PRAOMYS (MASTOMYS) NATALENSIS

IN AGING RESEARCH

with emphasis on

autoimmune phenomena

H. A. Solleveld

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STELLINGEN

1

Praomys (Mastomys) natalensis lijkt in verband met het voorkomen van een grote variëteit aan autoantilichamen een geschikter model te zijn voor de bestudering van algemene mechanismen ten grondslag liggend aan autoimmuniteit dan de tot nu toe bekende modellen.

2

Dat Praomys (Mastomys) natalensis een model zou zijn voor myasthenia gravis is ontzenuwd.

3

De "beperkende definitie" voor autoimmuniteit zoals gebruikt door Fudenberg, welke luidt: "Autoantibodies are antibodies present in the serum of man or infrahuman species, presumably produced by unaltered native antigen and which reacted preferentially with antigens of the host, compared with those of other individuals of the same species, and especially when compared with the same antigen from different species" is onbruikbaar.

Fudenberg, H.H. (1978) Scand.J. Immunol. 7, 351-355

4

Reëvaluatie van het gebruik van progestativa ter voorkoming van loopsheid bij teven is noodzakelijk gezien de nevenwerkingen van een aantal van deze stoffen.

Rijnberk, A. et al. (1980) JAVMA, 177, 534-537.
Os, J.L. van (1981)
The Vet.Quarterly, 3, 46-56

5

In een "clinical trial" dient nagegaan te worden of ovariectomie inderdaad een te verwachten gunstig effect heeft op het verloop van het mammacarcinoom bij de hond.

6

Het onbelemmerd kunnen invoeren van bijna alle exotische knaagdieren vormt een direkt gevaar voor de volksgezondheid.

7

Het toenemen van het aantal specialistische operaties in de kleine en grote huisdierenpraktijk maakt in de naaste toekomst een stage van ko-assistenten bij praktici noodzakelijk.

8

Als men spreekt van scharreleieren dient men zich te realiseren dat het voorvoegsel scharrel meer betrekking heeft op de leverancier dan op de producent.

9

Het *individualistisch karakter* van onderzoekers is één van de grootste bedreigingen voor het handhaven van de gezondheidsstatus van een proefdierkolonie.

10

Centralisatie van de fok van proefdieren in Nederland, zoal mogelijk, zal een negatieve invloed hebben op de verdere ontwikkeling van de proefdierkunde hier te lande.

11

Het huisvesten van dieren in schoollokalen dient in het algemeen te worden verboden.

12

Het gezegde "de ouderdom komt met gebreken" wekt de verwachting dat bij de huidige toenemende vergrijzing van de bevolking de *financiën* die beschikbaar worden gesteld ter bestrijding van deze gebreken aan deze toename gerelateerd zouden zijn.

13

Bejaardencentra en prostitutie hebben het ontbreken van nestwarmte met elkaar gemeen.

Pen, J. (1979) Kijk, economie over mensen, wensen, werk en geld. Uitgeverij Het Spectrum, Utrecht/ Antwerpen.

14

De wet AROB biedt de burger met een goede schoolopleiding, een goede wetskennis, een groot doorzettingsvermogen en veel vrije tijd of geld een goede bescherming tegen lichtvaardig gebruik van artikel 19 van de Wet op de Ruimtelijke Ordening.

15

Een politikus met toekomst heeft geen verleden.

Stellingen behorende bij het proefschrift "Praomys (Mastomys) natalensis in Aging Research; with emphasis on auto-immune phenomena",

Hendricus A. Solleveld,
Utrecht, 9 april 1981.

PRAOMYS (MASTOMYS) NATALENSIS IN AGING RESEARCH

with emphasis on autoimmune phenomena

PROEFSCHRIFT

ter verkrijging van de graad van doctor in de diergeneeskunde
aan de Rijksuniversiteit te Utrecht,
op gezag van de Rector Magnificus
Prof.Dr. M.A. Bouman,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op
donderdag 9 april 1981 des namiddags
te 4.15 uur

door

Hendricus Aart Solleveld

geboren op 25 december 1945 te Monster

1981

W.D. Meinema B.V. - Delft

PROMOTOR: PROF.DR. C.F. HOLLANDER

CO-REFERENTEN: PROF.DR. J. BOUW
PROF.DR. P. ZWART

Mature agouti-colored black-eyed male (right) and female (left) Praomys (Mastomys) natalensis of the Rijswijk colony.

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CHAPTER I

INTRODUCTION

1 Aging

Aging can be defined as a time-dependent process in which the body can no longer cope with environmental factors and change as easily as it could initially (Makinodan, 1977), ultimately leading to an increased risk of dying. The aging process is associated with deterioriation at different levels of organization: the molecular, subcellular, cellular, organ and tissue levels. In addition, due to the complex manner in which organs function, it is very difficult to differentiate between cellular and extracellular causes for functional impairment. This means that aging research has to include investigations at all levels, including that of the intact organism.

A decrease in functional capacity with age can lead to disease. In fact, many diseases in aging individuals might be complications of the aging process. Mackay et al. (1977) distinguish different categories of age-related diseases in man, among which are vascular diseases, neoplasia, autoimmune diseases, diseases characterized by degeneration and atrophy and infectious diseases.

To gain insight into the different aspects of aging, one has to realize that the aging process is not limited to the elderly but must be regarded as an extension of the growth and developmental phases. It is a dynamic process starting approximately at the age of 30 years in man, when most functions have reached optimum levels. To study this dynamic process one can make use of cross-sectional or longitudinal studies. In cross-sectional studies, groups of various ages are studied and age-related differences are sought. In longitudinal studies, serial prospective measurements are obtained on one group of subjects at specified intervals. Rowe (1977) discussed the advantages and disadvantages of the two methods and concluded that longitudinal studies have advantages over cross-sectional studies. However, the drawbacks of longitudinal studies in man are that they are often impractical due to the long life span and frequent mobility of humans. In addition, genetic and environmental factors may vary greatly from one person to another, so that the results eventually obtained from such a longitudinal study must be interpreted with caution. On the basis of these arguments, it can be stated that animal models represent an essential means of studying the basic mechanisms of aging as well as the age-related diseases. Dependent on the choice of the animal species, one can easily perform longitudinal studies and can limit the number of variables to a great extent. With regard to the question of extrapolation of data from animals to man, we must realize that the fundamental aspects of the aging process are the same in all mammals.

In studying the aging process in animals, one hopes to find means for the prevention or amelioration of at least some of the disabilities of old age in man.

2 Animal models for aging research

Some aspects of aging can be fruitfully investigated in non-mammalian animals such as fish (Woodhead, 1978), insects (Rockstein et al., 1976) or nematodes (Zuckerman, 1974). However, for practical reasons, it is often not possible to follow these kinds of animals longitudinally; it is also especially difficult to extrapolate observations made in those species to aging phenomena in mammals. Therefore mammals, especially rats and mice, are more frequently used in biomedical research. Some reasons for using rodents instead of higher mammals in gerontological research are their relatively short life span (which allows longitudinal studies to be performed), the availability of inbred strains, the possibility of housing and handling them under well-defined conditions and the relatively low costs of production as compared to other, larger laboratory animals. Rodent species other than rats and mice, such as the Syrian hamster and the Mongolian gerbil, have not yet found use in gerontological research on a large scale. The same can be stated for Praomys (Mastomys) natalensis, although this species seems to be very promising for aging research at present.

A prerequisite for developing animal models for aging research is the availability of animals of good health. Infectious diseases and parasites may, in addition to being life threatening conditions, also have influence on a number of functions. Some of these so-called non-age-related conditions which may affect the results of, for instance, immunological studies are reviewed by van Zwieten et al. (1981). They also discuss the desirable health status of animals for aging research as well as a microbial monitoring program.

The selection of an appropriate model for aging studies in either organ systems or intact organisms requires knowledge of both the survival characteristics as well as of age-associated neoplastic and nonneoplastic lesions occurring in the animals to be studied (Hollander, 1976; Hollander & Burek, in press). Such information is important to avoid the selection of a rodent strain which is short-lived because of a specific disease condition, thus preventing the characteristic age-associated multiple pathological changes from becoming manifest. Furthermore, knowledge of the age-associated lesions in the various species and strains will disclose to the investigator whether or not the target organ to be studied is diseased and whether the organ function is seriously impaired by lesions elsewhere in the body. Thus, especially studies on aging demand a good experimental design.

A number of strains of mice, rats and other animal species have proved to be useful models for studying fundamental aspects of aging and for studies on the relationship between aging, cancer and immunity.

Cancer is a major cause of death in aging humans. The overall incidence of cancer increases exponentially between the ages 45 and 80, doubling about every 9 years, and levels off after age 80 (Kent, 1977). Numerous animal models of human neoplastic diseases have been described in recent years (for a survey, see Mitruka et al., 1976).

A relatively new animal species in cancer research is Praomys (Mastomys) natalensis, hereafter referred to as Mastomys (*Mastos* = nipple, mammary gland; *Mys* = mouse) because this designation has far greater common usage than the complete scientific name. The first report on the spontaneous occurrence of neoplastic lesions in Mastomys dates from the early fifties, when Oettlé (1955, 1957) described the necropsy findings in 63 animals. In particular, the finding of carcinomas of the glandular stomach in 46% of the cases excited the interest of investigators active in the area of cancer research. Since that time, a great number of reports on spontaneously occurring neoplastic and nonneoplastic lesions in this animal species have been published (Fujii & Sato, 1972; Holland, 1970; Hollander & Higginson, 1971; Hosoda et al., 1976; Jobard et al., 1974; Kurokawa et al., 1968; Oettlé, 1955, 1957, 1961; Randeria, 1979; Rudolph, 1980; Rudolph & Thiel, 1976; Snell & Hollander, 1972; Snell & Stewart, 1965, 1967, 1969 a,b, 1975; Soga et al., 1969a, 1975a; Soga & Sato, 1977; Sokoloff et al., 1967; Solleveld, 1978; Stewart & Snell, 1968, 1974, 1975). From these studies, it appears that Mastomys differs in its disease pattern from other rodents. Some of the diseases are unique to Mastomys; others have been seen only rarely in other rodents. On the other hand, lesions frequently encountered in other rodents are rare in Mastomys. A number of lesions in this animal species are suggestive for autoimmune disease. This, together with the frequent occurrence of pathological changes in the thymus, makes Mastomys of interest not only for studying the relationship between aging and cancer, but also for the relationship between aging and immunity. It is well-known that many immune functions decrease with advancing age. This decline or even failure of the immune system has been associated with a number of diseases which are known to have an increased incidence in old age (Blankwater, 1978b); among them are autoimmune diseases.

Most experimental studies on autoimmunity have been performed in New Zealand mouse strains. These have been considered to be excellent models, particularly for systemic lupus erythematosus and autoimmune hemolytic anemia. These animal models have contributed greatly to the current knowledge of autoimmunity.

Nevertheless, one has to bear in mind that, in contrast to man, only a few autoantibody specificities have been found in these mouse strains; yet, much of the current knowledge regarding autoimmunity has been based on the findings in these few mouse strains. Hence, there is still a need for models which develop other varieties of autoantibodies than those found in the New Zealand mouse to gain more insight into the nature of incompletely or not understood autoimmune phenomena in man. Since Mastomys develops lesions with age suggestive for autoimmune disorders, it seems to be worthwhile to further study Mastomys in this branch of research.

3 Outline of the present study

The study can be roughly divided into three parts. The first part gives general information on Praomys (Mastomys) natalensis. The second part is devoted to specific information on animals of the Rijswijk colony. The last and major part deals with the pathology and certain immunological aspects of aged Mastomys of the Rijswijk colony.

The outline of these studies as presented in the various chapters is as follows.

Chapter II gives general information on Mastomys, in which the following items are discussed: the taxonomic status of this animal species (which has been in discussion since 1915 and is still not settled), the geographical distribution and habitat of Mastomys in the wild, the ancestry and distribution of laboratory colonies, its biological characteristics and its role as a vector of diseases in man. This chapter will end with a review of the use of Mastomys in biomedical research.

Chapter III is completely devoted to certain characteristics of Mastomys of the Rijswijk colony. These include a TG- and C-banding chromosome analysis, breeding data of animals of the random bred and inbred colonies, the extent of inbreeding, growth data of random bred and inbred animals and survival data of random bred animals.

Chapter IV is concerned with screening of serum samples from 145 aged Mastomys of the Rijswijk colony for the presence of autoantibodies. The screening was carried out by using an indirect immunofluorescent technique.

Chapter V gives a histopathological survey of the same animals which were screened for the presence of autoantibodies.

In Chapter VI, an attempt is made to correlate the pathological findings with the presence of autoantibodies.

Inasmuch as Mastomys exhibits certain immunopathological changes with age which resembles in some respects those seen in myasthenia gravis of man, an investigation was performed on the presence of antiacetylcholine receptor antibodies and the number of acetylcholine receptors at the neuromuscular junction in a well-defined group of Mastomys. The results are presented in Chapter VII.

The final chapter, Chapter VIII, contains a discussion, in which the value of Mastomys as a model for studying autoimmunity will be considered. This includes a comparison of this animal species with the so-called autoimmune-prone mouse strains.

CHAPTER II

GENERAL INFORMATION ON PRAOMYS (MASTOMYS) NATALENSIS

1 Taxonomic status of Praomys (Mastomys) natalensis (A.Smith, 1834)

Praomys (Mastomys) natalensis (A. Smith, 1834), commonly called the multimammate mouse, is a rodent belonging to the family Muridae and is intermediate in size between Mus and Rattus (Fig.1). It is one of the most common rodents in Africa south of the Sahara and also occurs in Morocco (Davis & Oettlé, 1958).

Fig. 1 From left to right Praomys (Mastomys) natalensis, (WAGxBN)F_1 hybrid rat and C57BL/KaLwRij mouse.

The taxonomic position of Mastomys among the other African Muridae has been modified considerably since the end of the last century. Some reasons for this difficulty in classifying the Muridae are:

a. the Murids represent one of the largest families among the rodents, and many species (and even genera) show only small differences. (Misonne, 1969);

b. previous studies were carried out on an uncoordinated regional basis instead of on a pan-African one, in which similar Murids were classified by several workers into widely differing categories (Isaäcson, 1975).

Despite considerable progress in clarifying the taxonomic status of Muridae during the last 3 decades by the introduction of parameters such as dental analysis, chromosome studies and cranial characters, the taxonomic position of Mastomys still presents a twofold problem. This problem can be traced back to the reasons outlined above, namely, whether Mastomys merits recognition as a genus or a subgenus and whether the taxon Praomys (Mastomys) natalensis represents the species on a pan-African basis.

These two questions will be dealt with in more detail by reviewing the literature on them chronologically.The current systematic classification is given in Table I.

TABLE I

TAXONOMIC CLASSIFICATION OF PRAOMYS (MASTOMYS) NATALENSIS

Phylum	:	Chordata
Subphylum	:	Craniata
Class	:	Mammalia
Subclass	:	Theria
Infraclass	:	Eutheria
Order	:	Rodentia
Suborder	:	Myomorpha
Superfamily	:	Muroidea
Family	:	Muridae
Subfamily	:	Murinae
Genus	:	Praomys
Subgenus	:	Mastomys
Species	:	natalensis

1.1 Mastomys: a genus or a subgenus?

Mastomys was first described in 1834. It was classified along with most other Murids as belonging to the Linnaean genus Mus according to Rosevaer (1969). In 1881, Trouessart (cited by Rosevaer, 1969) introduced a subgenus Epimys which included rat-like rodents, amongst which was Mastomys. However, Trouessart was unaware of the proposal made by Fischer in 1803 (cited by Rosevaer, 1969), who had suggested the subgenus Rattus for the same purpose. The subgenus Epimys was raised to generic rank by Miller in 1910 and in 1916 the name Epimys was replaced by Rattus (cited by Rosevaer, 1969). In 1915, Thomas divided African rats into a number of subgenera. His classification was based on the mammary gland formula. On the basis of this classification, Thomas (1915) preferred the generic rank for Mastomys, but it was never formally raised by him to the standing of a true genus, not even when he redefined and elevated a number of Rattus subgenera to genera in 1926. Nevertheless, in "A Key to the Families and Genera of African Rodentia" by St. Leger (1931), Mastomys was listed as a genus. On the other hand, in the "Checklist of African Mammals" compiled by Allen in 1939, Mastomys was listed as a subgenus of Rattus. In 1941, Ellerman made another

attempt to classify the Muridae and was the first to take into account all of the genera. He also regarded Mastomys as a subgenus of Rattus. This subdivision was essentially based upon cranial characters and, to a lesser extent, dental characters. Although he payed little attention to dental characters, he did note that the molars of Mastomys are well cusped, as a rule, and might be reminiscent of the Mus type. He suggested that Mastomys might be one of the primitive lines that may have given rise to Mus, on the one hand, or some members of Rattus on the other.

Until 1962, Mastomys was known as Rattus (Mastomys) natalensis, although various authors (Lundholm, 1955; Petter & Saint Girons, 1965; Roberts, 1951 and Setzer, 1956) were in favor of giving Mastomys a generic rank. The opinion of Lundholm (1955) was especially interesting because he discovered a number of cranial and dental characters which gave a better impression of the affinities of African Muridae. Lundholm (1955) compared these characters between Rattus-like rodents and Mastomys, but, unfortunately, Mus was not included. This was done by Davis & Oettlé (1958). They showed that Mus and Mastomys shared a number of cranial and dental characters. These are 3-rooted upper first molars as opposed to 5-rooted ones in the rat, forked mastoid process of the squamosal portion of the temporal bone, which is hook-shaped and undivided in the rat, and weak supra-orbital ridges, which are pronounced in Rattus. In addition, tubercle 1 of the first molar is backwardly displaced, which is a step towards the marked displacement of this tubercle in Mus. However, the subapical notch of the upper incisor which is present in Mus and which is one of the generic characters employed for separating Mus from Rattus is, as in Rattus, absent in Mastomys. Notwithstanding the resemblance of Mus and Mastomys with regard to these characters, Davis & Oettlé (1958) preferred to use the nomenclature existing at that time. In 1962, however, Davis unexpectedly used Praomys as the generic name for Mastomys. An explanation for this change of mind came in 1965, when he published an interesting article in which he described the alveolar-molar root formula. This formula was developed to be a practical device for clarifying the affinities of a number of controversial genera, subgenera and species. Applying this formula, the African genera of the subfamily Murinae were divided into groups called "complex" Rattus type and "simple" Mus type. Mastomys was grouped with Myomyscus and Hylomyscus as subgenera of Praomys and as a member of the Mus type group. The systematic name was therefore Praomys (Mastomys) natalensis. Misonne (1969) supported the opinion of Davis (1965), but Rosevaer (1969) was in favor of again elevating Mastomys to a genus. Rosevaer's opinion was based on a number of differences between Mastomys and Praomys, including cranial characters, and this view has been strengthened by a recent study of van der Straeten (1979), who analyzed cranial data submitted to multivariate biometric analysis. From this study, it appears that Myomyscus, Mastomys and Praomys are quite different from each other biometrically and can be considered as different genera. It is very attractive to follow van der Straeten

(1979) and to elevate Mastomys to a genus, but one has to realize that only skull measurements were taken as a criterion. From this review, however, it appears that the classification is dependent on the criteria used and, even when using the same characters, different conclusions have been drawn. An answer to the question of whether Mastomys is a genus or a subgenus can be given only when characters other than skull measurements are taken into account; this implies that the current designation Praomys (Mastomys) natalensis should still be maintained.

Fig. 2 Map of Africa showing collecting sites of members of the taxon Praomys (Mastomys) natalensis which were studied cytogenetically. The collecting sites in southern Africa are shown in Fig.3

1.2 Does Praomys (Mastomys) natalensis represent a single species on a pan-African basis?

The discussion concerning Mastomys is not limited to its generic status only; it also revolves around its position at the species level.

Until 1944, Praomys (Mastomys) natalensis (A. Smith, 1834) was known as Mastomys coucha (A. Smith, 1836). Roberts (1944) then discovered that Smith had described the same species twice. In such a case, it is mandatory to give the first used name priority, so that the current species name is natalensis.

Fig. 3 Map of southern Africa (representing the portion of the map within the rectangle in Fig.2) showing collecting sites of members of the taxon Praomys (Mastomys) natalensis which were studied cytogenetically.

The matter became more complicated when chromosome studies were introduced into taxonomy to provide additional discriminatory criteria for separating closely allied forms which cannot be correctly identified by external morphology alone (Misonne, 1969).

Matthey (1954,a,b, 1958, 1965, 1966) carried out chromosome studies in Praomys (Mastomys) natalensis from various regions of Africa and found three chromosome forms within the taxon. Two of these forms, 2n=32 and 2n=38, are sympatric in West Africa (Fig.2). The 38 chromosome form was described previously by Matthey (1958) as having 40 chromosomes, but the chromosome number was reconsidered in 1965 by him as 38. Petter (1957) concluded on taxonomic grounds that the 38 chromosome form was a separate species. It is now known as Praomys (Mastomys) erythroleucus. In South Africa, only a form with a diploid number of 36 was found and, in the Central African Republic, only one with 32 chromosomes (Fig.2). Matthey (1966) concluded that these forms had such different chromosome complements that they can no longer interbreed and must be considered as true species.

In 1962, Huang & Strong confirmed Matthey's finding that animals from South Africa had 36 chromosomes. They used the same source for their study as did Matthey (1954a), namely, laboratory bred Mastomys kept at the Medical Ecology Center in Johannesburg. This breeding colony originated from animals caught in Transkei, Rooiwal and Baragwanath (Davis & Oettlé, 1958). Most laboratory colonies of Mastomys in the world, among them the Rijswijk colony, were derived from this stock (see this chapter, Section 3).

Recently, reports (Hallett, 1977b; Lyons et al., 1977) which deal with the cytogenetics of Praomys (Mastomys) natalensis have been published. Hallett (1977b) described a cytogenetic study of Mastomys derived from various parts of southern Africa and Lyons et al., (1977) one of Mastomys from Zimbabwe. In contrast to Matthey (1954a) and Huang & Strong (1962), these authors found two forms: animals with diploid chromosome numbers of 32 and 36.

Hallett (1977b) showed that animals derived from the south and central interior (northern and eastern Cape Province, northern Orange Free State, southern Transvaal and Lesotho) had 36 chromosomes (Fig.3). Specimens from the northern Cape Province, however, differ from those of the other regions in fundamental number (the number of major chromosome arms in a karyotype): 58 for animals from the northern Cape Province and 60 for the other regions. This reduction in fundamental number may be the result of pericentric inversion (Fig.4) converting a pair of metacentric autosomes to a pair of acrocentric ones (Hallett, 1977b).

On the other hand, animals from the northern and eastern areas of southern Africa (Southwest Africa [Namibia], northeastern Transvaal, Natal and Zimbabwe) had 32 chromosomes (Fig.3). After comparing the karyotypes of the two groups, Hallett (1977b, 1978) concluded that this difference in chromosome number may be due to the occurrence of a centric Robertsonian fusion (Fig.5) between two non-

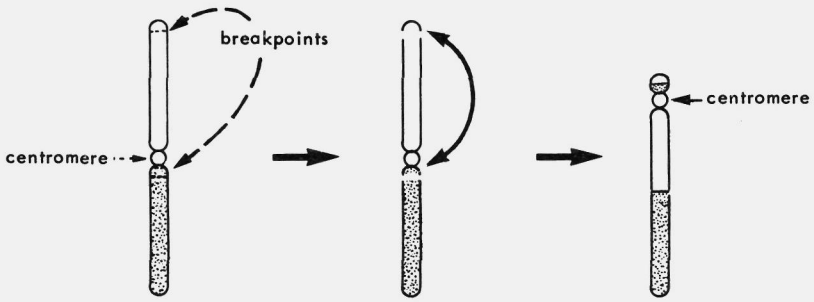

Fig. 4 Pericentric inversion converting a pair of metacentric autosomes to a pair of acrocentric ones.

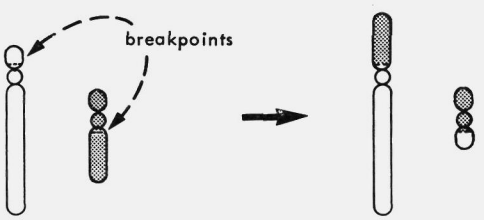

Fig. 5 A centric Robertsonian fusion between two nonhomologous acrocentric autosomes resulting in a submetacentric autosome. The small fragment is lost.

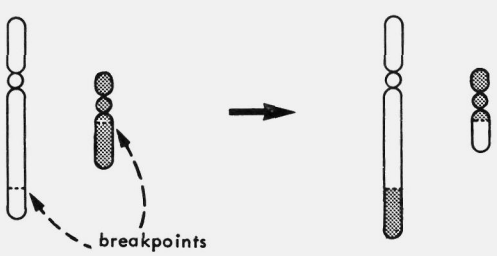

Fig. 6 Rearrangement in which the arm of an acrocentric chromosome is translocated onto the long arm of a submetacentric one. The small fragment is lost.

homologous acrocentric autosomes resulting in a submetacentric autosome and a reduction in chromosome number of one pair. The second reduction was possibly due to a rearrangement in which the arm of an acrocentric chromosome was translocated onto the long arm of a small submetacentric one (Fig.6). Unfortunately, it was not possible to substantiate this hypothesis, as banding techniques were not used. This can be seen as a distinct weakness of Hallett's study.

Between the two chromosome forms, differences in external morphology of the head and in behavior were observed. The 32 chromosome form had a slightly more tapered snout and was much more aggressive than the 36 chromosome form.

Hallett (1977b, 1978) crossed the two chromosome forms in the laboratory and F_1 hybrids with 34 chromosomes were produced. The hybrids obtained were sterile. Macroscopically, the male hybrids had smaller testes and the females had larger ovaries and uteri which were double in weight when compared to the respective organs of the two parents. Histologically, only early stages of meiotic cell division up to the pachytene stage of primary spermatocytes were present in the seminiferous tubules and atypical follicles were observed in the ovaries. These findings suggest that members of the two groups probably mate only within their respective groups under natural conditions. This suggestion is supported by Lyons et al. (1977) and Green et al. (1978), who also found the two chromosomal forms in Zimbabwe even occurring sympatrically (Fig.3); however, despite the presence of sympatric populations, no animals with 34 chromosomes were found. In addition, Green et al. (1978) determined that distinct hemoglobin phenotypes were correlated with each diploid number and Gordon (1978) mentioned the lack of homology between Giemsa-banded chromosomes of these forms; however, he did not present these data.

All of the above-mentioned authors came to the same conclusion: that there is enough evidence for regarding the 32 and 36 chromosome forms as two separate species. This opinion was based on chromosome polymorphism, the production of sterile hybrids and the absence of hybrids in sympatric populations. Applying the definition of a species as proposed by Mayr (1963) that "A species is a group of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups", one has to conclude that the conditions are fulfilled for these populations. This might mean that the two forms will be admitted as two separate species in the near future. However, they should perhaps be considered as being sibling species, since the two chromosome forms do not show clear differences in external morphological characters.

Such a decision will also have implications for the species name. Hallet (1977b) as well as Gordon (1978) found only animals with 32 chromosomes in Natal in the vicinity of Durban. This area is the type locality of Praomys (Mastomys) natalensis. If after a thorough survey no animals with 36 chromosomes are found in this area, the 32 chromosome form should then be given the species name natalensis and the 36 chromosome one would have to revert to the earlier species name, coucha. The type locality of coucha as described by Smith was between the Orange River and Bechuanaland. Only the 36 chromosome form has been found in that area up to now; therefore, it appears to be correct to use the species name coucha for this group. Furthermore, it would also appear that most of the laboratory Mastomys in the world should in fact be called Praomys (Mastomys) coucha. Since this subject requires further investigation, the species name natalensis will be retained in this monograph.

2 Geographical distribution and habitat

As stated in Section 1 of this chapter, Praomys (Mastomys) natalensis is the most widely distributed and one of the most common rodent species in Africa. It occurs from Knysna on the southern coast to Erithrea in the northeast and there is a population in Morocco in the northwest (Coetzee, 1975). It is most prevalent in the southern and northern savanna biotic zones (Isaäcson, 1975). It is absent from the extremely moist area of the Zaïre basin, the montane forests, the dry parts of the Karoo, Kalahari, the northwestern Cape Province and southern parts of Namibia, the Namib, Sahara and Somali deserts and from typical winter rainfall areas (Coetzee, 1975). This wide geographic and climatic range indicates that Mastomys has great capacity for adaptation. This can be illustrated from data obtained by Coetzee (1975), who found Mastomys in areas where frost occurs over 90 days per year and with a mean annual rainfall of less than 400 mm; on the other hand, it was also found in areas where the mean minimum temperature is 23°C and with a mean rainfall of 4,000 mm per annum.

Mastomys is found mainly in bush, scrub and cultivated areas and its population density is higher around human habitations than in open bush (Coetzee, 1975). This semicommensal habit makes it an important link between other wild rodent species and man, particularly in its role as a vector of zoonotic diseases.

Mastomys is nocturnal and shows two peaks of activity: one at dusk and a greater one at sunrise (Veenstra, 1958). It is omnivorous, but mainly granivorous, living on seeds of wild grasses, grass rhizomes, grain, maize and rice. Since Mastomys lives gregariously, extensive damage to crops may occur and, through its semicommensal habit as well as its fair amount of climbing activity, the animal has access to man's food storage. When food is plentiful, Mastomys can be found in daily association with other wild rodents (Coetzee, 1975) but, when competition leads to food scarcity, Mastomys gives way; this is also the case when Rattus rattus is present in large numbers (Davis, 1953; Isaäcson, 1975; Rosevaer, 1969; Shortridge, 1934). This suggests that its territorial instinct is rather weak. When food is scarce, it will travel several kilometers in search of nourishment. Mastomys is to some extent dependent on the presence of water, although open water is not necessary (Coetzee, 1975). It shows very little fear of water, however, and can be regarded as a good floater and swimmer, using the well-known dog-paddle method (Veenstra, 1958).

Mastomys uses deserted burrows of other rodents such as those of gerbils (Tatera brantsi and T. leucogaster) and the mole rat (Cryptomys hottentotus), but, if necessary, it can dig its own burrow; however, this occurs only where the soil is soft or cracked. The animals are able to make intricate intertwining systems of tunnels in soft soil without apparent order or a central chamber (Veenstra, 1958). The burrows serve mainly for nesting purposes. As nesting material, grass stems, rhizomes and leaves are generally used. Shelter and refuge, on the other hand, are taken in or under anything available, whether natural or man-made (Isaäcson, 1975).

The population density of Mastomys is dependent on the food supply. Ripening of crops normally takes place in the period following the rainy season and hence breeding activity shows a peak towards the end of the rainy season and the beginning of the dry period. The average litter size determined from various parts of Africa is 10. This is in agreement with the finding of Coetzee (1967), who found a mean corpora lutea count of 10.92 in a sample of 36 wild-caught females. The survival is dependent on the climatic conditions and on the population density. In most parts of southern Africa, the beginning of the dry period coincides with the beginning of the winter, which may have an adverse effect on the survival of this species. In addition, a high population density may lead to a disruption of the normal social structure and possibly to food scarcity; this again may have consequences for survival (Coetzee, 1965, 1975).

With regard to the maximum life span of free-living Mastomys, De Wit, referred to by Coetzee,1975 , has calculated this to be 339 days, but life spans of up to 38 months have been recorded for animals maintained under laboratory conditions (see Chapter III, Section 6.3.2.).

3 Ancestry and distribution of laboratory colonies

Laboratory colonies of Praomys (Mastomys) natalensis are descended from wild animals caught at various places of South Africa. In 1939, Davis (Davis & Oettlé, 1958) at the Medical Ecology Center, Johannesburg, started the laboratory breeding of wild animals which had been caught in the Glen Grey district, Transkei, eastern Cape Province. In 1940, animals from Rooiwal, Orange Free State, were added to this colony. This resulted in the establishment of two lines: Transkei and Rooiwal, as well as a cross between the two.

In 1946, the original stock was supplemented by seventeen wild pairs from Baragwanath, 16 kilometers southwest of Johannesburg. It cannot be excluded that interbreeding between the old and new stocks took place. Oettlé (1967) obtained animals of this stock in 1958 and, after 20 generations of brother-sister mating, called them the R-strain. The animals had a dark brown-gray coat with a light gray belly (agouti). The eyes were black, the ears and tail dark and the paws light pink. From this stock, animals were sent to the Walter Reed Army Institute of Research (WRAIR), Washington D.C., U.S.A. in 1950 and to the London Zoo, London, U.K. in 1952. The Animal Production Center at the National Institutes of Health, Bethesda, Maryland, U.S.A., obtained animals from WRAIR in 1954, which were lost when an attempt was made to inbreed them. The center started a new colony in 1959, again from stock from WRAIR. This colony provided the breeding stock for most other laboratories. A survey of the distribution of the agouti-colored Mastomys colonies is given in Fig.7. Many laboratories have attempted to establish inbred colonies of agouti-colored animals, but most have not succeeded. At present, two inbred colonies exist: one at the Institute of Medical Science,

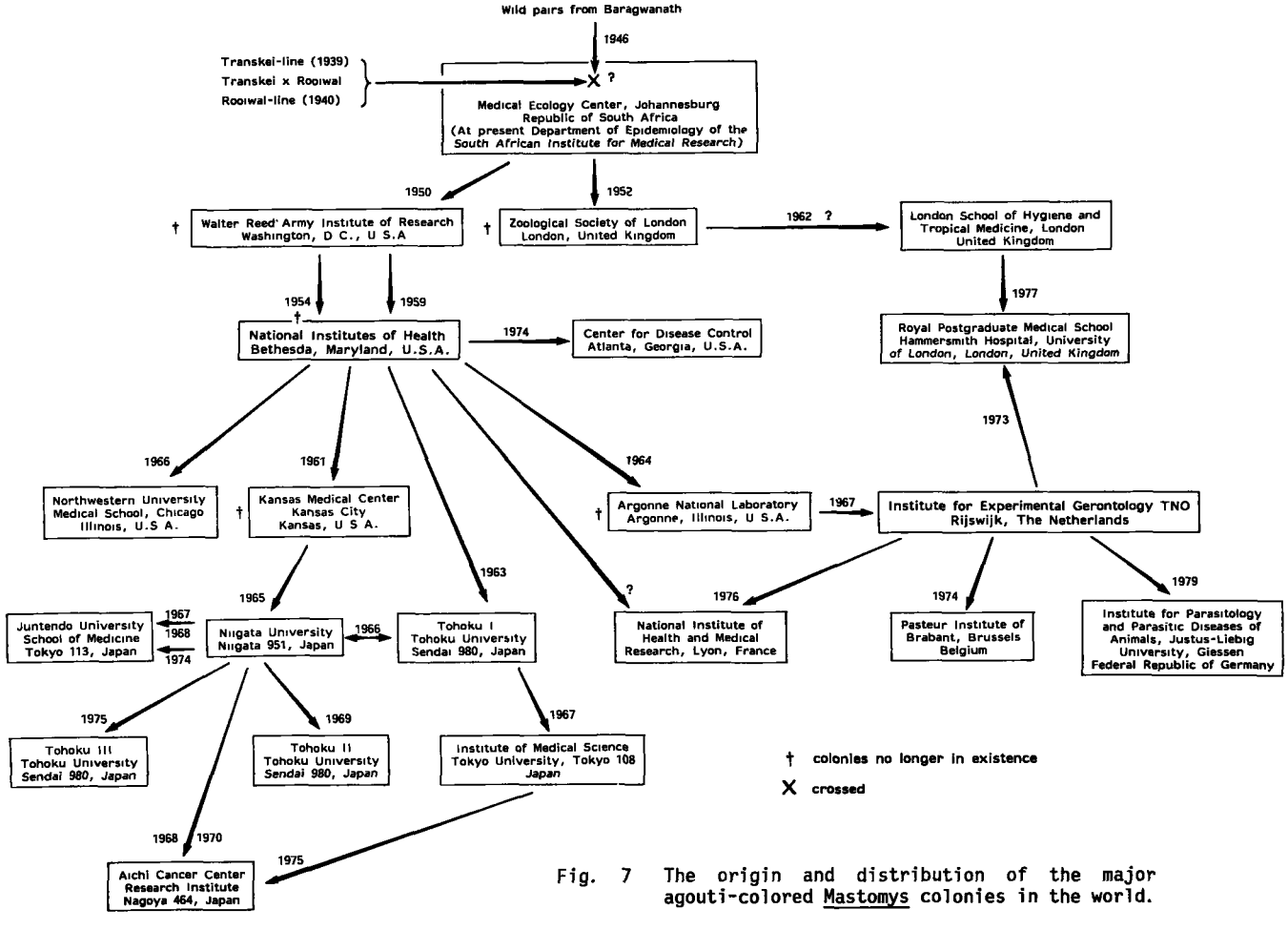

Fig. 7 The origin and distribution of the major agouti-colored Mastomys colonies in the world.

Tokyo, Japan, and the other at the Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands. Both colonies have now reached the 30th generation of brother-sister mating.

In 1953, mutants appeared in the Baragwanath line at the Medical Ecology Center, Johannesburg, Republic of South Africa. These animals had light gray coats, dark ruby eyes and pale gray-brown ears and tails. They are known as the Baragwanath dilutes.

In the fifties, Meester (1960) started with the laboratory breeding of wild animals caught at Iscor Steelworks, Pretoria. In 1958, Coetzee received a pink-eyed agouti-colored female mutant from this stock and mated her to one of her sons (Coetzee, 1980). Brother-sister mating was subsequently employed. Different coat and eye colors were observed during the process of inbreeding, but continuous selection has finally resulted in the so-called chamois-colored pink-eyed Mastomys, also known as the Y-strain (Coetzee, 1980; Randeria, 1978). The animals have a yellowish coat with a pale gray belly. Coetzee (1980) has suggested calling this subline "MEC-chamois Mastomys", because they were bred at the Medical Ecology Center, Johannesburg, Republic of South Africa. This laboratory has recently changed its name to Department of Epidemiology of the South African Institute for Medical Research, so that the possibility of another line or subline being developed there no longer exists. Therefore, the designation "MEC-chamois" can be used without objection. The major inbred colony of these chamois-colored pink-eyed animals is presently at the Cancer Research Institute, University of Durban-Westville, Durban, Republic of South Africa.

Crossing the Baragwanath dilutes and the chamois-colored pink-eyed Mastomys has given rise to the so-called Z-strain. This colony is also located at the Cancer Research Institute in Durban and has now reached 30 generations of brother-sister mating (Randeria, 1978). The animals of this strain have, like the chamois-colored pink-eyed Mastomys, a yellowish coat with a pale gray belly and pink eyes.

Between 1959 and 1961, numerous Mastomys from Roodepoort and Komatipoort, the Baragwanath stock and chamois-colored pink-eyed animals (Y-strain) as well as some with intermediate coat colors were sent to the Bilharzia Field Research Unit of the Medical Research Council, Nelspruit, eastern Transvaal. It is likely that intercrosses have taken place among these animals. In 1966, the Institute for Parasitology, Giessen, Federal Republic of Germany, received animals from this source. On inbreeding, mutants with a chamois-colored coat and light red eyes appeared. These mutants were bred further and are now known as the GRA-Giessen strain. This colony is partly inbred. These mutants are used all over the world for parasitological research. A scheme showing the origins of the various mutant stocks of Mastomys is given in Fig.8.

