CORRIGENDA TO VOL. 49 No. 2 (June 1978 Issue)

In the paper by B.A. Prior and C. Casaleggio entitled "The Microbiology of Polony" (pp. 115–119):

1. Add, on page 115, under the heading: MATERIALS AND METHODS, subheading: Isolation and counting of micro-organisms, after the sentence: "Pseudonomads were counted on the selective agar of Solberg *et al.*[20] after incubation at 25°C for 3 d." "*M. thermosphactum* were enumerated using the selective medium of Gardner[8] after incubation at 25°C for 5 d."
2. On page 116, in the heading to Table 2, read "LOG_{10}" instead of "LOG_{19}".
3. On page 118, in the legend to Fig. 1 read "▼, pH" instead of "▽, pH".
4. On page 118, in the legend to Fig. 2 read: "✪ , psychrophiles" instead of "●, psychrophiles"; "▼, pseudomonads" instead of "▽, pseudomonads"; and "□, micrococci" instead of "☆, micrococci".

JOURNAL OF THE SOUTH AFRICAN VETERINARY ASSOCIATION

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ALLE BRIEFWISSELING: Direkteur, SAVV

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ADVERTEERTARIEWE op aanso

Financial subvention by the Department of National Education and the Medical Research Council is gratefully acknowledged.

Geldelike steun deur die Departement Nasionale Onderwys en die Mediese Navorsingsraad word met dank erken

EDITORIAL

VAN DIE REDAKSIE

LABORATORY ANIMAL SCIENCE IN SOUTH AFRICA

South Africa has seen immense development in the field of laboratory animal science during the last decade. during this time, about ten large modern facilities, the cost of some running into millions, have been commissioned. Standards have been no less than that required for modern hospitals, with additional specifications such as precise temperature, humidity and lighting controls. Such developments have promoted the emergence of laboratory animal science as a distinct scientific discipline.

The first scientific meeting devoted exclusively to this subject was held in 1970 in Pretoria under the auspices of the CSIR and the newly created Medical Research Council*. In view of the success of the first meeting, the National Laboratory Animals Committee of the MRC resolved to hold a further meeting during September 1977. Both were three-day meetings, but the second was attended by twice as many delegates, more than double the number of papers were delivered: six overseas experts delivered 12 scientific papers at the second meeting. The escalating logistics of these two meetings and the enthusiasm displayed by many individuals from a wide variety of disciplines confirmed a general desire to attain higher standards of practice and the need for ongoing education in this country. Undoubtedly there is also an increasing awareness of the ethical responsibilities associated with the practice of the science.

Why, then, was animal experimentation so long confined to hushed activity in basements, attics and abandoned stables? The "emergence" may be due partly to increasingly complex requirements of investigators as specialization developed and partly as response to the need for larger volumes of more critical information. Undoubtedly the immense benefits of using a high standard of laboratory animal has been realised in many fields and a good rationale exists which equates the standard of laboratory animal science in a country to the general state of health in that country. On the other hand, it is a multi-disciplinary science and its rapid development has created considerable organisational questions. For instance, who must be responsible for its administration, its practice and effective control? It has even been said that laboratory animal science is an abandoned orphan between various scientific disciplines and administrative authorities. Undoubtedly the major need that has emerged concerns the field of laboratory animal medicine and more particularly the requirements of the medical profession.

How did the veterinarian become involved as a specialist in this field? Participation in biomedical research was a natural development, since the veterinarian is not only a trained expert on animals, but has a mandatory professional obligation to the welfare of animals, which he is trained to view as sensitive patients. Thus today the situation has been reached where all advanced biomedical research involving animals is invariably done under the direction of, or in collaboration with, the veterinarian. Happily this partnership arrangement with medical colleagues is well developed at all leading biomedical research centres in this country and is undoubtedly one of the factors which has lead to the immense improvement of laboratory animal science in South Africa in recent times.

Bureaucratic control has by no means caught up with the rapid development of the science in many countries. In South

PROEFDIERKUNDE IN SUID-AFRIKA

Gedurende die afgelope tien jaar was daar 'n uitgebreide ontwikkeling op die gebied van proefdierkunde in Suid-Afrika. Dit is veral gekenmerk deur die totstandkoming van ten minste tien moderne proefdiereenhede waarvan die koste van oprigting van sommige miljoene rande beloop het. Die standaard van bestuur is hoog en is vergelykbaar met dié van moderne hospitale. By komend hiertoe is egter ook die daarstelling van noukeurig gekontroleerde omgewingstemperatuur, humiditeit en dagliglengte. Hierdie gebeure het meegebring dat proefdierkunde plaaslik ontwikkel het tot 'n selfstandige wetenskaplike vakgebied.

Die eerste wetenskaplike byeenkoms in Suid-Afrika wat uitsluitlik oor dié vakgebied gehandel het, is in 1970 in Pretoria gehou onder beskerming van die WNNR en die toe nuutgestigte Mediese Navorsingsraad*. Na aanleiding van die verrassende sukses van die eerste byeenkoms, het die Nasionale Proefdierekomitee van die MNR besluit om 'n tweede simposium in September 1977 te reël. Beide geleenthede het oor drie dae gestrek maar die tweede is bygewoon deur dubbel die aantal afgevaardigdes. Daarbenewens is meer as dubbel die aantal referate gelewer as gedurende die eerste byeenkoms, terwyl ses buitelandse outoriteite twaalf wetenskaplike voordragte gelewer het. Hierdie gebeure, asook die groot entoesiasme van meeste van die afgevaardigdes, wat wyd uiteenlopende vakgebiede verteenwoordig, het die begeerte om 'n hoë standaard in die vakgebied te handhaaf, asook die noodsaaklikheid van deurlopende onderrig in die vakgebied ter plaatse, beklemtoon. Gepaardgaande hiermee was daar ook ongetwyfeld 'n toenemende besef van die etiese verpligtinge verbonde aan dié vakgebied.

Die onlangse snelle ontwikkel in die vakgebied van proefdierkunde mag deels toegeskryf word aan die toenemende kompleksiteit van ondersoeke as gevolg van toenemende spesialisasie, asook aan die groter vereiste vir noukeuriger en breedvoeriger navorsingsgegewens. Dat die baie groot voordele verbonde aan die gebruik van 'n proefdier van hoë gehalte algemeen aanvaar word, word weerspieël in die stelling dat die standaard van proefdierkunde in 'n bepaalde land nou verband hou met die algemene gesondheidstoestand van die mense van daardie land. Tog is die snelle ontwikkeling nie sonder probleme nie en bring die multidissiplinêre geaardheid van die vakgebied heelwat organisatoriese probleme mee. Dit is onduidelik wie verantwoordelikheid moet aanvaar vir die administrasie, uitvoering en effektiewe beheer. Juis om die rede word dit tans nog tot sy nadeel tussen verskillende wetenskaplike vakgebiede en administratiewe owerhede rondgeskuif. Die aspekte wat tans sonder twyfel die meeste aandag benodig, behels die gebied van 'proefdiere geneeskunde' en veral die vereistes van die mediese professie.

Die vraag ontstaan hoe die veaarts as spesialis betrokke geraak het in die gebied. Deelname in biomediese navorsing was 'n natuurlike ontwikkeling, aangesien die veearts nie net 'n dierespesialis is nie, maar besit hy ook weens die geaardheid van sy opleiding 'n ingeburgerde professionele verantwoordelikheid teenoor die welsyn van die diere wat hy as sensitiewe, lewende wesens bejeen. Tans word alle gevorderde biomediese navorsing in die R.S.A., waarby laboratoriumdiere betrokke is, uitsluitlik onder toesig van, of in samewerking met 'n veaarts uitgevoer. Dit is verblydend dat hierdie vennootskap met mediese kollegas by alle vooraanstaande

Africa there is no specific legislation controlling animal experimentation apart from the general terms of the Cruelty to Animals Act which some authorities feel is adequate. The immensely complicated British system of a huge inspectorate and various categories of licences for different types of animal investigations, seems, in the view of some, to be bogged down by formidable logistics. In the U.S.A., experience has shown that the most effective means of control is the mandatory requirement of "adequate veterinary care", a term which has now assumed a very specific legal connotation. Generally speaking, in this country, public opinion is now such that abuses are likely to lead to disastrous consequences for those responsible; let us hope that with awareness of the responsibilities involved, combined with true professionalism, a level which does not offend civilized standards will be maintained.

In this context it is gratifying to note that earlier emotive and divergent opinions between scientistis and animal welfare enthusiasts are now being replaced by co-operation between responsible welfare societies and ethically-minded scientists to the advantage of all, particularly the animals. Extremist organisations, who revel in displaying often irrelevant "horror" material, still exist and are best ignored.

It was not possible to publish all 46 papers delivered at the Second South African Laboratory Animal Science Symposium. The objective of the Symposium was to stimulate further technological improvements; here an important area is the field of specific pathogen-free technology. Expert papers on the topic in this issue crystallize the advantages and pitfalls. Clearly there is an urgent need for a single central producing facility, since duplication of such elegant facilities would be folly. The rather mundane topic of animal management was handled in a provocative manner, indicating clearly how handsome benefits can be derived from taking imaginative cognisance of local circumstances. A strange feature of animal experimentation is that the few species picked at its inception are still used; local research has already made inroads into this convention, but there is more scope. Primatology in Africa presents its unique problems of disease, management, production and conservation. Has the time not arrived for long-term planning and policy-making between users and conservation authorities? The possibilities are exciting.

*See this Journal, Vol. 41 nos 3 & 4 and Vol. 42, no. 1.

biomediese navorsinginrigtings in die land goed ontwikkel is en is dit ongetwyfeld een van die bydraende faktore tot die huidige snelle ontwikkeling van proefdierkunde in Suid Afrika.

In meeste lande het die daarstelling van amptelike beheermaatreëls nie tred gehou met die ontwikkeling van die vakgebied nie. In Suid-Afrika is daar geen spesifieke wetgewing wat betrekking het op die uitvoer van eksperimente op diere nie. Hier geld slegs die Wet op Dieremishandeling, wat deur sommige owerhede as voldoende aanvaar word. Daarenteen word die gekompliseerde Britse sisteem, wat met sy uitgebreide inspeksie-personeel en die daarstelling van verskillende kategorieë 'n beperking plaas op die geaardheid van dierenavorsing wat gedoen mag word, deur sommige mense as lomp en onuitvoerbaar beskou, juis weens die kompleksiteit van die sisteem. Uit ondervinding in die V.S.A. het dit geblyk dat die mees effektiewe kontrole uitgeoefen word deur die daarstelling van 'n wetlik gedefinieerde bepaling dat 'voldoende veartsenykundige versorging' beskikbaar moet wees. Plaaslik is die trant van die openbare mening oor die algemeen van so 'n aard dat wanpraktyke ongetywfeld katastrofiese gevolge sal meebring. Ons kan net vertrou dat die besef van die betrokke verantwoordelikhede en 'n ware sin van professionalisme 'n standaard van optrede sal verseker wat nie teen beskaafde norme indruis nie.

In dié verband is dit verblydend dat die vroeëre emosionele verskille tussen wetenskaplikes en voorstanders van dierewelsyn bygelê word en dat samewerking tussen verantwoordelike dierewelsynsorganisasies en navorsers met 'n duidelike etiese kode ontwikkel tot voordeel van veral die diere betrokke in die eksperimente. Die pogings van ekstremistiese groepe om deur die vertoon van hoogs ontstellende maar meesal irrelevante en verouderde navorsingsresultate verkry vanuit obskure bronne, fondse van die geskokte publiek af te rokkel, kom nog voor, maar moet liefs geïgnoreer word.

Dit was nie moontlik om al 46 referate, wat by die Tweede Suid-Afrikaanse Proefdierkundesimposium voorgedra is, te publiseer nie. Dit was die doelstelling van die simposium om verdere tegnologiese ontwikkeling te stimuleer, waaronder die veld van spesifieke patogeen-vrye tegnologie, een is.

Referate deur deskundiges op die gebied het die voor- en nadele van die sisteem duidelik omskryf en was dit ook duidelik dat, weens die gesofistikeerde geaardheid van inrigtings daarby betrokke, duplisering van fasiliteite onwys sou wees en die daarstelling van slegs een gesentraliseerde produksieeenheid die ideaal moet wees. Die alledaagse tema van diereversorging het 'n paarstimulerende gedagtes opgelewer en was dit duidelik dat, met 'n bietjie oorspronklikheid, plaaslike omstandighede met vrug aangewend kan word. Dit is eienaardig dat die enkele spesies van laboratoriumdiere, wat aanvanklik vir navorsing gebruik is, amper uitsluitlik nog in gebruik is. Alhoewel dié situasie plaaslik besig is om te verander, is daar nog baie geleentheid vir uitbreiding. Unieke probleme in Afrika is verbonde aan die gebruik van primate, veral ten opsigte van siektes, bestuur, produksie en bewaring en het die tyd moontlik nou aangebreek dat 'n aanvang met langtermyn vooruitbeplanning deur die gebruiker en bewaringsowerhede gemaak word. Die moontlikhede is veelvuldig.

*Sien hierdie Tydskrif, Jaargang 41 nr. 3 & 4, en Jaargang 42, nr 1.

PRINCIPLES OF CARE AND MANAGEMENT IN A LABORATORY ANIMAL FACILITY

C. J. JOUBERT

ABSTRACT: Joubert C.J. **Principles of care and management in a laboratory animal facility.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 153–154 (En), S.A. Bureau of Standards, Private Bag X191, 0001 Pretoria, Republic of South Africa.

The keeping of animals in an enclosed area and their use for scientific work goes back to the beginning of the Christian era. This activity gradually developed into the specific science we know today. Principles of housing, animal care, equipment and management according to which, it is felt a modern animal facility should be designed, equipped and operated, are discussed.

INTRODUCTION

Since World War II, development in the laboratory animal field has been rapid and of considerable magnitude. Although development here has been substantial, there is still much leeway to be made up and the discussion is therefore aimed at underlining certain principles of care and management which could be helpful in bringing us closer to the ideal. Reference is made to sophisticated housing, modern equipment, properly qualified staff, and a constant supply of high quality laboratory animals.

THE ANIMALS

It is becoming increasingly evident that uniform, disease-free animals must be used if worthwhile research is to be carried out economically. Regretfully most of the laboratories in South Africa are still using animals which do not comply with all these requirements. As a result, workers are forced to use excessive numbers of test animals, when carrying out assays or research. It is felt that the main cause of the problem is the fact that available animals are

(a) not bred properly and therefore genetically an unknown entity, and
(b) infected with a host of pathogens which influence test results.

The answer lies therefore in correct breeding methods as well as cleaning up the colony. By these means inherited differences as well as those caused by disease can be largely eliminated.

By correct breeding methods it is meant that the latest breeding techniques should be applied, to ensure that a genetically controlled animal, be it inbred or random bred, is produced. This can only be achieved if proper records are kept.

The cleaning up of the colony is achieved by eliminating all ecto- and endoparasites as well as all pathogens which could influence experimental results. This is done by means of caesarian derivation and the use of drugs.

In our own as well as in most overseas countries mice and rats make up upwards of 75% of the total number of experimental animals used. Unfortunately this does not mean that the work load per animal species is also divided in the same proportion. If this had been the case matters would have been much simpler, as the keeping and breeding of rats and mice is relatively speaking a much easier operation.

However, keeping primates, dogs, rabbits, guinea pigs, and lesser well known species such as fish, frogs, and reptiles calls for special diets which include cooked or raw foods, fresh fruit, and vegetables as well as mixed compounded diets with added vitamins.

Furthermore, the cleaning up and disease control of these animals, together with special treatment necessary because of stress caused by caging, makes their care a specialized activity.

Matching demand and supply is a factor which must be considered, as this is a constantly varying entity and affects the number of animals that are kept. Liaison with buyers and other users is important, so that planning for future breeding and supply is done well in advance, and to ensure that orders once placed are debited, whether the animals are taken or not.

HOUSING

It is accepted today that the housing of laboratory animals is highly sophisticated and very expensive. Consequently a critical analysis should be made of the functions to be served before embarking on the design and erection of an animal facility, and the re-designing of existing facilities should only be contemplated if modern acceptable principles can be achieved.

Consideration should be given to future development and the design should incorporate versatility of application, coupled with allowance for expansion.

From the viewpoint of the organization which intends creating an experimental animal facility it is important to realize that architects and engineers who have much experience and knowledge in this field are practically non-existent.

It is therefore important to appoint someone with the relevant knowledge to act as Technical Adviser to the design team. Preferably this should be the person who is going to take charge of the new facility. If he does not already have this knowledge, he should acquire this by working and visiting as many animal facilities here and overseas as possible.

The layout depends very much on personal taste and preference. The following main areas should, however, always be identified:

(a) breeding area,
(b) experimental area,
(c) service area, and
(d) staff facilities.

The size and design of each will depend on the type of facility envisaged. From personal experience it is felt that the clean/dirty corridor system is the one of choice. This is the one where the animal rooms are built between two passages, with a door leading into each passage.

There are, however, many designs which will be quite suitable for a given requirement, and this will be mainly influenced by finances, available area, number and species of animals to be housed, and personal preferance.

Fortunately no one has any doubt today that air-conditioning is a necessity in the animal facility. Its cost will be roughly 1/3 of the total bill. As a general rule the optimum temperature for small rodents is 22°-24°C, poultry, guinea pigs and rabbits 18°-20°C, and cats and dogs not more than 18°C. Relative humidity should be between 50%-60% and air changes at least 14-16 per h.

Fresh air should be brought into the animal room at floor level and taken out as high up as possible, without recirculation.

EQUIPMENT

(a) Caging should be robust, rust and stain-free, easily cleaned and sterilized without damage to it, if possible stackable and preferably on wheels. Fortunately we are living in the plastic era, and plastic cages are competitive in price and eminently suitable for most of the smaller species. For the bigger species glass fibre, stainless steel, aluminium or epoxy coated cages are the answer.

(b) The following ancillary equipment can be classified as essential in the modern animal facility:
   (i) A large vacuum autoclave ($\pm m^3$).
   (ii) An automatic cage washer.
   (iii) A vacuum system (for removing dust and soil), and
   (iv) an incinerator.

In addition a
   (1) bottle washing machine,
   (2) piped compressed air,
   (3) steam cleaning equipment, and
   (4) a freezer for dead animals

would be good investments.

MANAGEMENT

From the foregoing, it is clear that well qualified staff is a necessity in the modern animal unit.

The animal facility should be under the supervision of a well qualified person, with the various sections divided amongst properly qualified technicians.

The supervisor should have at least the same qualifications as a technician, but have in addition a lot of practical experience in this field, as well as being an above average organizer. If possible each technician should handle only one species. For obvious reasons this will allow the person in charge of, for instance the mouse colony, to become fully conversant with his part of the set-up, and allow him to get the most out of his colony. Where the facility is small, and a large number of species are held, this is sometimes impossible. In such cases it is preferable to divide the day or week in compartments, give attention to one species at a time, and not to move backwards and forwards from one to the other. It should also be a rule that when moving from one species to the other, outer clothing should be changed and hands washed with a suitable disinfectant soap.

The practice of keeping several species in one room is a bad one and must be avoided.

To ensure a constant flow of laboratory animals the supervisor should have the best possible facilities as well as the most competent staff, which apart from having the necessary qualifications should have the additional attribute of being able and inclined to work with animals even outside normal working hours.

It should not require a major effort on the part of the technical staff to feed, water, and clean their individual sections. They are much too valuable and should spend the bulk of their time running and improving their departments to obtain maximum benefits, or to assist in test and research work. The assiduous use of unskilled labour and some mechanized equipment is necessary to ensure this. There should also be time for reading and discussion with others in the same field.

CONCLUSION

It is, therefore, evident that the supply of high quality laboratory animals is no longer a haphazard activity, which forms part of the work of a scientific organization, a university faculty or zoological garden, and is carried out in unused or redundant space, which may or may not be designed for this purpose.

It is rather a well planned programme of activities carried out in a building or buildings specially designed for the purpose, by properly qualified and experienced staff, enabling the scientist to carry out research or test work with the minimum loss of time and at a reasonable cost.

REFERENCE

1. LANE-PETER W. & PEARSON A.E.G. 1971 *The Laboratory Animal – Principles and Practice.* London & New York: Academic Press.

EXPERIENCE OF DIRECTING A NATIONAL LABORATORY ANIMALS CENTRE

J. BLEBY

ABSTRACT: Bleby J. **Experience of directing a National Laboratory Animals Centre.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 155–156 (En), MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF, United Kingdom.

The challenges of managing a facility serving a new and developing science are outlined. Basic to success are the appropriate handling of aspects such as staff selection, line management, financial support, changing demands, training and relations with staff unions, welfare organisations and at the international level.

BACKGROUND

The Medical Research Council (MRC) Laboratory Animals Centre was started by the British Government in 1947 and would seem to be the first national centre to be established. The Centre came into being as a direct result of a memorandum produced by a large number of interested parties which included in particular the Research Councils, Scientific Societies, Government institutions and the pharmaceutical industry. Even prior to the Second World War, an increasing number of influential scientists was becoming concerned about the inadequate supply of high quality laboratory animals, but it was not until after the war that it was possible for any action to be taken. Indeed, the war had made the need for something to be done even more urgent, as the sulphonamides and antibiotics had begun to be developed during that time. So, in 1947, the Government accepted the recommendations contained in the report and instructed its Medical Research Council to set up a laboratory animal bureau which was renamed the Laboratory Animals Centre in 1958.

The Centre is located at Carshalton, an ideal site where we also can house some of the larger animals such as sheep and goats, and is within short distances from London and both Heathrow and Gatwick airports which is essential today with the international movement of laboratory animals by air.

TERMS OF REFERENCE OF THE CENTRE

Basically the Centre has 4 terms of reference, which are:

1. To act as a national and international centre.
2. Supply of animals: breeding nuclei; accredited breeders.
3. Research in laboratory animal science
4. Training: Graduate; Technical.

MANAGEMENT

Running any organisation, whatever its size or function, is primarily a matter of organising staff and facilities as efficiently as possible. It is surprising how many people think that their management problems are somehow unique, when in fact they are no different from those of anyone else.

Since 1965, the staff of the Centre has doubled with the establishment of new departments such as Environmental Physiology, Nutrition, Virology and Cryobiology. These developments, of course, have come about largely as a direct result of the development of laboratory animal science and not unexpectedly have brought their own problems with them.

For example, to start with, one has to persuade the authorities to approve and finance new developments. This may sound easier than it actually is, since you can fall between two stools which are (a) if the work has never been done anywhere before – is it worth doing? and (b) if it is being done or has been done elsewhere, then there is no need for us to do it! Although this attitude by administrators is amazing and sometimes downright infuriating, it is no good fighting it because this is the way they are trained to think, and in any case, they may be asked to justify the additional expenditure which they authorised.

The other major difficulty one has to deal with is what might be termed as "bureaucratic blank spots" because it is incredible how sometimes the most obvious proposals fall on stony ground. In our case, a classic example of this "syndrome" was the extreme difficulty we encountered in the authorisation of a scientist to deal with the detection of viruses, and yet almost since the Centre's inception the MRC had always recognised the importance of detecting bacteria and parasites. It took 5 years and numerous memoranda repeating the obvious *ad nauseum* before the appointment was finally approved. Within 6 months of the appointment being made, it paid for itself many times over, as the Centre had to bring under control and wipe out an epidemic of ectromelia in the United Kingdom. Such action would have been almost impossible without the assistance of a skilled serologist.

Having surmounted the many hurdles of getting an appointment authorised, the next difficulty is to find the right person to fill the post. The main problem here is that one is trying to locate someone who has never actually done the job before because, you will remember, if they had, the post would not have been authorised in the first place.

SELECTION OF STAFF

This then leads on to the selection of staff, and I have no doubt that it is probably the most difficult and yet most important task a Director has to perform. Select the wrong person and you are in trouble from the beginning because obviously the work does not develop as it should and you also have the embarrassment of getting rid of the person before you can begin finding a replacement. I am convinced, therefore, that it is far better to leave a post vacant until the right person comes along, rather than rush to fill it because you are anxious for the work to proceed. Staff selection, is of course, an art as well as a science, and although there are certain standard danger signals that one must be on the lookout for, such as someone always changing their jobs, at

the end of the day it comes down to a personal opinion – a gut feeling – as to whether a candidate is the right person for the particular job. A good personality is an essential quality, particularly at the Centre where teamwork is vital if its terms of reference are to be discharged quickly and efficiently. The Centre does not have any room for prima donnas.

LINE MANAGEMENT

In my view, if staff are to be efficient and happy they must have clearly defined terms of reference so that they know what their job is, who they are responsible to and who is responsible to them. One of the first changes I introduced to the Centre was a system of line management; all senior staff, including technicians, were provided with job specifications which, of course, were drawn up in consultation with the members of staff concerned. Without the introduction of these simple basic management tools it would have been much more difficult for the Centre to respond to the ever changing and developing field of laboratory animal science, which has grown increasingly complex. One only has to consider developments since 1965, such as specialised animal strains, in particular the nude mouse and rat, mandible analysis, environmental effects, virology and cryobiology, to appreciate how complex laboratory animal science is becoming. It is, therefore, absolutely essential, if confusion is not going to take over, for everyone on the staff to know absolutely within carefully defined limits, what their job is and then left to get on with it. This last point needs emphasizing as there is nothing more galling or frustrating than for staff to have someone looking over their shoulders. Once having been told what to do, they must be given every opportunity to use their own initiative and be judged by their results.

FINANCIAL SUPPORT

The development of the Centre is due almost entirely to the fact that it has been totally financed by the tax payer, and without which support most of the research would have been impossible. Some people are of the view that national centres should ultimately become self-financing through the sale of animals, but I am convinced that this is not the best approach as it eventually leads to too much attention and emphasis having to be given to animal production and sales, to the detriment of research. Without research, progress is severely curtailed to the ultimate detriment of all concerned. The policy adopted by the Centre is, therefore, that it undertakes work which is unlikely or cannot be done by commerce, but simultaneously encouraging commerce to take on work it can and should do. A clear example of this is the breeding and supply of animals for use, whereby the Centre develops and supplies defined breeding nuclei to commercial breeders who then undertake and finance the large scale production of animals. The Centre does, however, maintain the standards of quality of the animals produced by commercial breeders through a voluntary accreditation scheme, and thereby ensures that user's time and money is not wasted by being supplied with inferior animals. When one considers that the pharmaceutical industry is not only one of the main users but also one of the UK's major industries, one is dealing with millions of pounds.

Until about 1970, the Centre like most other government institutes enjoyed an almost uninterrupted growth in its financial support, but since then times have changed. In 1973, Lord Rothschild produced his radical report recommending that government supported research institutes should develop customer/contractor programmes. Twenty-five percent of the MRC's budget was thus subsequently transferred to the Department of Health and Social Security in order that they could "contract" the MRC to undertake research projects which they considered important.

Although the Centre has not, so far, received any direct DHSS commissions, it has gone out for contract work particularly with industry and the receipts now being generated help to meet about 5% of the Centre's costs. It is likely that this figure will grow, but one hopes never to the point whereby the Centre is totally dependant on contract money, for the reasons I have already mentioned.

Although many people were initially dubious about the customer/contractor principle, the Centre's experience has shown that it does have advantages as it provides an appropriate degree of staff stimulation and it also leads to increased efficiency as people become more cost conscious and practical. As contracts have clearly defined limits, especially in terms of time, they also bring about more precise attitudes to getting programmes underway and completed, as opposed to when there is just a continuous supply of tax payers' money. On balance, therefore, we welcome the development of contract work, provided that it is not allowed to interfere with Research and Development which, in my view, must continue to be totally supported by public funds. However, there is little doubt that following the effects of inflation and the recession, contracts offer almost the only hope for furnishing any future expansion as financial growth from public funds is now virtually nil. The only other alternative is to fund new developments at the expense of old – a most unpallatable pill which most people find difficult to swallow.

CHANGING DEMANDS

During the last 12 years the Centre has had to respond to ever changing demands which the staff usually find interesting and stimulating. After all, who wants to be employed in providing a service that is either diminishing or nobody wants? Without exception we have been able to offer scientists and technicians worthwhile careers in a new science, and I have taken much comfort in the fact that almost everyone we have taken on has either stayed with us or moved on to another post but in the same field.

Laboratory animal science is not a glamorous subject and the extent to which he relies on laboratory animals is usually not evident to the man in the street. The legislation that followed the thalidomide tragedy is wide and far reaching, and as we can see with recent action taken by the American FDA is likely to continue to be so. Moreover, Britain's entry into the EEC has also brought increased use of laboratory animals especially in the field of toxic hazards, and the introduction of the Health and Safety At Work Act is adding further to demands on us. The Centre has, therefore, had to devote considerable effort into, for example, determining the correct environmental conditions under which experimental animals must be maintained, which in turn has

had a direct bearing on the design and construction of animal houses. With the increase in toxicity testing we have tried to promote a much greater use of inbred strains but we have found this has been a long and difficult course to follow. However, this difficulty showed us that we had got to go to the user as the user was unlikely to come to us. The ignorance that can exist in some institutes, especially academic ones, is sometimes dumbfounding. In 1975, therefore, we appointed a User Liaison Officer whose task is to go out and talk to users about their requirements and problems. Apart from assisting users we have found that the "feed back" from the User Liaison Officer has been invaluable to the Centre. In my view this development is very important as we must try to get over to people in authority what they should know. Literature and publications alone will not do because we all have far too much to read anyway, and so publications just don't get read. However, a visit by someone who wishes to help you is another matter and usually yields results.

TRAINING

One of the main contributions to the improvement of laboratory animal husbandry and welfare has been the training of staff and in particular animal technicians. For too long the only people that were employed in animal houses were those that were unemployable elsewhere, and I am sorry to say that this practice still exists in many countries. In one country I was personally told by the Director of a large institute that he 'punished' members of staff by sending them to work in the animal house for varying periods depending upon the length of their sentence which was related to how naughty they had been!

Within 2 years of its establishment the Centre began actively encouraging the training of animal technicians through The Animal Technicians Association, later to become the Institute of Animal Technicians. During the last 12 years we have made it compulsory for all new animal technicians to undertake courses leading to certification by the IAT. This is usually accomplished by day release courses, but we expect the staff to use some of their free time as well. I would, however, like to introduce a word of caution here and that is that whilst the Centre has encouraged the training of animal technicians to equate with laboratory technicians, I think that in some cases we have gone too far. In any animal house a considerable amount of the routine work is simply feeding, watering and cleaning out, and it is therefore essential to have people who are content to do this and who have not been trained too far above this level. This is why I am inclined towards a 2 tier structure, namely, animal technicians who are expected to work directly with scientists on experimental projects, but having under them animal aides who take care of the routine duties.

TRADE UNIONS/STAFF ASSOCIATIONS

This paper would not be complete without some reference to Trade Unions and Staff Associations which, whether we like it or not, are now very much a fact of every day life. Until 1969, the MRC did not recognise any Trade Unions although many of its staff did belong to them. Eventually, in company with many other employers, Trade Unions were recognised by Council and this has resulted in the establishment of formal negotiating machinery both within the Centre and throughout the MRC, which has led to a considerable amount of senior staff time being spent on such matters. As with all new developments, mistakes were made on both sides, some of them quite traumatic and energy sapping, but everyone has learnt from their experiences and in recent years reasonably harmonious relations have been established. One fact, however, does emerge very clearly and that is that management must nowadays maintain an almost continuous dialogue with staff so that they become involved in decision making and know what is going on. Any Director who ignores these present day facts of life, ignores them at his peril. But here again one comes back to the importance of selecting the right people to appointments, because reasonable people with good personalities can usually resolve their problems without too much difficulty.

INTERNATIONAL RELATIONS

One of the interesting aspects of laboratory animal science is that it is international and the Centre has always been keen to play its part in helping other countries. The establishment of ICLA was the first major step towards some form of international liaison but it has suffered from being under-financed. However, I believe that if more positive and stronger measures are taken in the future, ICLA will successfully fulfill its function. During the last 12 years, the Centre's international activities have developed considerably and I think there is hardly a single country to which we have not supplied breeding nuclei, help or advice. It is significant that we have even supplied animals to the Peoples Republic of China and it seems likely that there will be increased contact with that country. In 1975, the Centre was designated a Collaborating Centre for Defined Laboratory Animals by the WHO in company with the NIH in the U.S.A. and Japan – a programme which is designed to increase the use of defined high quality animals throughout the world.

ANTIVIVISECTIONISTS/ANIMAL WELFARE ORGANISATIONS

Unfortunately, there is one unpleasant aspect of being Director of a national centre, at least in the U.K., and that is the activities of the Antivivisectionist Societies and certain members of Parliament. Also the premises of many of our Accredited Breeders have been attacked, leading to convictions for arson and malicious damage. These activities by fringe groups have caused considerable and often unpleasant work for members of staff, but I am afraid it is inevitable and we must learn to live with people opposed to experiments on animals and I try and look upon them as our conscience. However, during the last 10 years we have detected a definite change of direction by some antivivisectionists away from absolute opposition to all animal experiments, to the greater use of alternatives. Here, of course, the pity of the whole controversy is that there is no basic difference of opinion between us, as the Centre has always advocated the use of alternatives wherever possible. Unfortunately, many antivivisectionists do not believe or understand that the use of alternatives is extremely limited – but the public as a

whole do understand if they are given the correct information in an appropriate form. This is why in recent years the Centre has also appointed an Information Officer because we are firmly of the opinion that the public do have some right to know what we are doing (especially as they are paying for it) and we find that reasonable people when told the facts, accept experiments on animals provided that they can see and are reassured that everything is under control, and that the animals are well looked after.

HUMANE CONSIDERATIONS IN THE USE OF EXPERIMENTAL ANIMALS

W. N. SCOTT

ABSTRACT: Scott, W. N. **Humane considerations in the use of experimental animals.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 159–160 (En), Universities Federation for Animal Welfare, 8 Hamilton Close, Potters Bar, Hertfordshire, England.

Various considerations aimed at improving the welfare of experimental animals are discussed, with particular reference to the situation in the United Kingdom. These include, *inter alia,* codes of standards for accommodation and equipment, prevention or reduction to a minimum of pain, and the appointment of committees consisting of biological scientists, veterinarians, teachers and lay persons to advise on proposed experiments.

Over a hundred years ago Charles Darwin, who said that the thoughts of painful experiments kept him awake at night, together with Thomas Huxley, then President of the Royal Society, and other eminent biologists, made certain recommendations for putting a check on cruel experiments. These were supported by the British Association for the Advancement of Science and the British Medical Association. A Royal Commission was subsequently formed and the law which now obtains in Britain (the Cruelty to Animals Act 1876) was approved by Parliament. The Act is based on three principles:

1. The licensing of experimenters
2. The establishment of an Inspectorate
3. The Pain Rule

all of which are indispensable in any reasonable system of control.

At the same time the first anti-vivisection society roused opinion against experiments on animals. They demanded the total abolition of all experiments, asserted that experiments on animals cannot usefully advance medical knowledge and alleged that all experiments were cruel and all experiments heartless. This general abuse caused great resentment among the scientists who developed a persecution complex, and extremists on both sides held the day. Such fracas continued to occur regularly and in 1906 the Government appointed another Royal Commission which took the view that the pursuit of knowledge must recognise a limit to the pain which shall be inflicted on an experimental animal, but that it would be inconsistent and unreasonable to impose a greater restriction upon the infliction of pain for the advancement of knowledge than public opinion sanctions in the pursuit of sport, in carrying out such operations as castration and spaying, or in the destruction of rabbits and rats and other vermin by traps and painful poisons.

There can be no doubt that the antivivisection societies often put out propaganda that contained reckless and indiscriminate allegations against scientists and this caused some of them to react with passion. Opinions became polarised, tempers ran high and calm and reasonable discussions became impossible.

There is some evidence that in recent years the policies of the antivivisection societies have modified and that some are now seeking the active cooperation of scientists in developing alternative methods to animal experimentation. Yet most of the old animosities are still present and the divisive squabble is continued in the political arena with no remedial actions being taken by the government.

In 1963 the Secretary of State appointed a Departmental Committee of Enquiry into Experiments on Animals, which became known as the Littlewood Committee, and which made a series of recommendations most of which would, if adopted, require legislation. So far this has not taken place.

The vast increase in the use of laboratory animals (for example in 1883 there were 311 experiments, and in 1975 nearly 5½ million) suggests that the present Act is now rather archaic and inconvenient and can no longer adequately cope. If the law was clarified and streamlined in certain ways, both in the interests of animals, and in removing some administrative inconveniences which irritate the scientists, it would help the animals in many ways.

The question may be asked: "Are all these experiments necessary?" Let us consider the situation from this point of view. When a new product, a pharmaceutical preparation, a pesticide, a food product or a face cream is being developed it is necessary before marketing not only to prove that it is effective but also that it is safe in use and will not result in harmful effects to the consumers in the case of medicines, foodstuffs and toiletries and to those who apply the substance in the case of pesticides and poisons. Efficacy and safety do not always go together, moreover what is safe for one animal (including man) may not be safe for another. (Penicillin kills guinea pigs.) When the animal for which the preparation is intended is other than man, tests can be carried out at an early stage of development in the particular animal concerned – the target species, e.g., dog, cat, cow, sheep, pig. When, however, the target species is man himself, difficulties arise. Apart from the ethical problems involved in experimenting on human beings, even volunteering criminals, there are also the practical ones of obtaining suitable controls with which to compare results in a scientific way. Sex difference, age difference and basic physiological differences are all of importance, and identical human twins or triplets are not always forthcoming. Hence testing must be carried out on suitable animal models and the results then extrapolated to the possible effects on man; remembering at the same time that there is no real substitute for man and extrapolations can be inaccurate.

Thalidomide was an excellent tranquilliser and especially for the aged who were suffering from senile dementia. Unfortunately it also inhibited the vomiting centre. When it was prescribed for young pregnant women to overcome the nausea and discomfort associated with morning sickness, it was most successful in this respect. The deformities which it caused in the unborn were not at first realised. Thalidomide and certain other substances will also cause foetal abnormalities in rabbits and other animals which are dosed at certain levels. As a result of the thalidomide tragedy practically

all drugs which are intended for consumption by human beings are now tested on a multiplicity of animal species for teratogenic effects. Yet the fact remains that such tests in animals may have little relevance to results in pregnant women who are invariably in excellent health, and those who require medication only represent a very small minority of the human population. The Medicines Act of 1968 imposes on manufacturers the necessity to prove the safety of the products but there is nothing which is really safe and the negative results obtained after testing drugs on pregnant animals do not necessarily mean that there is no danger when such substances are given to pregnant women.

In the opinion of many scientists the testing of drugs for teratogenesis on its present scale is quite unnecessary unless the substance is specifically intended to alleviate some serious disorder which is normally associated with early pregnancy. Amongst commercial companies there is also a great deal of duplication of experimental tests. For example: a developmental chemist may offer a patented substance A to three different companies for experimental testing as to its ultimate potential. All three companies test the substance unknown to each other and the first company which is satisfied and makes the best financial offer then acquires the patent and develops the substance for marketing. A central testing agency to which companies could forward such substances for testing and report would also much reduce the overall competitive and duplicated research which at present obtains.

There are various other tests which are carried out in the development or safety testing of drugs and other substances, some of these such as the LD_{50} tests are of extremely doubtful validity especially when the substance is only very mildly toxic. Such tests, however, have become accepted as normal and established and the results continue to be submitted more or less as a matter of routine and without regard to their meaning.

Some of these experiments which are carried out in testing drugs are, in my opinion, not really necessary and their abandonment would considerably reduce the number of animals used. Some progress has already been made in this direction by the development of alternative methods which usually mean tissue culture techniques. Early examples of the use of tissue culture were the replacement of animal tests by chemical methods in the assays of digitalis glycosides and tubocurarine. The mouse brain test used in quality control to assess the potency of yellow fever vaccine has been replaced by a tissue culture method. Progress has also been made in chemotherapy research, diagnostic tests and in vaccine production and *in vitro* methods are being increasingly developed for the preliminary screening of certain drugs. At the present a member of Universities Federation for Animal Welfare's staff is studying at the University of Strathclyde a method of tissue culture for the initial detection of substances which are carcinogenic. If positives are obtained from tissue culture preparations it would probably then be unnecessary to undertake further tests in animals. In addition, tissue culture is more economical, hazards from bites and scratches are avoided, in the use of human material species variation between man and animals is eliminated, and the material can be reproduced under controlled conditions of experimentation. There are also limitations. If a drug has to be metabolised *in vivo* into an active, or a more toxic or less toxic form, then that form of the substance would have to be prepared first for *in vitro* testing. Also tissue culture is not a good model for studies in teratogenicity, and it cannot directly measure effects on fertility or on blood clotting, or on effects of analgesics and anaesthetics or drugs with tranquillising, hypnotic or psychological effects. It also does not provide a suitable model to study the development of tumours in animals. Nevertheless tissue culture has a role to play in reducing the number of animals required and is a useful adjunct which provides additional and valuable information.

The abolition of experiments of animals is both impracticable and improbable but the regulation of experiments on animals is both practical and possible.

To improve the welfare of experimental animals it is essential to cooperate in a practical way with those who work with them. It is to humane scientists that we must look for leadership and effective action, but laymen can also help by understanding the difficult parts of the problem and approaching the scientific world in an informed way. It is necessary to create an atmosphere of sanity, objectiveness and intellectual honesty in which the very difficult ethical problems which arise, can be discussed dispassionately and without the excitement and invective that the subject has come to be associated with in many minds.

REFERENCE

Universities Federation for Animal Welfare 1977 Proceedings of a Symposium *"The Welfare of Laboratory Animals, Legal, Scientific and Humane Requirements"*

CURRENT STATUS AND FUTURE OPTIONS FOR THE DEVELOPMENT OF LABORATORY ANIMAL TECHNOLOGY AND THE TRAINING OF LABORATORY ANIMAL TECHNICIANS

G. CREWE and J. C. AUSTIN

ABSTRACT: Crewe G., Austin J.C. **Current status and future options for the development of laboratory animal technology and the training of laboratory animal technicians.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 161–165 (En), MRC/University of Witwatersrand Dental Research Institute, 1 Jan Smuts Avenue, 2001 Johannesburg, Republic of South Africa.

Laboratory animal technology has evolved into a specialised field of expertise which is associated with the production, care and use of laboratory animals in biomedical teaching and research. A survey of laboratory animal facilities and supporting personnel was undertaken to assess the uses of laboratory animals in relation to the administrative and technical staffing of animal facilities. The results of this study indicate that there is a need for training in laboratory animal science at both the technical and professional levels. Options for the development of formal training in laboratory animal technology are reviewed.

INTRODUCTION

Laboratory animal science and technology have developed side by side in the European countries and North America for the last 30 years and have evolved into a specialised field of biology which is concerned with the selection, production, care and use of laboratory animals in teaching and research. A feature of this development has been the emergence of the laboratory animal technologist as a formally trained specialist with a well defined role in the staffing structure of educational and research institutions. These persons are professionals in their own right and they have made important contributions towards better management and housing of laboratory animals and to the advancement in knowledge of the biology of these species[3].

This development stemmed mainly from the increasing use of laboratory animals in biomedical research which began in the early 1950's. An American Association for Laboratory Animal Science was established in 1949. This association grew rapidly into a large organisation which concerned itself with promoting the exchange of scientific information in all fields of laboratory animal usage, with establishing standards for laboratory animal care and with the development of training programmes for animal technicians. In 1950 animal technicians in the United Kingdom set up an *ad hoc* committee to study the working conditions in animal houses. As the direct result of the recommendations of this committee an animal technicians' association which is now known as the Institute for Animal Technicians, was formed. This Association grew rapidly and made significant contributions to the advancement of laboratory animal technology by establishing a scientific journal devoted to laboratory animal technology, arranging congresses and symposia and by establishing formal courses of training for their members. Animal technicians in other parts of the world soon followed these leads and similar associations have since been established in many countries.

Another notable development which took place concurrently with the growth of laboratory animal science associations was the development of National Laboratory Animal Centres. In 1945 an unofficial committee comprised of members of a number of scientific societies produced a report on the supply of laboratory animals in Great Britain. This was the first survey on the production and utilisation of laboratory animals ever to be undertaken and published[1]. This survey gave rise to the recommendation that an advisory committee on laboratory animals be set up on a national basis, and that this task should be delegated to a laboratory animals bureau with a full time scientific director. In 1947 the British Medical Research Council created a new unit on the lines suggested. This unit grew from modest beginnings to become the present day British Laboratory Animal Centre. Other countries followed suit and there are now a number of national laboratory animal centres operating in Europe.

Neither of these developments have, to date, taken place in South Africa. Whilst significant numbers of people are currently being employed as laboratory animal technicians in South African institutions, their standard of training, ethical committments, legal status and the regulation of their mode of employment is undefined. The continuance of this state of affairs is hardly in the interests of the laboratory animals, the scientific community or the technicians themselves. There appears to be a need for the establishment of appropriate standards and expertise for employment in this field, for the development of a suitable programme of training and for the recognition of qualified technicians by certification and registration.

A national survey was recently conducted to gather information on the use of laboratory animals and laboratory animal personnel in South Africa in order to examine this problem in depth.

The purpose of this paper is to report on the results of this survey and to comment on options for the future development of laboratory animal science and the training of laboratory animal technicians.

SURVEY OF LABORATORY ANIMAL FACILITIES AND SUPPORTING PERSONNEL

The survey was conducted in July 1977 to provide information on the national usage of laboratory animals. Data was collected on the nature of organisations served by animal facilities, the specific types of work being conducted with laboratory animals, the numbers and species of the various types of animal being maintained in the animal facility, the staffing structure and the academic qualifications of the technical personnel employed in the facilities. Opinions on the need for formal courses of training for animal care personnel were also solicited.

SURVEY METHODOLOGY

The survey was carried out by means of a questionnaire which was circulated together with a covering letter explaining the purpose of the inquiry, to all persons and organisations listed in the South African Index of Laboratory Animals[2]. Respondents were not requested to identify their animal facilities in this survey and anonimity was maintained when data was analysed. The questions in this document were organised into 4 major categories with each category being divided into subsections. A series of carefully preselected questions were chosen so that respondents had only to record affirmative or negative responses. This format was chosen to minimise the questionnaire completion time and facilitate the analysis of results. The questionnaire was based on a questionnaire used by the Institute of Laboratory Animal Resources of the U.S. National Academy of Sciences for a national survey of animal resources which was conducted in the U.S.A. in 1967-68[4]. The following 4 categories of questions were used for the survey.

A. Organisations

Respondents were asked to identify the type of organisation to which the animal facility was attached under 2 subsections. Subsection 1 included the following categories: University, College for Advanced Technical Education, State Research Institute or laboratory, Provincial Research Institute or laboratory, Others (type to be specified). Subsection 2 required the field of biomedical activity or activities which the animal facility supported, within the following categories: Medicine, Dentistry, Veterinary Science, Pharmacy, Zoology, Behavioural Sciences, Public Health and Others (to be specified by the respondent).

