverse effect. Toxic symptoms were, however, seen when the dosage was increased to 30 mg/kg INH. Ten out of 19 Jersey cows refused the medicated feed during the first 10 days and a number of others showed stiffness in the hindquarters in the beginning. One group of 65 Friesian cows took 15 gr (30 mg/kg) INH well, while the same dosage was not tolerated in another herd with 170 Friesian cows. In this herd stiffness, trembling and weakness was seen, and a 25% drop in milk production occurred. The farmer gave a double dose (60 mg/kg) on Saturdays to make up for the interruption of dosing on Sundays, and he reported the loss of 7 animals. The affected animals could not get up on Sunday morning and the fatal cases apparently died from bloat.

An experiment was conducted to establish the toxic and lethal dose of INH for cattle, goats and sheep. The dose of 30 mg/kg was increased daily by 10 mg/kg until 100 mg/kg was reached and this dose continued for 3 days. Ataxia was seen after 80 mg/kg INH per os in 2 out of 5 cows and sheep, after 90 mg/kg in all 5 goats and after 70 mg/kg i.v. in all 6 cows. Death occurred after 100 mg/kg INH per os in 1 out of 5 cattle and sheep, 2 out of 5 goats, and 1 out of 6 cattle after 80 mg/kg i.v. INH. In all fatal cases the main pathological feature was a severe fatty degeneration of the liver. Other findings were cyanosis, tumour splenis, slight nephrosis, epi- and endocardial haemorrhages and mild lymphocytic meninigitis. Comparison of the onset of toxic effects in intravenously and orally dosed animals indicates that it is not the free INH but the breakdown products of the drug which are toxic. After intravenous injection extremely high blood and tissue levels are reached so that one would expect toxicity at a much lower level than after oral dosage. In all cattle regardless of the way of drug administration the onset of symptoms started about 5 to 8 hours after dosing, at a time when breakdown products accumulate in the body. Large amounts of ammonia produced during the breakdown of INH have been blamed for causing the neurotoxic effects and the liver damage([18]).

TUBERCULIN SENSITIVITY OF TREATED TUBERCULOUS CATTLE

In previous papers it was shown that a continuous dimunition of tuberculin sensitivity occurs which is due to the death and removal of the tubercle bacilli. Skin reactions tend to lose their typical characteristics of diffuseness, oedema, local heat, pain and adherence to the subcutaneous tissues. The reaction site becomes harder, flatter and more circumscribed and resembles a non-specific reaction. After the full treatment, only

TABLE 3.—TUBERCULIN SENSITIVITY OF ISONIAZID TREATED TUBERCULOUS CATTLE.

Identity of herd	Start of treatment		End of treatment			Results of subsequent tuberculin tests											
	1st & re-test		6–10 months			12–15 months			18–24 months			2½–3 years			3–4 years		
	Positive & suspicious cattle		Positive & suspicious cattle		Negative cattle	Positive & suspicious cattle		Negative cattle	Pos.& susp. cattle	Negative cattle		Pos.& susp. cattle	Negative cattle		Pos.& susp. cattle	Negative cattle	
	No.	Average Reaction mm.	No.	Average Reaction mm.	No.	No.	Average Reaction mm.	No.	No.	No.	%	No.	No.	%	No.	No.	%
De Wildt	10	10.9 mm	7	3.2 mm	1	8	3.7 mm	0	4	2	33%						
Elsburg	79	13.5 mm	55	5.3 mm	9	33	4.5 mm	15									
Residencia	44	13.0 mm	42	6.4 mm	2	41	6.3 mm	0	26	12	32%	17	14	45%	7	13	65%
Brakpan	68	11.6 mm	42	6.4 mm	15	19	5.0 mm	33	10	34	77%	8	33	81%	2	24	92%
Pinetown	60	15.0 mm	37	4.0 mm	15	34	4.2 mm	9	17	22	56%	17	24	59%			
Walkerville	157	10.6 mm	96	4.4 mm	33	73	4.4 mm	42	41	53	56%	15	69	82%	10	54	84%
Naboomspruit	153	8.4 mm	94	4.5 mm	27	40	4.6 mm	28	13	58	82%	5	47	90%	5	31	86%
Heidelberg	87	12.7 mm	66	7.1 mm	17	—	—	—	37	23	38%	30	8	21%	17	17	50%
Lion's River	60	15.0 mm	40	6.5 mm	11	18	5.5 mm	29	13	26	67%	8	29	78%			
Mount Ashley	58	19.5 mm	29	9.0 mm	16	15	6.6 mm	19	10	19	66%	3	12	80%			
Boksburg F	136	17.5 mm	104	9.4 mm	19	87	8.0 mm	27	66	28	30%	42	29	41%	19	23	55%
Henley-on-Klip	24	9.0 mm	10	4.0 mm	9	7	4.0 mm	8	3	8	73%	0	6	100%	0	6	100%
College	56	14.6 mm	24	7.0 mm	8	10	5.5 mm	19	8	19	70%	5	16	76%			
Ashburton	67	14.7 mm	33	6.5 mm	19	10	3.9 mm	30	6	28	82%	7	19	73%			
Merrivale	153	16.9 mm	75	8.6 mm	75	67	5.2 mm	61	35	65	65%	26	19	42%			
Kliprivier	69	9.5 mm	54	8.3 mm	1	35	4.6 mm	8	29	9	24%	13	22	63%			
Camperdown	31	17.6 mm	23	6.0 mm	3	18	6.8 mm	6	6	15	71%						
Paulpietersburg	26	15.9 mm	15	6.8 mm	2	4	2.3 mm	7	0	9	100%	0	6	100%			
Boksburg J	61	16.4 mm	52	8.0 mm	5	47	6.6 mm	6	38	11	22%	30	11	27%	3	4	57%
Grasmere	113	14.6 mm	67	5.9 mm	19	49	4.3 mm	34	24	40	63%						
Garsfontein B	18	17.4 mm	18	7.2 mm	0	10	6.0 mm	5	11	3	21%	6	3	33%			
Garsfontein W	171	11.5 mm	108	5.7 mm	59	82	5.0 mm	75	87	47	35%	54	60	53%			
Delmas	66	10.5 mm	53	4.3 mm	13	30	3.8 mm	30									
Springs	74	13.4 mm	50	8.2 mm	18	35	6.1 mm	34	7	39	85%						
Roodepoort	84	13.6 mm	17	9.4 mm	25	9	4.8 mm	14									
Stainbank	44	16.0 mm	21	8.0 mm	9	—	—	—	12	15	56%						
Olifantsfontein	65	13.2 mm	38	5.7 mm	10	30	5.1 mm	12									
Rustenburg	19	10.7 mm	16	5.9 mm	1	3	3.0 mm	5									
Holmdene	175	15.5 mm	133	5.2 mm	42												