For those interested in obtaining Mastomys for research, a partial listing of addresses of laboratories and institutes maintaining Mastomys colonies is given in the Appendix.

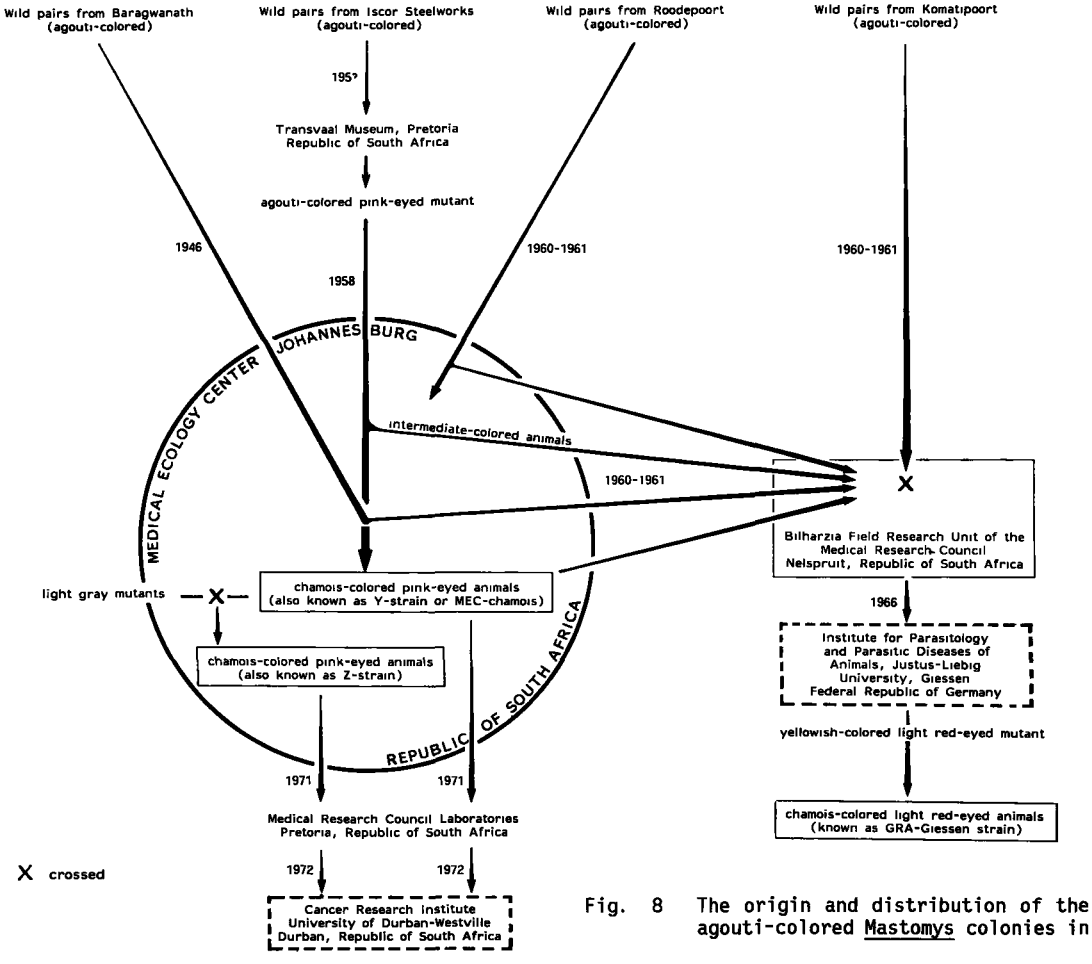

Fig. 8 The origin and distribution of the major non-agouti-colored Mastomys colonies in the world.

4 Biological characteristics

4.1 General description

The pelage of wild Mastomys varies with age. The coat color of subadults is gray, but cinnamon, brown and even reddish colors appear with adulthood. The eyes are black, the ears and tail dark and the paws are light pink. As discussed in the previous section, various coat and eye colors have been developed in laboratory bred Mastomys. Wild and laboratory kept adult males weigh up to about 100 g and females to about 80 g. The total length of the head and body of adult Mastomys is 115 to 135 mm, with a tail length of 112 to 120 mm. As previously stated, wild Praomys (Mastomys) natalensis may have 32 or 36 chromosomes, but most current laboratory colonies probably represent the 36 chromosome form only.

4.2 Behavior

Veenstra (1958) has given an informative account of the behavior of wild and laboratory kept Mastomys. In general, the behavior in the laboratory is compatible with its field habits. It is a nervous, irritable animal, immediately ready to bite and to escape and, therefore, difficult to handle. Frequent handling can assist in taming the animals. The mutants such as the chamois-colored pink-eyed Mastomys and the Baragwanath dilutes are tamer than the agouti-colored animals. Mastomys is very tolerant of other rodent species. However, with respect to its own species members, it frequently exhibits cannibalism. If males from various litters are caged together, there is a great chance that one of the cage mates will be attacked and eaten. This is sometimes restricted to nibbling away the ears and the tail, while the victim makes no attempt to escape. If brothers of the same litter are housed together, the aggressiveness is much less, just as among females and breeding pairs. With regard to the mating behavior, the male is always allowed by the female to remain near to her, but is permitted to mount only at estrus. If the male attempts to do so when the female is not in estrus, it is rejected by a kick from a hind paw. If a polygamous breeding system is used, the male may occasionally be attacked by the females and this may result in serious wounds. The cannibalistic tendencies are most pronounced in Mastomys females with first litters. Litter eating is a common phenomenon and may result in the loss of 96 per cent of all first litters (Oettlé, 1961,1967). Experiments with alternative objects for gnawing, food supplements and total darkness all appeared to be ineffective (Oettlé, 1967). The only successful way to prevent cannibalism seems to be strict genetic selection.

Another annoying characteristic is the wastage of food by Mastomys. It is particularly liable to pull into its cage large quantities of fragmented food pellets and to leave these fragments uneaten. Because of the wastage of food, the animals should be supplied with only a limited amount of food per day [approximately 10 grams standard rodent pellets per adult animal per day (Saito et al., 1977)].

4.3 Reproductive data

The reproductive data are summarized in Table II. Since different lines and sublines have been developed in the laboratory, a distinction is made between animals originating from Baragwanath and from Iscor. The latter was divided into the agouti-colored and the chamois-colored pink-eyed mutants; the latter is also known as the Y-strain. Data obtained from laboratory colonies derived from animals of the Baragwanath line are listed as such. From Table II, it appears that considerable differences have been found for some of the reproductive parameters, which is not so surprising when one realizes that housing, food, genetic selection procedures, inbreeding or random breeding, etc., may have an influence on these parameters.

Johnston & Oliff (1954) reported that the estrous cycle length is 8.8 ± 0.4 days and that proestrus and metestrus both last for 1.9 ± 0.15 days. Oettlé (1967) has reexamined the cycle and showed that the average cycle is 7 or 8 days. The various stages of the estrous cycle are: proestrus, 1.9 ± 0.15 days, showing abundant mucous streaks with nucleated squamous and mucous cells; late proestrus, large squamous epithelial cells with degenerating nuclei and cornified squamous and mucous cells; estrus, clumps of cornified nonnucleated squamous and mucous cells; early metestrus, few leukocytes and degenerating cornified squamous cells; late metestrus, abundant leukocytes, degenerating cornified and noncornified squamous cells and mucous cells; diestrus, abundant leukocytes, nucleated squamous and mucous cells, absence of cornified cells.

Except for the duration of the proestrus, Oettlé (1967) did not give the length of the stages of the estrous cycle.

4.4 Development

An excellent description of the development of Mastomys has been given by Meester (1960), from which the following data are taken.

Mastomys has an average weight of 2.2 grams at birth. The newborn animal is bright pink with a translucent skin. The entire body is covered with sparse hairs which are black on the dorsal side and white on the ventral side of the body. Vibrissae are present. Eyes are closed, the ear pinnae are folded down over the external ear and the teeth are unerupted. The toes of both fore and hind paws are fused.

The newborn animals are active after birth, and will suck at the mother's nipples as early as the first hour of life. The ear pinnae unfold soon after birth, usually on the first or second day. Response to sound starts at the 12th day. Body hair begins to grow rapidly after the 8th day. The toes become separated from each other before the 8th day. Teeth start erupting on about the 9th day and all are erupted by the 12th day. Between the 14th and 17th days, the eyes open, after which nursing takes place less frequently and probably from that time they start eating solid food. Mastomys can be weaned at 21 days after birth, when nursing occurs only sporadically. They have then reached an average weight of

TABLE II

REPRODUCTIVE DATA FOR *MASTOMYS*

	Origin		
	Baragwanath	Iscor	
	Agouti-colored	Agouti-colored	Chamois-colored pink-eyed mutant (also called Y-strain and MEC-chamois)
Perforation of the vagina (days)	76.0 ± 5.9 (c); 41-44 (e)	-	-
Body weight (g) at sexual maturity	40 (a,e); female 30 to 35 male 40 to 45 (i)	-	-
Estrous cycle length (days)	8.8 ± 0.4 (c); 7 or 8 (e)	-	-
Age at first estrus (days)	104 ± 9 (c)	-	-
Mean age at first conception (days)	76 (e)	-	-
Gestation period (days)	23.0 ± 0.7 (a,c,e)	-	-
Age at birth of first litter (days)	± 130 (c)	77 (d)	98-147 (g)
Post-partum estrus (days)	2.6 ± 0.5 (c)	-	-
Mean no. young born per litter	7.3 (f); 7.4 (a); 6.3 (b) 5.4 (h); 5.7 and 5.4 (j)	8.0 (d)	6.0-6.5 (g)
Mean interval between births (days)	61.5 (f); 64 (a)	33.1 (d)	121 (g)
Mean no. litters per lifetime	7.6 (a)	3.3 (d)	-
Sex ratio males/females	0.93 (f)	1.09 (d)	-
Mean age at birth of last litter (days)	277.3 ± 7.5 (e)	-	-
Maximum age at birth of last litter (days)	672 (a); 401 (e)	-	-

References:

a. Davis, 1963
b. Fujii et al., 1966
c. Johnston & Oliff, 1954
d. Meester, 1960
e. Oettlé, 1967
f. Oliff, 1953
g. Randeria, 1978
h. Soga et al., 1969a
i. Solleveld, unpubl.data
j. Solleveld et al., 1980a

11.7 grams. At the time of sexual maturity, the males weigh 40 to 45 grams and the females 30 to 35 grams (Solleveld, unpublished observations).

4.5 Anatomical characteristics

Despite the fact that Mastomys belongs to the same subfamily (Murinae) as do the laboratory mouse (Mus musculus) and the laboratory rat (Rattus norvegicus), it differs from these species in some anatomical features. Before discussing these differences, some general remarks will be made.

The skeleton of Mastomys differs very little from that of the mouse and the rat. For more details regarding skeletal descriptions, the reader is referred to the works of Greene (1959) and Hebel & Stromberg (1976) for the rat and to Hummel et al. (1966) for the mouse. To enable a comparison between Mastomys and these rodent species, a lateral and dorsoventral radiograph of a mature male Mastomys is included (Figs.9a,b). Some minor differences with respect to skull and dental characteristics were discussed in Section 1.1 of this chapter. With regard to the anatomy of the major organ systems, Mastomys more closely resembles the rat than the

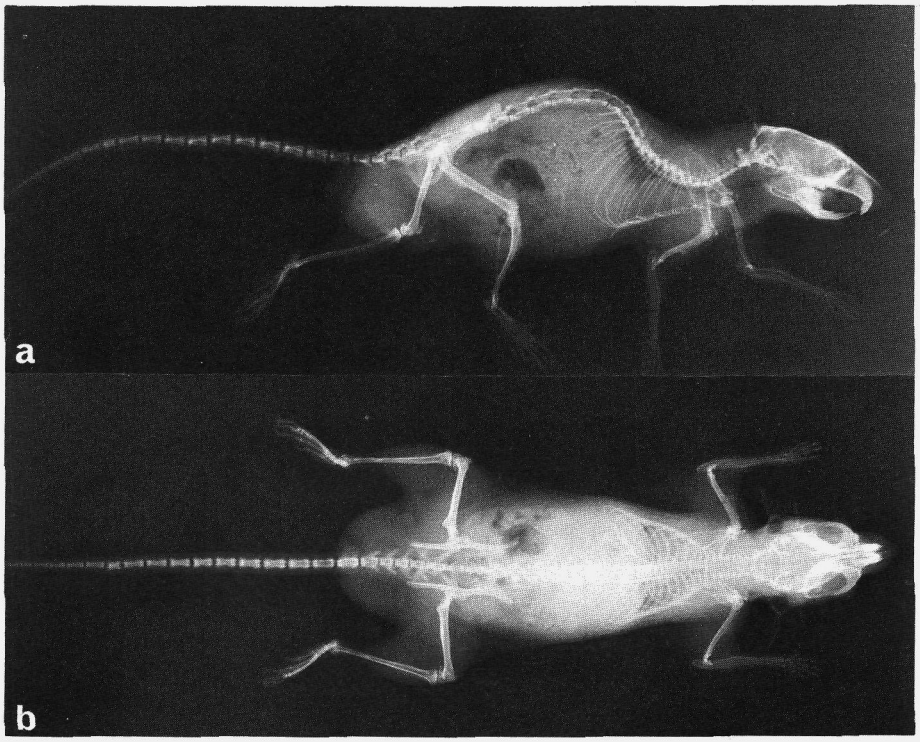

Fig. 9 **a**, lateral and
b, dorsoventral radiograph of a mature male Mastomys.

mouse, e.g., in having no gall bladder. However, it differs distinctly from both species with respect to the anatomy of the genitourinary system, which will be discussed below.

4.5.1 Some anatomical characteristics distinguishing Mastomys from the mouse and the rat

4.5.1.1 Prostate gland

The most distinctive characteristic of Mastomys is the presence of a well-developed prostate in the female (Brambell & Davis, 1940). The female prostate gland consists of two lobes lying caudal to and on each side of the bladder neck. Dorsally, these lobes are separated by the urethra and vagina. The localization corresponds to the ventral prostate lobes of the male (Ghanadian et al., 1975; Price & Williams-Ashman, 1961). With the light microscope, no differences can be observed between the male and female prostate. However, ultrastructurally, some differences have been noted at the subcellular level (Smith et al., 1978a). This particularly concerns the presence of prominent dilated Golgi vesicles in the apical region of the acinar cells of the male prostate, which are only occasionally present in the estrous female. The differences between the male and female prostate at the subcellular level may be due to different hormone levels. This suggestion is supported by the findings of Ghanadian et al. (1977) who found that serum testosterone values were 10 times higher in the male than in the female. The testosterone concentration in the male and female prostate was similar, but the dihydrotestosterone concentration in the female prostate was significantly lower than in that of the male. These findings justify the conclusion that the female Mastomys has a prostate gland which exists in a low androgen milieu (Ghanadian et al., 1977).

Despite all investigations performed up to now, the function of the female prostate is still obscure, although Holland (1970) mentioned that the female prostate is homologous to the human female Skene glands which serve to lubricate the distal urethra.

4.5.1.2 Preputial and clitoral glands

Preputial glands lying on either side of the distal part of the penis in the subcutaneous adipose tissue and opening into the preputial cavity near its orifice can be found in the mouse and the rat, but not in Mastomys. The same holds for the female homologue of the preputial glands, the clitoral glands.

4.5.1.3 Mammary glands

The female Mastomys has 7 to 9 mammary glands on each side as compared to 6 or less in the rat and the mouse, hence the origin of its common name, the multimammate mouse. In mice and rats, the thoracic glands are separated from the

abdominal glands. This separation is not found in Mastomys. The glands are more or less equally distributed along the mammary line.

4.5.1.4 Penis

In bats, dogs, cats, ferrets, mink, squirrels, cricetids, murids and many subhuman primates, a bone, the os penis or baculum, is present in the penis (McKeever, 1970). This bone adds rigidity to the penis. The os penis of each species has a characteristic shape (Burt, 1960). Mastomys has an os penis with a cordate anterior process instead of a broad one as in the rat or a lanceolate one as in the mouse.

5 Hematology and clinical biochemistry

This section provides normal data on the hematology and clinical biochemistry of laboratory kept Mastomys (2n=36). It is emphasized that normal values may vary widely depending on the animal's sex, strain, breed and age as well as on the sampling methods and analytical conditions. This means that these data can serve only as guidelines. The values given in this paragraph are expressed as SI units. For that reason, they can differ from those given by the various authors cited.

5.1 Hematology

The reader is referred to the excellent and detailed reports of Heitmann (1971, 1972) for the morphology of bone marrow and blood cells and the cellular composition of bone marrow of animals of the GRA-Giessen Mastomys strain.

Hematological values were determined by Martin & Rutty (1969). A summary of results for the various parameters studied by them is given in Table III. The animals used in this study were derived from the Baragwanath stock. Their ages varied from 10 to 20 weeks.

The red cell counts, hemoglobin and packed cell volumes were found to be significantly higher in male Mastomys. The values obtained are within the ranges described for various mouse (Harrison et al., 1978; reviewed by Russell & Bernstein, 1966) and rat (reviewed by Ringler & Dabich, 1979) strains.

In addition, Martin & Rutty (1969) confirmed the finding of Davis & Oettlé (1958) that the neutrophils of Mastomys resemble those of the mouse and differ from those of the rat in giving a negative alkaline phosphatase reaction.

TABLE III

HEMATOLOGICAL VALUES FOR MALE AND FEMALE MASTOMYS [a]

		Males (n=23)	Females (n=32)
Erythrocytes	($10.^{12}.l^{-1}$)	7.9 ± 0.4 [b]	7.3 ± 0.6 [b]
Hemoglobin	($nmol.l^{-1}$)	8.6 ± 0.6	8.0 ± 0.6
Packed cell volume	(%)	42.0 ± 3.4	38.8 ± 3.0
Mean corpuscular volume	(fl)	53.1 ± 3.4	53.5 ± 3.4
Mean corpuscular hemoglobin	(fl)	1.1 ± 0.1	1.1 ± 0.1
Mean corpuscular hemoglobin content	($nmol.l^{-1}$)	20.7 ± 0.8	20.5 ± 0.6
Reticulocytes	(%)	1.8 ± 1.3	2.2 ± 1.4
Thrombocytes	($10^{9}.l^{-1}$)	368 ± 127	289 ± 56
White blood cell count (WBC)	($10^{9}.l^{-1}$)	7.6 ± 1.7	7.5 ± 2.1
Neutrophils	(% WBC)	22.8 ± 9.2	18.4 ± 7.2
Lymphocytes	(% WBC)	73.2 ± 9.4	78.0 ± 7.9
Monocytes	(% WBC)	2.7 ± 1.6	1.9 ± 1.3
Eosinophils	(% WBC)	1.3 ± 1.3	1.7 ± 2.1
Basophils	(% WBC)	0	0

a) Recalculated from data of Martin & Rutty (1969)
b) Mean values ± S.D.

5.2 Clinical biochemistry

5.2.1. Serum proteins

The total serum protein concentration was determined in animals of the Rijswijk colony of various ages by van Pelt & Blankwater (1972). This colony originated from animals of the Baragwanath line. The total serum protein concentration ranged from 48 to 82 $g.l^{-1}$. The mean concentration increased during the first weeks of life from approximately 50 $g.l^{-1}$ between 1 and 5 weeks of age to 72 $g.l^{-1}$ at 26 weeks of age, after which it remained at a more or less constant level. No differences were observed between males and females. These authors also determined the relative concentration of the serum protein fractions in animals of both sexes at various ages. The same was done by Kozima (1977) in animals of the Niigata colony, which, like the Rijswijk colony, originated from the Baragwanath line. The results of the two studies are given in Table IV for male and in Table V for female Mastomys.

As can be seen in Tables IV and V, marked differences in some fractions are found between the two studies, particularly the α_2 and the combined β and γ globulin fractions. Van Pelt & Blankwater (1972) found that the α_2 globulin fraction in both sexes remained relatively constant with advancing age, while Kozima (1977) found a considerable decrease in this fraction. In females, the decrease occurred

TABLE IV

RELATIVE CONCENTRATION OF SERUM PROTEIN FRACTIONS IN MALE MASTOMYS AT VARIOUS AGES

Age weeks	Age months	Prealbumin + albumin (%)	α_1 globulin (%)	α_2 globulin (%)	β_1 globulin (%)	$\beta_2+\gamma$ globulin (%)	$\beta+\gamma$ globulin (%)	
1- 5		48.5 ± 1.0	10.0 ± 1.0	13.9 ± 5.8	14.6 ± 6.0	9.0 ± 12.1	-	a)
	1	55.4 ± 2.4	17.0 ± 4.0	20.0 ± 4.4	-	-	7.6 ± 1.3	b)
6-13		34.5 ± 5.0	26.0 ± 6.0	11.5 ± 3.0	9.5 ± 4.0	17.5 ± 1.0	-	a)
	2	39.0 ± 4.6	43.2 ± 5.0	10.2 ± 6.2	-	-	7.6 ± 1.0	b)
	3-5	42.1 ± 5.3	41.9 ± 5.0	8.0 ± 4.3	-	-	7.8 ± 1.6	b)
14-26		31.0 ± 2.0	30.0 ± 7.0	12.0 ± 3.5	16.0 ± 6.0	14.3 ± 7.0	-	a)
27-52		31.5 ± 5.5	21.0 ± 3.0	16.0 ± 3.5	10.0 ± 1.5	22.0 ± 2.5	-	a)
	11-15	27.4 ± 2.9	32.7 ± 3.7	33.0 ± 5.2	-	-	7.0 ± 1.1	b)
53-78		34.0 ± 6.0	17.5 ± 4.5	15.5 ± 2.0	10.5 ± 1.0	22.0 ± 4.0	-	a)
	19-38	42.7 ± 6.8	37.0 ± 8.1	4.5 ± 4.8	-	-	15.8 ± 3.5	b)
79 and older		36.0 ± 7.0	19.0 ± 3.0	13.0 ± 2.5	10.0 ± 4.0	21.5 ± 2.5	-	a)

References:
a) van Pelt & Blankwater (1972)
b) Kozima (1977)

TABLE V

RELATIVE CONCENTRATION OF SERUM PROTEIN FRACTIONS IN FEMALE MASTOMYS AT VARIOUS AGES

Age weeks	Age months	Prealbumin + albumin (%)	α_1 globulin (%)	α_2 globulin (%)	β_1 globulin (%)	$\beta_2+\gamma$ globulin (%)	$\beta+\gamma$ globulin (%)	
1- 5		46.5 ± 1.5	8.5 ± 1.5	12.9 ± 7.8	16.3 ± 4.4	15.4 ± 8.9	-	a)
6-13		47.0 ± 3.5	13.5 ± 2.5	12.0 ± 1.5	9.5 ± 2.5	20.5 ± 1.0	-	a)
	2	52.9 ± 6.8	11.1 ± 1.3	29.9 ± 4.9	-	-	8.0 ± 1.2	b)
	3- 5	49.6	10.9	31.4	-	-	8.2	b)
14-26		44.5 ± 1.0	10.0 ± 4.5	14.0 ± 3.5	9.0 ± 2.0	23.0 ± 4.0	-	a)
27-52		37.0 ± 6.5	10.0 ± 3.5	15.5 ± 4.0	11.0 ± 5.0	24.5 ± 3.0	-	a)
	11-15	43.0 ± 5.3	12.5 ± 1.3	36.5 ± 6.7	-	-	8.0 ± 1.3	b)
53-78		37.0 ± 6.0	11.0 ± 1.5	15.0 ± 4.5	13.0 ± 1.5	24.0 ± 3.0	-	a)
	19-38	55.7 ± 7.0	15.6 ± 4.1	10.0 ± 7.9	-	-	16.7 ± 4.7	b)
79 and older		38.5 ± 5.5	10.0 ± 3.0	13.0 ± 4.5	12.0 ± 4.0	26.5 ± 3.0	-	a)

References:
a) van Pelt & Blankwater (1972)
b) Kozima (1977)

between the ages of 11-15 and 19-38 months; in males, this occurred between 1 and 3-5 months of age and was followed by an increase between the ages of 3-5 and 11-15 months and again a strong decrease between 11-15 and 19-38 months.

For the β + γ globulin fraction, an expected increase was found with age in both studies. However, the difference in the relative concentration of this fraction between the two investigations is obvious. Kozima (1977) found a relative concentration of between 7.0% and 16.7% and van Pelt & Blankwater (1972) one of between 23.6% and 38.5% (Tables IV and V). The latter range corresponds to the values found in various mouse (reviewed by Bernstein, 1966) and rat (reviewed by Ringler & Dabich, 1979) strains and therefore seems to be more realistic.

A conspicuous finding in both studies was the sex-linked difference in α_1 globulins, which can be seen from the 6th week of age onwards. The prealbumin-albumin fraction decreased earlier in males, probably due to the increase in α_1 globulins. The component responsible for the sex-linked increase of the α_1 globulin fraction was identified as haptoglobulin (Kozima, 1977).

The observation made by Ootsu et al. (1976) must also be mentioned here. They observed polymorphic protein patterns showing three distinct phenotypes in the β globulin region of sera from Mastomys which were identified as transferrins. Transferrin is a nonheme iron binding protein. Its major function is to exchange iron between tissues. The serum transferrin phenotypes of Mastomys were designated as Trf-K, Trf-M and Trf-KM. This finding was confirmed by Kozima and Oite (1977). In addition, these authors reported on 2 other phenotypes: Trf-Km and Trf-kM. Genetic studies have indicated that the serum transferrin polymorphism is controlled by a pair of codominant allelic genes at a single autosomal locus. The phenotypes of serum transferrin are the only genetic markers discovered so far in Mastomys.

5.2.2. Other serum constituents

5.2.2.1. Serum urea nitrogen, creatinine and cholesterol

These constituents have been determined in animals of various ages of the Rijswijk colony (van Pelt & Blankwater, 1972; Zurcher et al., 1980). A summary of results on the various parameters is given in Table VI. The urea nitrogen and cholesterol values are approximations derived from curves.

Van Pelt & Blankwater (1972) observed the following changes in serum urea nitrogen and cholesterol levels with age. A slight increase in the serum urea nitrogen concentration occurred in both sexes with advancing age. The serum cholesterol was high in very young animals of 1 to 5 weeks of age and this was followed by a strong decrease at 13 weeks of age; after this, the concentration remained relatively constant in males and increased with age in females. It should be mentioned that the sera for the determination of the cholesterol concentration were not always collected at the same time of the day. The serum creatinine varied considerably, but was not related to the age of the animals (Zurcher et al., 1980).

TABLE VI

SERUM UREA NITROGEN, CREATININE AND CHOLESTEROL VALUES OF MASTOMYS

Constituent	Sex	Values (range)	Age range (weeks)	References
Urea nitrogen ($mol\ 10^{-3}.l^{-1}$)	males	9 - 16	1 - >78	van Pelt & Blankwater, 1972
	females	9 - 14	1 - >78	
Creatinine ($mol\ 10^{-6}.l^{-1}$)	males	98.2 - 154.0	30 - 112	Zurcher et al., 1980
Cholesterol ($mol\ 10^{-3}.l^{-1}$)	males	3 - 7	1 - >78	van Pelt & Blankwater, 1972
	females	4 - 7	1 - >78	

The serum urea nitrogen and creatinine concentrations are a factor 2 or more higher than those reported for the mouse (Harrison et al., 1978) and the rat (reviewed by Ringler & Dabich, 1979).

5.2.2.2 Enzymatic activity in serum

Several serum enzyme levels have been determined in the GRA-Giessen Mastomys strain by Schuster et al. (1972). They used animals of both sexes of between 4 and 8 months of age. The serum enzyme levels are given in Table VII.

TABLE VII

SERUM ENZYME LEVELS ($U.l^{-1}$) OF MASTOMYS *)

Enzymes	Males	Females
GPT **)	6.4 ± 1.9	7.3 ± 2.0
GOT	36.6 ± 8.8	48.3 ± 9.1
GLDH	1.6 ± 0.8	2.1 ± 1.1
SDH	3.4 ± 1.2	4.0 ± 1.3
LDH	352.3 ± 111.4	391.7 ± 119.0
LDH-1-isoenzyme (α-HBDH)	104.4 ± 35.0	116.9 ± 39.1
LAP	27.7 ± 8.7	25.7 ± 6.0
G-6-PDH	3.4 ± 2.5	4.4 ± 3.9
Alkaline phosphatase	65.6 ± 14.8	45.5 ± 11.4
Acid phosphatase	22.7 ± 6.3	16.7 ± 5.5

*) With permission from Schuster et al. (1972) and the publisher

**) Abbreviations:
GPT = Glutamic pyruvic transaminase
GOT = Glutamic oxaloacetic transaminase
GLDH = Glutamic dehydrogenase
SDH = Sorbit dehydrogenase
LDH = Lactic dehydrogenase
α-HBDH = α-Hydroxybutyric dehydrogenase
G-6-PDH = Glucose-6-phosphate dehydrogenase
LAP = Leucine aminopeptidase

The mean values for seven enzyme activities (GPT, GOT, GLDH, SDH, LDH, LDH-1-isoenzyme [α-HBDH] and G-6-PDH) were observed to be significantly higher in female Mastomys than in males, while the mean values for alkaline phosphatase and acid phosphatase were significantly higher in males than in females. In comparing these enzyme activities with those found in the mouse (Harrison et al., 1978) and the rat (Platt et al., 1973), it was found that the GPT and GOT levels are lower, and the LDH levels are higher in Mastomys.

Nakaya & Ishikawa (1977a,b) studied the isoenzyme patterns of lactic dehydrogenase and compared them with those in the mouse and the rat. Without giving levels, they reported that the isoenzyme patterns of lactic dehydrogenase in various tissues in Mastomys, mice and rats were quite similar. The V type isoenzyme was shown to be unstable when tissue homogenates were kept at -20°C, while the other isoenzymes were stable at that temperature.

6 Urine

Reported values for urine-related parameters are very sparse. Zurcher et al. (1980) found an age-related decline in urinary osmolality after water deprivation in male Mastomys of the Rijswijk colony. The osmolality determined at 7 months was 1852 ± 373 $mOsm.l^{-1}$ and decreased gradually to 716 ± 208 $mOsm.l^{-1}$ at 26 months of age. They also determined the urinary protein concentration, which increased from 261 ± 81 $mg.dl^{-1}$ at 7 months of age to 521 ± 156 $mg.dl^{-1}$ at 19 months of age and then decreased to 431 ± 139 $mg.dl^{-1}$ at 26 months of age.

7 Miscellaneous

The average rectal temperatures are 35.9°C in males, 36.9°C in nonlactating females and 37.5°C in lactating females (Prinsloo, 1963, cited by Oettlé, 1967).

Davis & Oettlé (1958) demonstrated that, as in the mouse, Mastomys can concentrate labelled iodine in the submaxillary glands. The ratio of gland radioactivity to that of the blood plasma ranges from 3 to 4 in the males and 2.75 in the females. This ratio seems to be higher than in the rat according to Davis & Oettlé (1958).

8 The role of Mastomys in the epidemiology of zoonotic diseases

Mastomys may be of real danger in transmitting diseases to man because of its semicommensal habit. It may act as an intermediate host between wild rodent populations and man or as a primary reservoir infecting man by direct or indirect contact. Diseases transmitted from animals to man (or vice versa) are called zoonoses. It has been proved that Mastomys plays a role in the epidemiology of at

least two zoonotic diseases: plague and Lassa fever. It plays a different role in each disease; therefore, they will be discussed separately.

8.1 The role of Mastomys in the plague cycle

Plague, caused by the bacterium Yersinia pestis, is an ancient disease originating in Central Asia and/or Central Africa (Burrows, 1968). It is primarily a disease of rodents, in which wild rodent populations act mainly as reservoirs. From these sources, it is transmitted to man by intermediate hosts which are predominantly commensal or semicommensal rodents. Although these rodents may be infected by feces and urine of infected wild rodents and by feeding upon wild rodents dying of the disease, the infection is most often transmitted by fleas. Plague in man most often appears in two forms: the bubonic or glandular form and the pneumonic form. The bacteria introduced by a flea bite enter the dermal lymphatics and are transported to the regional lymph nodes in which they cause buboes. From the buboes, the bacteria may spread via the circulation and give rise to hemorrhage and necrosis in organs. When embolic pneumonia develops, the infection may easily spread from man to man by droplets. In the terminal stage, cyanosis is common in patients with pneumonic plague, as a result of which the disease became known as the black death.

No infectious disease has caused greater human losses in the world than did plague. Only sporadic cases occur nowadays, but it must be borne in mind that it is not just a disease of the past since it is still endemic in some rodent populations in certain locales (Anon., 1980). Therefore, the disease can still occur in the future, even in epidemic form. An example is a recent outbreak in Zimbabwe, from which two deaths were recorded before the disease was identified and controlled (Lyons et al., 1977).

In southern Africa, gerbils (Tatera brantsi and, to a lesser extent, Tatera leucogaster) act as reservoir hosts of plague bacilli (Davis, 1948, 1953; Isaäcson, 1975). However, it cannot be excluded that other animal species also play an important role in the plague cycle in southern Africa (Hallett et al.,1970).

It has been shown that the flea Xenopsylla philoxera which is associated with Tatera brantsi and which has a low host specificity transmits the bacilli from Tatera to other rodents, among them Mastomys. When Mastomys is parasitized by infected fleas, different routes of transmitting the disease to man are possible (Fig.10). Infected Mastomys can excrete plague bacilli in feces and urine and can infect man by contamination of his environment. Other possibilities are that Mastomys infects rats through its excretion products or that it dies of the infection and is fed upon by rats. In both cases, rats can then infect man through their excretion products. Mastomys is most likely parasitized by infected fleas of gerbils, which can then infest and possibly infect rats and, due to the commensal habit of rats, the ectoparasites can be carried to man. However, Mastomys and rats are no more than so-called liaison rodents between the reservoir host, Tatera brantsi, and

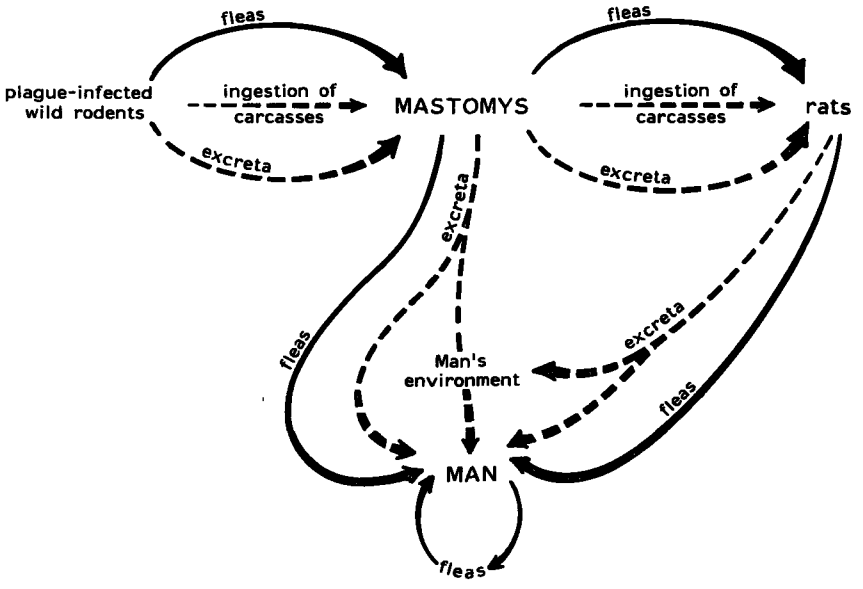

Fig. 10 The possible role of Mastomys in the plague cycle.

man. While the infection is initially carried to man by the rodent fleas or in some other way, epidemic bubonic plague in man is transmitted largely from man to man by human ectoparasites, notably the human flea Pulex irritans. It is of interest to note that this ectoparasite has also been found on Mastomys (Isaäcson, 1975), so that it cannot even be excluded that Mastomys may directly infect man via Pulex irritans.

8.2 The role of Mastomys in the epidemiology of Lassa fever

Lassa fever is a severe, acute systemic febrile disease with frequent hemorrhagic manifestations. It has been found so far in only 3 West African countries [Nigeria, Sierra Leone and Liberia] (Monath, 1974; Monath et al., 1974), although serological evidence of infection in man has been obtained in Guinea, Senegal, Upper Volta, the Ivory Coast, Central African Republic and Zaïre (McIntosh, 1979; Monath, 1974). The disease is caused by the Lassa virus, an enveloped RNA virus which is included in the taxon designated as arenaviruses to which belongs, among others, lymphocytic choriomeningitis (LCM) virus and viruses of the Tacaribe complex such as the etiologic agents of Bolivian and Argentinian hemorrhagic fever.

The public health importance of the disease in West Africa is emphasized by the high case fatality ratio (36-67%) in hospitalized cases (Monath, 1974). This percentage exaggerates the real picture, since mild or subclinical infections are not uncommon. The means by which the virus is spread has not yet been elucidated

(Monath, 1974). Related arenaviruses are known to be associated with rodents and bats. For that reason, Monath et al. (1974) centered their attention on wild vertebrates during an outbreak in Sierra Leone in 1972. They collected a total of 641 small vertebrates, including 15 rodent species, bats, monkeys and a turtle. The virus was isolated from the organs of 14 of 82 Mastomys tested. All other animals, including 268 rodents, were negative. The authors regarded Mastomys as a reservoir host of this virus in Sierra Leone but did not discover the mode of virus transmission to man, although transmission via infected urine or other excreta seems to be most likely. Demartini et al. (1975) found a difference in weight and length between infected and noninfected Mastomys. Virus positive Mastomys were lighter in weight and shorter in length. The age of the animals, however, could not be determined, so that their conclusion can be questioned. Robbins (1980) observed no differences in weight between infected and noninfected Mastomys. In addition, Demartini et al. (1975) found that virus-positive animals had higher frequencies of a number of lesions including myositis, myocarditis, lymphoid perivasculitis and splenic follicular hyperplasia. However, one must be very cautious in interpreting these data, because most of these lesions are also observed in laboratory maintained Mastomys in which it is almost certain that the virus is not present (Johnson, 1980). In 1975, Wulff et al. investigated animals which were trapped in or near human dwellings in Nigeria where no Lassa fever cases were known to occur at the time of collection. Lassa virus was isolated from blood specimens and pooled tissues from Praomys (Mastomys) natalensis, Rattus rattus and Mus minutoides. It has been proven that these two last mentioned species were misidentified (Robbins, 1980), so that Mastomys can be regarded as the main reservoir of infection.

Walker et al. (1975) infected squirrel monkeys, guinea pigs and Mastomys with Lassa virus. While clinical disease occurred in monkeys and guinea pigs, infection of neonatal Mastomys caused no clinical disease or pathological changes during the study of 74 days, despite isolations of virus from blood, lymph nodes, and other organs. Of prime importance from an epidemiological point of view was the presence of virus in urine and throat secretions. Infected adult Mastomys also remained normal despite the presence of virus in many of the organs. Virus persisted in one adult animal throughout a 103-day study period. It was isolated from lymph nodes, brain and urine of this animal. Histological examination revealed a mild meningoencephalitis in some animals. Walker et al. (1975) concluded from this study, that: "the pattern of infection and virus shedding in Mastomys is ideal for maintenance of the virus in nature. Together with the epidemiological field data this emphasizes the incidental nature of the exposure and infection of man."