B. Animal Facility

In this section respondents were asked to identify the purpose for which animals were used in the following categories: Teaching, Biomedical Research, Diagnosis and Testing, Vaccine Production and Others (to be specified). Details of the genera and species and numbers of each of the different types housed in the animal facility on the day the questionnaire was completed, were requested. A tabulated form was included in the questionnaire to facilitate recording of data under the following headings:

Rodents & Rabbits	mice, rats, hamsters, guinea pigs rabbits and others (specify)
Primates	baboons, monkeys and others (specify)
Avians	chickens, ducks, quail and others (specify)
Carnivores	dogs, cats and others (specify)
Ungulates	pigs, sheep, goats, cattle, horses and others (specify)
Other species	(specify)

C. Personnel

This category was divided into 3 subsections to determine firstly if the animal facility was administered by a designated director and what his academic status was, secondly to determine the numbers of technical staff and cleaners and their academic qualifications and, thirdly to determine the number of hours which each of the technical staff spent in the animal care programme of the facility each week.

D. Training

In this category respondents were asked whether any formal training in laboratory animal technology was being offered to their staff or if it was available within a commutable distance; whether a formal course in laboratory animal technology was needed and, if so, whether it was needed at the technical, or professional levels or at both levels.

RESULTS OF SURVEY

Eighty-six questionnaires were sent out and 55 were returned completed (64% return). Of the 55 replies received, only 46 contained data relevant to the survey. The 9 replies which were discarded were from persons or institutions which had either discontinued the use of animals or had reduced their stocks of animals to levels which could not be considered to be representative of a laboratory animal facility. The results of the survey are based on the data received from 46 animal facilities.

Table 1: NUMBER AND PERCENTAGE OF SURVEY RESPONDENTS IN RELATION TO THE TYPE OF ORGANISATION (n = 46)

	No. of Animal Facilities	%
University	24	52
State	12	26
C.A.T.E.	3	6,5
Provincial	3	6,5
Private	3	6,5
Others	1	2,5

Although respondents did not identify their animal facilities comparisons between the animal species listed in the South African Index of Laboratory Animals and the data recorded in the questionnaires returned suggested that: (1) all major users of laboratory animals in the country had participated in the survey, (2) that the results could be accepted as being reliable, valid and representative for the time at which the survey was undertaken.

A. Organisations

Table 1 presents the number and percentages of respondents who participated in the survey in relation to the types of organisations with laboratory animal facilities. From this table it can be seen that the Universities (52%) and State Institutions (26%) operate the largest number of animal facilities in the country.

In Table 2 the number and percentages of biomedical fields supported by the animal facilities are listed. Sixty-nine fields of activity were supported by the 46 animal facilities, indicating that the animal facilities in a number of instances were being used for more than one purpose. Medicine (25%) and Zoology (23%) were the most frequently supported activities in this category.

Table 2: NUMBER AND PERCENTAGE OF BIOMEDICAL FIELDS SUPPORTED BY THE RESPONDENTS' ANIMAL FACILITIES (n = 69)

	No. of Biomedical Fields	%
Medical	17	25
Zoology	16	23
Diverse	11	16
Public Health	7	10
Pharmacy	7	10
Behavioural Sciences	5	7
Veterinary Science	4	6
Dentistry	2	3

B. Animal Facilities

The number and percentages of respondents participating in different types of work are detailed in Table 3. Ninety-four different types of work were being supported by the 46 animal facilities, indicating that animal facilities had dual or multiple committments.

Table 3: NUMBERS AND PERCENTAGES OF RESPONDENTS PARTICIPATING IN VARIOUS TYPES OF WORK (n = 94)

	No. of Respondents	%
Teaching	26	56
Biomedical Research	25	54
Supply of Animals	12	26
Others	12	26
Diagnosis and Testing	11	24
Vaccine Production	8	17

The major 2 categories which were supported by animal facilities in this section were Teaching (56%) and Biomedical Research (54%).

The number and percentages of the various species of laboratory animals held in the respondents' facilities in July 1977 are detailed in Table 4. From this table it can be seen that most of the small and large laboratory animals were held in University or State Institute animal facilities. A breakdown of the numbers of the various species of rodents and rabbits held in the animal facilities is listed in Table 5.

Table 5: NUMBERS AND PERCENTAGES OF RODENTS AND RABBITS HELD IN THE RESPONDENTS' ANIMAL FACILITIES IN JULY, 1977

	No. of Animals	%
Mice	83 211	75
Rats	13 461	12
Guinea Pigs	9 901	9
Rabbits	2 527	2
Hamsters	1 114	1
Others	505	<1
Total	110 719	

C. Personnel

Administrative personnel

Thirty-five of the 46 animal facilities had designated directors of which 33 (72%) were university graduates (D.Sc. (6), Ph.D. (8), B.V.Sc. (6), M.B.B.Ch. (3), M.Sc. (4), B.Sc. (6) and two held technical diplomas (Medical Technology).

Technical personnel

A total of 91 persons were employed as technicians in the 46 laboratory animal facilities. The number and percentages of technical personnel employed in the various organisations and their academic qualifications are listed in Table 6. From this table it may be seen that the Universities (44%) and the State Institutes (34%) are the major employers of laboratory animal technicians. There were 22 university graduates (all in Science subjects) and 27 persons with technical diplomas amongst the persons employed as technical staff. The technical diplomas in the latter group were held in Medical Technology (6), Microbiology (5), Veterinary Technology (4), Agriculture (6), Nursing (2), Nature Conservation (1) and Laboratory Animal Technology (3). The 3 persons in the latter group all held British Institute of Animal Technicians' qualifications. The remaining 42 staff members in this category had secondary school education at various levels ranging from below standard 8 to matriculation.

The weekly working time of the technical staff is also listed in Table 6. An inverse relationship existed between time spent in the animal programme and academic qualifications in the technical staff.

Table 4: NUMBERS AND PERCENTAGES OF LABORATORY ANIMALS HELD IN THE RESPONDENTS' FACILITIES IN JULY 1977

Organization	Small lab. animals; mice, rats, hampsters, guinea pigs, rabbits	Large lab. animals			Birds	Amphibians
		Primates	Carnivores	Ugulates		
	n = 110719	n = 1322	n = 327	n = 7214	n = 22632	n = 907
	%	%	%	%	%	%
University	14	43	71	32	2	89
C.A.T.E.	1	0	0	0	0	11
State Inst.	71	34	23	66	98	0
Prov. Inst.	<1	1	6	<1	0	0
Private Inst.	13	19	0	1	0	0
Others	<1	3	0	<1	0	0

Table 6: NUMBERS AND PERCENTAGES OF TECHNICAL PERSONNEL EMPLOYED BY VARIOUS ORGANISATIONS WITH ACADEMIC QUALIFICATIONS AND MEAN NUMBER OF HOURS SPENT PER WEEK IN THE ANIMAL CARE PROGRAMME

Organization	Technicians Employed n = 91 %	Academic qualifications of technicians: Graduates n = 22 %	Technicians n = 27 %	Matriculation n = 24 %	Std. VIII n = 11 %	>Std. VIII n = 7 %
Universities	44	45	37	46	36	86
C.A.T.E.	3	5	7	0	0	14
State Inst.	34	36	37	33	45	0
Prov. Inst.	9	9	11	4	19	0
Private Inst.	8	0	4	0	0	0
Others	2	5	4	17	0	0
Mean hours/week spent in the animal care programme		18 h	19 h	26 h	40 h	40 h

Cleaning personnel

There were 226 cleaners employed in 43 of the 46 animal facilities (3 had no cleaners). The educational standards which had been reached by these cleaners varied from no formal education to matriculation. Only 3 of the cleaners had passed standard 8 and 3 were matriculants.

D. Training requirements for laboratory animal personnel

Formal training in laboratory animal science was conducted in only one institution in South Africa. This was a 2 year part time in-service training course run for technical staff employed in a University. Technical staff from 7 of the animal facilities which participated in the survey attended this course.

Respondents indicated that there was a need for formal training courses in laboratory animal science and technology at both the technical and professional level. Eight percent of the respondents wanted courses at a professional level only, 53% at a technical level only and a further 39% felt that courses should be established at both academic levels. The sum of these responses indicated that 92% of the respondents felt that there was a need for technical training and 47% a need for professional training in order to improve the animal care programme in their animal facilities.

DISCUSSION

The data collected in this survey provides a useful guide for evaluating the purposes for which laboratory animals are used in South Africa, for identifying the major animal users and for examining the administrative and staffing structure of animals facilities. Most surveys of this kind examine the data we have collected in relation to annual usage of animals and animal care costs. This approach was specifically avoided since much of this type of data would have been either confidential or difficult to extract from financial reports of large institutions. Suffice it to say that the production and maintenance of animals for teaching and research is an expensive operation particularly with the current trend towards the use of animals which are genetically, microbiologically and nutritionally and environmentally defined. Several large new animal facilities are presently being built and others are currently being planned for major teaching and research institutions in the country. These facilities will be required to provide better teaching and research support and people will need to be trained to staff them.

Apart from future requirements the present survey indicates that there is a widespread need for training of personnel in the existing animal facilities. This was clear from the 92% of survey respondents who indicated that a formal course in laboratory animal technology would improve their animal care programme.

Other data in the survey would also support this viewpoint. A look at the administrative and staffing structures of the laboratory animal facilities surveyed indicate that 72% of these facilities have designated directors. The decision to designate a director of laboratory animal care in an organisation with animal facilities is usually associated with an awareness of the necessity of operating institutional animal facilities on a sound management basis.

The high academic qualification of the 33 designated directors who were graduates, in relation to the technical staffing structures, suggests that in some instances these persons may have held these appointments in a part time capacity and had other full time duties in the organisations in which they were employed. An indication of the hours which they spent in the animal care programme would have clarified this aspect.

An examination of the technical staffing complement and academic qualification of the graduate and diplomate personnel employed in the animal facilities and mean hours/week which they spent in the animal care programme suggest that they too were not really full time laboratory animal technicians (see Table 6). Even the matriculant group did not appear to be employed as full time animal technicians. The only technicians who were fully engaged in animal care were those who had not completed their secondary schooling.

These findings suggest that the animal care programmes are largely being conducted on a part time basis by technical personnel who have not had formal training in laboratory animal technology.

There is clearly a need for the creation of more full time career opportunities for trained laboratory animal technicians in South Africa. This viewpoint is also supported by the diversity of animals which are maintained in our institutional animal facilities. The trained animal technician should have a good enough grounding in theoretical and practical biological subjects to be able

to be versatile enough to care for and handle the range of animal species which are housed in these institutional facilities.

With these points in mind the question may be asked which organisations would have a major interest in the acquisition of trained laboratory animal technicians. From the data collected in the survey it is the Universities and State Institutes which operate the largest number of animal facilities; most of the animals used in biomedical teaching and research in South Africa. The problem of training animal technicians would thus be of foremost concern to these 2 groups of organisations. Support for the establishment of personnel should be sought in these quarters.

OPTIONS FOR THE FUTURE TRAINING OF LABORATORY ANIMAL PERSONNEL

Two kinds of training are employed in most countries in which courses in laboratory animal technology are offered[1]. These are practical in-service training and formal, or tutorial training. Practical training is undertaken through day to day supervision and experience in carrying out duties. Formal training consists of lectures, demonstrations and tutorials together with practical tasks which are associated with such teaching.

The formal training in most countries is carried out on a part time basis for personnel who are employed in a full time capacity as trainee technicians. The 2 aspects of the training are given concurrently because of the practical nature of the expertise which constitutes laboratory animal technology.

The formal training is usually given by colleges for advanced technical education. In South Africa for new courses to be run at colleges of advanced technical education approval from the Department of National Education is first required. Such courses have to be shown to be viable in terms of attracting at least 30 students per class per year to be considered for inclusion in the educational programme of colleges for advanced technical education.

An immediate objective should be to train the technical personnel who are presently in employment as animal technicians. There are presently 70 technicians with educational qualifications which would permit them to participate in such courses. Such courses would have to be run within commutable distance of the place of employment of these technicians.

Since the anonimity of respondents was maintained in the survey it is not known whether the personnel who are eligible for further training could form large enough groups to justify the running of courses in the technical colleges situated in our major cities. An alternative approach may be considered in view of the practical problems which exist in this regard. This is the use of a correspondence course for technicians who are being trained whilst they are in full time employment in animal facilities. These correspondence courses could be combined with full time attendance of formal courses of instruction several times a year.

Whatever the mode of training be, it should be carried out under the auspices of an institution for advanced technical training as an accredited course which is recognised by the Department of National Education. The overall goal should be to train technicians who will be prepared to accept the disciplines which will enable them to fulfil their obligations to scientists and educationalists and to provide professional care for laboratory animals. The challenge now is to develop a training programme which has some meaning, which develops and recognises technical expertise and is fair to all individuals involved.

The realisation of this goal may perhaps best be achieved by the formation of a laboratory animal science association with these objectives in mind.

ACKNOWLEDGEMENTS

We are indebted to the many persons who participated in this survey and co-operated by providing the data presented in this paper. Appreciation is also expressed to Mrs. H. Ball for typing the manuscript.

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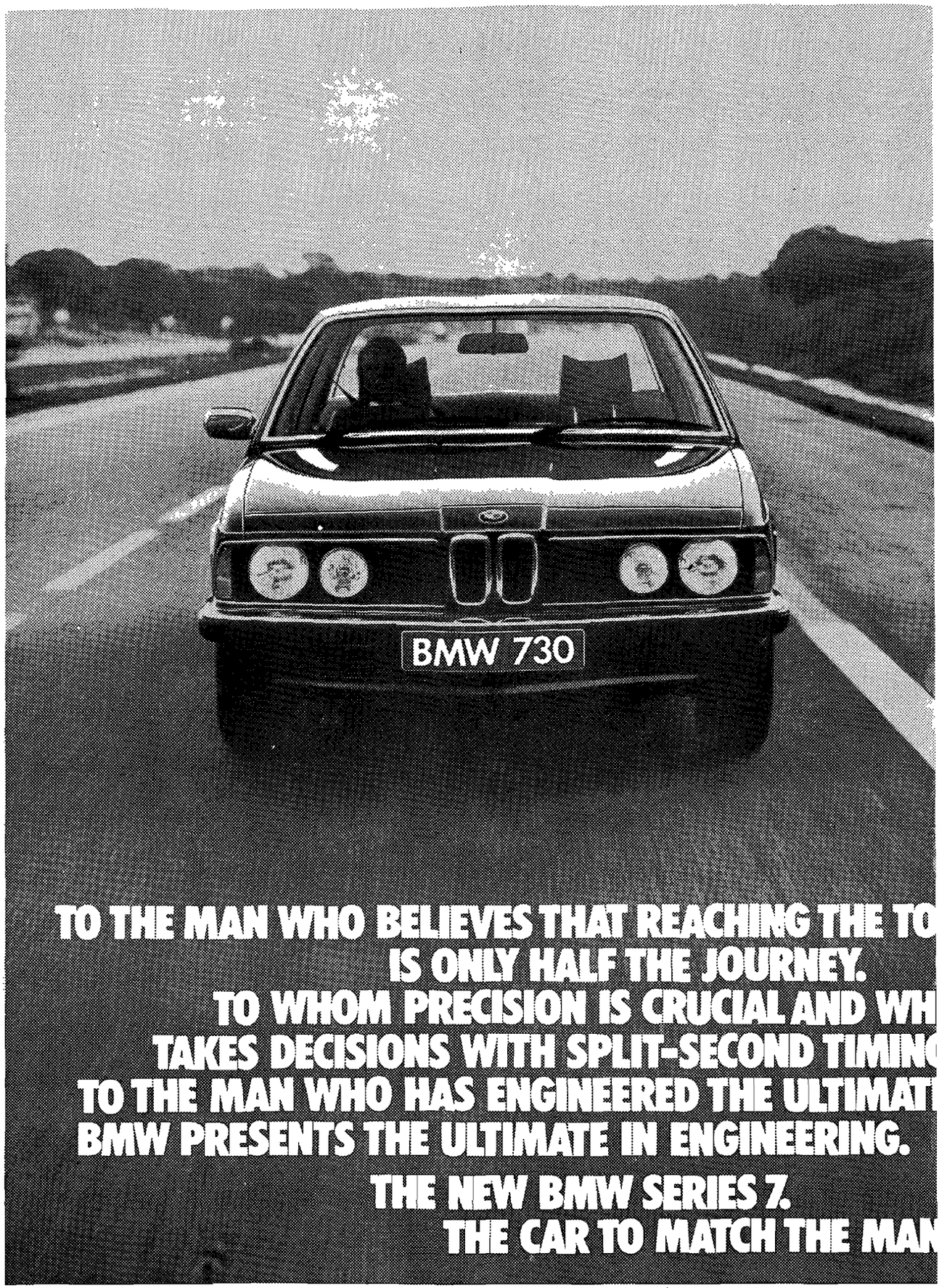
INLIGTING

INFORMATION

Emeritus Professor Douw G. Steyn has recently written a Technical Communication (No. 136) for the Department of Agricultural Technical Services concerning "*Modern trends in methods of food production, food processing and food preparation which constitute a potential hazard to human and animal health*". It refers mainly to food of vegetable origin. This publication appeared in September 1977 and is available, post free on request, from the Director, Department of Agricultural Information, Private Bag X144, 0001 Pretoria.



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ELIMINATION OF CONTAMINATION WITHOUT CAESARIAN DERIVATION – SUCCESS OR FAILURE?

J. BLEBY

ABSTRACT: Bleby J. **Elimination of contamination without caesarian derivation – success or failure?** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 167–169 (En), MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF, United Kingdom.

Unfortunately, only successes tend to be published, so we are denied knowledge of partial successes and failures regarding SPF unit breakdowns. If more workers were prepared to be open about their partial successes, a combination of various techniques as appropriate to the case, could prove to be most successful. Each SPF colony is different and must be considered on its merits, nature of contamination, species and strains of animals and so on, but it would seem that caesarian derivation is not now always the only course of action.

INTRODUCTION

The Specific Pathogen Free (SPF) concept has been in use now for about 20 years and without any doubt it has been the major development in laboratory animal science during this period. It has been responsible for the marked improvement in the health status of laboratory animals. However, the SPF concept does have one major drawback and that is that contamination of barrier maintained colonies is inevitable, irrespective of the precautions that are taken to prevent its occurrence. Anyone who says that their SPF unit never breaks down is either incredibly lucky, or does not yet know that it has broken down.

The usual method for eliminating all but vertically transmitted viruses and some other infections is to rederive the colony by caesarian derivation into germfree isolators and re-populate the SPF unit from germfree stock. But, rederivation is a nuisance to say the least, and is not always feasible, especially where there are many different stocks and strains involved, or large numbers of production animals are required. Under these circumstances there is the conflict of maintaining supplies whilst simultaneously rederiving colonies into decontaminated rooms without the risk of cross contamination.

Perhaps, therefore, we should think more about trying to develop alternative methods of dealing with contamination and the purpose of this paper is an attempt to make us think along new lines. The paper is not, however, intended to provide a panacea for eliminating contamination and some of the examples that are quoted are not necessarily foolproof. But I think they offer alternative ways of dealing with the problem.

CONTAMINATIONS

Following a study of the main causes of contamination in LAC Accredited Colonies, Sparrow[5] showed that the mean colony life of Category 4 mice was 24 months and Category 3 – 35 months. Rats presented a similar picture.

The most frequent contaminant was *Pasteurella pneumotropica* and although it is considered by some to be of low pathogenicity, it nonetheless is serious, particularly in some of the newer and more delicate strains that are being developed. For example, in the nude mouse, *P. pneumotropica* causes serious subcutaneous abscesses. The LAC is now also developing the nude rat and it is likely that it will also be similarly susceptible to *P. pneumotropica.*

Other examples of commonly occurring contaminants are the nematode *Syphacia,* a common pinworm infestation, and several virus infections, e.g. Sendai virus in guinea-pigs. Sendai is also a serious and often fatal infection of nude mice.

However, it may be possible to eliminate these single organism contaminations, of usually low pathogenicity, by resorting to methods of isolation and chemotherapy. In all cases a very high standard of husbandry and management and laboratory monitoring is required, such as is usually present in SPF units, together with a full understanding of the nature and behaviour of the infecting organisms. Here, unfortunately, there are still gaps in our knowledge and continual monitoring, however good, does not always detect the presence of infections of low incidence or the presence of carriers.

VERTICAL ISOLATION

Many infections are transmitted from the adults to the young while in the nest and then spread within the weanlings when these are held in holding pens. Thus for those infections which are not detected before infection reaches 100%, control can be exerted by isolating the breeding stock in separate rooms, or preferably filter racks of boxes. This situation is of particular use when many different inbred strains of rats and mice are held, as these invariably show differences in susceptibility to the infecting agent. For example, from our own limited experience the inbred rat strains, WA, WAC, LH and Porton are highly susceptible to Kilham virus. In mice, strain A is particularly susceptible to ectromelia virus, whereas NZB is not, and with *Mycoplasma pulmonis,* strain BalbC mice are very susceptible. With careful study therefore, it may be possible to separate the strains of animals with clinical disease from those with non-clinical infections and thus greatly reduce the number of strains which require treatment or rederivation. This has been the experience of the LAC with a recent contamination with *P. pneumotropica.* Our colonies of mouse strains had been harbouring *P. pneumotropica* as a low grade infection but without signs of overt disease. The situation only became a serious problem when our nude mice became infected with the organism and developed subcutaneous abscesses, particularly post-orbitally. Therefore, we then knew that this strain was particularly susceptible and developed evidence of clinical disease, and as a result we are now isolating the nude mice from our other mouse strains, and rederiving this particular colony by caesarian derivation.

Vertical isolation, including the keeping of litters separately is also an additional aid where chemotherapy is undertaken.

CHEMOTHERAPY

(a) Parasitic

The increasing effectiveness of broad-spectrum antibiotics and anti-parasite agents makes the possible use of chemotherapy worth investigating. Chemotherapy is probably most readily of use in the case of parasites, where sampling for the presence of eggs can be carried out on the live animal, and the reappearance of eggs in event of failure of treatment can also be readily detected.

The LAC's recent experience with *Syphacia* infestation has shown that with extended treatment this nematode can be eliminated (to date) from a mouse colony. Because of the delay in getting one chemotherapeutic agent, thiabendazole, incorporated into the diet, immediate treatment with piperazine in the drinking water at 2 g/ℓ was introduced with the aim of reducing the number of eggs laid. Treatment with the thiabendazole at 0,1% in the diet was continued for 5 months to allow for all eggs in the environment to become uninfective or to be ingested, develop and be killed. In addition chemotherapy was aided by the careful cleaning of cages, racks and rooms etc. to reduce the possibility of recontamination. Another outbreak which was treated may be compared with this example, in this case in one of our breeder's colonies. Here, the offending organism was the tape-worm *Hymenolepis nana.* The parasite was eliminated by separating off a small colony and treating these animals with the anthelmintic Niclosamide through several generations. The resulting animals were then used successfully as breeding nuclei, and the disease problem was completely eliminated. Yet a further example is provided by Sebesteny[4] who reported high losses in a colony of C57 BL mice due to a massive infection with *Hexamita muris.* Treatment with 0,5% dimetridazole (Emtryl soluble) given in the drinking water to all freshly weaned mice for a period of 14 days proved successful in clearing the infection.

Meshorer[2], however, in describing an outbreak of hexamitiasis found that treatment with dimetridazole, nitrofurazone and oxytetracycline produced little or no effect on the clinical condition of the mice – A success and failure.

(b) Bacterial

Chemotherapy for bacterial diseases is complicated by the nature of these infections, some surviving in biologically inaccessible places, e.g., gall bladder in the case of *Salmonella* and the upper respiratory tract for many of the important organisms of laboratory animals including *Pasteurella* spp., *Bordetella* and *Mycoplasma.* It is difficult to eliminate organisms from such sites and also to sample from them to confirm elimination, but it may be possible in some cases with the use of vertical isolation and sampling. However, it is unlikely to be successful on a large scale and may only be justified in the cases of small colonies or one which is being experimentally used and near the end of its experimental period.

However, Hunter[1] has reported the successful control and apparent eradication of Tyzzers disease *(B. piliformis)* from a barrier maintained mouse colony by the use of oxytetracycline. It is perhaps fortunate that oxytetracycline is available as a syrup containing 60% w/v sucrose which facilitates its easy administration to mice in the drinking water at a dose of 10 mg/kg body weight. Syrup was added to the drinking water for 5 consecutive days and then repeated for a further 5 days after a lapse of 7 days. Subsequent examination of livers failed to show the presence of *B. piliformis.* A success story.

(c) Viral

Virus infections, which are now becoming increasingly important, present particular problems. So far, there is no chemotherapy which is effective, but, perhaps when new agents against viruses are developed, (and there are hopeful signs), the elimination of specific monocontaminations may be possible.

Recently, however, there was an interesting report at a meeting of the Laboratory Animal Science Association when Ashe of ICI Pharmaceuticals Division reported the successful elimination of Sendai virus from a guinea-pig colony. As the guinea-pig is not the natural host of Sendai virus it was therefore considered likely that the infection would be self-limiting. A policy was therefore adopted whereby the susceptible young were continuously removed until all the adults had become infected and had developed immunity. It is worth considering therefore that where infections are known to be self-limiting, a system of infecting the adults and eliminating the young may succeed.

VACCINATION

Vaccination of stocks has long been used particularly against ectromelia, but it is a 2 edged sword as it produces carrier problems. In 1975 the U.K. suffered a serious outbreak of ectromelia following the importation of vaccinated carrier animals, and the LAC does not therefore recommend vaccination against ectromelia.

However, Nikkels and Mulling[3] have reported an outbreak of *Bordetella bronchiseptica* pneumonia in 2 SPF colonies of guinea-pigs and its successful elimination by administration of a single dose of vaccine mixed with Freund's incomplete adjuvant. Bearing in mind that *B. bronchiseptica* is a common inhabitant of the upper respiratory tract of the rabbit and rat as well as the guinea-pig, surely here is a valuable example of a alternative method that should be investigated further, particularly as it does not seem to cause carrier problems.

OTHER METHODS

So far, I have only mentioned traditional methods as an alternative to caesarian derivation, but what about other possibilities? For example, Ward[6] describes the elimination of *Pasteurella multocida* from rabbits by the transfer of fertilised ova to *Pasteurella* free foster mothers. In this case, the original pedigree of the strain was maintained without laborious caesarian rederiva-

tion and hand-rearing, which is particularly difficult in the rabbit. Ova transplantation then offers another alternative and should be considered where appropriate.

ACKNOWLEDGEMENT

The assistance of Dr. M. Lancaster in the preparation of this paper is gratefully acknowledged.

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OPERATING PROCEDURES, EQUIPMENT AND HOUSING FACILITIES TO MAINTAIN SMALL LABORATORY ANIMALS UNDER SPF CONDITIONS IN LARGE BREEDING COLONIES AND IN EXPERIMENTS

W. HEINE

ABSTRACT: Heine, W. **Operating procedures, equipment and housing facilities to maintain small laboratory animals under SPF conditions in large breeding colonies and in experiments.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 171–174 (En), Zentralinstitut für Versuchstiere, Lettow-Vorbeck-Allee 57, D-3000 Hannover 91, Federal Republic of Germany.

The procedures and techniques to maintain specific pathogen free animals in modern barrier-type animal quarters and the improvement of existing conventional facilities, are discussed.

INTRODUCTION

In animal experimentation conventionally raised animals are being increasingly replaced by specific pathogen free (SPF) animals. Colonies of small laboratory animals free of species specific infectious diseases can be developed successfully by using gnotobiotic methods and by exposing germ-free animals to a proper bacterial "cocktail". SPF mice, rats, guinea pigs and rabbits are available today from several different sources.

Like all laboratory animals, SPF animals must be maintained under standardized environmental conditions. They can be kept without difficulty, provided they are protected against species specific pathogenic micro-organisms. It is necessary to have special barrier type animal facilities available for the development and maintenance of healthy colonies of small laboratory animals and for fastidious laboratory animal experimentation under standardized conditions. However, modern laboratory animal quarters are as expensive as modern hospitals. Therefore the construction of such facilities is often postponed while existing rooms have to be improved temporarily. In this paper 2 ways in which to achieve proper hygienic conditions are discussed:

(a) the operation of specially designed, barrier-type animal houses, and
(b) the improvement of conventional facilities for the maintenance of laboratory animals.

A. PRINCIPLES FOR THE MAINTENANCE OF SPF CONDITIONS

Since 1958 we have designed, built and maintained 4 generations of animal houses. The principles on which the construction of the 2 houses of the third generation completed in 1967 and the 2 of the fourth generation in operation since 1972 are based, are the same and are still considered to be the ideal. Several modern animal facilities, not only in our country, have been designed according to these principles[2 4].

In order to maintain SPF animals, the animal houses are equipped with special devices and operated according to special procedures. By means of these methods an effective hygienic barrier is established.

Air conditioning

Independent air conditioning systems for the different animal rooms, inner corridors and shower wings are equipped with efficient filters to prevent air-borne microbial contamination. In the animal rooms a positive pressure (*ca.* 15mm H_2O) is maintained as an additional safety measure. There is furthermore a pressure drop of 5 mm H_2O between the animal rooms and clean corridor, another 5 mm drop between corridor and shower tract, and finally, another 5 mm decrease between the shower tract and outside.

Staff routine

The four-doored shower tracts have 3 gastight inner doors each with a doorstep of approximately 50 cm high. The staff uses their own soap and nailbrushes and shower following disinfection of their forearms, hands and feet. The shower has a greater psychological than germicidal effect. It is, however, necessary to indicate the barrier. The staff wears sterilized clothing, face masks, caps, slippers and sometimes cotton gloves.

The staff works 8h per day, 5d per week. It is not permitted to eat and smoke behind the barrier. Hence there is a break after 4 hours during which time they leave the system. The same procedure is followed on re-entry. The flushing system of the toilet inside the barrier is connected to the central disinfection line.

Sterilization and pasteurization

Man is the weakest link in the hole system. Apart from him, all other components can be controlled. Food, water, bedding, cages, etc. are sterilized or pasteurized.

Dietary constituents, bedding, cages and bottles are autoclaved for 10 minutes at 105°C. The clean-side door of the autoclave can be opened only after running a cycle. Untreated tap water should not be used for SPF-animals. The common asbestos filters and the ceramic-type filters are unsatisfactory since they only function well as long as there is a constant flow of water through them. Intermittent flow allows bacterial growth on the filter material with the consequence that filtered water is an even worse source of bacteria than is unfiltered water.

Drinking water is preferably pasteurized at 105°C in a steam operated pasteurizing plant such as those normally used on dairy farms to pasteurize milk. Unfiltered water is pasteurized automatically and stored in stainless steel tanks. In addition, the pasteurized water is acidified with hydrochloric acid to a pH of 2,5 and

stored in stainless steel tanks. Acidification ensures that the drinking water in the drinking bottles remains free of bacteria while on the cages. This system has been used for 5 years without any noticeable effect on the animals[5].

The question of whether an automatic watering system with drinking nipples is preferable to bottles has not been resolved. We prefer bottles since the tubing of an automatic system is rather difficult to clean.

Smaller items like pencils, paper and occasionally an instrument like a scale, are disinfected after being cleaned carefully in a small but powerful UV-cabinet outside the barrier system. As far as possible, instruments are dismantled and treated overnight or even over a weekend in the UV-chamber. The components are then sealed in similarly treated plastic bags and passed into the barrier system through a dunktank.

Sterilization can also be done in an ethylene oxide chamber. If this sterilizer has no double-end doors and is not fitted into the barrier, material can be sealed in polyethylene bags. Polyethylene oxide penetrates polyethelene and sterilizes the contents. The treated bags can then be passed into the barrier system through a dunktank.

Another useful installation through which to pass material into a barrier system, is a formaldehyde chamber. Built into the barrier, the chamber has an outer and inner door which are electronically interlocked. The inner door can only be opened after a disinfecting cycle has been completed. A specific amount of formaldehyde is automatically sprayed or introduced as an aerosol. At the end of the cycle the formaldehyde vapour can be neturalized by ammonia. Subsequent to this, the chamber is ventilated either with air from inside the barrier system or by filtered air from outside. The air exhaust is operated by an automatic gastight motor valve which opens only after the ventilation system has been in operation and a safe positive pressure has built up in the chamber. We use formaldehyde chambers to pass cardboard shipping boxes into the system. It can also be used for other purposes.

Cleaning and sterilization of buildings

Before commissioning a barrier system and before repopulating an animal facility, the buildings should be cleaned thoroughly.

To detect any air leaks in the buildings and installations, the following procedure should be followed: The fans in the ingoing airducts are set into operation while the outgoing airducts are closed. As a result a relatively high positive pressure is built up in the system. Smoke candles are now burned in order to detect air leaks in the hygienic barrier, building and technical equipment. Leaks are carefully sealed with plastic material. While using the smoke candles, the air filters should be removed to prevent clogging by smoke particles.

The rooms are next fumigated with a suitable insecticide while the air conditioning system is still not in operation. Twenty-four hours later large quantities of formaldehyde (diluted 1:10 = 4%), is sprayed on all surfaces in the system. The formalin should remain in the building for at least 24 hours but preferably for 48 hours, before the air conditioning system is set into operation. It is not necessary to treat the airducts since the atmosphere will penetrate these remote areas. Fumigation must always be performed by 2 employees working together inside the barrier. They must wear protective rubber garments and gas masks. After this treatment the inner surfaces and the interior of the building is monitored for the presence of microbes by using swabs and open petri dishes. No microbes should be present.

Introduction and maintenance of SPF animals

To pass the developed or purchased SPF animals into the system, we use portholes as used for isolators. Several of these portholes should be built into the barrier of the building. Isolators with animals or shipping containers can be attached directly to these portholes. The animals are passed into the barrier system by using approved techniques[3].

If the pretreatment procedures have been performed carefully the microbial pathogen-free status of the introduced animals can be maintained for years. We have maintained SPF colonies free of undesirable contamination for 5 years. During this period the associated flora is mainly increased by ubiquitous germs brought into the system by man. However, some of these ubiquitous bacteria may be facultative pathogens. Because the flora we supplied to the germ-free animal is still rather incomplete, additional germs sometimes cause transient imbalances of the flora. This gives rise to clinical signs and alterations which can be classed as "animal house hospitalism".

It is well known that the microbiological contamination of an SPF colony can only increase.

One should attempt to keep the degree of contamination as small as possible by ensuring that no additional animals are allowed to pass into the system after the colony has been started; no change in the trained and trustworthy staff working inside the system takes place and that the barrier equipment should be operated continuously by the same skilled and reliable staff. This is comparatively easy in an SPF breeding colony. However, it can become a frustrating problem at a central animal laboratory for animal experimentation where many different and frequently changing animal strains from different sources and of varying microbial status, have to be maintained under one roof. If there is only one barrier system, in other words if there is no possibility to separate and isolate animals of different microbial status, that of the whole population will be as poor as that of the most inferior animals.

Furthermore, in this case, caesarian derived SPF animals often seem to be the poorest animals. They show severe clinical signs and pathological lesions because they simply have never been in contact with the introduced pathogenic and facultatively pathogenic germs.

In the past we have had several unpleasant contaminations of some of our colonies and we culled the animals in order to re-establish the infected strains. At the end of 1976 one of the animal houses was contaminated by *Pasteurella pneumotropica* and *Mycoplasma pulmonis*, due mainly to the following 3 reasons[1]:

(a) Insufficient screening of new introductions.
(b) Introduction of animals into a very large facility – 11 000 cages of 900 cm² each. In a large system like this the barrier cannot be controlled as properly as in smaller systems. The large number of technicians working behind the barrier and the involved fluctuation of the staff is an added danger.

(c) Neglect of personal hygiene, due to over-confidence in the function of the technical barriers. Well-trained and reliable staff working according to strict and detailed hygienic procedures is as important, or even more important, than a good barrier system.

In central animal units for animal experimentation with frequent fluctuation of animals of different strains and species from different sources on the one hand, and fastidious medium-range and long-term investigations under controlled environmental conditions on the other hand, several completely independent barrier systems should be available. In addition, sufficient staff should be available to obviate the interchange of employees between the different barrier systems.

B. IMPROVING CONVENTIONAL ANIMAL HOUSES

Despite efforts to establish modern animal laboratories, most scientists throughout the world still have to work in conventional animal facilities. In order to improve the maintenance and care, and with this the quality control in laboratory animals, we suggest 21 measures which should increase the reliability of animal experimentation in conventional animal quarters. The application of these 21 rules, however, will not eliminate the necessity of modern barrier type animal laboratories.

1. Skilled care, control and hygienic measures

1.1 Leave the care for breeding and care of the animals – if at all possible – to specially trained experts. This is a prerequisite for good economics and success of animal experimentation.

1.2 Restrict admittance to the animal rooms to authorized personnel only, i.e., only persons who either have to care for the animals, or have to work with them, are allowed to enter the animal room.

1.3 Change overalls before entering an animal room. Each animal room has its separate and specially marked overall which may not be worn outside the room.

1.4 Wash hands before entering the animal room. Place a hand basin, soap, disinfecting solution, nailbrush, paper towels and a waste bin beside the entrance door in the animal room. Behave as if entering an operating theatre.

1.5 Place a mat soaked with disinfecting solution on the floor at the door of the animal room. A plastic foam mat in a shallow plastic bowl and overshoes are acceptable.

2. Food and bedding

2.1 Protect animal rooms, groups of rooms or buildings against wild rodent. Mount a 50 cm high removable slab in the door frame as a doorstep.

2.2 Use rations of proven formulas compiled by reliable manufacturers. Never change the diet formula and/or supplier during an experiment. The food should be free of pathogenic micro-organisms.

2.3 Provide bedding from reliable sources. Be sure no wild rodents, cats, etc. have been in contact with the bedding material. If uncertain, sterilize the bedding. The material should not contain chemicals, e.g. preservatives.

2.4 Do not use pretreated animals in experiments unless required by the investigation programme. Insecticides in the bedding or other drugs may interfere with the experiment and its result.

3. Cleaning, disinfection and sterilization

3.1 The animal room must be easy to clean and disinfect. Walls, floor and ceiling should be smooth, jointless and never made of wood. A cheap and easily renewable paint is better than an expensive one which cannot be disinfected by steam.

3.2 The equipment of the room must be easy to clean and to disinfect. Never use wooden racks. Remove all unnecessary equipment.

3.3 Use cages easy to clean, disinfect or sterilize. Do not use wooden cages because they are difficult to clean; rather those of metal and concrete. With respect to temperature conductivity and cleanliness, plastic is ideal.

3.4 Use drinking bottles which are easy to clean, disinfect or sterilize. Long nipples are difficult to clean; conical caps are better. Automatic watering systems should be completely disinfectable.

4. Air conditioning, ventilation and lighting

4.1 Provide environmental temperature control in the animal room. Install thermostats at radiator valves or the air conditioning box in a window. Metal cages with high temperature conductivity and cages with wiremesh bottoms require highly effective temperature control.

4.2 Ensure sufficient ventilation and air-exchange in the animal room. Two window-fans with adjustable diaphragms and interchangeable rotary direction, installed in 2 distant windows may improve the conditions.

4.3 Keep the rooms insect-proof. Install wiremesh to prevent insects from entering through opened windows, ventilation holes, etc.

The precautions 4.1, 4.2 and 4.3 cannot substitute for an automatic air conditioning system with proper filters!

4.4 Provide a regular light-dark cycle in the animal room. Use an automatic switch and darkening devices for the windows.

5. Provision of laboratory animals

5.1 Accept animals only for known, reliable breeding colonies and directly from the breeder. Never buy animals from a retailer because the sources of the animals are obscure, the groups are mostly genetically undefined and they will be of poor health status.

5.2 Use animals of known, defined colonies, either of defined inbred strains or animals bred according to acknowledged outbred schemes.

5.3 Make sure the animals are of defined health status. The more precisely the health status is defined the more is known about the presence of potential interfering factors. The supplier should specify the health status of the animals or the absence of pathogenic micro-organisms, respectively.

5.4 Strictly avoid contact between animals of different health status, especially between animals of the same or of closely related species. Animals from healthy, defined colonies may show severe symptoms of

diseases and mortality when maintained together with apparently healthy but latent infected animals.

These rules can help to improve the quality of laboratory animals, especially when a modern barrier type animal house is not yet available. They can help to make animal investigation more fastidious and its results more reliable. And, finally, the observation of these rules is in conformity with our ethic obligation to protect the animals entrusted to us. Relatively fewer animals are needed in investigations if the animals are healthy and maintained under proper conditions.

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TWELVE YEARS EXPERIENCE IN ESTABLISHING LARGER COLONIES OF SMALL LABORATORY ANIMALS UNDER SPF CONDITIONS BY MEANS OF GERMFREE TECHNOLOGY

W. HEINE

ABSTRACT: Heine W. **Twelve years experience in establishing larger colonies of small laboratory animals under SPF conditions by means of germfree technology.** *Journal of the South African Veterinary Association* (1978) **49,** No. 3, 175–177 (En), Zentralinstitut für Versuchstiere, Lettow-Vorbeck-Allee 57, D-3000 Hannover 91, Federal Republic of Germany.

The special procedures and techniques to develop poly-associated gnotobiotic animals in order to start large SPF colonies of small laboratory animals are discussed.

RATIONALE OF USING SPF ANIMALS

In animal experimentation the laboratory animal is the basic platform and foundation upon which the investigator builds his programme. The quality of the animals used in animal experimentation is one of the main determinants of the reliability of the results obtained.

A large number of known, less known and as yet unknown environmental factors do interfere with the animal, the experiment and its results. One important complex group of factors is the microflora, particularly the pathogenic and facultative pathogenic micro-organisms of which the laboratory animal should be free. Moreoever this status should be defined or specified (hence the term SPF: Specified Pathogen Free) to ensure that no latent infection will disrupt the experiment and influence the results.

For technical and economic reasons it is impossible to use poly-associated gnotobiotic animals – that is animals with a completely known and defined flora – for the vast majority of animal experiments. As a compromise the SPF animal of high quality is the recommended platform for fastidious animal experimentation.

The total microbial flora in SPF animals is unknown. However, SPF animals are routinely examined for the absence of certain specified species of micro-organisms. The number of pathogens which do not occur is used as an indication of the quality of an SPF animal. By using SPF animals, an investigator knows which species of germs will not interfere with his experiments.

The argument that, because there is no SPF human being, the utilization of SPF animals is the incorrect way in which to obtain experimental results applicable to man, is wrong. Man, at least in developed areas of our world, is specified free of some pathogens.

In man the mean life expectancy has shown a considerable increase during the last 100 years. While in 1870 man lived an average of 35 years, today the mean life expectation in certain countries goes beyond 70 years. During the same time the infant mortality dropped from an average of 40% to 2% and the spectrum of diseases has changed. With few exceptions the great epidemics like plague, leprosy and small pox have vanished. Contagious infections like whooping cough, scarlet fever, measles, diphtheria, typhus and tuberculosis have shown a significant decrease. On the other hand typically age-related maladies have increased. Today cancer, in general a disease of people older than 40, is one of the main challenges of modern biomedical research. Other diseases like diabetes mellitus, arthritis, rheumatism and maladies of the heart, the nervous system and the vascular system, have also increased. Apart from modern therapeutics, the decrease of human specific contagious diseases and the increase in life expectation may mainly be ascribed to the development and progress of hygiene. Hygienic measures and precautions particularly, have freed man from the above specified pathogens.

As in man, the life expectancy of the laboratory animal has increased due to improved hygiene. The result is that comparatively less animals are needed to start an experiment, because less animals die of intercurrent disease during the experiment. Not only is the risk of an untimely death reduced by using SPF animals, but also – like in man during the last 100 years – a change of the spectrum of diseases has taken place. Instead of the lifespan-reducing animal plagues like, e.g., ectromelia in mice or the pneumonia complex in rats, the typical age-related maladies have increased.

METHODS OF DEVELOPING SPF ANIMALS

In the first place it is conceivable to find animals free of certain pathogens and to select and propagate these animals under proper conditions. Such methods, however, leave too much to chance.

The second method is decontamination by means of therapy, combined with a high standard of hygiene. In animal colonies this method is time consuming, expensive and yields unsatisfactory results. Incidentally, therapy and hygiene are currently the main determinants of human health status.

Reyniers proposed utilizing gnotobiotic technology, and this is the most successful method of developing pathogen-free animals. He suggested associating germ-free animals with a proper "cocktail" of non-pathogenic germs and to use these animals as a basic breeding nucleus to establish large colonies of healthy laboratory animals. This method has become a common technique, not only in laboratory animal medicine.

Satisfactory isolators are required to develop and to maintain germ-free animals. The most common isolator in use today is the Trexler type flexible plastic isolator with a chamber made of vinyl film. In combination with peracetic acid as an effective sterilizing agent, the flexible film isolator can be used in any laboratory provided an autoclave is available to sterilize the supply cylinders with the supply material.

HYSTERECTOMY DERIVATION

Germ-free mice, rats, guinea pigs and rabbits are established by means of hysterectomy. The more complicated technique of caesarian section is used only for

larger animals where it may be necessary to keep the valuable mother alive.

For hysterectomy in small animals, the dam is killed mechanically shortly before natural delivery is expected. This method ensures that the young animals are not encumbered by anaesthetic drugs. The dam is prepared for hysterectomy by employing common aseptic surgical techniques. The abdominal cavity is opened and the uterus is placed on a sterile sheet covering the abdomen. The *os uteri* and the Fallopian tubes are clamped and the uterus dissected and placed in an isolator. It is important to perform the hysterectomy as close as possible to natural time of delivery. In animals with a very short gestation period, a few hours can be decisive. In small laboratory animals, oestrus and copulation can be triggered by utilizing suitable mating methods and by making use of the Bruce or Whitten effect. By these methods and by checking for the vaginal plug, the time of copulation can be determined and time of parturition predicted. However, the time of impregnation and of nidation remains unknown. Likewise, it is difficult to predict the length of gestation, which in mice is normally 21 d, but may vary from 18 to 30 d. The use of hormones to delay parturition has been suggested as a further method to ensure foetal maturity. We prefer to predict delivery time by palpation of the pregnant dam. With experience and training the number of errors can be less than with other methods.