4.5% reactors developed reactions with an increase of skin thickness of 8 mm or more. Other conditions and factors which may cause diminished positive tuberculin reactions in cattle can be excluded as a reason for this phenomenon. Table 3 gives the results of tuberculin tests at the start and end of treatment, and subsequent tests at 6 to 12 month intervals. A reaction was only regarded as negative when the measured increase was less than 2 mm. Applying this strict interpretation, 60% of the reactors which were still in the herd were negative 2¼ to 3 years after the start of therapy, and 73% were negative after 3 to 4 years. At that time, due to normal turnover and selected slaughter, only one third of the original reactors were still in the herd. In some herds a non-specific factor complicated the picture and kept the reaction of certain cows above 2 mm.

PATHOLOGY

The reaction of the tuberculous lung tissue to treatment consisted chiefly of demarcation of the diseased parts, encapsulation of necrotic foci, transformation, disintegration and removal of debris by the action of enzymes and macrophages, and very marked fibrosis and collapse of lobuli. In the later stages, the advanced transformation of diseased tissue often made it difficult to recognise it as a former tuberculous lesion, especially if necrotic foci were absent. The altered area was always surrounded by normally functioning lung tissue. Mainly four distinct pictures occurred in lung lesions i.e. encapsulation of necrotic foci, healing of cavities, collapse of lobuli and fibrotic induration. More or less typical tuberculous necrotic material was found in 80% of cases. The contents of the lesions showed great variation in consistency. During the first months of treatment it was still soft, creamy or semi-calcareous, later becoming chalky, gritty, rubbery, or even gelatinous and always much drier. The colour ranged from slightly yellowish to yellow and greyish. In a number of lesions the centres were chalky, whereas the necrotic material near to the capsule showed collagenisation. Another tissue reaction was observed when the formation of necrotic material was apparently not yet far advanced in the diseased lobuli i.e. acinar tuberculosis pneumonia. The colour, consistency and appearance of this tissue, seen in 58% of infected lungs was very similar to that of lung atelectasis. Entire lobuli, usually in sharp contrast to surrounding normal lung tissue were collapsed and reddish grey with absence of normal alveoli. These

areas varied in size from 1 to 10 cm in diameter. As the result of treatment tuberculous lymph nodes showed the same general trend as lung tissue, namely the disappearance of the specific tuberculous tissue and debris, encapsulation of necrotic material by connective tissue and scar formation. With the exception of small, old necrotic foci a very marked and complete encapsulation was present with walls of whitish fibrous tissue ranging in thickness from 0.5 to 5 mm.

The compactness of the necrotic material usually allowed the removal of the nodule as a whole with little debris in the inner surface of the clearly defined capsule. The colour varied from whitish grey to intense yellow, the consistence from rubbery and semi-calcareous or chalky to hard and calcified. The cut surface was either smooth or gritty. Bigger necrotic masses were sometimes penetrated by anthracotic or greyish connective tissues. No signs of hyperaemia or inflammation in the surrounding normal lymphatic tissue could be noticed.

The necrotic material in the lesions of treated cattle contained 8.6% Phosphorus, 16.1% Calcium, 0.52% Magnesium. The figures for untreated controls were 2.6% P, 3.5% Ca and 0.17% Mg (averages). The chemotherapy resulted in a significant increase of the calcium and magnesium salts i.e. $Ca_3(PO_4)_2$, $Ca\ CO_3$ and $Mg_3(PO_4)_2$. The concentration of P and Mg trebled and that of Ca was four times higher in treated cattle.

BACTERIOLOGY

Bacteriological studies provide a very decisive criterion of the value of an anti-tuberculosis drug. The bacteriological and biological results obtained with tuberculous material of 213 cattle treated for different periods are summarised in Table 4. The categories shown in Table 4 were set up according to the bacteriological and biological properties of the tubercle bacilli recovered. The animals grouped under "highly attenuated *M. bovis* only", harboured only isoniazid resistant organisms, which caused but local lesions when injected into guinea pigs. The group with non-cultivable and non-infectious bacilli only, and the fourth group with sterilized lesions or no visible lesions, can be regarded cured from the bacteriological point of view. Pooled lymphnodes, collected from all no visible lesion cases were negative on culture, which is not surprising, after a treatment which even rendered necrotic lesions non-infectious.

In Table 4, 20 cattle are included which had received 20 mg/kg INH for the first 4 months, and also 5 cattle which received 15 mg/kg INH for 10 months. Of those 25 cattle, 3 did not present lesions, 1 harboured fully virulent *M. bovis*, and the others were either sterilised or contained non-viable bacilli only. This ratio of 24 bacteriological cures against 1 case with virulent *M. bovis* would indicate an improvement of therapeutic results with the higher dose.

The origin of the animal influenced the result slightly, insofar that the most meticulous farmers obtained the highest rate of cure, and animals from farms with a lower standard of management had a correspondingly lower rate of cure.

EPIZOOTOLOGY

Theoretically, the future spread of the disease in a treated tuberculous herd, would be influenced by the following factors — the detection of all infected cases by the first two tests, the use of chemoprophylaxis or separation of infected and non-infected stock, the accuracy of treatment, the removal of the remaining reactors, and tuberculin testing of all introductions. In practice, however, the overall picture of the epizootology was directly

TABLE 4.—BACTERIOLOGICAL RESULTS IN RELATION TO DURATION OF CHEMOTHERAPY

Characteristics of Tubercle Bacilli Isolated	Period of treatment			
	2–3 months	4–6 months	7–8 months	9–10 months
Virulent M. bovis	6	3	3	2
Highly attenuated M. bovis only	4	8	19	10
Non-cultivable, non-pathogenic acid fast bacilli only	3	7	27	11
Sterilised lesions only and 23 cases without lesions	7	10	52	33
Number of cattle examined	20	28	101	56
Bacteriological cure	50%	61%	78%	79%
Reactivation possible	30%	11%	3%	3.6%

Figures represent bovine animals.

correlated to the standard of management. Very few new cases occurred in T.B. free stock during the first and second years except in the Naboomspruit Afrikaner herd consisting of 1,000 head, in which cattle were dying of the disease before therapy was started. Thus the accuracy of the tuberculin test was truly demonstrated.