Since Praomys (Mastomys) natalensis in West Africa has been found to have 32 chromosomes, it would be of interest to investigate whether the 36 chromosome form is also susceptible to Lassa virus and whether there are differences in chromosome pattern between the 32 chromosome form found in West and Central Africa and that found in southern Africa. This might be an explanation for the fact that

clinical disease in man has not yet been observed in Central and southern Africa.

Wulff et al. (1977) and McIntosh (1979) recently reported that, during field studies in 1972 on arboviruses at Mopeia, central Mozambique, rodents were trapped and tested for viruses. Virus was recovered from Mastomys and shown to be immunologically related but not identical to Lassa virus. Lassa and the "Mopeia" virus also differ in their pathogenicity for laboratory mice. Four hundred and nine sera were collected from school children at Mopeia to search for the presence of "Mopeia" virus antigens by an indirect immunofluorescent technique. Eleven percent of the 409 sera were positive. It is of importance now to determine whether this virus is pathogenic for man and whether it is a new arenavirus or an antigenic variant of Lassa virus. It is also of importance to determine whether Mastomys of West Africa and southeast Africa are identical with respect to their susceptibility to "Mopeia" virus.

9 The use of Mastomys in research

In 1940, Davis and co-workers (Davis, 1963) began to test Mastomys for their suitability in routine diagnostic and experimental work on plague. It appeared that Mastomys was more sensitive than the guinea pig in routine testing of plague-suspect material. In addition, Mastomys substituted for the white mouse as the standard test animal for evaluation of vaccines.

Oettlé (1955), who did a number of necropsies on animals dying in the Mastomys colony of Davis, found a tumor of the stomach in the first animal he examined. It appeared later on that Mastomys spontaneously developed a high incidence of stomach cancer. About one-third of the males and half of the females over the age of 12 months appeared to have this tumor, which was diagnosed as an adenocarcinoma of the glandular stomach (Oettlé, 1957). This diagnosis was reconsidered in 1969 when Snell & Stewart (1969a) proved that this tumor was a malignant argyrophilic carcinoid. In addition to the gastric carcinoids, Oettlé (1957) found a wide variety of other neoplastic lesions and called Mastomys a museum of spontaneous tumors. This stimulated the interest of research workers in the areas of cancer and aging research. About the same time, Lurie & de Meillon (1956) discovered that Mastomys was an excellent laboratory animal for studying bilharziasis. This discovery gave rise to extensive use of Mastomys in protozoological and helminthological research. Furthermore, the presence of a well-developed prostate in the female makes Mastomys a valuable experimental model for studying hormonal effects on the prostate. Literature data on the use of Mastomys in various areas of biomedical research are summarized in Tables VIII to XIV.

TABLE VIII

A SURVEY OF VIROLOGICAL STUDIES PERFORMED IN MASTOMYS

Family	Virus	Spontaneous or experimental condition studied	References
Papovaviridae	Polyomavirus	Induction of sarcomas of kidney (83%), liver (18%), heart (12%) and of subcutaneous tissue (12%).	Rabson et al., 1960
	Papillomavirus	Spontaneous development of benign and malignant skin tumors (3%) from which the virus was isolated (GRA-Giessen strain).	Müller & Gissmann, 1978
		Seventeen spontaneous skin tumors were found in 4 agouti-colored Mastomys. Papilloma-like virus particles were observed electron microscopically.	Rudolph, 1980
	SV 40	Induction of papillary ependymomas (80%).	Rabson et al., 1962
Adenoviridae	Human adenovirus type 12	Induction of undifferentiated malignant tumors (6%).	Rabson et al., 1964
Parvoviridae	Rat virus	Acute fatal disease in experimentally infected newborn animals.	Rabson et al., 1961
Rhabdoviridae	Rabies virus type 1	Resistant to intracerebral infection. Resistance develops between 3 and 21 days after birth.	Müller & Schoop, 1976
		Natural subject of a persistent asymptomatic infection. Virus spread was shown to occur by vertical transmission.	Schoop, 1977
Togaviridae	Chikungunya, H 336, Rift Valley fever	Experimental infections resulted in viremia and antibody formation, except to H 336; no deaths occurred.	McIntosh, 1961
	Simbu, Spondwoni	Nonsusceptible after experimental infections.	McIntosh, 1961

Arenaviridae	Lassa virus	Lassa virus isolation from captured Mastomys in Sierra Leone.	Monath et al., 1974
		Pathological examination of naturally infected and noninfected Mastomys from Sierra Leone. The presence of Lassa virus, small body size, myocarditis and lymphoid perivasculitis appeared to be interrelated.	Demartini et al., 1975
		No clinical signs or pathological changes in experimentally infected neonatal animals; infection of adult animals resulted sometimes in a mild meningoencephalitis.	Walker et al., 1975
		Isolation of an arenavirus closely related to Lassa virus from captured Mastomys in southeast Africa which produced illness, paralysis and death in suckling mice.	Wulff et al., 1977
Retroviridae	C-type oncornavirus	Viral antigens detectable in glomeruli of aged animals, not in young ones. Infectious virus was demonstrated in cell-free spleen extracts.	van Pelt et al., 1976
	MSV-H	Induction of erythroblastic splenomegaly and severe anemia (70%), angiomatous sarcomas in diaphragm, peritoneum, subcutaneous tissue and splenic mesentery (29%); fibro(angio)sarcomas and reticulum cell sarcomas in the spleen (64%).	Harvey, 1968
	MSV-H	Malignant tumors (50%), particularly rhabdomyosarcomas.	Hirano et al., 1977
	F-MuLV	Nonsusceptible after experimental infection.	Harvey, 1968

TABLE IX

A SURVEY OF BACTERIOLOGICAL STUDIES PERFORMED IN *MASTOMYS*

Bacterial agent		Experimental condition studied	References
Family	Genus and Species		
Bacteriaceae	*Yersinia pestis*	Highly susceptible; suitable for evaluation of plague suspected material and live-attenuated vaccines.	Amies, 1951; Davis, 1963 Hallett, 1977a; Pirie, 1927
Corynebacteriaceae	*Listeria monocytogenes*	Fatal encephalitis.	Pirie, 1938
Mycobacteriaceae	*Mycobacterium microti*	Moderately susceptible; lesions in minority of animals.	Grasset et al., 1946
Treponemataceae	*Borrelia duttoni*	Prolonged bacteremia without development of clinical signs.	Zumpt, 1959

TABLE X

A SURVEY OF PROTOZOOLOGICAL STUDIES PERFORMED IN *MASTOMYS*

Protozoal agent		Experimental condition studied	References
Family	Genus and Species		
Babesidae	*Babesia rodhaini*	Parasitemia with fatal course.	Gunders, 1956 cited by Davis & Oettlé, 1958; Raether, 1971
Trypanosomidae	*Trypanosoma brucei*, *T.gambiense*, *T.evansi* and *T.equiperdum* (laboratory strains)	High levels of parasitemia with acute, lethal course.	Raether, 1971
	Trypanosoma equinum, *T.cruzi* and sometimes *T.rhodesiense* (laboratory strains)	Chronic or latent infection.	Raether, 1971
	Trypanosoma congolense	Parasitemia with fatal course.	Gunders, 1956 cited by Davis & Oettlé, 1958
	Trypanosoma brucei gambiense	Parasitemia. Polymorphic trypanosome strains could be isolated from pigs and dogs using *Mastomys* as recipient.	Mehlitz, 1978
	Leishmania donovani	Highly susceptible. Further information is lacking.	Bouillez, 1917 cited by Davis & Oettlé, 1958
	Leishmania mexicana mexicana	Susceptible to intradermal inoculation of amastigotes. Lesions at the site of infection up to 3 months.	Pereira et al., 1978
Plasmodidae	*Plasmodium berghei*	Fatal course after infection.	Vincke et al., 1953 cited by Davis & Oettlé, 1958
Eimeriidae	*Toxoplasma gondii*	Development of numerous and rather large cysts in the brain. Test animal for chemotherapeutic studies.	Matuschka, 1977 , 1978 Werner & Egger, 1974
		Used to passage organism for infectivity studies in pigs.	de Meuter et al., 1975, 1978

TABLE XI

A SURVEY OF HELMINTHOLOGICAL STUDIES* PERFORMED IN MASTOMYS

Helminths		Spontaneous and experimental condition studied	References
Family	Genus and Species		
Schistosomatidae	Schistosoma mansoni (South African and Egyptian strains)	Experimental infection caused development of granulomas in liver and spleen. Egyptian mansoni infection also produced bile duct proliferation and lesions in the lung.	Lurie & de Meillon, 1956
		Experimental infection did not influence the incidence of malignant gastric carcinoids. However, hepatomas were found in 55% of the animals, while none appeared in control animals.	Oettlé et al., 1959
		Mastomys experimentally infected with S.mansoni can be regarded as a useful animal for testing potential schistosomicidal compounds.	Lämmler & Petrányi, 1971
	Schistosoma mansoni; S. mansoni var.rodentorum S.rodhaini and S.mattheei	Sporadic natural infections observed in wild caught Mastomys. However, this animal species plays no role in the epidemiology of schistosomiasis.	Pitchford & Visser, 1960 and reviewed by Pitchford & Visser, 1962
Ancylostomidae	Ancylostoma caninum	After oral infection with third stage larvae, living larvae could be found only in skeletal muscle, where they remained without further development. Mastomys was regarded as a suitable animal species for the evaluation of potential nematodicidal compounds for larvicidal activity.	Lämmler et al., 1970; Lämmler & El-Gendi, 1978; Matsusaki, 1951
Filaroididae	Angiostrongylus cantonensis	Mastomys proved to be a useful model for the comparative evaluation of the larvicidal activity of anthelmintics against some extraintestinal nematodes.	Lämmler & Weidner, 1975
Filariidae	Brugia malayi	After subcutaneous infection 81% of the animals consistently or intermittently showed detectable microfilariae. An average of 13.1 live adult worms were found in lungs, testes and lymphatics.	Petrányi et al., 1975

	Brugia pahangi	After experimental infection, development of relatively high titers of homocytotropic antibodies showing reagin-like characteristics.	Benjamin & Soulsby, 1976
	Wucheria bancrofti	Infection with infective stage larvae resulted in fourth stage larvae in the heart-lung system until the 175th day postinfection. The conclusion was that Mastomys was not a suitable host for the West African strain of W. bancrofti.	Zielke, 1979
	Litomosoides carinii	Suitable host for the laboratory maintenance of this parasite. In addition, Mastomys can be used with satisfactory results for the first evaluation and standardization of the chemoprophylactic activity of filaricidal compounds.	Lämmler, 1977; Lämmler et al., 1977; Pringle, 1974 Pringle & King, 1968
		A marked production of homocytotropic antibodies analogous to those of IgE of the rat and man was found after experimental infection.	Soulsby et al., 1976
		Rejection of transplanted L. carinii adults by naive Mastomys, which can be abrogated by splenectomy of the recipient animal.	Weiner & Soulsby, 1976; 1978
Capillariidae	Capillaria hepatica	Natural infections observed in wild Mastomys.	Cochrane et al., 1957
		The dynamics and duration of egg production of the parasite in experimentally infected animals proved to be dependent on the infective dose. Foci of liver cell necrosis were found after heavy infection within the prepatent period. Increase in weights of liver and spleen were observed with the beginning of egg deposition.	Zahner et al., 1976

* Lämmler et al. (1968,1977) have also reported successful experimental infections in Mastomys with Fasciola hepatica, Opisthorchis felineus, Schistosoma haematobium, Schistosoma japonicum, Schistosoma intercalatum, Dicrocoelium dendriticum, Syphacia obvelata, Aspiculuris tetraptera, Heterakis spumosa, Nippostrongylus muris, Dipetalonema viteae and Trichinella spiralis. In addition, Mastomys can be regarded as an experimental intermediate host for Ascaris suum, Toxocara canis, Echinococcus granulosus, Echinococcus multilocularis and Taenia crassiceps infections. Susceptibility of Mastomys was incomplete for Hymenolepis diminuta, Hymenolepis fraterna and it was completely nonsusceptible to Paramphistomum microbothrium.

TABLE XII

A SURVEY OF CHEMICAL CARCINOGENESIS STUDIES PERFORMED IN MASTOMYS

Carcinogen	Dose and route of administration	Lesions developed	References
Azaserine	30 mg.kg^{-1} body weight i.p. First treatment at 28 days of age and subsequently each week for 5 weeks. Animals were killed at 6 months of age.	Large numbers of atypical pancreatic acinar cell nodules and 2 of 4 males had pancreatic acinar cell adenomas.	Roebuck & Longnecker, 1979
BNUR (N-n-butyl-N-nitrosourethan)	300 ppm in drinking water daily for 17 to 19 weeks.	Esophageal carcinomas (87.0%).	Soga et al., 1975b
DMBA (7,12-dimethylbenz (α) anthracene)	0.1 ml of 1% DMBA in the thighs of one-year-old animals.	Sarcomas detectable by 150 days after injection. The chromosome pattern of tumors varied from diploid to hypertetraploid.	Huang & Strong, 1963
DMNA (N-nitrosodimethylamine)	A total dose of 2 to 8.8 mg for 10 to 44 weeks given subcutaneously.	Mainly peliosis hepatis and cholangiocarcinomas.	Fujii & Sato, 1970
	10 mg.kg^{-1} body weight intraperitoneally. The total dose singly or in twofold.	Benign (95%) and malignant (6%) hemangioendotheliomas.	Wayss et al., 1979
ENNG (N-ethyl-N'-nitro-N-nitrosoguanidine)	50 mg.l^{-1} admixed with Tween 60 as 0.4% of drinking water for 6 months.	Malignant lymphomas of the mesentery (62.5%).	Kawasaki et al., 1976

MNNG (N-methyl-N'-nitro-N-nitroso-guanidine)	20 $mg.l^{-1}$ in drinking water for 56 to 235 days and 40 to 160 $mg.l^{-1}$ for 140 days In addition, 6 to 12 mg as total dose intragastrically.	Adenomatous hyperplasia of the glandular stomach (14%) and duodenal adenocarcinomas (12.5%). The other main lesions were squamous cell papillomas and carcinomas of the forestomach and liver tumors.	Kurokawa et al., 1975a
	Glandular stomach cells were treated in vitro in doses of 0.25 to 8.00 $\mu g.ml^{-1}$ and injected in several ways.	In approximately 50% of the cases, take and development of mesenchymal tumors. Argyrophilic cells were scattered in small numbers in the tumors.	Kurokawa et al., 1975b
3-Methylcholanthrene (3-MC)	Initial dose of 0.2 ml of 1% 3-MC in olive oil intragastrically followed by 4 doses of 0.5 ml at weekly intervals.	Slight increase in tumors of the glandular stomach (61%) as compared with control animals (50%).	Randeria, 1980
N-OH-FAA (N-hydroxy-N-2-fluo-renyl-acetamide)	0.06% and 0.1% in the diet for 32 to 52 weeks.	Liver cirrhosis, bile duct adenomas and carcinomas and liver parenchymal cell tumors.	Yamamoto et al., 1968
2,7-FAA (N.N'-2,7-fluorenylenebis-acetamide)	0.025% in 10% corn oil in the diet for the entire life span.	Slight increase in papillomas of the small intestine, pancreatic carcinomas and cholangiocarcinomas.	Hollander & Higginson, 1971
	0.025% and 0.3% in the diet for the entire life span.	Two to 2.5 times higher incidence of liver cell tumors (39.0%).	Kanahara et al., 1972

TABLE XIII

A SURVEY OF TUMOR TRANSPLANTATION STUDIES PERFORMED IN MASTOMYS

Type of tumor transplanted and derived from Mastomys	Effects	References
Granulosa cell tumor	First transplant in male Mastomys resulted in atrophy of the testes and, in females, in enormous hypertrophy of the uterus. During successive subpassages, the tumor lost its hormonal activity.	Hosoda, 1977
Malignant gastric carcinoid	Mastomys with growing transplants excreted 11.3 times more histamine and 4.4 times more 5-hydroxyindole-3-acetic acid in the urine than control animals. All transplantable carcinoids contained histamine and 5-hydroxytryptamine, whereas primary carcinoids contained only histamine.	Hosoda et al., 1979
Diffuse lymphosarcoma	Failed to grow in untreated Mastomys.	Snell & Stewart, 1969b
Plasma cell myeloma	Early generation transplants in Mastomys were composed exclusively of neoplastic plasma cells. Portions of later transplants additionally had sarcomatous areas with interlacing bundles of spindle-shaped cells and occasional deposits of osteoid and bone.	Snell & Stewart, 1969b
Well-differentiated reticulum cell sarcoma	Failed to grow in irradiated and non-irradiated Mastomys and newborn mice.	Snell & Stewart, 1969b
Lymphoepithelial thymoma	Failed to grow in recipient Mastomys during a 12-month observation period.	Stewart & Snell, 1968
	Failed to grow in nude mice.	Solleveld, unpubl. data

TABLE XIV

A SURVEY OF PROSTATIC RESEARCH PERFORMED IN MASTOMYS

Type of study	Results	References
Determination of the uptake and retention of androgens	Uptake and retention of androgens by Mastomys is similar to that reported for the rat. The uptake of ^{3}H testosterone by the male Mastomys prostate is similar to that by the female Mastomys prostate.	Ghanadian et al., 1975;1976
Determination of the androgen concentrations in prostate and serum	Serum testosterone levels were 10 times greater in the males than in females. The concentration of testosterone in the female prostate was similar to that in the male, but the dihydrotestosterone concentration in the male prostate was much higher than in the female prostate.	Ghanadian et al., 1977
Testing the effect of testosterone, prolactin and diethylstilbestrol on the female prostate	Testosterone caused enlargement of the prostate due to proliferative changes and abscess formation. Stilbestrol caused cystic changes, probably due to squamous metaplasia of the ducts. No consistent response occurred in animals given prolactin.	Holland, 1970
Identification and characterization of the receptor protein for androgen	Demonstration of a receptor protein in the cytosol of the male Mastomys prostate which binds to dihydrotestosterone and is comparable to that of the rat prostate.	Smith et al., 1978a
Electron microscopic studies on the male and female prostate	Differences in subcellular morphology and hormone dependence between the male and female prostate. Most obvious are the prominent dilated Golgi vesicles and electron-dense mature secretory granules seen in the apical region of the cells of the male prostate. These features are occasionally present in the estrous female.	Smith et al., 1978b

CHAPTER III

GENERAL CHARACTERISTICS OF MASTOMYS OF THE RIJSWIJK COLONY

1 General Introduction

After a brief summary of the origin of Mastomys of the Rijswijk colony, this chapter will deal with a number of unrelated topics, all concerned with Mastomys of the Rijswijk colony. These include the following:

a. a TG- and C-banding chromosome analysis;
b. breeding data of random bred and inbred Mastomys;
c. the extent of inbreeding;
d. growth data of random bred and inbred Mastomys;
e. survival data of random bred Mastomys.

2 Origin of the Rijswijk colony

The historical facts related to Mastomys of the Rijswijk colony are given in Fig.7. The most important data can be summarized as follows. Mastomys from the stock of Davis (Davis & Oettlé, 1958), originating from wild animals caught at Baragwanath, were sent outside the African continent in 1950. Among others, a breeding colony was established at the Walter Reed Army Institute for Research, Washington D.C., U.S.A. From this stock, animals were sent to the National Institutes of Health, Bethesda, Maryland, U.S.A., in 1959. This nucleus developed into a large breeding colony and offspring were distributed to several laboratories, including the Argonne National Laboratory, Argonne, Illinois, U.S.A., in 1964. This laboratory supplied the Institute for Experimental Gerontology TNO with 4 breeding pairs in 1967. Shortly after the establishment of a random bred colony, an inbred colony was initiated using animals from the random bred stock. The inbred colony has now reached the 30th generation of brother-sister mating.

3 TG- and C-banding chromosome analysis of Mastomys

3.1 Introduction

Several investigators (Hallett, 1977b; Huang, 1968; Huang & Strong, 1962; Matthey, 1954a) have studied the cytogenetics of laboratory kept Mastomys from the stock of Davis (Davis & Oettlé, 1958). Inasmuch a TG- and C-banding chromosome analysis of animals of this stock had not been carried out, we were prompted to initiate such a study in Mastomys of the Rijswijk colony with the aim of comparing our findings with those of Lyons et al. (1977), who performed such a study in wild caught animals from Zimbabwe. Recently, however, a TG- and C-banding chromosome analysis of laboratory kept Mastomys became available (Lee & Martin, 1980). The animals in that study probably also originated from Mastomys

of the Davis stock. It is not surprising that so few banding studies have been performed in laboratory kept Mastomys, as most chromosome studies in these animals were done before 1970 and chromosome banding technique were introduced only in 1970. Caspersson et al. (1970) then demonstrated that chromosomes stained with quinacrine dyes produced characteristic and reproducible patterns of fluorescence when activated with UV light, enabling the positive identification of chromosomes. This technique is known now as the Q-banding (Q=quinacrine) technique. The principle of this technique is still not completely understood, but there are indications that the banding pattern is determined by the sequence of the bases in DNA (Ellison & Barr, 1972; Weisblum & de Haseth, 1972). Simpler, alternative techniques with the same effect have been developed since 1970, one of which is the TG-banding technique. This technique has been so called because pretreatment of cells takes place with trypsin (T) followed by staining with Giemsa (G).

Another technique is C-banding, which demonstrates certain types of constitutive (C) heterochromatin. Constitutive heterochromatin differs from euchromatin in its condensation and replication pattern as well as in its genetic content (Goodenough, 1978). Its precise role in chromosomal and nuclear organization is not yet completely understood. Constitutive heterochromatin is located mainly in the centromeric regions of the chromosomes in many mammals.

The introduction of these banding techniques has made it possible to investigate in detail karyotypic arrangements among closely related species. As stated previously the situation at the species level is still confusing in Mastomys due to the existence of different chromosome numbers and variations in the number of chromosome arms (see Chapter II, Section 1.2). Therefore, it was of interest to compare the chromosomal banding pattern of animals from the Rijswijk colony (2n=36) with those of wild caught animals from Zimbabwe (2n=36) and, additionally, to compare those with laboratory kept animals (2n=36) which probably have the same origin as those of the Rijswijk colony. Moreover, this will offer the opportunity to study the relationship between the 32 and 36 chromosome forms in the near future.

3.2 Materials and Methods

Chromosome preparations were derived from bone marrow cells of male and female Mastomys of the Rijswijk colony. The cells were obtained by flushing the femurs with Hanks balanced salt solution. After filtering the bone marrow suspension through a nylon sieve, the cells were centrifuged at 225 x g for 10 minutes at 15°C. They were resuspended in 1 ml of the supernatant and treated for 15 min with 5 ml 0.075 M KCl in a waterbath at 37°C. Immediately thereafter, a few drops of fixative (methanol: acetic acid 3:1) were added and the cells were again centrifuged under the same conditions as described above. They were then resuspended in 1 ml of the supernatant and freshly prepared fixative (5 ml) was carefully added drop-wise along the wall of the centrifuge tube with constant agitation of the tube. This procedure was repeated 3 times. Cell preparations were

made by dripping the cell suspension from a distance of about 1 meter onto slides cooled on dry ice. The slides were then dried by the use of gentle heat. The preparations were first treated according to a modified TG-banding technique of Seabright (1971). They were incubated in 3% H_2O_2 in demineralized water for 15 min at room temperature, rinsed in tap water and submerged for 5 to 45 seconds in 0.005% trypsin solution in phosphate buffer, pH 6.8 and rinsed again in tap water. Subsequently, the slides were stained in a 3% Gurr's Giemsa R 66 in Gurr's pH 6.8 buffer (Searle, High Wycombe, UK). The excess stain was finally removed with tap water and the slides were air dried. Photographs were made with a photomicroscope (10x ocular and 63x objective lens + green filter) using a 17 din Agfa Copex Pan film and an exposure time of 20 seconds. Photos were developed in Promicrol (May & Baker Ltd., UK)

The same slides were then rinsed (xylol; alc.100%, 90%, 70%; phosphate buffer, pH 6.8) and stained for C-banding according to a modified technique of Marshall (1975). For this purpose, slides were incubated in 1% $Ba(OH)_2.8H_2O$ for 15 min at room temperature, rinsed in distilled water and treated for 17 min at 65°C in 2xSSC pH 7.0 (1xSSC=0.15 M sodium chloride, 0.015 M trisodium citrate). The slides were rinsed afterwards with tap water and stained in a 2% Gurr's Giemsa R 66 in Gurr's pH 6.8 buffer (Searle, High Wycombe, UK) for 10 to 15 min; the excess stain was removed with tap water. Karyotypes were made from at least 10 cells of each male and female Mastomys.

All chromosomes of 20 karyotypes were measured and expressed as a percentage of the haploid autosomal complement.

3.3 Results

A metaphase chromosome spread showing C-banding is given in Fig.11 and a karyotype stained by a TG-banding technique in Fig.12.

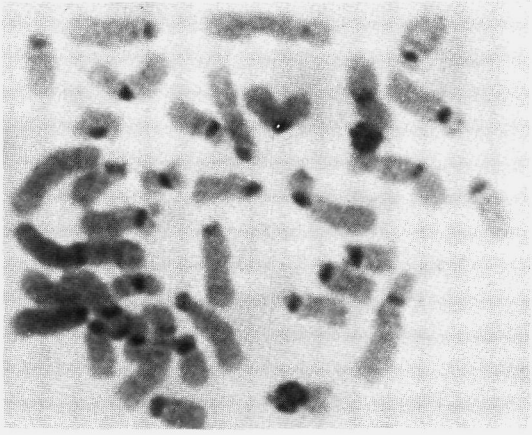

Fig. 11 Metaphase spread of a Mastomys cell. The chromosomes are stained by a constitutive heterochromatin banding technique.

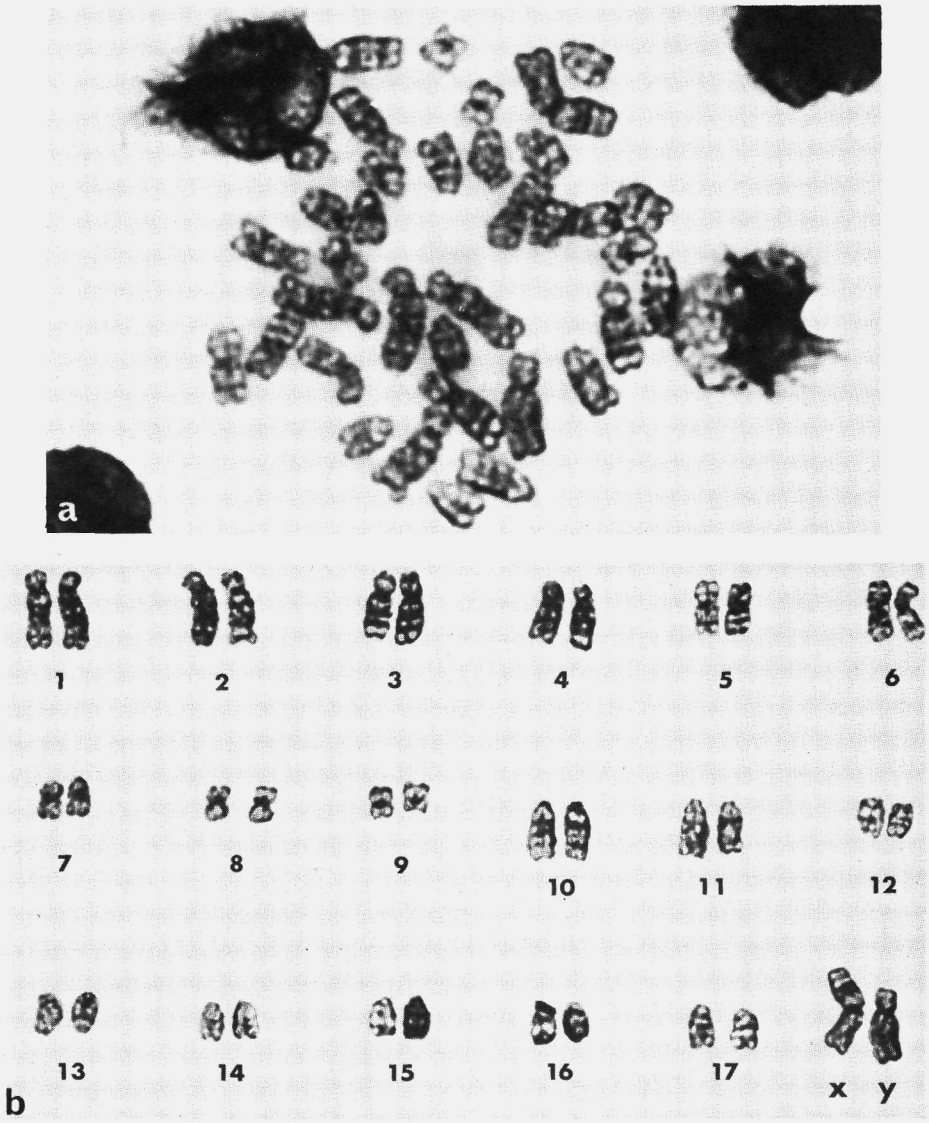

Fig. 12 a, Metaphase spread of a Mastomys cell. The chromosomes are stained by a trypsin-giemsa banding technique.
b, Mastomys karyotype stained by a trypsin-giemsa banding technique.

TABLE XV

MEAN CHROMOSOME LENGTH EXPRESSED AS A PERCENTAGE OF THE HAPLOID AUTOSOMAL COMPLEMENT OF MASTOMYS CELLS

Chromosome number	Per cent of haploid complement	± 1 S.D.	Shape
1	9.70	0.59	
2	9.13	0.47	submetacentric
3	7.74	0.40	
4	6.68	0.37	
5	6.58	0.36	
6	6.27	0.60	
7	4.15	0.32	metacentric
8	3.93	0.32	
9	3.84	0.35	
10	6.77	0.47	
11	6.41	0.74	
12	5.01	0.55	telocentric or
13	4.80	0.23	acrocentric
14	4.93	0.17	
15	4.83	0.46	
16	4.55	0.31	
17	4.72	0.25	
X	11.31	0.98	metacentric
Y	8.87	0.90	submetacentric

The karotypes consisted of 17 pairs of autosomes and two sex chromosomes resulting in 2n=36. The relative length of each individual chromosome is given in Table XV. This table also gives the groups to which the chromosomes are assigned according to shape or size. An idiogram based on all karyotypes was constructed and is presented in Fig.13.

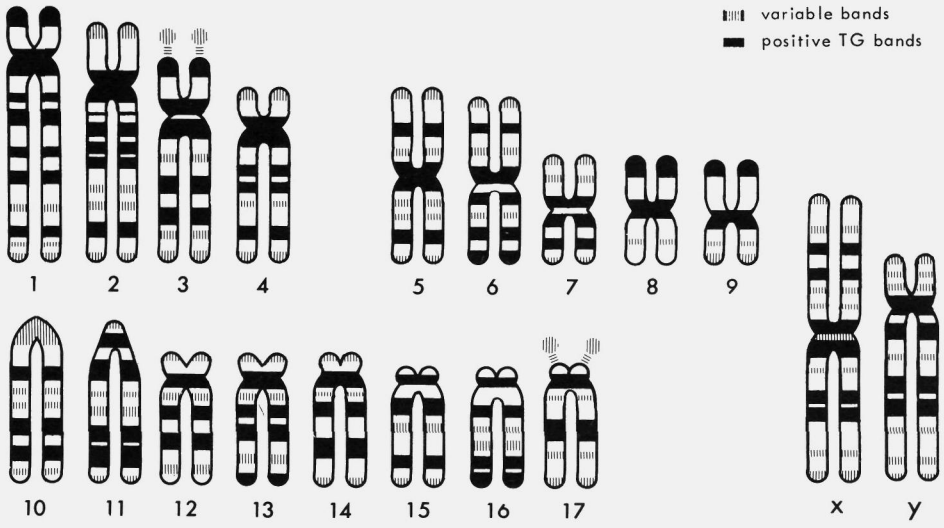

Fig. 13 Idiogram of Praomys (Mastomys) natalensis (2n=36) of the Rijswijk colony.

3.4 Discussion

In 1977, Lyons et al. presented an idiogram of Praomys (Mastomys) natalensis (2n=36) derived from animals caught in Zimbabwe. The locations of the C-bands we described are in accordance with the positions of the centromeres in Lyons' idiogram. In general, we found more distinct TG-bands, which may be due to the fact that colchicine was not used in our chromosome preparation technique. Treatment with colchicine results in considerable shrinkage of the chromosomes (Bosman, 1976) and therefore less separation of the bands. Furthermore, Lyons' idiogram depicted chromosome 10 of Mastomys with a heavily stained top (very broad TG-band). In our karyotypes, the staining of the top of this chromosome was much less intensive and sometimes even difficult to detect.

When we compared our results with those of Lee & Martin (1980) we found no essential differences in the TG- and C-banding patterns. Apparent minor differences existed in the arrangement of chromosomes in the karyotype and in the terminology applied to several types of chromosomes.

Huang (1968) found a substantial difference in size between the two X chromosomes of female Mastomys. The larger X chromosome averaged 11.1 μ and the other 9.2 μ. Our measurements of 19 XX chromosome pairs did not show this difference: 15 pairs were of the same length and 4 pairs showed a slight difference in size; however, all values were within the normal range found for the autosomal chromosomes. The length we found corresponded to the length of the largest X chromosome described by Huang (1968).

Unfortunately, we had neither Mastomys with 32 chromosomes nor Giemsa stained slides of chromosomes from these animals which could be decolorized and used again for TG- and C-banding. Otherwise, it should have been possible to investigate with the banding technique the hypothesis advanced by Hallett (1977b) that the 2n=32 specimen developed from the 2n=36 by a centric Robertsonian fusion between two nonhomologous acrocentric autosomes and by a rearrangement in which the arm of an acrocentric chromosome was translocated onto the long arm of a small submetacentric one.

4 Breeding data of animals of the Rijswijk colony

4.1 Introduction

Breeding data of an inbred colony of agouti-colored Mastomys are lacking at present. This is not surprising if one realizes that only a few laboratories have succeeded in establishing such a colony. In this section, breeding data of animals from our inbred colony will be given and compared with those of our random bred colony and with those of other random bred colonies.

4.2 Materials and Methods

4.2.1 Colony husbandry

The animals were housed under conventional conditions (Solleveld, 1978) in rooms maintained at 21 ± 1°C with a relative humidity ranging from 60 to 65 per cent. The rooms were kept under positive air pressure and ventilated with 15 exchanges of filtered (insect and dust filters) air per hour. A light schedule of 12 hr light/12 hr dark was used. The animals were maintained on autoclaved pine shavings in 35x23x16 cm polycarbonate cages. Pregnant females were provided with aluminum nesting boxes and shredded paper for nesting material. The cages were changed and washed twice per week in water at 80-90°C, with the exception of cages housing newborns less than 1 week old. The animals were fed a standard rodent pellet (AM II®, Hope Farms, Woerden, The Netherlands) ad libitum. The composition and analysis of this diet has been published (Burek, 1978). Water was provided ad libitum in bottles with sipper tubes and was changed three times a week. Floors were cleaned and disinfected with formalin soap once a week.

4.2.2 Mating system

Inbreeding and random breeding was accomplished by using a polygamous system with one male being placed with a maximum of 3 females. For inbreeding, brother-sister mating was followed; for random breeding, a special rotational schedule (Falconer, 1976) was employed to avoid mating of related animals as much as possible. When a female became pregnant, it was removed from the breeding cage and caged individually for parturition and nursing. After 3 weeks, the offspring were weaned and the female returned to the breeding cage of origin, if it originated from the inbred colony or to another breeding cage for animals of the random bred colony. If a litter died before the age of 3 weeks, the dam was transferred back to the mating cage immediately after the death of the litter. At weaning, the young were sexed and males and females were housed separately. At the age of 8-12 weeks, males and females were placed together for breeding. A breeding record including information on breeding pair identification codes, dates of birth and weaning of litters, the number of offspring born and weaned, the sexes of the offspring and the identification codes of the offspring, was kept for each male and female.

4.3 Results

The breeding data from generations 5 and 6, 10 and 11, 15 and 16, 21 and 22 and 25 and 26 were analyzed in order to compare the breeding results in the different stages of inbreeding. Two generations were combined at each of the 5 periods so as to have sufficient numbers of females at each period for analysis. The five groups each consisted of about 60 females.

It can be seen in Table XVI that, with each succeeding pregnancy, the pro-

ductivity of the females decreased in terms of litters produced.

In general, no distinct differences were found among the five groups. From these data, it seemed justified to replace females that had produced three litters, although one could consider breeding further only those females which produced litters at regular intervals. A period of approximately 60 days between introduction of the female to the male until parturition was considered acceptable (Table XVII).

TABLE XVI

PERCENTAGE OF FEMALES PRODUCING LITTERS IN DIFFERENT GENERATIONS OF BROTHER-SISTER MATING

No. of litters produced	Generation no. 5 and 6	10 and 11	15 and 16	21 and 22	25 and 26
1	72	91	83	82	88
2	59	64	66	68	73
3	45	25	40	45	41
4	30	13	17	23	22
5	18	4	-	8	8
6	7	-	-	-	-

TABLE XVII

LITTER INTERVAL* IN DIFFERENT GENERATIONS OF BROTHER-SISTER MATING

No. of litters produced	Generation no. 5 and 6	10 and 11	15 and 16	21 and 22	25 and 26
1	49	44	48	43	50
2	52	55	49	37	54
3	44	37	52	60	35
4	48	45	38	47	30
5	43	64	-	58	35
6	44	-	-	-	-

* Time in days from introduction to the male until parturition

Table XVIII lists the average number of young born and weaned per female per litter in different generations and the preweaning loss in per cent. These data indicate that the preweaning loss in the fifth litter was so high that breeding any group for more than 4 litters was inefficient. The average productivity per generation group derived from the data in Table XVIII is given in Table XIX. The average preweaning loss in all groups was very high and varied between 54 and 68 per

cent. Obvious is the decline in the average number of young born per litter when comparing the data of the 5th and 6th generations with those of the 25th and 26th generations.

TABLE XVIII

PRODUCTIVITY* OF FEMALES BY LITTER NUMBER IN DIFFERENT GENERATIONS OF BROTHER-SISTER MATING

No. of litters produced	Generation no. 5 and 6 born	weaned	10 and 11 born	weaned	15 and 16 born	weaned	21 and 22 born	weaned	25 and 26 born	weaned
1	7.0	4.3	4.9	2.4	5.6	2.1	4.7	2.2	4.4	1.7
		39**		51		63		53		61
2	6.1	2.6	4.5	1.9	5.5	1.6	5.2	2.7	4.1	2.0
		57		58		71		48		51
3	6.2	2.3	3.9	1.1	5.1	1.2	5.1	1.7	4.2	0.8
		63		72		76		67		81
4	5.8	2.4	5.7	1.4	3.9	0.6	4.6	2.0	3.5	1.2
		59		75		85		57		66
5	4.3	1.3	5.5	0.0	-	-	5.3	1.0	4.0	1.0
		70		100		-		81		75
6	5.6	0.0	-	-	-	-	-	-	-	-
		100		-		-		-		-

* calculated for productive females only
** preweaning loss in per cent

TABLE XIX

AVERAGE NUMBER OF YOUNG BORN AND WEANED PER PRODUCTIVE FEMALE IN DIFFERENT GENERATIONS OF BROTHER-SISTER MATING

	Generation no. 5 and 6	10 and 11	15 and 16	21 and 22	25 and 26
young born	6.2	4.7	5.3	4.9	4.2
young weaned	2.9	1.9	1.7	2.2	1.6
preweaning loss (%)	53	60	68	55	62

The breeding performance of Mastomys from different colonies is compared in Table XX. Since breeding data on other inbred colonies are lacking, we compared the data from random bred colonies of other institutions with those of our inbred and random bred colonies.