The pregnant uterus is passed into the isolator through a germicidal trap filled with a disinfecting solution at a temperature of about 40°C. We use a device which allows us to convert each isolator to a surgical isolator. The outside cap of the isolator port is replaced with a cap fitted with a sealed plexiglass tube. The free end of the tube is plugged by a rubber stopper like the nipples in the usual outside cap. The surgery cap is sealed to the port and sterilized by peracetic acid. Then the plugged end of the tube is immersed in disinfecting solution at 40°C in a bucket. We use undiluted "Wescodyne", an iodine solution. Beneath the surface the plug is removed, then the inner cap, and a small plastic receptacle with a perforated bottom, fixed on a long stainless steel wire, is pushed through the tube into the disinfecting solution. The pregnant uterus with the 3 clamps is placed into the receptacle and an assistant pulls the receptacle into the isolator. In the isolator the uterus is laid on cotton material which is heated by an electric pillow placed under the isolator and the cotton material. The uterus is opened and the babies are removed. The umbilical cords are torn and the babies rubbed and massaged vigorously until they breathe. Pink and breathing babies are placed in a prepared cage with a foster mother. Weak and cyanotic babies are removed from the isolator together with the uterus, the surgical instruments and the cotton pads.

FOSTERING

If no foster mothers are available the young must be raised by hand feeding. However, this laborious procedure is no longer necessary in mice and rats because suitable germ-free breeding females, which can be used as foster mothers, are available from other institutions.

Foster mothers should be vigorous and robust animals of strains which normally have a good reproduction rate. For good fostering results these animals should have raised a litter of their own in the isolator provided as foster isolator. In addition, the foster mothers should have raised 3 or 4 other litters, so that they are accustomed to their duties. If it is not feasible to familiarize the selected foster animal to the isolator, she should be placed into the isolator in the cage with which she is familiar.

Foster mothers of mice, rats, guinea pigs and rabbits should have a litter of about 24 hours older than the babies to be fostered. The cage of the foster mother and her litter should not have been cleaned for some days, and the cage should be identified and occupied by the animal. Shortly before putting the babies to be fostered into the foster cage, the foster mother with a corresponding number of her litter should be removed from the cage. The new babies are put into the cage, mingled with the remaining original babies and left for a few minutes to acquire the smell of the cage after which the foster mother is replaced with the mixed litter.

There is no generally valid formula for the timing of surgical delivery, nor for details of successful fostering. All this mainly depends on the skill, experience, patience and ideas of the staff. Successful fostering of about 50% of the hysterectomy-delivered babies must be considered as excellent.

Today the development of germ-free mice and rats and the propagation of these species in the isolator is no problem. However, the cost is comparatively high.

PRECAUTIONS TO AVOID INFECTIONS

One problem involved in raising germ-free animals is vertically transmitted infection by means of penetration of the placental barrier by certain micro-organisms and consequent intrauterine infection of the foetus. Virusses particularly may penetrate the placenta, but intrauterine infection by some species of bacteria and parasites has also been described.

Because of this, it can be dangerous to transfer animals raised by gnotobiotic technology directly into healthy SPF colonies. We have made this mistake. Infections were passed unnoticed with the animals into our breeding colonies.

For too long we believed in the reliability of gnotobiotic technology and disregarded the possibility of vertical infection. We operated a barrier type animal house with large colonies of SPF mice and rats of excellent health status. Additional strains were transferred into the house by means of germ-free technology, without checking the animal properly before and after the gnotobiotic procedures. As a result we unwittingly introduced *Pasteurella pneumotropica* and *Mycoplasma pulmonis* together with the animals.

We therefore recommend that animals be screened bacteriologically, virologically and parasitologically before they are passed through the isolator into an animal house. It is important to examine the raised germ-free animals in the isolator carefully.

ASSOCIATION WITH CULTURED SYMBIOTIC INTESTINAL BACTERIA ("GERM-COCKTAILS")

In the isolator we introduce the germ-free animals to a "cocktail" of several cultured species of bacteria. Up to 1970 we exposed mice and rats to only 6 species, including only one anaerobic organism. Today we have realized that the intestinal flora in mice and rats consists

mainly of anaerobic bacteria; hence we have attempted to compile a more suitable "cocktail".

We currently feed the following organisms in the indicated sequence:

Days 1 - 4: Several times with *Lactobacterium lactis*
Day 5: *Fusobacterium fusiformis*
Bacteroides fragilis
Bacteroides distasonis
Bacteroides thetaiotaomicron
Day 12: *Clostridium ramosum* CRF_1
Eubacterium moniliforme 73/9
Propionibacterium freudenreichii 73/1
Eubacterium aerofaciens 73/2
Day 14: *Eubacterium* ss 73/15
Clostridium difficile
Eubacterium moniliforme 73/4
Fusobacterium rusii 73/12
Day 15: *Peptostreptococcus anaerobicus*
Fusobacterium symbiosum 73/18
Fusobacterium symbiosum 73/21

As with the composition of the "cocktail", the sequence of introduction of the bacterial species is still subject to alterations. It might be preferable to provide the animal daily or at even greater intervals with only one strain in order to give the bacteria a better chance to settle.

As already mentioned the animal should be examined carefully for undesired contamination. Following this they are either propagated in the isolator or, with consent of the microbiologist, transferred to the prepared animal house.

OVUM TRANSPLANTATION

Recently there has been considerable emphasis on the investigation of deep-freeze conservation and on the transplantation of fertilized ova of mice and rats. The initial aim of this programme was to store as many animal strains as possible at comparatively low costs for a long time. Furthermore, genetic drift which occurs even in highly inbred strains would be reduced. In our institute we also investigate this area, but in addition I hope to adapt this technique to develop germ-free and SPF animals. Today we use hysterectomy or caesarean section to obtain germ-free animals, but possibly it can in future be replaced by the transplantation of fertilized eggs to germ-free foster mothers. When this method becomes a standard operation procedure it might be more simple than a hysterectomy and, above all, the chance of vertically transmitted infections will be far less.

GENETIC IMPLICATIONS

A special problem in using gnotobiotic technology to establish healthy animal colonies is the unavoidable reduction of the gene pool in outbred strains. The development of SPF inbred strains entails no genetic problems. Because all animals of an inbred strain are isogenous, only the offspring of one or two breeding females need be passed through the germ-free status in an isolator. The strain started with the associated gnotobiotic animals will be identical with the original strain.

The procedure is quite different in outbred strains. Several elaborate breeder rotation schemes are applied in order to avoid the mating of related animals and to maintain the gene pool. The expense of gnotobiotic technology, however, precludes an unlimited number of hysterectomies, hence an irreparable loss of genes can hardly be avoided.

I do not know how many hysterectomies are necessary to maintain the gene pool of an outbred strain: the more the better. One hundred hysterectomies might be desirable but with increasing numbers of hysterectomies the danger of vertically transmitted infections is increased. Ten litters are certainly not enough.

We have to accept that a hysterectomy-derived SPF outbred strain will differ in a number of characteristics. Consequently the newly developed strain may no longer be suitable for some research programmes. For outbred strains the isolator can be considered as an eye of a needle.

ECONOMICS

As already pointed out the majority of laboratory animals used should be of good SPF quality. Furthermore I have discussed the necessity of using gnotobiotic methods in order to develop healthy animal colonies. However, because of the expense, the equipment and the necessary skilled staff an isolator division can not be operated by every small research institute or breeding unit. In our country even the 6 larger private breeders are not in a position to run their own isolator sections. Therefore it seems reasonable to have in a country one or several centralized and governmental supported institutions which are capable of providing scientific institutions and private breeders with hysterectomy-derived healthy breeding stock. Beyond that departments with isolators at research units should be established only if research on gnotobiotic animals under gnotobiotic conditions is planned.

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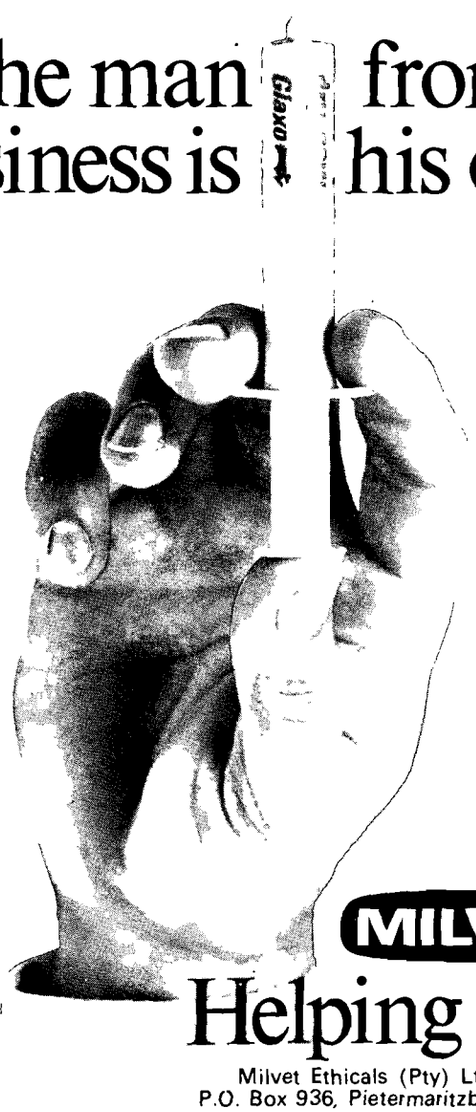

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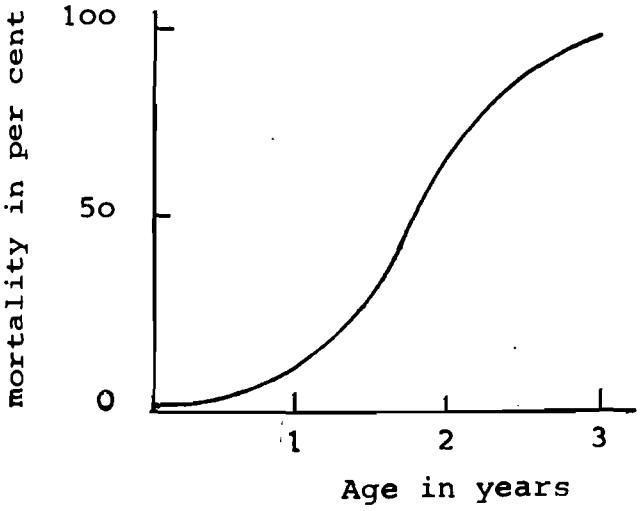
THE EFFECT OF GERMFREE AND SPF MAINTENANCE CONDITIONS ON THE LIFESPAN AND DISEASES OF SMALL LABORATORY ANIMALS

F. DEERBERG and W. PITTERMANN

ABSTRACT:Deerberg F., Pittermann W. **The effect of germfree and SPF maintenance conditions on the lifespan and diseases of small laboratory animals.** *Journal of the South African Veterinary Association* (1978) 49 No. 3, 179–182 (En), Zentralinstitut für Versuchstiere, Lettow-Vorbeck-Allee 57, D-3000 Hannover 91, Federal Republic of Germany.

The mortality rates and spontaneous diseases defining the course of natural mortality graphs in colonies of Han: NMRI mice kept under conventional, SPF and germfree maintenance conditions are reported. A specific problem in SPF colonies is caused by ubiquitous germs. The lesions and possible reasons for such types of infections are discussed.

The mortality rate in a colony of laboratory animals is influenced by endogenous and exogenous factors. Ideally the mortality graph of a mouse colony will take the following course: after a low death rate of the young animals up to weaning, only sporadic deaths occur in the first year; thereafter the mortality rate increases. The mean length of life is between the 20th and 24th month of age. The maximum lifespan is about 3 years (Fig. 1).



The mortality rates of conventionally maintained colonies differ from this ideal case. Like snapshots they only represent the momentary situation of those colonies with little relevance to the mortality rates of past and future generations. Exogenous factors largely determine the course of the mortality graph. Such factors are mainly germs which may infect the conventional animals easily. During the suckling age a high mortality rate of the young animals may be expected, mainly due to infantile diarrhoea, which is widely distributed throughout conventional mouse colonies in Western Europe at least[6]. Losses among the suckling animals are not always constant but mortalities of 50% and more are not unusual especially amongst first litters.

A variable but regular motality rate occurs amongst the weaned animals in colonies with chronic diseases like pneumonia or infectious catarrh, which are found in most conventional mouse and rat colonies[7]. Acute outbreaks of the infections may rapidly change the course of the mortality graph at any time. In this way the mortality rate of conventionally maintained stocks or strains of laboratory animals becomes irreproducable (Fig. 2).

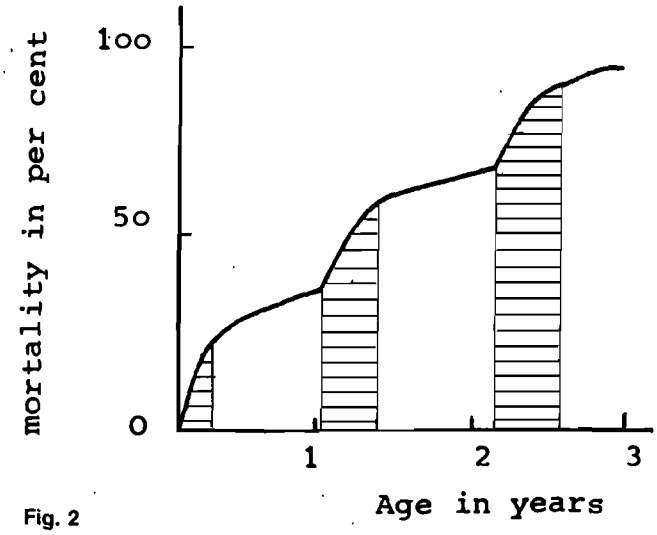


Fig. 2

The development of SPF breeding colonies and the maintenance of these animals in barrier type animal houses are specifically designed to eliminate and to prevent such infections. If this can be achieved and other exogenous factors can be eliminated, the mortality rate of a SPF colony should be constant for each generation.

The ideal situation as outlined above is not necessarily fully attained. Even in SPF colonies at certain times peaks will appear in the course of the mortality graph depending on the animal stock or strain. Such peaks will be associated with defined diseases, which will appear in all other generations as well, due to endogenous influences. They will also occur in conventional animals but often they will overlap other processes. The specific endogenously influenced diseases determine the course of the mortality graph in colonies with high health standards. They definitively influence the lifespan of those stocks and strains.

The mortality rates of germfree rats and mice will differ from those of the SPF colonies of the same strains in so far as pathogenic germs are present in the SPF animals. Based on our experience, the characteristic diseases determining the course of the mortality graphs in high standard SPF colonies will appear even in germfree animals. This does not exclude the possibility that other diseases occur sporadically, but they are not significant determinants of the mortality rate.

On this theoretical basis we have investigated the influence of the different maintenance conditions in Han:NMRI outbred mice. These conditions are conventional maintenance in open animal houses, the SPF status in barrier type houses and the germfree maintenance in isolators as described by Prof. Heine.

SPF animals in contrast to germfree and gnotobiotic animals are not defined regarding their total germ status. The absence of certain specified pathogenic germs – the characteristic of SPF animals – may be important for the health status of the animals and under certain circumstances even more important for the scientists using the animals, but such a defined absence of pathogenic germs does not go far enough to qualify the health status of the animals completely. From this point of view the results of a comparison between SPF and conventional animals are not transferable to other colonies kept in the same manner. Such results may differ from one study to another, more often depending on the presence than on the absence of certain pathogenic germs.

Considering the foregoing aspects, the following observations should only be interpreted as a report of our own experience.

Fig. 3 shows the mortality rates of NMRI mice maintained under SPF and conventional conditions developed between 1968 and 1971[2]. This study includes 1 200 SPF and 1 200 conventional female mice maintained from weaning until the natural end of life. The columns represent the summarized percentage mortality per month.

The black columns represent the conventional and the white columns the SPF animals. In both maintenance groups there are individuals which reached the 36th month of life. There is a large difference in the mortality rate between weaning and the 30th month. This difference results in a mean length of life of 23,7 months for the SPF and of 19,8 months for the conventional mice. That means a nearly 20% higher lifespan in the SPF animals.

The mortality graph as shown in Fig. 3 seems to be ideal. Here are almost no peaks as mentioned in the beginning of this report. The impression changes if the results of the mortality rate are not represented as accumulative totals. In Fig. 4 the deaths rates are expressed as mortality in per cent per month without summarizing the results.

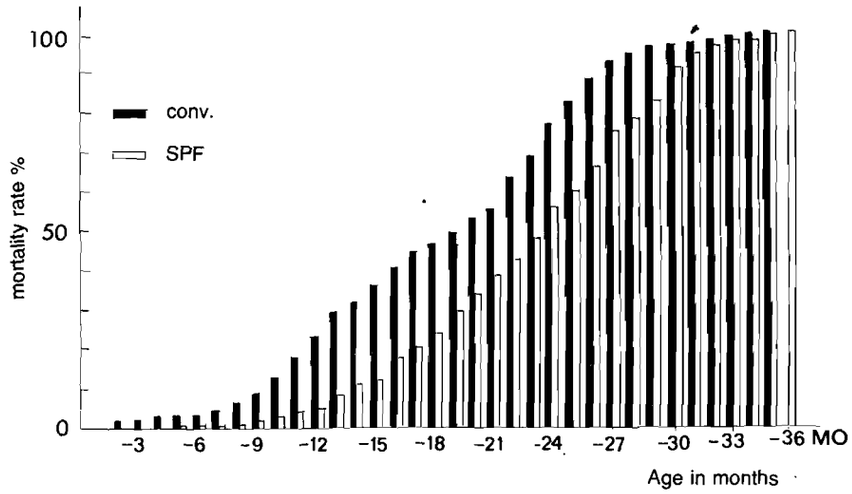


Fig. 3 Longevity study in Han:NMRI mice. Percentage mortality (summarized)

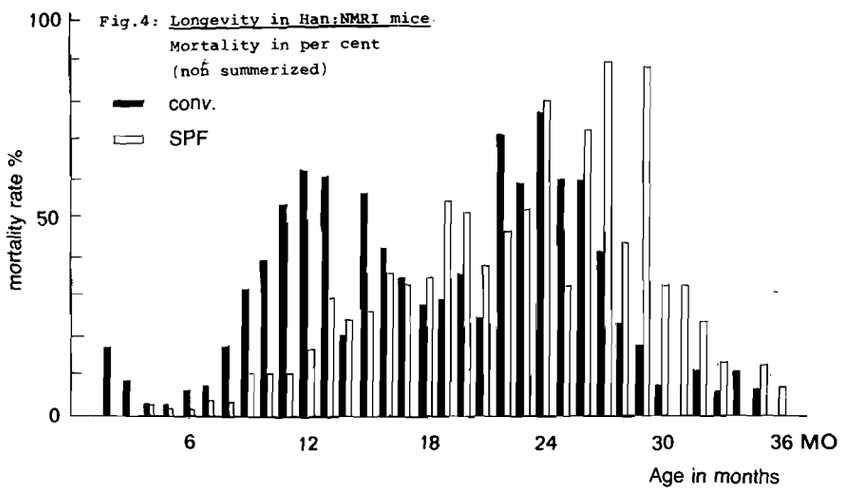


Fig. 4 Longevity in Han:NMRI mice. Percentage mortality (not summarized)

In the mortality graph of the conventional animals there is a small peak within the second and third month of life. During this time no deaths were observed in the SPF animals. A second peak in the death rates of the conventional mice includes the time from the 6th to 16th month of age. Even in this period the mortality of the SPF mice remains distinctly lower. A third peak between the 22nd and 26th month of life in the conventional mice appears to be strongly mimmicked in the SPF mice between the 27th and 29th month of life.

Fig. 5 shows the same death rates for the conventional and SPF animals but with each bar representing a quarter. The mortality rate of the conventional animals again shows the three peaks mentioned above. The distribution within the SPF animals seems to be normal. The columns contain some different parts, each of which represents a particular disease as percentage of the quarterly death rate. The black parts represent tumours, the dotted parts the rates of leukaemia and the hatched ones that of glomerulonephritis. These three kinds of diseases are characteristic for the Han:NMRI mice. The remaining parts of the columns represent all other diseases.

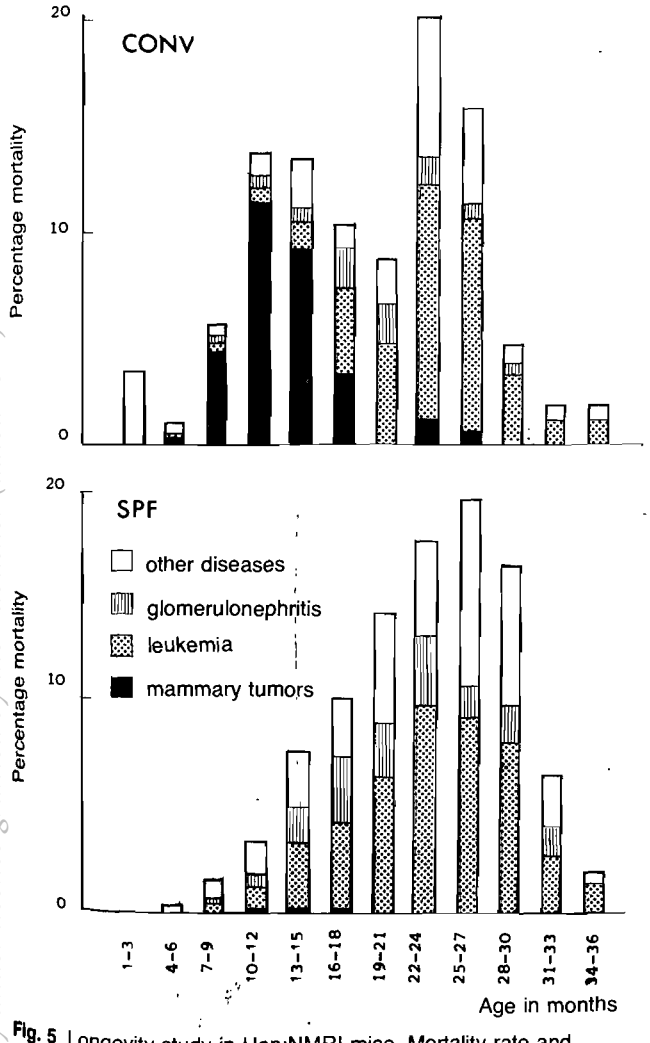


Fig. 5 Longevity study in Han:NMRI mice. Mortality rate and characteristic diseases

The peak of the mortality rate of the conventionally maintained animals within the first 2 months of life consists of a certain disease which we diagnosed as a delayed consequence of infantile diarrhoea[6]. The animals survived the acute stage during the suckling period but died as a result of the stresses of weaning and growing.

The second peak of the mortality rate of the conventional group is caused by a high number of mammary tumours marked as the black parts of the columns. Until the 18th month of life 30% of these mice had mammary tumours, while only 3 carcinomas of the mammary gland were recorded in SPF animals between the 12th and 16th month of life. The decline of mammary tumours which are caused by viral agents and influenced by hormonal and genetic factors may be explained with some reservations. The galactogenic transfer of mammary tumour virus was stopped by the technique of caesarian derivation followed by a germfree phase and uterine infection has not or only in some cases occurred. Three adenocarcinomas in the SPF animals and a slow increase of the mammary tumour frequency within our SPF colony of Han:NMRI mice may support this hypothesis.

The mortality rate caused by leukaemia and glomerulonephritis (the dotted and hatched parts of the columns) is relatively low in the conventional group between the 7th and 15th month of life. These diseases overlap the high number of mammary tumors within this age period. During the same time leukaemia and glomerulonephritis are the main cause of deaths of the SPF mice.

After the 20th month of life leukaemia becomes an increasingly important determinant of the mortality rate in both maintenance groups. In the 3rd year of life leukaemia and some other neoplastic diseases exert the most important influence on the mortality rate.

In our SPF colony of NMRI mice the mortality in the first year of life is very low with a death rate around 1% and is determined by sporadic diseases mainly caused by infections with ubiquitous germs. In the second year of life glomerulonephritis – an immune-complex-glomerulonephritis of unknown origin[4] – together with leukaemia and in the third year of life leukemia together with a number of age dependent neoplastic alterations are the diseases determining the mortality graph in this colony.

It is not practical to keep large numbers of mice under germ-free conditions, hence our data was obtained from 62 germ-free NMRI mice kept until the natural end of life[3]. Some of these animals reached the 3rd year of life. Of this group, 30% died in the 2nd year of life from glomerulonephritis. In 26% of the germ-free animals leukaemia was the natural cause of death, and 18% of the germ-free mice died from severe atonia of the caecum which was often followed by spontaneous rupture of the caecum wall or intestinal haemorrhage.

With exception of the atonia of the caecum, the only specific disease of germ-free mice, there was a clear tendency that glomerulonephritis and leukaemia observed frequently in conventional and SPF NMRI mice were also the determining factors of the mortality graph in the germ-free colony.

The results of all these studies were obtained in the first years after starting the SPF colonies. At that time many colleagues of our institute concurred with the opinion that the main problems in maintaining laboratory rats and mice on a large scale were solved and that maintenance of such colonies in barrier type animal houses would stabilize the health status of such animals for a long time. More recently we have learned that there are new problems associated with this kind of maintenance which lessen the results described above.

In face of great success in eliminating such important enzootic diseases as infantile diarrhoea of suckling mice, ectromelia and chronic respiratory diseases from the mice and rat colonies respectively, it is understandable that the importance of small but self-reliant problems of the SPF colonies has not been foreseen. This problem consists mainly of infections caused by ubiquitous germs[9 11]. Many references describe similar observations in other SPF colonies established in the same manner [1 10 13]. Generally the germs were transferred into the animal house by the animal technicians. Excluding *Pasteurella pneumotropica* in our institute, all the noted germs have been isolated from one or more of the technicians. References in the literature indicate that such a mode of introduction is also possible for *P. pneumotropica*[14].

After starting our SPF colonies nearly all infections were caused by *Staphylococcus aureus* and *Streptococcus faecalis*. Later other germs were isolated such as *Proteus* species, *Escherichia coli* and *P. pneumotropica*.

Infections caused by *S. aureus* manifest as abscesses in many of the tissues and organs regardless of the age of the animals. Areas particularly affected are the head, especially the orbita and mandibles and the preputial glands in male mice. Purulent dermatitis in the area of the ears and the neck can also be caused by these bacteria. Portal of entry of bacteria which cause head lesions are often traumatic gingivitis induced by rough food ingredients in the autoclaved diet.

Mastitis in nursing rats is usually caused by *Streptococcus* species. After an acute stage the total mammary complexes become demarcated and ulcerated.

During the last 4 years infections with *E. coli* 07:K1:H7 have caused severe purulent inflammations and abscesses, mainly in the urogenital organs, and presenting as pyelonephritis, endometritis and ovarian abscesses which are sometimes followed by septicaemia[12].

Apart from abscesses in nearly all the organs, *P. pneumotropica* causes a slow subclinical and chronic tracheitis and rhinitis. Such animals are not suitable for inhalation studies.

The incidence of all the described diseases registered during autopsies and histological examinations is very low compared to the total animal production. The economic effect is negligable.

It should be considered, however, that virtually all the animals of such colonies are infected with ubiquitous germs, not only those which subsequently develop abscesses or other purulent processes. This induces permanent stimulation of the immune system, morphologically expressed by follicular hyperplasia in the lympho-reticular tissues. Even in healthy looking animals the stimulation of the immune system may cause certain differences in the immunological response of the animals. These alterations may interfere with particular types of animal experiments[5].

SPF colonies seem to offer quite favourable conditions to ubiquitous germs even though maintenance is in barrier type animal houses under highly hygienic standards. In conventionally maintained colonies of rats and mice infections with ubiquitous germs constitute a lesser problem. This may be related with the artificially supplied intestinal flora and its qualitative and quantitative composition which may diminish the antagonistic effects against ubiquitous germs.

We started with a very simple artificial flora. In the following years it was supplemented by several species of germs[8]. Some of them are strictly anaerobic bacteria. Experience during the last few years indicates an increasing efficacy of the supplemented flora. This may be due to prevention of the penetration of ubiquitous germs or by increased resistance to purulent alterations. In our opinion the further improvement of the artificially supplied intestinal flora seems to be the only solution to this problem.

After the outstanding success of the elimination of a large number of specific enzootic diseases from the conventionally maintained colonies by using gnotobiotic techniques we hope to find a way to control disease caused by ubiquitous germs in SPF colonies.

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THE BREEDING AND USE OF SPECIFIC PATHOGEN-FREE (SPF) RATS

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ABSTRACT: Goldstein, B. **The breeding and use of specific pathogen-free (SPF) rats.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 183 (En), National Research Institute for Occupational Diseases, South African Medical Research Council, Box 4788, 2000 Johannesburg, Republic of South Africa.

Bronchiectasis is common in older rats and is a serious complication when the lungs are required for research purposes. SPF rats do not develop this complication, but special isolated accommodation must be provided and care of the animals must avoid introduction of outside micro-organisms. Animals removed from the isolated accommodation for experimental work are not returned, but can be kept in the general animal house provided there are no conventionally-bred rats.

INTRODUCTION

Chronic lung disease (syn. enzootic bronchiectasis; chronic murine pneumonia) in rats is caused by *Mycoplasmas* and other organisms such as *Haemobartonella muris, Alcaligenes bronchosepticus, Pasturella multocida muris* and *Streptobacillus moniliformis*[2]. If affects a large proportion of old rats and often the dilated bronchi fill with thick pus and the lungs become grossly diseased, rendering them unsatisfactory for experimental work.

SPF rats do not develop this complication and they also have a longer life-span than do conventionally-bred rats. They have, however, not been available in South Africa and must be bred from imported stock, which can be obtained from the Imperial Chemical Industries Laboratories in England or the British Medical Research Council Laboratories in Surrey.

These laboratories will dispatch a breeding colony of 4 males and 12 females in special isolation containers and by air freight the animals arrive on the following day. Written permission to import the rats must, however, be obtained from the local State Veterinary Officer and a clearing agent should be engaged to deal with the airport formalities.

ACCOMMODATION

In order to breed the animals and to maintain a colony in a specific pathogen-free condition, special isolated accommodation is necessary. The accommodation must have double doors at the entrance and exit, with rubber lining the door frames to give an air-tight fit, and the windows must be sealed. Air is supplied through a Vokes absolute filter by a fan with a capacity of 0,5 m^3/sec. This will also maintain a slightly raised pressure in the area to prevent entry of unfiltered air. Access to the fan for maintenance must be from outside the area to avoid contamination. The area should include a change room for the attendant as well as storage and washing-up space. If possible, there should be an autoclave with an opening to the outside and another door opening inside the sterile area, to receive sterilised materials.

Before introducing any animals, the accommodation must be sterilised. The most satisfactory method is by formalin gas generated by boiling formalin diluted with water[1]

CARE OF ANIMALS

Only the animal attendant has access to the isolated area and this attendant must have a knowledge of sterile techniques. Before entering the area the attendant puts on sterile clothing and also changes into rubber boots which are kept in the change room in a shallow pool of disinfectant. The attendant feeds and waters the animals daily. The food consists of rat pellets which are autoclaved in a double paper bag and only the inner bag is taken into the animal section. These pellets are rather hard but are still readily eaten by the rats. Ordinary tap water is used and Vit. C. (100 mg/bottle) is added twice a week. Vitamin K ("Synkavit", Roche), 0,5 mℓ/bottle, is also given to pregnant and nursing females and Vitamin E ("Ephynal", Roche), 50 mg/bottle, is added twice a week for 2 weeks before mating.

After feeding the animals the attendant follows a weekly schedule to ensure that the cages, racks, trays and water bottles are cleaned and sterilised regularly. The trays below the cages are covered with autoclaved vermiculite which is changed 3 times a week.

Breeding is carried out by mating 3 females with 1 male. Litters of up to 10 or 12 can be obtained but about 20% die. The mortality rate amongst the more mature animals is, however, very low. Careful breeding records and details such as age, deaths and usage of the animals must be maintained.

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BREEDING MACAQUES FOR RESEARCH

MARIA NELLY GOLARZ DE BOURNE

ABSTRACT: Golarz de Bourne, M. Nelly. **Breeding macaques for research.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 185–188 (En), Yerkes Primate Research Center, Emory University, Atlanta, Georgia, 30322, U.S.A.

A breeding programme or rhesus macaques was established to produce animals with minimal disease and satisfactory behavioural patterns. Problems of design, management, health care and dietary requirements are described.

The rhesus macaque *(Macaca mulatta,* Zimmerman, 1780) is frequently selected as the human analogue for a variety of biomedical studies because this primate shows many biological parameters similar to man. It has a body size not too large for laboratory housing, but not too small to allow safe biological sampling. Not the least important characteristic is the fact that the rhesus macaque is a hardy, high spirited, cooperative animal which makes him an excellent team member in research projects.

Where subtle changes are expected to result from the experimental conditions, the use of animals born and raised under controlled conditions is imperative. In imported wild-born rhesus macaques we have observed considerable histopathological evidence of disease present even in those animals acclimatized under laboratory conditions for several years. Parasitic invasions, as well as viral and bacteriological infections and the resulting pathological changes have been demonstrated in wild caught rhesus macaques. Particularly extensive were changes which we observed in the skeletal musculature, a system which we specifically wanted to study under weightless conditions[3].

Rhesus macaques can be bred under a variety of conditions. Our requirement was to obtain, economically, healthy animals with a normal complement of microorganisms and free of behavioural abnormalities.

Before the restrictions on export of rhesus macaques were imposed by the Indian government, tthe breeding of macaques in laboratories was carried out for certain limited research needs. This was done mostly in individual cages; the male and female would remain together only during the mating period. This method allows timed pregnancies, but in our experience it is costly, and often the infants to not develop normally. This is a method suitable for projects requiring the separation of mother and infant soon after birth.

At the Bionetics Laboratories in Virginia, U.S.A., where a breeding programme of rhesus monkeys has been established for a long time, their experience in housing 2 to 4 females with a single male, even in a commodius cage, proved unsatisfactory for breeding purposes, but the more spacious gang cages in which single pairs of animals were housed together, was more effective[7].

In 1969, when our colony was planned, there were no other long established projects for the extensive breeding of macaques as research animals. The wild caught supply of macaques was abundant and the only large groups maintained in the American continent were mostly used for behaviour-related observations. The best known of these are the free ranging groups established in the islands of Cayo Santiago, La Parguera, and Sabana Seca in Puerto Rico and less known is the colony formed in the 1940s in the island of Pinheiro near Rio de Janeiro, Brazil[2].

In addition to the problem of deciding the best way to raise in captivity, psychologically and physically normal animals, the rate of reproduction has to be considered. It is known that success in reproduction in rhesus colonies, as with other animals, is a function of the living conditions to which the animals are exposed. Not only conception, but the course of pregnancy, successful delivery, and proper postnatal care of the infant are all affected by the diet and the environment in which the animals are kept.

The different possible methods of breeding rhesus macaques were extensively investigated. We decided that in order to economically produce animals reasonably free of disease and at the same time behaviourally normal, the open compound or corral system should be used. A large number of animals are housed in spacious outdoor enclosures where the infants can be raised in the care of their mothers, in family groups more closely resembling their natural free ranging social structure. At the same time, the open air corral requires the least expensive structural facilities and the most economical upkeep of a large number of macaques.

The original breeding colony was established in 1970 at the Field Station of the Yerkes Primate Research Center in a compound measuring 38 m × 38 m which was enclosed by a 4,3 m high fence constructed of chain-link fencing with the top lined with sheet metal to prevent the animals from climbing out. A double gate, safety area provides access.

The outside enclosure communicates with a den building, 12 m long, 3 m wide and 2,4 m high with ambient control, perches and water drinkers as well as a working area.

Inside the enclosure all the underbrush was cleared and the trees were reduced to a height of 3,04 m since we had indications that some miscarriages were due to high jumps. A few months after the colony was assembled all the remaining vegetation was destroyed by the animals.

A climbing structure was built of redwood to replace the trees, and unsuccessful attempts have been made to grow rye grass. More successful was the planting of weeping love grass *(Eragrostis curvula),* a plant with a strong root system which survived well the monkeys' abuse and provided a soft cushioned ground. Unfortunately, we lost two animals due to large bezoars formed by the tough indigestible blades of the love grass in the stomach and upper intestine.

A number of cement culverts 0,9 m in diameter provide cover in the open. To reduce overeating and obesity of the dominant animals, crib cages were built which

allow access to supplemental food only by infants and the smaller adults.

During the more severe cold spells in the winter months, heating is provided in the outside enclosure by directing hot air from a kerosene space heater, situated outside the fence, into a 5 m wide and 6 m long metal pipe placed inside the enclosure. The animals sit on or near this pipe for warmth.

It was initially calculated that a group of 50 animals could be housed in the size enclosure to be used. This group was to be composed of 45 females and 5 males. Most of these animals were wild caught imports, acclimitized under controlled conditions during several years in various locations in the United States. Upon arrival at our facilities, they were quarantined for 30 days in individual cages and tuberculin tested 3 times at 2-week intervals before being released simultaneously into the compound. Some days of turmoil and fighting followed, resulting in many injured animals and a few deaths, the latter were mainly due to injuries inflicted by the long canines of the males. The canines are now routinely removed from all the adult males. This has reduced the incidence of fatal injuries. The aggressive interaction subsided after a few weeks and a social organization evolved with the typical role patterns of different levels of dominance characteristics in rhesus macaque communities. The animals established themselves in hierarchies with an alpha male and various subdominant males; the females have organized similarly. Once the hierarchy was formed, the rigid organization with the alpha male has remained in control. This may last for several years, until the leader is challenged by one of the lower rated males. The closeness of the community with its rigid social structure makes it very difficult to add other animals to the colony.

Males which were added after the hierarchy was established were mangled within a very short time. Females that were added were often badly injured. On 2 occasions, females taken out for caesarean delivery because of dystocia were severely injured after the reintroduction to the group and had to be removed permanently from the colony. In these cases, the females had been absent from the colony for only a few weeks. For such animals and other newly acquired macaques, which could not be placed in the main group, 2 subsidiary breeding colonies were set up in converted "corn cribs" which are cylindrical structures made of a meshwork of thick metal wire and a solid roof which are commercially available for the storage of ears of corn. These measure 4,5 m in diameter and are 3 m high. They were set in a concrete foundation and sloping floor equipped with doors, perches, water drinkers, and a small den in the form of a heated box attached to one side. Such structures provide good housing accommodations for small groups of animals.

When the infants are born, they remain in the family group for one year. The weaning process is not completed in the rhesus monkey until a few weeks before the next delivery, so these animals are just weaned when they are finally separated from their mothers approximately at 12 months of age.

Otherwise, the group remains undisturbed except for daily sanitation and feeding and scheduled medical examinations or required treatment of illness and injuries.

Once a year during the early fall the infants born during the previous year are removed to a "corn crib" enclosure. They remain together for another year and are subjected to more frequent medical examinations, clinical tests, and close observation to establish their health parameters.

At the end of the second year they are removed from this open-air housing and transferred to a specially designed modular housing located at the main Yerkes Center where they are placed in pairs of similar age and compatible temperaments in spacious cages, 1,8 m tall by 76 cm by 61 cm. They are subjected to detailed clinical studies and their access is limited to highly trained personnel which observe high standards of hygiene to minimize infections. The animals are provided with a television set to give them visual and auditoray stimulation during the day.

The diet provided in the breeding compounds and in the various stages following the removal of the infants, is fundamentally the same. A commercial monkey chow and water are offered *ad libitum*. In addition, each animal receives half an orange a day, together with vegetables, such as carrots and cabbage. The monkey chow is mainly whole ground grain and is adequate in vitamin B complex and also in vitamin E; vitamins A, D and C are added. More recently, however, we have been supplementing it with additional vitamins and corn oil. Most of the infants so far born under the above described method are in excellent physical condition and do not show the extensive evidence of diseases found in wild-born animals.

Table 1: YEARLY BREEDING DATA OF MACAQUES BRED IN CAPTIVITY

Year	Adults		Live births					Stillbirths/abortions			Known conceptions	
			Dying under 1 month		Surviving over 1 mo.		Total	% per no. of adult females				Total	% per no. of adult females
	M	F	M	F	M	F			M	F	?		
1970	8	41	2	0	8	4	14	34,1%	1	0	2	17	41,4%
1971	8	50	1	4	13	6	24	48%	0	0	2	26	52%
1972	6	46	0	0	12	13	25	54,3%	2	1	1	29	63%
1973	9	56	4	4	15	10	33	58,9%	1	1	0	35	62,5%
1974	13	98	3	2	28	21	54	55,1%	2	3	2	61	62,2%
1975	11	67	4	3	20	12	39	58,2%	3	2	3	47	70,1%
1976	11	73	1	2	20	22	45	61,6%	0	3	5	53	72,6%
To Aug. 1977	12	68	2	1?	20	14	37	54,4%	1	–	1	38	55,8%
Totals			17	15	136	102	271					326	

1974: Two additional groups formed which accounts for large number of females
1975: One group disrupted due to burning of the den building.
1976–77: Extremely cold winter affecting birth rate of 1977

The breeding colony has produced 271 young between January, 1970 and August, 1977, which represents an average of 38,7 animals per year (Table 1). Another interesting fact is that there have been 153 males and 117 females born. A neighboring colony of rhesus monkeys at the Field Station belonging to another project showed a preponderance of female births during the first 2 years but over the last 5 years had averaged to equal numbers of male and female births. The Cayo Santiago colony[5] produced over a 4-year period a ratio of 81 to 118 males for each 100 females.

Table 1 also shows also that a total of 35 out of the 326 pregnancies resulted in abortion or stillbirth. This represents a 10,7% mortality figure at birth; the cage-bred rhesus macaques of the Bionetics Laboratory[7] had a 15% mortality rate.

Van Wagenen[8] described the vital statistics of an indoor rhesus colony raised in cages in the Department of Obstetrics and Gynaecology, Yale University School of Medicine, since 1935. Breeding females were contained in individual cages and the male was placed with the female on the 11th day of her menstrual cycle. During the period of 1935 to January 1, 1969, there had been 583 pregnancies in this colony. These pregnancies produced 15 neonatal deaths, 42 stillbirths, 33 abortions, and 3 maternal deaths. There were 308 live births, which represent 76-78% of the pregnancies; neonatal deaths, stillbirths, and abortions totalled 22,4%. The discrepancy between the above figures may be due to the fact that under the controlled conditions of mating caged animals, close observation is possible during early pregnancy and any resulting abortions are noticed while in animals housed in compounds in large groups, this may sometimes occur unobserved.

Koford[5] estimated about a 4% stillbirth rate for the Cayo Santiago free-ranging group of rhesus. The observed figure was only 2,7%, but he points out that some mothers have their dead babies at sites where they are difficult or impossible to find. This refers only to stillbirths since losses at earlier stages of pregnancy would not be possible to assess in their island colony.

The female rhesus monkey has a mentrual cycle resembling that of human females: it lasts 26-32 days with a mode of 28 days. MacDonald[6] found a mean of 29,2 days. The extent of the menses normally ranges from 2 to 5 days[7]. The menstrual cycle stops when the animal becomes pregnant. The period of normal pregnancy has been established as $165,7 \pm 5,15$ days[4 6]. In the wild condition most rhesus monkey births occur in March, April and May.

The breeding of captive rhesus macaques also appears to be seasonal even under laboratory conditions. Over a 3 year period, the Bionetics Laboratories in Virginia found that most of the births took place in February, March, April and May, which placed the mating season from September to October. Their breeding schedule was prepared once a week and all matings were planned in advance and based on menstrual history of each female[7].

There is some evidence[6] that the period of fertility during the menstrual cycle is from Day 11 to Day 15 inclusive of the cycle, calculated from the first day of menstruation. In one study monkeys bred in cages[6] exhibited some seasonal breeding, although they appeared to be moving toward all year round breeding[6].

The best studied free-ranging groups of rhesus monkeys are those established in the island of Cayo Santiago, a 40 acre island off the east coast of Puerto Rico. This colony was established by Carpenter in 1938[1]. In 1965, there were 400 monkeys living there which were descendents of the original group. According to Koford[5] reproduction was seasonal and practically all the infants were born during the first 6 months of the year.

In the southern hemisphere, in the group of rhesus macaques established on the island of Pinheiro near Rio de Janeiro, the births in 1974 and 1975 occurred during the spring season (October, November and December), which corresponds to the northern hemisphere period of April, May and June.

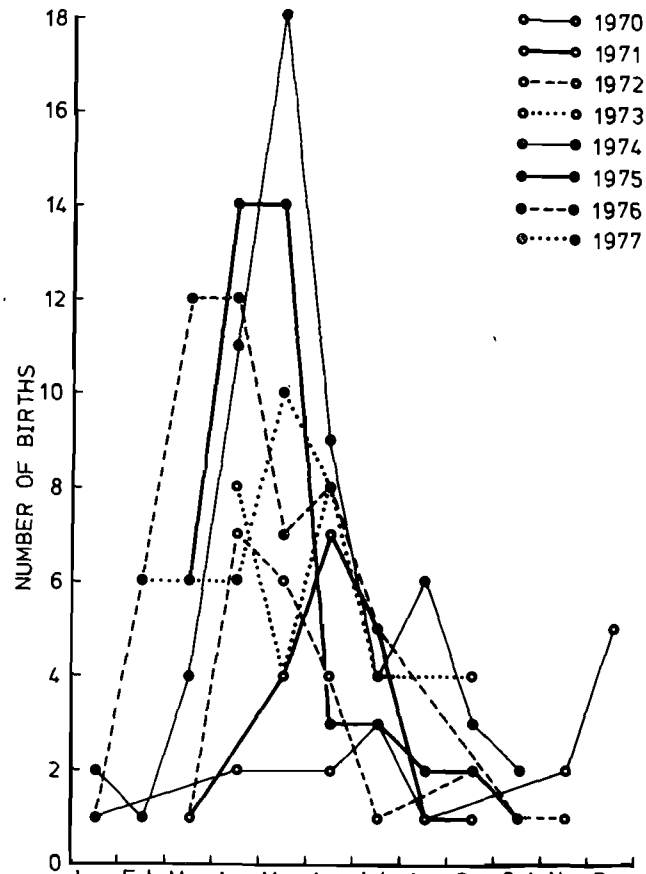

Fig. 1

Combining the results we have obtained during 7 years of study (Fig. 1) we find that May is the most prolific month since 65 young have been born in this month. In June there were 41 births, in April 46, in July 18, in August 14 and in September 12. Very few (5 and 3 respectively) were born in October and November, but there were 5 births in December of 1970; 7 animals were born in February and 23 in March.

The procedure of breeding rhesus monkeys in large open-air compounds has been found to be an economical way to produce animals of high quality, relatively free of disease and behaviourally adequate. This procedure has also been successful in the number of animals it has produced and at the time of this writing, 7 years after the establishment of the colony it has nearly tripled its original number.

However, as we gain experience changes are being introduced. The original enclosures of each were divided in half, housing 2 smaller groups which allowed closer observation of the individual animals. Because of the close monitoring of these animals we have learned of the complexity and difficulties in obtaining animals completely free of pathogens.

Nevertheless, the animals born in our colony are much hardier specimens for experimental purposes. Their known life and clinical history make them a much more reliable human analogue. At autopsy, we have observed no or a greatly reduced incidence of disease. This we expect to reduce even more in the future generations by careful selection of the adult breeders.