Isoniazid as a prophylactic agent was useful in heavily infected herds. In many herds, prophylaxis was used when the farmer was not able to separate the tuberculous from the non-tuberculous cows nor identify them so that the tuberculous ones could be accurately treated. Some farmers could not trust the attendants to distinguish between the two groups and preferred to treat all cows in spite of higher costs. Full separation was exercised during the first 8 or 12 months in two herds only. Except on two farms, the dosing was done satisfactorily by the farmers. In two other cases, the wrong dose was administered. There was a general tendency to overdose the cattle rather than underdose them.

The arrest of further spread of the disease can be readily explained. Autopsies showed that all the tuberculous lesions were well encapsulated within three months of starting treatment, open cavities were empty and diseased lung tissue collapsed and fibrotic.

When it was realised, however, that virulent organisms did survive in 3% of long term treated cattle, farmers were advised to gradually remove all cattle which remained tuberculin positive for more than 2 years after start of therapy. It should be noted that the farmers were under minimal veterinary control.

Some farmers did not want to lose valuable animals and wished to treat chronic reactors for another period of 3 to 5 months. This was consequently allowed in 8 herds with a total of 192 animals. It is difficult to assess the effect of this retreatment upon the subsequent epizootology, but the authors believe that such an animal under INH dosage will not excrete virulent tubercle bacilli.

Judgement of apparent cure was based entirely on the measurements of skin reactions. As long as an animal showed a trend of decreasing sensitivity or became completely negative, it was regarded as apparently cured. Two negative tuberculin tests in the second or third year after treatment indicated bacteriological cure.

In 28 herds in which the epizootology has been studied for between two and five years, 118 new positive reactors were found in approximately 4000 non-infected animals after treatment had been completed. The majority of these cases occurred in three major breakdowns in the herds Merrivale, Boksburg F and Klipriver which accounted for 78 of the cases. For full details see Table 5.

TABLE 5.—EPIZOOTOLOGY AFTER FULL COURSE OF ISONIAZID TREATMENT
INCIDENDE OF BOVINE TUBERCULOSIS BASED ON SINGLE INTRADERMAL TEST.

Identity of Herd	Start of Experiment		2 Years later			3 Years later			4–5 Years later		
	TB	TB Free	Size of herd	Previous positive cases	New positive reactors	Size of herd	Previous positive cases	New positive reactors	Size of herd	Previous positive cases	New positive reactors
De Wildt	10	15	35	0	0						
Elsburg	79	50	80	46	0						
Residencia	44	313	339	43	0	342	21	4	261	19	4
Brakpan	68	75	138	40	0	134	34	0	130	23	0
Pinetown	60	3	59	39	1						
Walkerville	157	160	477	99	4	475	67	0			
Naboomspruit	153	865	842	49	8‡	939	41	0	1100	38	0
Heidelberg	87	152	223	54	0	261	37	1			
Lion's River	60	210	244	39	1	258	37	0			
Mount Ashley	58	253	188	29	0	246	15	0			
Boksburg F	136	131	262	94	4*	243	49	3*	289	30	19
Henley-on-Klip	24	198	167	11	2	194	6	0	225	6	0
College	56	104	104	27	1‡	102	21	1			
Ashburton	67	83	94	34	0	86	26	0			
Merrivale	153	351	363	100	2	399	81	43			
Kliprivier	69	81	100	40	1	101	16	16*			
Camperdown	31	37	125	21	0						
Paulpietersburg	26	28	41	9	0	39	6	0			
Boksburg J	61	99	227	49	0	241	41	0	271	11	0
Grasmere	113	156	216	64	3						
Garsfontein B	18	36	92	10	0						
Garsfontein W	171	305	488	116	0						
Delmas	66	99	165	52	1						
Springs	74	294	287	46	3‡						
Roodepoort	84	206	81	23	0						
Stainbank	44	37	74	27	0						
Olifantsfontein	65	247	292	42	0						
Rustenburg	19	24	20	8	0						

Figures represent bovine animals.

* = All or part new introductions.
‡ = One or more negative on post mortem.

Animals were introduced without the consent of the authorities in eight herds. In three herds, introduced animals were found to be tuberculous and subsequently substantial numbers of clean stock became infected in two of them (Boksburg F and Kliprivier). There was, however, no possible method of determining whether the breakdowns were due to the introduced animals or to reactivation of tuberculosis in treated reactors. In one case the evidence appeared to point to the latter source. In the Merrivale herd, the new outbreak mainly concerned calves which became infected from milk from a treated case of mastitis. This was a large, badly infected herd, where cows were dying of miliary tuberculosis before treatment. The identification of animals was not satisfactory, the owner did not co-operate in culling properly before treatment and removing positive cases after treatment. On another farm, a treated animal with a continuous skin reaction, was kept in isolation and the disease progressed to involve most of both lungs. In the Residencia herd, the picture was complicated by non-specific reactors, and interpretation of tests became so difficult that after 5 years of constant control, the herd was still not free from tuberculosis and a few new cases were detected. If all animals which maintained a certain level of tuberculin reactivity or showed an upward trend were removed, the herds could be freed of all reactors within 3 years. Some farmers started removing uncured cases only in the fourth year but no spread of the disease was seen in later years.

Discussion

In advising on an eradication scheme, one must consider the economic, organisational and agricultural structure of a region or herd. The ideal measure — the slaughter of all reactors in a very short time — might be excessively expensive. One should strive towards measures suited to the particular agricultural and economic situation.

Isoniazid could play an important role in an eradication scheme, particularly in the early stages, when there are not enough tuberculosis-free herds to provide replacement for the slaughter programme. It is especially suited for dairy herds with infection rates of 20% to 70%, where slaughter of all reactors would interfere severely with the economy of the farm.

The field studies leave no doubt that any responsible farmer can protect his non-infected stock, cure most of his infected stock and continue farming and producing without major changes. The appropriate combination of chemotherapy and selected slaughter, both at the beginning and end of a 3 or 4 year eradication scheme, could be fitted to meet the farmer's needs, local conditions and the available financial resources. Infected cattle could be saved for normal production of milk and calves, and most of them could remain in the herd for a normal productive life span. Separation is not necessary during, and for a long time after treatment. Unnecessary slaughter of non-specific reactors could be avoided as they could be studied during the control period.