TABLE XX

BREEDING PERFORMANCE OF MASTOMYS IN DIFFERENT COLONIES

	Tohoku colony* Japan Random bred	Niigata colony** Japan Random bred	Rijswijk colony The Netherlands Random bred		Inbred	
	1966	1969	1969	1977	1969	1978/79
Average number of young born per litter	6.3	5.4	6.1	5.7	6.2	4.2
Average number of young weaned per litter	2.3	3.2	3.3	4.2	2.9	1.6
Preweaning loss (%)	63	41	46	26	53	62

*
** Fujii et al.(1966)
Soga et al. (1969a)

Comparing the data from the random bred colonies, it seemed that the results improved as time passed, which is in contrast to the situation in our inbred colony. Note the marked difference in preweaning loss between our random bred (26%) and inbred (62%) colonies when comparing the most recent data.

4.4 Discussion

From our data, it appeared that the major problem in establishing and maintaining an inbred as well as a random bred Mastomys colony was the occurrence of a large number of preweaning deaths. The high loss was due primarily to cannibalism by the mothers. The first days after birth appeared to be the most critical period. It was found to be important to protect the mother and offspring against any possible disturbances. Excitement appears to stimulate a latent aggressive behavior in the mother which results in cannibalism. To decrease the loss of animals due to cannibalism, strict selection against this undesirable trait was necessary. In addition, animal caretakers who were to work with Mastomys had to be carefully selected. In our experience, nervous or noisy persons were not suited for this job; they excited the animals too much and this resulted in increased cannibalism.

Another selection criterion should be a regular litter interval. Females not becoming pregnant within 60 days after introduction to the male should be culled from the breeding colony. In terms of breeding efficiency, females should not be allowed to produce more than four litters each and only those which produce litters at regular time intervals of less than 60 days should be maintained.

Comparing the breeding results in successive stages of inbreeding, a decline in the number of young born per female can be observed, indicating that inbreed-

ing depression, one of the consequences of inbreeding, was manifested (Falconer, 1976).

The higher weaning rate in the random bred colonies with time might be explained by increasing domestication of Mastomys on the one hand and improved methods of maintenance on the other (Soga, 1977c). These arguments, however, did not hold for the difference in preweaning loss between our random bred and inbred colonies. The breeding results of our inbred colony also did not improve with time. When we started the inbreeding of Mastomys, strict selection against cannibalism was not possible, as we needed all available females for breeding in view of the low percentage of weaned animals. Selection against this trait was achieved more easily in our random bred colony. This fact might explain the difference in preweaning loss between the two colonies.

At present, we have reached the 30th generation of brother-sister mating of Mastomys but breeding problems still remain. Similar difficulties at other laboratories may explain the existence of only a few inbred colonies of agouti-colored Mastomys in the world and it would seem prudent to distribute animals of these few inbred colonies among other institutions to diminish the risk of losing the existing inbred colonies.

5 Determination of the extent of inbreeding

If one starts with 4 breeding pairs for establishing a random bred colony, the question of whether one may still speak of random bred animals may arise. This prompted us to investigate serum transferrin phenotypes and to perform skin transplantations in animals of the random bred and inbred colonies. Since there is no correlation between transferrin phenotypes and histocompatibility (Kozima, 1977), these two criteria can be considered as additional indicators of the degree of inbreeding or lack thereof.

To determine transferrin phenotypes, serum samples were taken at random from animals of both colonies and analyzed by Prof. Kozima, University of Niigata, Niigata, Japan, according to a method described previously (Ootsu et al., 1976). The results are given in Table XXI.

TABLE XXI

PHENOTYPES OF SERUM TRANSFERRIN IN RANDOM AND INBRED MASTOMYS

	Mastomys	
Phenotypes	Random bred (n=6)	Inbred (n=6)
K	-	+ (6)
M	+ (3)	-
KM	+ (2)	-
kM	+ (1)	-
Km	-	-

Clearly different transferrin phenotypes (M, KM and kM) were found in the animals of the random bred colony, whereas only one phenotype (K) was identified in the inbred animals.

For skin grafting, full thickness skin transplantation was performed according to the technique described by Balner (1964). Skin grafts were exchanged among random bred and inbred animals in several combinations. Skin grafting from random bred to random bred animals, from random bred to inbred animals and vice versa resulted in rejection within 10 to 12 days. However, skin grafting between inbred animals gave different results. Some animals showed a permanent take of the graft, while others rejected it in approximately 10 to 12 days. Autologous transplants were not rejected, so that technical failures can be excluded. This means that complete homozygosity has not yet been reached.

These data indicate that, based on the two above-mentioned generally recognized criteria, the breeding program applied to the random bred animals was successful and that to the inbred colony only partly successful in spite of 30 generations of brother-sister mating.

6 Growth and survival data

6.1 Introduction

Baseline data on growth and longevity are of value only if they are regularly updated, since minor changes in genetic and environmental factors may have a profound influence on both parameters (Cohen, 1968; Lesser et al., 1973; Poiley, 1972). This also means that comparison of such data from different laboratories must be treated with caution, since there will also inevitably be substantial differences in breeder selection criteria, husbandry, nutrition, etc. among the different laboratories.

Furthermore, body weight and longevity are interrelated. It is well-known that dietary restriction (Barrows & Kokkonen, 1978) results in a longer life span. This has been demonstrated for mice (Fernandes et al., 1976; Leto et al., 1976 a,b; Stuchlíková et al., 1975; Weindruch et al., 1979), rats (reviewed by Cohen, 1979) and hamsters (Stuchlíková et al., 1975).

In aging research, survival data are of importance in defining an aged animal and for calculating the cost of an experiment (Zurcher et al., in press). In addition, survival data and body weight may give a crude indication of the health status of an animal colony. A great number of infectious diseases will lead to less rapid growth, an arrest of growth or even weight loss as well as mortality. The latter may have consequences for the 90%, 50%, 10% and maximum survival ages, data which are frequently used in aging research. Survival curves can be constructed from survival data and the shape of such curves may indicate whether random loss is an important factor in the mortality observed in a certain population. However, one must be cautious in relying on the shape of a survival curve

to exclude random losses. For an aging population, one would expect a more or less rectangular curve (Comfort, 1979). However, a curve with a similar shape but exhibiting an earlier decline and decreased maximum life span may be observed when, for instance, a rapidly fatal infectious disease affects a group of animals which had been previously free of disease (Hollander, 1973; van Zwieten et al., 1981). To differentiate between aging and intercurrent diseases, one must perform complete necropsies and histopathological examinations. The hallmark of aging is the presence of multiple pathological changes. Hence, the shape of the survival curve and the presence of multiple lesions are incorporated into the definition of an aged animal. This definition is as follows: an aged animal is one past the 50% survival age derived from a population which has a more or less rectangular survival curve and in which the pathological changes are characterized by multiple lesions (van Zwieten et al., 1981; Zurcher et al., in press).

In this section, growth data of random bred and inbred male and female Mastomys as well as the survival data of random bred male and female Mastomys are given. Survival data of inbred animals are not available, since all inbred animals were needed for breeding purposes. To determine the influence of aggressive behavior on longevity in Mastomys, particularly in males, survival data from two groups of random bred Mastomys were also collected and compared. One group of animals was housed individually and the other in groups of 5 per cage.

6.2 Materials and Methods

6.2.1 Husbandry conditions

In general, the husbandry conditions were the same as those described in Section 4.2.1 of this chapter. If the conditions differed from those described, they will be mentioned in the text.

6.2.2 Body weight

As part of a larger experiment on the determination of autoantibody levels in Mastomys with age body weights were obtained from four groups of virgin Mastomys: 1) random bred males, 2) random bred females, 3) inbred males and 4) inbred females. All animals were housed individually in polycarbonate cages (22x15x14 cm). The first two groups consisted of 100 animals each and the third and fourth ones of 25 animals each. The initial body weights were taken at the ages of 3, 4 or 5 weeks. They were subsequently weighed at 2, 4, 6, 9 and 12 months of age. A total period of 1 year was chosen, as most small rodents attain maximum growth between 6 and 9 months. Beyond this point, measurements reflect excessive development of adipose tissue or progressive debilitation (Poiley, 1972). When the animals were weighed, blood samples were collected by orbital sinus puncture for the determination of autoantibodies. The animals were transferred to fresh, clean cages once weekly.

6.2.3 Survival data

Survival data were collected from 479 random bred virgin male and from 321 random bred virgin female Mastomys. The animals were housed 5 per polycarbonate cage (35x23x16 cm) from the age of 3 weeks. On the death of an animal, the surviving cage mates were left in the cage without the introduction of another animal. In addition, survival data from two groups consisting of 27 and 100 random bred virgin male Mastomys were collected and compared. One group of animals (n=27) was housed individually in polycarbonate cages (22x15x14 cm) and the others (n=100) were housed 5 per polycarbonate cage (35x23x16 cm). Again, no animals were added when one of the animals in a cage died. The animals of these two groups were 7 months old at the start of the experiment.

Survival curves were constructed by plotting the percentage surviving against time.

6.3 Results

6.3.1 Body weight

The mean body weights (± 1 standard deviation) of random bred male and female Mastomys in grams are given in Table XXII and those of inbred male and female Mastomys in Table XXIII.

TABLE XXII

GROWTH TABLES FOR RANDOM BRED MALE AND FEMALE MASTOMYS

Males				Females			
No.of animals	Age (weeks)	Weight (grams) Mean ± 1 S.D.		No.of animals	Age (weeks)	Weight (grams) Mean ± 1 S.D.	
76	3	12.6	2.3	54	3	11.8	2.6
4	4	16.9	1.7	22	4	14.1	2.0
20	5	24.7	2.7	24	5	19.8	2.7
100	8	41.7	5.6	100	8	29.2	3.6
100	17	53.0	6.2	100	17	33.9	3.7
100	26	57.8	7.8	100	26	42.0	5.8
100	39	67.8	8.3	100	39	45.0	4.8
100	52	79.0	8.9	100	52	51.2	7.2

TABLE XXIII

GROWTH TABLES FOR INBRED MALE AND FEMALE *MASTOMYS*

Males				Females			
No. of animals	Age (weeks)	Weight (grams) Mean ± 1 S.D.		No. of animals	Age (weeks)	Weight (grams) Mean ± 1 S.D.	
10	3	15.4	2.6	10	3	11.0	1.5
10	4	17.5	3.6	10	4	15.3	2.4
5	5	24.2	3.0	5	5	22.3	1.7
25	8	41.3	5.4	25	8	32.5	5.0
25	17	51.3	7.8	25	17	39.5	4.1
25	26	58.5	8.8	25	26	43.6	4.9
25	39	69.7	6.7	25	39	51.6	5.3
25	52	80.0	8.0	25	52	58.0	5.6

The mean body weights of the four groups are also shown graphically in Fig.14. It appears from these data that there was no difference between random bred and inbred male *Mastomys*. A small, not significant, difference was found between random bred and inbred females. The inbred females were slightly heavier than the random bred females from 8 weeks of age onwards. Random bred and inbred males were significantly heavier than the corresponding females from the age of 17 weeks onwards.

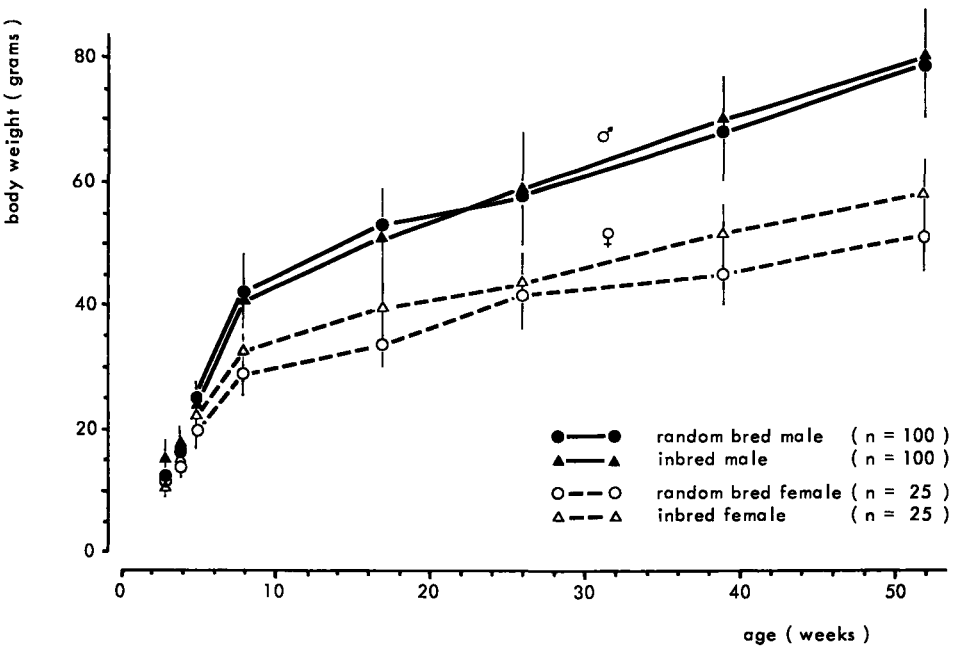

Fig. 14 Mean body weights (± 1 standard deviation) in grams of random bred and inbred male and female *Mastomys*.

6.3.2 Survival data

The survival curves of 479 random bred male Mastomys and 321 random bred female Mastomys housed 5 per cage are shown graphically in Fig.15. The respective values of 90%, 50%, 10% and maximum survival ages for the two groups are given in Table XXIV.

TABLE XXIV

SURVIVAL DATA OF RANDOM BRED MALE AND FEMALE MASTOMYS

Sex	Number	Age in months at different survival values 90%	50%	10%	Maximum age (months)
male	479	5.8	20.1	29.3	34.6
female	321	13.6	26.8	33.2	38.3

From the survival data, it appears that females are older than males at each survival value from the age of 3 months onwards. Another obvious finding is the more or less linear shape of the survival curve for males, while the shape of that for females approaches a more rectangular one.

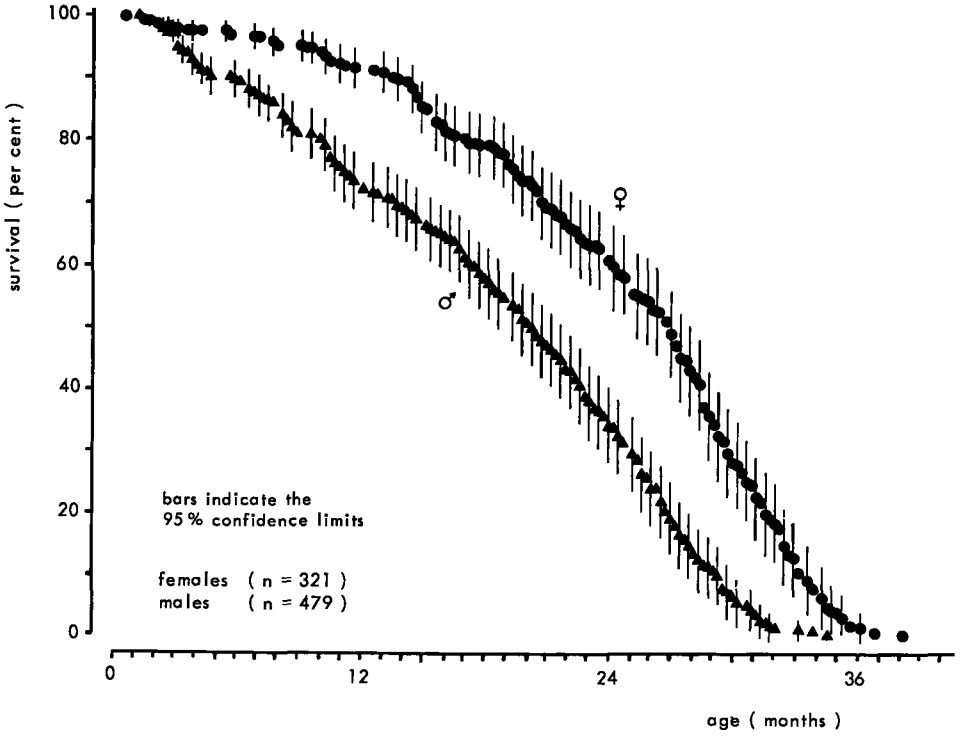

Fig. 15 Survival curves of random bred male and female Mastomys housed 5 per cage.

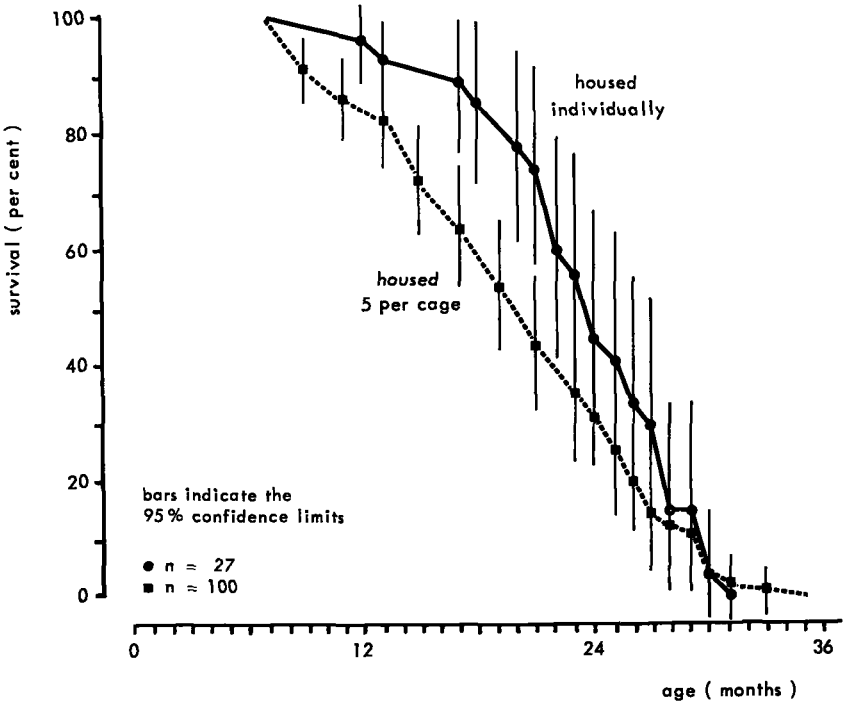

Fig. 16 Survival curves of random bred male Mastomys housed individually and in groups of 5 per cage.

The survival curves of males housed individually and in groups of 5 per cage are given in Fig.16. Interpretation of the two survival curves should be made with a certain amount of caution, in view of the fact that the animals were 7 months old at the start of the experiment and the small number of animals involved in the group housed individually. However, they clearly give an indication that the number of animals per cage plays an important role in the longevity of male Mastomys.

The shape of the survival curve changed from linear to more rectangular, resulting in older 90% and 50% survival ages for animals housed individually. In contrast to the 90% and 50% survival ages, the maximum survival age was less than in the animals housed in groups of 5 but, because of the small number involved, little value should be attached to the maximum age.

6.4 Discussion

The difference in body weight between males and females is a common finding in animals and man. Whether or not the regular blood sampling of our animals influenced body weight is not known but, when comparing the mean body weights of our animals with those of animals of the Niigata colony in Japan (Soga, 1977c), no obvious differences were found between the two colonies.

Regarding the survival data, the difference in survival between males and females (Fig.15) is also a common finding in rats and mice. In general, males have a shorter life span than females (Comfort, 1979; Festing & Blackmore, 1971). However, this cannot be regarded as a universal rule, as has been shown by Burek (1978), Kunstýř & Leuenberger (1975), Smith et al., (1973), and Storer (1966).

The most probable explanation for the increased mortality in male Mastomys maintained 5 per cage as compared with those kept individually is that this is due to fighting, which is a well-known phenomenon among male Mastomys (see Chapter II, Section 4.2). This is readily apparent from the survival curve, which lacks the characteristic of a more or less rectangular form according to the definition of a prerequisite for an aging population. This finding also implies that the 50% survival age of male Mastomys housed in groups is not the real 50% survival age, as is clearly demonstrated in Fig.16. Comparing the two curves, the 50% survival age of the animals housed in groups corresponds with the 80% survival age of the animals which were housed individually. This means that the males of the former group which are past the 50% survival age cannot be regarded as old, despite the presence of multiple pathological changes.

The conclusion must be that a real estimation of the survival ages of male Mastomys is possible only if the animals are housed individually. Whether this also holds true for females, which are known to be much less aggressive, needs to be investigated.

The findings obtained here stress once again the importance of establishing baseline data for the particular animal colony to be investigated. In addition, the collection of such data must take place under the same conditions as are maintained for any experimental group.

CHAPTER IV

NATURALLY OCCURRING AUTOANTIBODIES IN AGED MASTOMYS OF THE RIJSWIJK COLONY

1 Introduction

Over the past years, several authors (Snell & Stewart, 1969b; Solleveld, 1978; Stewart & Snell, 1968) have suggested that a number of pathological changes observed in aged Mastomys may have an autoimmune basis. These changes include lymphoplasmacellular thyroiditis, myositis, myocarditis, sialoadenitis and lymphoepithelial thymomas. In addition, some evidence for the presence of autoantibodies in this animal species has been provided by Strauss et al. (1968), who demonstrated serum immunoglobulin reactivity against striated muscle in all of the 14 aged Mastomys tested, and by van Pelt & Blankwater (1972), who found antinuclear factor positive sera in approximately 14% of the males and 21% of the females older than one year.

These findings prompted us to conduct a more extensive search for autoantibodies in aged Mastomys. The results are described in this chapter.

2 Materials and Methods

2.1 Animals

Eighty-five female and sixty male Mastomys were used in this study. The age of the females ranged from 20 to 39 months with a mean of 28.8 months, while that of the males ranged from 18 to 37 months with a mean of 26.2 months. The animals were randomly bred and housed individually at our Institute and were maintained under the same conditions as described in Chapter III, Section 4.2.1

The animals were allowed to complete their life spans and were killed when moribund, after which a complete necropsy was performed. Detailed histopathological data of this group of animals will be given in Chapter V.

2.2 Sera

Blood samples were collected by orbital sinus puncture when the animals were moribund. In addition to the terminal sampling, serial blood samples were collected from 10 of the 60 male Mastomys at 7, 13, 20, 27 and 31 months of age for the purpose of obtaining information on the age at which the various autoantibodies appeared. Serum was separated and stored at -20°C. The serum samples were diluted 1:10 in phosphate buffered saline (PBS) for screening and Ig class determinations; for determination of antibody titers, they were serially diluted from 1:10 to 1:2560 before use.

2.3. Substrates

Tissues from normal 10-week-old male WAG/Rij rats were snap-frozen in liquid nitrogen. The choice of the tissues used as substrates was based on findings in a previous histopathological study (Solleveld, 1978) and on the results of a pilot study. Fixed or unfixed 4 µm cryostat sections of the following organs were used as antigens: Liver, to detect antinuclear factors (ANF), antibodies to cytoplasmic antigens of parenchymal cells and antibodies to erythrocytes (present in blood vascular spaces); Thyroid, to detect antibodies to cytoplasmic antigens of follicular epithelial cells and to colloid; Kidney, to detect antibodies to mesangial components, to arterial vessel walls and to cytoplasmic and brush border antigens of renal tubular cells; Heart and diaphragm, to detect different types of antibodies to striated muscle; Stomach, to detect antibodies to parietal cells, other epithelial cells and to smooth muscle. Tests for antibodies to smooth muscle antigens were considered positive when gastric muscle was stained (Andersen, 1977; Gabbiani et al., 1973). Antibodies to parietal cells were considered to be present when only these epithelial cells of the glandular stomach were stained. Furthermore, the fact that five other tissues (thyroid, kidney, heart, diaphragm and stomach) were used as antigens in addition to liver enabled us to study the tissue distribution of anti-nuclear factors among these different tissues. In a few instances where Mastomys substrates as well as rat substrates were used comparable results were obtained.

2.4. Antisera

To screen for the presence of autoantibodies and to determine their titers, two antisera were used. These were rabbit-anti-mastomys immunoglobulin (RAM/Ig) prepared as previously described (van Pelt et al., 1976) and horse-anti-rabbit immunoglobulin conjugated with fluorescein isothiocyanate (HAR/Ig/FITC) obtained from the Central Laboratory of the Dutch Red Cross Blood Transfusion Service, Amsterdam, The Netherlands. For determination of Ig classes, use was made of distinct cross-reactivity between antisera raised against mouse immunoglobulins and Mastomys immunoglobulins (van Pelt & Blankwater, 1972). IgG fractions of specific rabbit-anti-mouse IgA and IgM sera conjugated with fluorescein isothiocyanate (FITC) and tetramethylrhodamine isothiocyanate (TRITC), and TRITC conjugated rabbit-anti-mouse IgG_1 and IgG_2 immunoglobulins (RAM/IgG/TRITC) were kindly provided by Dr.J.Radl of our Institute (Bloemmen et al., 1976; Radl, 1981). An FITC labelled IgG fraction of goat anti-mouse IgG_1 and IgG_2 immunoglobulins (GAM/IgG/FITC) was purchased from Nordic Immunological Laboratories, Tilburg, The Netherlands.

2.5. Immunofluorescent technique (IFT)

Indirect immunofluorescent staining was performed as recently reported (Coolen et al., in press) by adapting some conditions previously described (Feltkamp & van Rossum, 1968). The cryostat sections were dried under a fan for 30 minutes. Kidney and stomach sections were fixed with acetone and air dried.

Liver sections were used unfixed but were irradiated for 30 minutes with a UV light source (Philips T UV bulb, 15W) at a distance of 30 cm to reduce cytoplasmic autofluorescence. Sections of thyroid, heart and diaphragm were used unfixed and unirradiated. The preparations were incubated for 30 minutes at room temperature in a moist chamber with the diluted serum under test. The sections were washed for 30 minutes with 3 changes of an appropriate buffer. For liver, Coon's buffer, pH 7.2, at 37°C (Anderson et al.,1971) was used to prevent the diffusion of nuclear material; for thyroid, use was made of phosphate buffered saline (PBS), pH 7.2, at room temperature to prevent the washing out of colloid; for heart, diaphragm, kidney and stomach, PBS, pH 7.2, at 37°C was employed. For screening and determination of titers, the sections were then incubated with RAM/Ig for 30 minutes at room temperature in a moist chamber and, after washing as indicated above, incubated with HAR/ Ig/FITC for 30 minutes at room temperature. For Ig class determinations, incubations were performed with the conjugates against mouse Ig classes. After a final washing procedure, the slides were mounted in 50% glycerol in PBS, sealed with paraffin and read with a Leitz Orthoplan microscope. An epiilumination system was used with a mercury arc HBO 100W/2 light source. The filters used were KP 500 + 1 mm GG 455 and K 515 for FITC and K 530 + KP 560 + 2 mm BG 36 and K 580 for TRITC.

Photographs were taken with a Leitz Orthomat on Ilford HP 5 film and Kodak Ektachrome 200 ASA daylight film (with special processing for 800 ASA)

The intensity of fluorescence was rated on a scale from 0 to 4 by two independent observers. All doubtful results were regarded as negative.

2.6 Determination of the number of autoantibodies per animal

To gain an impression of the multiplicity of autoantibodies in individual animals, the following 12 autoantibodies were evaluated: antibodies to nuclear antigens, cytoplasmic antigens (of liver parenchymal cells, thyroid follicular cells, renal proximal tubular cells, all epithelial cell types of the glandular stomach and striated muscle of the intermyofibrillar type), gastric parietal cells only, erythrocytes, colloid, renal distal tubular cells, glomerular mesangium, striated muscle mitochondria, gastric smooth muscle and to striated muscle resulting in 3 staining patterns (zebra and two striational patterns).

2.7 Statistical evaluation

To determine whether positive staining using one substrate was related to results obtained with other substrates, chi-square testing was used.

3 Results

3.1 Autoantibodies with various organ and tissue specificities

A survey of the types and incidences of naturally occurring autoantibodies in aged Mastomys is given in Table XXV.

TABLE XXV

AUTOANTIBODIES IN AGED MALE AND FEMALE MASTOMYS

Autoantibodies to:		Males (n=60) no. positive	%	Females (n=85) no. positive	%
Liver:	nuclei	16	27	13	15
	cytoplasm	29	48	33	39
	erythrocytes	1	2	6	7
Thyroid:	cytoplasm	24	40	35	41
	colloid	5	8	14	16
Kidney:	proximal tubules	24	40	36	42
	distal tubules	0	0	5	6
	glomerulus (mesangium)	5	8	6	7
	arterial vessel wall	31	52	33	39
Diaphragm:	intermyofibrillar type	10	17	17	20
	striated "	5	8	3	4
	zebra "	13	22	10	12
	mitochondrial "	1	2	1	1
	myasthenia gravis "	9	15	13	15
Heart:	intermyofibrillar type	15	25	22	26
	striated "	16	27	8	9
	mitochondrial "	2	3	2	2
	myasthenia gravis "	18	30	17	20
Stomach:	parietal cells	16	27	40	47
	all epithelial cells	24	40	17	20
	smooth muscle	20	33	14	16
Mean age (months) (range)		26.2 (18 - 37)		28.8 (20 - 39)	

Antinuclear antibodies were detected in 13 (15%) of the females and 16 (27%) of the males. Various patterns of nuclear fluorescence were observed (Fig.17). A homogeneous pattern (Fig.17a) was found in the majority of cases (12 females and 13 males); in a minority, a speckled (1 female and 2 males) or a nucleolar (1 male) pattern was seen (Fig.17b,c). The tissue distribution of antinuclear factors among different tissues is shown in Fig.18. It is shown that, in 38 of the total of 57 positive cases, the ANF was demonstrated with renal tissue as nuclear antigen as compared with a frequency of 29 out of 57 when liver or gastric mucosa was used. Thyroid, diaphragm and heart showed a frequency which was between that of the previously mentioned tissues. In 14 of the 57 cases, all 6 tissues reacted positively. In another 14, ANF reacted with only one substrate: 6 with liver, 5 with kidney, 2 with gastric mucosa and 1 with thyroid. In all other cases (29 of the

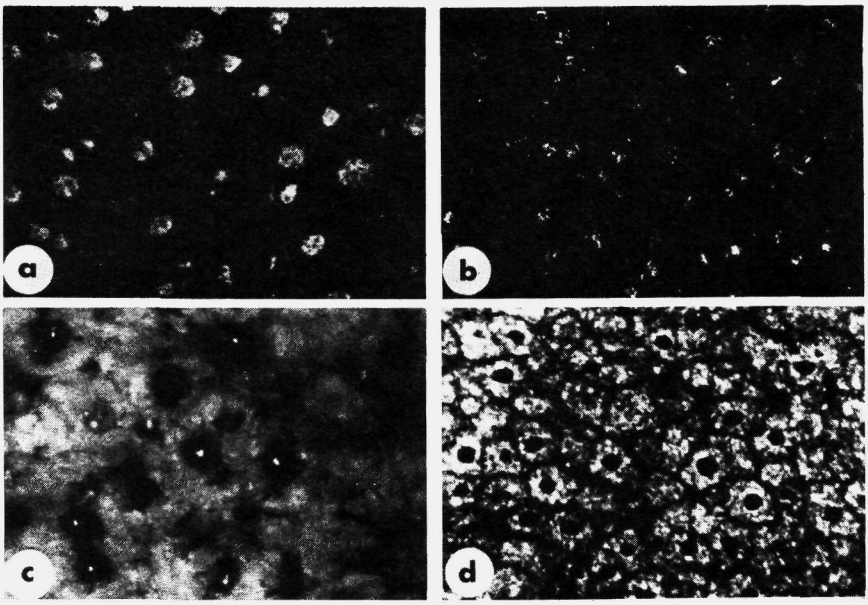

Fig. 17 Staining patterns obtained after incubation of normal rat liver with 1:10 dilutions of aged Mastomys sera.
a. Homogeneous staining pattern of nuclei (235x);
b. Speckled staining pattern of nuclei (235x);
c. Nucleolar staining pattern (360x);
d. Diffuse fine granular cytoplasmic staining of hepatocytes (235x).

57), ANF was detected by more than one but less than 6 tissue substrates. Various combinations were found in these cases.

Antibodies to cytoplasmic antigens were encountered most frequently (up to 50% of the cases). Cytoplasmic staining of liver parenchymal cells (Fig.17d), renal proximal tubular cells, thyroid follicular cells (Fig.19a) and all epithelial lining cells of the glandular stomach in males and females coincided more frequently than would be expected by chance ($p<0.001$). In females, such cytoplasmic staining was often associated with intermyofibrillar staining of striated muscle (Fig.20a).

Antierythrocyte antibodies were detected in only 7% of the females and 2% of the males. These autoantibodies did not react with intact red cells from young Mastomys when tested by the indirect Coomb's test (data not shown).

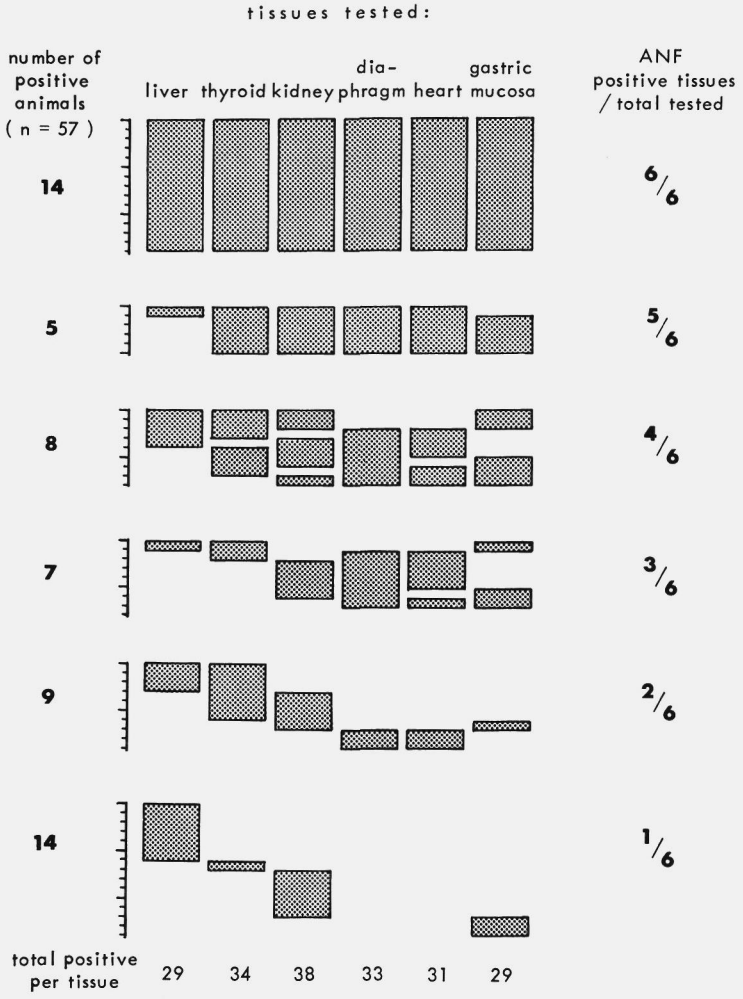

Fig. 18 Tissue distribution of ANF reactivity in 57 Mastomys sera positive for ANF on at least one tissue substrate. The vertical scale indicates the number of serum samples tested and each subdivision represents one single serum sample.

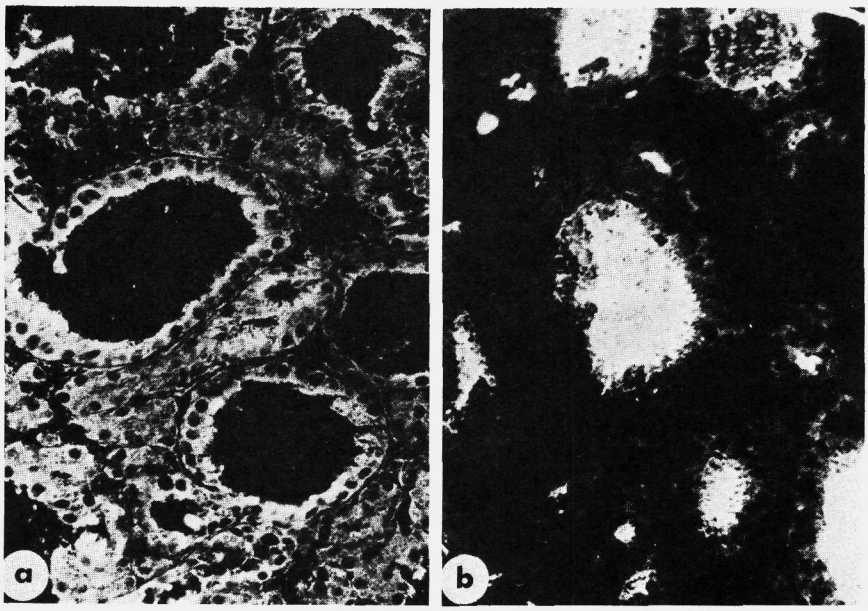

Fig. 19 Staining patterns obtained after incubation of normal rat thyroid with 1:10 dilutions of aged Mastomys sera.
a. Staining of thyroid follicular cell cytoplasm (115x);
b. Staining of thyroid colloid (115x).

Autoantibodies to colloid were observed more frequently in females (16%) than in males (8%). The antibodies gave uniform staining of colloid (Fig.19b).

Besides autoantibodies to cardiac and skeletal muscle of the intermyofibrillar type, other staining patterns of striated muscle were also observed (Fig.20). These were the zebra type (Fig.20b), the mitochondrial type and two striational patterns (Fig.20c,d). The majority of those with a striational staining corresponded to that found in human patients with myasthenia gravis. The so-called myasthenia gravis type is characterized by broad stained bands with an unstained central zone. This unstained central zone is not present in the striated type. The coarse granular staining pattern of antimitochondrial antibodies could be easily distinguished from the interrupted linear staining of the intermyofibrillar type. Antimitochondrial antibodies observed in striated muscle were not correlated with positive staining of the distal tubules of the kidney, which was an unexpected finding.