ACKNOWLEDGEMENT

The establishment and continued work with the above described colony was made possible by Grant No. NGR-11-001-016 of the National Aeronautics and Space Administration.

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THE LESSER BUSHBABY *(GALAGO SENEGALENSIS)* AND THE CHACMA BABOON *(PAPIO URSINUS)* AS LABORATORY RESEARCH ANIMALS

G. A. DOYLE

ABSTRACT: Doyle G.A. **The lesser bushbaby** *(Galago senegalensis)* **and the Chacma baboon** *(Papio ursinus)* **as laboratory research animals.** *Journal of the South African Veterinary Association* (1978) **49,** No. 3, 189–190 (En), Primate Behaviour Research Group, University of the Witwatersrand, 2001 Johannesburg, Republic of South Africa.

The 2 species are evaluated as far as adaptibility, economics, reproductive performance and behaviour are concerned. An important limitation is availability which makes conservation and breeding programmes imperative.

Until about 10 years ago very little was known of the Lesser Bushbaby and much of the available information was inaccurate. It was at this time that our Research Group at the University of the Witwatersrand began studying it and most of the available information on this animal today, at least on its behaviour, comes from our own studies in the laboratory and in the field[1 2 3]. Fig. 1 shows 2 adult, laboratory-born bushbabies of the type indigenous to South Africa.

Fig. 1 Two laboratory born adult bushbabies, *Galago senegalensis moholi*

Our thoughts have never really turned to the bushbaby as a possible subject for bio-medical research although it is used as such in many countries including South Africa to a very limited extent. Our interests have been primarily on the animal itself, its position in the natural scale of evolution and the extent to which it can throw light on the probable course of primate evolution as a whole. It is widely recognised that primates, because of their close evolutionary affinity with man, are essential for most types of biomedical research. The Great Apes, the most suitable primates from this point of view, are all endangered species, are expensive and in short supply and should not be used in terminal research of any kind. Biomedical scientists have tended to concentrate on the Rhesus macaque, certain South American monkeys and, to a lesser extent, the baboon, but possibly for too long we have been using these animals at a rate exceeding their natural reproductive rate in the wild.

One solution, as a believer in the conservation of our natural resources, I put to you with some misgivings, and that is that we must turn our attention to a more readily available, smaller, cheaper, and quicker breeding primate like the bushbaby.

The Lesser Bushbaby is widely distributed throughout Africa and the Southern variety, *Galago senegalensis moholi,* is found in suitable pockets in the thornveld areas of the Transvaal, Botswana and South West Africa. How widely distributed it is and how dense the population is not known, but suffice it to say that, like all primate species, and indeed most animals living in their natural habitat, it is endangered as a result of the slow but sure destruction of its natural habitat to make way for ever increasing and largely human populations.

Since the Bushbaby is a primate it has many features in common with man and as such it is a suitable model for many, but by no means all, biomedical research programmes. I stress this point because, for obvious reasons, it cannot replace the baboon entirely or the Rhesus monkey as a model for many types of research.

The Lesser Bushbaby, *G. s. moholi,* is a small animal weighing less than the laboratory rat (Table I). It adapts very readily to good laboratory conditions (large semi-natural environments approximately 1,5 m × 2 m × 2 m – not small cages) in which they breed freely. Most females will produce 2 sets of twins a year, the young reaching breeding maturity before 1 year of age. They are easy to feed and will eat almost anything in the way of fruit and vegetables but they do require a high protein component in the form of live insects (mealworms, locusts, cockroaches, etc.). They also tolerate a wide temperature variation. Under good dietary and hygienic conditions infant mortality rate is low.

Table 1: BODYMASS OF ADULT *G.S.MOHOLI* UNDER LABORATORY CONDITIONS

Sex	N	No. of determinations	Mass in grams Range	Mean	Age range in years
Male	7	47	188–301	235	2–6
Female	10	161	150–252	195	2–6

Detailed records on 28 laboratory gestations have thus far been obtained. The gestation period was 123 ± 2 days. In the wild births are seasonal but in the laboratory breeding was purposely restricted and births were recorded throughout the year. Twelve single births, particularly amongst first gestations, were observed and all 16 multiple births were twins except for

Table 2: VITAL BIRTH MASS STATISTICS FOR BOTH SEXES AND MEAN TIME TAKEN TO DOUBLE, TRIPLE AND QUADRUPLE BIRTH MASS

Sex	N	Birth mass in grams Range	Mean	Mean time in days taken to multiply birth mass X2	X3	X4
Male	28	8,6–15,5	11,9	7	13	20
Female	28	8,5–14,8	11,7	7+	−14	21+

one instance of triplets. Table 2 summarises vital birth weight statistics for the Lesser Bushbaby.

Many will appreciate that, for the reasons given, which may be subsumed under the general rubric of economic reasons, the Lesser Bushbaby would provide a suitable model for much of the research on which they are presently engaged. Our own research has been concerned entirely with the behavioural biology, including ecology, of this species and much of it has been directed, explicitly or implicitly, at problems of conservation in the firm belief that this species must be conserved and that conservation programmes will only succeed to the extent that we understand the total biology of the animal in question. This brings me to the second solution to our problem, the laboratory breeding of the Chacma baboon, the most popularly used research primate in South Africa.

Chacma baboons are becoming more difficult to capture and as year by year we have to go further and further afield to capture them, wild-caught baboons are becoming less economical. The scales are slowly dipping towards the necessity of large scale breeding programmes as more animals are being required and less are available. Other countries with their own primate populations such as India, Japan, Thailand, are already firmly committed to such breeding programmes for the same reasons as they are being advocated here. A similar breeding programme will soon be underway in Kenya.

The breeding of the Chacma baboon, however, is not as simple as it is with the bushbaby. They are more subject to the stress of captive conditions largely because they are kept in small cages which are much more economical than large semi-natural corals, where they can live in natural groups, analogous to the type of semi-natural environment in which we keep the Lesser Bushbaby. Under captive conditions male baboons tend to become aspermatic and the natural cycle of the female undergoes changes not conducive to breeding. Suffice it to say that given our present knowledge of the problems of breeding baboons in captivity, breeding programmes will prove prohibitively expensive.

Biomedical science has made remarkable progress in the last few years using nonhuman primates in research programmes directed towards the solution of many of man's afflictions from cancer to drug addiction. Much of our research on primates is concerned with programmes directed towards increasing our fundamental knowledge of the nonhuman primates themselves, but within such programmes many other important problems are being solved – colony management, nutritional requirements, reproduction, behaviour, disease and many other issues pertinent to the problems of conservation.

Research programmes directed towards either the acquisition of fundamental knowledge or towards the solution of human health problems need not be contrary to the interests of primate conservation. The solution to the problem is the creation of research environments which allow both types of programmes, those concerned with conservation and those concerned with basic and applied research, to work in close collaboration and harmony. Unless we adopt such an economic, multi-disciplinary approach through the establishment of suitable primate research centres, we will be judged in the future to have acted contrary to the interests of both man and of the nonhuman primates.

Our research laboratories in the foreseeable future must become self-sufficient by breeding to meet their own needs. Only then will it become apparent that research involving nonhuman primates is not endangering these species, as it is now in South Africa. For many of the ideas expressed in the latter half of this paper I am indebted to Hunt[4] and to Kalter *et al*[5].

ACKNOWLEDGEMENT

I am indebted to the Human Sciences Research Council and the University of the Witwatersrand, Johannesburg, for their continued interest and financial support.

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SOCIAL CONTACT INFLUENCES ON THE MENSTRUAL CYCLE OF THE FEMALE CHACMA BABOON *(PAPIO URSINUS)*

M. E. HOWARD-TRIPP* and C. BIELERT**

ABSTRACT: Howard-Tripp M.E., Bielert C. **Social contact influences on the menstrual cycle of the female Chacma baboon** *(Papio ursinus). Journal of the South African veterinary Association* (1978) **49** No. 3, 191–192 (En), Central Animal Unit, University of the Witwatersrand, 2001 Johannesburg, Republic of South Africa.

The menstrual cycle characteristics of 2 groups of adult female Chacma baboons were compared. Out of a group of 27 female baboons, 41% (11/27) failed to cycle during the 6 month study period but were all under the stresses of fairly severe experimental manipulation. Data on the menstrual cycles of the remaining 16 unmanipulated females was collected and carefully analysed. These 16 females were individually caged and denied social contact with other baboons. Their menstrual cycles were then contrasted to those of a group of 8 individually caged females which received 24 minute mating tests with vasectomized males on alternate days during their cycles. The females denied the social contact of the mating tests showed significantly longer cycles as a result of significantly lengthened periods up to the time of perineal detumescence.

INTRODUCTION

It is well documented that female baboon menstrual cycles are influenced by a number of factors. Gillman & Gilbert[1] noted that baboons became acyclic on an inadequate diet, and under the stress of capture and transport. Rowell[4] in a study on the anubis baboon noted that psycho-social stress resulted in lengthened follicular phases in her animals. She further noted that 2 factors, (1) isolation, and (2) repetative cycling, worked in a manner so as to increase the maximal perineal swelling size in her animals, and suggested that exaggerated swellings might be indicative of underlying physiological changes.

With this in mind an examination was carried out on the menstrual cycle characteristics of a group of long term, individually caged, and social contact isolated female Chacma baboons. For purposes of comparison these females were contrasted to another group of long term individually caged females who were receiving male social contact on alternate days during their menstrual cycles.

MATERIALS AND METHODS

Animals and Husbandry

A group of 35 wild caught adult female chacma baboons comprised the subject pool. Twenty seven of these were singly caged in open air runs of the following dimensions: 6 m long × 3 m high × 1 m wide. Opening into each run was a small 0,9 m square, cubicle for shelter and protection from the elements. The remaining 8 females were housed in a separate building, in individual cages of 1,5 m wide ×2 m long × 2 m high, with a cubicle like section of 0,8 m long × 1,5 m wide × 0,8 m high. In both cases although singly caged the females were in visual, auditory, and olfactory contact with other baboons. In addition the group of 8 females each received contact with an adult vasectomized male baboon during 24-minute mating tests carried out on alternate days throughout 4 successive menstrual cycles. All the animals were fed on a prepared commercial baboon pellet (Epol (Pty.) Ltd.). This diet was supplemented 3 times weekly with fruit and vegetables to ensure adequate supplies of ascorbic acid and roughage. Water was available to all animals *ad libitum*. The cages were hosed out twice daily, at 08h00 and again at 14h00. Medication was occasionally carried out to superficial wounds, and terramycin powder* with vitamins was administered as prophylactic medication when slight diarrhoea was noted.

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Cycle Assessment

All females were visually inspected daily at 09h00 in order to determine menses. In addition to checking for any signs of bleeding, the females were rated on a 3 point scale with regard to the degree of perineal swelling. Females who showed no signs of swelling were rated as "1". Females who were either increasing or decreasing in perineal tumescence were rated as "2", and females with a fully turgid perineum were rated as "3". The females differed in the maximal perineal swelling size but the observers were familiar with the individual females and their particular swell patterns.

RESULTS

The group of 27 social contact isolated females were examined daily for a period beginning on March 10, 1977 and ending on August 31, 1977. Forty one per cent (11/27) of the females never showed menstrual bleeding, or showed only one period of menses thus making cycle demarcation impossible. These 11 females differed in their histories and as a result will only be mentioned in passing. Six had been experimentally manipulated severely enough to disturb cyclicity, as an example, one animal was experimentally euthyroid. The remaining 5 females had either never been manipulated or had received only minor treatments, yet all were followed for a mean of 111 days and did not show the 2 periods of menstrual bleeding which are required for cycle determination.

The menstrual cycle data for the 16 females that did show at least 2 periods of menstrual bleeding during the examination period are shown in Table I. For purposes of comparison the data from these 16 females will be contrasted to that of the group of 8 females which had

*Pfizer (Pty) Ltd.

Table 1: MENSTRUAL CYCLE DATA ON INDIVIDUALLY CAGED FEMALE BABOONS

	Number of Cycles examined for each female	Duration of menses in days	Period of quiescence before sex skin swelling in days	Period fully turgid in days	Period prior to sex skin detumescence in days	Period from detumescence to next menses in days	Cycle length in days
Females denied social contact N=16	$\bar{x}$ = 2,93 SD ± 1,12	$\bar{x}$ = 3,35 SD ± 1,20	$\bar{x}$ = 7,79 SD ± 9,90	$\bar{x}$ = 13,56 SD ± 12,91	$\bar{x}$ = 28,72* SD ± 15,70	$\bar{x}$ = 12,24 SD ± 3,65	$\bar{x}$ = 40,95* SD ± 13,40
Females allowed male social contact N=8	4,00	$\bar{x}$ = 3,81 SD ± 1,04	$\bar{x}$ = 3,50 SD ± 1,53	$\bar{x}$ = 8,59 SD ± 2,93	$\bar{x}$ = 21,34* SD ± 3,08	$\bar{x}$ = 13,62 SD ± 2,44	$\bar{x}$ = 34,97* SD ± 1,84

*difference between the means of the two groups significant ($p \leq 05$, one tailed "t" test with unmatched samples)

been checked daily from December 22, 1975 to June 22, 1976 and who in addition received 24 minute mating tests on alternate days of their cycle. The data from these 8 females are also shown in Table I.

As can be seen from Table I the 2 groups differed significantly ("t" tests; McCall[3]) in terms of the period of sex skin swelling and turgidity prior to detumescence and in total cycle length. Differences are also evident in the period of sex skin quiescence before sex skin swelling and in the period of time that the sex skin was fully turgid. In both cases the measures just failed to reach a level of significance. No differences were supported statistically for the duration of menses or for the period of detumescence and inactivity that preceded the next menstruation.

DISCUSSION

The cycle data collected in this study agree fairly well with those previously collected by Gillman & Gilbert[1]. This is particularly true for the measure of cycle length where Gillman & Gilbert in an examination of 507 cycles reported a cycle length of 39,63 ± 16,2 days ($\bar{x}$ ±S.D.). This takes on greater significance when one considers the fact that Gillman & Gilbert collected their data from individually caged or social contact isolated females.

Our data are also consistent with those of Rowell[4] with regard to the variability involved in the follicular cycle phase. In our examination only an accurate determination of ovulation could have allowed us to determine exact follicular phase time periods, however, laparotomies on female Chacma baboons have documented that ovulation precedes sex skin detumescence by 2-3 days (Gillman & Gilbert[1]). We thus feel confident that the period prior to detumescence is roughly equateable with the follicular phase. It is perhaps interesting to note here that as little exposure as 24 minutes on alternate days was sufficient to result in a difference between our 2 groups of females. Although the 2 groups were checked at different times, the diet remained constant and there was considerable overlap in terms of the months of examination. It is possible that some seasonal factors may have had differential influences on the two groups, but Gilbert & Gillman[2] reported conceptions at all seasons of the year for the Chacma baboon and until a systematic study is carried out to determine possible effects of seasonality on the female menstrual cycle an accurate decision about its effects is difficult. It is impossible to ascertain whether the male-female contact was the critical factor here since Rowell[4] has suggested that female-female contact may also serve as a stress reducer, and Gillman & Gilbert[1] observed that females procured from the wild would start cycling sooner if caged with an already established and cycling colony member. It is clear, however, that group living female baboons are capable of showing menstrual cycle responses to the mere presence of adult males[5]. Additional work would have to be done in order to properly assess the differences which male and female social contact opportunities may have on the female baboons menstrual cycle.

Studies such as that of Trollope[6] on cage stereo-types have made it clear that social contact is a very important factor with regard to the individual well being of primates, and that this factor takes precedence over others such as; cage complexity, lighting, and access to inside or outside space.

Although it was impossible to determine if any of the examined female baboon cycles were ovulatory, it may well be that the difference observed between the females allowed social contact and those denied such contact has a physiological relevance to the events of ovulation. Such differences become increasingly important when breeding efforts come to be considered and it is hoped that future work will allow a consideration of the way in which social contact opportunities may affect female baboon fertility.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the University of the Witwatersrand whose financial support of the Central Animal Unit made this work possible. Additional financial support is acknowledged from the University Council to the Primate Behaviour Research Group. Assistance from the staff of the Central Animal Unit helped make this study possible.

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A SEMI-SYNTHETIC DIET FOR GROWING BABOONS

D. B. DU BRUYN and W. A. DE KLERK

ABSTRACT: Du Bruyn D.B., W.A. de Klerk. **A semi-synthetic diet for growing baboons.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 193–195 (En), National Food Research Institute, CSIR, Box 395, 0001 Pretoria, Republic of South Africa.

A semi-synthetic diet capable of sustaining good growth in young baboons and containing casein as the only protein source, is proposed. Growth rate data and problems encountered, such as soft stools and hair loss, are discussed. The main shortcomings of the diet are its lack of bulk (fibre) and the amount of time and effort which its preparation requires.

INTRODUCTION

During our studies on the relationship between dietary mineral imbalances and calcinosis in baboons *(Papio ursinus)* we compounded a semi-synthetic diet which we regard as suitable for use in a wide range of nutritional studies on baboons. The results obtained with this diet over a feeding period of 20 months will be described.

Much of the data used to formulate the composition came from a review article by Kerr[1] and a book, edited by Harris[2], dealing extensively with the feeding of non-human primates. Also consulted were the pamphlets of the U.S. National Academy of Sciences on the nutrient requirements of laboratory animals[3], and those of the Food and Nutrition Board on the recommended nutrient allowances for humans[4].

MATERIALS AND METHODS

The diet contained 15% casein (protein content *ca* 90%; N × 6,25) as the sole protein source, 10% white cane sugar and 58% dextrin, as the carbohydrates and 10% sunflower seed oil as the fat source. The diet also contained a vitamin pre-mixture which included choline chloride and inositol. Agar-agar and a mineral premixture were also included and in the formulation of the latter, the high phosphorus content of the casein *(ca* 600 mg/100 g) was taken into account.

Further details regarding the diet are given in Table 1. In the second column of this table the nutrient levels are given in relation to the energy value of the diet (100 g diet = *ca* 1 670 kJ) and in the third column nutrients are expressed per 100 g air-dry mixture.

Thirty components are at present known to be essential for optimal nutrition and all of these were included in the diet. The adventitious nutrients such as chromium, fluorine, selenium, molybdenum, silicon, tin and vanadium were not added as we assumed these to be present as impurities in the chemicals and in the non-synthetic dietary components such as the casein and dextrin.

The diet had a low fibre content because no fibre was added and only 2% agar-agar was included as a bulking agent. Fibre was excluded from the diet because originally we were not aware of its possible benefit to primate health. The high level of vitamin D was chosen because the baboons were kept indoors for the greater part of the experimental period and also because the amount of vitamin D synthesized in the skin of the baboon is unknown.

The ascorbic acid level of 42 mg/1 000 kJ, used in the diet under discussion, is also considerably higher than that recommended for human diets, but was added

Table 1: COMPOSITION OF A SEMI-SYNTHETIC DIET WHICH HAS PROVED SUCCESSFUL IN THE FEEDING OF YOUNG BABOONS IN CAPTIVITY

Nutrient	Nutrient level: Per 1000 k J intake	Per 100 g ration (air-dry basis)
Protein (g)	8,1	13,5
Carbohydrate		
Sugar (g)	6,0	10,0
Dextrin (g)	34,5	58,0
Fat		
Total fat (g)	5,4	9,0
Essential fatty acids (g)	3,0	5,0
Vitamin A (i.u.)	360	600
Vitamin D (i.u.)	120	200
Vitamin K (μg)	120	200
Vitamin E (mg)	42	72
Vitamin B_1 (μg)	300	500
Vitamin B_2 (μg)	479	800
Vitamin B_6 (μg)	479	800
Vitamin B_{12} (μg)	3,6	6,0
Nicotinic acid (mg)	6	10
Folic acid (μg)	150	250
Biotin (μg)	30	50
Ascorbic acid (mg)	42	70
Choline (mg)	90	150
Inositol (mg)	120	200
Calcium (mg)	240	400
Phosphorus (mg)	186	310
Magnesium (mg)	50	84
Sodium (mg)	263	443
Potassium (mg)	168	280
Chloride (mg)	180	300
Inorganic sulphur (mg)	150	260
Iron (mg)	3,6	6,0
Iodine (μg)	18,0	30,0
Zinc (mg)	1,4	2,4
Copper (mg)	0,9	1,5
Manganese (mg)	1,2	2,0

in accordance with the findings of De Klerk *et al:*[5] these workers reported that feeding 20 mg ascorbic acid/kg body mass/day to captive baboons was necessary to maintain serum ascorbic acid levels comparable to those of free-living baboons (1 to 1,5 mg ascorbic acid/100 mℓ).

A stiff porridge was prepared before each feeding by adding the sunflower seed oil and cold water to the premixed ingredients. A bolus of porridge was then fed to each baboon. As fresh porridge was prepared before each feeding and because the dry premix was stored for a short period (usually less than 4 days), it was not considered necessary to add anti-oxidants to prevent rancidity.

Five male and 5 female baboons, each less than 10 weeks old, were placed on the diet. The animals, when captured, were less than 5 weeks old. They were

brought to our Institute and then raised according to the regimen for feeding and rearing in captivity as has been described elsewhere[6]. For 70 weeks the animals were housed in individual cages suspended in a room, the temperature of which was not allowed to decrease to below 20°C. Thereafter they were moved to spacious outdoor cages with heated concrete floors. Food was offered twice daily in excessive quantities and water was made freely available from automatic watering devices.

The baboons were weighed weekly without the aid of drug-immobilization. At the end of the 20-month experiment, the animals were immobilized with phencyclidine hydrochloride and killed with an overdose of sodium pentobarbitone. Post mortem examinations were conducted and specimens were taken for histological examination.

RESULTS AND DISCUSSION

The baboons remained in good physical condition throughout the experiment. Growth curves compiled from individual body mass records are shown in Fig. 1. It can be seen that the rate of growth was virtually constant for 70 weeks.

Table 2: ESTIMATED INITIAL BODY MASS AND GROWTH RATE OF THE BABOONS BASED ON A LINEAR REGRESSION OVER A 70 WEEK-PERIOD

Males Estimated initial body mass	Growth rate (kg/week)
2,39	0,11
2,04	0,122
2,08	0,102
2,79	0,115
1,66	0,1
2,19 ± 0,19*	0,11 ± 0,004*
Females	
1,51	0,088
1,48	0,101
1,85	0,101
1,22	0,106
0,75	0,106
1,36 ± 0,18*	0,1 ± 0,003*

*Average ± SE

The average growth rate (kg/week) of each baboon was estimated by fitting a straight regression line to a plot of body mass versus time. Mean growth rates of 0,11 kg/week for females (see Table II) were obtained. Mean initial body-mass was 2,19 ± 0,19 kg for male baboons and 1,36 ± 0,18 kg for females. Linear projections of these growth curves suggested that male baboons should reach a body mass of *ca* 40 kg by 6 or 7 years of age, and that females should reach *ca* 20 kg by 3 or 4 years. These projections indicate that the baboons would have attained their normal body mass at maturity.

Food consumption appeared to be good but was impossible to measure accurately because wasted food could not be retrieved from the cages. However, the growth rates (see Table II) and good physical condition of the animals, indicate that the food intake was adequate and that the diet tested is suitable for the feeding of young baboons in captivity.

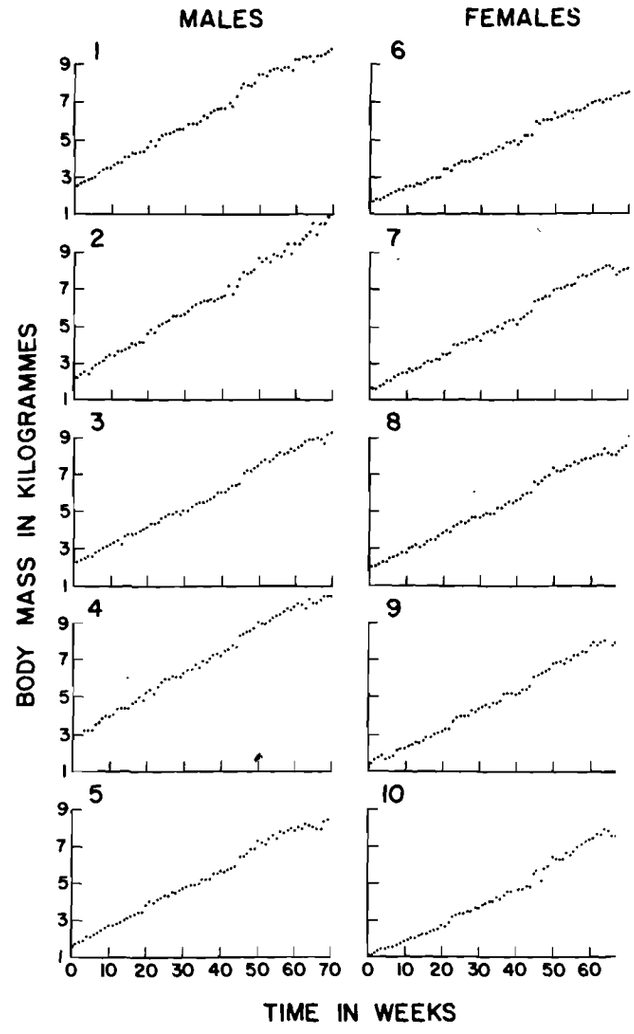

Fig. 1 Growth curves of 5 male and 5 female baboons fed on a semi-synthetic diet over a 70 week-period

Two minor problems were encountered during the experiment. Firstly, slight hair loss (alopecia) occurred on the lateral aspects of the thighs of all the baboons. The cause of this was not established but hair-plucking might have played a role. Secondly, the animals passed very soft, almost liquid, stools. Although this apparently had no ill effects on the baboons, we feel that a suitable bulking agent should be added to the diet, either to replace or to supplement the agar-agar. Apart from the fact that the latter product was not effective when used at the 2% level, it is also very expensive. Carboxy-methyl-cellulose (CMC) at a 2% level was used without success in the diet of another group of baboons, while good results were obtained by adding wheaten bran. Bran, however, cannot be used in semi-synthetic diets because it contains many nutrients in varying and unknown quantities as well as an anti-nutrient (phytic acid). Only a purified bulking agent can be considered. Cellulose products such as Solkafloc are not available locally and we have not yet been able to test its effects in baboon diets. Other objections to the cellulose products are that these products are partly digested and that large quantities (*ca* 15%) are needed in the diet before they are effective as bulking agents.

Post mortem and histological examination did not reveal any disease conditions, such as rickets or soft tissue calcification, which can be related to inadequate nutrition but all the baboons had lesions of chronic typhlitis localised in the vicinity of the ileo-caecal valve. The possible role of the low fibre content of the diet as a causative factor in this condition has been discussed elsewhere[7].

The vitamin D level in the baboon diet (120 i.μ/1 000 kJ) was very high relative to that recommended for man (30 and 60 i.μ/1 000 kJ for adults and 1 to 3 years respectively). That this high intake was not excessive, is indicated by the absence of soft tissue calcification – a symptom of vitamin D poisoning.

Preparation of fresh porridge on a twice daily basis is both time and labour consuming and an investigation of methods to overcome this problem is required. We are at present investigating the possibility of preparing the mixture in the form of a baked loaf using a chemical leavening system. The results of this study will be reported at a later stage.

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THE INBREEDING OF THE Y AND THE Z STRAINS OF *PRAOMYS NATALENSIS* WITH SPECIAL REFERENCE TO THE LABORATORY USES OF THE MASTOMYS

J. D. RANDERIA

ABSTRACT: Randeria J. D. **The inbreeding of the Y and the Z strains of** *Praomys Natalensis* **with special reference to the laboratory uses of the Mastomys.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 197–199 (En), Cancer Research Institute, University of Durban – Westville, Private Bag X54001, 4000 Durban, Republic of South Africa.

The Y and the Z strains of *Praomys natalensis (Mastomys)* are being successfully inbred, as far as is known for the first time. Spontaneous tumours of the glandular stomach have been found to develop in these rodents. Evidence is provided of the great potential of the laboratory Mastomys for use in medical research on cancer, plague, bilharziasis, and more recently in the research on the Lassa fever virus.

INTRODUCTION

Praomys natalensis (syn. *Mastomys coucha, Rattus natalensis* Smith), popularly called the Mastomys or multi-mammate mouse[9] is found in most parts of the African continent[21]. The animals have a body mass of less than 100 g and live on cultivated land where they do great damage to grain crops. In the young the coat is slate, becoming agouti in mature and drab agouti when old[23].

The Mastomys, although believed to be a genuine mouse[5] and a close relative of the common rat[21], belongs to the genus *Praomys* Thomas[2]. Some of the anatomical and physiological features of the Praomys are rat-like, others mouse-like, and still others appear to be unique such as the development of a prostate gland in the female.

ORIGIN OF THE LABORATORY MASTOMYS

P. natalensis (Mastomys) captured in the Glen Grey district[1] of the Transkei were bred for the first time in a laboratory in 1939 at the South African Institute for Medical Research (SAIMR) in Johannesburg. In 1946, 17 wild pairs caught in the Baragwanath district of Johannesburg were added to the original stock[3]. After 12 generations in the laboratory the field behaviour of the Mastomys[12] was found to be unchanged. They are essentially amicable towards members of their own species and exhibit a marked escape reaction towards possible danger sources such as man[23]. The laboratory Mastomys must be constantly handled otherwise they revert to their natural wild behaviour. In the Johannesburg stock 95% of the litters were cannibalised[8]. Pairs of breeding animals were kept together for life, if one died its partner was discarded. Because of the cannibalistic tendencies only one 'inbred' line reached the 13th generation by 1961. These animals remained mostly heterozygous.

In 1971 the Mastomys colony at the SAIMR in Johannesburg was moved to the Medical Research Council laboratories in Pretoria (W. A. de Klerk, personal communication). From this colony, animals of the Y and the Z strains, derived from the original stocks of the *P. natalensis (Mastomys)* of Dr. A. G. Oettle, were brought to us in Durban. The Y strain is a mutant from Iscor stock[1]. The animals have light red eyes and a yellowish coat with darker belly fur. The Z strain (formerly DBU) was selected for double dilution from the Iscor dilute and the Baragwanath dilute lines (P. C. King, personal communication). The Z strain animals also have yellowish coats and red eyes.

INBREEDING OF THE MASTOMYS

Prior to 1972 the Mastomys of the Y and the Z strains were "forced bred", i.e. brother-sister had been allowed to mate, produce litters and remate at the first post-partum oestrus. Thus females often delivered litters from second, third or fourth pregnancies while still suckling their previous young ones. The present programme of inbreeding in our laboratory started with the forced bred litter-mates from Generation 26 of the Y strain and from Generation 31 of the Z strain. In our laboratory, 1 male is caged with 1 or more sisters. The pregnant females are isolated before parturition and throughout the lactation period.

Biological behaviour during inbreeding

Two lines are being developed from each of the Y and the Z strains. The first 7 generations have been reared successfully. The animals will be inbred after 19 generations. The specific data on the patterns of behaviour of the breeding pairs, the pregnant females, the nursing mothers, the suckling litters and the weaned litters recorded for the first 7 generations of inbreeding of the Y and the Z strains of the *P. natalensis (Mastomys)* are as follows:

Breeding pairs

One adult male with its sisters is housed per cage at the age of 10-12 weeks. Fighting is minimised by handling the animals several times a day. Conception generally occurred readily and the first litters were born between 14-21 weeks of age; the average age being 17 weeks. The duration of pregnancy was approximately 3 weeks. The males were reintroduced 4 weeks after parturition. The second litter may be born at an age of 20 to 32 weeks with an average of 27 weeks.

The pregnant female and the nursing cage

The isolated pregnant female readily makes a nest of the material supplied in the cage. At birth the litter is cleaned and nursed by the mother. Provided necessary precautions are taken and the female is left undisturbed, cannibalism of the litters rarely occurs. Nursing is equally good for the first and the subsequent litters. Although the number of pups in a litter varies from 3 to 12, there is no constant difference between the number of pups at the first, second or third pregnancy. The average number of pups born at first and second pregnancies were 6 and 6,5 respectively. The offspring

thrived and could be weaned at 3 weeks of age. We uniformly weaned every litter at 4 weeks of age and returned the mothers to the mating cages. Male and female weanlings were separated and housed in different cages.

Weaned litter mates

The weanlings are wild and aggressive but become tame with frequent handling. They were earmarked at the age of 8 weeks, utilising a simple numbering system. The litters born of the second pregnancies are being used for raising consecutive generations.

LABORATORY USES OF THE MASTOMYS

The Mastomys has become of increasing importance in medical research. They are transmitters of bubonic plague and have been used in plague research since 1940. The animals are also used in bilharzia research due to their susceptibility to *Schistosoma mansoni.* Among laboratory rodents, with the exception of a single strain of inbred mice (STR/IN), Mastomys is the species most susceptible to osteo-arthritis[20]. Severe degenerative joint disease of diarthroses and intervertebral discs develops spontaneously during the second year of life. Spontaneous lesions of glomerulonephritis, comparable to that in man, have also been described[18]. More recently Mastomys has been implicated as a carrier of Lassa virus[7]. Chronic Lassa infection with shedding of the virus in the urine has been demonstrated in old outbred Mastomys in a colony in the U.S.A. (K.M. Johnson: personal communication).

A large variety of spontaneous tumours have been found in older laboratory Mastomys. Among those reported in the Johannesburg strains[1 8] were carcinoid tumours of the glandular stomach (originally called adenocarcinomata), granulosa cell tumours of the ovary, adrenal cortical adenoma, pheochromocytoma, pituitary chromophobe adenoma, bile duct papilloma, papilloma[10], carcinoma of the vagina, hepatoma, leukaemia, papillomas and squamous carcinoma of the skin. Multiple skin papillomas of basal type[11], occasionally metastasising, with high, keratin forming cutaneous horns, were also found and were attributed to infestation with *Psorergates oettli*[1].

In the Japanese stock multiple tumours of the stomach, lung, liver, ovary and thymus have been reported in old animals[6]. In the random bred colony at the National Institute for Health, USA, established in 1955, malignant argyrophilic carcinoids of the glandular stomach, renal tumours, thymomas and thymic hyperplasias as well as a variety of hematopoietic neoplasms categorized as lymphosarcoma, type B reticulum-cell sarcoma and plasma cell myeloma were found to develop spontaneously[16 19]. A rare adenocarcinoma of the female prostate gland has also been recorded[17]. The spontaneous thymic tumours, unlike those in other rodents, bear a striking resemblance to human thymic lymphoepitheliomas associated with myaesthenia gravis[22]. Mastomys has thus been proposed as an experimental model for the study of auto-immunisation to striated muscle in relation to thymoma, myaesthenia gravis and polymyositis.

The majority of experimental studies on the induction of neoplasia with Mastomys deal with the enhancement or prevention of its spontaneous neoplasms. New-born Mastomys injected with SV-40 virus develop tumours distally in the brain[14]. Splenomegaly and splenic sarcomas were induced after injection of the new-born animals with MSV-H virus[4]. Renal and heart sarcomas as well as liver angiomas developed in new-born Mastomys inoculated with 0,05-0,1 mℓ of polyoma virus[13]. An extract of prickly pears has been used medicinally in parts of Australia and South Africa for the treatment of neoplasia. However, dried spineless cactus *(Maxima* sp.) given as an infusion in lieu of drinking water to Mastomys (J.F. Murray, personal communication) had no effect on spontaneous tumours in the Mastomys[11]. The Mastomys of the Y and the Z strains treated intragastrically with small doses of 3-methylcholanthrene have yielded tumours of the cardiac and the glandular stomach[15].

Of the spontaneous tumours, gastric carcinoids in some colonies develop in 71% of females and 44% of males over 18 months of age respectively[8]. The incidence greatly exceeds that in man[21] and Mastomys is thus an unique experimental model for the study of the aetiology and pathogenesis of gastric carcinoma, which has been hampered by the lack of a suitable experimental animal. The species is furthermore useful in the testing of suspected gastric carcinogenesis including environmental nitrosamines and in determining the factors associated with 'spontaneous' carcinogenesis.

With these aims and objects in mind, the Y and the Z strains of the Mastomys, selectively derived from the original wild stocks maintained as random bred rodents, are being inbred for use in medical research on Lassa fever, plague, bilharziasis and particularly cancer.

ACKNOWLEDGEMENTS

This programme is supported partly by an annual grant from the National Cancer Association. The author is grateful to Prof. J.F. Murray, President, National Cancer Association of South Africa, to Prof. J.V.O. Reid, Head, Department of Physiology and Dean, Medical School, University of Natal for the various facilities provided. Technical assistance of Miss N. Hari, Miss S. Sankar and Miss D. Munsamy in the rearing and care of animals is acknowledged.

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JOURNAL REVIEW

TYDSKRIF-RESENSIE

Journal of Fish Diseases

Vol. 1, No. 1., Jan. 1978
Editor: R. J. Roberts, Unit of Aquatic Pathobiology, University of Stirling, Scotland
Blackwell Scientific Publications

The policy of the editor of the Journal of Fish Diseases is to provide an international journal in which scientific papers, short communications, review articles and book reviews are published. The journal expects to cover all the aspects of disease in wild and cultured fish and shellfish. Papers are criticized by reviewers in whose field the paper lies and where appropriate, reviewers other than those on the Editorial Board are consulted.

The Editorial Board consists of scientists who have a long history as prominent researchers in various aspects of aquaculture and fish diseases and each is an acknowledged expert in his field.

From the first number of the first volume it is clear that the Editors set a high standard in their choice of material. The presentation is in a readable print and duplication of black and white illustrations and photographs is excellent. One would, however, like to see an improvement in the quality of the colour plates as these tend to be rather hazy.

Articles which appear in the first number of this journal include one on skin tumours of Pacific and Atlantic flatfish, cytological studies on a new species of Rickettsia of marine bivalve molluscs and a beautifully illustrated analysis of the Saprolegnias of salmonid fishes.

This journal is strongly recommended to people and institutions interested in the various aspects of fish diseases. In South Africa especially, where very few of the diseases of fish have been studied, this journal could make an important contribution, both in training and updating the researchers in fisheries sciences.

J. B.

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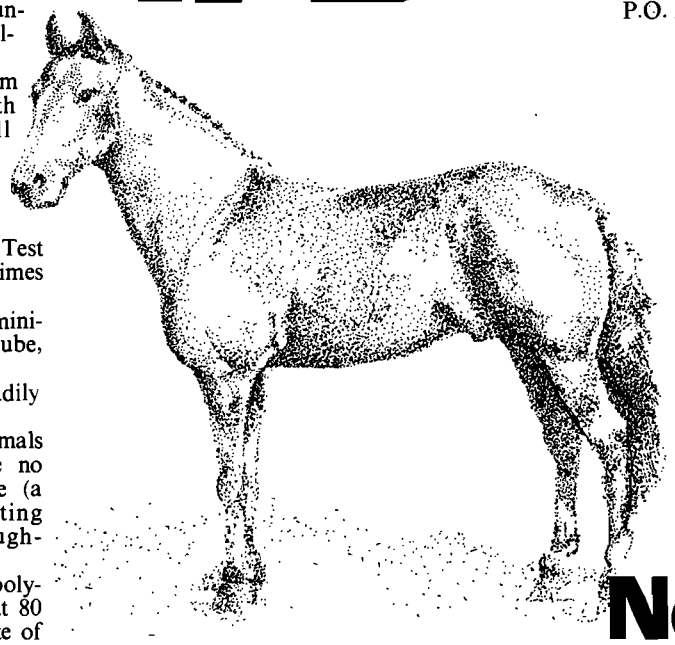

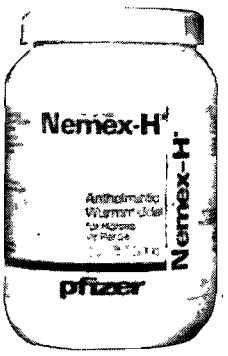

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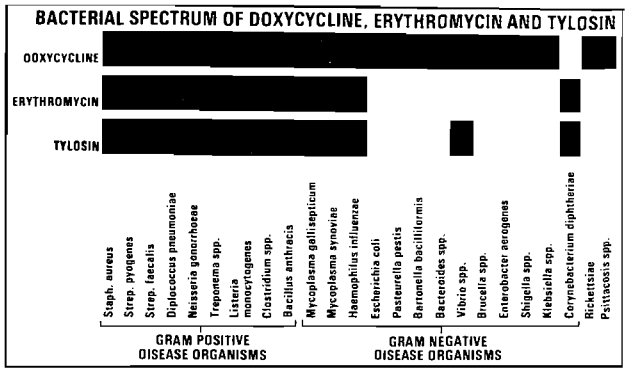

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THE JAPANESE QUAIL *(COTURNIX COTURNIX JAPONICA)* AS A LABORATORY ANIMAL

RENÉ ICHILCIK and J. C. AUSTIN

ABSTRACT: Ichilaic, René, Austin, J.C **The Japanese Quail** *(Coturnix coturnix japonica)* **as a laboratory animal.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 203–207 (En), MRC/University of the Witwatersrand Dental Research Institute, 1 Jan Smuts Avenue, 2001 Johannesburg, Republic of South Africa.

The use of the Japanese quail as a laboratory animal was first reported in 1959. Since then this species has been increasingly used as an avian model for biomedical research. A quail colony was recently established in the Dental Research Institute to provide adult birds for teratogenicity and toxicity studies. These birds are not widely used for biological research in South Africa at present. The housing, husbandry, breeding and nutrition of this species is described, together with the biomethodology which is applicable to this species.

INTRODUCTION

Japanese quail *(Coturnix coturnix japonica)* have been kept as domesticated birds for the last 800 years in Japan[1]. They were mainly prized as song birds. At the beginning of this century they became economically important for meat and egg production in the Far East[6]. These birds are fast growing, efficient food converters and prolific egg producers (Fig. 1).

Fig. 1 Adult male *Coturnix coturnix japonica*.

In the late 1940's and early 1950's quail were introduced into England and the U.S.A. for food production purposes. Their usefulness as research animals was soon recognised. A close anatomical and physiological resemblance to domestic chickens and their small size made them ideal models for poultry research[9 16]. The use of Japanese quail in biomedical research increased rapidly in the next 2 decades and they were employed in studies in the fields of embryology[7 10], toxicology[8], physiology, nutrition[12], endocrinology[15], genetics[6 13], cancer research[11] and others. The main advantage of using this species as an avian animal model is the small size. When fully grown, the males weigh 100-120 g and the females 120-150 g. They are easily managed under laboratory conditions and have a high rate of egg production (up to 300 per year) and a short generation interval. Quail eggs hatch in 16-18 days and the chicks grow rapidly thereafter and attain sexual maturity at 6-8 weeks of age in contrast to the domestic chicken which requires at least 22-25 weeks to begin producing eggs. Four to 5 generations of quail can easily be raised in a year.

Interest in the quail as a research animal was initiated in our laboratory by a search for suitable animal models for studying the biological effects of long term exposure to anaesthetic gas pollution. A colony of these birds was established to provide both adults and embryos for teratogenicity and toxicity studies on nitrous oxide.

Coturnix quail are not widely used in biomedical research in South Africa and this paper has been prepared to review the biology of this species, to provide guidelines for the establishment of a laboratory colony of these birds and to detail the basic biomethodology which is applicable to this species.

ORIGIN OF STOCK

Breeding stock from long established genetic lines which have been propagated in closed colonies may be obtained from the United States and the U.K. In these colonies a heterogeneous genetic pool has been maintained by minimal inbreeding programmes. Quail are extremely sensitive to inbreeding depression[13] and sibling mating should be avoided if reproductive performances are to be maintained.

Importation of quail into South Africa is subject to veterinary regulation and import permits must be obtained from the Division of Veterinary Services. A mandatory quarantine period of 30 to 60 days is also applicable depending on the country of origin of the birds. The importation of fertile eggs is prohibited.

To avoid inbreeding problems we were advised by geneticists, of British Medical Research Council's Laboratory Animal Centre, to start our colony with 20 breeding pairs. Since importation of breeding stock from abroad involved both considerable delay and costs it was decided to establish a colony from an undefined local source of supply for our initial toxicological screening studies.

The clinically healthy adult breeding pairs were purchased from a local breeder of exotic birds* to establish a small colony for initial studies.

HOUSING, CAGING AND SANITATION

Quail may be kept either in communal groups in pens or rooms on deep litter, or singly or in small groups in battery type caging systems. The latter method of hous-

*R. Heynecke, 154 Eloff St., Eloffsdal, Pretoria.

ing was adopted as it can be operated on a more intensive basis. This facilitates the managements of birds under laboratory conditions. Animal rooms in which quail are housed should be well ventilated to control the strong odour generated by these birds. A single 5,0 × 2,0 m room was used for housing juvenile and adult birds, and a chick brooder and incubator. The room was equipped with heaters but these were not often required as heat generated by the brooder and incubator usually kept the room temperature between 21-25°C throughout the year. Quail are extremely sensitive to the influence of a daily photoperiod. The length of daylight and wavelength of light have been shown to influence both the age at which sexual maturity is attained and the rate of egg production[18]. A 14-16 hour daylight favours early maturity (6-8 weeks) and high egg yields. A time switch was installed to provide a 14 hour light and 10 hour dark cycle.

Many different types of cages have been used for housing quail. In some laboratories plastic rat cages with grid floors have been successfully used. Our initial attempts to use these types of cages failed as the birds in the breeding nucleus were introduced from a free ranging mode of life. The birds kept attempting to escape by flying upwards and head injuries resulted from collision with the mesh cage top. This behaviour ceased when mesh walled cages were tried. Quail do not require much space for growth maturation and reproduction. Space recommendations for adult birds are 200 cm^2/bird with a cage height of 200 mm^3.

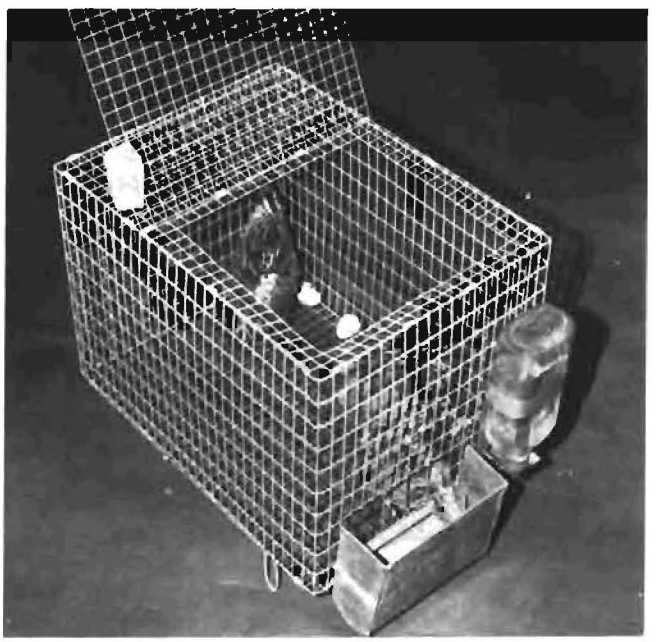

Fig. 2 Standard weldmesh cage used for rearing and egg production.