Separation of infected and clean stock can rarely be achieved in South Africa without building new stables and erecting new fences and water troughs. In this case chemoprophylaxis is of use, mainly in the first 2 months, when some infected cows are still excreting bacilli. It is a cheap and effective substitute for separation during the initial period. In preventing further spread of infection, it is as effective as slaughter or complete isolation of all tuberculosis cases. It can be recommended for all valuable animals, especially in conjunction with chemotherapy of infected animals. One farmer preferred to build a new stable, some fences and water troughs, at a cost of R2,000 for the isolation of 100 clean cows. If he had used isoniazid treatment, he could have cured his herd at far less expense.

The factors influencing the success of chemotherapy can be summarised as follows. Firstly the farmer's co-operation with the veterinarian and the general management are most important. Identification, keeping of records, regularity of dosing, follow-up by testing and removal of uncured animals are facilitated by good management. Secondly, the veterinarian must determine which herds are suitable for chemotherapy. He must decide which cattle must be removed before treatment, and instruct the farmer on treatment and dosage of cattle, and decide which animal to remove after treatment. The control of cattle movement, the enforcement of permanent identification and a certain standard of hygiene, and the supervision of effective disinfection of premises by the veterinary authorities would be similar in any campaign. The State would have to prevent the indiscriminate use of the drug, control the sale of the drug and the sale of partly treated cattle. The level and length of administration of isoniazid should not be less than 10 mg/kg and not shorter than 7 months. A daily dose of 20 mg/kg is superior to the 10 mg/kg dose. Theoretically 30 mg/kg during the first month or two, should be even better, although side-effects may be encountered at this level. Valuable animals would justify the expense of a longer period up to 10 months and a higher dosage up to 30 mg/kg.

Under the present circumstances, treatment was economical for most farmers, because no compensation was paid for positive reactors, but expenses for the State were higher than in herds freed by slaughtering the reactors. Factors other than the cost of the drug and the value of the milk, calves and cattle cured must be considered. To the farmer, there is the cost of administering the drug, which is low in the case of dairy farmers feeding concentrates, but considerable if cattle have to be dosed. Dosing of ranching cattle in a crushpen, as done in one herd, can therefore not be recommended. To the State, the costs which would have to be borne are the testing of cattle, the salaries of staff (including office staff and stock inspectors), the mileage travelled, and the tuberculin used. With treatment there are probably twice as many tuberculin tests necessary, than with slaughter-out. The extra testing and the length of control periods would require more staff than the "slaughter-out" method.

The classification of stock would be complicated because instead of merely having "T" branded reactor-cattle, there would be untreated reactors, partially treated reactors, treated cured reactors and treated uncured reactors. With treatment, unscrupulous farmers would have to be prevented from getting rid of tuberculous cattle whatever their condition or reactivity might be. The main difficulty in instituting an eradication programme in South Africa is the shortage of professional staff. The position would be aggravated by using the more time consuming treatment. The interpretation of the tuberculin test is more difficult after treatment. Each case has to be followed up over the whole period. When the stage is reached where treated animals have only a low residual sensitivity, and a concurrent non-specific sensitisation problem exists in the herd, it is difficult to decide whether the reactions to bovine P.P.D. are due to surviving *M. bovis,* or cross sensitivity due to *M. avium-like* bacilli.

The fear that an animal might still be tuberculous and later become a clinical case and reinfect the herd, should not lead to retreatment of the case but its immediate removal. The danger of INH resistant organisms infecting cattle or man is remote, as long as treatment is restricted to well controlled herds. As far as spread by air droplets is concerned, the typing of 2000 strains of tubercle bacilli from pulmonary disease of man, has only revealed a single case caused by *M. bovis*[19], showing that lung infection of man even by fully virulent *M. bovis* is very rare in this country. Calves were infected by different routes with large numbers of INH resistant *M. bovis* and progressive disease could not be produced. If, however, cases of tuberculosis mastitis are overlooked in the beginning of treatment and milk is not pasteurised, there is a possibility of infection of children or calves. One should prevent the dispersal at sales of treated stock, still infected. Only closed herds are suitable for INH treatment, but many tuberculous herds are of the type that do not breed replacement stock, but into which cows and heifers are continually being brought.

It was also seen in Europe that eradication was more difficult in the bigger herds with large numbers of tuberculous cattle. The veterinary control in our experimental herds was hampered by our inability to enforce the removal of animals and to prevent the introduction of untested animals within the limits of a voluntary eradication scheme. If treatment is applied within the framework of an eradication campaign, suitable legislation should be introduced and farms strictly controlled.

In conclusion, it becomes apparent that individual farmers can greatly benefit by treatment of their tuberculous animals, but for the purposes of eradication by state action, the slaughter-out method will be speedier and easier to control.

ACKNOWLEDGEMENTS

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LETTERS TO THE EDITOR

The Editor,

S.A.V.M.A. Journal.

I feel that few of our members are aware of the pioneering role played by Joan Morice, both as the first woman graduate from Onderstepoort and also as the first Onderstepoort graduate to go into private practice.

Joan qualified in 1927 and set up practice in her home town, Johannesburg, during 1928. Her venture was eminently successful. When she and M. C. Robinson married in 1930 they carried on the practice together until he joined the Johannesburg Municipality in 1935. Mike Robinson was therefore the second Onderstepoort graduate to blaze the trail for following practitioners. Joan died in November, 1944.

After Joan qualified it was 19 years before we had another woman graduate, Maud Bales in 1946. Since then we have seldom been without at least one woman student in the faculty and this year there are ten.

As the years roll by and our numbers increase it is well for us to recall the part played by our graduates who did much to establish the profession in South Africa.

R. C.

SERUM-AGGLUTININ RESPONSE IN FOWLS IMMUNIZED WITH LIVE ATTENUATED *SALMONELLA* VACCINE

It has been brought to our attention that fowls immunized with Onderstepoort live, polyvalent *S. enteritidis* var. *gallinarum-typhimurium* vaccine, sometimes give positive or suspicious reactions when subjected to the whole blood agglutination test. These reactions have been and are still, wrongly, attributed to either a carrier-state or a reaction stimulated by the live vaccine, especially since one or both vaccinal strains have been isolated from such reactors.