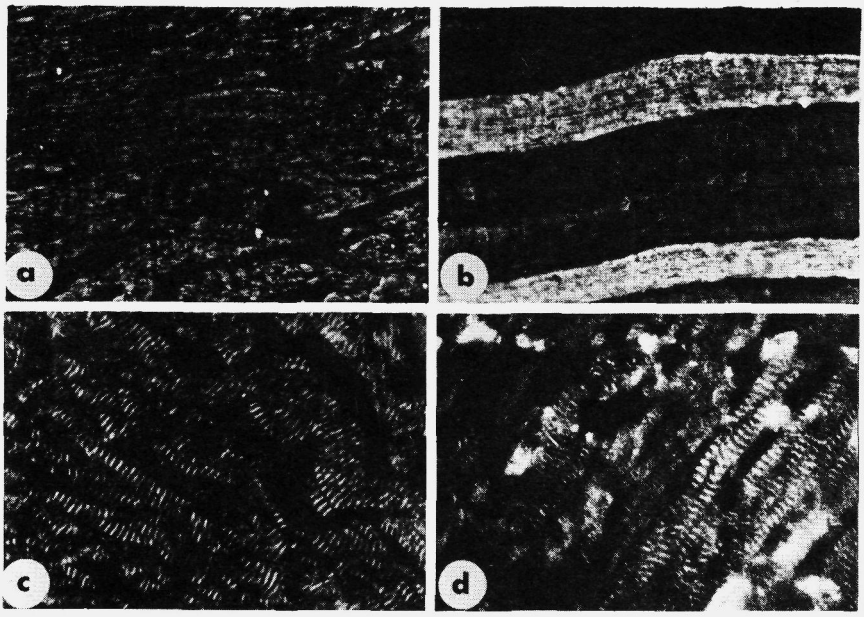

Fig. 20 Striated muscle staining patterns obtained after incubation of normal rat diaphragm with 1:10 dilutions of aged Mastomys sera.

a. Intermyofibrillar type. Interrupted linear, fibrillar staining of muscle fibres (115x);
b. Zebra type, showing a nonstriational fibrillar type of staining of red fibres (115x);
c. Striated type showing narrow stained bands alternating with broad unstained stretches of individual fibrils (235x);
d. Striational staining of the myasthenia gravis type showing broad stained bands with a central unstained zone alternating with unstained stretches of individual fibrils (235x).

Autoantibodies to gastric parietal cells (Fig.21a) were found in 47% of the females and in 27% of the males. No association was found between the presence of these autoantibodies and autoantibodies to cytoplasmic antigens in other tissues. Staining of the brush borders of renal tubular cells or polygonal staining of hepatocytes as observed with antiactin antibodies was never observed.

Autoantibodies to gastric smooth muscle (Fig.21b) were found in a higher percentage in males (33%) than in females (16%). It was expected that staining of renal arterial vessel walls would be found in approximately the same percentages. However, much higher percentages were found for this site, namely, 52% in males and 39% in females.

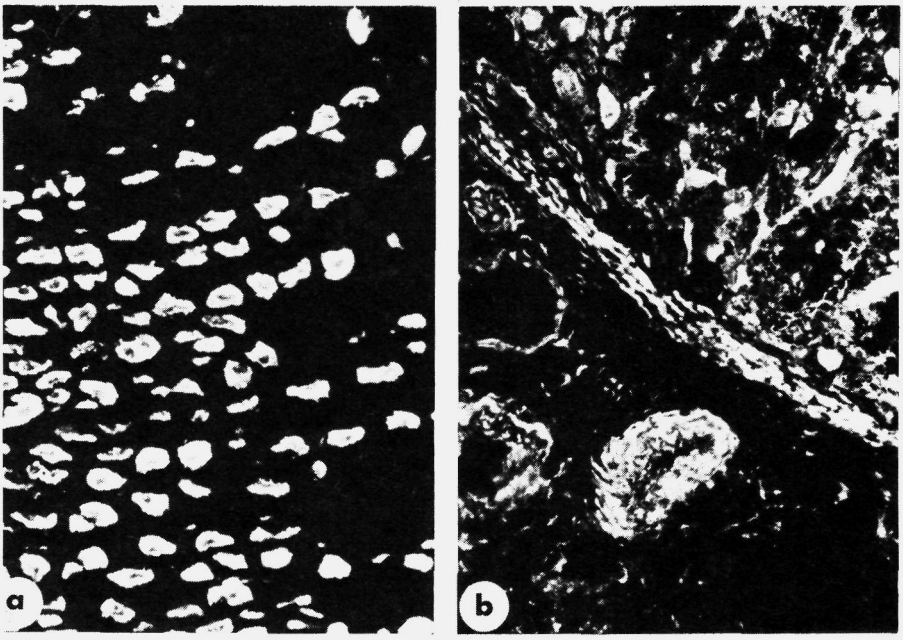

Fig. 21 Staining patterns obtained after incubation of normal rat glandular stomach with 1:10 dilutions of aged Mastomys sera.
a. Staining of gastric parietal cells (115x);
b. Staining of the muscular layer of the gastric mucosa and submucosal arterial vessel walls. The fluorescence present in the mucosa (upper part) is partly due to autofluorescence (115x).

3.2 Age at which autoantibodies appeared

The age at which the various autoantibodies were detected in the 10 male Mastomys studied longitudinally is shown in Fig.22. ANF, antistriated muscle antibodies of the intermyofibrillar and myasthenia gravis types and autoantibodies to gastric parietal cells were present as early as 7 months of age. One animal with autoantibodies to gastric parietal cells which was positive at 7 months of age was found to be negative at 13 months. It was positive again at 20 months of age. Two types of autoantibodies to striated muscle (the striated and zebra types) and autoantibodies to glomerular mesangial components appeared late in life, namely, between 27 and 31 months of age. Most other autoantibodies developed between 7 and 20 months of age. An intermittent pattern with regard to antistriated muscle antibodies of the intermyofibrillar type was also found in one animal. This type of

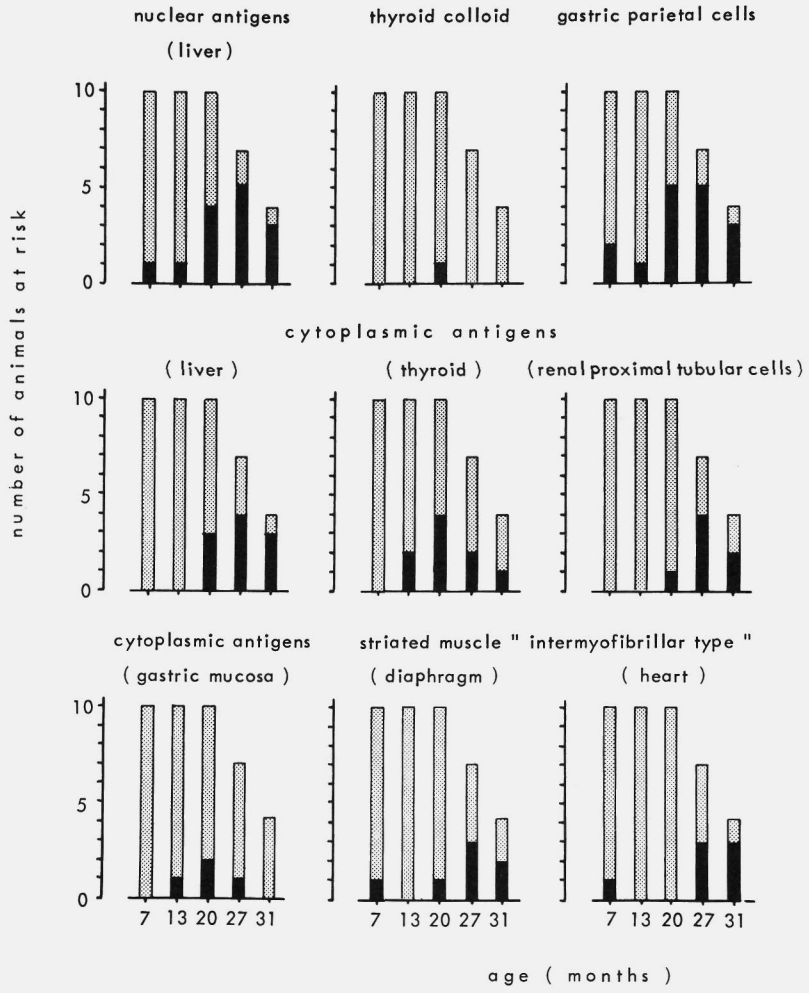

Fig. 22 The age at which the various autoantibodies were detected in 10 male Mastomys. These animals were bled at 7, 13, 20, 27 and 31 months of age. Three animals died between 20 and 27 months of age and three between 27 and 31 months of age. The black area represents the number of animals with the particular autoantibody and the shaded area the number of animals without the particular autoantibody.

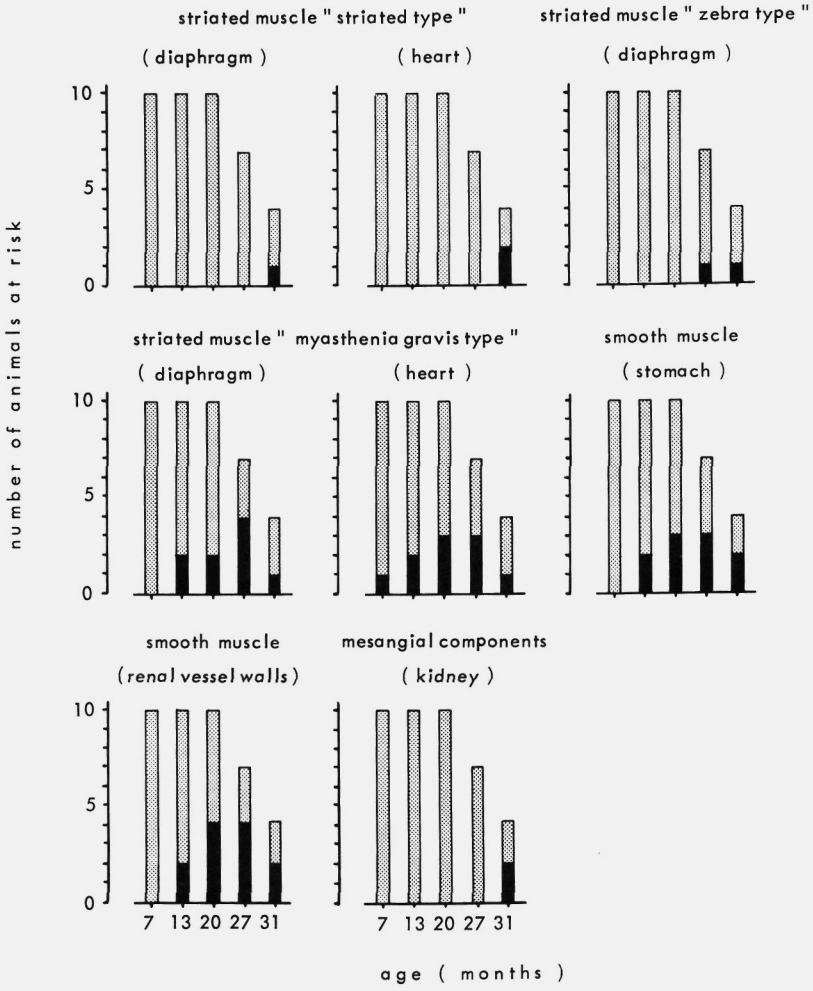

Fig. 22 continued

antibody was already present at 7 months of age, but absent in both substrates (heart and diaphragm) at 13 months. It was found to be present again in heart at 20 months and in diaphragm at 27 months of age. The remaining autoantibodies persisted to the death of the animals.

The age at which autoantibodies to mitochondria, erythrocytes and renal distal tubular cells appeared could not be determined, since these antibodies were not observed in the series of 10 male Mastomys tested longitudinally.

3.3 Titers and class distribution of autoantibodies

To obtain information on the titers and the distribution of Ig classes of the various autoantibodies, a random selection of positive sera (31 sera for the determination of titers and 16 for the distribution of Ig classes) of the total of 145 serum samples was made. The titers showed a rather wide range but were generally high (Table XXVI). This was particularly true for autoantibodies to cytoplasmic antigens, colloid and striated muscle of the intermyofibrillar and myasthenia gravis types. The majority of the various autoantibodies (Table XXVI) were of the IgM class. Only those to colloid and to striated muscle of the myasthenia gravis type were of the IgG class. Autoantibodies to erythrocytes and to striated muscle of the intermyofibrillar type belonged to both the IgG and IgM classes.

TABLE XXVI

TITERS AND Ig CLASSES OF AUTOANTIBODIES IN AGED MASTOMYS

Auto-antibodies to:	number tested	mean titer	range	Ig class
Liver:				
nuclei	12	151	10 - 640	IgM
cytoplasm	12	305	10 - 1280	IgM
erythrocytes	3	480	320 - 640	IgG + IgM
Thyroid:				
cytoplasm	11	144	20 - 640	IgM
colloid	14	545	160 - 1280	IgG
Kidney:				
proximal tubules	18	≧ 158	10 - ≧ 640	IgM
distal tubules	5	34	10 - 80	IgM
Diaphragm:				
intermyofibrillar type	2	≧1600	640 - ≧2560	IgG + IgM
zebra "	2	340	40 - 640	IgM
myasthenia gravis "	5	≧ 645	10 - ≧2560	IgG
Heart:				
intermyofibrillar type	2	≧1440	320 - ≧2560	IgG + IgM
myasthenia gravis "	8	≧ 650	40 - ≧2560	IgG
Stomach:				
parietal cells	19	≧ 170	20 - ≧ 640	IgM
all epithelial cells	8	90	10 - 160	IgM
smooth muscle	20	45	10 - 160	IgM

3.4 Multiplicity of autoantibodies

The number of autoantibodies in individual male and female Mastomys is given in Fig.23. In both males and females, the number of autoantibodies per animal varied from 0 to 6; however, animals with 3 types of autoantibodies were most common.

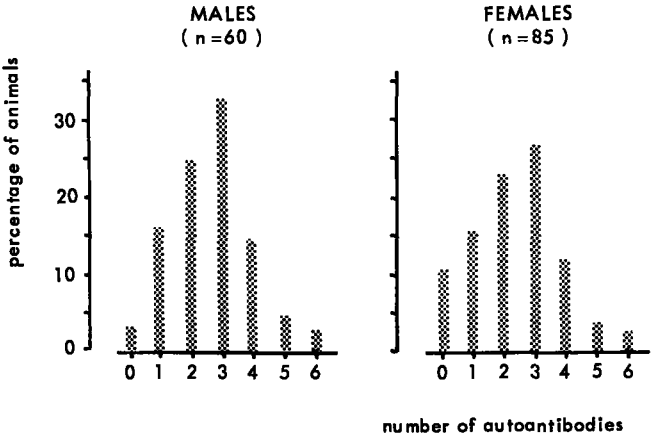

Fig. 23 Number of different types of autoantibodies in individual male and female Mastomys.

4 Discussion

Spontaneously occurring autoantibodies have been described in many species of domestic and laboratory animals. Of these, the NZB mouse and its hybrids and the more recently developed mouse strains, the MRL and BxSB, have been studied most extensively. The spectrum of autoantibodies in Mastomys found in this study seems to be much broader than that in other animal models for spontaneous autoantibody formation (Andrews et al., 1978; Lewis, 1974; Talal, 1976). However, the reaction of Mastomys sera with many tissue substrates does not necessarily imply the presence of autoantibodies with as many different specificities. Nontissue-specific antibodies such as antimitochondrial, antiribosomal and antimicrosomal ones (Doniach et al, 1971; Homberg et al., 1974; Labro et al., 1978; Mathy et al., 1980; Rizetto et al., 1973) may react with their respective antigens in a variety of tissues. Antibodies directed to actin or other components of the cytoskeleton (Fairfax & Gröschel-Stewart, 1977; Kurki et al, 1978a,b; Labro et al., 1978) may explain several of the staining characteristics of Mastomys sera. For instance, antiactin antibodies may react with smooth muscle of vessel walls and with the muscular layer of the stomach as well as with striated muscle, mesangial

components of the renal glomeruli, liver parenchymal cells, the brush border of renal proximal tubular cells and gastric parietal cells (Toh, 1979). In this series of Mastomys sera tested, neither polygonal liver cell staining nor brush border staining of renal proximal tubular cells compatible with antiactin activity was observed.

As mentioned previously, antismooth muscle antibodies were considered to be present when the serum reacted with gastric muscle; 29 of the positive sera also reacted with renal arterial vessel walls, 5 reacted only with gastric muscle and 35 reacted only with renal arterial vessel walls. A similar inconsistency between staining of gastric muscle and arterial vessel walls was also observed in human sera by Andersen (1977). In addition, no correlation was found between the presence of antismooth muscle antibodies and that of antistriated muscle antibodies. This seems difficult to explain when antibodies to a common antigen such as actin or myosin are involved. However, antibodies to smooth muscle myosin do not necessarily cross-react with myosin from striated muscle (Fairfax & Gröschel-Stewart, 1977). Furthermore, a disparate reactivity of sera with tissues which supposedly contain the same antigens may also be explained by differences in antigen concentration, antigen accessibility or state of polymerization (Andersen et al., 1979; Toh et al., 1978). The cytoplasmic staining of liver cells and its association with the staining of renal proximal tubular cells, thyroid follicular cells and all epithelial lining cells of the glandular stomach could be due to nontissue-specific antimicrosomal antibodies. Antibodies to gastric parietal cells were classified separately when only these epithelial cells of the stomach were stained. This was to exclude reactions due to nontissue-specific antimitochondrial or antimicrosomal antibodies. However, an identical staining pattern can be the result of antismooth muscle antibodies (Ceredig & Toh, 1977). Therefore, in the presence of such antibodies, special procedures may be necessary to exclude this possibility (Ceredig & Toh, 1978).

Nevertheless, even if some of the staining patterns observed with Mastomys sera should be ultimately shown to be due to antibodies directed against actin, myosin or some other antigen common to muscle and nonmuscle tissues, several groups of autoantibodies such as antimitochondrial, antimuscle (smooth plus striated), anticolloid, antierythrocyte and antinuclear antibodies and possibly also antimicrosomal antibodies will remain.

In addition, it cannot be excluded that even more antibodies may be found in Mastomys, since only a selected number of tissues were used as substrates in this study. However, in spite of the limited number of substrates used, the variety of autoantibodies found is already greater than that described in other species and is comparable only to the situation in man.

With regard to the tissue distribution of ANF, it appeared that its frequency of occurrence depended on the substrate used, which was also found in man (Feltkamp, 1966; Feltkamp & van Rossum, 1968). Antinuclear antibodies generally lack tissue or species specificity (Nakamura & Tan, 1978). However, it is well known that differences in the detection of ANF may be due to a different concen-

tration of various nuclear proteins in cells of different tissues. This may be one explanation for the observed differences in frequency among the various tissues. Another explanation may be that, in a number of cases, it is a question of different nuclear antigens. No further investigations to specify the nuclear antigens of the various tissues responsible for demonstrating the antinuclear factors have yet been performed. Furthermore, Nakamura & Tan (1978) noted that the substrate used for demonstrating antinuclear factors should be fixed in acetone, since no fixation results in loss of some of the nuclear constituents. In our study, unfixed as well as fixed tissues were used. Fixation or not does not seem to be of major importance, as the frequency of ANF demonstrated in fixed stomach sections was as high as in unfixed liver sections.

Autoantibody titers appeared to be rather high in Mastomys, although antinuclear antibody titers were much lower than those found in the so-called autoimmune mouse strains (Accinni & Dixon, 1979). In these mouse strains, titers up to 1:12,288 were found. On the other hand, the highest titer of autoantibody to colloid in the BUF rat (Noble et al, 1976) was found to be 1:32 in the indirect immunofluorescence test, while that in Mastomys was 1:1280. As in the BUF rat (Noble et al, 1976), these anticolloid antibodies belonged to the IgG class. Antibodies to striated muscle of the myasthenia gravis type were also of the IgG class, which is comparable to the situation in man (Strauss, 1963). As in NZB mice (DeHeer et al, 1978), antierythrocyte antibodies proved to be of the IgG and IgM classes. Antinuclear antibodies, however, were of the IgM class only, while, in mice (Talal, 1976), they are of the IgG as well as IgM classes. Most of the other autoantibodies belonged to the IgM class. No IgA autoantibodies were observed.

The exact time of appearance of the various autoantibodies could not be determined in this study, but most developed before 20 months of age; some were present at the early age of 7 months. The inconsistency found in one animal with respect to autoantibodies to gastric parietal cells and in another to autoantibodies to striated muscle of the intermyofibrillar type, both of which were positive at 7 months of age, then negative and later positive again, may be the result of the grading system used. In the negative samples of these cases, the fluorescent staining was interpreted as doubtful and they were scored as negative according to the grading system used.

The modal number of autoantibodies. (3) in individual male and female Mastomys corresponds with that found in the autoimmune mouse models (Solleveld et al.,1980b) but the variety of autoantibodies is greater in Mastomys.

In summary, it can be stated that Mastomys is not only a museum of tumors (Oettlé, 1967) but represents a museum of autoantibodies as well.

CHAPTER V

A HISTOPATHOLOGICAL SURVEY OF 145 AGED MASTOMYS OF THE RIJSWIJK COLONY

1 Introduction

A survey of pathological changes in animals that are allowed to live out their natural life spans is of prime importance for long-term studies. Such a survey is of importance not only for determining the usefulness of an animal species, strain or sex for a particular study but can also give information on age-associated pathological changes. These age-associated changes are generally grouped as neoplastic and nonneoplastic lesions, the latter including a heterogeneous group of lesions having a degenerative, inflammatory or autoimmune basis.

This histopathological study of aged Mastomys had two objectives. The emphasis was placed on the detection of autoimmune lesions. As stated in the Introduction to Chapter IV, reports which suggested that a number of pathological changes in Mastomys might have an autoimmune basis have been published. The purpose then was to make a survey of these lesions in Mastomys of the Rijswijk colony and to relate them to the autoantibodies detected in this group of animals (see Chapter VI).

The second aim was to make and summarize a survey of pathological lesions found in Mastomys. During the past 25 years, substantial work has been done on the pathology of a number of organs in this animal species. Reports have been published in many different journals, some of which are difficult to acquire. However, no extensive survey of neoplastic and nonneoplastic lesions occurring in Mastomys has been published up to now. Inasmuch as a number of lesions has already been adequately characterized, only brief summaries of such will be given here, with reference to the original reports. Those not previously described will be discussed in more detail. It is the intention that this survey will serve as a reference point for future studies. A periodic updating of pathology data is necessary to ensure that the animals under study are still appropriate for the purpose for which they were chosen. It is well-known that genetic and environmental changes can influence the incidence and expression of lesions. Furthermore, the "normal" histology of old animals differs from that of young ones. What is regarded as normal in aged animals may be abnormal in the young. Except to a limited extent, the normal histology of old Mastomys will be not discussed in this chapter.

2 Materials and Methods

2.1 Animals

Histopathological studies were made on the same 60 male and 85 female Mastomys in which autoantibodies were determined. These animals were subdivided into four age groups in order to study age-related pathology. However, it must be borne in mind that the youngest animals in this study were 18 months of age, so that a selection did take place. The age groups and the number of Mastomys studied per age group for each sex are given in Table XXVII.

TABLE XXVII

AGE GROUPS AND THE NUMBER OF MASTOMYS PER GROUP STUDIED HISTOPATHOLOGICALLY

Age groups (months)	Number of Mastomys studied	
	males (n=60)	females (n=85)
18-23	18	11
24-29	27	36
30-35	14	31
36-41	1	7

For more details concerning the animals involved in this study, see Chapter IV, Section 2.1.

2.2 Necropsy procedure

All animals were moribund when euthanized with ether after which a complete necropsy was performed according to a standard pathology protocol (Burek, 1978). Tissues routinely examined were skin, salivary glands, trachea, lungs, heart, esophagus, stomach (squamous and glandular portions), duodenum, ileum, cecum, colon, liver, pancreas, kidneys, urinary bladder, spleen, lymph nodes (superficial cervical, axillary/brachial, anterior mediastinal, mesenteric and inguinal), thymus, sternum, thyroid, parathyroid, adrenals, brain, pituitary gland, testes, seminal vesicle, prostate (male and female), ovaries, uterine horns, cervix, vagina, lumbar spinal cord and lumbar vertebrae, knee joint, skeletal muscle and any additional gross lesions.

All tissues were fixed in 10% phosphate-buffered formalin or Tellyesniczky's fluid, trimmed, embedded in paraplast, sectioned at 5 µm and routinely stained with hematoxylin-phloxin-saffron (HPS). Other stains were prepared when required.

In a number of cases, certain tissues routinely sampled were not found

during the histological examination due to prosector error or accidental loss during trimming. This was taken into account in calculating the incidence of the lesions, with the exception of the thymic lesions. It is a known fact that the thymus undergoes a gradual atrophy with age which results in such a small tissue remnant that, unfortunately, it is sometimes improperly removed during necropsy or mal-positioned during embedding. However, hyperplastic and neoplastic thymus tissue can be easily recognized at necropsy. If one now takes into account only thymic tissue present in the tissue sections, an overestimation of the percentage of hyperplasia and thymomas will be the result. For that reason, it was decided to regard the thymus as atrophic when no thymus tissue was present in the tissue sections and no gross abnormalities in the thymic region were observed.

2.3 Statistical evaluation

To determine whether various lesions were related to each other, chi-square testing or 2-tailed exact Fisher test was used. Specifically, the relation between parathyroid lesions, fibrous osteodystrophy and renal lesions and between thymomas and other lymphoreticular lesions was investigated.

3 Results

A wide variety of neoplastic and nonneoplastic lesions was found in the 60 male and 85 female Mastomys. The types and overall incidence of neoplastic lesions and nonneoplastic lesions are tabulated in Tables XXVIII and XXIX, respectively. The lesions will be discussed by organ system. In discussing an organ system, the types and incidence of lesions will be given per age group. Generalized lesions will be discussed under the organ system in which they originated.

TABLE XXVIII

NEOPLASTIC LESIONS IN 60 MALE AND 85 FEMALE MASTOMYS

	Males	Females	Total incidence (per cent)
Skin and adnexa			
Squamous cell papilloma, skin	2/58 (3)*	1/85 (1)	2
Squamous cell carcinoma, skin	3/58 (5)	0/85 (0)	2
Adenocarcinoma, unknown origin	0/58 (0)	1/85 (1)	1
Musculoskeletal system			
Osteoma, tibia	1/57 (2)	0/84 (0)	1
Osteosarcoma, ribs or sternum, knee joint	3/57 (5)	1/84 (1)	3
Pleomorphic sarcoma, skeletal muscle	2/57 (4)	2/84 (2)	3

* number of lesions / number of tissues examined (percentage)

cont'd TABLE XXVIII

Digestive system			
Gastric squamous cell papilloma	2/58 (3)*	3/80 (4)	4
Gastric argyrophilic carcinoid	2/58 (3)	4/80 (5)	4
Hepatocellular adenoma	8/60 (13)	37/84 (44)	31
Hepatocellular carcinoma	3/60 (5)	12/84 (14)	10
Mesothelioma, abdominal cavity	0/60 (0)	1/85 (1)	1
Cardiovascular system			
Hemangioma, uterus		1/79 (1)	1
Endocrine system			
Pituitary adenoma	4/35 (11)	5/65 (8)	9
Pituitary carcinoma	0/35 (0)	1/65 (2)	1
Parathyroid adenoma	5/38 (13)	3/46 (7)	10
Adrenocortical adenoma	19/60 (32)	6/84 (7)	17
Adrenocortical carcinoma	12/60 (20)	2/84 (2)	10
Pheochromocytoma, adrenal medulla	1/60 (2)	1/84 (1)	1
Ganglioneuroma-pheochromocytoma, adrenal medulla	1/60 (2)	0/84 (0)	1
Pancreatic islet cell adenoma	1/59 (2)	4/82 (5)	4
Pancreatic islet cell carcinoma	1/59 (2)	0/82 (0)	1
Lymphoreticular system			
Malignant lymphoma, different types	2/60 (3)	15/85 (18)	12
Granulocytic leukemia	0/60 (0)	1/85 (1)	1
Histiocytic sarcoma	0/60 (0)	3/85 (4)	2
Lymphoepithelial thymoma	23/60 (38)	35/85 (41)	40
Urinary system			
Cystic renal adenoma	1/60 (2)	0/84 (0)	1
Renal adenocarcinoma	1/60 (2)	1/84 (1)	1
Reproductive system			
Leydig cell tumor, testis	1/59 (2)		2
Papilloma, rete testis	2/59 (3)		2
Mesothelioma, tunica vaginalis	1/59 (2)		1
Carcinosarcoma, seminal vesicle	1/48 (2)		2
Prostatic adenocarcinoma	0/44 (0)	4/43 (9)	5
Granulosa-theca cell tumor, ovary		7/80 (9)	9
Luteoma, ovary		1/80 (1)	1
Endometrial stromal polyp		8/79 (10)	10
Uterine stromal sarcoma		2/79 (3)	3
Uterine leiomyosarcoma		1/79 (1)	1
Vaginal/Cervical squamous cell carcinoma		1/79 (1)	1

* number of lesions / number of tissues examined (percentage)

TABLE XXIX

NONNEOPLASTIC LESIONS IN 60 MALE AND 85 FEMALE MASTOMYS

	Males	Females	Total incidence (per cent)
Skin and adnexa			
Dermatitis	4/58 (7)*	9/85 (11)	9
Folliculitis	1/58 (2)	1/85 (1)	1
Epidermal inclusion cyst	1/58 (2)	2/85 (2)	2
Musculoskeletal system			
Degenerative joint disease	53/57 (93)	83/84 (99)	96
Fibrous osteodystrophy	11/57 (19)	2/84 (2)	9
Myositis	4/57 (7)	9/84 (11)	9
Osteomyelitis	1/57 (2)	1/84 (1)	1
Respiratory system			
Tracheitis	2/58 (3)	1/78 (1)	2
Alveolar histiocytosis, lung	6/60 (10)	6/83 (7)	8
Fetalization, lung	1/60 (2)	3/83 (4)	3
Focal granulomatous pneumonia	2/60 (3)	0/83 (0)	1
Focal interstitial pneumonia	1/60 (2)	1/83 (1)	1
Cardiovascular system			
Fibromyxomatous change of cardiac valves	53/53 (100)	73/73 (100)	100
Myocardial degeneration and fibrosis	55/60 (92)	63/85 (74)	81
Myocarditis	9/60 (15)	21/85 (25)	21
Cartilaginous metaplasia, atrium and aorta	1/60 (2)	1/85 (1)	1
Pericarditis	3/60 (5)	5/85 (6)	6
Periarteritis nodosa	3/60 (5)	4/85 (5)	5
Fibrinoid necrosis, arteries	5/60 (8)	7/85 (8)	8
Telangiectasia, uterus		1/79 (1)	1
Digestive system			
Sialoadenitis	22/60 (37)	18/83 (22)	28
Atrophy, salivary gland	9/60 (15)	4/83 (5)	9
Cytomegalic acinar cell hypertrophy, salivary gland	10/60 (17)	21/83 (25)	22
Atypical ductular cell hyperplasia, salivary gland	1/60 (2)	0/83 (0)	1
Herniation esophageal mucosa	3/60 (5)	1/85 (1)	3
Esophagitis	0/60 (0)	1/85 (1)	1
Hyperkeratosis, stomach	27/58 (47)	41/80 (51)	49
Horn cysts, stomach	15/58 (26)	8/80 (10)	17
Erosions/ulcerations, stomach	11/58 (19)	24/80 (30)	25
Gastritis	0/58 (0)	2/80 (3)	1
Squamous cell hyperplasia, stomach	13/58 (22)	9/80 (11)	16
Glandular hyperplasia, stomach	6/58 (10)	8/80 (10)	10
Mucosal herniation, colon	0/60 (0)	1/84 (1)	1

* number of lesions / number of tissues examined (percentage)

cont'd TABLE XXIX

Digestive system (cont'd)			
Enteritis	7/60 (12)*	4/84 (5)	8
Mucosal hyperplasia, cecum	2/60 (3)	0/84 (0)	1
Fatty change, liver	7/60 (12)	5/84 (6)	8
Sinusoidal dilatation, liver	1/60 (2)	10/84 (12)	8
Cysts, liver	6/60 (10)	33/84 (39)	27
Liver cell necrosis	9/60 (15)	7/84 (8)	11
Hepatitis	16/60 (27)	19/84 (23)	24
Liver cell hyperplasia	7/60 (12)	5/84 (6)	8
Bile duct proliferation	3/60 (5)	5/84 (6)	6
Atrophy, pancreas	1/59 (2)	1/82 (1)	1
Ductular hyperplasia, pancreas	1/59 (2)	1/82 (1)	1
Acinar hyperplasia, pancreas	1/59 (2)	0/82 (0)	1
Atypical proliferation of pancreatic acinar cells	12/59 (20)	18/82 (22)	21
Endocrine system			
Sinusoidal dilatation, pituitary gland	1/35 (3)	1/65 (2)	2
Pituitary hyperplasia	0/35 (0)	4/65 (6)	4
Thyroiditis	6/57 (11)	18/77 (23)	18
Follicular thyroid cell hyperplasia	3/57 (5)	3/77 (4)	4
Parathyroid hyperplasia	5/38 (13)	4/46 (9)	11
Adrenocortical hyperplasia	2/60 (3)	3/84 (4)	3
Medullary hyperplasia, adrenal gland	0/60 (0)	2/84 (2)	1
Lymphocytic infiltration of pancreatic islets	0/59 (0)	3/82 (4)	2
Pancreatic islet cell hyperplasia	3/59 (5)	1/82 (1)	3
Lymphoreticular system			
Hemorrhage, spleen	0/60 (0)	2/85 (2)	1
Cysts, lymph node	13/60 (22)	5/85 (6)	12
Mesenteric disease	0/60 (0)	1/85 (1)	1
Lymphadenitis	2/60 (3)	5/85 (6)	5
Lymphoid hyperplasia	9/60 (15)	21/85 (25)	21
Thymic hyperplasia	10/60 (17)	7/85 (8)	12
Urinary system			
Glomerulonephropathy			
grade 1	1/60 (2)	22/84 (26)	16
grade 2	21/60 (35)	38/84 (45)	41
grade 3	38/60 (63)	24/84 (29)	43
Nephritic scars	28/60 (47)	41/84 (49)	48
Renal cysts	3/60 (5)	5/84 (6)	6
Hydronephrosis	4/60 (7)	2/84 (2)	4
Granulomatous nephritis	0/60 (0)	1/84 (1)	1
Pyelitis	3/60 (5)	1/84 (1)	3
Cystitis	4/54 (7)	0/75 (0)	3
Ulceration, urinary bladder	1/54 (2)	0/75 (0)	1
Reproductive system			
Testicular atrophy	44/59 (75)		75
Orchitis	1/59 (2)		2
Epididymitis	6/59 (10)		10
Seminal vesiculitis	18/49 (37)		37

* number of lesions / number of tissues examined (percentage)

cont'd TABLE XXIX

Reproductive system (cont'd)			
Papillary hyperplasia, seminal vesicle	3/49 (6)*		6
Prostatitis	16/44 (36)	3/43 (7)	22
Prostatic hyperplasia	10/44 (23)	23/43 (53)	38
Bursal cyst, ovary		2/80 (3)	3
Bursal hemorrhage, ovary		2/80 (3)	3
Parovarian cyst		2/80 (3)	3
Ovarian atrophy		73/80 (91)	91
Cystic follicle, ovary		2/80 (3)	3
Hemorrhagic follicle, ovary		1/80 (1)	1
Cholesterol granuloma, ovary		2/80 (3)	3
Smooth muscle hyperplasia, ovary		1/80 (1)	1
Hemorrhage, uterus		1/79 (1)	1
Hydrometra, uterus		1/79 (1)	1
Squamous metaplasia endometrial epithelium		1/79 (1)	1
Endometritis, cervicitis, vaginitis		5/79 (6)	6
Stromal proliferation, uterus		3/79 (4)	4
Endometrial hyperplasia, uterus		3/79 (4)	4
Mucoid metaplasia vaginal epithelium		3/79 (4)	4
Nervous system			
Vacuolization, brain	41/46 (89)	59/69 (86)	87
Mononuclear cell infiltration, meninges	2/46 (4)	5/69 (7)	6
Spinal cord compression	40/47 (85)	30/74 (41)	58

* number of lesions / number of tissues examined (percentage)

Neoplastic lesions found in considerable percentage were lymphoepithelial thymomas (40%), hepatocellular neoplasms (41%), adrenocortical neoplasms (27%), malignant lymphomas (12%), parathyroid adenomas (10%) and endometrial stromal polyps (10%). The incidence of adrenocortical tumors was six times higher in males than in females, while the situation was the reverse for hepatocellular neoplasms and malignant lymphomas, which were found in a three and six times higher frequency, respectively, in females than in males. Lymphoepithelial thymomas were found in an equal frequency in males and females. Notable was the occurrence of prostatic adenocarcinomas in female Mastomys, while no prostatic tumors were found in males.

The most common nonneoplastic lesions were fibromyxomatous changes of cardiac valves (100%), degenerative joint disease (96%), ovarian atrophy (91%), vacuolization of brain tissue (87%), moderate to severe glomerulonephropathy (84%), myocardial degeneration and fibrosis (81%), testicular atrophy (75%), spinal cord compression (58%), hyperkeratosis of the squamous part of the stomach (49%), nephritic scars (48%), prostatic hyperplasia (38%) and seminal vesiculitis (37%).

Most of these lesions occurred with equal frequency in males and females. Differences in incidence between the sexes were found only for spinal cord compression, prostatic hyperplasia and moderate to severe renal lesions. Compression

of the spinal cord was seen twice as often in males as in females, while prostatic hyperplasia was found two times more frequently in females than in males. Generally, the renal lesions were more severe in males than in females.

3.1 Integumentary system

Lesions found in the skin and adnexa (excluding the mammary gland) are tabulated in Table XXX. The mammary gland is anatomically a part of the integument; however, it will be considered with the reproductive system of which it is a functional part. Lesions such as hyperkeratosis, parakeratosis and acanthosis, often the result of a mite infestation, were not tabulated but were common.

TABLE XXX

SKIN AND ADNEXAL LESIONS IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=58)				Females (n=85)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	18	26	13	1	11	36	31	7
Dermatitis	1(6)*	3(12)	-	-	3(27)	5(14)	1(3)	-
Folliculitis	-	-	1(8)	-	1(9)	-	-	-
Epidermal inclusion cyst	-	-	1(8)	-	-	1(3)	-	1(14)
Squamous cell papilloma	-	1(4)	1(8)	-	-	-	1(3)	-
Squamous cell carcinoma	1(6)	1(4)	1(8)	-	-	-	-	-
Adenocarcinoma unknown origin	-	-	-	-	-	-	1(3)	-

* number of animals with lesion (percentage)

Nonneoplastic and neoplastic lesions were recognized in a small percentage of the cases. No age-association of these lesions was apparent due to the small numbers of animals with a lesion. Ulcerative pyogranulomatous dermatitis was diagnosed in three males and three females, suppurative dermatitis in one male and mononuclear dermatitis and folliculitis in seven females and one male. The granulomatous processes were considered to be reactions to bedding material present in

the skin, presumably as a result of trauma; the etiology of the other inflammatory processes was not discovered. Epidermal inclusion cysts were found in one male and two females. They appeared as small nodules which were located in the dermis. The cyst wall was composed of flattened squamous epithelium and the contents consisted of keratinous material.