Simple, inexpensive cages were constructed with 1,6 mm thick galvanised welded mesh* (Fig. 2). Cage floors were made of 13 mm × 13 mm mesh and the walls and cage top were made of 13 mm × 25 mm mesh. For convenience and economy of usage of the standard 1 200 mm wide welded mesh available, a cage size of 250 mm (width) × 350 mm (length) × 250 mm (height) was adopted to hold 4 adult birds. Welded mesh panels were precut to size and joined with clips. A hinged cage top was added. Sections of 5 alternate vertical wires and a horizontal wire were removed from the front panel of the cage to form 5 feeding openings 50 mm × 25 mm in size. An externally mounted food hopper with raised back and side walls and a turned over front lip was used to reduce food wastage. Quail feed vigorously and large amounts of food can be wasted by being scattered from conventional poultry feed troughs. A drinking trough which protruded through the wire mesh was also mounted on the front cage panel. The water level in the trough was maintained by an inverted 180 mℓ medical flat bottle. Water troughs are rapidly contaminated with feed from the birds beaks and the troughs and water bottles have to be washed on a daily basis.

Three 50 mm long wire feet were soft soldered to the front of the 2 side panels and the middle of the rear panel respectively to raise the cage floor and provide a self cleaning cage system. Galvanised sheet metal trays were provided to hold the cages. The trays were supported on a metal wall racking system (Fig. 3) and lined with newspaper to facilitate sanitation and daily removal of faeces.

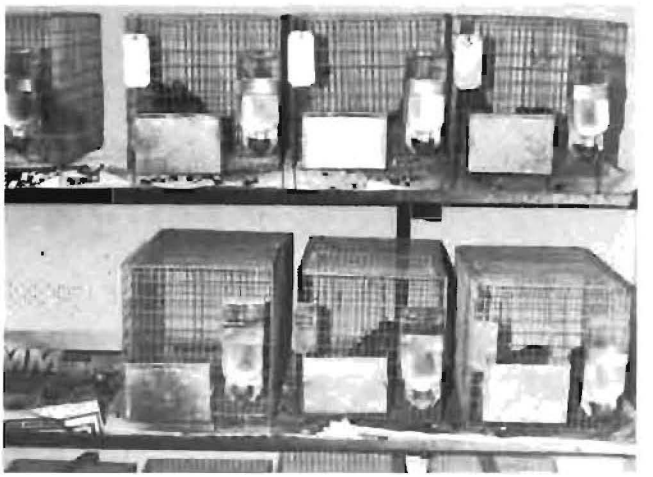

Fig. 3 Cages on metal trays on the wall racking system.

NUTRITION

Commercially available poultry feeds were used for feeding. All mash laying mash* was fed to young birds after they left the brooder and to all adult stock as a standard maintenance ration. This contained 15% protein and appeared to be satisfactory, although it has been reported that such low protein diets result in a loss of condition as the birds age[5]. Chicks were fed from hatching to the end of brooding (3 weeks) on turkey starter mash* with a protein content of 28% which is necessary for the early rapid growth rate from 7-50 g in the first 3 weeks of life. By 6 weeks of age quail reach 90-95% of their mature body mass. A study on the growth rate of 10 male and 10 female quail was carried out on the feeding regimen. The birds were marked individually and weighed at weekly intervals from hatching to 6 weeks of age. Little difference was found between the growth rate of males and females during this period and the mean rate of growth of the pooled sam-

*Bonnox (Pty) Ltd., Pretoria.

*Epol (Pty) Ltd., Springs, Transvaal.

ple of 20 birds is shown graphically (Fig. 4). This growth rate compared favourably with rates reported in other studies[6 11]. Food conversion ratios are reported to range between 2,3-3:1 dependent on the ratios fed and the strain[14].

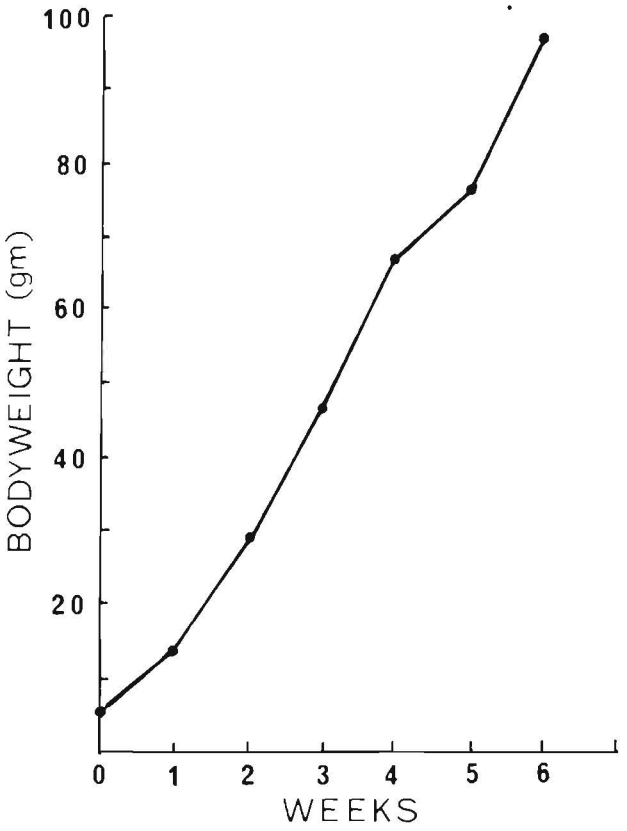

Fig. 4 Growth rate of Coturnix quail.

BREEDING AND REARING

After an initial 7-10 day period of adaptation to small cages and the indoor environment females in our breeding nucleus began to lay regularly. Breeding pairs were placed together in separate cages. Quail exhibit sexual dimorphism and can be easily sexed by their plumage characteristics from 3 weeks of age. Males have light brownish red breast feathers in contrast to females which exhibit speckled gray and black breast feathers. Males also have a large cloacal gland which discharges a foamy secretion when the cloaca is examined. The faeces from male birds is coated with this secretion when it is voided. Younger birds can be sexed from a day old using a cloacal examination method[4].

Several of the initial breeding pairs of quail were found to be incompatible and these birds had to be separated to prevent injury from bullying and pecking. Aggressive birds were debeaked and again paired together. When our breeding stock had reached maturity a standard breeding ratio of 1 male to 2 females was adopted. This has been reported to yield the highest fertility rates[17].

Eggs were laid directly onto the floor mesh of the cages and these were collected twice daily. Minimal breakages were experienced. Quail eggs average 10 g in weight and vary from pale blue to a speckled blue and brown colour. Many of the females regularly laid one egg per day. Eggs were collected and stored for up to 5 days in a cool room. The eggs were stored in vermiculite with their larger ends uppermost. Hatchability has been reported to fall off in quail eggs stored for longer than 7 days[3].

Fig. 5 Quail eggs being incubated in cardboard rings.

Batches of eggs were incubated in a small still air chicken egg incubator* at 38°C (Fig. 5). Batches of eggs were separated using cardboard rings and the eggs were turned by hand twice daily. A relative humidity of 60-65% was maintained in the incubator until hatching. The relative humidity was raised to 90% by spraying the eggs with water as the chicks pipped. Quail eggs hatched in 17,5 days and chicks were left in the incubator for 6-12 hours to dry out before being transferred to a brooder. A hatchability ranging between 40-60% was achieved. This is average for this species.

A simple brooder was constructed with 13 mm × 13 mm weldmesh. This was 250 mm × 150 mm (height) in size (Fig. 6). The side walls were covered in wire fly screening to prevent the chicks from escaping from the brooder. The brooder was supported on an asbestos sheeting shelf and was placed on newspaper to facilitate cleaning. The chicks were brooded under a 250 watt infra red heater** which was suspended

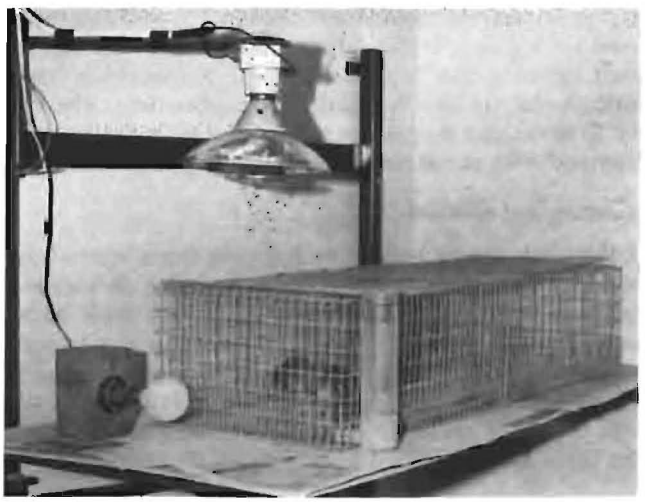

Fig. 6 Weldmesh brooder with heater and light.

*"OVO", John F. Marshall (Pty(Ltd., Johannesburg.

**Softglow (Pty) Ltd., Birnam Park, Transvaal.

300 mm above the floor of the brooder over one end of the cage. A 25 watt light bulb was continually burning alongside the brooder to prevent panic and smothering of chicks when the main room lights were switched off. Turkey starter mash was fed in petri dishes and water was provided by means of plastic bird cage drinkers mounted on the side of the brooder. Newly hatched chicks frequently drown if open water dishes are provided.

At 3 weeks of age chicks were routinely debeaked prior to being transferred to growing cages. This debeaking was found to be necessary to prevent cannibalism and head pecking which occurred when groups of birds reached sexual maturity at 6-8 weeks of age. Half of the upper beak, as measured from the tip to the nares, was removed with a guillotine type dog toenail clipper. The cut end was lightly cauterised with a heated scalpel blade to arrest haemorrhage. Cannibalism and feather pulling often increased as the birds get older than 6 months and results in loss of condition and damage to the plumage over the heads and backs of the birds. Attempts to control this problem by managemental methods have been unsuccessful and problem birds are either culled or housed alone.

BIOMETHODOLOGY

The use of the Coturnix quail in experimental biology involves the application of techniques for handling and restraining the birds, and the implementation of experimental treatments and techniques for the collection of samples to enable experimental data to be monitored and recorded. Some of these basic techniques are detailed below:

Identification

Individual birds may be marked by using the conventional techniques which have been developed for marking cage birds and poultry. Plastic leg bands (for canaries and budgerigars) are suitable for marking newly hatched chicks up to adulthood. Canary rings must be used with care and must be removed before they become too tight on the leg. If they are not removed strangulation of the distal limb will occur. After 14 days of age shortened aluminium pigeon leg bands can be used for leg banding and the unmodified bands can be used for wing banding. Delta rings* for marking domestic chicks can also be used for wing banding. The latter 2 items can be purchased in batches of 100, prestamped with serial numbers.

Handling and restraint

Adult birds are picked up by grasping them around the body with the neck held between the index and second finger. The birds are lifted and turned on their backs and the neck is lightly squeezed between the fingers. Birds thus restrained will lie flaccidly in an open hand for examination and blood sampling. Handling of the legs provokes struggling and should be avoided when the birds are restrained.

Body Mass Determination

This is easily accomplished by determining that of individual birds in a brown paper bag.

*John F. Marshall (Pty) Ltd., Johannesburg.

Intubation

Liquids and suspensions are easily administered per os by means of direct intubation of the crop using a bulbous tipped 100 mm long 18 gauge hypodermic needle.

Blood collection

Small quantities of blood may be collected in heparinised microhaematocrit tubes by puncturing the brachial vein with a hypodermic needle. Larger quantities of up to 3 mℓ may be collected from adult birds by jugular venipuncture.

Anaesthesia

A variety of anaesthetic agents which have been employed for avian anaesthesia were tested on the quail. These included xylacine*, alphaxalone•, ketamine hydrochloride‡, and metomidate§ administered by intramuscular injection at varying dosage rates. The most effective agent was found to be metomidate administered intramuscularly into the thigh at a dosage rate of 20 mg kg body mass. Anaesthesia was induced in 5 m and lasted for 30 m. None of the other agents produced satisfactory relaxation and anaesthesia in this species.

Euthanasia

The administration of carbon dioxide in a large glass jar was found to be a rapid and convenient method for killing quail.

DISEASES

Although quail are susceptible to a variety of poultry diseases such as salmonellosis, coccidiosis and respiratory viruses[2], no outbreaks of recognisable disease have occurred in our colony. Deaths have resulted from cannibalism, accidents and occasional vent prolapse in old females.

CONCLUSION

The Coturnix quail is easily managed and propagated under laboratory conditions using relatively simple equipment and commercial poultry diets. The birds are hardy and inexpensive to maintain and are worthy of further consideration as a standard avian model for teaching and research in South Africa.

ACKNOWLEDGEMENTS

We are grateful to Mr. R. Heynecke for his advice and assistance with the establishment of our colony, to Mr. A. Moepi for technical support and to Mrs. H. Ball for typing the manuscript.

* Rompun – Bayer S.A. (Pty) Ltd.
• Saffan – Glaxo-Allenburys S.A. (Pty) Ltd.
‡Ketalar – Parke-Davis Laboratories Ltd.
§Hypnodil – Ethnor Laboratories (Pty) Ltd.

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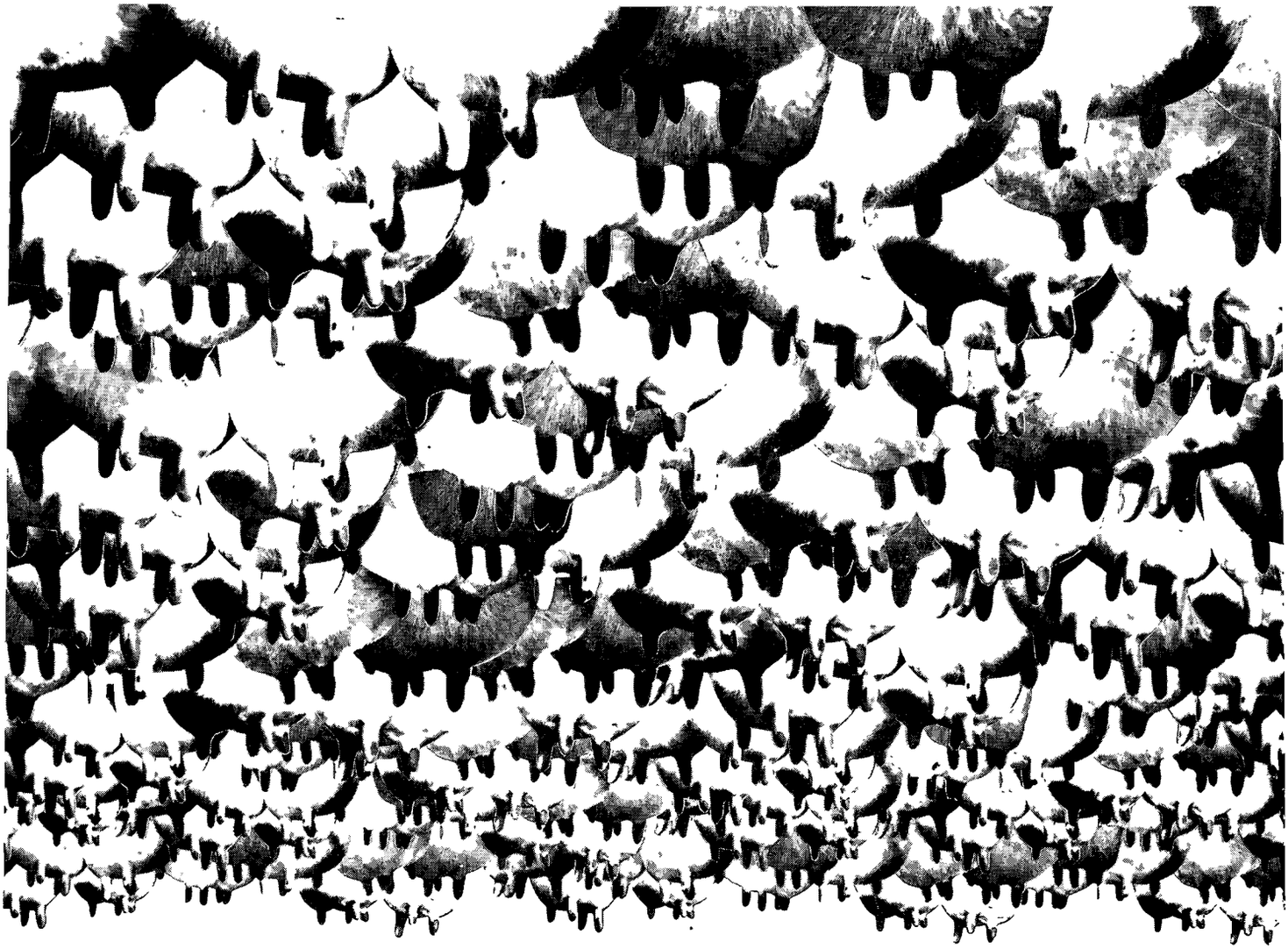

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BATES-WELLS ROSTRON

THE YERKES REGIONAL PRIMATE RESEARCH CENTER

G. H. BOURNE

ABSTRACT: Bourne, G.H. **The Yerkes Regional Primate Research Center.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 209–210 (En), Yerkes Primate Research Center, Emory University, Atlanta, Georgia, U.S.A.

The development and organisation of the centre which now has over 1 500 non-human primates representing more than 30 species, including great apes, is described. Important projects are mainly in the neural and behavioural field, including language acquisition, but also on aspects of experimental pathology.

INTRODUCTION

The Yerkes Center is one of the oldest continuously operated centre for primate research in the world. The founder, Dr. Robert M. Yerkes, conceived the idea of a laboratory to study the psychological and biological development of the great apes while he was a student at Harvard University. In 1923, Dr. Yerkes began working with 2 young chimpanzees on his farm in New Hampshire and by 1930 he had acquired 2 additional pairs of chimpanzees. Some of the animals at Yerkes today are descendants of one of these first 6 chimpanzees.

In 1930, the Yale University scientist moved his small colony of apes to Orange Park, Florida. Over the next 35 years, the Yerkes Laboratories of Primate Biology became world-famous for its studies of ape psychology and the implications for human mental processes. Eventually other primate species were added to the Yerkes colony including a group of orangutans and another of gorillas. Ultimately the Center became one of 7 divisions of the Woodruff Medical Center of Emory University.

In the late 1950's there was a growing interest in primate research on the part of the National Institutes of Health, leading to the allocation of federal funds for 7 primate centers, and the Florida installation evolved into the Yerkes Regional Primate Research Center. In the summer of 1965, the entire Yerkes complement – apes, monkeys and people – moved from Orange Park to the Emory campus in suburban Atlanta, Georgia where new facilities had been built at a cost of more than $1 800 000 funded by a grant from the National Heart Institute of the National Institute of Health. The main building was dedicated October 27, 1965, at which time then Emory President, Dr. Sanford S. Atwood called it "the formal beginning of a great research operation in the field of biology".

The research programmes of the Yerkes Primate Research Center are directed towards 2 missions identified for the Center by the National Institutes of Health: (1) Neural and behavioural research, and (2) Experimental Pathology with emphasis on neoplastic and degenerative diseases.

Research projects involving a wide range of disciplines including anatomy, histology, pathology, sociobiology, psychobiology, neurophysiology and biochemistry are being conducted at the Center. Among the projects are comprehensive behavioural studies of great apes involving the animals' social patterns, habits, cross-modal perception, and language formation; electrophysiological analyses of the behaviour of nonhuman primates in social environments using techniques of telemetry and telestimulation; studies of the social organization of selected primates; studies of the reproductive physiology of the great apes, including artificial insemination; and the ability of test animals to sustain prolonged weightlessness in space.

Some of the findings and applications of the work carried out at the Center are as follows:

1. *Cross-modal transfer of information (integration of information across modalities).* In normal humans various senses such as hearing, vision, touch etc. seem to function in an integrated way. In other words, information obtained through these modalities can easily be transferred to the others. Some young children do not show this ability and some adults with brain damage have difficulty in performing such tasks. It has been claimed that the great apes do not have this ability and even that they do not have the necessary neurological equipment for this purpose. Visual-tactual cross-modal perception has, however, been established at the Yerkes Center for chimpanzees and orangutans. These animals shown an object or even a photograph or a drawing of an object can then select the object by touch without seeing it and vice versa. It has been suggested that cross-modal ability is a function of language and that animals could not perform it because they had no language ability. Dr. Richard Davenport, who has been carrying out this work at the Yerkes Center, suggests that the inability of children to read (dyslexia) may be related to an inability to transfer information cross-modally.

2. *Language acquisition by chimpanzees.* Language is another ability said to be unique to man, but Dr. Duane Rumbaugh at the Center states that "apes can learn vocabulary and basic rules for the concatenation of those words into novel and meaningful sentences". Apes do not have vocal equipment for speech so another method of communication must be used. We have used a keyboard with symbols (lexigrams) from a specially designed language "Yerkish". The keyboard is connected to a computer which carries out the requests made by the chimpanzee (Lana). At first Lana only had to depress the key which represented a particular item of food or drink for the computer to deliver it. Later she had to embody the request in a sentence such as "Please machine give piece of apple", and the machine would only deliver the apple if every word was in the correct position. Lana now has a vocabulary approaching 150 words and can make up her own 10 word sentences from them using the correct syntax. She can request not only various items of food and drink but also movies, lantern slides, music, opening the window, the technician to come into her room and play with her or groom her, the names of objects she does not know and so on. She will also agree with the exper-

imenter if he or she gives the animal a piece of information she knows to be wrong. Scientists are now argueing that Lana's performance explodes all previous ideas about the uniqueness of the human uses of language.

The computer system used in communicating with Lana has now been set up at the Georgia Retardation Center and is being adapted with great success to communicate with children who, for various reasons, have failed to develop language skills of their own.

3. In the *medically oriented part of the programme* at the Center, Drs. R. Metzgar and H. Seigler have used the chimpanzee as a model to study the rejection mechanisms in kidney and heart transplants and more recently to use these animals to produce anti-cancer sera. The Yerkes group was the first to demonstrate that many of the histocompatibility antigens that were being defined in man by human reagents were also present in chimpanzees and other apes. These antigens have many similarities to blood group antigens, but they are responsible for the rejection of foreign grafts such as those of the skin, heart or kidneys and are not responsible for the reactions which occur in blood transfusion. The antigens responsible for normal organ and tumour rejection are very similar if not identical between man and apes. Such a model is most valuable for testing immunosuppressive drugs.

Drs. Metzgar and Seigler have also produced from chimpanzees anti-leukaemia and anti-melanoma sera. With these sera they have established a specific serodiagnosis of these 2 diseases. The anti-leukaemia serum is being tested for its therapeutic value in association with anti-leukaemia drugs. In the case of melanoma a preliminary study has demonstrated that conjugating an alkylating chemotherapeutic agent with the melanoma antibody caused regression of a disseminated tumor. The investigators have also developed a radioisotope labelled chimpanzee anti-human melanoma antibody. This will permit the development of a specific radio-scanning technique for identifying metastatic areas in the body.

4. Another area in which the Yerkes Center has found chimpanzees to be a useful model is in the *area of human female reproductive biology*. It has been found that the endocrine patterns of chimpanzees during the sexual cycle and pregnancy are more or less identical. These hormonal changes have been correlated with body temperature (using an implanted telemeter), with uterine and vaginal histology, sexual skin swelling and ovulation (by endoscopy). The changes closely resemble those of the human female and indicate that the chimpanzee is an excellent model for the human in the study of female reproductive physiology and pathology and also in the development of contraceptive devices.

5. Studies are also carried out on rhesus monkeys which are of importance to *space travel.* Since the changes that take place in humans in bedrest are similar to those of weightlessness the Center has been studying the changes which take place in monkeys in enforced bedrest. Changes in heart and skeletal muscle and even liver changes develop in such animals. Rhesus monkeys are specially bred for these studies and for possible flights in the Space Shuttle in the 1980s.

The examples quoted in this article represent only a small fraction of the researches carried out at the Yerkes Primate Center, but they will serve to give some idea of the type and range of the projects under way.

The Yerkes Center has 32 full and part-time scientists on its staff. Specialities range from gross anatomy and physical anthropology to pathology, sociobiology, psychobiology and immunology.

Three staff veterinarians are responsible for the health of the animals; a veterinary pathologist provides pathologic and laboratory diagnostic services to the Center.

Supporting the investigators are research scientists, technicians, laboratory aides, animal caretakers, secretaries and clerks. On the staff are a librarian, medical illustrator, x-ray technician, registered nurse, photographer, electron microscope technician and various other skilled technicians.

Each year visiting scientists and students use Yerkes facilities for their research. All programmes are designed to provide an optimal environment wherein resident and visiting scientists or students may actively pursue their work and correlate accumulated data with appropriate information from other institutions. In addition to supporting a multi-national visiting scientist programme, Yerkes furnishes blood and other biological specimens to researchers all over the world. The Center represents an integrated scientific community without geographic limitations.

The Yerkes Center conducts its operations at 2 locations – the main site in a secluded part of the Emory University Campus, and a field station 30 miles north of Atlanta. In the main building on campus are executive and business offices, library, conference rooms, research laboratories, a surgical suite, x-ray facilities, a clinical pathology laboratory, and animal areas. The field station provides limited office space, some caging facilities, and spacious outdoor animal compounds. The station is designed primarily for breeding experiments and studies of social behaviour in medium-size groups.

The Yerkes Center is unique in that it has the largest collection of great apes in the world. The colony contains over 1 500 non-human primates representing more than 30 species. Of these, 178 animals are great apes including 123 chimpanzees, 3 pygmy chimpanzees, 35 orangutans and 17 gorillas. Among the other primates represented are gelada, mandrill and drill baboons, a variety of macaques, cercopitheques, and new world monkeys, and representatives of some of the rarer genera.

The Center has been very successful in breeding apes, over 300 have been born in the last 40 years, giving an average of 7 or 8 per year over this period. Thirty-six orangutans have been born, a record for breeding these animals in captivity. There have been 5 gorillas born recently including 3 which were all born in the one month of March 1976.

The Center Director is responsible for administration to the Vice President for Business of Emory University and to the Vice President for Health Affairs for the scientific activities of the Center. The Center participates with the scientific faculty of Emory University in many research and training projects.

The 7 primate centers are administered at a national level by the Primate Centers' Section, Animal Resources Branch of the Division of Research Resources of the National Institutes of Health.

CARDIAC SURGICAL RESEARCH AND HEART TRANSPLANTATION IN *PAPIO URSINUS*

JACQUES G. LOSMAN

ABSTRACT: Losman J.G. **Cardiac surgical research and heart transplantation in** *Papio Ursinus. Journal of the South African Veterinary Association* (1978) **49** No. 3, 211–215 (En), Cardiac Surgical Research Unit, Department of Cardiac Surgery, Medical School, University of Cape Town, 7925 Cape Town, Republic of South Africa.

The use of non-human primates stems from the concern for employing an animal genetically closely related to man. During a 3 year period over 300 baboons were handled in the Cardiac Research Unit and more than 60 heterotopic cardiac transplantations were performed. The baboon presents multiple advantages if compared with the problems arising from the use of the dog or the pig in cardiac surgical experimentation. This experimental work illustrated these advantages and demonstrated that heterotopic cardiac transplantation is a valuable model for treatment of patients in end stage cardiac failure. The procedure has been performed successfully in 16 patients, 12 being presently alive, 1-36 months following transplant.

INTRODUCTION

The goal of all medical research with experimental animals is to establish models which are able to define methods of treatment of metabolic or anatomic disorders, or models of possible treatment.

The use of non-human primates in the laboratory stems from the concern for employing an animal genetically very close to man. The relation is significantly greater than for most other laboratory animals. It has been established that the use of non-human primates allows more reliable extrapolation of data than the data derived from using other animals[17 19 30].

This close relation between man and simians is also seen in the nature of their pathology[16 27]. For example, the baboon resembles man in its pattern of cholesterol metabolism [7 35] and arteriosclerosis and myocardial infarctions have been observed in freeliving baboons[10 13 21 22 28].

ADVANTAGES OF THE BABOON FOR EXPERIMENTAL WORK

The baboon displays special attributes which make him an elected animal for research. The animal is still available in relatively large numbers and is at present inexpensive in South Africa.

Baboons are easy to maintain in captivity[4 6]; we are keeping them in large cages of about 2 m^3 in volume and 2 m in height. They are fairly easy to handle by immobilizing them with short acting anaesthetics (Diagram I).

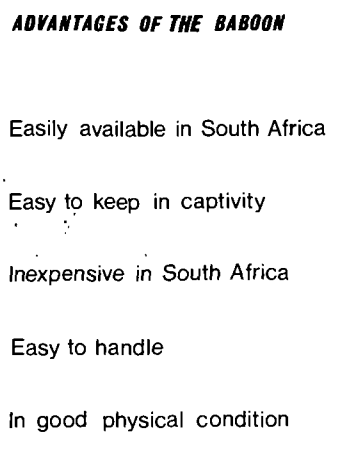

Diagram 1 Advantage of the baboon as an experimental animal

In our experience, in spite of the fact that many reports have mentioned that an animal such as a baboon is far from its optimal condition of fitness in the wild[18 29], we have found that our animals were healthy and could be conditioned in a few days to become excellent experimentation specimens. The dogs obtained locally are in comparison usually in miserable condition, undernourished, full of parasites and often present major pathology, in particular chronic glomerulo-nephritis.

The pressure of natural selection and survival of the fittest is probably an important mechanism in keeping the wild baboon in healthy condition. This is one of the reasons for selecting to work with this animal. For the cardiac surgeon the baboon has also some favourable characteristics, the first one being the great similarity of its cardiac and thoracic anatomy compared to human anatomy. The cardiac circulatory function of this animal is well established[8 9 14 15 31 34 36 37].

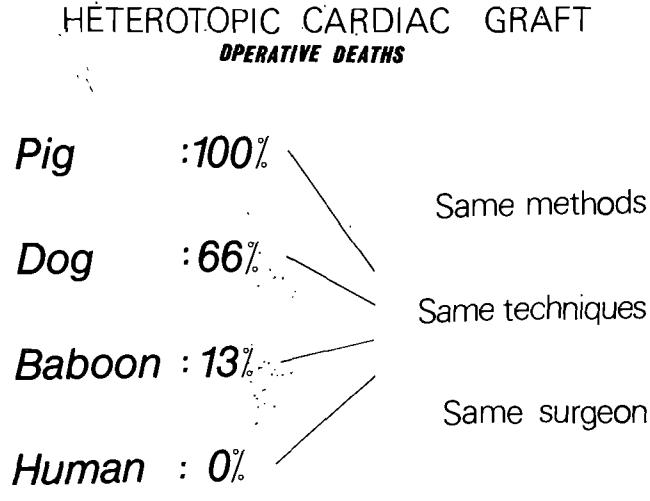

Diagram 2 Operative mortality following heterotopic cardiac graft in pig, dog, baboon and human

Following cardio-pulmonary surgery most animals suffer a high mortality or morbidity from pulmonary complications. The baboon has a large vital capacity in relation to its total body mass and furthermore, his habit of sitting most of the time improves his respiratory function immediately following thoracic surgery.

On the other hand, if we compare our personal experience and that of others with extra-corporeal circulation between pigs[5 33], dogs[25], and baboons there is no

doubt that the baboon tolerates extra-corporeal circulation much better (Diagrams 2 & 3). He presents much fewer problems, in particular in regard to surgical quality of tissues and post-operative haemostasis.

Heterotopic Cardiac Graft

OPERATIVE DEATHS

	PIG	DOG	BABOON
ANATOMICAL FIT	100%	25%	0%
HAEMORRHAGE	-	62,5	22
CNS DAMAGE	-	12,5	33
BYPASS ACCIDENT	-	-	22
NARCOSIS DEATH	-	-	22
CASES	**4/4**	**8/12**	**9/68**

Diagram 3 Causes of operative death following heterotopic cardiac graft in pigs, dogs and baboons

In the field of cardiac transplantation we have also found that the immuno-suppressive drugs given at similar dosage per kg body mass had a similar efficiency to the one they have in human beings[1 20]. The baboons of these series were treated following regimes similar to the ones given to the human patients following cardiac allografts.

DISADVANTAGES OF THE BABOON AND PATHOLOGY

One of the major disadvantages is that this fierce animal is dangerous and cannot be handled, examined or investigated without major sedation or full anaesthesia. This is a significant problem in some haemodynamic studies where the drug given to immobilize the animal may interfere with delicate evaluations. A further problem is the fact that wild baboons are often carriers of various pathogens, in particular shigella, salmonella, and less often of dangerous simian herpes viruses[3 26 32]. (Diagram 4).

DISADVANTAGES OF THE BABOON

Dangerous

Investigations under Narcosis

Carrier of Shigella,

Salmonella and

B virus

Diagram 4 Disadvantages of the baboon as an experimental animal

The only serious pathology encountered over a period of 7 years in over 500 baboons were episodes of severe gastro-enteritis, often progressing towards a picture of haemorrhagic colitis. Less than half the stool cultured showed a responsable pathogen (usually shigellae), and we attributed most of these cases to the stress of capture and changes in diet. If not treated vigorously, this condition has a high mortality. The best treatment in our hands, is to stop oral feeding and rehydrate the animal vigorously with 100 to 200 mℓ/kg/day of an electrolyte solution given intravenously and to add vitamin supplements and a 6 day course of chloramphenicol (150 to 200 mg/kg body mass/day). This treatment resulted in a salvage of 70 to 80% of the affected animals.

HETEROTOPIC CARDIAC TRANSPLANTATION IN BABOONS

Our research work on heterotopic cardiac transplantation in baboons was initiated by the fact that following orthotopic cardiac transplantation for irreversible left ventricle disease often a normal or hypertropic right heart is removed at the same time as the severely diseased left ventricle[2 23]. This is of particular importance in patients with severe pulmonary hypertension. Their own right ventricle is trained to cope with this high pulmonary resistance but a normal heart is not. During the early experience in cardiac transplantation, many patients died in the post-operative period due to excessively high pulmonary hypertension. Severe pulmonary hypertension is now an absolute contra-indication to orthotopic cardiac transplantation[11 12].

Another life threatening problem in orthotopic cardiac transplantation is rejection. Following rejection after a renal transplant the patient can be maintained alive by haemodialysis, and rejection can be treated, but until now we do not have such a device to support the failing donor heart during rejection. By leaving the recipient heart *in situ* and connecting the transplanted heart in parallel we aimed for a model where the recipient heart is kept as a possible assistance device.

In the experimental protocol we did not attempt to achieve long term survival. Our major aim was first to find out the possible problems of anatomical fit of the second heart in the chest of a primate, to study the importance of synchronising the 2 hearts, and to evaluate the haemodynamics of such a model. Immunosuppression was given only to delay or minimise the effect of rejection until the animal was submitted to repeated haemodynamic investigation[24].

MATERIAL

Large Chacma baboons *(Papio ursinus)* with a body mass of 21,5 ± 1,1 kg were paired for mass, (donor recipient difference being less than 5%) after being matched for A, B and AB blood groups. Operation was performed within a few weeks of catching. The animals were starved overnight and anaesthetized with ketamine 5 mg/kg and subsequently with morphine 1,5 mg/kg and pancuromium bromide 0,5 mg/kg.

Anaesthesia was maintained after intubation by intermittent positive pressure ventilation with a Bird Mark 8 with a closed circuit receiving 3ℓ/min. of oxygen and 6ℓ/min.of nitrous oxide and 0,5% of fluothane intermittently. Ventilation was monitored by repeated

determination of arterial pCO_2, pO_2 and pH. Extracorporeal circulation was established in the conventional way using a pediatric Bentley bubble – oxygenator with 1 000 mℓ of an electrolyte solution (Plasmalyte B – Baxter), 500 mℓ of fresh heparinized homologous baboon blood and 200 mℓ of human albumin. Fifty grams of dextrose and 50 g of mannitol were added to the priming solution.

SURGICAL TECHNIQUE

The surgical technique for the removal of the donor heart is only slightly different from that for total heart transplantation. The cranial and caudal venae cavae are ligated, and the aorta is divided distally to the subclavian artery in order to provide enough to extend from the recipient's right chest cavity across the cranial vena cava to the lateral wall of recipient ascending aorta. The chest of the recipient is entered through a median sternotomy. The right pleural cavity is opened widely. The right half of the pericardium sac is turned down as a flap. There is then enough space cranial to the hilum of the right lung and in the right cardiophenic angle to harbour the donor's heart without unduly compressing the right lung's caudal lobe. The baboon is connected to the heart-lung machine by cannulating the cranial and caudal venae cavae through the right atrial wall for venous drainage and cannulating the ascending aorta for arterial return.

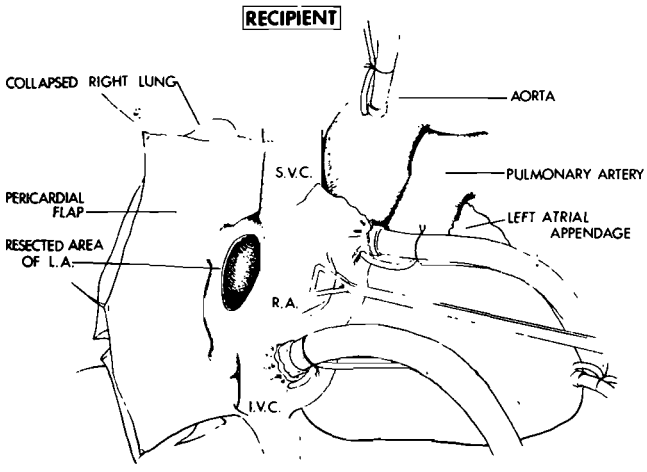

Fig. 1 Diagram of recipient's heart in situ, with ventricles and right atrium lifted towards the left. The right half of the pericardial sac is turned down as a flap. Note the opening created in the right lateral wall of the patient's atrium

An opening of the size of a normal mitral valve orifice is created in the recipient's left atrial wall, and a similar size opening is made in the donor's left atrium including the entrance of the 2 left pulmonary veins; a side to side anastomosis is now performed between these 2 openings creating a common left atrium (Fig. 1 & 2). Similar openings and anastomoses are made between both right atriae, at the level of the junction between cranial venae cavae and right atria. An end to side anastomosis is performed between the end of the donor aorta and the side of the recipient aorta. The donor pulmonary artery is then anastomosed to the pulmonary trunk of the recipient heart. This is achieved by using a piece of donor thoracic aorta as a free graft (Fig. 3).

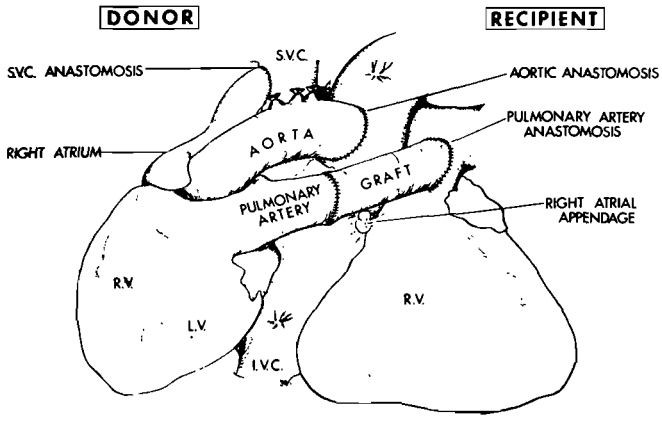

Fig. 2 Diagram illustrating connection between the left atria of donor and recipient

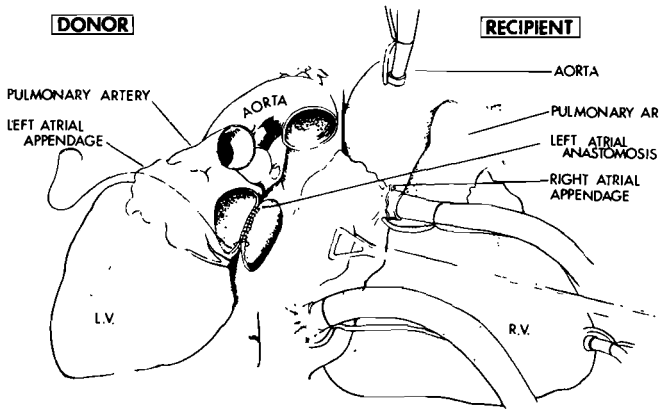

Fig. 3 Diagram illustrating the bypass of both the patient's left and right ventricles by the use of a cardiac allograft

The donor heart usually starts beating spontaneously in sinus rhythm once coronary circulation is re-established. The recipient heart which has been infarcted before the transplantation procedure requires usually one electric shock (Fig. 4). The animals have excellent circulation immediately following surgery. During 6-8 hours continuous monitoring is maintained on the operating table; measurements are done to assess car-

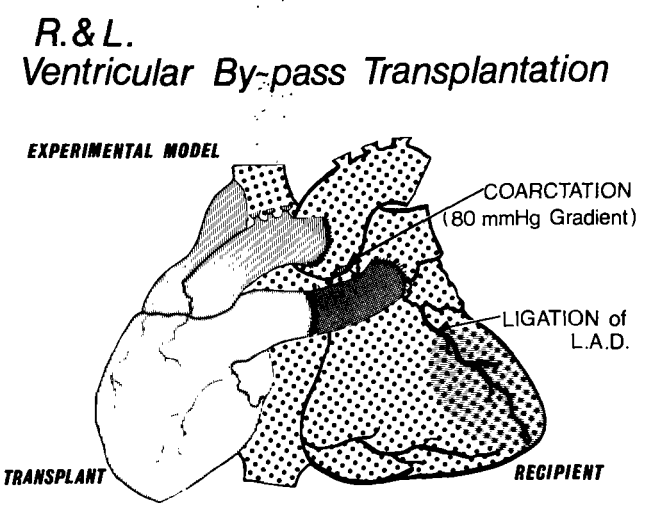

Fig. 4 Diagram of our experimental model showing the method of damage of the recipient heart and the cardiac graft in position

diac function and eventually the animal is closed in conventional way. Anaesthesia is reversed, the animals awakened rapidly and then placed in their cage. Following an uneventful procedure the baboon sits up immediately and starts drinking (Fig. 5).

Fig. 5 Baboon in its cage two hours following heterotopic cardiac transplantation

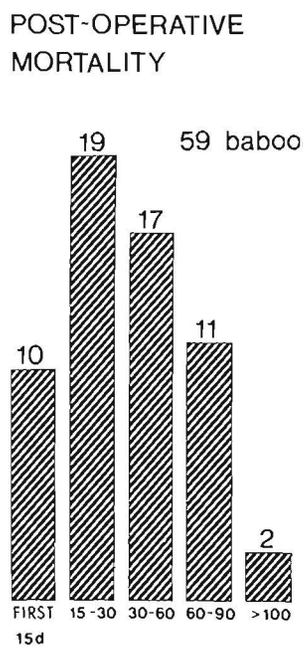

Diagram 5 Post-operative survival and mortality following heterotopic cardiac transplantation in 59 baboons

Each animal receives 1 g of Keflin intra-muscularly 3 times a day for 5 days and the immunosuppressive regime consists of Azathioprine 3 mg/kg/body mass per day and methyl-prednisolone 5 mg/kg per day. This treatment is given to delay rejection and no efforts were made in this series to achieve long term survival of the animals (Diagram 5). The mean survival time was 35,2 days the longest survivor living 127 days. The cause of death is presented in Diagram 6.

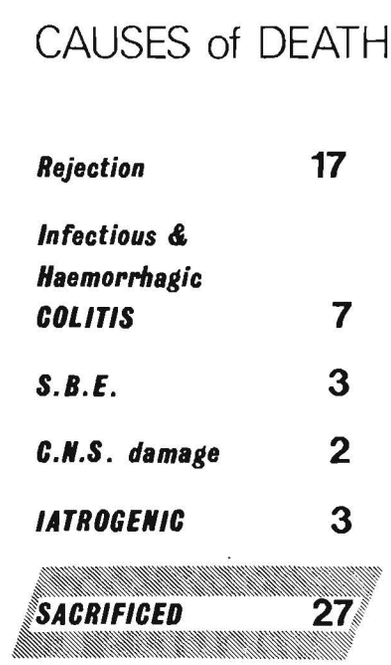

Diagram 6 Causes of death following heterotopic cardiac transplantation in 59 baboons

An important point to mention is that extensive and prolonged haemodynamic studies of these animals resulted in severe morbidity and subsequent mortality, due to the techniques themselves and to contamination during cardiac catheterization or open chest studies.

HAEMODYNAMIC EVALUATION

Extensive investigations were done to assess the function of this system and are reported in detail elsewhere[24]. In summary they demonstrated that an allograft heart in an heterotopic position has normal haemodynamics and is capable of supporting normal circulation, independently of the degree of the myocardial damage of the recipient heart. The inter-action of the recipient and donor heart upon each other are extremely complex. Pre-load and after-load are changing from beat to beat.

Using synchronous pacing of both hearts a dynamic status is created, the 2 hearts being in maximum competition for load and ejection. The damaged heart has reduced filling and increased pressure load.

Using pacing of both hearts at the same rate but with a 180° phase shift alternate beating was obtained. Filling of the recipient heart was significantly increased and immediate pre-ejection aortic pressure reduced. Alternate pacing did not change total output of the system but resulted in improvement of recipient performance.

In the whole series there were no complications directly related to the design of the surgical model and heterotopic cardiac transplantation proved itself to be a safe procedure in spite of lethal recipient heart damage.

CONCLUSIONS

Because of its size, its anatomy and its physiology the baboon is an ideal animal for cardio-vascular and pulmonary research. Compared with dogs and pigs the Chacma baboon was in our hands and for our type of research the optimal laboratory animal. The information obtained from this non-human primate was indispensable in the development of our transplantation programme.

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TYDSKRIF VAN DIE SUID-AFRIKAANSE VETERINÊRE VERENIGING 49(3) 1978

BLOOD CHEMICAL AND HAEMATOLOGICAL CHANGES IN DIARRHOEIC BABOONS *(PAPIO URSINUS)*

D. G. STEYN

ABSTRACT: Steyn D.G. **Blood chemical and haematological changes in diarrhoeic baboons** *(Papio ursinus). Journal of the South African Veterinary Association* (1978) **49** No. 3, 217–218 (En), Dept of Surgery, Faculty of Veterinary Science, University of Pretoria, Box 12580, 0110 Onderstepoort, Republic of South Africa.

Blood was collected for blood chemical and haematological determinations from untreated surviving and non-surviving diarrhoeic baboons. The significance of changes in blood chemical and haematological values is discussed and the importance of supportive treatment is emphasized.