It has been shown that the vaccinal strain persists for a considerable period after immunization, without causing symptoms, pathological lesions or stimulation of serum-agglutinins specific to smooth antigen. The vaccinal strain has been isolated from the internal organs of up to 5 per cent of clinically normal fowls, 6 months after vaccination. This incidence may be considerably higher within 6 weeks to 10 weeks after immunization.

A large percentage of vaccinated fowls, when exposed, invariably developed O-agglutinating antibodies, the titre of which was in direct correlation to the severity of exposure and not necessarily to the state of prevailing immunity. These vaccinated fowls reacting to the whole blood agglutination test were completely immune. Repeated efforts to recover virulent *S. enteritidis* var. *gallinarum* or any other virulent *Salmonella* from their organs were unsuccessful. In the majority of cases, the vaccinal strain was a constant isolate from both the unexposed, vaccinated fowls without a titre and from the exposed, vaccinated fowls possessing detectable serum-O-agglutinins.

On one particular farm where severe losses due to fowl typhoid occurred, immunization with the live vaccine was followed by whole blood agglutination tests at regular intervals. Although cessation of mortality followed immunization, reactors, to the coloured antigen test, continued. Up to 5 per cent of vaccinated fowls reacted. These were removed but the following test also showed a comparable number of reactors. Bacteriological examination carried out, on one of these reactors produced the rough vaccinal strain, only.

These "reacting" fowls therefore are not carriers of infection but are immune and serve as a barometer of a persisting infection on the plant.

These results therefore confirm the inability of the whole blood agglutination test to differentiate between immune reactions following exposure of vaccinated fowls and the actual carrier of the virulent infective strain.

In recommending a solution for this delicate problem, I would suggest quantitative bacteriological examinations to be carried out on a percentage of reactors in a vaccinated flock, before the B.W.D.-free certificate is finally withdrawn.

H. J. W. BOTES. ONDERSTEPOORT.

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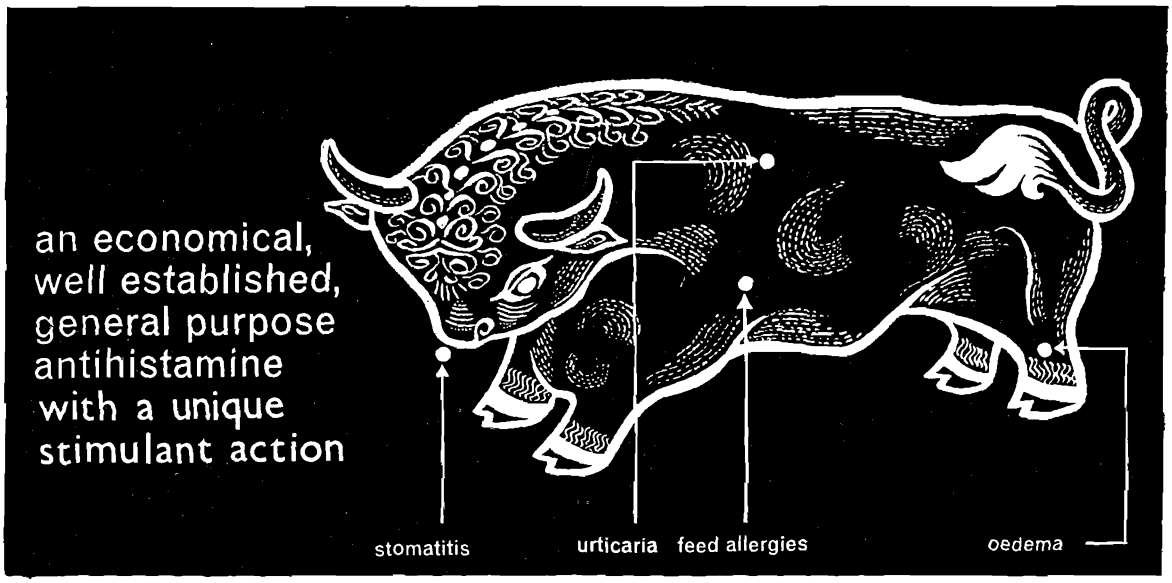

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COCCIDIA AND COCCIDIOSIS

BY L. P. PELLERDY,
Akadémiai Kiadó Budapest, Hungary.
657 pp. 197 figs. Price: R4,11.

This beautifully produced book is indispensable as a work of reference to the protozoologist working in this field. It has no index, but if used in conjunction with Pellérdy's *Catalogue of Eimeriidea* (1963) this difficulty can be obviated. Pellérdy has a refreshing approach to his subject of which he is obviously a master.

Part I deals briefly with the classification, life cycles, the morphology, biology and cytochemistry of developmental stages, host-parasite relationships, immunity and host-specificity of coccidia. In Part II the various genera and species of the suborder Eimeriidea (i.e. coccidia) are described in detail under the headings of their respective hosts. Detailed information is furnished on the morphology of the oocysts and other stages, developmental cycle, pathogenesis and pathology, epizootiology and chemotherapy of the various species, especially the coccidia of domesticated animals.

"Globidial schizogony", is defined as the occurrence in the life cycle of only one generation of large schizonts visible to the naked eye. This is said to occur in *Eimeria bovis, E. leuckarti, E. arloingi* and *E. parva,* in spite of the fact that the author is apparently aware of the recent discovery of small, second generation schizonts in the life cycle of *E. bovis* (p. 538).

Pellérdy is not prepared to assign *Globidium gilruthi* to a specific *Eimeria* sp. on the grounds that there are at least three *Eimeria* spp. of sheep with large schizonts that are macroscopically visible.

The author also mentions the difficulty of differentiating between oocysts of *E. arloingi, E. crandallis* and *E. ahsata.* This serves to emphasize the dire necessity for studying clones in order to establish the validity of these and some other species of mammalian coccidia.

R. D. BIGALKE.

CASE REPORT

Stomatitis caused by the Flower Heads of *HORDEUM MURINUM L.*

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Hordeum murinum L. (See fig. 1) commonly known as False Barley, Barley grass, "Kruipgras", Mouse Barley, Wall Barley grass, Wild Barley or "Wildegras" is an introduced weed in South Africa(1). It occurs on sandy wastes and other disturbed areas in the Southern and South Western Coastal districts of the Cape Province(2). The bristly heads are said to be apt to cause irritation and injury to the mouths of stock(1),(2).