Neoplastic skin lesions were diagnosed in five males and one female. Three animals, two males (28 and 32 months of age) and a 33-month-old female, had squamous cell papillomas and three males (23, 28 and 34 months of age) had well-differentiated squamous cell carcinomas. One papilloma and one squamous cell carcinoma were found in the external ear canal, one squamous cell carcinoma was located at the base of the ear, two papillomas in the pectoral region and one squamous cell carcinoma in the posterior abdominal region.

The skin tumors in Mastomys have been described in detail by Rudolph (1980) and Rudolph & Thiel (1976). The incidence found by us (4%) was in agreement with that (3%) observed by Müller & Gissmann (1978) in chamois-colored Mastomys of the GRA-Giessen strain. While we found only a single skin tumor per animal, others (Rudolph, 1980; Rudolph & Thiel, 1976) have observed multiple tumors per animal. Rudolph (1980) noted 17 skin neoplasms in four agouti-colored Mastomys and Rudolph & Thiel (1976) 54 skin neoplasms in 20 chamois-colored Mastomys of the GRA-Giessen strain. Müller & Gissmann (1978) demonstrated that the causative agent of these neoplastic skin lesions was a papilloma virus (MnPV).

A poorly differentiated adenocarcinoma of unknown origin was found in the subcutaneous tissues of a 35-month-old female. The main part of the tumor consisted of sheets of large polyhedral cells with acidophilic cytoplasm and vesicular nuclei varying in shape and size. Tubular or ductal structures were present in some areas of the tumor. The cells of these tubules resembled the other cells of the tumor, but were more regular in shape and size. Areas of necrosis and polymorphonuclear leukocytes were abundantly present. Extensive sectioning failed to reveal the local origin of the neoplasm and metastasis from an undisclosed site cannot be excluded, although no other primaries (resembling this neoplasm) were found.

3.2 Musculoskeletal system

According to the standard pathology protocol, the sternebrae, the lumbar vertebral column and the knee joint were routinely examined. Only in two cases were none of these parts of the skeleton included for histopathological examination and, in another two cases, it was impossible to review them microscopically due to a processing artifact. In all other cases, one or more of the three tissues could be evaluated histologically. The nonneoplastic lesions found in our series are tabulated in Table XXXI.

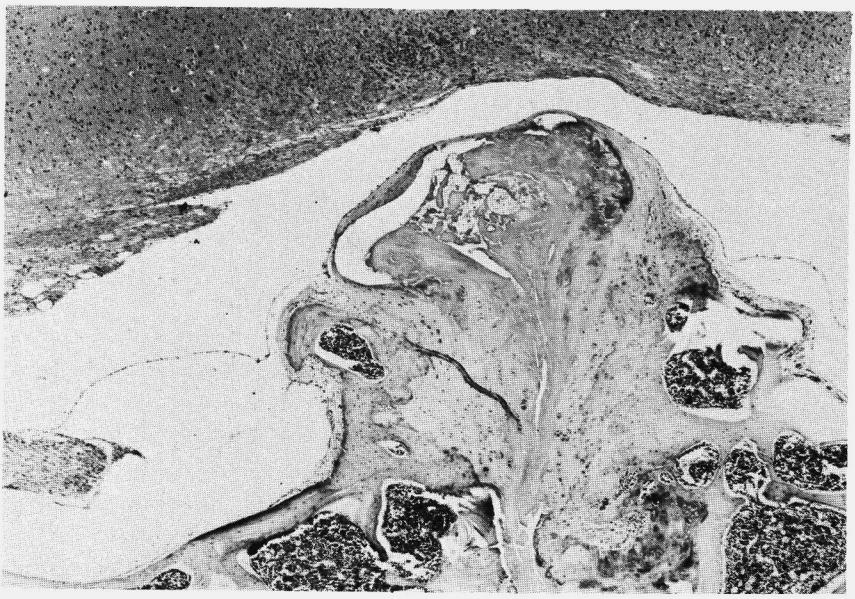

Fig. 24 Protrusion of intervertebral disk material causing compression of the spinal cord in a 32-month-old female. HPS, x 37

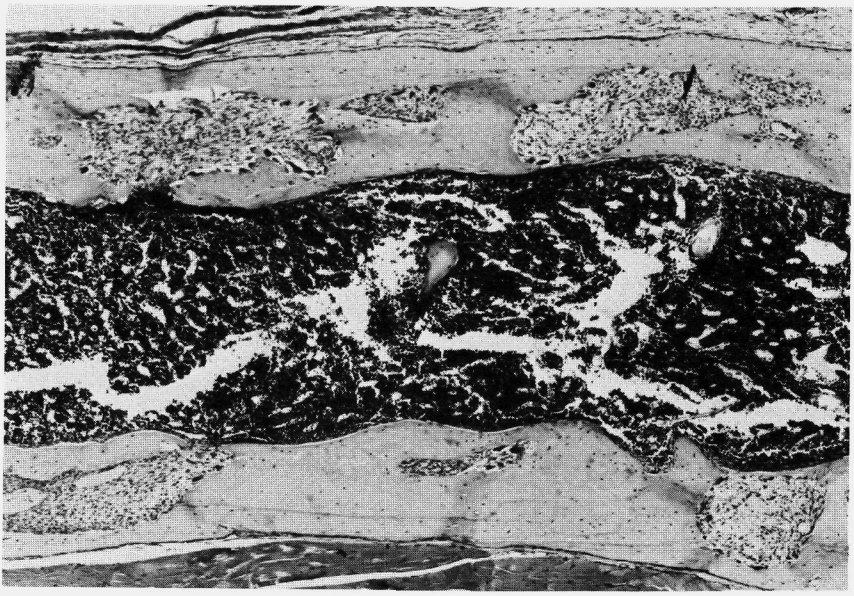

Fig. 25 Section of tibia from a 27-month-old male showing fibrous osteodystrophy. Cortical bone is partly replaced by fibrous connective tissue. Numerous osteoclasts are present along the irregular endosteal bone surface. HPS, x 45

TABLE XXXI

MUSCULOSKELETAL LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=57)				Females (n=84)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	25	14	-	11	35	31	7
Degenerative joint disease	17(94)*	23(92)	13(93)	-	11(100)	34(97)	31(100)	7(100)
Fibrous osteodystrophy	3(17)	4(16)	4(29)	-	-	-	2(6)	-
Myositis	-	3(12)	1(7)	-	1(9)	6(17)	2(6)	-
Osteomyelitis	-	1(4)	-	-	-	1(3)	-	-
Osteoma	-	1(4)	-	-	-	-	-	-
Osteosarcoma	1(6)	1(4)	1(7)	-	-	-	1(3)	-
Pleomorphic sarcoma	-	2(8)	-	-	1(9)	-	1(3)	-

* number of animals with lesion (percentage)

With the exception of four males and one female, all Mastomys showed moderate to severe degenerative joint disease. The disease was characterized by aseptic necrosis of the lumbar and sternal epiphyses, degeneration of intervertebral disks associated with protrusion of intervertebral disk tissue and osteoarthritic changes consisting of aseptic necrosis and erosion of articular cartilage, proliferative changes in the synovial membrane and mucoid and cystic changes in the joint capsule. The disease occurred in about the same frequency in all age groups in both sexes. Protrusion of degenerated disk material was more common in males (45 of the 47 cases examined) than in females (35/74), while no sex differences were found in the frequency and severity of diarthrodial lesions. Protrusion of disk material was associated with spinal cord compression (Fig.24) in 40 of the 45 males and in 30 of the 35 females. If severe compression of the spinal cord had occurred, the animals showed paresis and paralysis which led to their death due to inability to reach their food and water. Muscular atrophy was a common finding in animals showing paresis or paralysis. For more details on degenerative joint disease in Mastomys, reference is made to the excellent report of Sokoloff et al. (1967).

Fibrous osteodystrophy (Fig.25) was found in eleven males and two females. A relation was sought between fibrous osteodystrophy, renal lesions and para-

thyroid gland changes. Fibrous osteodystrophy was correlated significantly ($p < 0.001$) with the presence of parathyroid hyperplasia and adenoma. However, the parathyroid lesions were not correlated with the severity of the renal lesions. Therefore, it is an attractive possibility that, in some of these cases at least, fibrous osteodystrophy may be the result of primary hyperparathyroidism.

Mild focal or multifocal myositis (Fig.26) was found in four males and nine females. In one of these animals, a 26-month-old female, suppurative myositis due to trauma was found; the other 12 animals showed a mixed inflammatory cell infiltrate (although predominantly lymphocytic), muscle degeneration and necrosis.

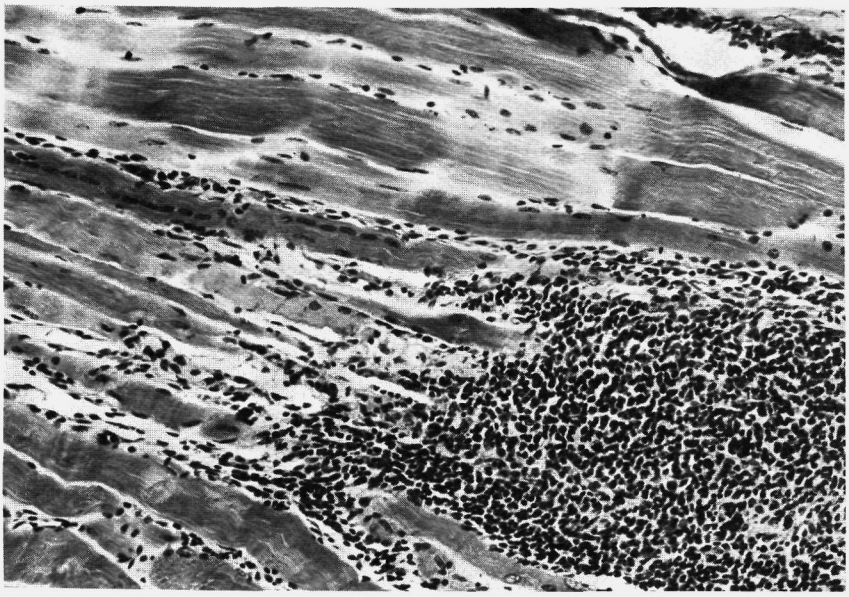

Fig. 26 Lymphocytic myositis associated with vacuolization, fragmentation and loss of muscle fibers in a 29-month-old female. HPS, x 160

A perivascular lymphoreticular cell infiltrate was also seen in some of these animals and was related to hyperplasia or neoplasia of the lymphoreticular system.

A 27-month-old male and a 25-month-old female showed osteomyelitis which resulted from a severe ulcerative dermatitis.

Neoplastic lesions occurred relatively frequently in our series. An osteoma was present in the diaphysis of the tibia of a 19-month-old male. Four fibroblastic osteosarcomas were diagnosed in three males (21, 28 and 30 months of age) and in one 31-month-old female. Two of the osteosarcomas arising from the ribs or sternum had infiltrated the thoracic musculature, the diaphragm, the liver, the pancreas and the renal capsule. One of the two had metastasized to the lungs. The other two osteosarcomas were located at the knee joint and had infiltrated the surrounding skeletal muscles. Pleomorphic sarcomas were recognized in two males

(24 and 26 months of age) and two females (21 and 34 months of age). They were located in the hindlegs in the males and in the forelegs in the females. The sarcomas resembled rhabdomyosarcomas but special stains did not reveal cytoplasmic striations; they were therefore classified as pleomorphic sarcomas. In the two males, the sarcomas had metastasized to the lungs and in one of these also to an abdominal lymph node and the adrenal cortex.

Spontaneously occurring neoplastic lesions of the musculoskeletal system which were not found in our material but described by others are a chondromyxoma in one of the limbs (Soga, 1977b) and a rhabdomyosarcoma located in the region of the ear (Oettlé, 1955). Additional soft tissue tumors not occurring in our animals but reported by others include a ganglioneuroma and a neurofibrosarcoma found in one of the limbs (Soga, 1977b), and three lipomas, a liposarcoma and two fibrosarcomas arising from the flank (Hollander & Higginson, 1971).

3.3 Respiratory system

The skulls of our Mastomys were not sectioned, so that lesions of the nasal cavity and paranasal sinuses could not be determined.

3.3.1 Trachea

Suppurative tracheitis was the only lesion encountered and was found in two males (3%) and one female (1%). There was no evidence for involvement of one of the common murine respiratory pathogens or of bacteria of unknown significance described as occurring in the respiratory tract of rats (van Zwieten et al., 1980).

3.3.2 Lungs

Lung lesions were relatively uncommon in Mastomys (Table XXXII). The most frequent finding, although not exceeding 8% in frequency, was focal accumulation of foamy macrophages (alveolar histiocytosis), located primarily in subpleural alveoli. Macroscopically, such accumulations appeared as grayish-white spots and these have been considered to be normal findings in rats (Bullock et al., 1968; Coleman et al., 1977; Giddens & Whitehair, 1969; Yang et al., 1966). Flodh et al. (1974) mentioned that these macrophages contained fatty acids, cholesterol and phospholipids. Cholesterol clefts in association with macrophages and giant cells were sometimes seen. They may have resulted from previous hemorrhage or necrosis, but these initial changes were not seen in our cases.

Reactive alveolar lining cells assuming a cuboidal appearance (fetalization) were present focally in one male and three females. The cause of this reaction is unknown. Focal granulomatous pneumonia compatible with focal aspiration pneumonia was seen in a 23- and a 27-month-old male. Focal interstitial pneumonia was observed in a 29-month-old male and a 30-month-old female. The lesion was characterized by a focal mononuclear infiltrate in the alveolar septa. Diffuse pulmonary

infiltration or focal peribronchial and perivascular aggregations of lymphoreticular cells were seen in cases of hyperplasia or neoplasia of the lymphoreticular system. In addition, polymorphonuclear leukocytes were frequently seen in the alveolar

TABLE XXXII

PULMONARY LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=60)				Females (n=83)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	27	14	1	11	35	30	7
Alveolar histiocytosis	2(11)*	4(15)	-	-	2(18)	2(6)	2(7)	-
Fetalization	-	1(4)	-	-	1(9)	2(6)	-	-
Focal granulomatous pneumonia	1(6)	1(4)	-	-	-	-	-	-
Focal interstitial pneumonia	-	1(4)	-	-	-	-	1(3)	-
Tumor metastases	1(6)	3(11)	1(7)	-	-	3(9)	-	1(14)

* number of animals with lesion (percentage)

septa. This finding was not related to pneumonia; instead, it was always associated with extensive granulopoiesis in the spleen and liver, often as a result of an inflammatory process elsewhere in the body, particularly in the accessory sex glands.

As regards neoplastic lesions, no primary pulmonary tumors were observed in our series. These tumors seem to be rare in Mastomys, since only two spontaneous lung tumors, an alveolar cell carcinoma and a leiomyosarcoma, have been described in these animals (Soga, 1977b). Tumor metastases were recognized in the lungs of nine animals. These involved metastases of a hepatocellular carcinoma in a 31-month-old male and three females (24, 28 and 36 months of age), an adrenocortical tumor in a 19-month-old male, a pleomorphic sarcoma in a 24- and a 26-month-old male, a mesothelioma in a 24-month-old female and an osteosarcoma in a 28-month-old male.

3.4 Cardiovascular system

3.4.1 Heart

The most common lesion was fibromyxomatous change of cardiac valves (Fig.27) that often resulted in severe thickening of the valves. This was seen in various degrees of severity in all heart sections in which one of the cardiac valves was present. A few animals also had chronic valvulitis and one showed necrosis of the valvular attachment site. Whether one of the cardiac valves was more affected than the others could not be determined, since only the mitral valves were present in most cases.

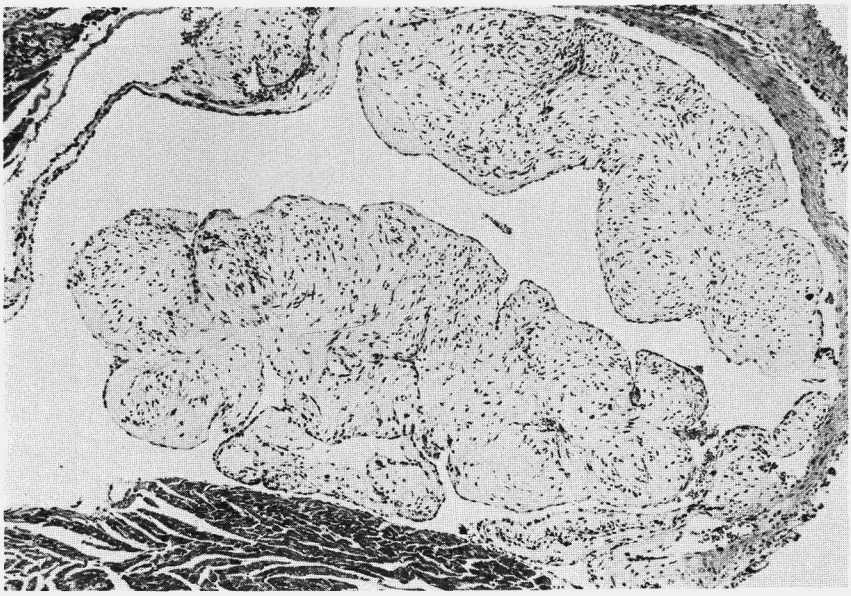

Fig. 27 Severe myxomatous change of mitral valves of the heart of a 21-month-old female. HPS, x 54

Another frequently observed lesion was mild to moderate myocardial degeneration and fibrosis. The changes consisted of muscular degeneration, atrophy, fibrosis and the presence of a variable number of inflammatory cells. The changes were most distinctive in the left ventricular wall and were seen in 92% of the males and in 74% of the females. Comparable changes are seen in mice and rats. In rats, they occur more frequently in males than in females (Fairweather, 1967). The onset in rats is usually after one year of age and the most striking increase occurs after 18 months. This also seems to apply to Mastomys (Table XXXIII). There is still discussion on whether myocarditis, chronic ischemia caused by coronary arteriosclerosis or severe renal disease is the precursor of myocardial

degeneration and fibrosis in rats (reviewed by Burek, 1978). Lesions of the coronary arteries were rare in Mastomys, in contrast to myocarditis and severe renal lesions. Serial killing experiments may be helpful to obtain insight into the pathogenesis of this lesion in Mastomys.

Chronic myocarditis (Fig.28) was seen in nine males (15%) and nineteen females (22%). The infiltrate consisted of lymphocytes, plasma cells, histiocytes and a few polymorphonuclear leukocytes and was associated with muscular degeneration and necrosis. The infiltrates were seen throughout the heart. Most animals showing this lesion had a thymoma, an association which was also found by Stewart & Snell (1968). These authors considered this association to be indicative of autoimmune disease. Demartini et al. (1975) found chronic myocarditis in 39% of wild Mastomys with no evidence of it being associated with a thymic lesion. Chronic myocarditis must be distinguished from the presence of subendocardial and subepicardial nodular aggregates of lymphoreticular cells which were often present concurrently.

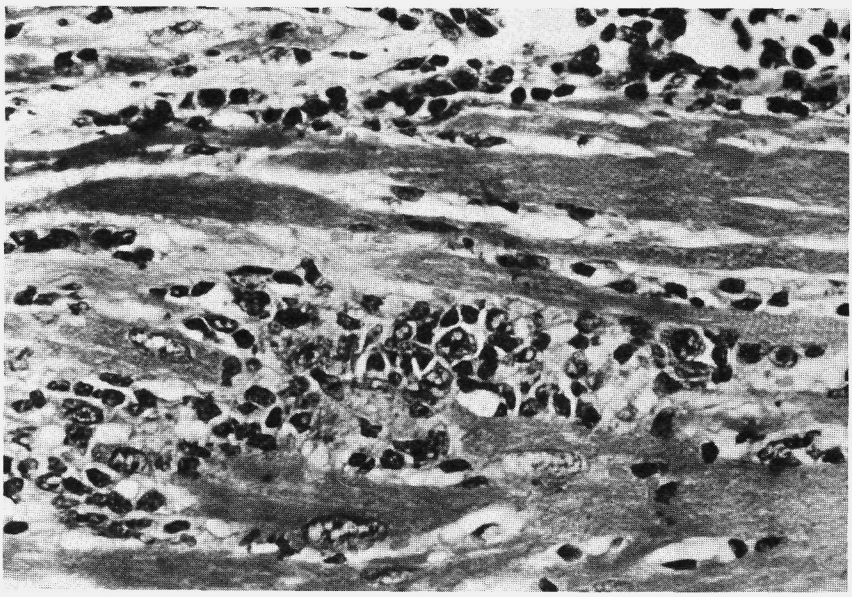

Fig. 28 Chronic myocarditis associated with destruction of cardiac muscle fibers. The infiltrate consists of small and large lymphocytes and histiocytes. Male, 25 months. HPS, x 400

Moreover, both lesions were often found in association with generalized proliferative changes of the lymphoreticular system. The nodular aggregates consisted of a monomorphic cell population and no other signs of an inflammatory reaction were seen. In the cases diagnosed as myocarditis, a mixed inflammatory cell population along with other changes associated with inflammation such as edema, fibrosis

and cellular destruction were observed. In addition, a focal acute necrotizing myocarditis was seen in a 24-and a 33-month-old female.

Chronic pericarditis was diagnosed in three males and five females (Table XXXIII). In one male and four females, it was regarded as a reactive process due to the presence of a large thymoma; the cause was obscure in the other three cases.

TABLE XXXIII

MAJOR CARDIAC AND VASCULAR LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=60)				Females (n=85)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	27	14	1	11	36	31	7
Cardiac lesions								
Fibromyxomatous change of valves**	(100)*	(100)	(100)	(100)	(100)	(100)	(100)	(100)
Cartilaginous metaplasia, atrium	1(6)	-	-	-	-	-	-	-
Myocardial degeneration and fibrosis	15(83)	25(93)	14(100)	1(100)	6(55)	29(81)	23(74)	5(71)
Myocarditis	4(22)	2(7)	3(21)	-	3(27)	11(31)	7(23)	-
Pericarditis	3(17)	-	-	-	1(9)	3(8)	1(3)	-
Vascular lesions								
Cartilaginous metaplasia, aorta	-	-	-	-	-	-	1(3)	-
Periarteritis nodosa	1(6)	2(7)	-	-	-	2(6)	2(6)	-
Fibrinoid necrosis	2(11)	2(7)	-	1(100)	-	2(6)	3(10)	2(29)
Telangiectasia, uterus					-	1(3)	-	-
Hemangioma, uterus					-	-	1(3)	-

* number of animals with lesion (percentage)
** cardiac valves were not found in all heart sections; for that reason, only the incidence in percent is given.

Cartilaginous metaplasia was seen in the atrial wall of a 21-month-old male and in the wall of the aorta in a 35-month-old female.

Primary tumors of the heart were not found and have not been reported by others in Mastomys. Metastasis of a hepatocellular carcinoma was observed in the heart of a 31-month-old male. Extensive outgrowth had occurred, causing the ventricular lumen to be completely obliterated. Tumor cells of a mesothelioma found in the abdominal cavity were recognized in the lumen of the right ventricle of a 24-month-old female.

3.4.2 Vascular system

Acute to chronic periarteritis nodosa (Fig.29) was seen in three males and four females. Multifocal involvement of the arteries of several organs was found in a 19-month-old male and a 34-month old female, while only the vessels of one organ were involved in the other animals. These organs included the heart (3x), the testis (1x) and the salivary gland (1x).

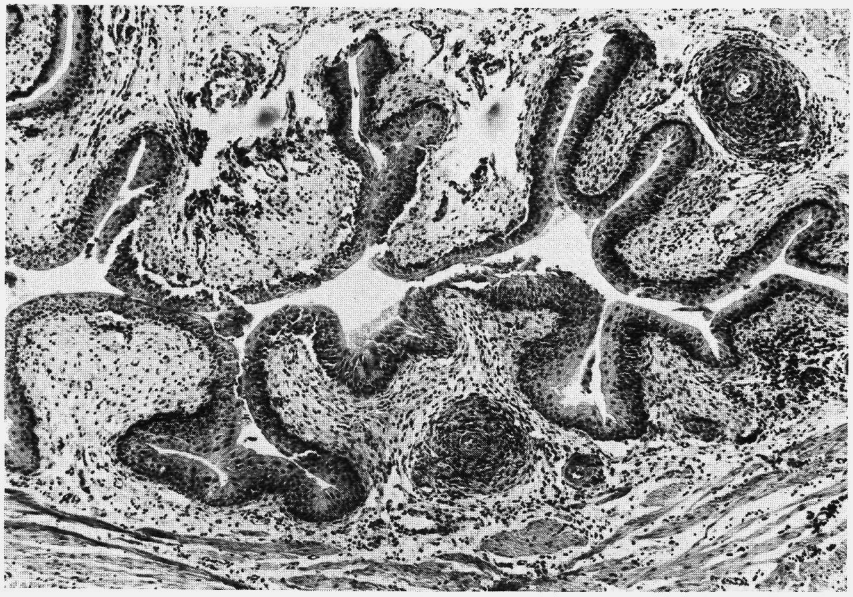

Fig. 29 *Periarteritis nodosa of small arteries in the submucosa of the urinary bladder of a 19-month-old male. HPS, x 54*

Fibrinoid necrosis of the media of small- and medium-sized muscular arteries without a cellular reaction (Fig.30) was observed in five males and seven females. In a 27- and a 30-month-old female, the lesion was seen in various organs and, in a 20-month-old male, in the spleen and testis, while it was restricted to arteries of the heart (2x), the ovary (2x), the bladder (2x), the cecum (1x) the spleen (1x) and the testis (1x) in the other animals.

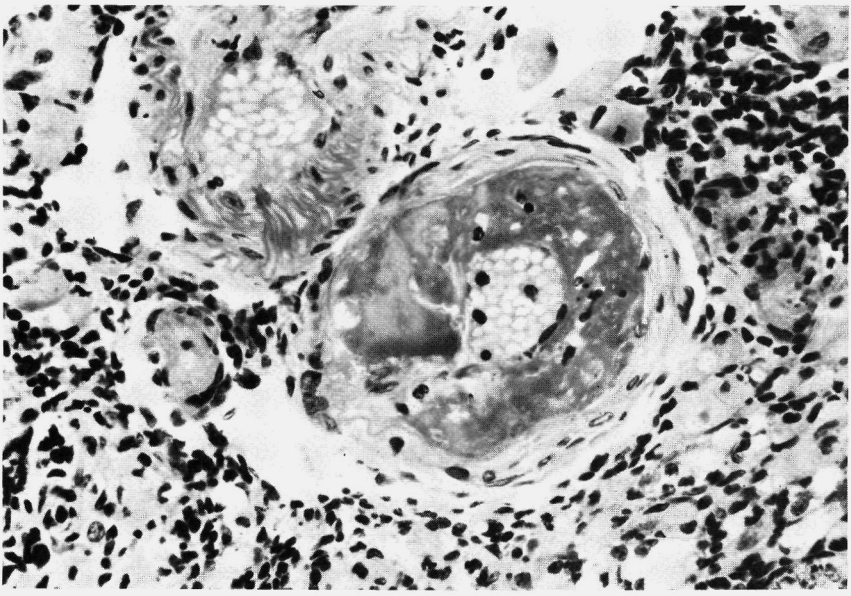

Fig. 30 Fibrinoid necrosis of an ovarian artery of a 34-month-old female. HPS, x 330

Hyalinization of small arteries, particularly in the testes, was often seen but not recorded, as was lymphocytic infiltration of the pulmonary veins in a number of animals bearing a thymoma. Other lesions encountered only once were vasculitis or perivasculitis of pulmonary vessels, proliferative inflammation around a coronary artery, telangiectasia and a hemangioma. The latter two lesions were found in the uterus.

3.5 Digestive system

3.5.1 Oral cavity

No gross lesions were observed in the oral cavity and, since the head was not routinely examined histopathologically, data on microscopic lesions are lacking.

3.5.2 Salivary glands

Inflammatory lesions of the salivary glands, particularly the submaxillary glands were common (Table XXXIV). Both suppurative and nonsuppurative lesions occurred independent of the age of the animals. The suppurative lesions were characterized by the presence of small foci of polymorphonuclear leukocytes and

TABLE XXXIV

SALIVARY GLAND LESIONS IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=60)				Females (n=83)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	18	27	14	1	11	34	31	7
Suppurative sialo-adenitis	6(33)*	8(30)	7(50)	-	1(9)	5(15)	7(23)	-
Lymphocytic sialo-adenitis	-	1(4)	-	-	1(9)	2(6)	2(6)	-
Atrophy	2(11)	2(7)	4(29)	1(100)	-	3(9)	1(3)	-
Cytomegaly	3(17)	5(19)	2(14)	-	-	6(18)	11(35)	4(57)
Atypical ductular cell hyperplasia	-	1(4)	-	-	-	-	-	-

* number of animals with lesion (percentage)

acinar and ductular cell destruction (Fig.31a). A lymphocytic inflammation was observed in one male and five females. It was generally a mild inflammation, with the exception of one female in which an extensive infiltration was seen (Fig.31b). Perivascular aggregrations of lymphoreticular cells were seen in animals showing hyperplasia or neoplasia of the lymphoreticular system.

Atrophy of the submaxillary gland characterized by atrophy of acinar tissue, dilatation of ducts and an increase in connective tissue between acini was found in nine males (15%) and four females (5%). Occasionally, small calculi were recognized within ducts. Both changes were regarded as results of inflammation. Foci of cytomegalic acinar cells (Fig.32) were observed relatively frequently in the parotid glands of male and female *Mastomys*. These foci consisted of hypertrophied cells with large nuclei and bluish instead of acidophilic cytoplasm. This change was also observed in the submaxillary glands of wild-caught *Mastomys* (Demartini et al., 1975). It was concluded that this finding was highly suggestive of cytomegalic inclusion disease because amphophilic intranuclear inclusions were observed in five of the 28 *Mastomys* examined. The inclusions were seen in ductal cells of the submaxillary, sublingual and parotid glands. Although cellular and nuclear pleomorphism was obvious in our animals, inclusion bodies were not found.

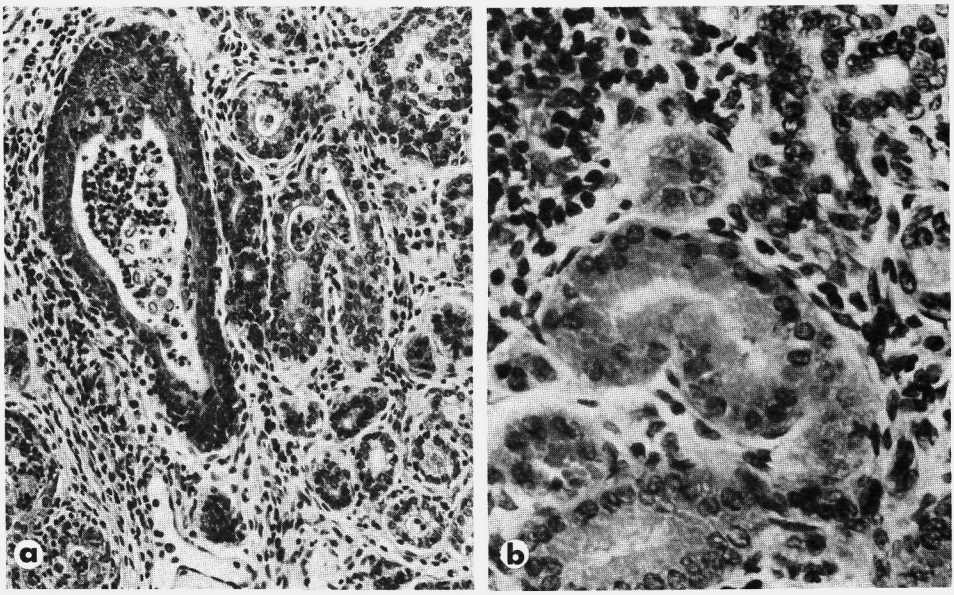

Fig. 31 a. Suppurative sialoadenitis associated with squamous metaplasia of salivary gland ducts. Female, 20 months. HPS, x 160
b. Lymphocytic sialoadenitis. Female, 24 months. HPS, x 330

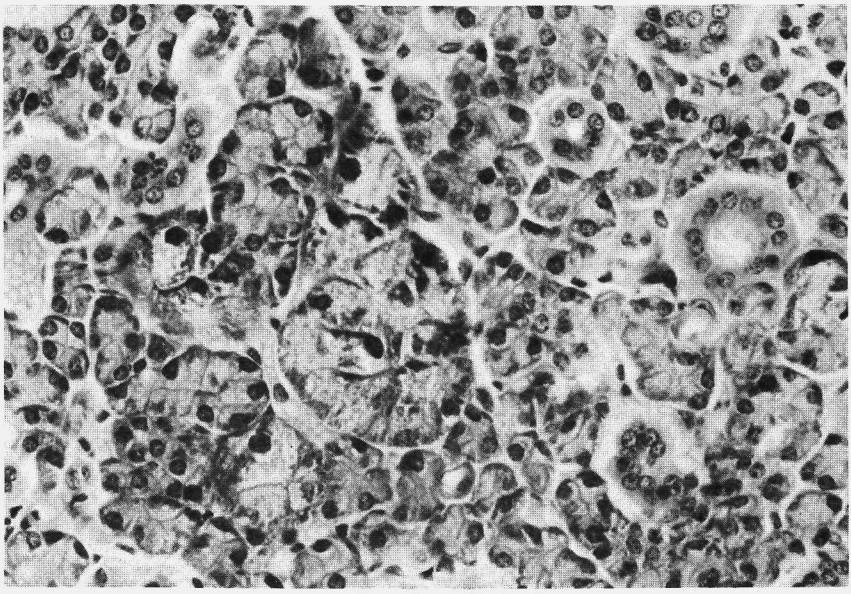

Fig. 32 Focus of cytomegalic acinar cells in parotid gland of a 24-month-old female. HPS, x 350

In a 29-month-old male Mastomys, a focus of atypical cells forming ductular structures was recognized in the submaxillary gland. The cells exhibited an acidophilic cytoplasm and a large darkly-stained nucleus. The cellular aspect was so different from that of normal ductular cells that the diagnosis of atypical ductular hyperplasia was made.

3.5.3 Esophagus

Herniation of the esophageal mucosa through the muscular tunics was seen in three males (5%) and one female (1%). One of these males also had a megaesophagus. The only other lesion encountered was chronic esophagitis in a 31-month-old female.

3.5.4 Stomach

Lesions were common in the squamous and glandular portions of the stomach and are tabulated in Table XXXV.

TABLE XXXV

GASTRIC LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=58)				Females (n=80)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	26	13	1	7	35	31	7
Squamous portion								
Hyperkeratosis	6(33)*	14(54)	7(54)	-	2(29)	16(46)	16(52)	7(100)
Horn cysts	3(17)	9(35)	3(23)	-	1(14)	3(9)	4(13)	-
Squamous cell hyperplasia	1(6)	6(23)	6(46)	-	-	2(6)	4(13)	3(43)
Squamous cell papilloma	-	2(8)	-	-	1(14)	-	2(6)	-
Glandular portion								
Gastritis	-	-	-	-	-	1(3)	1(3)	-
Glandular hyperplasia	1(6)	2(8)	3(23)	-	-	3(9)	3(10)	2(29)
Argyrophilic carcinoid	1(6)	1(4)	-	-	-	1(3)	3(10)	-
Transition squamous/glandular								
Erosions/ Ulcerations	5(28)	4(15)	2(15)	-	1(14)	9(26)	9(29)	5(71)

* number of animals with lesion (percentage)

Hyperkeratosis of the squamous portion of the stomach was the most common finding. This occurred in 47% of the males and in 51% of the females and was most obvious at the limiting ridge. Horn cysts located beneath the squamous epithelium were found in 26% of the males and 10% of the females. These cysts were visible through the serosa as rounded white elevations. They were filled with squames of keratinized material. Squamous cell hyperplasia, also occurring at the limiting ridge, was found in 22% of the males and 11% of the females. The epithelial layer was thickened and hyperkeratotic and showed many infoldings. In the same region, squamous cell papillomas were found in two males (24 and 27 months of age) and three females (23, 33 and 35 months of age). The tumors appeared as branching structures consisting of a central core of connective tissue covered with acanthotic, hyperkeratotic epithelium.

The transitional zone between the squamous and glandular portions was generally infiltrated by inflammatory cells, predominantly polymorphonuclear leukocytes, which was associated with the presence of erosions or ulcerations in 11 males (19%) and 24 females (30%). In addition, two females had chronic gastritis in the fundic region of the stomach not associated with ulceration or erosion of the mucosa. Small aggregations of lymphoreticular cells were often situated in the basal part of the lamina propria adjacent to the muscularis mucosa and these were sometimes increased in size in cases of lymphoreticular hyperplasia or neoplasia. Hyperplasia of cardiac glands was diagnosed in six males (10%) and eight females (10%). The glands were increased in number, irregular and often dilated. This change was likely to have been the result of the inflammation present in this region. Of all these lesions, only hyperkeratosis, squamous cell hyperplasia and glandular hyperplasia were age-associated

Argyrophilic carcinoid tumors of the glandular stomach were found in a 23- and a 26-month-old male and in four females varying in age from 24 to 31 months. The incidence found in our series (4%) is extremely low if compared to the findings of others (Hollander & Higginson, 1971; Oettlé, 1955, 1957; Sato & Fujii, 1967; Sato et al., 1969, 1974; Soga et al., 1969a and Snell, 1965). The incidences found by these authors varied from 12 to 68 per cent. Some investigators found a higher incidence in females, others in males and still others found no sex predilection at all. An excellent description of this type of tumor has been given by Soga (1977a); therefore, it is only briefly described here. Grossly, the tumors found in our series were visible as nodules in the greater curvature of the stomach. These measured from 5 to 12 mm in diameter. Microscopically, they were all well-developed tumors according to the classification of Soga (1977a) and the largest mass of the tumor was found in the submucosa (Fig.33). The overlying mucosa was either edematous or ulcerated. The tumor cells were either arranged in nests or in a trabecular pattern. Mitoses were common. The malignancy of these tumors was evidenced by invasive growth into the submucosa and gastric muscular wall and by the presence of metastases. One tumor in our series had metastasized to the pancreatic

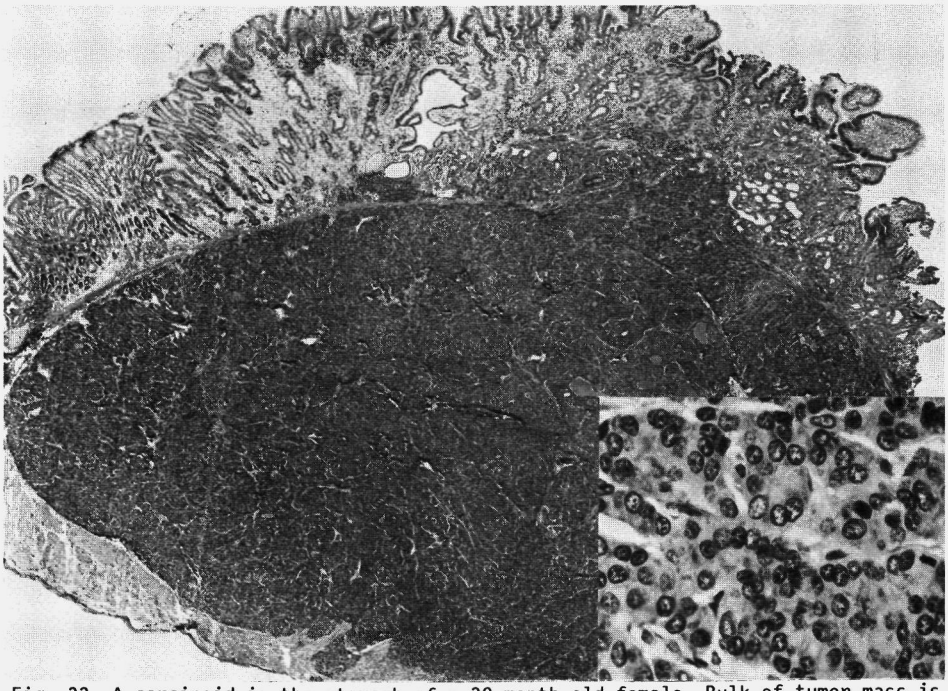

Fig. 33 A carcinoid in the stomach of a 32-month-old female. Bulk of tumor mass is present in the submucosa of the stomach. Tumor cells have infiltrated the gastric muscular wall. HPS, x 21. Inset: Higher magnification to show uniform appearance of carcinoid cells and arrangement of cells in nests.