INTRODUCTION

Diarrhoea or dysentery is one of the most serious diseases encountered in non-human primates particularly during the conditioning period. *Shigellae, Salmonellae* and *Escherichia coli* have been isolated as causal organisms from diseased animals. Dehydration and inanition are presumably major factors in this disease[2]. Because supportive treatment plays such an important role in the treatment of disease in non-human primates it was decided to investigate the effect of bacterial diarrhoea on the blood chemistry and haematology of the chacma baboon. The studies described in this communication were made on baboons which received no treatment during the course of the disease. The effects of the disease on the fluid and electrolyte balance were therefore not complicated or masked by intravenous infusion of fluids and electrolytes.

MATERIALS AND METHODS

The baboons maintained at the primate colony at Karl Bremer Hospital, Bellville, Cape are captured by farmers in various regions of the Cape Province. From the farms the animals are transported in a specially designed vehicle to the colony where they are conditioned and kept for medical research purposes. The housing management and feeding of animals maintained at this primate colony have been described previously[5].

The diseased baboons were anaesthetized with pencyclidine hydrochloride* on the first day of showing evidence of diarrhoea. Blood was collected from the femoral vein for blood chemical and haematological determinations and arterial blood was collected from the femoral artery for blood gas determinations. All determinations were done according to standard laboratory procedures[4]. Food and water were available at all times during the experiment. The animals received no treatment and the various parameters were measured daily until death from diarrhoea or dysentery took place or until there was obvious recovery from the disease.

The animals were divided into 3 groups to facilitate comparison of results. Group 1 included 7 animals that died after 3 days of illness. A similar group of 7 animals which recovered after only 3 days of illness was included in Group 2. Group 3 was made up of 9 animals that recovered after 5 days of illness.

RESULTS

The results for the blood chemical determinations are tabulated in Table 1. The total plasma protein (TPP) values did not change significantly in any of the groups. The plasma albumen values recorded in Group 1 decreased significantly ($P<0,05$) from the first to the third day whereas the alpha-1 and alpha-2 globulin values increased significantly ($P<0,01$) between day 1 and day 3 in the Group 1 animals.

Significant electrolyte changes occurred only in Group 1 animals. The serum sodium values *and the serum chloride values* decreased significantly ($P<0,05$

Table 1: BLOOD CHEMICAL DETERMINATIONS IN DIARRHOEIC BABOONS

	Group 1			Group 2			Group 3				
TPP	5,53	5,49	5,63	5,86	5,84	5,47	6,12	5,83	5,57	5,54	5,60
Alb	2,67	2,21	2,04	2,52	2,51	2,18	2,82	2,69	2,39	2,30	2,43
Alpha-1	0,19	0,28	0,35	0,33	0,27	0,26	0,22	0,24	0,27	0,27	0,24
Alpha-2	0,70	1,03	1,20	0,94	0,91	0,81	0,87	0,95	0,90	0,96	0,86
Beta	0,77	0,79	0,79	0,82	0,86	0,86	0,88	0,79	0,80	0,79	0,85
Gamma	1,24	1,18	1,24	1,31	1,30	1,28	1,33	1,16	1,27	1,21	1,27
K	3,33	3,59	4,26	2,80	3,22	2,97	3,22	3,38	3,50	3,33	3,38
Na	137,86	136,29	126,43	130,33	133,56	135,67	136,44	136,56	131,89	138,78	138,89
CL	96,86	93,00	85,71	92,22	93,00	95,89	92,22	92,89	92,67	97,00	95,11
CO_2	24,57	19,71	21,29	26,00	29,22	28,00	25,89	29,00	28,11	30,22	31,78
Urea	50,86	105,43	207,71	54,78	52,67	51,11	69,89	62,56	61,56	64,22	51,56
Chol	95,86	89,43	104,00	119,44	118,33	102,33	99,56	106,67	109,44	108,44	115,11
SGPT	14,86	15,86	24,00	11,33	18,11	12,44	12,78	18,22	13,22	12,67	16,89
SGOT	18,14	26,71	78,43	17,22	22,67	23,44	20,56	23,00	22,67	23,67	23,44
LDH	308	310	461	248	281	306	259	274	286	353	386
SAP	713	660	896	462	505	418	496	408	401	419	486

*Sernylan – Parke Davis and Co.

Table 2: HAEMATOLOGICAL AND BLOOD GAS DETERMINATIONS IN DIARRHOEIC BABOONS

	Group 1			Group 2			Group 3				
WBC	10,84	8,60	7,60	6,56	7,47	8,43	10,10	6,50	6,52	6,34	7,34
RBC	5,53	5,89	6,31	5,36	5,20	4,78	5,70	5,54	5,48	5,11	4,89
Hgb	14,59	15,14	16,17	13,58	12,91	11,98	14,89	14,16	12,34	12,93	12,24
Haemat	42,86	44,14	46,86	40,33	39,22	36,11	43,56	41,56	40,78	37,89	36,56
PCO_2	38,29	33,57	36,57	38,33	45,89	42,67	38,22	42,56	40,22	41,11	44,44
pH	7,42	7,41	7,38	7,44	7,41	7,43	7,44	7,44	7,46	7,47	7,44
PO_2	114,86	108,71	118,14	111,67	96,33	118,33	84,67	99,78	100,78	90,44	89,00
BE	0	–3	–3	+2	+3	+3	+2	+4	+4	+6	+5

and <0,01) from the first to third day. The values recorded on the third day of Group 1 animals were significantly lower than the third day values of Group 2 (P<0,05) and the fifth day values of Group 3 (P<0,01).

The most dramatic changes were recorded in the urea values of Group 1. The values increased from 50,-86 ± 33,31 mg/100 mℓ on the first day to 207,71 ± 64,51 mg/100 mℓ on the third day (P<0,01). The values for Group 2 and Group 3 were within normal limits and showed little fluctuation. Cholesterol values blood sugar levels and serum enzyme activity with the exception of serum alkaline phosphatase (SAP) did not show any significant changes. The SAP values for Group 1 were much higher than the values recorded in the other groups. The third day values of Group 1 animals were significantly (P<0,01) higher than the third day values of Group 2 and the fifth day values of Group 3.

The results for the haematological and blood gas determinations are presented in Table 2. No significant changes occurred in the total white blood cell count. In Group 1 the erythrocyte count haemoglobin concentration and haematocrit level increased significantly (P<0,01) but these values decreased significantly (P<0,01) in Groups 2 and 3. A significant increase in the erythrocyte sedimentation rate (ESR) could only be found in Group 3.

Blood gas determinations did not reveal any significant changes. The blood pH decreased slightly in Group 1 animals and negative base excess values (BE) were recorded for Group 1 animals whereas positive values were determined for Groups 2 and 3.

DISCUSSION

The results indicated that a significant incidence of hyponatraemia hypochloraemia and uraemia occurred in those baboons that succumbed to the effects of dysentery. Similar but not significant findings were also recorded in Groups 2 and 3. The considerably higher potassium values recorded in Group 1 animals could be explained by tissue destruction due to the colitis and dehydration due to fluid loss. Similar increases occurred in the red blood cell count haemoglobin concentration and haematocrit level. This is in contrast to the marked decreases in these values which occurred in the surviving animals. The lower haematological values could be attributed to the blood loss during the course of the disease.

The electrolyte changes in Group 1 animals were in a direction opposite to the changes in the haematological values. The decrease in the electrolyte values was therefore a reflection of the severe faecal and electrolyte losses which resulted from the severe dysentery. In this group the loss of erythrocytes in the faeces was masked by the dehydration resulting from severe fluid loss.

The significant increase demonstrated in the serum urea values was probably due to the lowered excretion by the kidneys as a means to conserve water. The ureamia in man is said to develop owing to an increased back diffusion of urea from the renal tubules which contain a scant volume of concentrated urine. The catabolism due to a negative protein balance may also have contributed to increased urea formation in the liver. Fisher[1] also demonstrated significantly higher blood ureas in dying calves than in normal and diarrhoeic calves that recovered from the disease.

The animals showed little alterations in their acid-base balance. The slight decrease in pH, carbon dioxide content and serum sodium value and the negative base excess values were characteristic of a compensated acidosis. Fisher[1] stated that an uncompensated acidosis is produced only in the very young animal. Although death results from diarrhoea in older animals uncompensated acidosis is not a feature.

From the above findings it is evident that the substitution of electrolyte losses and the control of blood loss are of utmost importance in the treatment of diarrhoeic baboons to minimize losses.

ACKNOWLEDGEMENTS

The technical assistance of the staff of the Primate colony and members of the Departments of Chemical Pathology and Haematology at Tygerberg Hospital, Tygerberg, is gratefully acknowledged.

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TUBERCULOSIS IN LABORATORY ANIMALS

P. B.FOURIE and H. H. KLEEBERG

ABSTRACT: Fourie P. B., Kleeberg H. H. **Tuberculosis in laboratory animals.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 219–221 (En), Tuberculosis Research Institute, Private Bag X385, 0001 Pretoria, Republic of South Africa.

Tuberculosis can cause great losses in captive colonies of various animal species. In South Africa the culprit is the human type of tubercle bacillus. Interspecific transmission of tuberculosis infection amongst laboratory animals, notably primates, is known to occur, and often handlers and caretakers act as the source of infection. The need for preventive measures in laboratory colonies and procedures for case-finding and treatment of tuberculous animals are discussed. Indiscriminate destruction of diseased animals is opposed. The South African situation as revealed by questionnaire survey is described.

INTRODUCTION

In many countries tuberculosis in animal colonies remains a major problem. It presents a health risk to humans, creates enormous financial losses and jeopardises costly experiments in the process. In the United States of America, for example, an estimated $443 200 is expended annually on readily identifiable costs related to detection and control of tuberculosis in nonhuman primates and their handlers[6]. This paper deals mainly with tuberculosis in those animal species used as standard biological models in research laboratories and which are known to be susceptible to natural infection with tubercle bacilli. The importance of adequate control measures is stressed and the effectiveness of diagnostic procedures is evaluated.

PREVALENCE OF TUBERCULOSIS IN LABORATORY SPECIES

Tuberculosis occurs in representative species of all vertebrate classes. The incidence in fish, amphibians and reptiles, however, is negligibly low, while birds and particular mammals show high susceptibility to infection.

The three mycobacterial species responsible for tuberculosis in animals are *Mycobacterium tuberculosis* (naturally maintained by humans and monkeys), *M. bovis* (maintained by cattle and buffalo) and *M. avium* (maintained by the domestic fowl)[8]. All 3 species can incidentally be hosted by almost any mammalian species, including man.

Primates

Despite the absence of tuberculous infections in primates in their natural environment there can be little doubt that the monkey in captivity is the most susceptible of all animals to tuberculosis. Incidence figures of 1,7%[6 20] and 1,9%[7] tuberculous infections in captive colonies have been reported. Infections due to *M. tuberculosis* account for 73%[11] to 79%[6] of cases. It is obvious that the threat of laboratory animals as a reservoir for human infections cannot be disregarded. Epizootics of tuberculosis in primate colonies usually reach dramatic proportions before they are discovered and brought under control. Typical examples are outbreaks which respectively involved 31% (25/80), 27% (61/230) and 28% (41/149) of animals in 3 colonies[5 7 9]. Of 55 institutions surveyed in the USA[6], 24 reported a total of 324 cases of simian tuberculosis in 1972, including 8 outbreaks ranging in size from 5 to 92 cases in single animal groups.

South Africa has a relatively low incidence of primate tuberculosis, as judged by way of a questionnaire survey. Out of 20 institutions, 7 house or use primates in large numbers. Only one outbreak, involving 59 cases in a colony of 121 vervet monkeys *(Cercopithecus aethiops)* occurred in recent years. Two other confirmed cases in a vervet monkey zoo colony of 20 were reported. *M. tuberculosis* infection accounted for 98% (60/61) and *M. avium* for 2% (1/61) of cases.

Although approximately 50% of primates used in South African research institutions are baboons *(Papio ursinus),* an extremely low tuberculosis incidence rate is evident. Out of 8 000 baboons necropsied over 9 years by one laboratory, only one was tuberculous. One case from a colony of 98 animals of at least 7 years' standing was reported. Necropsies on 100 and 150 baboons respectively revealed no tuberculous lesions. Collectively taken over 9 years, it represents an annual attack rate of 0,003% (2/8 348). No information on the mycobacterial species involved was available.

Carnivores

Snider[15] reviewed the world literature published between 1890 and 1969 on tuberculosis in canine and feline populations. Necropsy accounts disclosed prevalences that ranged from 0,1% to 13,5% in the dog and from 10% to 13,2% in the cat. The dog is equally susceptible to bovine and human tubercle bacilli while the cat shows a much greater susceptibility to *M. bovis* than to *M. tuberculosis.* Carnivores are known to contract tuberculosis from humans easily, while transmission of human or bovine tubercle bacilli from carnivores to man is extremely rare. None of the South African institutions questioned had any tuberculosis experience in their carnivore populations.

Other Laboratory Species

In spite of their large-scale usage as laboratory animals, rabbits, hamsters, mice and rats rarely have natural tuberculous infection. No reports of tuberculosis in any of these species in South African laboratories were received.

Avian tuberculosis has been reported in so many species of wild birds as to suggest that none is immune. Birds seldom form part of an experimental colony in South Africa and do not present a major threat to the health of other laboratory animals and humans.

PATHOGENESIS OF ANIMAL TUBERCULOSIS

The course of events in tuberculosis is essentially the same for all species hitherto mentioned. The main difference between man on the one hand and non-human

primates, carnivores and birds on the other is that early dissemination to organs other than the lung and progression to tertiary disease in the latter group usually represents the rule, unlike in humans[5 15].

The route of infection with *M. tuberculosis* is usually aerogenous, and less often through ingestion of live bacilli. The opposite holds true for infection with *M. bovis* and *M. avium*.

DETECTION OF TUBERCULOUS CASES

A variety of procedures has been developed and standardised to detect early stages of tuberculosis in humans. These include tuberculin skin testing, radiology and clinical investigations. In addition to these, the active advanced case is confirmed by the demonstration of tubercle bacilli in smears or cultures from bronchial mucus and other excretions. Disease symptoms play an important role in diagnosis. Most of these methods show limited usefulness when applied to animals. Tuberculin skin testing is most commonly used and is based on the delayed type hypersensitivity to tuberculin. Oedema, erythema and induration at the site where the tuberculin was injected 48-72 hours earlier constitutes the tuberculin reaction.

Primates

Clinical signs in primates are not striking until disease is far advanced[18]. Even considerable coughing is not indicative of tuberculosis[11]. Regular screening practises to avoid outbreaks are thus essential.

Much uncertainty exists about the most reliable detection procedures. Radiography and sputum or faecal investigation appear unreliable[11 18]. Serological tests[20 18 3 12] hold promise but need more trials. Factors that could influence the tuberculin reaction, such as diluent[4] and type of tuberculin[10] were also investigated. The tuberculin test, however, must still be considered the most reliable of all available techniques[17].

Carnivores

No reliable results with serologic, tuberculin, radiographic or bacteriologic tests have been described for cats or dogs[15]. In a recent study[16], however, intradermal tuberculin testing of dogs with USDA tuberculin and concentrated strengths of PPD and OT at 250 tuberculin units (TU) were found to be satisfactory antigens. In minks the iodine agglutination test was found to have 93% correspondence with pathologic findings[14].

CONTROL AND TREATMENT OF TUBERCULOSIS

Screening of Animals

The screening method provisionally advocated by the AAZV Committee on Infectious Diseases and Tuberculosis[2] in 1976 is that primates should be tested with 0,1 mℓ old tuberculin injected intradermally in an abdominal site and in one of the eyelids. As far as possible animals must be immobilised. All palpebral tests are read at 24, 48 and 72 hours.

Mulder[11] suggests the use of 0,1 mℓ 1:10 dilution USDA mammalian tuberculin (which represents 15 000 TU mℓ/undiluted) injected intradermally into the eyelid, using a 25- to 27-gauge 1,5 cm needle. The test is read at 24, 48 and 72 hours. A positive reaction is detected by partial to complete closing of the eye. Any degree of reaction is positive. When animals are first acquired they must pass 5 consecutive negative tuberculin tests at two-week intervals. Thereafter they are tested every 3 months. Open colonies are tested once a month.

This last method is preferred here, although the use of 0,1 mℓ human PPD at a strength of 5 000 TU/mℓ would be desirable. It must be emphasised that the reliability of the tuberculin test also depends on the sensitivity of the site, the specificity and potency of the tuberculin, the precision of the injection and of the technique employed and the knowledge and experience of the testing personnel in the interpretation of the reaction.

Chemoprophylaxis and Immunoprophylaxis

Chemoprophylaxis is highly effective and should be considered for all animals which are at risk of being infected with *M. tuberculosis* or *M. bovis*. Its main advantage is that it is immediately effective and its disadvantage is that its protective value ceases the moment treatment is discontinued. Successful chemoprophylaxis also leaves a fully susceptible individual.

A daily dosage of 2,5 mg/kg body mass Isoniazid (INH) was claimed to be sufficient to challenge infection with *M. tuberculosis* in rhesus monkeys provided it is administered in single doses with food in original preparations. Commercially prepared Isoniazid Diet® cannot be recommended as it may fail to produce isoniazid serum concentrations[13].

A daily dosage of 5 mg/kg body mass INH for all laboratory primates and carnivores is regarded as fully protective.

Immunisation with BCG vaccine is effective for only 3 months in primates but is recommended for cats and dogs.

Treatment of Diseased Animals

Even though some authors still advocate the "kill rather than treat" motto[20 11 16] the value of the animal must be taken into account and treatment considered. While killing in exceptional cases is sometimes unavoidable, indiscriminate depopulation measures are unacceptable.

Successful chemotherapy was administered to baboons in doses of 40 to 80 mg/kg INH daily[1], to monkeys in doses of 5 to 20 mg/kg daily[19] and to various Great Apes by a combination of INH, para-amino salicylic acid (PAS) and vitamin B_6[14]. Recently a dramatic response of tuberculin positive and clinically ill monkeys to treatment with a new drug was reported[5]. Combined chemotherapy using streptomycin, phthivazid, and a preparation named OHA-5 (N-amino-acetyl-N^1-isonikotinoil-hidrazin-dihidrobromid), showed a high therapeutic effectiveness when it was given to 8 sick monkeys, including 2 cases with early cavernous tuberculosis. The combination of INH at 10-20 mg/kg with the antibiotic Rifampicin at 10-15 mg/kg is considered to be bactericidal.

Additional Preventive Measures

The safeguarding of animal caretakers against infection from diseased animals, and vice versa, is no great

undertaking. A regular twice-yearly screening programme for personnel in contact with animal colonies should consist of a chest X-ray and an intradermal tuberculin (Mantoux) test using 2 TU PPD. Tests are read at 72 hours and any induration over 10 mm regarded as positive. Suspects are further investigated by sputum culture and treated. Negative reactors are immunized. It must be stressed that a known case of tuberculosis is better than an unknown one. An infected person is safe to employ provided he is regularly examined. As far as possible, an authoritative person at the laboratory should be responsible for ensuring that employees take the necessary precautions.

General hygiene in and around the colony is important. Protective clothing, shoes, caps and masks for handlers are desirable. When animals are transported the containers must be sterilised afterwards. The urine, feces and hair must be considered infectious and all living quarters must regularly be cleaned thoroughly.

The South African Situation

In 6 of the 20 institutions surveyed, handlers of colonies wear protective clothing or face masks. Screening of staff for tuberculosis forms regular practice in 14. One institution mentions an annual incidence of 1% open cases detected by regular screening programmes. Quarantine periods ranging from 21-90 days is common practise for all institutions housing primates and SPF colonies. Screening of animals for tuberculosis is by tuberculin testing in 2 institutions and tuberculin testing and culture investigation in another.

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SOME INFECTIOUS DISEASES OF WILD VERVET MONKEYS *(CERCOPITHECUS AETHIOPS PYGERYTHRUS)* IN SOUTH AFRICA

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ABSTRACT: Kaschula, V.R., Van Dellen, A.F., de Vos, V. **Some infectious Diseases of Wild Vervet Monkeys** *(Cercopithecus aethiops pygerythrus)* **in South Africa.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 223–227 (En), National Institute for Virology, Private Bag X4, Sandringham, 2131 Johannesburg, Republic of South Africa.

Our surveys indicate that nearly all batches of vervet monkeys arriving at the National Institute for Virology from various areas of South Africa, are infected with foamy viruses and there is evidence that intra-uterine infection also occurs. Monkeys from certain areas of South Africa are apparently infected with the protozoal parasite *Entopolypoides macaci* in a sub-clinical state which becomes active when the monkeys are splenectomised. Serological studies indicate that infections with schistosomes, tick-bite fever *(Rickettsia conori),* chlamydsiae and occasionally by leptospirae, occur in monkeys in the Kruger National Park. Complement fixation tests for antibodies to *R. mooseri* and *R. prowazeki* were also sometimes positive; a finding of doubtful importance. There was a high percentage with positive antibody tests to chikungunya virus in a certain age group suggesting that there had been an outbreak of this disease in the Kruger Park in the late summer and autumn of 1976. This coincided with a human outbreak. Large numbers of vervet and baboon sera were tested for antibodies against SA 12 virus, a polyoma virus. A high percentage of baboons but only a small number of monkeys was found to be positive and it is concluded that SA 12 is probably a baboon virus which is occasionally transmitted to monkeys by contact. It is pointed out that these studies only indicate diseases from which monkeys recover and they do not indicate prevalence of severe disease causing grave illness or death.

INTRODUCTION

Vervet monkeys *(Cercopithecus aethiops pygerythrus)* are widely used at the National Institute for Virology mainly for poliomyelitis vaccine production and for primary kidney cell cultures used for diagnostic purposes. More than 1 000 monkeys are used annually.

The tissues used for poliomyelitis vaccine production are carefully screened for the presence of contaminating organisms especially viruses. Malherbe *et al*[3] have described the 15 different viruses they encountered in such examinations. While most viruses are rare contaminants, foamy viruses are frequently present and cause a serious loss of poliomyelitis vaccine. During the period, 2 February 1972 to 20 June 1973, some 240 tissue batches were examined in the Safety Testing Section of the Institute, then the Poliomyelitis Research Foundation. One hundred and four batches were found contaminated with foamy viruses (43%) and therefore rejected on the first examination. Further tests revealed an additional number of contaminant foamy viruses so that the final amount of vaccine discarded because of this contamination was 60%. There was a general impression that when monkeys arrived at the Institute and were kept in close confinement, this favoured the spread of foamy viruses. This then became a subject of investigation, and monkeys arriving at the Institute were examined for the presence of foamy viruses. Later an opportunity arose to conduct a study of the diseases of monkeys in the field in the Kruger National Park (KNP) when monkeys were captured and autopsied locally without the stress of a long journey or confinement. The results are the subject of this report, but a fuller report will be made by Van Dellen *et al.* on the spectrum of all diseases affecting monkeys in the KNP.

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METHODS AND MATERIALS

Collection of monkeys

Field trapped monkeys, caught in the wild in many different areas of South Africa, were brought to the Institute either by train or truck. Throat swabs were taken at the time of arrival and they were then housed in their respective groups for 3 months or more before being used. They were screened for tuberculosis infection. During a period of 15 years only one has been found to be infected with *Mycobacterium tuberculosis* and this infection was traced to contact with an infected keeper.

Those trapped or drugged in the KNP were caged and brought to the laboratory at Skukuza where a comprehensive examination was made and an autopsy done within a few days. Thus far 50 monkeys have been examined. Swabs were taken from the throat and rectum and organ specimens were suspended in antibiotic solutions before centrifugation and seeding of material into Vero and RK 13 tissue cultures containing coverslips. After 14-15 days coverslips were stained and examined for cytopathic effects.

In addition to these routine tissue culture procedures nerve tissues were examined for the presence of herpes viruses. A pool of cranial cervical and lumbar ganglia, brain and lumbar cord tissue was made and the material was injected intradermally into rabbits and in tissue cultures.

Some duodenal tissues were also collected in transport media and examined by Dr. B. Schoub for the presence of rotaviruses (SA 11) by indirect immunoflourescence and tissue culture methods. All these tests for herpes and rotaviruses were negative.

Serological Tests

The examination of serums was an important part of this investigation. Monkeys were exsanguinated, the sera frozen at -20°C and processed for serology. The reason for the large number of anti-complementary reactions is not understood but sera had sometimes been stored at -20°C for 4-6 weeks before being examined. H.I. (Haemagglutination-inhibition) tests for arbovi-

ruses were done at the National Institute for Virology by Dr. B. M. McIntosh as follows: The antigens of all viruses were prepared from infected baby mouse brain excepting Rift Valley fever which was prepared from infected adult hamster liver. These antigens were diluted in a mixture of bovine plasma albumin and borate saline buffer at pH 9,0. The erythrocytes used were male goose red cells at pH 6,3 for Sindbis, Chikungunya and Banzi, at pH 6,4 for Middelburg, at pH 6,6 for Wesselsbron and Yellow fever and pH 5,7 for Rift Valley fever. The tests for parasitic diseases were done by Dr. B. Wolstenholme of the Tropical Diseases Department of the S.A. Institute for Medical Research. Some of the pertinent data are given in Table 1.

Table 1: SOME OF THE ANTIGENS USED IN THE TESTS FOR PARASITIC DISEASES

Disease	Test	Antigen	Source
Bilharzia	CFT	cercaria of *S. mansoni*	SAIMR
		schistosomes of *S. mansoni*	
		ova of *S. mansoni*	
	FAT	cercaria of *S. mansoni*	SAIMR
		antihuman fluorescent antibody	Wellcome commercial
Malaria	FAT	*P. falciparum* in human blood	SAIMR
		antihuman fluorescent antibody	Wellcome commercial
Amoebiasis	FAT	unknown strain of amoeba	Wellcome commercial
		antihuman fluorescent antibody	Wellcome commercial
Trypanosomiasis	FAT	*T. brucei* in bovine blood	SAIMR
		antihuman fluorescent antibody	Wellcome commercial
Toxoplasmosis	CFT	*T. gondii* in egg yolk sac	SAIMR
	FAT	*T. gondii* mouse exudate or VK TC	SAIMR
	SFD	Sabin-Feldman dye test using mouse exudate	Wellcome commercial
Epidemic typhus	CFT	*R. prowazeki* in egg yolk sac	SAIMR
Murine typhus	CFT	*R. mooseri* in egg yolk sac	SAIMR
Tick-bite fever	CFT	*R. conorii* in egg yolk sac	SAIMR
Q fever	CFT	*C. burnetti* in egg yolk sac	SAIMR
Chlamydia group	FAT	chlamydia organisms in yolk sac	SAIMR
		antihuman fluorescent antibody	Wellcome commercial
Leptospirosis	CFT	*L. canicola* in vitro culture	SAIMR
	CFT	*L. icterohaemorrhagiae in vitro* culture	SAIMR
	CFT	*L. pomona in vitro* culture	SAIMR

Abbreviations used:
CFT = complement fixation tests
FAT = fluorescent antibody technique
SAIMR = South African Institute for Medical Research, Box 1038, Johannesburg
Wellcome commercial = Wellcome Reagents Ltd., Beckenham, England

Baboon sera were obtained from 4 farms in the Mica area of the north-eastern Transvaal and from the Western Cape and tested together with a large number of vervet sera at John Hopkins University, Baltimore, maryland, by immuno-fluorescence and neutralization tests[5].

RESULTS

Viruses

(a) *Foamy viruses* To test the theory that foamy viruses were largely disseminated in monkeys after their arrival at the Institute and when kept confined for periods of several months, a project was started in March 1975, in which a representative sample of 5 monkeys in each batch on arrival from the field was tested for foamy viruses by examining throat swabs from them. Details of the tests done are given in Table 2.

Table 2: ISOLATIONS OF FOAMY VIRUSES FROM THROAT SWABS OF VERVETS TAKEN ON ARRIVAL FROM THE FIELD

Batch No.	Source	Date	No. in batch	Contam.	Positive	Negative
1	Bedford, C.P.	24.3.75	5	0	2	3
2	Brits, Tvl.	7.4.75	5	R5R	0	5
3	Buffelsdrift, Tvl.	4.4.75	60	1	3	1
4	Vaalwater, Tvl.	10.4.75	10	0	2	3
5	Bedford, C.P.	25.4.75	5	0	2	3
6	Bedford, C.P.	5.5.75	5	0	2	3
7	Bulge Rivier, Tvl.	9.5.75	13	3	1	1
8	Buffelsdrift, Tvl.	9.5.75	84	2	3	0
9	Buffelsdrift, Tvl.	23.5.75	80	0	3	2
10	Buffelsdrift, Tvl.	4.6.75	49	0	2	3
11	Buffelsdrift, Tvl.	4.6.75	50	0	2	3
12	Buffelsdrift, Tvl.	4.7.75	59	1	4	0
13	Bothaville, O.F.S.	1.8.75	5	1	0	4
14	Bothaville, O.F.S.	1.8.75	11	3	0	2
15	Skukuza, K.N.P.	22.7.76	12	0	4	8
16	Skukuza, K.N.P.	7.11.76	12	0	3	0
17	Skukuza, K.N.P.	28.3.77	12	0	5	7
18	Letaba, K.N.P.	11.7.77	7	0	1	6
19	Pafuri, K.N.P.	11.7.77	5	0	1	4
			489	16	42	67

K.N.P. = Kruger National Park
R = repeated

The results given in Table No. 2 indicate that only monkeys from Brits (Batch 2) and Bothaville (Batches 13 and 14) were negative. The swabs from the Brits monkeys however, had been stored for 2 weeks at -20°C and re-examined because of contamination. This may have resulted in loss of virus. The Bothaville monkeys, however, seemed to be completely foamy virus-free. All the Bothaville monkeys were again tested after 40 days in isolated confinement and were found to be still free from the viruses.

It therefore appears that monkeys mostly arrive infected and that spread within the quarantine quarters

does not play an important role in the prevalence of the virus. It is possible, however, that the stresses caused by capture, travel and confinement may play some role in the potency of the virus.

Peries & Todaro[4] reported on a foamy virus isolated from the placenta of a rhesus monkey. The isolation from this site was interesting and in July 1977, when an opportunity arose to examine the foetuses of 3 pregnant monkeys we were able to isolate a foamy virus from the placenta and amnion of a 6 weeks old foetus. This is positive proof that at least in some cases intrauterine transmission of infection occurs. This aspect of the epizootiology will be examined later.

It therefore seems that foamy virus infection are acquired in the field and that at least in some cases the transmission takes place before birth.

(b) *Other viruses* Only one other virus was isolated from the 50 monkeys tested in the KNP. This was an adenovirus isolated from a throat swab, while 14 isolations of foamy viruses were made. No other viruses were isolated.

Serological studies for the presence of various arbovirus H.I. antibodies are listed in Table 3.

is probably a common non-lethal viral infection of baboons *(Papio ursinus)* since a high percentage of all baboons tested in the Mica area of the north-eastern Transvaal and the Western Cape had antibodies and infection in monkeys is acquired by contact with baboons.

A discussion of natural monkey diseases should mention Marburg virus or the related Ebola virus which has been associated with these animals elsewhere. Over a long period of time thousands of monkeys have been examined by many people in South Africa but these viruses have never been encountered as natural diseases of monkeys in South Africa and their carrier hosts have yet to be found.

2. Protozoal and other diseases

During routine splenectomy of vervets for the purpose of cultivating *Plasmodium fieldi* another contaminating blood parasite was encountered.

In this procedure monkeys were splenectomised and then infected with *P. fieldi* after a period of a month or more. The appearance of a preponderance of ring-form parasites in the blood of 3 of these monkeys from

Table 3: H.I. TITRES OF VERVET SERA AGAINST 8 ARBOVIRUSES

Virus	Neg.	H.I. titres of sera 1/10	1/20	1/40	1/80	1/160	1/320	1/640	Interpretation
Sindbis	54	1	–	–	–	–	–	–	All negative
Chikungunya	28	1	1	2	12	8	2	2	26 pos./28 neg.
Middelburg	36	8	12	–	–	–	–	–	12 trace
Banzi	50	3	2	–	–	–	–	–	2 trace
Wesselsbron	52	3	–	–	–	–	–	–	All negative
West Nile	51	1	–	–	–	–	1	–	1 pos./52 neg.
Yellow fever	55	–	–	–	–	–	–	–	All negative
Rift Valley fever	55	–	–	–	–	–	–	–	All negative

The above results indicate that nearly 50% of monkeys showed positive titres to Chikungunya virus. The first 4 monkeys captured in January 1976 did not have antibodies. Also young monkeys about 6 months of age captured in July 1977, were negative, but most adult monkeys captured in the period July 1976 to July 1977 were positive. It therefore seems that there had been an epizootic in the lowveld in the late summer of 1976. This corresponds to the epidemiological evidence collected by Dr. B.M. McIntosh from the National Institute for Virology, who showed that there had been an outbreak of Chikungunya in both man and baboons in the Mica area near Phalaborwa in the later summer of 1976 (unpublished results). Our survey in monkeys indicated that the epizootic had occurred in all areas of the KNP from Skukuza to Pafuri in the far north. One monkey also exhibited high antibodies to West Nile virus.

The serums of these monkeys from the Kruger Park and elsewhere in South Africa as well as baboons from 2 areas of South Africa, were tested for antibodies against SA 12 which has been characterised to be an SV-40 related papovavirus probably of baboon origin[5]. Most monkeys were negative except those from an area near Thabazimbi in the north-western Transvaal which had antibodies but in this case there was close contact with wild baboons. It has been concluded that this virus

October 1974 to February 1975, after malaria infection caused suspicion that another parasite may also have been present. Therefore in the case of the next 4 monkeys which were splenectomised in March and April 1975, before infection with *P. fieldi,* a careful study was made of the blood picture and it was found that in all cases a babesia-like parasite began to appear 7-10 days after splenectomy. The parasitaemia became very severe in 2-3 months when it was nearly 100% and they then recovered after several months.

These monkeys originated from the Buffelsdrift area of the Limpopo Valley of the north-western Transvaal and later another one monkey out of 6 from the Skukuza area of the KNP developed a parasitaemia of the babesia-like organism which then became severe after splenectomy as in the other cases mentioned. Two monkeys from Vaalwater, western Transvaal, and 2 from Phalaborwa, north-eastern Transvaal, which were splenectomised did not develop this parasitaemia. This parasite is apparently similar to or perhaps identical with that described by Hawking[1] who named it *Entopolypoides macaci (cercopitheci)* and the condition in South Africa has been reported by Fripp *et al.*[2].

Large numbers of blood smears from vervets have been examined and found to be negative except the one monkey from Skukuza mentioned above which was positive before splenectomy. Unsuccessful attempts

Table 4: ANTIBODY LEVELS AGAINST PARASITIC INFECTIONS

Disease	Test	AC	0	+	++	+++	++++	Interpretation
Bilharzia	CFT	28	8	9	2	2	2	6 positives
	FAT	—	32	10	1	1	—	2 positives
Malaria	FAT	—	42	6	—	—	—	6 trace
Amoebiasis	FAT	—	41	6	1	—	—	6 trace, 1 suggestive
Trypanosomiasis	FAT	—	40	6	—	—	—	6 trace
Toxoplasmosis	FAT	—	39	7	1	—	—	7 trace, 1 positive
	SFD	—	48	—	—	—	—	All negative
	CFT	39	11	3	—	1	1	3 trace, 2 positives

Abbreviations used:
CFT = complement fixation test
FAT = Fluorescent antibody test
SFD = Sabin Feldman dye test
AC = anti-complementary

were made to develop an FA test using hyper-immune serum from rabbits and positive monkeys. The study of this disease has therefore been handicapped as there was no serological test available. It is thought that monkeys acquire the infection at an early age probably from tick-bites and a carrier state develops. It appears to be a non-lethal infection.

In the survey on monkey diseases being conducted in the KNP active diseases were rare but serological studies showed that the monkeys had been exposed to a number of infections during their lifetime. The results of studies of antibodies against parasitic infections are given in Table 4 and those against rickettsiae, chlamydiae and leptospirae are given in Table 5.

The studies of antibody levels given in Table No. 4 indicate that there was considerable exposure to schistosomes, probably from cercaria of infections in other animals such as the hippopotomi, antelopes, birds, etc. No active schistosomiasis was seen in any of the monkeys autopsied. There was probably also occasional exposure to toxoplasmosis carried by other animals. As the level of antibodies to other parasitic diseases was only at a trace level it can be concluded that the exposure was only slight.

Antibody levels against rickettsiae, chlamydiae and leptospirae listed in Table No. 5 suggest also that monkeys in nature are exposed to a number of these infections during their lifetime. It is not surprising that most monkeys had been infected with tick-bite fever *(R. conorii)* probably at an early age, but this organism probably causes only a transient infection which gives a lifelong immunity. The 2 positive cases for epidemic typhus *(R. prowazekii)* and the one for *R. mooseri* indicates that there had been exposure to this organism or one closely related immunologically. The large number of positives to chlamydiae indicate that there is a source of contact with this organism which may be wild birds or their eggs harbouring psittacosis, antelopes which may be suffering from an ovine-abortion-like disease or related diseases. It did not appear to be a primary disease of monkeys. The low level of Q-fever *(Coxiella burneti)* antibodies is surprising as this is prevalent in some other animals.

Similarly the low level of antibodies against leptospirae was unexpected as these organisms are likely to be present in certain species of animals. The low incidence can probably be explained by lack of contact with carnivores and rodents which are probably the most frequent carriers.

Wild vervet monkeys are generally healthy animals especially under the good conditions in which they live in the Kruger National Park. Although serological evidence suggests that during their lifetime they become exposed to a number of diseases, probably mostly of a non-lethal type, these diseases appear to be of a transient nature and the monkeys recover from them without becoming seriously ill.

When these animals become seriously ill they cannot

Table 5: ANTIBODY LEVELS AGAINST RICKETTSIAE, LEPTOSPIRAE AND CHLAMYDIAE

Disease	Test	AC	0	+	++	+++	++++	Interpretation
Rickettsiosis:								
R. prowazeki	CFT	22	30	2	–	1	1	2 trace, 2 positives
R. mooseri	CFT	22	31	2	1	–	–	2 trace, 1 positive
R. conorii	CFT	22	0	5	1	9	19	5 trace, 29 positives
C. burnetti	CFT	22	30	2	–	–	–	2 trace
Chlamydia group	CFT	19	17	2	4	5	4	2 trace, 13 positives
Leptospirosis								
L. canicola	CFT	20	14	1	–	–	–	1 trace
L. ecterohaemorrhagiae	CFT	20	14	–	–	–	–	All negative
L. pomona	CFT	20	8	2	–	–	1	2 trace, 1 positive

Abbreviations used:
CFT = complement fixation test
AC = anticomplementary
0 = negative
+ = trace
++ = highly suggestive
+++ = positive
++++ = strongly positive

survive attacks from predators or other enemies, even their own kind, and only the strong and healthy will survive. These survivors are the animals which we received for examination.

If the carcases of sick, dying or dead animals could have been examined then probably many more and different diseases would have been encountered but under natural conditions this is almost an impossibility. Therefore a survey such as this, in which diseases in healthy animals are studied, will reflect only the mild diseases from which the animals recover or from which they do not become seriously ill. In general it does not give much information about the diseases which they have but rather about those to which they have been exposed to and from which they have recovered.

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LETTER TO THE EDITOR — BRIEF AAN DIE REDAKSIE

CANINE SYSTEMIC LUPUS ERYTHEMATOSIS

Sir,

An 8 year old Golden Cocker Spaniel was presented with acute and complete anorexia, panting and a temperature of 39,5°C. Pain was present on deep abdominal palpation and the dog walked with difficulty, seeming sore on both front legs. There appeared to be hepatomegaly and splenomegaly. Differential white blood cell count revealed neutrophilia. A blood smear was negative for *Babesia canis* parasites.

Treatment with a potentiated sulphonamide* was instigated together with 20 mg prednisolone intra-muscularly. Response was reported to be good initially but a relapse 5 days later occurred. The anti-microbial drug was changed to lincomycin• together with 20 mg prednisolone intra-muscularly on the first day. Response was again very good but a second relapse occurred 6 days later. Epistaxis of 24 h duration was observed at this stage. The patient was hospitalized for further tests.

Urine analysis revealed severe proteinuria. Other urine tests were negative. On the history, presentation of signs and symptoms, response to therapy and a process of elimination, a preliminary diagnosis of systemic lupus erythematosis (SLE) was made and blood taken for further tests. The sheep cell agglutination test (SCAT) and latex test were both negative for Rheumatoid Factor. The antinuclear antibody test (ANA) was also negative. Anti-DNA activity was tested at 220 μg DNA bound/mℓ serum (normal range 0-5 μg). This confirmed the diagnosis of SLE and the patient was discharged on a daily oral dose of 15 mg prednisolone. This was reduced within 2 weeks to 5 mg daily. The dog has remained stable for 4 months, active and eating, except for 2 flare-ups which occurred at intervals of 2 weeks. The relapses were treated successfully with an additional 5 mg prednisolone for 2 days.

The case is interesting in that it differs somewhat from those described by Moore[1]. Difference include: (1) Epistaxis. Thrombocytopoenia was seen in 2 cases described by Moore but was not associated with haemorrhage or bruising as described by Hall[2]; (2) Positive ANA latex tests were obtained in all the cases described by Moore. In this case the ANA latex test was negative; (3) Except for Case no. 2, all dogs treated by Moore recovered within 4 weeks of the initiation of treatment. This case has presented us with symptoms for more than 3 months and relapses occur as soon as medication is decreased or discontinued.

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ACKNOWLEDGEMENTS

Prof. Meyer and staff of the Clinical Immunology Laboratory of the Medical School at Groote Schuur are thanked for their interest and the haematological determinations.

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*"Bactrim", Roche
•"Lincocin", Upjohn

WILD RODENTS AS LABORATORY MODELS AND THEIR PART IN THE STUDY OF DISEASES

H. J. KEOGH and M. ISAÄCSON

ABSTRACT: Keogh H.J., Isaäcson M. **Wild rodents as laboratory models and their part in the study of diseases.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 229–231 (En), Department of Epidemiology, SAIMR, Box 1038, 2000 Johannesburg, Republic of South Africa.

This paper describes the use of 14 South African wild rodent species as experimental models and demonstrates the proven value of many of these as laboratory animals in research on bacterial infections (plague, relapsing fever), rickettsial infections (tickbite fever, louse typhus), viral infections (poliomyelitis, Rift-Valley fever and other arbovirus infections, Lassa fever), fungal infections (histoplasmosis), parasitic infections (schistosomiasis) and in diabetes mellitus and cancer research.

INTRODUCTION

Dr. D.H.S. Davis established stocks of laboratory adapted wild rodent species, and the first colony of rodents he reared successfully at the South African Instutute for Medical Research was that of the multimammate mouse, *Mastomys natalensis* (A. Smith), in 1939[1]. Since then our ecology laboratory has always been on the look-out for small wild mammals for experimental purposes and many of these have played a rewarding part in medical research[2 3 4 5 6 7].

The maintenance of wild rodent colonies

The following species have been successfully bred in the SAIMR animal house: *Aethomys chrysophilis, Aethomys namaquensis, Thallomys paedulcus, Mastomys natalensis, Rhabdomys pumilio, Mus minutoides, Saccostomus campestris, Malacothrix typica, Mystromys albicaudatus, Tatera afra, Tatera brantsi, Tatera leucogaster, Gerbillurus paeba, Desmodillus auricularis.*

Characteristic features of each of these 14 species are summarised in Table 1. The colonies are housed in temperature controlled rooms (21°C). There is no special lighting control and animals are, if necessary, moved into cages with controlled lighting cycles. Metal cages measuring 30 × 15,5 × 12,5 cm are used for breeding. Sawdust and woodwool are supplied as bedding although cotton wool is used for some of the smaller species. Modern caging facilities are not always conducive to successful breeding of wild rodents. Some admit too much light for nocturnal species, and cages with rungs over removable trays are a hindrance to nesting for some wild species. Standard mouse cubes and weekly carrots, cabbage and potatoes, form the basic diet which is varied or supplemented according to the feeding needs and habits of each species (see Table 1). Monogamous mating is used, but to establish newly adapted species other ways of forming breeding units may be necessary.

LABORATORY USES AND EXPERIMENTAL RESULTS

Mastomys Natalensis

Plague: The role of *Mastomys* as a source of plague to man from wild rodents in South- and East Africa is well known[9]. Hallett (1971) showed its value in studies on plague vaccines[10].

Cancer: *Mastomys* has proved especially useful in cancer research since a spontaneously occurring tumour was first demonstrated in 1954 in the SAIMR *Mastomys* colony[6].

Bilharzia and Relapsing Fever: *Mastomys* is highly susceptible to experimental infection with *Schistosoma mansoni*[8], and with *Borrelia duttoni*[12]. Banzi virus was isolated from *Mastomys* and it was shown to have antibodies to this virus and to the Witwatersrand virus. From this and other data it is concluded that wild rodents are the vertebrate maintenance hosts of these viruses[13].

Lassa Fever: *Mastomys* is assuming greater importance than before in research on the arenaviruses since its recognition as the reservoir host of Lassa fever in 1972[14 15].

Mystromys Albicaudatus

Mystromys needs careful handling but is an excellent laboratory animal because of its longevity and suitability for long term experiments. It was used as a passage animal in the early attempts to attenuate poliovirus[16]. It has proved highly susceptible in both direct cave exposure tests and for indirect soil sample isolation of *Histoplasma capsulatum* (SAIMR report 1960, R. Borok pers. comm.). Spontaneous diabetes developed in a colony of *Mystromys* in the U.S.A[17] and it is used in diabetes mellitus research at the Cape Town Medical School.

Saccostomus Campestris

S. campestris is used in bilharzia research and was the only field rodent to die when tested for susceptibility to Rift Valley fever. McIntosh showed that strains of the Banzi virus circulate at high concentration in the blood of this species.

The Gerbils

The *Tatera* species and *Desmodillus* are docile and easy to handle but do not breed very readily in the laboratory. *D. auricularis* has been successfully bred when given a 16 hour light regime[18]. It has been used in tick bite fever studies, and shows partial resistance to *Yersinia pestis,* while *T. brantsi* and *T. afra* were used during World War II for typhus vaccine production[3].