While investigating a complaint by farmers about sores in the mouths of sheep in the Somerset East district cases of complete blockage of the mouth cavity in stable fed rams, where the grass occurred in the lucerne hay, were seen. There was severe irritation with foul smelling necrosis at the point of entry of the rigid awns into the mucous membrane of the mouth. The animals were hollow in the flanks and obviously hungry. When food was placed in front of them they showed interest, but had difficulty in prehension. They were kept under observation for two hours and only one animal attempted to ruminate but the ingesta ran out of the mouth and nose.

Cases could be prevented by regular cleaning of the mouths of the animals.

The plant has since been found to be fairly widespread in the districts of Pearston, Cradock, Grahamstown, Bedford and Adelaide.

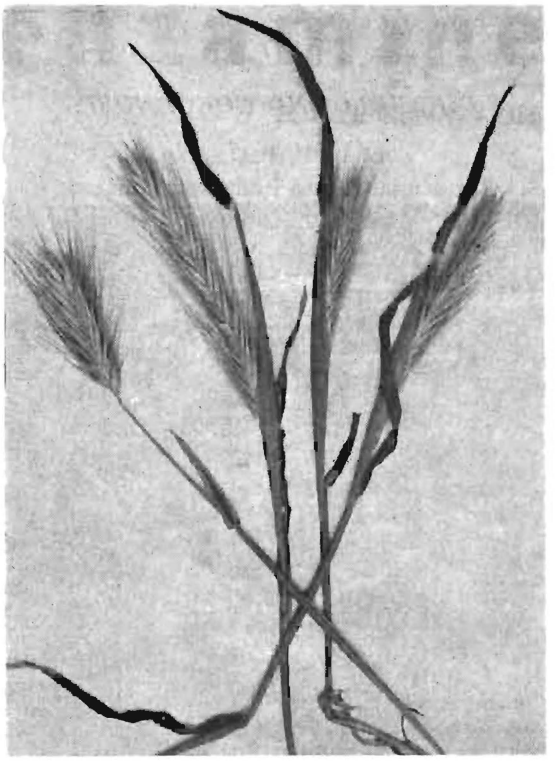

Fig. 1

ACKNOWLEDGEMENTS.

My thanks are due to Mr. M. Wells, Senior State Botanist, Grahamstown, for the identification of the grass, to Prof. T. Adelaar and Dr. M. Terblanche for their interest and advice, to Mr. A. du Bruyn for excellent photography and to the Chief Vet (Field) for his permission to publish this report.

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COLLECTION OF SALIVARY GLAND SECRETION FROM THE ARGASID *ORNITHODOROS SAVIGNYI* ADOUIN (1827) BY THE USE OF A PHARMOCOLOGICAL STIMULANT.

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The salivary gland secretion of ticks is of major importance whenever man or animals are subjected to attack by these parasites. Transmission of disease is effected mainly by this route, the causal organisms being transferred into the tissues of the host along with the salivary secretion. However, the introduction of the secretions of a large number of tick species is responsible for conditions that are not the result of pathogens transmissable from diseased to normal animals. The causal agents of these diseases appear to be "toxins" derived from the complex of biological products secreted by the salivary cells.

"Tick paralysis" was recorded as early as 1904 by Malley (cit. Ross[1]) in South Africa, which he found to occur in sheep as the result of bites of the tick, *Ixodes pilosus*. It has been shown subsequently that the tick species involved was *Ixodes rubicundus* (Theiler[2]). A similar condition was found in man, sheep and dogs in Australia, Canada and Crete, and was described from these countries during the years 1912 to 1924 (Ross[1]). Subsequently tick paralysis has been described and investigated many times, generally implicating the genera *Ixodes* and *Dermacentor*. Paralysis affecting lambs as a result of attack by *Rhipicephalus evertsi* was recorded in South Africa by Clark[3] while the same species was shown to be capable of causing paralysis in adult Merino sheep by Neitz and Jansen[4].

Rhipicephalus simus is incriminated in the aetiology of tick paralysis in man by Zumpt[5] as was *Hyalomma truncatum* (*transiens*) by Swanepoel[6]. In South Africa the available records appear to indicate that not only are strains of certain genera normally associated with the condition, but other ticks under certain conditions may also be involved.

Neitz[7] found the vector of "sweating sickness" (La Dyhydrose tropicale) to be *Hyalomma transiens* and that a toxin of some type was the causal agent. The toxins responsible for paralysis and sweating sickness have not yet been identified.

"Tick toxicosis", a descriptive term for the physiological and pathological changes brought about by severe infestation with ticks led van Rensburg[8] to conclude that the extensive damage observed in bovines by the genus *Rhipicephalus* could be caused by a potent cytotoxic substance as reflected by lymph-node changes. Emulsions of salivary glands were prepared by Hughes and Philip[9] in an attempt to produce experimental tick paralysis with rather negative results. Gregson[10] found that small quantities of oral secretion could be collected from ticks recently removed from a host by applying a capillary tube over the hypostome and chelicirae of *Dermacentor andersoni*.

The presence of haemolysins in the salivary secretion of argasid ticks suggested by Pavlovsky and Chodukin (cit. Chinery[11]) could not be substantiated by Lavoipierre and Riek[12] in their observations on the feeding habits of argasid ticks.

Arthur[13] described the presence of cytolysins in the salivary secretion of *Ixodes* ticks as observed in histological sections of tissue surrounding tick bites. Chinery[11] demonstrated the presence of certain polysaccharides, proteins, lipids and nucleic acids in the salivary glands of *Haemaphysalis spinigera* stained histochemically.

Our own observations at Onderstepoort on *Ornithodoros savignyi* have shown that laboratory animals vary in their resistance to toxicosis caused by the bites of these arthropods, and that the guinea pig is highly susceptible to whatever agent is responsible. It has been found that to allow *three* females of this species to feed on a guinea pig will cause its death. The white rat is more resistant, while albino rabbits can tolerate large numbers of these parasites over a long period without any symptoms apart from localised erythema.

Reported cases of the death of bovines overnight as the result of attack by these ticks appear to indicate that a highly potent toxin may be involved.