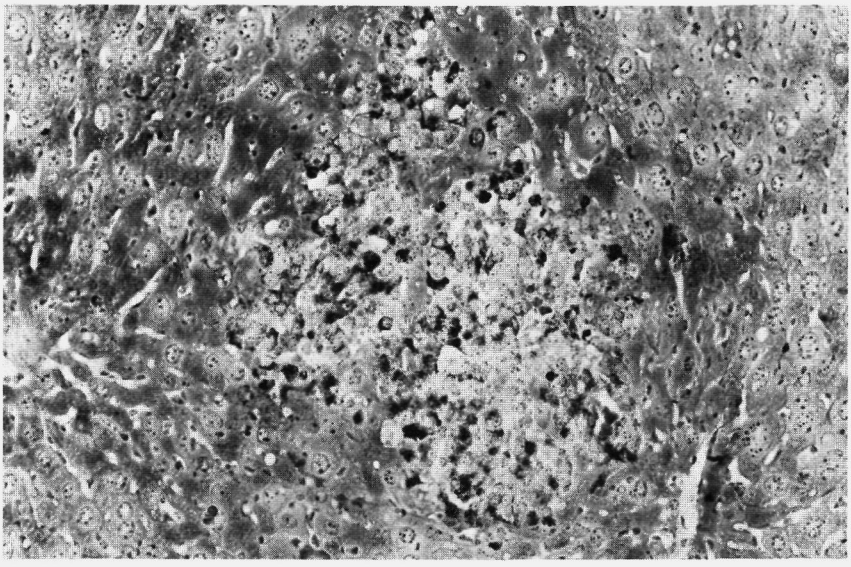

Fig. 34 Hepatic metastasis of gastric carcinoid. Note the characteristic coal-black argyrophilic granules in tumor tissue. Sevier-Munger method, x 170

lymph node and another to the pancreatic lymph node and to the liver (Fig.34). The Sevier-Munger method revealed coal-black granules in the cytoplasm of the carcinoid cells. The Fontana-Masson method gave negative results. Based on the same findings, Snell & Stewart (1969a) classified these tumors in Mastomys as non-argentaffin argyrophilic cell carcinoids. Before that time, they had been classified as adenocarcinomas (Oettlé, 1955, 1957). The diagnosis carcinoid was confirmed by electron microscopic studies (Capella et al., 1973; Håkanson et al., 1973; Sano, 1975; Soga et al., 1969b). Biochemical tests on primary tumors and transplants revealed large amounts of histamine (Fukushima et al., 1974; Hosoda et al., 1970) and histidine decarboxylase activity (Håkanson et al., 1973; Hosoda et al., 1971). These findings suggested histamine production by these tumors. This would also be in agreement with the observations of Stewart & Snell (1975), who found multiple ulcers of the stomach, duodenum and upper jejunum in transplanted animals. The animals died of intestinal hemorrhage, perforating ulcers or peritonitis. No such lesions were observed in our animals bearing a carcinoid. Recently, Hosoda et al., (1979) found histamine and serotonin production in a transplantable carcinoid tumor.

Of interest are the recent reports of Randeria (1979, 1980) on the spontaneous occurrence of antral adenocarcinomas in the Y- and Z- strains of Mastomys. However, she also mentioned the existence of mixtures of carcinoid and adenocarcinoma in a number of animals. Since the diagnoses seem to have been based on light microscopic examination only, the question of whether the adenocarcinoma may be a variant of the carcinoid arises. For a definitive diagnosis, it is necessary to apply special stains and other techniques (e.g., electron microscopy) to prevent a misinterpretation similar to that which occurred in the past when carcinoids in Mastomys were diagnosed as adenocarcinomas.

3.5.5 Intestine

Only nonneoplastic lesions were found in the intestinal tract. With the exception of mucosal herniation occurring in a small area of the colon of a 30-month-old female, all other lesions concerned inflammation of the intestinal tract. Suppurative inflammation of the duodenum was observed in a 30- and a 31-month-old female and a 30-month-old female had a perforated ulcer in the duodenum. None of these lesions was associated with the presence of a gastric carcinoid. The cecum was most commonly inflamed. Suppurative typhlitis was found in three males (20, 30 and 32 months of age) and in a 33-month-old female. In two males, glandular hyperplasia was present in the inflamed region. Ulcerative typhlitis with an acute suppurative reaction was recognized in a 19- and a 22-month-old male. Abscesses in the cecum were seen in a 29-month-old male, which were due to pinworms which had invaded the mucosa. In addition, a suppurative colitis was seen in a 20-month-old male.

Pinworms were recognized in the cecum, colon or both of almost every animal, but these generally seemed to be harmless. In only one case were these parasites found in the mucosa of the cecum; in all the other cases, no reaction to the presence of the parasites was seen.

3.5.6 Peritoneum

Peritonitis was observed in some animals, with severe inflammation of the accessory sex glands, ulcerative enteritis and, in some cases, a tumor or tumor metastasis in the abdominal cavity. Tumor cells of a mesothelioma were found throughout the abdominal cavity in a 24-month-old female. The anatomic site of origin of this tumor was not known. Other tumors or tumor metastases found in the abdominal cavity are discussed under the organ system in which they originated.

3.5.7 Liver

Many nonneoplastic and neoplastic lesions were found in the livers of the aged Mastomys. Only the major lesions observed in the liver of an animal were tabulated, as it was generally not practical to list all changes, particularly in animals with a neoplastic lesion. In cases of neoplasia, a wide variety of changes such as degeneration, regeneration, fatty change, sinusoidal dilatation, thrombosis, cysts, necrosis, etc., were seen. This means that the incidence of a number of lesions may be higher than is shown in Table XXXVI. Liver lesions were generally more common in females than in males. Most lesions seemed not to be age-related.

In approximately 25% of the animals, multiple small foci consisting of cell destruction and polymorphonuclear leukocyte infiltration were seen and this condition was diagnosed as suppurative hepatitis. Special stains such as periodic acid Schiff's, Grocott's methenamine silver, Brown and Brenn tissue Gram stain and Giemsa did not reveal microorganisms in these foci. A granulomatous inflammation, the cause of which was not discovered, was recognized in a 34-month-old female. The general term necrosis (Table XXXVI) included coagulative necrosis of a lobule or several lobules and individual liver cell necrosis. Coagulative necrosis was observed in a 20-month-old male and in three females (22, 23 and 27 months of age) and individual liver cell necrosis in 13% of the males and 5% of the females. Two types of cysts were recognized: hepatocellular cysts which were lined by flattened parenchymal cells and filled with proteinaceous fluid or blood and empty biliary cysts lined by flattened bile duct cells. The hepatocellular cysts appeared

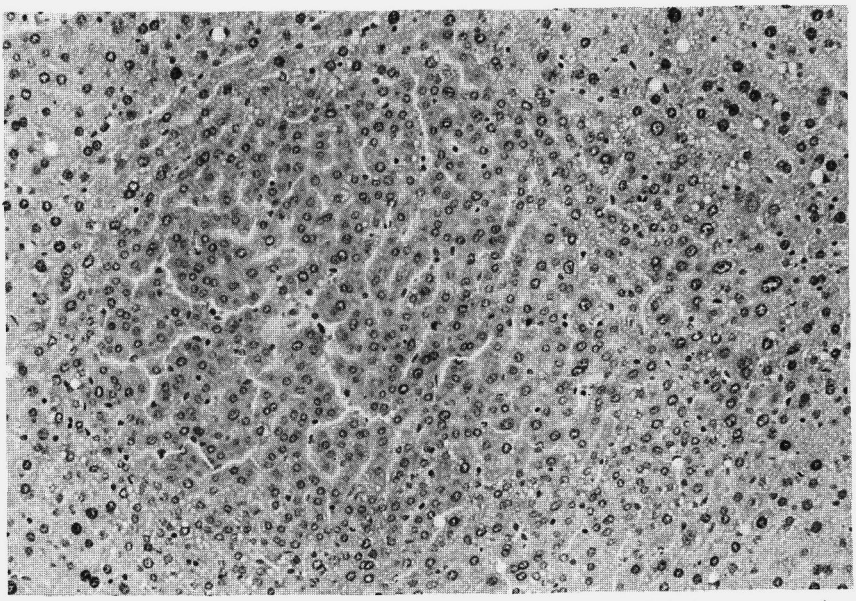

Fig. 35 Focus of hyperplastic liver parenchymal cells. Slight compression of adjacent normal liver parenchyma is visible (upper left). Male, 19 month. HPS, x 140

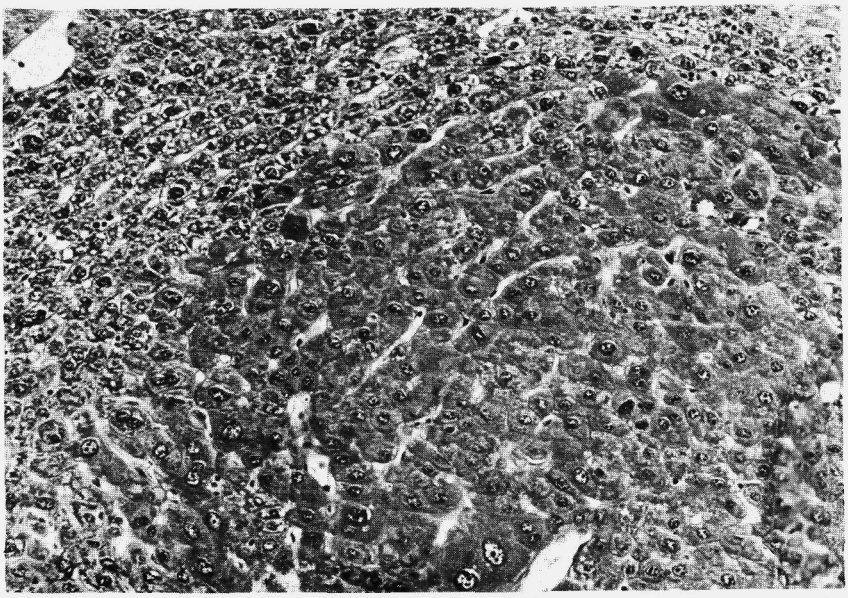

Fig. 36 Hepatocellular adenoma. Tumor tissue compresses the adjacent normal liver parenchyma. Male, 30 months. HPS, x 140

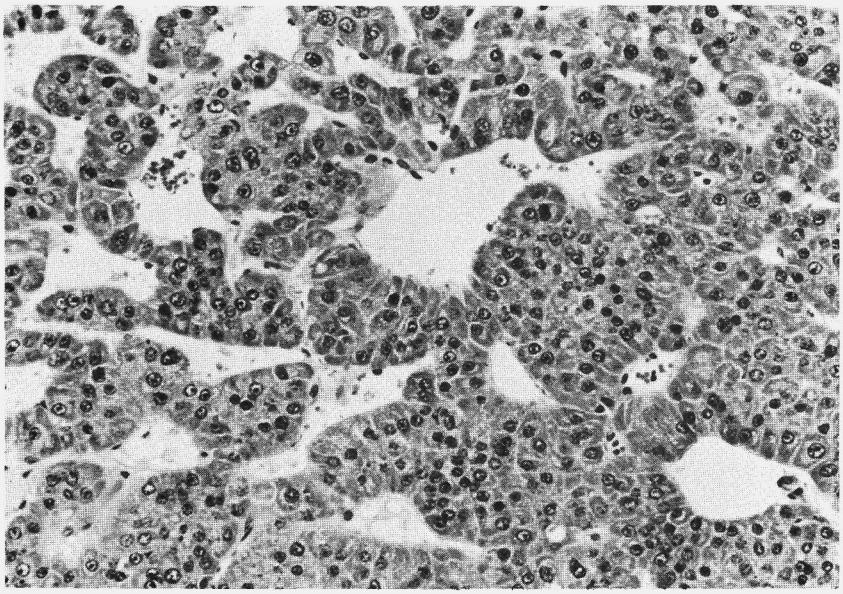

Fig. 37 Trabecular structures in a well-differentiated hepatocellular carcinoma. Female, 33 months. HPS, x 160

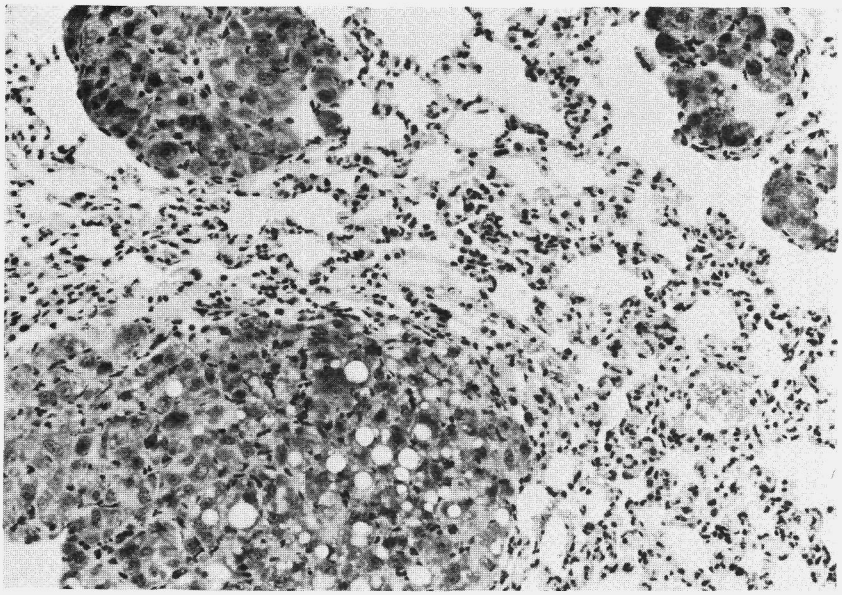

Fig. 38 Lung of a 36-month-old female with metastases of hepatocellular carcinoma. HPS, x 140

TABLE XXXVI

LIVER LESIONS IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=60)				Females (n=84)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	18	27	14	1	11	36	30	7
Fatty change	3(17)*	1(4)	3(21)	-	-	3(8)	2(7)	-
Suppurative hepatitis	3(17)	10(37)	3(21)	-	3(27)	10(28)	5(17)	-
Granulomatous hepatitis	-	-	-	-	-	-	1(3)	-
Necrosis	4(22)	5(19)	-	-	3(27)	3(8)	1(3)	-
Bile duct proliferation	2(11)	1(4)	-	-	-	2(6)	2(7)	1(14)
Sinusoidal dilatation	1(6)	-	-	-	-	3(8)	5(17)	2(29)
Cyst	1(6)	4(15)	1(7)	-	2(18)	15(42)	12(40)	4(57)
Hyperplasia	1(6)	2(7)	4(29)	-	1(9)	2(6)	2(7)	-
Adenoma	1(6)	4(15)	3(21)	-	3(27)	13(36)	18(60)	3(43)
Carcinoma	-	2(7)	1(7)	-	1(9)	6(17)	3(10)	2(29)

* number of animals with lesion (percentage)

to result from hepatocellular atrophy and/or necrosis. The overall incidence of cysts was 10% in males and 39% in females. The higher percentage in females was primarily due to a higher incidence of hepatocellular cysts rather than biliary cysts. This may be explained by the higher incidence of neoplasia in females, which was often associated with necrosis.

The proliferative lesions were subdivided into hyperplasia, adenoma and carcinoma. Hyperplasia was diagnosed when an increase of cells not giving distinct compression of the surrounding parenchyma was seen (Fig.35). The foci were often not larger than a lobule and were sharply demarcated from the normal parenchymal cells by the appearance and staining reactions of their cells. Two distinctive cell types were recognized in these foci. These were small cells with basophilic cyto-

plasm and a darkly-stained nucleus and large cells with acidophilic cytoplasm and a vesicular nucleus. Adenomas always resulted in compression of the surrounding parenchyma (Fig. 36). The normal hepatic structure in these tumors was lost; e.g., portal triads were absent. The cells varied in size and mitotic figures were often seen. Fatty change, necrosis, cysts and dilated sinusoids were also present. If plates of hepatocytes three or more cells thick were observed, the diagnosis carcinoma was made (Fig.37). Cellular pleomorphism, mitotic figures and the secondary changes were more obvious in carcinomas than in adenomas. When various proliferative lesions were seen, only the most severe lesion was tabulated.

Hyperplastic lesions were seen more frequently in males (12%) than in females (6%), while adenomas and carcinomas were more common in females. Adenomas were found in 13% of the males and 44% of the females. The male/female ratio of about 1:3 was also found by Soga et al. (1969a) and Kanahara et al. (1972), although they found a lower overall incidence of 13.5% as compared with 31% in our series. Carcinomas were diagnosed in 5% of the males and 14% of the females, while Hollander & Higginson (1971) found percentages of 2.7 and 3.4, respectively. One of the three carcinomas in males (33%) and three of the twelve in females (25%) had metastasized to the lungs (Fig.38).

Hepatic metastasis of a gastric carcinoid was observed in a 32-month-old female and infiltrative growth of an osteosarcoma originating from the sternum or ribs was seen in a 21-month-old male. Diffuse infiltration or aggregations of lymphoreticular cells was frequently seen in animals with hyperplasia or neoplasia of the lymphoreticular system and will be discussed in that section.

3.5.8 Exocrine pancreas

Interstitial fatty infiltration between pancreatic acini was found in most animals and appeared to be independent of the age of the animals, since it was also seen in series of young animals (2-6 months of age) (Solleveld, unpublished observations). The most obvious lesion was atypical proliferation of pancreatic acinar cells (Fig.39) as described by Hosoda et al. (1976). This was found in 20% of the males and 22% of the females and appeared not to be age-related. Hosoda et al. (1976) found the same percentage in males, but one twice as high in females. Depending on the degree of pleomorphism, the cells retained an acinar arrangement or lost this architecture. The cells were usually polygonal with a central acidophilic granular area and a peripheral basophilic fibrillar zone. The eccentrically located nucleus was enlarged and hyperchromatic. The most atypical lesions consisted of large bizarre cells with one or more nuclei and polychromatophilic cytoplasm. Two hyperplastic acinar nodules adjacent to each other were observed in a 28-month-old male. In this case the acinar cells resembled normal ones but were increased in number. Slight compression of the surrounding parenchyma had occurred. Additionally, atrophy of acinar cells was seen in a 28-month-old male

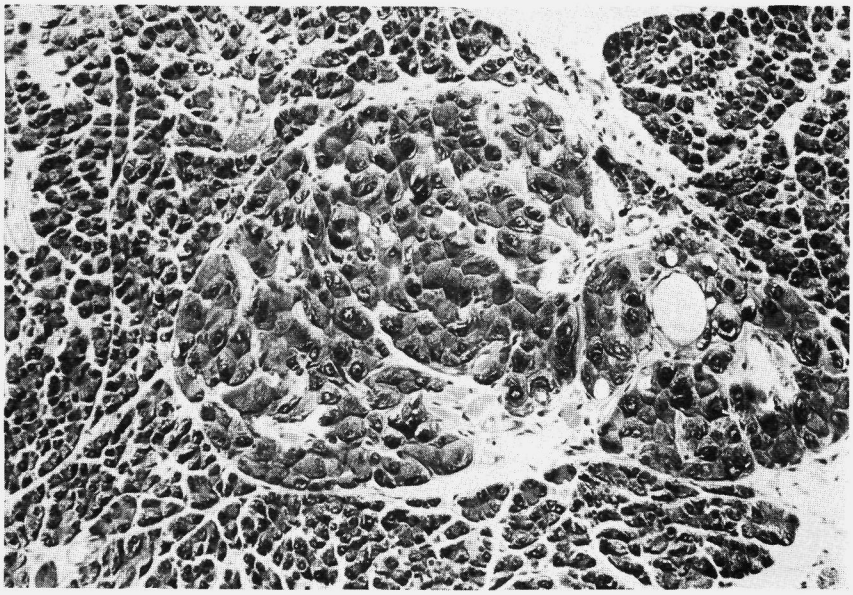

Fig. 39 Focus of atypical nodular proliferation of pancreatic acinar cells. Male, 22 months. HPS, x 140.

and a 27-month-old female and ductular hyperplasia was observed in a 20-month-old male and a 24-month-old female. Acinar cell tumors were not found, although Hosoda et al. (1976) reported such tumors in 19% of animals older than two years. Infiltrative growth of an osteosarcoma arising from the sternum or ribs was observed in a 28-month-old male.

3.6 Endocrine system

3.6.1 Pituitary gland

The intermediate lobe of the pituitary gland is quite prominent in Mastomys. It is larger than that of the mouse, in which this lobe is known to be larger than of most other animals (Liebelt, 1979).

Pituitary gland lesions were not common in Mastomys, as can be seen in Table XXXVII.

Besides the presence of sinusoidal dilatation in the anterior lobe of a male and a female, the other changes were proliferative in character. The proliferative lesions were found in the anterior and intermediate lobes and consisted of hyperplasia (4%), adenoma (9%) and carcinoma (1%). As a criterion for separating hyperplasia from adenoma, compression of the adjacent tissue was used. If compression was observed, the diagnosis adenoma was made. The diagnosis carcinoma of the

TABLE XXXVII

PITUITARY GLAND LESIONS IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=35)				Females (n=65)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	11	13	10	1	6	29	24	6
Anterior lobe								
Sinusoidal dilatation	1(9)*	-	-	-	1(17)	-	-	-
Hyperplasia	-	-	-	-	-	1(3)	2(8)	-
Adenoma	2(18)	1(8)	1(10)	-	-	-	3(13)	-
Carcinoma	-	-	-	-	-	1(3)	-	-
Intermediate lobe								
Hyperplasia	-	-	-	-	-	1(3)	-	-
Adenoma	-	-	-	-	-	-	2(8)	-

* number of animals with lesion (percentage)

anterior pituitary gland was made only in a 29-month-old female and was based on ingrowth into the pars nervosa, cellular and nuclear pleomorphism and the presence of mitoses. Mitoses were not observed in the adenomas. The separation between lesions of the anterior and intermediate lobes was based on the anatomical localization and on the growth pattern. No special stains or techniques were applied to differentiate between cells of the two lobes. Grossly, the adenomas of the anterior pituitary gland were large, varying from 3 to 8 mm, and were pale reddish-brown and very friable. Three of the largest tumors caused compression of the brain. Microscopically, they were circumscribed nodules or masses consisting of round to polyhedral cells with scanty pale staining cytoplasm or abundant acidophilic cytoplasm. The nuclei were densely stained or vesicular. The hyperplastic and adenomatous changes in the pars intermedia were microscopic lesions. The cells also had pale staining acidophilic cytoplasm instead of the pale staining agranular basophilic cytoplasm of the normal cells and a densely stained or vesicular nucleus. The growth pattern was nodular and a whorled arrangement of cells was evident.

Soga et al. (1969a) and Fujii & Sato (1972) found adenomas of the anterior pituitary gland in 2 to 3% of *Mastomys* examined, which is three to four times lower than found in our series.

3.6.2 Thyroid gland

Only a few changes were found in the thyroid glands, the major ones of which are shown in Table XXXVIII.

TABLE XXXVIII

THYROID GLAND LESIONS IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=57)				Females (n=77)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	18	25	13	1	10	32	28	7
Lymphoplasma-cellular thyroiditis	-	3(12)*	2(15)	1(100)	2(20)	8(25)	6(21)	2(29)
Follicular cell hyperplasia	-	2(8)	1(8)	-	-	2(6)	1(4)	-

* number of animals with lesion (percentage)

The most obvious lesion encountered in the thyroid gland was lymphoplasma-cellular thyroiditis (Fig.40a,b). It was more common in females than in males, with incidences of 23% and 11%, respectively. It was characterized by a lymphoplasma-cellular infiltrate in either a diffuse or nodular pattern and was often associated with destruction of follicles. Some lesions were focal and limited to several adjacent follicles, while others were extensive and involved the entire gland. The severity of the cellular infiltrate was graded according to a scoring schedule previously established by Kite et al. (1969), where 1+ corresponds to involvement of up to 25% of the total histologic cross-sections, 2+ to 25%-50%, 3+ to 50%-75% and 4+ to 75%-100% involvement of the histologic cross-sections. The scoring and number of positive animals per age group are given in Table XXXIX. In addition to a lympho-plasmacellular infiltrate, aggregations of cells of a generalized lymphoreticular tumor (malignant lymphoma, immunoblastic (1x) and heterogeneous (2x) types) were present in the thyroid glands of 3 females. These aggregations were included in the scoring of the lesions, since the two infiltrations were intermingled.

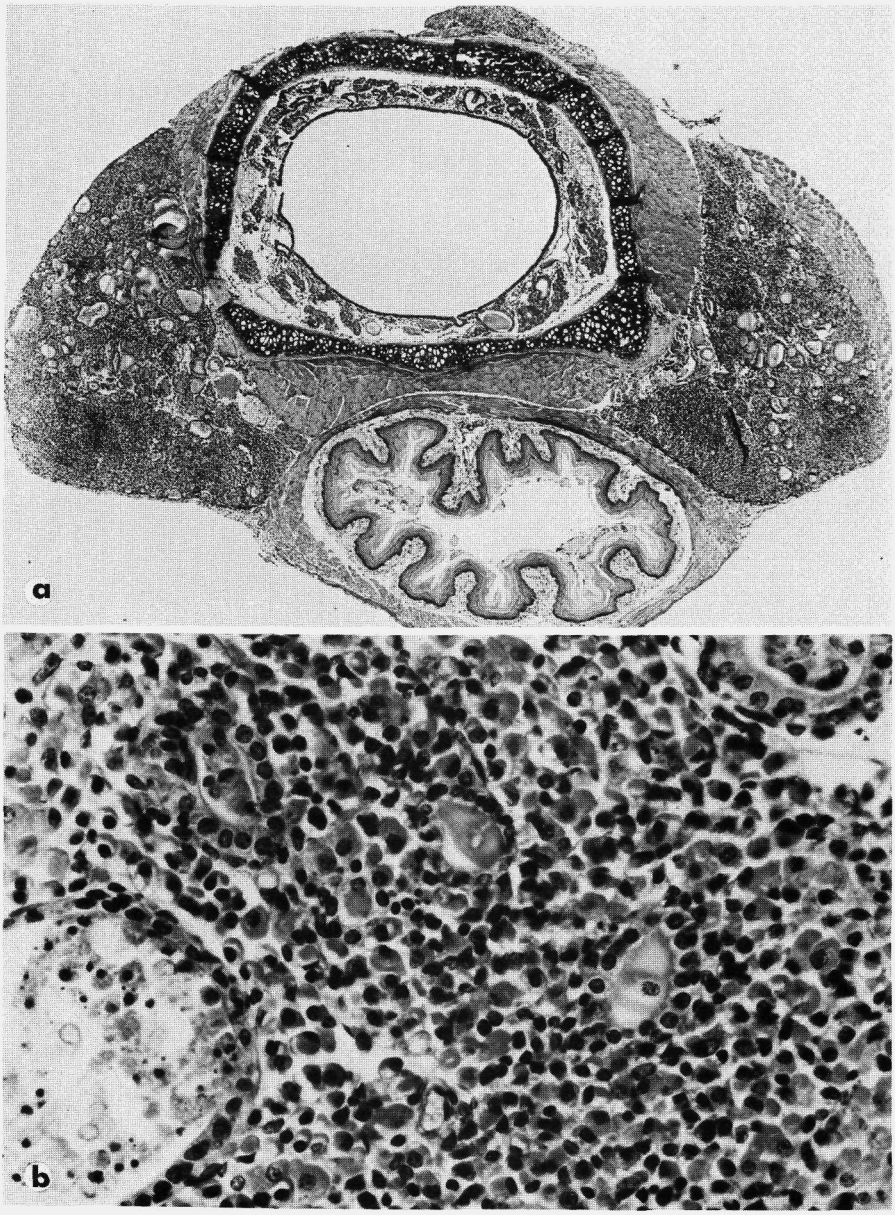

Fig. 40 a. Diffuse plasmacellular infiltration of the thyroid gland of a 27-month-old female. HPS, x 27
b. Higher magnification showing destruction of thyroid follicles and massive infiltration of plasma cells. HPS, x 330

TABLE XXXIX

SEVERITY OF LYMPHOPLASMACELLULAR THYROIDITIS GRADED PER AGE GROUP IN MALE AND FEMALE Mastomys

Age groups (months)	Males (n=6) Severity 1+	2+	3+	4+	Females (n=18) Severity 1+	2+	3+	4+
18-23	-	-	-	-	-	2	-	-
24-29	3	-	-	-	4	1	1	2
30-35	-	1	1	-	1	2	2	1
36-41	-	1	-	-	2	-	-	-

Neither a peak incidence nor an increase in severity with age was found, as shown in Tables XXXVIII and XXXIX. Lymphoid follicles with germinal centers such as can be found in Hashimoto's disease in man (Volpé, 1978), dogs (Gosselin et al., 1978) and in the obese strain (OS) of chickens (Wick et al., 1974) were not observed in Mastomys. Fibrosis as present in variable amounts in humans with Hashimoto's thyroiditis (Volpé, 1978) was seen focally in one female Mastomys with thyroiditis and never in animals not showing the lesion.

The only other significant lesion was mild papillary hyperplasia of the thyroid follicular epithelium in three males and three females. Cystic follicles were common in the aged Mastomys but were not tabulated.

Only one neoplastic lesion of the thyroid gland has been mentioned in the literature; this was an adenocarcinoma (Oettlé, 1955).

3.6.3 Parathyroid gland

Parathyroid hyperplasia and adenoma were the only lesions of this gland encountered in Mastomys.

Normal parathyroid glands (Fig.41a) of Mastomys consist of cords and nests of uniform chief cells possessing small, compact, mostly elongated hyperchromatic nuclei and a poorly defined faintly acidophilic cytoplasm. An inconspicious supportive stroma is present between the cell cords. Unlike in the mouse (Dunn, 1979a) and the rat (Lindsay & Nichols, 1969), intracellular vacuoles were observed in parathyroid chief cells of aged Mastomys.

In our series of 145 Mastomys, parathyroid glands from 84 animals were evaluated histologically. Seventeen of these glands showed lesions, ten of which were diagnosed as hyperplasia and seven as adenoma. In two animals, a

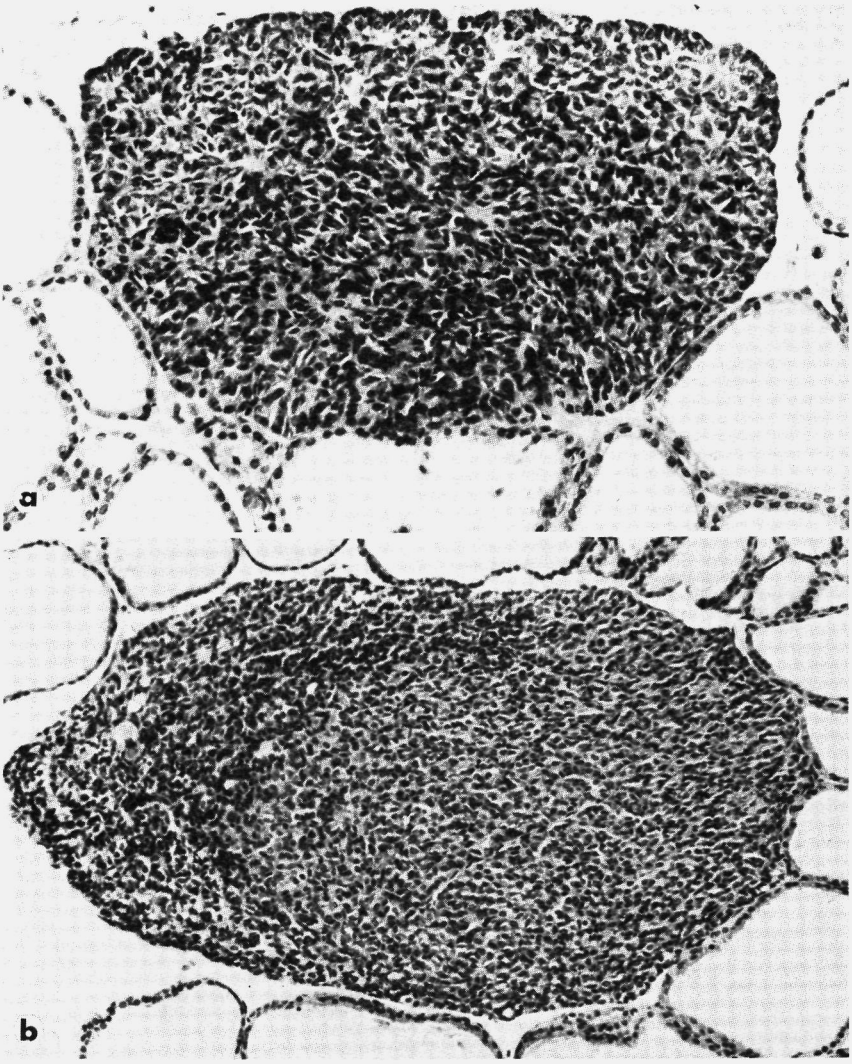

Fig. 41 a. Normal parathyroid gland of a 6-month-old female. HPS, x 190
b. Hyperplastic parathyroid gland of a 28-month-old male. Compression of normal parathyroid cells (left) is not apparent. HPS, x 140

bilateral parathyroid lesion was found. One showed hyperplasia in one gland and an adenoma in the opposite one; the other showed bilateral hyperplasia. The incidence of hyperplasia and adenoma was higher in males than in females. Both lesions were found mainly in animals of between 24 and 35 months of age (Table XL).

TABLE XL

PARATHYROID LESIONS* IN AGED MALE AND FEMALE *MASTOMYS*

	Males (n=38)				Females (n=46)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	13	16	8	1	5	15	22	4
Hyperplasia	2(15)**	1(6)	2(25)	-	-	1(7)	3(14)	-
Adenoma	-	2(13)	3(38)	-	-	1(7)	2(9)	-

* bilateral lesions are tabulated only once and the most severe one is listed.
** number of animals with lesion (percentage)

Of the ten hyperplastic glands, three were enlarged. One caused compression of the adjacent thyroid tissue and the others bulged into the surrounding loose areolar connective tissue. The other seven glands were not significantly enlarged (Fig.41b). In the eight cases of adenoma, all glands were larger than normal and were generally two or three times normal size. Parathyroid hyperplasia was either diffuse or focal and adenoma involved a portion of the gland or the entire gland. In the cases of focal hyperplasia, compression of adjacent parenchyma was not prominent, while it was in the cases of focal adenoma. Generally, the hyperplastic and adenomatous chief cells were slightly enlarged and showed an acidophilic, finely granular cytoplasm and less densely stained nuclei than normal. In one hyperplastic and one adenomatous parathyroid gland, acinar structures were an obvious feature (Fig.42). The acini were filled with a pink-staining material that simulated the colloid of thyroid tissue. These acinar structures were also occasionally seen in normal parathyroid glands of aged *Mastomys*. In two cases of parathyroid gland adenoma, multiple nodules consisting of serpentine cords were present. The cells were clearly enlarged and characterized by acidophilic cytoplasm and vesicular nuclei. Pleomorphic cells and multinucleated cells as well as mitoses were present in one of these two cases. Cystic changes were found in a hyperplastic and an adenomatous gland (Fig.43) and three hyperplastic glands were composed of spindle-shaped cells with elongated nuclei.

No conclusion could be drawn as to whether or not bilateral parathyroid gland lesions were common. Only in five cases were both glands present in the tissue section. Of these five cases, two bilateral lesions were seen, while no lesions were found in the three other cases. Whether the parathyroid lesions could be related to other pathologic changes, particularly renal lesions and fibrous osteodystrophy, has been discussed in this chapter under Musculoskeletal system.

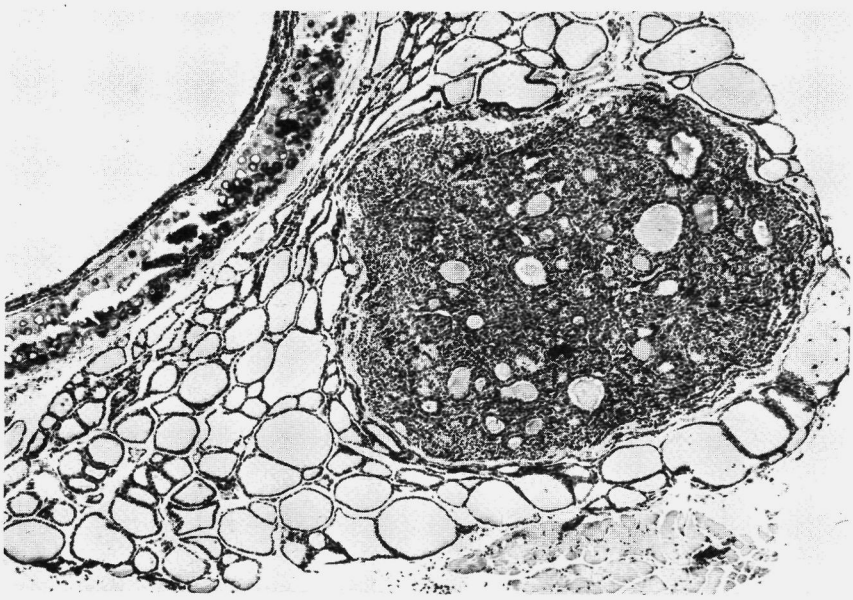

Fig. 42 Parathyroid adenoma causing compression of the adjacent thyroid tissue. Some tumor cells form acinar structures which contain colloid-like material. Female, 24 months. HPS, x 54

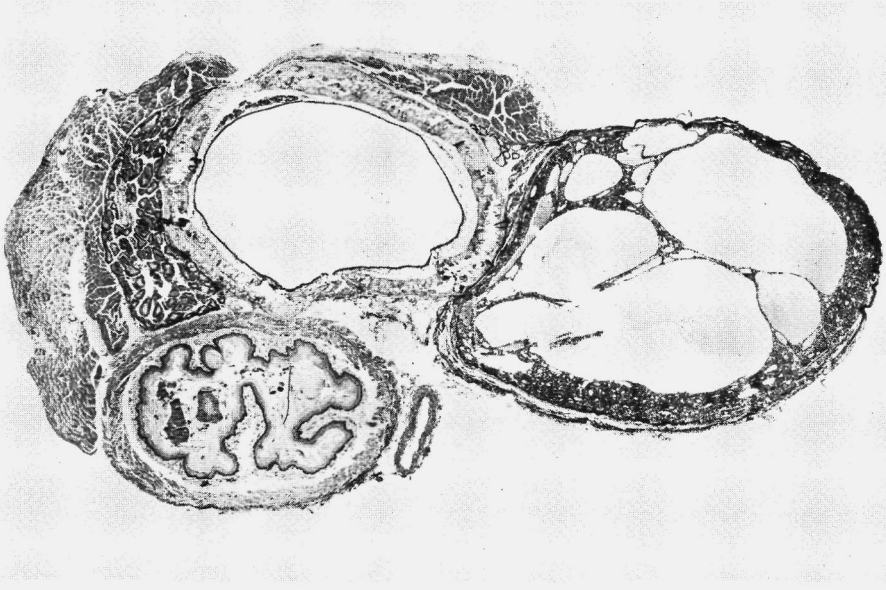

Fig. 43 Cystic parathyroid adenoma in a 32-month-old female. HPS, x 21

Two aberrant parathyroid glands were found. They were embedded in the thymic tissue and were fully developed. This localization is not so surprising, since the parathyroid glands arise from the cephalic portions of the third and fourth pharyngeal pouches and the thymus from the caudal portions of these pouches (Russfield, 1967).