Aethomys Chrysophilus

The African bush rat breeds freely in the laboratory, although it is not easy to handle. *A. namaquensis* has

Table 1: DISTINCTIVE FEATURES AND LABORATORY BREEDING NOTES ON 14 SPECIES OF RODEN

Species	Lab. handling	Lab. breeding	Colouring and other distinctive features	Food and bedding requirements* (water available at all times)
MURINAE *Aethomys chrysophilus*	F	G	Underparts covered in grey-based hairs and not pure white. Upperparts vary but generally reddish-brown or reddish yellow with white feet. Tail about half overall length.	Standard mouse cubes and greens once a week. Thrive in normal laboratory conditions. Once breeding has become established make excellent laboratory animals.
Aethomys namaquensis	F	P	Underparts pure white, but not always: with trace of grey-based hairs on upper chest and flanks. Dorsally brownish grey with rufous colouring on flanks. Scales on tail closely spaced. Tail longer than ½ overall length.	Standard mouse cubes and greens once a week. Become laboratory adapted but no litters reared at SAIMR animal house.
Mastomys natalensis	F	G	Underparts dark grey, with individual hairs grey based with whitish tips. Upperparts dark grey with brownish tinge. Tail approximately half overall length. Finely ringed with scales.	Thrive on standard mouse cubes and greens once a week. Become readily adapted to laboratory conditions.
Thallomys paedulcus	P	P	Pure white underparts, pale yellowish or greyish-yellow upperparts. Dark patch between nose and eye continuing as a dark line towards base of ears. Tail longer than head and body. Dark coloured. Arboreal in nature.	Standard mouse cubes and greens as food. Require tree leaves for nesting material. Difficult to handle but become adapted to laboratory conditions. Should be housed in large type cages.
Mus minutoides	G	F	Pure white underparts. Upperparts vary from reddish brown to buffy brown. Small size of adult distinguishing feature. Tail less than half overall length.	Thrive on bird seed and greens. Seem to breed best when housed in colonies, not pairs, in wooden boxes with wire-mesh tops. Cotton wool makes ideal nesting material.
Rhabdomys pumilio	P	P	Underparts yellowish white or grey. Easily identified by four dark stripes running from head to base of tail. Tail dark on top, lighter underneath. Diurnal.	Standard mouse cubes and greens as food. Tend to moult dorsally if not supplied regularly with grass nesting. Lively and best handled in large steep sided container.
Saccostumus cumpestris	G	G	White underparts. Upperparts vary from dark grey to light grey. Characteristic short tail – about a quarter of overall length. Cheek pouches are distinctive.	Diet of standard mouse cubes and greens supplemented regularly with sunflower seeds. Appear to breed better when cages are placed in lighter parts of the laboratory. Docile and easy to handle.
DENDROMURINAE *Malacothrix typica*	G	P	Underparts pale grey; dorsally varies from yellowish grey to buffy grey. Darker markings distinctive. Ears large. Tail less than half overall length.	Standard mouse cubes and greens supplied but usually only eats sunflower seeds and bird seed. Can be kept in pairs or colonies in wooden boxes. Bedding cotton wool.
CRICETINAE *Mystromys albicaundatus*	G	G	Underparts greyish/white. Upperparts vary from dark to light grey. Individual hairs sometimes black interspersed with grey hairs. White tail less than half overall length.	Thrives on standard diet supplemented with sunflower seeds. Readily eats wheat-germ when added to diet to stimulate breeding.
GERBILLINAE *Desmodillus auricularis*	G	P	Underparts white. Upperparts vary from light greyish to dark grey or rufous colour. White spots behind ears distinctive. Tail shorter than half total length.	Eats mainly sunflower seeds and greens although will take to standard mouse cubes. Seldom has litters in laboratory conditions unless given extra light hours.
Gerbillurus Paeba	G	P	Underparts white. Bright orangy/brown upperparts. Small size of adult distinctive. Tail over half total length. Soles of feet hairy.	Eats bird seed, sunflower seeds and greens. Hoarding instinct is strong. Housed in normal cages but have only bred when given extra light hours.
Tatera brantsi	G	F	Underparts white. Upperparts vary but mostly beigy/brown or darker or lighter brown. Tail lacks scales, hairy, has dark stripe but never to tip. Tail length approximately half total length.	Normal diet of mouse cubes and greens has to be supplemented with sunflower seeds. Should be kept in larger type cages.
Tatera leucogaster	G	P	Underparts white. Upperparts variable from reddish brown to darker brown. Tail striped to tip with terminal tuft of hair. Tail length approximately half total length.	Same as above. Poor breeders in laboratory conditions.
Tatera afra	G	P	Underparts white. Dorsally dark brown or brown with darker hairs interspersed. Tail hairy, half total length.	Same as above. Poor breeders in laboratory conditions.

G: Good F: Fair P: Poor
* Personal observations

not bred freely in the laboratory although it survives well in captivity.

Mus Minutoides and *Malacothrix typica*

These 2 species breed best when housed in wooden boxes, and in colonies rather than pairs.

Rhabdomys Pumilio

This rodent does not breed freely in the laboratory and is active and awkward to handle.

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RODENTS AS LABORATORY HOSTS FOR SCHISTOSOMES

P.J. FRIPP

ABSTRACT: Fripp, P.J. **Rodents as Laboratory hosts for schistosomes.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 233–234 (En), Bilharzia Res. Unit, S.A.I.M.R., Box 1038, 2000 Johannesburg, Republic of South Africa.

The rodent species used at the South African Institute for Medical Research for the culture of mammalian species of schistosomes are listed. Emphasis is given to the advantages of wild South African species which have been adapted to laboratory conditions, and infection techniques are discussed.

For many years the white mouse has held the leading place as the most used rodent for the laboratory maintenance of most species of mammalian schistosomes. A mouse can tolerate the presence of 100-200 adult *Schistosoma mansoni* worms in the mesenteric blood vessels into the chronic condition without overt distress, and can tolerate an even greater number of individuals of the rodent parasite *S. rodhaini* which, when we consider the relative sizes of host and parasite, is quite an achievement.

In a number of laboratories overseas white mouse strains are used exclusively in schistosomicide drug screening tests in which when the parasite is usually *S. mansoni.* They are also very popular in studies of the host-parasite relationship. Thus the mouse-*S. mansoni* combination has become widely used by immunologists who, for example, show that the size of the host's granulomatous reaction around eggs is a measure of the immunological state of the mouse. This type of response does not occur in the hooded rat where an initial high worm burden is almost completely eliminated 10-12 weeks after exposure. Although somewhat out of context, I mention this as it is important to emphasize that the response to a particular species of parasite is a characteristic of the host, and the assumption that 2 host species may respond to the same schistosome species in a similar fashion is often fallacious.

White mice are poor or inadequate hosts for *S. haematobium,* the causative agent of urinary bilharzia in man, and overseas the golden hamster *(Mesocricetus auratus)* usually replaces the white mouse. However the snail hosts are also difficult to maintain in the laboratory, and consequently *S. haematobium* is not as popular as *S. mansoni* as a parasite for experimental work. Moreover as the aim of most laboratory work (to judge by applications for financial support) is the unravelling of a human situation by analogy, the fact that the schistosomes do not inhabit the vesical plexus to the great extent that they do in human (and baboon) infections further reduces the impetus for the study of *S. haematobium* in rodents.

Surveys during the 1950's in East Africa[4], the Transvaal[5] and to a lesser extent, Zaire and Egypt produced records of wild rodents carrying natural infections of *S. rodhaini, S. bovis, S. mattheei, S. mansoni* and, in one instance, *S. haematobium.* The *S. mansoni* infections may or may not have been fortuitous, but the low prevalence of this species in wild rodents in hyperendemic areas strongly suggests that rodents are unimportant as reservoir hosts. The rodents found infected with human schistosomes included the savannah genera *Mastomys* in South and East Africa, *Otomys* (different species in the 2 regions) and the swamp rat *Dasymys* in East Africa.

These findings led to collaboration between the then CSIR (now MRC) Bilharzia Field Unit, Nelspruit, and the Medical Ecology and Bilharzia Research Units of the SAIMR in studies on the susceptibility of South African rodents to South African strains of both human and non-human mammalian schistosomes[3]. A number of rodent species were found to be susceptible to *S. mansoni, S. haematobium* and *S. mattheei,* and a short list of the most susceptible included *Mastomys natalensis* the multimammate mouse, *Saccostomus campestris* the pouched mouse, *Aethomys chrysophilus* the bush rat, *Tatera brantsi* the highveld gerbil, and *Mystromys albicaudatus* the white tailed mouse. The final selection for further studies was then determined more by practical laboratory factors such as ease of breeding and handling, rather than susceptibility to infection.

Before the rodent species is chosen as a laboratory host, the aim of infecting the animal should be considered. First, is it to maintain the life cycle of the parasite when production of live, viable ova is the prime consideration, or secondly, to obtain sufficiently large numbers of adult worms, for instance for further biochemical or histochemical studies or use as an antigen in routine immunodiagnostic procedures or, thirdly, as a host in the study of pathogenetic processes *with reference to the human situation?* To take the last first: white mice, as has been mentioned, produce a response too far removed from what is known of human pathogenic processes to be of much use. *Mastomys* is better in regard to *S. mansoni* infections for the acute disease has features vaguely similar to that of the human disease, but in this context we prefer to use the Vervet monkey *Cercopithecus aethiops pygerythrus* as both acute and the established schistosomiasis *mansoni* closely resembles human disease. Moreover, *Mastomys* has a life span far shorter than the average for the worm which on epidemiological evidence seems to be about 7 years. *S. haematobium* adults tend to inhabit the hepatic portal system of rodents whereas their predilection site in man is the vesical plexus, although some eggs of *S. haematobium* may be found in bladder tissues of golden hamsters.

When large numbers of adult worms are required, *Mastomys* can be used for the majority of species of schistosome parasites of mammals (except *S. haematobium,* where we use *Saccostomus)* as it can maintain as many as 400 developing worms, but harvesting the worms must take place at about 10 weeks after exposure to the cercariae, just as they become mature, otherwise the massive egg-laying programme by the female worms produces pathological responses which rapidly cause the death of the animal. The worms can be easily and cleanly harvested by deeply anaesthetizing the animal with pentobarbitone sodium ("Sagatal",

Maybaker) and heparin, opening the body cavity, inserting a needle into the descending aorta and perfusing the viscera with warm citrated saline. The worms are washed out of a cut made in the hepatic portal vein into a urine flask. This technique was originally developed by Duvall & de Witt for white mice[1], but can be used with equal facility on *Mastomys* and the larger *Aethomys chrysophilus* and *Saccostomus* although a larger needle – an 18 rather than 21 gauge – may be needed. Perfusion of golden hamsters is less successful without ligating some of the major vessels first.

When maintenance of the parasite life cycle is the prime consideration, the worm burden and hence the egg production must be reduced to a level at which the infection can be tolerated by the rodent, whilst ensuring that an adequate number of eggs can be harvested. The number of infecting cercariae should not exceed about 250 per rodent. In white mice where large numbers of viable eggs of *S. mansoni* and *S. rodhaini* are excreted in the faeces, the faeces can be collected *en bloc* in a plastic tray containing saline placed under a communal cage[2] and the miracidia hatched from them preferably with the aid of a special side-arm conical flask. With other combinations of species of host and parasite, eggs tend to be trapped in the liver and intestinal wall and the usual procedure is to kill the rodent 12-14 weeks after exposure to cercariae and hatch the eggs from the macerated viscera.

The initial screening of possible schistosomicides takes place with rodents, often in acutely infected animals. The response by the parasite to the drug in the acute phase, whilst it is asserting its position, may be different from that in the chronic phase, when the invasion has consolidated and the host-parasite relationship is more balanced. Therefore, candidate schistosomicides should preferably be assayed on animals with chronic as well as acute infections, although financial considerations usually prevent this. It should be noted that drugs may affect some parasite species differently in different host species. Notable in this regard are the diphenoxy alkanes, which were found to be most efficient destroyers of *S. mansoni* in white mice[6], but had a negligible effect on the same parasite strain in monkeys.

Infecting the rodents is not difficult and no special apparatus is needed. If the rodents have long tails, the rodents can be restrained in a small cylindrical cage of wire mesh and the tails allowed to dangle in a suspension of cercariae[2]. However, adequate infection rates can be obtained by letting the rodents paddle in the infected water for about 45 minutes. There does not seem to be any loss of infection rates if the rodents excrete in the water. We usually infect rodents individually in commercial 500 g "honey jars" but batch infections give surprisingly even rates of infection.

At the present time our laboratory uses *Mastomys* and *Aethomys chrysophilus* for the bulk production of adult *S. mansoni* worms, and *Mastomys* for the routine maintenance of *S. mansoni, S. rodhaini* and *S. mattheei*. *S. haematobium* is maintained almost exclusively in *Saccostomus* with a back-up or golden hamsters whilst the West African human parasite *S. intercalatum* and the two interesting buck schistosomes *S. margriebowiei* and *S. leiperi* are carried in both *Mastomys* and *Saccostomus*.

The use of African rodents as laboratory animals in bilharzia research is not restricted to our laboratory. Several other laboratories in Southern Africa also maintain their schistosome colonies in veld rodents. In addition *Mastomys* have been exported to countries in Europe and to the United States where they have been introduced into various research programmes with success.

ACKNOWLEDGEMENT

The Bilharzia Research Unit is financially supported in part by the South African Medical Research Council.

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DESIGN AND USE OF AN INHALATION CHAMBER FOR AIR POLLUTION STUDIES IN SMALL ANIMALS

J. C. AUSTIN, P. E. CLEATON-JONES and E. G. VIEIRA

ABSTRACT: Austin J.C., Cleaton-Jones P.E., Vieira E.G. **Design and use of an inhalation chamber for air pollution studies in small animals,** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 235–238 (En), MRC/University of the Witwatersrand Dental Research Institute, 1 Jan Smuts Avenue, 2001 Johannesburg, Republic of South Africa.

Environmental chambers provide a convenient means of studying the toxicology of gases in experimental animals. The specialised equipment needed for these studies is not commercially available. This paper reports on the design, construction and performance of a simple, and inexpensive, inhalation chamber and gas mixing unit for air pollution studies in small laboratory animals. Provision has been made for controlling the mixing of gases, the ventilation rate, the even distribution of gases in the chamber and for the removal of vented gases to prevent the hazard of air pollution in the laboratory animal facility. No significant differences were found between the food and water consumption and body mass gain of a group of rats housed in the chamber for 5 days and a control group housed in similar cages located in a rodent room and managed under conventional laboratory conditions. The chamber fulfills all the requirements for an animal inhalation system.

INTRODUCTION

The biological effects of breathing air containing particulate and gaseous pollutants are currently being studied in many different fields of medical science. This task cannot be undertaken without the use of laboratory animals. Research in this field usually involves the exposure of experimental animals to pollutants under carefully controlled conditions in inhalation chambers[6]. In dentistry and medicine occupational exposure to trace levels of anaesthetic gases has been associated with an increase in the prevalence of foetal abnormalities and spontaneous abortion and hepatic and renal disease in women working in operating rooms[4 8].

The recent increase in the use of nitrous oxide/oxygen sedation in dental practices using open circuit administration without scavenging systems for the disposal of waste gases has awakened interest in the problem of dental surgery pollution[2 3 7 9]. We recently initiated a series of animal studies on the biological and teratogenic effects of exposure to low levels of nitrous oxide (N_2O) pollution for which inhalation chambers were needed. This specialised equipment is not commercially available and was specifically developed for this project.

The purpose of this paper is to report on the design, construction and performance of a simple, inexpensive inhalation chamber suitable for air pollution studies on small laboratory animals.

DESIGN REQUIREMENTS

The basic requirements which have to be fulfilled for an animal inhalation system include the following factors:

1. The experimental animals need to be housed and managed in a non-stressful environment in a caging system which is readily accessible for daily servicing.
2. The chamber holding the animal cages should be designed to ensure that the animals are exposed to uniform levels of pollution.
3. Variable control of the ventilation rate and mixing of the gases is needed to enable the range of pollution levels recorded in human environments to be simulated.
4. Provision should be made for sampling of the environment in the cages so that the gas pollution concentrations can be monitored whilst the chamber is in operation.
5. All waste gases require to be removed to prevent the hazards of exposure of personnel and other experimental animals present in the vicinity of the chamber to air pollution.

DESIGN AND CONSTRUCTION

For convenience the design of the chamber was based on the use of a commercially available rat cage and its cage racking system*. The polypropylene cage used had a wire mesh floor and cage top and was equipped with a food hopper, water bottle and removable waste tray.

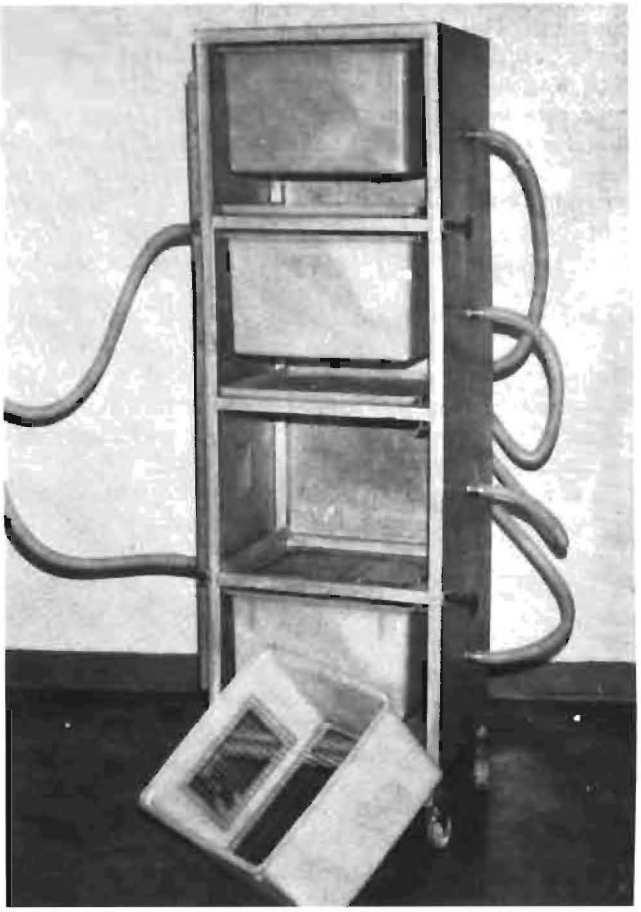


Fig. 1 Open inhalation chamber showing air supply and extraction system and modified rat cage.

*Code 707, Labotec (Pty) Ltd., Industria, Transvaal.

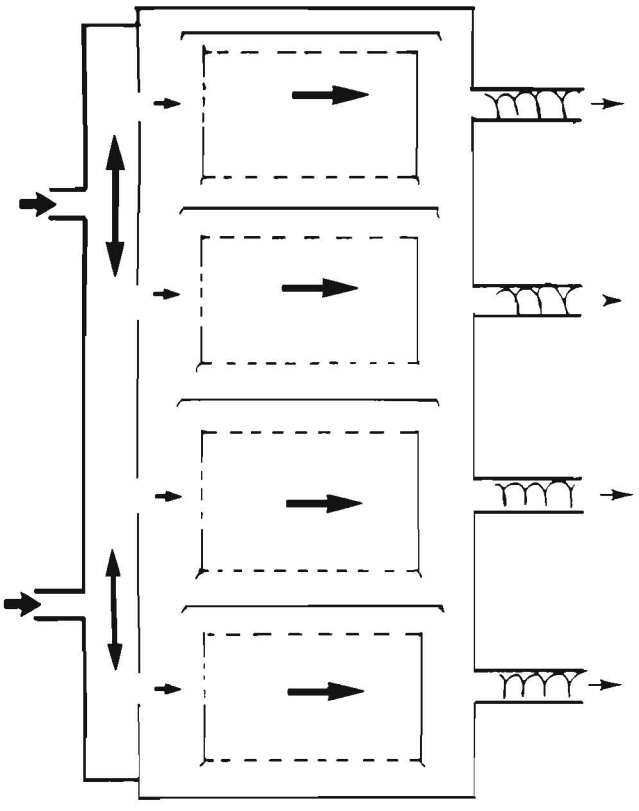


Fig. 2 Air flow diagram in the inhalation chamber.

The experimental design of our studies required rats to be housed in batches of up to 20 in the inhalation chamber. This requirement was met by using 4 rat cages tiered one above the other in a metal rack. This rack was constructed by cutting the standard 20 unit cage rack up into short sections and welding on additional supports so that it held a single vertical row of 4 cages. This rack then formed the framework of the inhalation chamber and it was covered on the top, bottom, back and 2 sides with 22 gauge galvanised sheet iron. A perspex door was constructed to seal the open front of the chamber. This door was seated on a sponge rubber gasket which was bonded to the door frame. Two rubber securing straps were mounted on either side of the door to maintain a gas tight seal between the door and rubber gasket when the chamber was in operation. Air sampling nozzles with screw caps were mounted on the perspex door at the level of each cage to enable gas samples to be withdrawn from inside each of the cages with a long needle for gas analyses.

An air supply distribution duct was mounted vertically along one side wall of the chamber. Rectangular ports were cut into this side wall at the level of each cage in the duct. Corresponding rectangular openings were cut in the sides of the plastic cages adjacent to the air supply ports to effect a direct air flow from the distribution duct to the interior of the rat cage. The openings were covered with wire mesh to prevent the rats from escaping from their cages. Provision was made for supplying premixed gases to the air distribution duct and for the extraction of exhaust gases from the chamber by means of corrugated plastic tubing. Two tapered tubing mounts were attached to the distribution duct for the air supply and 4 tapered mounts were attached to the opposite side of the chamber, for air extraction. The completed chamber (Fig. 1) was mounted on castors for mobility.

The air flow from the distribution duct was directed through the side opening in the rat cages against the solid panel of the opposite side wall of the cage. Air left the cage through the top and bottom mesh grids and was exhausted from the chamber through the extraction vent opposite each cage (Fig. 2). This air flow pattern was selected to create air turbulence in the cages for continuous mixing of air to ensure an even distribution of the fresh gases supplied to each cage in the chamber.

A gas metering and mixing unit was specially constructed to control the ventilation rate and facilitate accurate mixing of air and pollutant gases. Since the accurate metering of gases is most easily achieved with rotameter type flow meters these were adopted for the regulation of flow rate of air and, in our case, the pollutant gas N_2O.

A pressurised source of gases was required to operate these flow meters. Oil-free compressed air was generated with 2 small twin cylinder diaphragm type compressors* which each had an air delivery capacity of 192 ℓ of air/minute on open circuit. The compressors were located 20 metres away from the animal rooms to prevent noise disturbances. Their pooled air output was carried in pressure tubing to the gas mixing unit (Fig. 3). This consisted of a tubular air receiver mounted vertically on a castor base. Two horizontal arms fixed at the top of the air receiver. Each arm carried air flow meters and a small centrifugal gas mixing fan. This provided a dual metering and mixing system which enabled 2 chambers to be operated simultaneously for the accommodation of control and experimental groups of rats for each experiment.

Fig. 3 Inhalation chambers with central gas metering and mixing apparatus.

All exhaust gases from the chambers were vented to the exterior of the building in corrugated plastic tubing by means of an air extraction system operated by a centrifugal fan.

The chambers were designed to be operated on a continuous basis for several months with a brief daily 5 m shut-down period for changing the waste collection trays and replenishing food and water. The rubber diaphragms and valves and valve springs in the compressors needed to be replaced after every 8-12 weeks of continuous operation. This was a simple procedure and the replacement parts were inexpensive.

*Model 440H, W. R. Brown Corporation, Chicago, U.S.A.

ENVIRONMENTAL CONSIDERATIONS

Environmental conditions can exert a profound effect on physiological responses in animals subjected to experimental procedures. Consideration was also given to this aspect in the design of the inhalation chamber. The provision of a perspex door and the use of transluscent plastic cages enabled a regular photoperiod to be maintained in the cages. The daily dark/light cycle in the animal room which had external windows was not specifically controlled. The temperature and relative humidity in the chambers was monitored with internally mounted thermometers and hygrometers and was controlled within reasonable limits (21-24°C; R.H. 45-65%) by adjusting the air flow to the chambers. The volume of the chambers is 245 ℓ. The air supply was varied between 50-100 litres/min. according to the animal stocking rate (12-20 rats) and ambient temperature and relative humidity. This range of flow rates provided 12-24 air changes per hour which was equivalent to an air supply of 2,5-5 ℓ of air/m/rat. These flow rates prevented the build up of water vapour, ammonia and carbon dioxide. Noise from the air compressors did not reach the animal room and vibration from the gas mixing fans was not transmitted to the inhalation chamber through the flexible plastic air supply tubes.

TESTING THE CHAMBER

(a) Assessment of the Chamber Effect on the Rats

Prior to the adoption of the inhalation chamber for our animal studies tests were undertaken to determine if the chamber environment had any effect on food and water consumption and mass gain in rats. Ten male Wistar Albino rats, with a body mass of 200-250 g were divided into 2 groups of 5 by random selection. A control group was housed in an unmodified rat cage and placed in a cage rack and managed in a conventional manner in a rodent holding room whilst the other group was housed in a modified rat cage in the environmental chamber which was ventilated with air at a flow rate of 35 ℓ/m. Both groups of rats were provided with a cubed commercial diet* and water *ad libitum*. The food and water intake and individual bodyweights were recorded on a daily basis for 5 days. No statistically significant differences were found between nutrient intake and body mass gain in the 2 groups during this period. The chamber environment did not appear to affect the nutritional status of rats housed therein.

(b) Uniformity of Distribution of a Standard Air/N_2O Mixture in the Chamber

The distribution of premixed gases was studied in the inhalation chamber stocked with 16 200-250 g rats (4 per cage). A 0,5% V/V concentration of N_2O in air was supplied to the rats by passing 60 ℓ of air/min. and 300 mℓ of N_2O/minute into the chamber for 1 hour prior to gas sampling. Twenty millilitre aliquots of air were then collected from the centre of each rat cage at hourly intervals for 5 hours. Samples were collected at a height of 50 mm above the cage floor grid with a long stainless steel sampling needle. The needle was inserted through the gas sampling nozzles and a corresponding small hole in the front panel of each rat cage. Air was twice aspirated and expelled with a 20 mℓ plastic syringe• prior to the collection of each gas sample. This was transferred to a nylon gas sampling bag[1] for storage prior to analysis. The gas samples were analysed with a gas chromatograph* operated under the conditions listed in Table 1.

*Epol Rat Cubes, Epol Group, Johannesburg

Table 1: DETAILS OF THE CONDITIONS UNDER WHICH THE GAS CHROMATOGRAPH WAS OPERATED

Gas Chromatograph	Pye Unicam GCV
Column	2 m × ¼" O.D. Glass packed with Porapak Q 80/100 mesh
Column Temperature	80°C
Injector Temperature	150°C
Detector	Thermal Conductivity Detector
Detector Oven Temperature	100°C
Detector Filament	150°C
Carrier Gas	Helium at 30 ml/min
Measurement of Peaks	Peak areas measured with a Pye Unicam DP88 Computing Integrator

The concentration of N_2O in air was comparable in each cage during the sampling period and was maintained within a narrow range throughout this period (n = 20 $\bar{x}$ ± S.D. = 0,49 ± 0,03%). These results confirmed both the accuracy of the gas mixing method and even distribution of premixed gases in the chamber.

(c) Ventilation Rate in the Rat Cages

After the last hourly sample had been withdrawn the ventilation rate was examined in the cages using N_2O as a tracer gas. The air flow rate was reduced to 35 ℓ/m and 175 mℓ of N_2O/min. was added to produce a 0,5% V/V mixture of N_2O in air. The chamber was run for 1 hour to stabilise the gas concentrations and then the N_2O supply was switched off. The fall in N_2O concentration in all 4 cages was monitored by simultaneous sampling of the air at 3 minute intervals for 30 minutes.

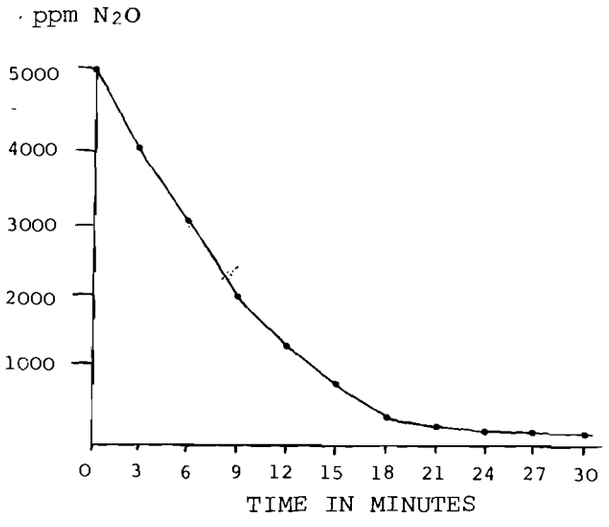
The decreasing N_2O concentrations recorded at the 4 levels in the chamber were comparable. The mean reduction in N_2O concentration with time is shown in Fig. 4.

After terminating the N_2O supply the N_2O concentration fell to about half the original concentration in the first 7,5 minutes and by a little less than half again in the next 7,5 minutes. After 15 minutes of supplying air alone only trace concentrations of N_2O were detected in the air samples. These results indicate that at a flow rate of 35 ℓ/min, i.e. one air change per 7 minutes, a complete turnover of air only took place every 14 minutes. This is attributed to the air flow pattern which was designed to produce air turbulence for mixing and even distribution of the air supply. This mixing produced recirculation of air in the inhalation chamber. The persistence of trace levels of N_2O after 15 minutes was probably also due to this recirculation effect.

It may be assumed that the N_2O concentration would rise at the same rate if N_2O was added to the air flow of 35 ℓ/min. On this basis it can be calculated that N_2O concentrations are restored to their original levels within 15 minutes when the chambers are put back into operation after the daily 5 minute shutdown for sanitation and food and water replenishment.

•Jintan Terumo

*Pye Unicam GCV



This inhalation chamber and air mixing system is currently being used for studying the effects of low levels of N_2O on laboratory rats and has proved to be a reliable low cost system which fulfills all the important criteria for inhalation studies.

ACKNOWLEDGEMENTS

We are indebted to Mrs. R. Shaw and Mr. R. Crichton for their technical assistance and Mrs. H. Ball for typing the manuscript.

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THE ROLE OF LABORATORY ANIMALS IN DENTAL RESEARCH

P. CLEATON-JONES and J. C. AUSTIN

ABSTRACT: Cleaton-Jones P., Austin J.C. **The role of laboratory animals in dental research.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 239–241 (En), MRC/University of the Witwatersrand Dental Research Institute, 1 Jan Smuts Avenue, 2001 Johannesburg, Republic of South Africa.

In dental research, in common with other forms of scientific research, the use of laboratory animals is necessary to carry out many investigations that may not be undertaken on man, and to control biological variability. In South Africa the main experimental animals used in dental research are rats and vervet monkeys, although other species such as dogs and baboons have been used. This paper lists examples of published dental research carried out in laboratory animals in South Africa in the fields of bone healing, dental caries, dental materials, growth studies, oral cancer, oral mucosa and periodontal disease investigations.

INTRODUCTION

As in many other branches of medical science, it is frequently necessary in dental research to use experimental models consisting of laboratory animals. Also, as in other disciplines, the answer obtained from the use of the experimental models will be only as good as the question posed. This in turn is dependent on sound use of scientific method in recognising problems, in gathering information related to these, in constructing a hypothesis, in testing the hypothesis in a suitable model and finally in the drawing of conclusions from the results of experiments.

When animal models are used it is essential that the investigator fully understands the model, a fact that has been emphasised by Navia[32]. He has stated that the effective use of animal models in research can never be improvised, it demands the expert handling of a body of knowledge that must be acquired. This is as true in dental research as in any other branch of science.

WHY USE ANIMAL MODELS?

This is a question often asked. In reply it is clear that there is a need for a biological system other than man, in which toxic or even lethal procedures can be used without endangering the health or life of a human being. Secondly, it is essential to have a simple but sufficiently designed model to avoid complications that might arise from the existence of multiple factors or from an interaction between these factors[32].

In choosing whether to carry out an investigation in man or in an experimental animal model Baker[3] has suggested three questions that should be answered:

1. Are the conclusions to be derived from the proposed study specific to man or would data derived from the study of other species be applicable to man?
2. Are the procedures required to control experimental variables applicable to human subjects?
3. Does the research procedure impose unreasonable hazard to the life, health or comfort of human subjects?

Although all 3 questions are important, the third is probably the most vital. Should there be any hazard to the life or health of a human subject then preliminary experimentation in animals is obligatory.

In dental research the threat to human life is minimal but there are many situations in which it is essential to decrease variability, e.g., by replication of experiments or by using a high risk or homogeneous population. Under these circumstances animal models must be used.

There are many avenues in dental research in which animal models may be used. This paper will list a number of these and give examples of published work from South African investigations.

BONE HEALING STUDIES

Included under this heading are studies on the implantation of biomaterials, replantation and transplantation of teeth and the healing of bony defects. Lemons[25] has written that in these types of studies the specific objectives of the investigation must be clearly defined before deciding on the experimental approach. For this reason cytotoxicity studies of new implant materials are usually carried out in inexpensive and readily available animals such as rodents and rabbits. In contrast, non-human primates are often selected for research on surgical implant designs.

Rodents, because of their small size, are often of limited use in certain types of tissue replacement studies. One such example is that of tooth replantation, so within South Africa studies on replantation have been carried out in the vervet monkey[4].

Rodents however are very useful for studies of the healing of experimentally induced bony defects. South African studies of this type have included investigations of the normal healing of defects in rat mandibles[34], long bones[28 44], on defects filled with frozen irradiated bone[15], with alginate[2], or with polytetrafuorethylene[27] or other substances[29], as well as the effect of nerve damage on defect healings[35]. Rodents have also proved useful in experiments to investigate the induction of bone formation in haematomata and the rat femur has proved a convenient size on which to study the role played by defects in the mechanical weakening of the bone[43].

DENTAL CARIES

Dental caries is a multifactorial disease which in essence requires an interaction for a sufficiently long period between teeth, bacteria and fermentable carbohydrates. The carbohydrates are broken down by bacteria into acids which decalcify tooth material. This damages tooth structure which is then further destroyed by proteolytic enzymes from the bacteria. The same groups of aetiological factors that are responsible for dental caries in man operate in animals.

Not all animals are susceptible to dental caries but rodents such as the rat and the non-human primate do develop dental caries. Dogs, cats, mice and guinea pigs do not easily develop dental caries, if at all, and are thus unsuitable as animal models.

In South Africa little work has been done on experimental dental caries although Ockerse & de Jager[34] have described examples of dental caries in the vervet monkey. More recently Louw *et al.*[26] studied dental plaque in the baboon and found cariogenic streptococci to be present.

DENTAL MATERIALS

Besides subcutaneous cytotoxicity studies the effect of dental materials on the dental pulp is an important avenue of investigation because any filling material used in tooth restoration needs to cause as little irritation of the pulp as possible. With this in mind the American Dental Association has drawn up a standard code of practice for the examination of the effects of dental materials on the dental pulp in laboratory animals[31].

In South Africa ready access to the non-human primate has enabled numerous studies of the effects of dental materials on the dental pulp to be carried out in the vervet monkey, which is an excellent model for such studies[38]. Materials that have been tested include composite resin filling materials[6 37], cavity sedative dressings[5], etching solutions[39 40] and systemic proteolytic enzyme mixtures[24]. The close resemblance between the teeth of man and of the vervet monkey enables a direct comparison of the results obtained in the 2 species to be made.

GROWTH STUDIES

The normal development and growth of the structures of the head, in particular the development of the secondary palate and the variations in this development that contribute towards the formation of cleft palates are important avenues in dental research. The experimental animals most frequently used in these types of studies are rodents including the mouse, rat and hamster. These animals may easily be bred under the controlled conditions necessary in growth and development investigations.

South African developmental studies have concentrated on the formation of the rat secondary palate. These include investigations using the light microscope[8 11], autoradiography[14], and the scanning electron microscope[15]. Abnormal development of the rat palate may be induced but cleft palates also occur spontaneously in certain species of dog including Boxers, Bulldogs and Pekinese. Examples of these animals have been raised in Johannesburg and their behaviour patterns examined[19].

Investigations on the development of other portions of the head have also been carried out. Such studies in the vervet monkey have included the eruption of the dentition[33] and the development of the periodontal ligament[45], while in the rat they include the mandible[30] and mandibular condyle[42].

ORAL CANCER STUDIES

The most common types of oral malignancies include the squamous cell carcinoma and tumours of the salivary glands and submucosa. Although spontaneous development of oral tumours have been reported in several species, induction of tumours remains the commonest avenue of investigation. Dogs, horses, cows, sheep, swine, monkeys and rabbits have been utilized but the laboratory animal *par excellence* is the hamster.

Dimethylbenzanthracene has been used in South Africa to induce salivary gland carcinomata[7] in order to study the effects of zinc supplements on the development of this tumour.

ORAL MUCOSA

The epithelium of the oral cavity is the first line of defence against disease in that region, thus much attention has been focussed on it. Numerous species have been studied but in South Africa most investigations have been carried out on the rat and the vervet monkey. They comprise studies on the normal light microscopic structure of keratinized and non-keratinized oral epithelium in the rat and vervet monkey, as well as elastic fibres in the lamina propria[21]. Coupled with these are ultrastructural studies using scanning and transmission electron microscopy of intact[12 17 22] and separated epithelium[9]. Other varieties of investigations include mitotic studies using colchicine and autoradiography[13] and the effect of controlled mechanical loading of selected areas of the oral mucosa to simulate functional stresses which occur in the oral cavity during mastication of food[23].

PERIODONTAL DISEASE

In the study of periodontal disease a wider range of laboratory animals is available and dogs, rats, marmosets, baboons, monkeys, hamsters, gerbils and ferrets have been employed. No one model has, however, fulfilled the requirements of researchers. Different techniques must be employed with each species to induce the disease and the resulting disease pattern varies within each model system.

In South Africa the vervet monkey has been used. Studies have been undertaken on the development of the periodontal ligament[45], on its detailed structure[41] and also on the structure and position of the gingiva in this species[44].

CONCLUSIONS

From the large numbers of papers listed in this article it can be seen that laboratory animals in dental research play an extremely important role in fundamental research on the biology of oral tissues and research which is directed towards resolving clinical problems in all fields of dentistry.

ACKNOWLEDGEMENT

We thank Mrs. H. Ball for typing the manuscript.

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GENETIC ASPECTS OF THE HORMONAL REGULATION OF SOME TESTIS ENZYMES DURING PUBERTAL DEVELOPMENT OF THE RAT

E. H. H. MEYER

ABSTRACT: Meyer E. H. H. **Genetic aspects of the hormonal regulation of some testis enzymes during pubertal development of the rat.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 243–245 (En), Animal and Dairy Science Research Institute, Private Bag X2, 1675 Irene, Republic of South Africa.

Using a rat model, it was shown that the synthesis of certain testis enzymes during pubertal development is under hormonal control, which acts as regulatory mechanism for gene expression during eukaryotic differentiation. Esterase activity and its electrophoretic banding pattern can be specifically induced by human chorionic gonadotrophin (HCG). Alcohol dehydrogenase and 3β-hydroxysteroid dehydrogenase are independently induced by HCG, and are apparently coded for by 2 different genetic cistrons.

INTRODUCTION

Laboratory animals are most suitable for studies on developmental genetics in higher organisms, and especially for studies on the mechanism of genetic control during differentiation. We selected a rat model, and studied some genetic aspects of the hormonal induction of certain testis enzymes during pubertal differentiation.

Previous studies by Engel and Frowein[2] showed that the genes for enzyme synthesis in the testis are already active in the embryo under influence of maternal hormones. This external stimulus for enzyme synthesis disappears at birth, and the enzyme activity declines after birth. The present study describes how enzyme synthesis is re-induced during puberty under the animal's own hormonal stimulus, as well as the genetic specificity of this process. The enzymes studied were alcohol dehydrogenase (ADH; E.C. 1.1.1.1), 3β-hydroxysteroid dehydrogenase (3βOH-StDH; E.C. 1.1.1.51) and the unspecific carboxyl esterases (EST; E.C. 3.1.1.1).

MATERIAL AND METHODS

Rats of the SIV-50 strain were used. In the induction experiments prepubertal male rats were injected daily with 100 IU human chorionic gonadotrophin (HCG) intraperitoneally from the 17th day onwards. A detailed description of the procedures for the preparation and homogenization of the testis, enzyme activity measurements, enzyme purification and electrophoresis is given elsewhere[4].

RESULTS AND DISCUSSION

As indicated in Fig. 1, the EST activity in control animals increases from the 26th day onwards. Under HCG treatment a similar induction pattern is observed. However, the enzyme activity already increases three days after the onset of hormone injections. A similar time lag was observed for 3βOH-StDH[3], whereas ADH is only induced after 6 days of HCG treatment[1]. The increase in testis weight in hormone treated and control animals closely resembles the EST activity pattern[4].

The electrophoretic banding pattern of EST on polyacrylamide even exhibits an individual regulation of single bands during pubertal development, as indicated in the schematic diagram and densitometric curves presented in Fig. 2. Especially in the middle anodal region II, a number of weak bands reveal an increased staining activity upon induction, while an additional strong band

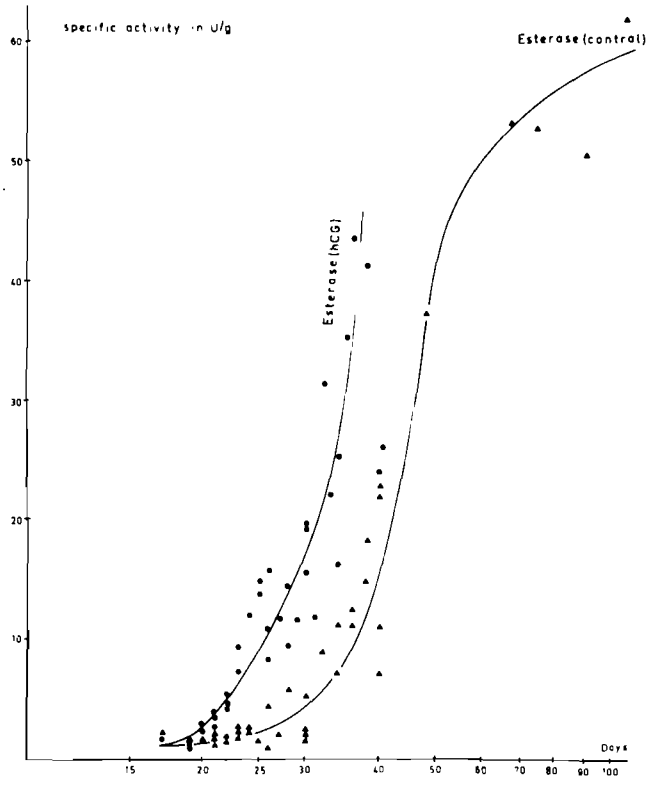

Fig. 1 Stimulation of testis nonspecific carboxyl esterase by chronic intraperitoneal injections of hCG from day 17 onwards. △ Esterase in controls, ● Esterase in treated animals.

also appears (Fig. 2, b, d, e). Furthermore, a new band shows up in the slowest migrating region III. A similar pattern can be induced in prepubertal rats by HCG treatment (Fig. 2c).

Therefore, the induction of EST activity and its electrophoretic banding pattern can be controlled by HCG; it is an example of the hormonal regulation of the genetic code's expression during eukaryotic development.

Although there is a different time lag of 2 and 6 days respectively for 3βOH-StDH and ADH in the induction response to HCG treatment[1,3], the alcohol and steroid activity of ADH isolated from adult rat testis by precipitation between 35% and 75% ammonium sulphate saturation is inseparable by column chromatography and electrophoresis in starch gel[4]. However, an alcohol inactive 3βOH-StDH could be isolated from adult rat testis at 35% ammonium sulphate saturation. As shown in Table 1, this 3βOH-StDH could be induced exclusively in the immature rat testis by means of

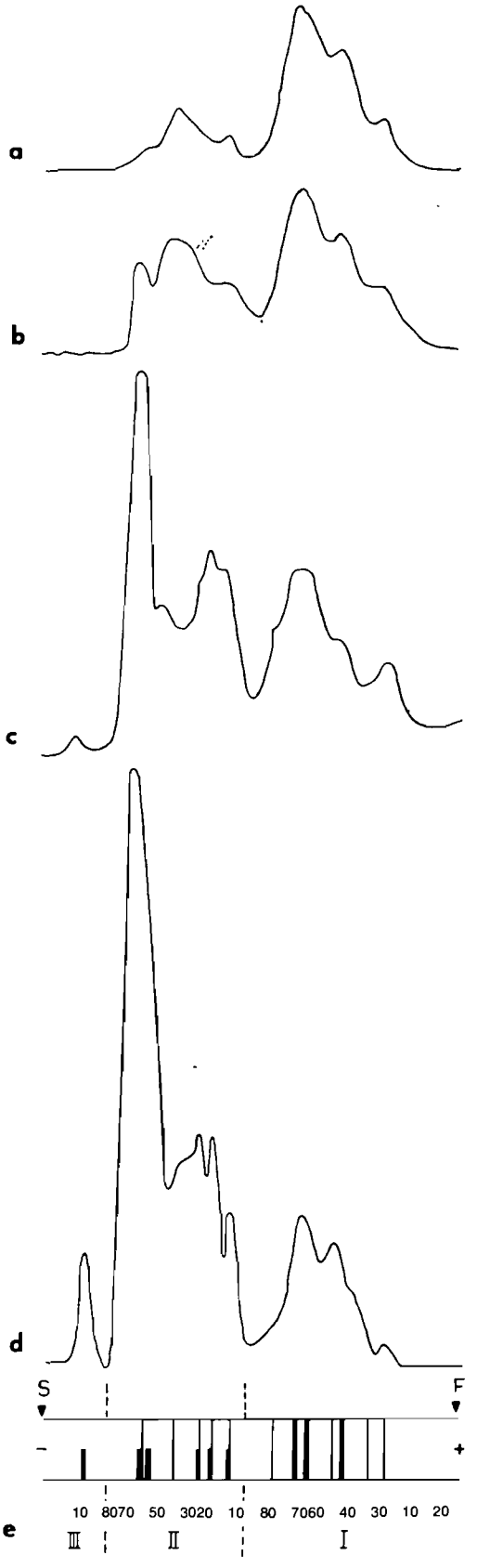

Fig. 2a Control, 19 days.

Fig. 2b Control, 26 days.

Fig. 2c HCG, 26 days.

Fig. 2d Control, 350 days.

Fig. 2e Schematic diagram of esterase bands.

upper half: control, under 22 days of age.

lower half: HCG treated immature or non-treated adult rat.

S: start; F: front.

Fig. 2 Densitrometric pattern and schematic diagram of non-specific carboxyl esterases in rat testis after electrophoresis on polyacrylamide.

Table 1: THE PREPARATION OF ADH AND 3BOH–StDH FROM IMMATURE RAT TESTIS AFTER 6 DAYS OF 100 IU HCG INJECTIONS

Fractions		ADH Specific activity (mU/mg Prot.)	ADH Times purified	3BOH–StDH Specific activity (mU/mg Prot.)	3BOH–StDH Times purified
So	(homogenate)	0,8942		1,1060	
P20	20% Amm. sulph.	0,0000		1,6473	1,49
S20	saturation	1,3209	1,48	1,7832	1,61
P35	35% Amm. sulph.	0,7536	0,84	54,4826	48,36
S35	saturation	2,1078	2,35	0,2848	0,26
P75	75% Amm. sulph.	0,4400	0,49	0,2249	0,20
S75	saturation	0,0000		0,0000	

a limited HCG treatment for 6 days, while ADH attained non-induced activity values.