A thorough study of the salivary secretion of various species of ticks, transmitting infectious

agents or producing other pathological conditions, might elucidate a number of problems as well as form a basis for prophylactic and therapeutic research. A prerequisite, however, is the collection of the oral secretion in quantities sufficient for biochemical and biological study.

During the latter half of 1965 several methods of procuring the salivary gland secretion of the argasid tick *Ornithodoros savignyi* were investigated. This particular species was selected because of the problem it presents over a large area and the relative ease with which large numbers could be collected within a short space of time by means of traps baited with solidified CO_2 (dry ice), (Nevill[14]).

The elaboration of a method by means of which salivary gland secretion could be obtained in a pure state from this argasid could later be adapted to the species of *Ixodidae* which are of importance but less easily obtained or maintained in the laboratory.

Initially both male and female argasids were fed through membranes of various types, and on a variety of different media held at body temperature, since they are stimulated by the heat of the medium and, within certain limits, will attempt to feed on practically any liquid. Males were later discarded as they do not feed very readily, even on laboratory animals, in contrast to females which engorge avidly. Some results were obtained by using chicken-skin or polythene membranes with either water or saline media, but feeding was erratic and the membranes and media became contaminated by the coxal fluid and rectal-sac contents expelled by the arthropods while engorging.

Emulsification of salivary glands by dissection of the ticks proved to be laborious and time consuming. Frequently the glands became soiled with intestinal contents, and the time and trouble involved in cleaning them did not warrant the result obtained.

Finally the approach to the problem adopted by Kato and his co-workers of physiologically stimulating live larvae of *Chironomus thummi* to secrete into a medium suggested that a tick could possibly be induced to do the same by introducing a suitable stimulant into its haemocoele (Kato et al[15]). A variety of available parasympathetic stimulants were used, several of which gave very satisfactory results. Different methods of introducing the drug into the haemocoele of the arthropod were investigated, including injection by the use of fine-bore needles, but none proved to be equal to the method of inserting a blunted needle through the genital orifice. Due to the relative rigidity of the integument, needle punctures seal off slowly thus allowing some of the stimulant mixed with haemo-lymph as well as other body constituents to escape.

Materials And Methods

Owing to the relatively small quantity of salivary secretion produced by the individual female tick, two hundred females are used daily in the laboratory. To handle such numbers efficiently, each individual is attached to a microscope slide, ventral side up, by means of quick-drying adhesive so that the hypostome faces one end. At this edge, a piece of cork sheet about four mm thick, two and a half cm in height and the same width as the slide, is glued. This is provided with slots, cut with a fine sharp blade, on the upper edge.

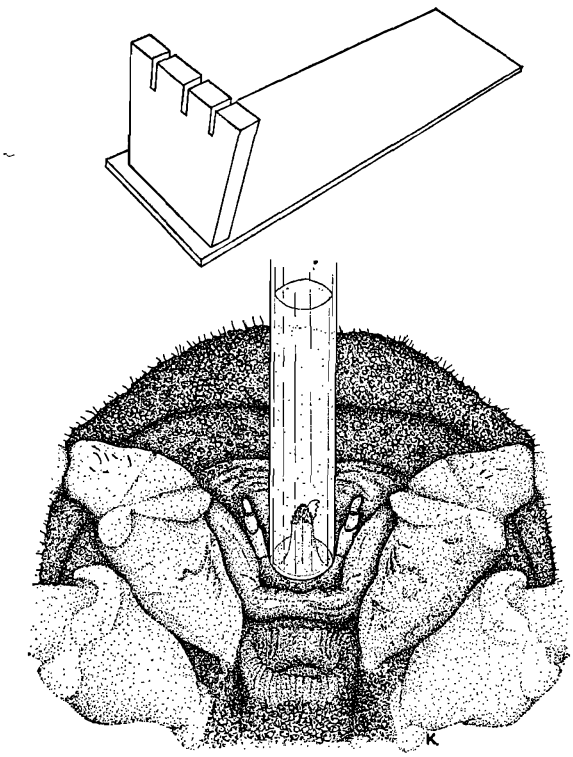

Fig. 1

A capillary tube is placed over the hypostome and chelicerae, once the process of secretion is initiated and is wedged into a slot, a number being provided for the sake of lining up the tube with the position of the mouthparts.

Introduction of the selected drug is achieved by using a one millilitre tuberculin syringe fitted with

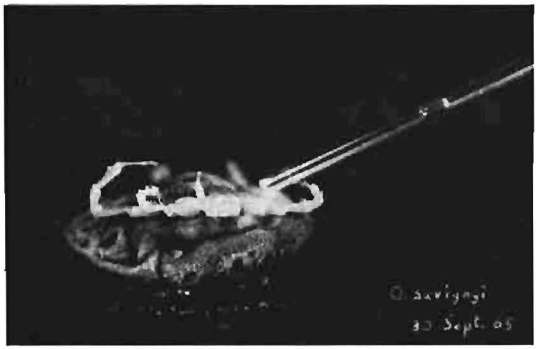

Fig. 2

a fine-bore blunted needle, bent at a right angle on itself. When inserting the needle into the sexual orifice the blunted point is directed slightly toward the posterior end of the argasid. By manipulating the syringe the needle is turned into a vertical position and with slight pressure rupture of the spermathecal wall is effected. This requires some experience, which is soon acquired and little other trauma results. The quantity of drug injected varies with the bulk and weight of the individual and is normally about 0.03 ml for adult *O. savignyi* females. The stimulant in routine use is Pilocarpine hydrochloride at 0.2% w/v of which a sufficient quantity is prepared weekly.

Daily weight records of some 20,000 females taken over many months, show the average weight per female to lie between 125 and 215 milligramme. The drug acts rapidly when administered correctly, and active movements of the chelicerae take place within about half a minute. After introduction, the chelicerae can be observed making antero-caudad penetrative movements, and on attaining maximum extension, performing an abaxial semicircular cutting movement involving only the tip or digital teeth before they are retracted again. This rythmic alternate pushing and cutting motion continues for as long as stimulation lasts and is accompanied by expulsion of small quantities of clear slightly viscid salivary secretion.

These movements are undoubtedly the method employed when the parasite penetrates the skin of a host, and are very likely accompanied by the secretion of a salivary constituent simulating a local anaesthetic, as the bite is not painful until after withdrawal of the mouthparts of the parasite. Most frequently salivary secretion ceases with termination of cheliceral movement, and unless the capillary tube is removed part or all of the fluid will be re-absorbed by the tick. Collection of the secretion from the individual capillary tubes into a small test tube is easily effected by expelling the fluid using air-pressure with a ball shaped rubber teat.

Observations:

1. The percentage of females reacting to pharmocological stimulation varies between 83% and 99%. The reason why a certain percentage of argasids will not respond is obscure, but such factors as age, trauma and physiological status undoubtedly play a role.
2. There appears to be a linear weight to secretion ratio of 0.000048 ml per milligramme of tick weight. The yield of salivary secretion in the weight range 123 to 215 milligramme per individual varies from 0.0059 to 0.010 ml.

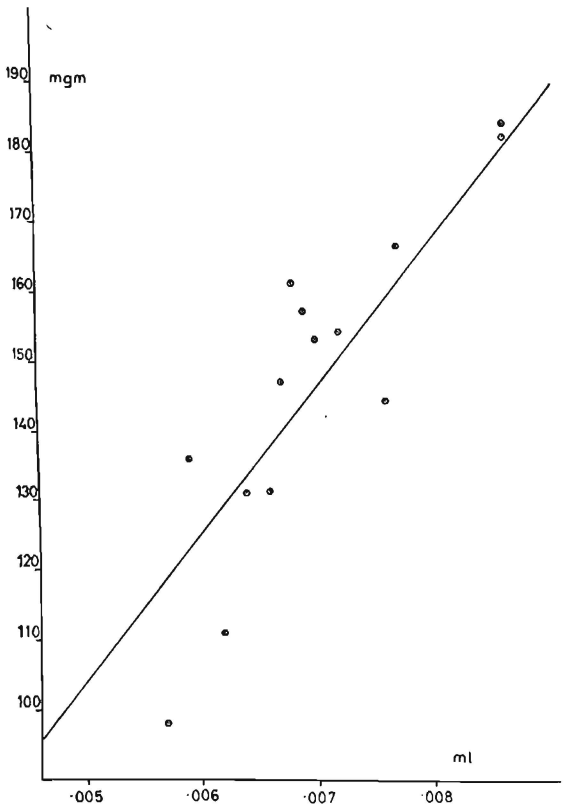

Fig. 3

3. The arthropods are kept at a temperature of 23°C and a relative humidity of 90% in the laboratory where 1,000 females per week are used for production of salivary secretion. The weekly production normally averages 6.8 ml.
4. There is no appreciable difference in quantity of salivary secretion produced between engorged

and unengorged females. The former are difficult to handle, however, as they void large quantities of coxal fluid which may adulterate the salivary fluid and be drawn up into the capillary tube.

5. The ticks are adversely affected by temperature fluctuation while subjected to treatment, and any drop in temperature results in a much reduced yield.
6. Various stimulants were tested for the production of salivary secretion of which only "Pilocarpine hydrochloride" gives constantly good results. Used at 0.20% to 0.30% the response is rapid and the stimulation lasts for about 45 minutes.

"Physostigmine", both the sulphate and salicylate, as well as "Prostigmine" (Neostigmine) gave mediocre results in *O. savignyi* the response being poor and erratic. The reason for this difference in reaction to the different drugs of the same group is not clear.

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FATAL INFESTATION OF THE BALD IBIS, *GERONTICUS CALVUS*, WITH *TROPISURUS AMERICANUS*

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INTRODUCTION

According to Roberts, MacLachlan and Liversidge(1), Geronticus calvus, the bald ibis or wilde kalkoen of the North Eastern Free State belongs to the family Threskiornithidae. These are mostly wading birds, fairly large with rather short legs for waders, robust bodies, moderately long necks and long slightly down-curved bills with a blunt tip.

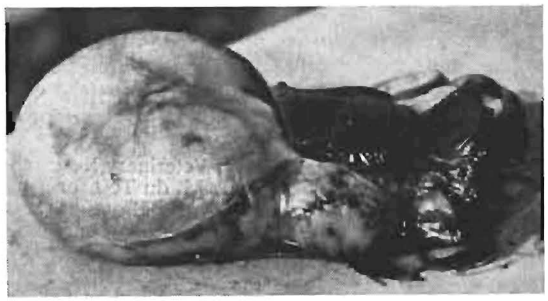
The bald ibis is generally green with conspicuous red on head, red iris, legs and bill, naked part of neck and head being thinly covered with greyish feathers. The bird is peculiar to South Africa and Basutoland. Its breeding area is limited to the rocky mountains of the Drakensberg. Its food consists of worms, grasshoppers, caterpillars and carrion.

The breeding season extends from July to October. The nest is made in the mountains in bushes growing on ledges or in crevices of krantzes and consists of a flat platform of sticks lined with grass. The nests and the nest site are much fouled by the droppings of the birds.

MATERIALS AND METHODS

Young birds of the abovementioned species were collected from nests in the Harrismith District and hand-raised in the Pretoria Zoo on minced meat, supplemented by minerals and vitamins. Some of the birds flourished on this diet but four of them developed anorexia, inanition and anaemia. They regurgitated the food which had been forcefed to them. They eventually died in an extremely emaciated and anaemic condition.

Post mortem examinations revealed several minute red spots in the proventricular wall. These red foci were visible on the outside of the organ. (Fig. 1) Subspherically shaped, red parasites with four grooves, arranged parallel to the longitudinal axis of their bodies, were recovered upon incision of these lesions. They were identified as adult



females of *Tropisurus americanus*. In South Africa *Tropisurus americanus* has been recorded from poultry but not from wild birds as has been the case in North and South America. In the Americas this parasite also occurs in poultry. This is the first report of this parasite from the bald ibis in South Africa.

Histopathological examination of the affected proventriculi revealed that the parasites were confined to the glands of these organs. Affected glands had lost some of their secretory cells and the parasites were sometimes in direct contact with the basement membrane of the glands. Vascular changes were not very prominent in the affected areas but accumulations of eosinophils, lymphocytes and histocytes were observed.

DISCUSSION

In South Africa certain orthopteran insects, which occasionally feed on the faeces of bald ibises, may serve as intermediate hosts of *T. americanus*. The nests of bald ibises are usually fouled by their own excreta and that may facilitate the accumulation of insects and completion of the life cycle of this tetramerid parasite. *T. americanus* may also be responsible for mortality among young bald ibises in nature.

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