Consequently, the differential induction response for ADH and 3βOH-StDH is due to an independently controlled induction by HCG. It can be assumed that the 2 enzymes are coded for by 2 different genetic cistrons, and that they are independently regulated by gonadotrophins during development.

ACKNOWLEDGEMENTS

The assistance of Prof. U. Wolf, Dr. W. Engel and Prof. O. v. Deimling of the University of Freiburg (German Federal Republic), as well as financial support by B.P. Southern Africa (Pty) Ltd. is gratefully acknowledged.

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THE INDUCTION AND TRANSPLANTATION OF HEPATOMAS IN WISTAR AND BD IX RATS

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ABSTRACT: Albrecht C.F., Dijkstra J.D. **The induction and transplantation of hepatomas in Wistar and BDIX rats.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 247–248 (En), Department of Pharmacology, Univ. Stellenbosch, Box 63, 7505 Tygerberg, Republic of South Africa.

Hepatomas were induced by feeding rats laboratory chow containing 0,6375 g of 3'-methyl-4-dimethylaminoazobenzene per kg for 3 to 5 months. DAB-1 was a hepatoma induced in randomly bred Wistar rats and was transplanted for 3 years after which it failed to grow *in vivo* but not *in vitro*. DAB-2 and DAB-3 are new transplantable hepatoma lines established in highly inbred DBIX rats. DAB-3 has a metaplastic morphology showing, *inter alia*, goblet cells, cartilagenous areas, duct-like structures and glandular follicles. This is unlike DAB-1 and DAB-2 which showed poorly differentiated trabecular or anaplastic carcinomatous patterns.

INTRODUCTION

In order to do meaningful experiments with cancerous tissue it is generally necessary to have a constant supply of material which does not differ significantly from experiment to experiment. This basic requirement for experimental cancer research can be satisfied by either working with cancer cells adapted to tissue culture and/or working with transplantable tumours. Morris has developed a family of transplantable hepatomas which have been used in numerous biochemical studies[6]. It would thus seem logical that work on hepatoma tissue in South Africa should also be conducted on Morris hepatomas because of the impressive body of normative data available. There is one serious drawback however, and this is the fact that Morris hepatomas only grow in Buffalo rats which would need to be established and maintained as an inbred strain. Such an ongoing endeavour can only be executed by an organization with sufficient momentum and motivation – i.e. hardly by one worker with inadequate facilities.

Originally our studies were aimed at analysing the critical molecular events during azodye carcinogenosis. We were thus interested in studying tumours induced by the carcinogen 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB), and not tumours induced by N-2-fluorenylphthalamic acid – which was used by Morris[6]. We therefore decided to try and induce a transplantable hepatoma with 3'MeDAB in Wistar rats. This was successful, however, after 3 years and about 75 serial transplants, the tumour failed to grow *in vivo*. It is interesting that this failure occurred almost simultaneously in 4 different laboratories in South Africa towards the end of 1975.

Early in 1977 it was decided to reinitiate transplantable tumours for experimental cancer research using the highly inbred DBIX rats. These rats were chosen because – although not proven – it was suspected that the abrupt termination of the Wistar transplantable hepatoma (DAB-1) was due to immunological incompatibility resulting from the fact that the recipient rats were randomly bred and thus had genomes that were subject to genetic drift.

Some preliminary studies of the characteristics of the new lines of transplantable hepatomas in BDIX rats will be discussed here.

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MATERIAL AND METHODS

The strain of Albino Wistar rats used in the experiments were originally obtained from Glaxo Laboratories in England in 1932. Since then the strain was maintained at the Veterinary Research Institute, Onderstepoort, the Council for Scientific and Industrial Research and now at the Bureau of Standards in Pretoria. These rats were randomly bred and male rats weighing about 150 g were used.

The highly inbred DBIX rats were obtained from the National Research Institute for Nutritional Diseases which imported them 10 years ago from Professor H. Druckrey of the Research Group for Preventative Medicine in Freiburg, West Germany. These rats have been inbred since 1937 and are now in the 46th generation. Male rats weighing 150 g were used.

Hepatoma induction and transplantation have been described previously[2 4].

RESULTS

Out of a total of 15 primary hepatocellular carcinomas induced in Wistar rats, only one managed to grow after transplantation. This tumour – designated DAB-1 – was transplanted at intervals of about 2 weeks. After about 75 transplantations the tumour ceased to grow in rats and if it grew, it became rapidly and excessively necrotic. A tissue culture line – designated DAB-1(TC) – was started at the end of 1972 and is still growing. (Both DAB-1 and DAB-1(TC) have been characterized before[1 2].)

Out of a total of 12 primary hepatocellular carcinomas, 2 managed to grow in DBIX rats. The first to be transplanted is now designated DAB-2 and is also growing in tissue culture. DAB-2 like DAB-1 is transplanted every 2 to 3 weeks. After 73 days it became obvious that another tumour was growing after transplantation. This slow growing tumour is designated DAB-3. DAB-2 and DAB-3 have been studied histologically and it was found that DAB-2 presented as a solid mass of cells divided into lobule-like islands by a delicate stroma. In general the cells have a loose appearance and appear individually discrete. Some necrosis was also noticed. DAB-3 appeared to be a mixed tumour containing glandular cell patterns, cartilagenous metaplasia, goblet cells and squamous cells lining acini.

DISCUSSION

Although DAB-1 can no longer be transplanted into Wistar rats, for unknown reasons, this tumour was useful and was used in a number of studies[3-5]. The resulting cell line DAB-1(TC) is being used by a number of workers in South Africa. DAB-2 and DAB-3 are now available to workers in South Africa and it is hoped that because these tumours are growing in inbred rats, they will survive better than DAB-1 which was grown in randomly bred rats.

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THE USE OF THE RAT IN THE EXPERIMENTAL INVESTIGATION OF THE PORPHYRIAS

L. EALES and G. H. BLEKKENHORST

ABSTRACT: Eales L., Blekkenhorst G.H. **The use of the rat in the experimental investigation of the porphyrias.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 249–251 (En), Department of Medicine, University of Cape Town, 7925 Observatory, Republic of South Africa.

The patterns of urinary porphyria excretion and hepatic porphyrin accumulation in hexachlorobenzene-treated rats is similar to that observed in overt porphyria cutanea tarda and may be attributed to a decrease in activity of hepatic uroporphyrinogen (UROGEN) decarboxylase. The 3,5 diethoxycarbonyl-1,4-dihydrocollidine (DDC)-treated rat has been evaluated as a model to test the porphyrinogenicity of drugs.

INTRODUCTION

The rat has probably been the most used experimental animal in porphyria research, as judged by the large number of reports in which it has featured. Although we have used other animals, we have found the rat to be the most suitable species for the experimental investigation of the human porphyrias, not only because it is a mammal, but also because pure bred strains are procurable and it has a number of desirable innate qualities: omnivorousness, tractability and high fecundity. The conveniently large size of the liver, spleen and kidneys are additional advantages. It has been demonstrated that an experimental porphyria develops in rats treated with hexachlorobenzene (HCB)[12 13] which biochemically closely resembles human symptomatic porphyria (SP)[7 9]. We have studied the development of HCB-induced porphyria in the rat in order to elucidate the pathogenesis of SP.

Human variegate porphyria (VP) is a genetically determined hepatic porphyria, the exacerbation of which is usually brought about by barbiturates and other drugs[8]. South Africa has the highest prevalence in the world of VP, with over 8 000 patients. The incidence of acute attacks has been materially reduced by the effectiveness of our family surveys which have detected the often asymptomatic but biochemically positive affected members among the immediate relatives of patients, and has enabled us to warn affected members to avoid the potentially hazardous drugs known to precipitate acute attacks. However, the danger of acute fatal VP still exists in view of the continued marketing of new drugs.

Three, 5-Diethoxycarbonyl-1,4-dihydrocollidine (DDC), when administered to experimental animals, produced a hepatic porphyria resembling VP in that protoporphyrin (PROTO) is the main porphyrin that accumulates in the liver[3]. Rats given a relatively small dose of DDC exhibit a porphyria closely resembling VP in its latent phase, since they become very sensitive to drugs that can exacerbate the metabolic disorder, changing the biochemical picture to that typical of the human attack[5]. We have studied the DDC-treated rat to determine if this model may be useful to assess the porphyrinogenicity of drugs, and whether they might be regarded as potentially hazardous to use in the human hereditary porphyrias.

MATERIALS AND METHODS

Treatment of rats

Female Wistar rats weighing between 160 and 190 g were fed with a diet of powdered rat cubes containing 0,2% HCB. Rats were starved 24 hours prior to determination of the various parameters. Male Wistar rats weighing between 190 and 240 g were starved for 24 hours, then 100 mg/kg DDC was administered intraperitoneally with simultaneous administration by gastric intubation of either 0,05 mg/kg ethyl morphine, 500 mg/kg propanidid, 500 mg/kg griseofulvin, 150 mg/kg phenylbutazone or 50 mg/kg sodium phenobarbitone. Rats were killed 17 h after treatment and the livers excised.

Enzyme and chemical assays

Urinary uroporphyrin (URO) and coproporphyria (COPRO) excretion was determined by the method of Rimington & Sweinsson[11]. The urinary porphyrin profiles were obtained by scanning the talc-extracted methyl esters which had been separated on silica gel according to the method of Sears *et al.*[14].

Hepatic porphyrin content was determined by direct esterification of the porphyrins contained in approximately 100 mg homogenised liver, followed by extraction, chromatographic separation and scanning. URO was quantitated by comparison of the peak area of the URO (8 GOOH) ester peaks with that of a known quantity of chromatically pure URO ester applied to the plate.

The activity of hepatic uroporphyrinogen-(UROGEN-I) decarboxylase and the levels of hepatic cytochrome P450 in whole liver homogenates were determined by previously published methods[2 10]. Hepatic amino laevulic acid (ALA) content was determined by the method of De Matteis[4], and hepatic porphobilinogen (PBG) content according to that of De Matteis & Gibbs[6].

RESULTS

Urinary URO and COPRO excretion in HCB-treated rats at 15 day intervals is shown in Fig. 1a. By Day 30 porphyrinuria was evident and had reached significant levels by Day 45, at which time URO was the predominant porphyrin, as was the case of Day 60. The fluorometric scans shown in Fig. 2a indicate the shift towards the urinary excretion of the higher carboxylated porphyrins becoming increasingly pronounced at Days 45 and 60 of treatment with HCB, at which time the intermediate, 7-, 6- and 5-COOH porphyrins were also present in significant quantities.

After 15 days feeding with HCB, small increases were noted in the levels of hepatic URO and COPRO, with small quantities of 7-COOH being detected (Fig. 2b). After Day 30 the excess hepatic porphyrins were

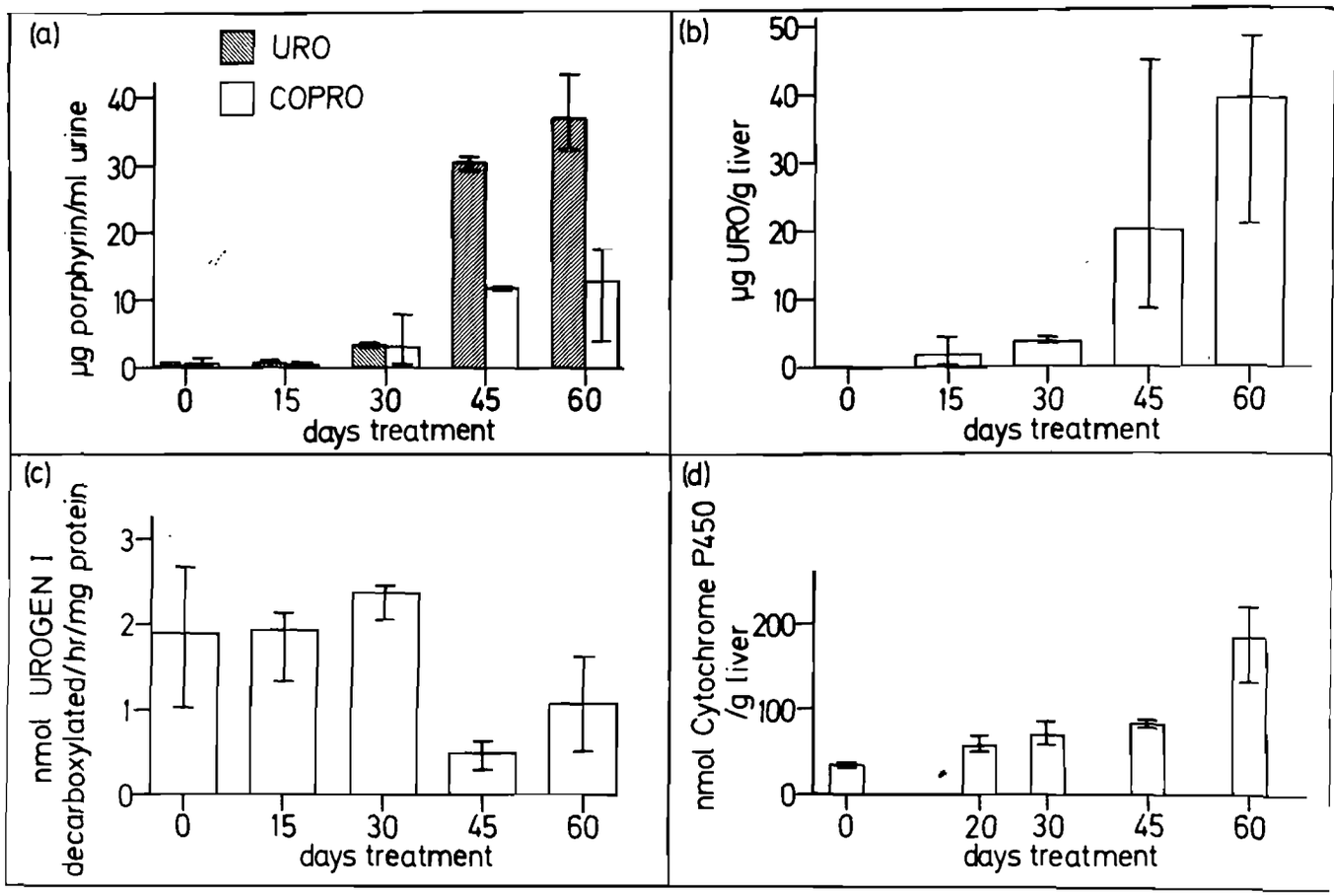

Fig. 1 The effect of HCB-feeding of rats at various time intervals on (a) urinary excretion of uroporphyrin and coproporphyrin; (b) hepatic uroporphyrin content; (c) hepatic uroporphyrinogen I decarboxylase activity, and (d) hepatic cytochrome P450 content. Histograms indicate the means from 3 observations, with bars indicating the range of values. URO = Uroporphyrin; COPRO = Coproporphyrin; UROGEN = Uroporphyrinogen decarboxylase

almost entirely URO and 7-COOH porphyrin (Fig. 2b). The hepatic URO content continued to increase after 45 and 60 days treatment (Fig. 1b).

As shown in Fig. 1c, up to 30 days feeding with HCB did not significantly affect the activity of UROGEN-1 decarboxylase; there was a significantly decrease in activity which increased somewhat at Day 60 and was still significantly different from that obtained for untreated rats.

The amount of hepatic cytochrome P450 was increased after 20 days treatment with HCB, with a further increase noted after 30, 45 and 60 days treatment (Fig. 1d).

It will be seen from Table 1 that DDC treatment alone elevated hepatic ALA and PROTO levels when compared to the levels in untreated rats. Ethyl morphine, which is known to be safe in patients with VP, did not potentiate the porphyrinogenic effect of DDC. Propanidid caused an almost 2-fold increase in PBG content and increases in URO, COPRO and PROTO when compared to that in rats treated with DDC alone. The mean hepatic content of all five haem precursors was markedly increased when DDC was given in combination with griseofulvin, phenylbutazone or phenobarbitone, which are known to be hazardous when administered to patients with VP.

Table 1: THE EFFECT OF THE SIMULTANEOUS TREATMENT OF RATS WITH DDC AND ETHYLMORPHINE, PROPANIDID, GRISEOFULVIN, PHENYLBUTAZONE AND PHENOBARBITONE ON THE HEPATIC CONCENTRATIONS OF HAEM PRECURSORS[1]

Treatment	ALA nmol/g liver	PBG nmol/g liver	uroporphyrin µg/g	coproporphyrin µg/g	protoporphyrin µg/g
None	13,7 (7,3–18,6)	2,82 (0,6–5,5)	0,04 (0,02–0,07)	0,15 (0,09–0,19)	0,20 (0,18–0,25)
DDC	24,0 (16,3–29,8)	1,4 (1,1–1,9)	0,04 (0,02–0,07)	0,22 (0,14–0,33)	0,37 (0,15–0,49)
DDC + ethylmorphine	18,4 (16,1–31,4)	3,5 (0,5–7,4)	0,05 (0,01–0,08)	0,20 (0,18–0,28)	0,25 (0,19–0,32)
DDC + propanidid	18,0 (9,3–48,9)	5,8 (0,4–26,2)	0,19 (0,03–0,82)	0,48 (0,29–0,91)	0,95 (0,06–1,62)
DDC + griseofulvin	36,4 (17,2–62,7)	30,7 (0,0–62,7)	0,25 (0,0–0,82)	4,04 (0,22—7,14)	2,01 (0,56–3,20)
DDC + phenylbutazone	41,5 (33,1–57,0)	24,8 (0,9–48,9)	0,20 (0,0–0,51)	1,25 (0,13–3,67)	1,09 (0,60–2,32)
DDC + phenobarbitone	60,4 (18,9–84,9)	63,8 (6,2–92,2)	0,56 (0,03–1,73)	1,12 (0,82–1,40)	2,13 (1,08–2,95)

1 The mean value for 6 observations is given with the range in parenthesis.

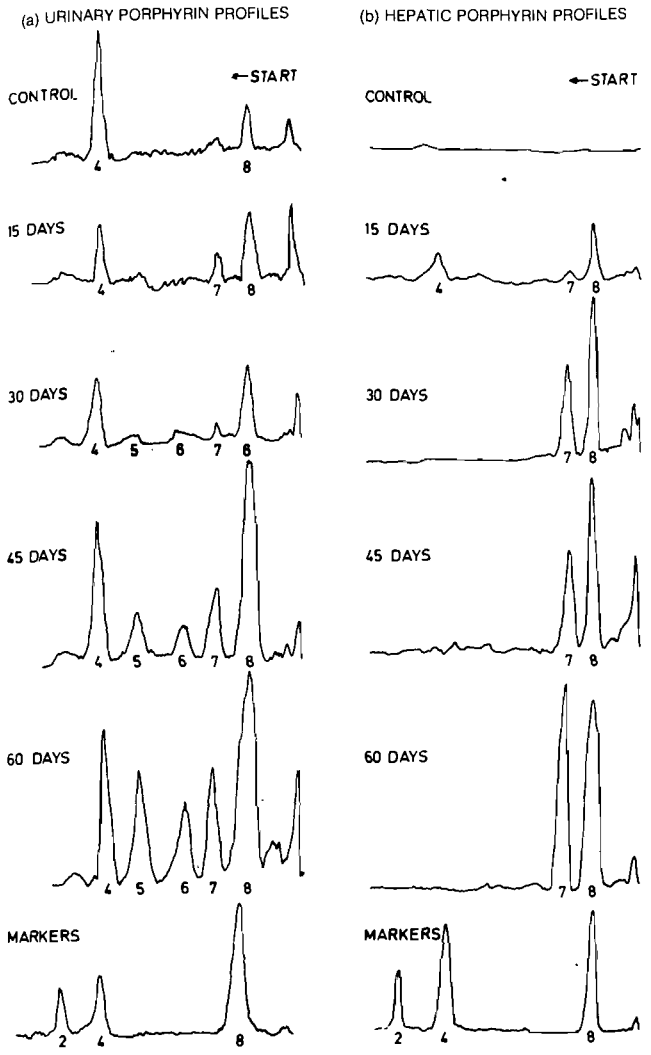

Fig. 2 The effect of HCB-feeding of rats at 15 day intervals on (a) urinary porphyrin composition and (b) hepatic porphyric composition. Porphyrins were extracted, esterified, chromatographed and the fluorometric scans using a Vitatron TLD 100 densitometer are shown. Numerals below each peak refer to the number of carboxyl groups of the porphyrins.

DISCUSSION AND CONCLUSIONS

The patterns of urinary porphyria excretion and hepatic porphyria accumulation in the HCB-intoxicated rat are similar to that observed in overt porphyria cutanea tarda (PCT). Furthermore, as the activity of UROGEN-I decarboxylase decreases, porphyrins corresponding to the substrate for, and intermediates of, the reaction catalysed by the enzyme became progressively more prominent in the urine. These results suggest that the porphyria is due to the decrease in the activity of this enzyme. Hepatic UROGEN-I decarboxylase has been reported to be deficient in patients with PCT and further investigations in HCB-treated rats are under way in our laboratory to elucidate the mechanism whereby the activity of this enzyme is decreased, and whether this is applicable to PCT. The finding of elevated levels of hepatic cytochrome P450 in HCB-fed rats is similar to that reported in PCT[10], and a metabolic basis to explain hepatic cytochrome P450 elevation, despite a block in haem systhesis at the level of UROGEN decarboxylase, has been reported in depth elsewhere[1].

The inherent difficulty in applying the DDC-treated rat model to screening drugs for porphyrinogenicity is that there is considerable variation in the individual response of the rats to the drugs. We are at present investigating the time course of the potentiating effect of drugs on rat porphyria, since maximal stimulation of the porphyrinogenic effect may not necessarily occur at 17 hours after administration of the various drugs.

ACKNOWLEDGEMENTS

The skilled technical assistance of Miss E. Cook is gratefully acknowledged. This work was funded in part by the South African Medical Research Council, University of Cape Town Staff Research Fund, Atomic Energy Board and Ethical Drug Association Foundation.

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Die mees belangrike 21 dae in 'n koei se jaar.

Die Probleem.

'n Hoë persentasie koeie het 'n residuele infeksie aan die einde van laktasie.

Daarby is alle koeie vatbaar vir 'n nuwe infeksie gedurende die vroeë stadia van die droë periode. Met 'n paar uitsonderings sal dit gedurende die eerste 10 tot 21 dae voorkom.

Die Antwoord.

Orbenin Droë Koei is ontwerp om beide hierdie probleme te oorbrug. Dit is geformuleer as gevolg van aanhoudende navorsing, beide in die laboratorium en in die veld.*

Orbenin Droë Koei is bakteriedodend teen streptokokke, penisillien sensitiewe en penisillien weerstandbiedende staphylokokke, die mees oorsaaklike organismes wat in residuele en nuwe infeksies gevind word.

Dit was duidelik uitgewys dat infeksie tydens kalwing gewoonlik van NUWE oorsprong en meesal 'n omgewings, of Gram -we infeksie is. Behandeling tydens daardie tyd met 'n breëspektrum Lakterende Koei produk soos Ampiclox L.K. is nodig om genoegsame antibiotiese konsentrasies te bereik om mastitis tydens kalwing te voorkom.

Orbenin Lakterende Koei en Ampiclox L.K. is bedoel om mastitis gedurende laktasie te beheer. Ampiclox word veral aanbeveel wanneer coliforme vermoed word.

Orbenin (kloksasillien) en Ampiclox (ampisillien/kloksasillien) is Beecham handelsmerke.

*Die jongste kliniese proef het 507 kuddes oor 'n tydperk van 3 jaar ingesluit – Brander G.C., Watkins J.H., en Gard R.P., Vet Rec. (1975) 97. 300-304.

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Afdeling van Beecham Pharmaceuticals (Edms) Bpk., Posbus 347, Bergvlei, 2012.

BA 4138

MORTALITY OF SUCKLING ATHYMIC NUDE MICE ASSOCIATED WITH *STAPHYLOCOCCUS AUREUS*

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ABSTRACT: Du Plessis J. L., Meyer-Scharrer Elizabeth, Verwoerd D. W., Henton M. M. **Mortality of suckling athymic nude mice associated with** *Staphylococcus aureus*. *Journal of the South African Veterinary Association* (1978) **49** No. 3, 253–255 (En), Veterinary Research Institute, 0110 Onderstepoort, Republic of South Africa.

Serious problems of suckling mortality were encountered in a breeding nucleus of athymic nude mice introduced into the Republic. The mortality could in all probability be ascribed to infection with *Staphylococcus aureus*.

INTRODUCTION

The production of athymic nude *(nu/nu)* mice may be hampered by their comparatively low reproduction potential[1 7 8], their increased susceptibility to known and facultative pathogens and their proneness to develop a wasting syndrome [7 9 14]. These obstacles may largely be overcome by keeping the mice in a specified-pathogen-free (SPF) or germ-free environment. Under these conditions male fertility increases significantly[8 13] and the lifespan is extended[13]. Such mice are much less prone to develop the wasting syndrome[6] and they remain free from viral and bacterial diseases[4 8]. However, even in a protected environment, their husbandry can be problematical to the inexperienced.

MATERIALS AND METHODS

Animals.

A breeding nucleus of 5 homozygous nude males and 10 heterozygous females was obtained from the Laboratory Animals Centre, Carshalton, England. These mice had been derived from pairs mated at random within the 8th backcross generation of the nude gene onto a BALB/C inbred background.

Husbandry and Breeding.

The mice were kept in a Trexler-type isolator under controlled environmental conditions of temperature and light. They were fed standard mouse pellets sterilized by autoclaving, supplemented by a vitamin concentrate introduced into the drinking water and sterilized by millipore filtration. Nude males were randomly bred to the heterozygous females. In some of the litters the heterozygous offspring were killed at birth. Females were remated after their nude progeny were weaned or had died.

Microbiological control.

Bacteriological cultures were made from anal swabs taken at weekly intervals. Later nalidixic acid[2] was added to the blood-tryptose agar medium to suppress its overgrowth by Gram-negative bacteria (particularly *Proteus)*, which at the beginning presumably hampered the isolation of *Staphylococcus aureus*. From time to time blood-tryptose agar plates were placed in the isolator.

Specimens.

Some of the moribund sucklings were submitted for bacteriological examination and others were autopsied. Specimens for histology were fixed and processed, and sections were cut and stained with haematoxylin and eosin. Two females and one male from among those originally received as well as 3 second generation females were sacrificed for bacteriological examination.

Serum prophylaxis.

Fifty-four nude sucklings from 15 litters were injected subcutaneously with 0,1-0,3 mℓ normal mouse serum from one day – 3 weeks after birth or until they died.

Conventional outbreeding.

One of the original nude males was mated under conventional conditions to 6 outbred conventionally bred females. First generation heterozygous progeny were subsequently outbred and 15 litters including 31 nude progeny were obtained.

RESULTS

Reproduction.

During the course of 10 months, 58 litters comprising 188 nudes and 239 heterozygotes were obtained from 20 females. The average litter size was 7,4.

Suckling mortality.

Only 15 nude offspring survived. The other 173 died or were killed in a moribund state from 8-18 days after birth. Eight of the survivors had been injected with normal mouse serum. None of the heterozygous offspring raised in the isolator or any of the nudes raised conventionally died or showed clinical signs.

Nudes that died or runted showed clinical signs of weakness, emaciation and runting from 6-10 days after birth for several days. At autopsy there was cachexia and peri-renal oedema. Histopathological examination showed conspicuous hydropic degeneration of hepatic and renal tubular epithelial cells and extensive neuronal degeneration and karyorrhexis.

Bacteriological findings.

Anal swabs. The following bacteria were isolated: *Proteus mirabilis, Streptococcus faecalis, Micrococcus* sp.,

Escherichia coli, Enterobacter liquefaciens, Citrobacter sp., Acinitobacter sp., *Enterobacter aerogenes, Alcaligenes* sp. and *Klebsiella* sp. Subsequent to the addition of nalidixic acid to the medium, *S. aureus* was isolated from the anal swabs of several heterozygous females and nude males in the isolator as well as from 2nd and 3rd generation heterozygous females and nude males in the isolator as well as from 2nd and 3rd generation heterozygous females and nude males obtained by conventional outbreeding.

Plates. The bacteria isolated on agar plates exposed in the isolator were *P. vulgaris, Neisseria* sp., *Micrococcus* sp., *P. mirabilis* and *E. coli.* Subsequently *S. aureus* was isolated as well.

Mouse organs. S. aureus was isolated from the liver, spleen, lung, myocardium and gut of several moribund nude sucklings as well as from the lung of one, the spleen of another and from the gut of all 5 females which were sacrificed.

DISCUSSION AND CONCLUSION

Whereas the reproductive capacity of both the females and males was satisfactory, the high pre-weaning mortality of the homozygous progeny seriously hampered their use.

The isolation of *S. aureus* from the organs of several moribund nude sucklings, from the anal swabs of both hetero- and homozygous adult mice and eventually also on agar plates placed in the isolator, suggest that mortality can probably be ascribed to staphylococcosis. Although Koch's postulates had not been fulfilled, the isolation of *S. aureus* from the liver, spleen, lung and heart of several sucklings, prove that a septicaemia was present. The beneficial effect of normal mouse serum is not unexpected because the serum of conventional mice probably contains antibodies against *S. aureus.*

Although the histological lesions described are not specific, they are compatible with a low-grade toxaemia associated with septicaemic staphylococcosis. The clinical and pathological picture described did not point to a pyogenic microbe such as *S. aureus* and accounts for the fact that more determined and more frequent attempts at bacterial isolations were not made.

The cachectic state of the moribund mice at autopsy, the absence of specific lesions and the extended course of the condition led one to suspect the wasting syndrome associated with athymic mice, but the early appearance of the disease ruled out this possibility.

The frequent isolation of *S. aureus* from the anal swabs newly taken from several adult mice as well as from agar plates only just placed in the isolator, but not from those exposed initially, showed that the entire isolator was eventually contaminated and that there was probably a gradual build-up of staphylococci.

Nude mice are generally maintained under SPF conditions[6] [10] but also in isolators[4] [10]. In view of our observations that adult healthy mice carried *S. aureus* in their gut, the possibility of a build-up of this microbe in an isolator is perhaps greater than under SPF conditions. The absence of clinical signs and mortality in our conventionally-outbred nude progeny is compatible with this and suggests that the nude progeny in the isolator were possibly exposed to an unduly large number of staphylococci.

Although *S. aureus* could not be isolated from moribund nude sucklings born after the arrival of the breeding nucleus, it seems probable that the original imported mice were carriers of this pathogen, as the clinical and pathological picture at that time was identical with that observed subsequently.

As none of the heterozygous litter mates showed clinical signs or died, the question arises why the athymic nude sucklings developed a sub-acute toxaemic staphylococcosis. As the resistance to staphylococcal infection depends upon phagocytosis assisted by opsonizing antibodies and antitoxins[5], it may be that the deficient immune response of athymic mice to T-cell dependent staphylococcal antigens accounts for their lowered resistance.

No account of the significance of *S. aureus* in nude mice could be traced in the literature. This microbe does, however, colonize SPF mice[3] [12] and *S. aureus* infection of the bulbourethral glands of barrier-maintained mice has been described[11]. Furthermore, *S. aureus* is recognized as one of the so-called ubiquitous microbes which, in association with contaminants such as *E. coli* and *Yersinia enterocolytica,* are held responsible for purulent processes in SPF mice (F. Deerberg, personal communication). Man being a carrier of this pathogen[5], barrier-maintained nude mice are therefore exposed to infection in more ways than one.

In conclusion it can be stated that our observations suggest that: 1. *S. aureus* may in all probability be responsible for a disease condition in nude sucklings characterized by high morbidity and mortality, loss of weight and cachexia. 2. Histological lesions of hydropic degeneration of hepatic and renal tubular epithelial cells and neuronal degeneration suggest a subacute toxaemia due to sub-acute staphylococcal septicaemia. 3. A staphylococcal build-up in an isolator should be borne in mind in the husbandry of nude mice.

ACKNOWLEDGEMENT

We wish to thank Dr. C. M. Cameron for valuable advice.

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LETTER TO THE EDITOR

BRIEF AAN DIE REDAKSIE

SURGICAL CORRECTION OF PREMATURE CLOSURE OF ULNAR GROWTH PLATE

Sir

I wish to compliment Dr. L.B. Evans on his case report entitled 'Surgical Correction of Premature Closure of the Ulnar Growth Plate in the Dog'; *Journal South African Veterinary Association* (1977) **48**:287. The report concerned a 5 month old Great Dane bitch with premature closure of the distal ulnar physis. Dr. Evans described the surgical correction of the deformity through the use of orthopaedic staples and ulnar osteotomy. It is a timely report as the problem which he describes is one which is becoming of increasing significance in many parts of the world following on the increase in the numbers of dogs of the larger breeds. May I make some comments on this case and on the management of dogs which are presented with premature closure of the distal ulnar physis.

The normal increases in diaphyseal (shaft) lengths of the radius and ulna are due to the contributions of the proximal and distal radial physes and in the ulna, the distal ulnar physis alone. The proximal ulnar physis only contributes to the length of the olecranon. If the distal ulnar physis is prematurely closed, normal elongation of the radius may result in subluxation at the carpus, as seen in the present case. It has been our experience and that of others [1-4] that some dogs with valgus carpal deformity due to premature closure of distal ulnar physis also have subluxation of the elbow joint followed by instability and secondary osteoarthritis. Skaggs *et al.*[4] in a review of 14 cases of dogs with trauma induced premature closure of the distal ulnar physis, reported that 10 had moderate to severe subluxation of the elbow joints. The severity of the subluxation was not related to the animal's age at the time of injury, contrary to what might be expected when one considers that the relative contribution to growth by the proximal radial physis reduces with age. The relative contribution to growth of the radius from the proximal physis varies from 43,2% to 25%, becoming less as the dog gets older because this physis closes before the distal radial physis[3]. However, the severity of subluxation may be due to marked shifting of the radius in relationship to the ulna, which occurs during growth[3]. Because of the possibility of elbow joint subluxation, clinical assessment of joint function and radiography of the elbow at the time of presentation, and at regular intervals until radius growth ceases at 6-9 months of age[5] would seem advisable.

Stapling of the distal radial physis is eminently suited to correction of the valgus deformity and cranial bowing as borne out by the excellent clinical result achieved by Dr. Evans. I agree that corrective wedge osteotomy is difficult, but prefer to select the surgical treatment for premature closure of distal ulnar physis on the following criteria.

1. Dogs in which the distal radial physis is still growing

Stapling of the medial side of the distal radial physis to correct valgus carpal deformity is used together with an ulnar ostectomy. The removal of 2-3 cm of the shaft of the ulna seems to delay union, which will occur within 2-3 weeks of an osteotomy. The ostectomy is performed with an oscillating saw* driven by nitrogen or a gigli wire saw. The distal shaft of the ulna may split if an osteotome or chisel is used. The ostectomy is repeated as necessary until growth ceases. A staple is placed in the craniomedial aspect of the distal radial physis if cranial bowing of the radius is present. Any residual deformity should be corrected by wedge osteotomy after growth ceases.

2. Dogs presented with valgus carpal deformity and rotation of the paw when the proximal and distal radius physes have closed and growth is complete.

A corrective wedge osteotomy is the only treatment. However, a corrective wedge osteotomy is contra-indicated in the growing dog, not because of the difficulty, but because further growth after the surgery will result in additional deformity.

Growing dogs with premature closure of the distal ulnar physis should not be left untreated until maturity because of the instability and secondary osteoarthritis of the carpus which are likely to develop.

Shortening of the foreleg after stapling is usually compensated for by extension of the shoulder and elbow joints by the dog. However, I have found shortening to be a problem, particularly when closure of the distal ulnar physis occurs at a very young age. Shortening of the foreleg can be avoided by performing only an ulnar ostectomy, repeated if necessary, without the use of staples (Furneaux 1977). However, I have not had experience with this technique.

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*ASIF Oscillating Bone Saw, Synthes Australia Pty Ltd.

METHODS FOR INVESTIGATING INTESTINAL ABSORPTION OF AMINO ACIDS IN CHICKEN INTESTINE *IN VITRO*

R. M. GOUS

ABSTRACT: Gous, R. M. **Methods for investigating intestinal absorption of amino acids in chicken intestine** *in vitro*. *Journal of the South African Veterinary Association* (1978) **49** No. 3, 257–258 (En), Faculty of Agriculture, University of Natal, Box 375, 3200 Pietermaritzburg, Republic of South Africa.

Certain techniques for measuring amino acid absorption are considered, their specific roles are defined and both theoretical and practical considerations are used to evaluate each system. Some methodological considerations are presented.

INTRODUCTION

One of the most important functions of the gastrointestinal tract involves the transfer of materials across membranes. These transfer phenomena are involved not only in absorption of nutrients by the intestine but also in numerous secretory processes. The study of absorption of amino acids by the intestine of the chicken has proved to be of value in both the study of transport phenomena such as competition, sodium dependence and saturation kinetics, and in the investigation of different methods for studying such transport processes.

In this paper some of the techniques for measuring amino acid absorption are considered; their specific roles are defined; and both theoretical and practical considerations are used to evaluate each system. Some methodological considerations are presented to demonstrate the complexity of the techniques used to measure amino acid uptake.

INTESTINAL PREPARATIONS

1. The everted sac[14]

This preparation has numerous disadvantages due primarily to fluid transport through the intestinal tissue resulting in solvent drag[9]. This vigorous fluid movement may cause considerable distension with resultant leaks or unfolding of the villous surface. Distension of uneverted sacs does not affect fluid transfer[4]. Parsons[11] suggests that closed sacs should not be used in intestinal transport studies, but favours a modified technique[2] using cannulated sacs filled so as to limit the serosal excess hydrostatic pressure.

2. Rings of whole wall[1]

These may be cut from everted or uneverted segments of intestine. If everted, the rings are transformed into miniature sacs due to retraction of the circular and longitudinal muscle coats. Short (1 cm) uneverted rings are favoured as they fold outwards thereby exposing a large mucosal surface to the bathing medium. Strips of intestine[8] should not be used as contraction of muscle coat causes apposition of villous surfaces in particular areas, hindering access of oxygen and substrate.[11]

3. Isolated mucosa

Such a preparation is particularly fragile, but is useful when used in an Ussing chamber[13]. In fact, steady state uptake by the epithelium should only be measured using such a system[10].

4. Isolated intestinal epithelial cells[6]

Because the polarity of the enterocyte is eliminated isolated cells cannot be used to separately characterize the 2 parts of the cell membrane. The functional integrity of the cell as an amino acid accumulating unit is markedly obstructed. Also, when measuring initial rates of uptake, very short (0,5 min) incubation periods must be used. These drawbacks do not preclude the use of isolated cells for studies of cellular equipment of transport mechanisms or enzyme studies[10].

5. The Ussing Chamber[13]

A membrane is clamped between 2 chambers containing identical bathing solutions and a short-circuit current can be applied to the two chambers. This system allows measurement of chemical and electrical fluxes simultaneously, thereby facilitating the demonstration of active inward transport of sodium ions, efflux of preloaded mucosal cells and steady state uptake by the epithelium.

METHODOLOGICAL CONSIDERATIONS

1. Measuring influx and efflux

Unless initial velocity is measured when using gut sacs, rings or cells, the nett accumulation of substrate by the tissue is in fact being measured and not necessarily the transporting ability of the tissue in any one direction. Influx can be measured directly[6], whereas efflux can be studied by preloading mucosal tissue with substrate then measuring efflux to the serosal side[5]. Such a method is more accurate than using everted sacs to measure accumulation.

2. Absorption or adsorption?

The most commonly applied tests for demonstrating the involvement of a carrier in solute transport are structural specificity, saturation kinetics and competition. However, these characteristics apply equally to various adsorption theories[7], to transport systems involving binding of substrate to fixed sites rather than to carriers[12], and to facilitated diffusion[12] involving mobile carriers. Only the phenomenon of counterflow can confirm the presence of mobile carriers, and this has not yet been demonstrated with amino acids using chicken intestines[3].

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EXTRAPOLATION TO MAN OF PHARMACOLOGICAL RESULTS OBTAINED ON ANIMALS

A. C. DREYER

ABSTRACT: Dreyer A.C. **Extrapolation to man of pharmacological results obtained on animals.** *Journal of the South African Veterinary Association* (1978) **49** No. 3, 259–260 (En), Dept. Pharmacology, Univ. of Potchefstroom, 2520 Potchefstroom, Republic of South Africa

Drug development relies on the assumption of a high degree of correlation between the effects of drugs in animals and man. While a good correlation exists as far as the pharmacodynamic action of the drug is concerned it is usually the unpredictable pharmacokinetic properties of the drug responsible for obscuring proper correlations.

INTRODUCTION

The laboratory animal remains the most important tool in the hands of the pharmacologist in the development of new therapeutic agents. Laboratory animals are used in early phase I and phase II studies in the development of drugs and information gathered in these animal tests lays the foundation for further testing in humans. It is therefore essential that the pharmacologist, and other disciplines involved in the development of therapeutic agents, remains cognizant of the fact that experimentation in animals cannot insure complete predictability in man but that knowledge of the differences may at least render it easier to extrapolate from animals to humans.

PHASES OF DRUG ACTION

The series of events involved in drug action can conveniently be divided in three phases viz. the pharmaceutical phase, the pharmacokinetic phase and the pharmacodynamic phase (Scheme 1).

It is important to remember that, but for a few exceptions, animal testing serves as a satisfactory means to predict the pharmacodynamic action of the drug in man, but it is often the pharmacokinetic properties of the drug which may be different in the animal to that found in man. This is usually the greatest single factor obscuring proper correlations between data obtained in animals and man.

SPECIES DIFFERENCES

In drug testing on animals a careful selection of the type of test animal need to be made as not all animals react similarly to the same drug. Species differences in drug response may be attributed to differences in the rate of absorption or excretion of the drug, the amount of binding to plasma proteins, the rate and route of metabolism and possibly even the nature of the receptor. The differences in metabolism may be in respect of the speed at which the metabolism occurs and also the enzymatic control for the different chemical conversions or metabolic pathways.

Chemical compounds demonstrating no species differences in metabolism are usually strong polar compounds, e.g., strong acids or bases which are not metabolised but excreted unchanged. Table 1 shows examples of such compounds.

Table 1: COMPOUNDS ELIMINATED WITHOUT UNDERGOING SIGNIFICANT BIOCHEMICAL CHANGES

Strong acids	Strong bases	Non-polar
Methotrexate	Hexamethonium	Ether
Disodium chromoglycate	Aminoglycosides	DDT
Frusemide	Pentolinium	

Scheme 1: THE VARIOUS PHASES OF DRUG ACTION

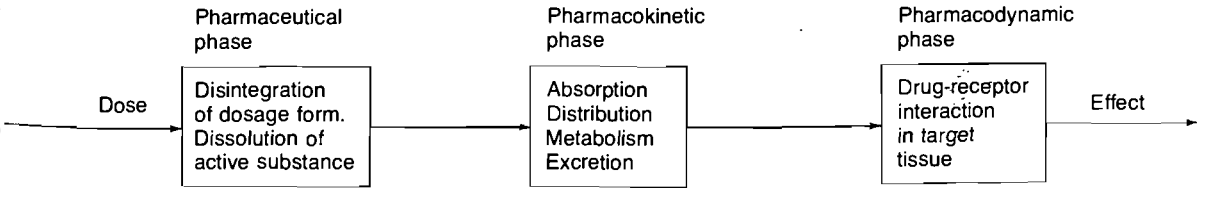

Table 2: EXAMPLES OF SPECIES DIFFERENCES IN METABOLISM

Demethylation is the major metabolic pathway in the dog, while for the rat it happens to be hydroxylation.
The cat is unable to conjugate certain compounds with glucuronic acid, e.g., phenol, paracetamol, etc.
The cat has certain catabolic deficiencies and retains chlorpromazine, diphenylhydantoin and dismethylimipramine for days after a single dose.
Aromatic amines that usually undergo acetylation reactions in most animal species are not acetylated in dogs.
Rats seem to be relatively insensitive to the spasmogenic action of histamine on smooth muscle, a response displayed by most other mammalian species whereas, on the other hand, rats seem to be more sensitive towards serotonin than other species.
Rats are less sensitive to glucocorticoids than either mice or rabbits.
Amphetamine is rapidly deaminated to phenylacetone in rabbits whereas in rats very little, if any, amphetamine is deaminated.
Phenylacetic acid is converted to its glycine conjugate in most mammalian species but is converted to its glutamine conjugate in old world monkeys.
Test animals feeding on grass have a more alkaline urine which effects the renal excretion, and thus the half-life, of acidic and basic compounds.

Table 3: EXAMPLES OF SPECIES DIFFERENCES ENCOUNTERED IN TOXICOLOGICAL STUDIES

Oral administration of thalidomide produce teratogenic lesions in rabbits but rats seem to be insensitive, probably due to poor enteral absorption.
The hamster appears to be completely resistant to the teratogenic effects of oral and parenteral administration of thalidomide.
DDT has been reported to be a weak carginogen in the mouse, but not in the hamster.
Malathion, a potent agricultural insecticide, is metabolised to inactive products much more rapidly in higher animals and man than in insects, and consequently is less dangerous to man and mammals at insecticidal concentrations.

When using the laboratory animal to gain data in respect to metabolic routes and metabolites for drugs, the investigator encounters such numerous frustrations of species differences in metabolism that it would seem an impossible task to predict a species as an appropriate metabolic model for man on the basis of chemical structure. Examples of such unpredictable inter-species variabilities are given in Table 2.

Toxicity studies in animals also demonstrate this phenomenon of species variations. Well-known examples of this are given in Table 3.

DISSIMILARITY TO MAN

The thalidomide disaster once again demonstrated that no laboratory animal would respond to drug treatment exactly like man. It has, for instance, been shown that morphine produces marked excitement in the horse, goat and cat whilst it produces analgesia and sedation in man. Another potent dependence producing analgesic, pethidine, is metabolized in dogs so fast that dosages related to its analgesic effects in man does not show any signs of drug dependence. The rabbit reveals tolerance to the toxic effects of the belladonna alkaloids, most probably due to the presence of atropine esterase. Hexobarbital has a half-life of several hours in man but only a few minutes in mice. Oxyphenbutazone has a 3 day half-life in man but only 30 minutes in the dog. These examples are but a few of a long list of unpredictable differences existing between the effects of drugs on animals and man.

Another problem pharmacologists are often faced with is that many drugs cannot be properly evaluated in animals in respect to their clinical usefulness as certain pathological conditions in humans have no satisfactory equivalent in common laboratory animals. No suitable "models" are thus available to mimic commonly encountered disorders such as rheumatic diseases, myocardial infarction, neurological and psychiatric diseases, etc.

CONCLUSIONS

Although it must be accepted that no amount of experimentation in animals will ensure absolute predictability in man, considerable progress in the handling of this problem has been achieved during the last 25 years. Unfortunately the choice of species as a model for man is often not based upon comparative anatomical, physiological or biochemical criteria, but upon such factors as availability, convenience and cost.

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