3.6.4 Adrenal gland

3.6.4.1 Adrenal cortex

The adrenal cortex of male and female Mastomys is characterized by its prominent zona reticularis. The zone was sometimes so marked in the aged animals that the diagnosis hyperplasia was occasionally considered. In aged females, the cells of the zona reticularis often showed hydropic degeneration and "brown degeneration", also referred to as ceroid cell degeneration or ceroidogenesis. This change is characterized by a collection of distinctive brown pigmented cells of uncertain origin (Dunn, 1979b). The pigmented cells often encircled the medulla and extended outward between cells of the zona fasciculata. Only a few pigment-laden cells were observed in aged males. The difference between the sexes was so evident that one could easily predict the sex of the animals on the basis of the quantity of pigment-laden cells present.

Extramedullary hemopoiesis, foci of hypertrophic adrenocortical cells and cysts were occasionally observed in the cortex. In a 31-month-old female, one adrenal gland was completely necrotic and the opposite one partly so. Both glands were surrounded by tumor cells from a histiocytic sarcoma which was found widespread throughout the abdominal cavity.

In contrast to young Mastomys, aged animals showed well-defined pericapsular adrenal nodules in the majority of cases. These nodules consisted of normal appearing cortical cells of the zona glomerulosa and zona fasciculata. Only in two males and three females ranging in age from 25 to 33 months was the diagnosis hyperplasia made on the basis of these nodules. The diagnosis was based on one or more of the following criteria: poorly circumscribed boundaries, cellular and nuclear pleomorphism and mitoses.

Neoplastic lesions of the cortex were observed in 39 of the 144 animals examined (27%). Thirty-three (52%) tumors were found in males and eight (10%) in females. Two of the males had bilateral tumors. The tumor incidence is much higher than reported by others. Soga et al. (1969a) found an incidence of 10.9%, Hollander & Higginson (1971) 6.7%, Sato et al. (1974) 13.2% and Soga & Tazawa (1977) 11.8%.

A difficulty was to differentiate between adenoma and carcinoma, as was also encountered in rats (Hollander & Snell, 1976) and in mice (Dunn, 1970, 1979b). Dunn (1970, 1979b) stated that, in the mouse, there is a progression from hyperplasia to adenoma to carcinoma with time. Nevertheless, probably somewhat

arbitrarily, we made a separation between adenoma and carcinoma based on size, as was suggested by Dunn (1970, 1979b). Large tumors extending outside the capsule were diagnosed as carcinomas. Mitoses and arrangement of the tumor cells could not be used as criteria because no differences were observed between small and large tumors with regard to those criteria. All tumors showed a trabecular pattern, uniformly appearing cells, evenly-sized nuclei and mitoses. The larger ones contained foci of necrosis and hemorrhage and often cystic spaces filled with blood or fibrin. One of the larger tumors found in a 19-month-old male had metastasized to the lungs. It could not be distinguished histologically from the other tumors.

To ascertain whether there was an age-associated incidence of adenomas and carcinomas, the incidence of these lesions per age group was calculated. These data are presented in Table XLI.

TABLE XLI

INCIDENCE PER AGE GROUP OF ADRENOCORTICAL TUMORS* IN MALE AND FEMALE *MASTOMYS*

	Males (n=60)				Females (n=84)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	18	27	14	1	11	36	30	7
Adenoma	1(6)**	15(56)	3(21)	-	1(9)	1(3)	3(10)	1(14)
Carcinoma	2(11)	4(15)	6(43)	-	-	-	2(7)	-

* bilateral tumors are tabulated only once and the most severe one is listed.
** number of animals with lesion (percentage)

In males, the adenomas were found predominantly in the age group of 24-29 months and the carcinomas in the 30-35 month age group. This may be regarded as support for the statement made by Dunn (1970, 1979b) that there is a progression from hyperplasia to carcinoma with time. The numbers in females were too small to justify such a conclusion. A 28-month-old male had bilateral adenomas and a 34-month-old male had a carcinoma in one gland and an adenoma in the opposite one.

Hollander & Higginson (1971), Tazawa et al. (1971) and Soga & Tazawa (1977) studied the adrenocortical tumors in *Mastomys* histochemically and electron microscopically. They concluded from these studies that the tumors arose from the zona glomerulosa. The observation of Hollander & Higginson (1971) that several

Mastomys bearing a large adrenocortical tumor showed extreme dyspnea suggested that some of these tumors, at least, produce aldosterone.

Additionally, metastases of a pleomorphic sarcoma arising in the hindleg were found in the adrenal cortex of a 24-month-old male.

3.6.4.2 Adrenal medulla

Only a few proliferative changes were observed in the adrenal medulla. In two females (33 and 35 months of age), medullary hyperplasia was seen. In one of these, a diffuse increase in normal appearing medullary cells was noted; in the other, a small nodule adjacent to the normal medulla was present. A pheochromocytoma was seen in a 30-month-old male and in a female Mastomys of the same age. Microscopically, the cells were smaller than, but still resembled, normal medullary cells and were arranged in nests filling up the major part of the adrenal gland. The male also had a cortical adenoma. A ganglioneuroma-pheochromocytoma, as described by Reznik et al. (1980) in rats, was found in a 23-month-old male Mastomys (Fig.44). The neoplasm was composed of typical large and small ganglion cells with large, pale eccentrically located nuclei and neoplastic pheochromocytes. Supporting cells, including Schwann cells and capsule or satellite cells, were not seen.

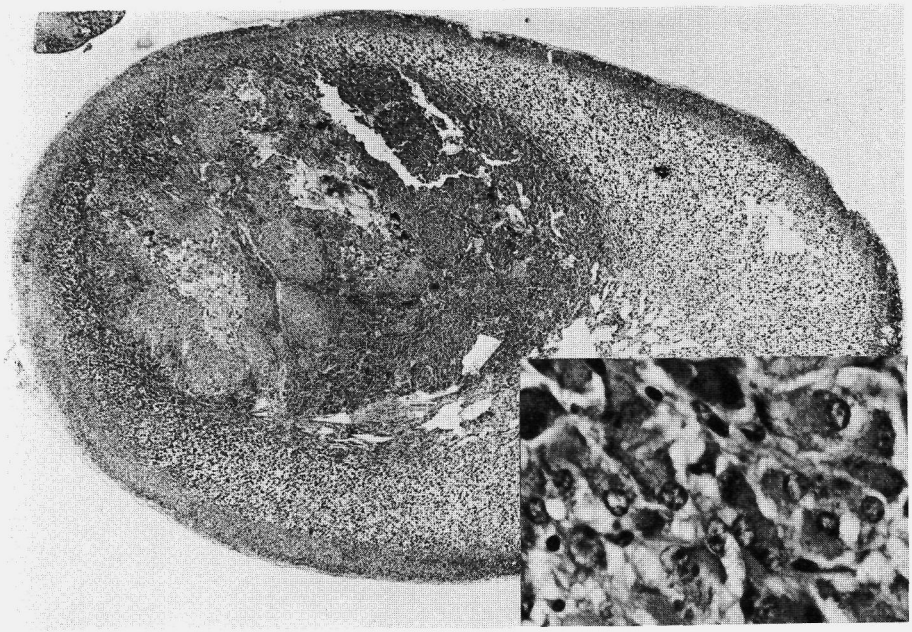

Fig. 44 Ganglioneuroma-pheochromocytoma arising in the adrenal medulla of a 23-month-old male. HPS, x 16. Inset: Higher magnification showing large ganglion-like cells with eccentrically located vesicular nuclei. HPS, x 330

3.6.5 Pancreatic islets

Islets of Langerhans varied greatly in size in individual Mastomys. When such a wide range in size exists, it is difficult to distinguish hyperplasia from normal large islets, since it is known that, e.g., in the mouse (Cardesa et al., 1979), hyperplastic and even adenomatous islets have a structure similar to that of normal ones. Despite this difficulty, the diagnosis hyperplasia was made in three males and one female ranging in age from 21 to 31 months. The diagnosis in the three males was based on the large size of the affected islets and on the irregular zone of contact with adjacent acinar parenchyma. The cells were relatively uniform in size and appearance and were arranged in the normal "ribbon and festoon" pattern. Dilated vascular channels filled with blood were sometimes present in hyperplastic islets. A small nodule consisting of pleomorphic islet cells was present in a normal islet of a 31-month-old female. The nodule slightly compressed the adjacent normal islet cells and was diagnosed as nodular hyperplasia.

Islet cell adenomas were found in one male and four females, all older than 29 months, and a carcinoma was seen in a 23-month-old male. All of these tumors had the same structure and appeared as solitary lesions. They consisted of nests of large polyhedral cells with centrally located nuclei and faintly acidophilic cytoplasm. The nests of cells were separated by thin connective tissue trabeculae. Dilated vascular channels were prominent. One of the adenomas had a central large, blood-filled cavitation which compressed the surrounding adenoma cells.

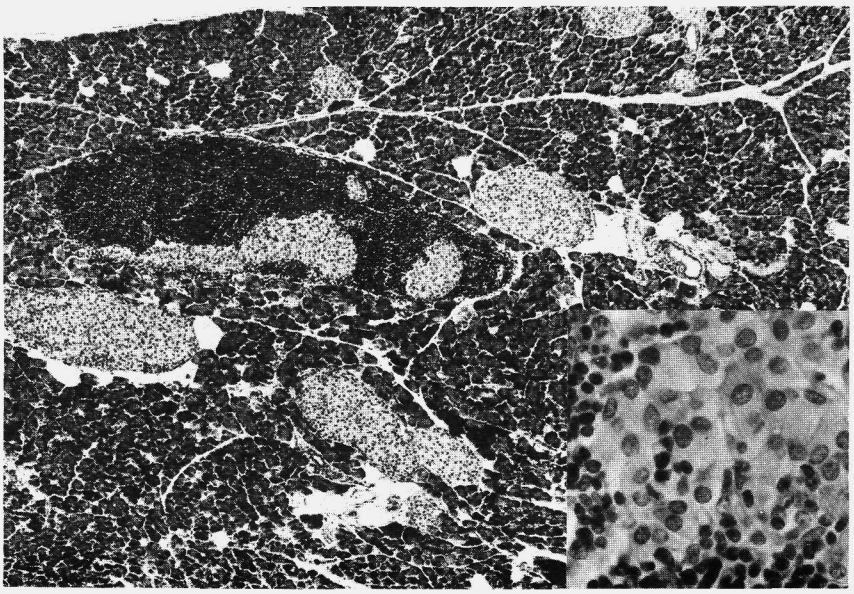

Fig. 45 Lymphocytic infiltration of pancreatic islet of a 30-month-old female. HPS, x 54. Inset: Higher magnification of pancreatic islet cells infiltrated by lymphocytes. HPS, x 330.

All adenomas were encapsulated but ingrowth into the capsule was observed, as were tumor cells outside the capsule. The diagnosis carcinoma was based on the presence of pleomorphic cells, invasion of the capsule by tumor cells, growth into blood vessels and the presence of tumor cells far beyond the tumor. In none of the cases was an attempt made to classify the component cells of the tumors with special stains, as most stains have a poor selectivity for any particular cell type and especially for tumor cells (Larsson, 1978).

In three of six cases (two adenomas and one carcinoma), lymphocytes were present at the periphery of the tumors; this was also seen around normal islets in three females (29, 34 and 36 months of age). Lymphocytic infiltration of a normal islet (Fig.45) was observed in three females (30, 35 and 38 months of age).

3.7 Lymphoreticular system

According to Carr et al. (1977), the lymphoreticular tissue comprises the bone marrow in its nonhemopoietic function, the spleen, the lymph nodes, Peyer's patches, the thymus and scattered lymphocytes and histiocytes elsewhere. In this section, the bone marrow, the spleen and lymph nodes and the thymus will be discussed separately and generalized lymphoreticular lesions will be dealt with under spleen and lymph nodes. The nonneoplastic and neoplastic lesions of the lymphoreticular system are listed in Table XLII.

3.7.1 Bone Marrow

Lesions of the bone marrow in its nonhemopoietic function were generalized and are discussed under spleen and lymph nodes. Only two other lesions which affected the hemopoietic compartment of the bone marrow were encountered. Necrosis of bone marrow cells in the epiphysis was found in a 30-month-old female, which also had severe degenerative changes of the femorotibial joint; multiple small foci of proliferating myelocytic and monocytic cells were observed in the bone marrow of the sternebrae of a 24-month-old male. This proliferative lesion was not seen elsewhere.

3.7.2 Spleen and lymph nodes

Obvious in the aged Mastomys was the large proportion of plasma cells, including cells with Russell's bodies in the spleen, lymph nodes and Peyer's patches. According to Snell & Stewart (1969b), plasma cells are observed in these organs in Mastomys before one year of age and they gradually increase in number with age. In aged animals, lymphoplasmacellular accumulations were frequently found in the interstitium of the accessory sex glands without being associated with cell destruction. Another striking finding was the presence of nodular aggregations of lymphoreticular cells in the wall of the urinary bladder and in the ovaries.

These aggregations were also seen in young animals (Solleveld, unpublished observations) and were regarded as a normal finding in Mastomys.

Focal hemorrhage in the spleen was seen in two females (22 and 25 months of age). Empty cysts or cysts filled with proteinaceous fluid were observed in lymph nodes of thirteen males and five females. They were most often found in one of the abdominal lymph nodes. Neither an inflammatory reaction nor fibrosis was seen around the cysts, so that they were regarded as representing dilated lymph sinuses. Mesenteric disease as described in mice by Dunn (1954) was observed in a 28-month-old female. Lymphadenitis was diagnosed in two 27-month-old males and in five females varying in age from 23 to 32 months. One male and four females had an acute lymphadenitis. One of the females had ulcerative dermatitis in the region drained by the lymph node which could explain the lymph node inflammation. However, no cause was found in the other four animals. One male with ulcerative dermatitis showed granulomatous lymphadenitis which was a reaction to bedding material present in that lymph node and one female showed chronic lymphadenitis which was associated with the existence of chronic peritonitis. Of all the lesions discussed above, only the cystic changes seemed to be age-related.

The proliferative lesions were classified as hyperplasia, atypical hyperplasia and neoplasia. The latter included different types of malignant lymphomas, granulocytic leukemia and histiocytic sarcoma. In cases of hyperplasia and atypical hyperplasia, the white pulp of the spleen and/or at least one of the lymph nodes were increased in size, although the normal architecture of these organs was still recognizable. In hyperplasia, either cortical hyperplasia with germinal center formation or paracortical hyperplasia was seen. The diagnosis atypical hyperplasia was made when the white pulp of the spleen and/or the lymph nodes was populated either by immunoblasts with or without plasmacytoid differentation or by a mixture of cells, including lymphoblasts, small and large lymphocytes, plasma cells, granulocytes and histiocytes. Atypical hyperplasia was regarded as a precursor of malignant lymphoma of immunoblastic or heterogeneous type (see below). Hyperplasia in spleen and lymph nodes was often associated with hyperplasia of Peyer's patches and with an increase in lymphoreticular cells in the urinary bladder, ovaries, periportal areas of the liver and bronchus associated lymphoid tissue. Lymphoreticular cell accumulations were also sometimes seen in the peripelvic area of the kidney and beneath the endocardium and epicardium. Hyperplasia was observed in 12% of the males and in 16% of the females and atypical hyperplasia in 3% of the males and 9% of the females. In the cases of neoplasia, the original architecture of the spleen and the lymph nodes was generally completely lost and these organs were markedly increased in size.

In a 29-month-old male, a diffuse malignant lymphoma of poorly differentiated lymphocytic type was diagnosed. The malignant cells in this tumor, in contrast to the residual small lymphocytes, possessed large pleomorphic nuclei, large nucleoli and more abundant cytoplasm. Neoplastic tissue involved the spleen, the abdominal

TABLE XLII

NONNEOPLASTIC AND NEOPLASTIC LESIONS OF THE LYMPHORETICULAR SYSTEM IN AGED MALE AND FEMALE MASTOMYS

	Males (n=60)				Females (n=85)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	27	14	1	11	36	31	7
Spleen								
Hemorrhage	-	-	-	-	1(9)*	1(3)	-	-
Lymph node								
Cysts	2(11)	7(26)	4(29)	-	-	2(6)	3(10)	-
Mesenteric disease	-	-	-	-	-	1(3)	-	-
Lymphadenitis	-	2(7)	-	-	1(9)	3(8)	1(3)	-
Spleen and lymph nodes including generalized lesions								
Lymphoid hyperplasia	2(11)	3(11)	2(14)	-	2(18)	4(11)	7(23)	-
Atypical lymphoid hyperplasia	1(6)	1(4)	-	-	-	3(8)	3(10)	2(29)
Malignant lymphoma:								
lymphocytic poorly differentiated type	-	1(4)	-	-	-	-	-	-
immunoblastic type	-	-	-	-	1(9)	3(8)	1(3)	1(14)
heterogeneous type	-	-	1(7)	-	-	3(8)	5(16)	1(14)
Granulocytic leukemia	-	-	-	-	-	-	1(3)	-
Histiocytic sarcoma	-	-	-	-	1(9)	-	2(6)	-
Thymus								
Hyperplasia	3(17)	6(22)	1(7)	-	-	2(6)	5(16)	-
Thymoma	6(33)	9(33)	7(50)	1(100)	4(36)	18(50)	10(32)	3(43)

* number of animals with lesion (percentage)

lymph nodes, the mesentery, the bone marrow, the liver (periportal) and lung (perivascular). Necrosis of neoplastic tissue was an obvious finding.

Malignant lymphoma of immunoblastic type was diagnosed in six females. The tumors (Fig.46) were characterized by a diffuse spread and consisted of rather large cells with a moderate to abundant amount of cytoplasm and bulky round or oval, sometimes lobulated, nuclei with a large central nucleolus. A number of cells showed an eccentrically located nucleus, similar to the position of that in a plasma cell. In most cases, the neoplastic tissue involved the spleen, the lymph nodes, the liver (periportal and diffuse in sinusoids), the ovaries, the urinary bladder, the pancreas, the bone marrow and the lung (perivascular and diffuse in alveolar septa). Obvious was a diffuse infiltration of tumor cells into the intestinal tract in three of the six females.

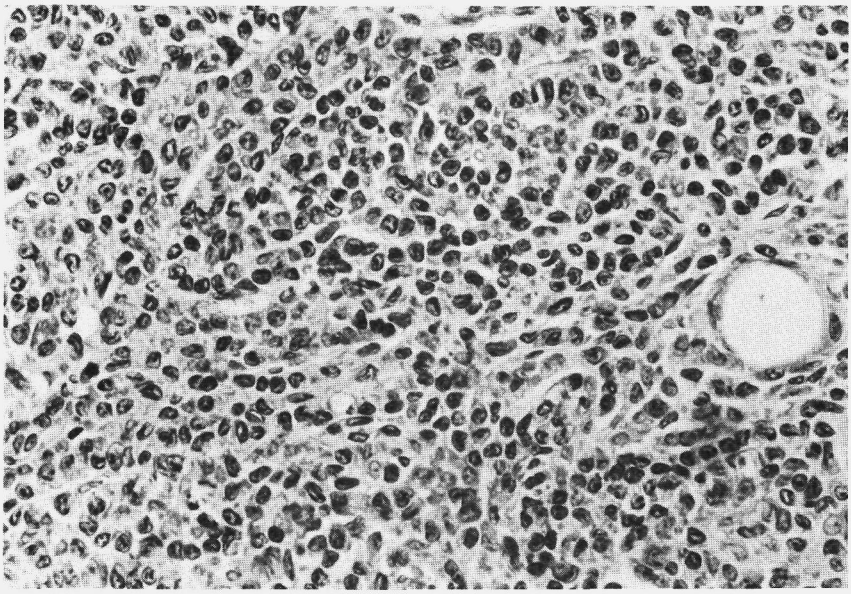

Fig. 46 Malignant lymphoma, immunoblastic type, involving mesenteric lymph node. The cells have a moderate amount of cytoplasm and round to oval nuclei, which are sometimes indented or lobulated. Centrally located nucleoli are prominent. Female, 26 months. HPS, x 330.

Malignant lymphoma of heterogeneous type was seen in one male and nine females. The tumors resembled reticulum cell sarcoma type B (Dunn, 1954), which is also known as composite lymphoma (Della Porta et al., 1979), in mice. Involvement primarily included the spleen, mesenteric and superficial cervical lymph nodes, Peyer's patches, urinary bladder, ovaries and sometimes the liver (periportal), kidney (peripelvic) and the lung (perivascular). The growth pattern was more or less nodular in the spleen (Fig.47) and diffuse in the lymph nodes.

The neoplastic tissue was composed of a mixture of cells, including small and large lymphocytes, histiocytes and granulocytes. For a detailed description of this type of tumor, reference is made to Snell & Stewart (1969b).

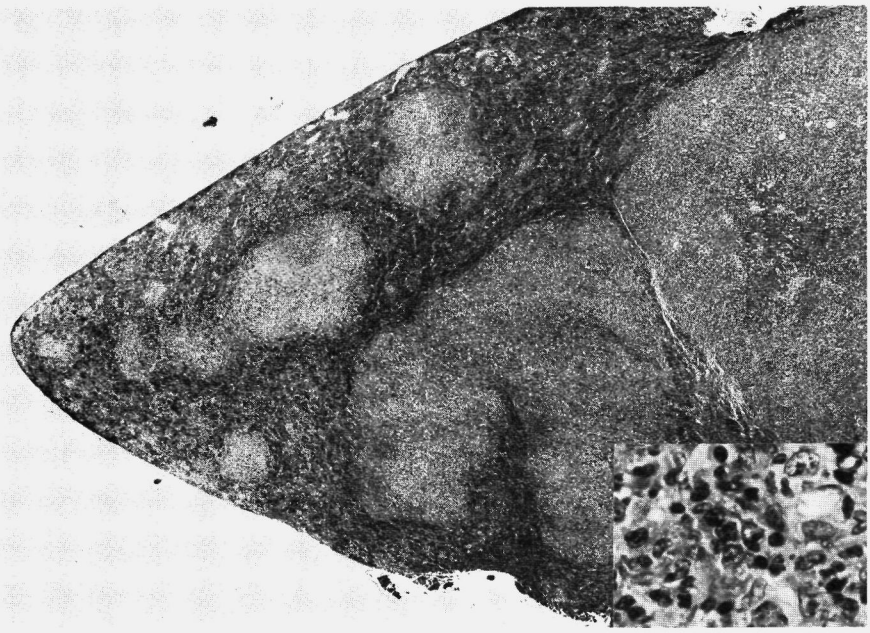

Fig. 47 Nodular growth pattern of malignant lymphoma, heterogeneous type in the spleen of a 33-month-old female. HPS, x 15. Inset: Higher magnification showing the mixture of cell types characteristic of this type of tumor. HPS, x 500

A histiocytic sarcoma was diagnosed in three females. In a 35-month-old female, the tumor was manifested as a large nodule in the caudal pole of the spleen and consisted entirely of large cells with abundant acidophilic cytoplasm and large vesicular nuclei. Erythrophagocytosis was evident. In a 22-month-old female, diffuse infiltration of the spleen, lymph nodes, bone marrow, lung, heart, liver (Fig.48), kidney, uterus and ovaries by neoplastic histiocytes was seen. The monomorphic cells were smaller than in the previous case and were more spindle-shaped and had basophilic oval nuclei. A pleomorphic histiocytic sarcoma was found in a 31-month-old female. The normal cell population of the abdominal lymph nodes was replaced by tumor cells. Neoplastic cells were also present in the spleen, bone marrow, liver and lung and were found to be widespread throughout the abdominal cavity, surrounding all viscera and sometimes invading them.

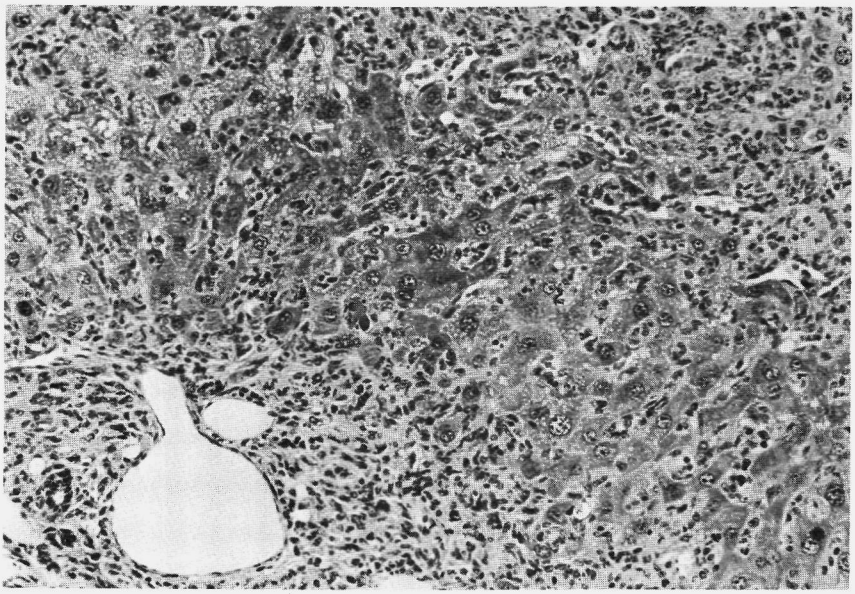

Fig. 48 Histiocytic sarcoma growing diffusely in the liver of a 22-month-old female. HPS, x 140

Granulocytic leukemia was observed in a 30-month-old female. Immature and predominantly mature granulocytes, including eosinophils, were abundant in the lymph nodes and in the red pulp of the spleen. In addition, large accumulations of leukemic cells were seen around portal vessels in the liver, in the peripelvic area of the kidney, in the alveolar septa of the lung, in the adrenal glands, in the pancreas, in the cecum and in the bone marrow. Leukemic cells from the bone marrow had invaded the musculature which surrounded the vertebrae and limbs. Numerous mitoses were present in leukemic cells. This animal had an extensive ulcerative typhlitis, which led to the speculation that the leukemia was possibly the result of a severe leukemoid reaction.

3.7.3 Thymus

In our series of aged Mastomys, we distinguished the following thymic changes: atrophy, hyperplasia and thymoma. Of these, atrophy should be regarded as a normal physiological process in aged animals and man. The incidences of hyperplasia and thymomas per age group are given in Table XLII.

Atrophy of the thymus was characterized by a decreased size, loss of cortical lymphocytes and single or small clusters of epithelial cells diffusely distributed throughout the gland and intermingled with lymphocytes. Occasionally, cysts lined by ciliated cuboidal epithelial cells were seen. A thymus was classified as

hyperplastic when the gland was larger than would be expected for the animal's age but the relationship of medulla to cortex normally seen in the noninvoluted thymus was more or less maintained (Fig.49) or when nodular or diffuse proliferation of epithelial cells was observed in an enlarged gland (Fig.50) without apparent distortion of the normal architecture. As stated by Hadlow (1978), little is understood about thymic hyperplasia and its histological expression in animals and how it is distinguished from, e.g., delayed involution. It cannot be excluded that what was interpreted as hyperplasia in a number of our cases, particularly in those where the relationship of medulla and cortex was more or less retained, was possibly delayed involution. In any case, hyperplasia as interpreted by us must be distinguished from the type of thymic hyperplasia most often encountered in man, which is commonly associated with myasthenia gravis (Levine & Rosai, 1978). In these cases, the thymus is seldom actually enlarged and shows lymphoid follicles with germinal centers.

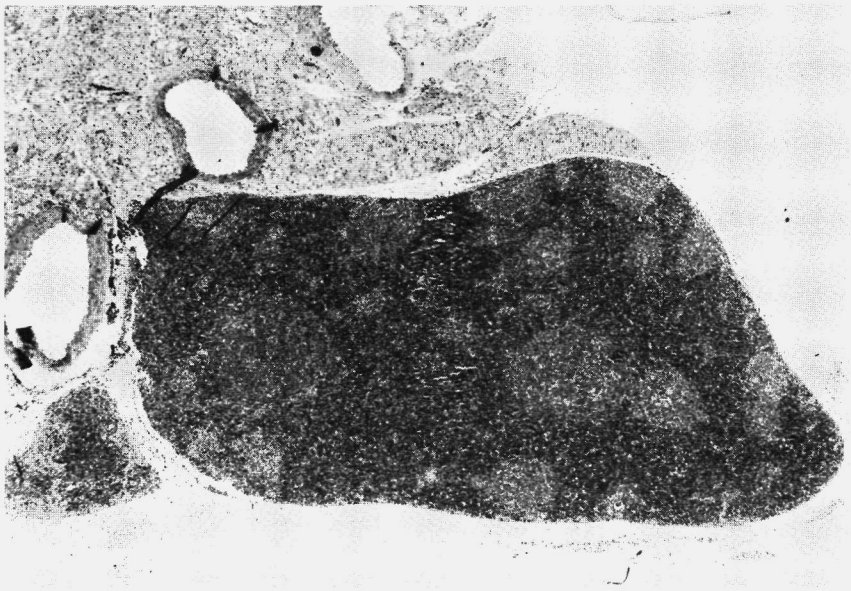

Fig. 49 Hyperplasia of the thymus of a 31-month-old female. The normal architecture is more or less retained. HPS, x 29

Macroscopically, thymomas varied in size but all were clearly enlarged and easy to recognize at necropsy. The tumors were generally firm, well-encapsulated and the surface was usually smooth. The largest tumors caused displacement of the heart and compression of the lungs (Fig.51a), which was the major cause of

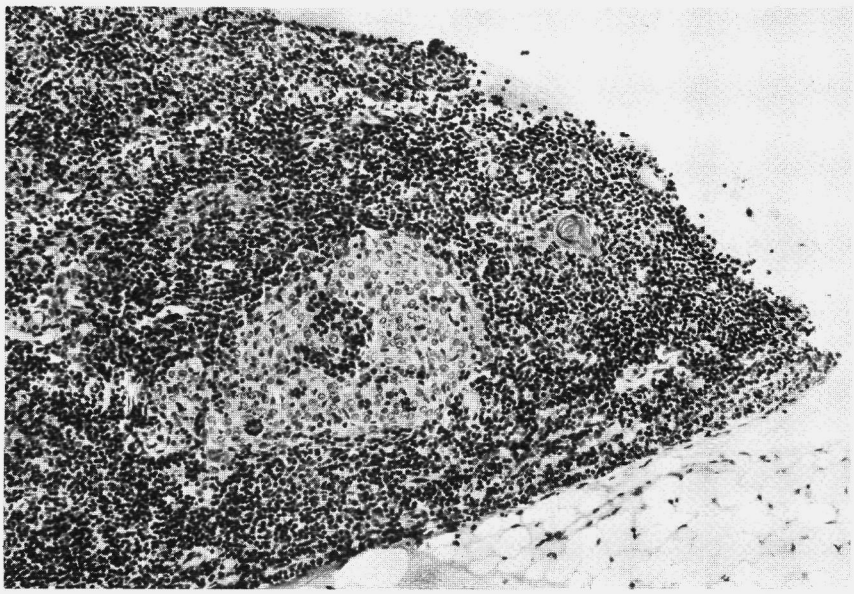

Fig. 50 Focal nodular epithelial cell hyperplasia in the thymus of a 23-month-old male. HPS, x 140

death in these animals. Microscopically, all thymomas showed distortion of the normal architectural pattern. Lymphocytes and epithelial cells appeared in abnormal proportions and configurations (Fig.51b,c,d) (Stewart & Snell, 1968). Cystic changes and necrotic areas were commonly present. Infiltrative growth outside the capsule and extending into the mediastinal fat was present in several instances. For a more detailed description of thymic lesions in Mastomys, see the reports of Kurokawa et al. (1968) and Stewart & Snell (1968).

Thymomas (40%) were more common than hyperplasia (12%). In contrast to hyperplasia, thymomas were found with approximately equal frequency in females (41%) and in males (38%). A relation with age was not apparent for either lesion. The incidence of thymomas found here is much higher than that observed by Kurokawa et al. (1968), who reported an overall incidence (males and females) of 8% and by Stewart & Snell (1968), who noted an overall incidence of 24%. A greater frequency in females was observed by them. The ages of the animals in both studies were comparable with those in ours.

A number of lesions encountered in our animals, such as lymphocytic and lymphoplasmacellular infiltrations in the salivary glands, thyroid glands, islets of Langerhans, skin and adnexa, skeletal muscle and cardiac muscle, is suggestive for an autoimmune disorder. This and the frequent occurrence of thymomas made it of interest to determine the relationships between thymomas and these lesions. It

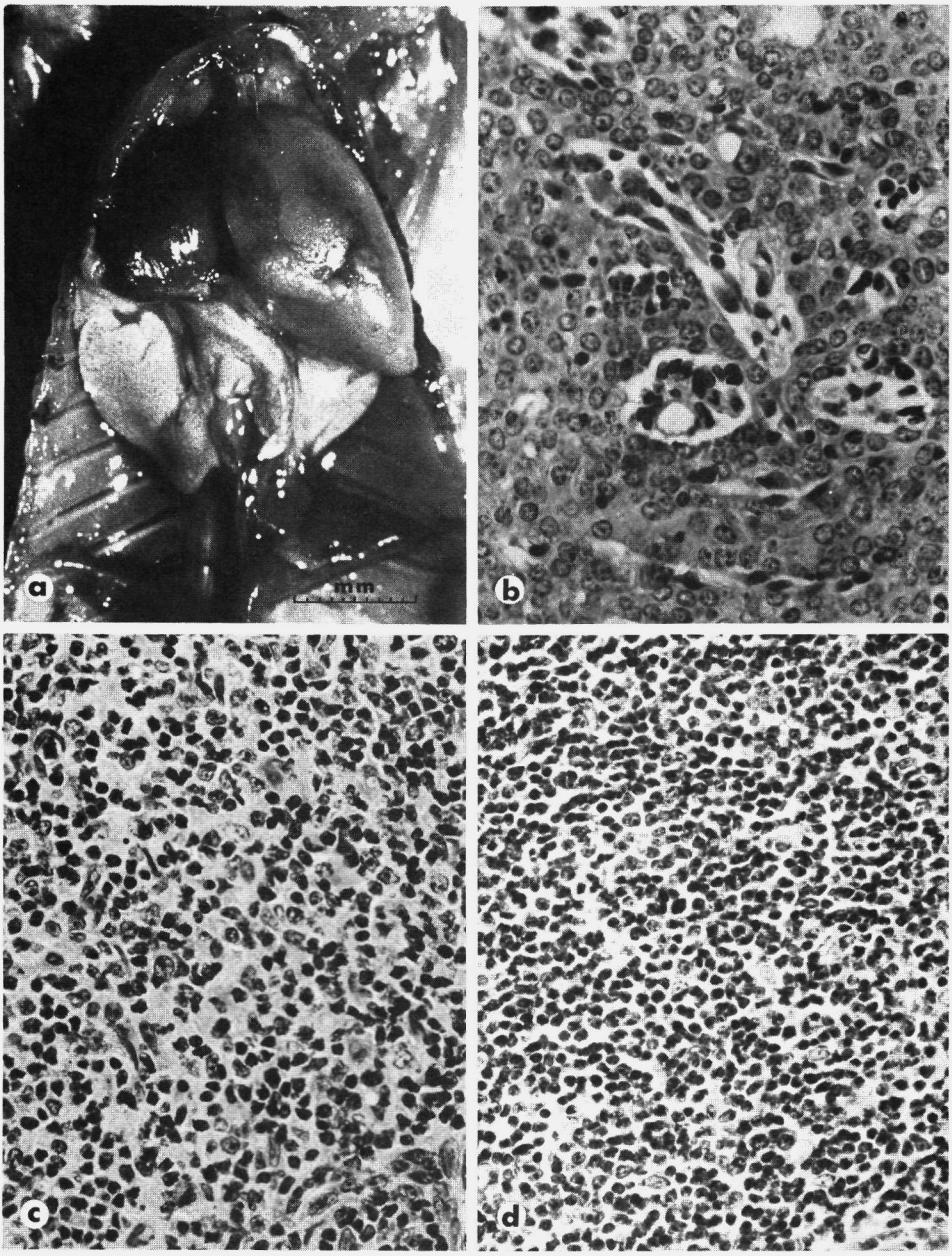

Fig. 51 a. Large thymoma in a 30-month-old male causing dextroposition of the heart and compression of the lungs.
b. Thymoma, predominantly epithelial. HPS, x 330
c. Thymoma, mixed epithelial and lymphocytic. HPS, x 330
d. Thymoma, predominantly lymphocytic. HPS, x 330

appeared that myositis ($p < 0.05$) and myocarditis ($p < 0.05$) in each sex were correlated with the presence of thymomas, as was thyroiditis in males ($p < 0.05$). The other lesions were not correlated with the presence of thymomas. Also no correlations existed between the occurrence of different types of malignant lymphoma and thymomas.

3.8 Urinary system

3.8.1 Kidney

All male and female Mastomys had glomerulonephropathy which varied only in extent and severity from animal to animal. The severity of the glomerulonephropathy was graded according to a modified grading system used by Zurcher et al. (1980) (Table XLIII).

TABLE XLIII

GRADING SYSTEM USED FOR SCORING THE SEVERITY OF GLOMERULONEPHROPATHY IN MASTOMYS

Severity	Glomeruli*	Tubules**	Interstitium***
1	mild	-	-
2	moderate	mild	mild
3	severe	moderate/severe	moderate/severe

* Glomerular lesions : increase in mesangial matrix, thickening of capillary loops, increased cellularity, glomerulosclerosis
** Tubular lesions : tubular dilatation, tubular atrophy and protein casts
*** Interstitial lesions : cellular infiltrate, fibrosis

In addition to the glomerulonephropathy, other types of renal disease and renal changes were found. The types and incidences of lesions are tabulated in Table XLIV.

From Table XLIV, it appears that severe (grade 3) glomerulonephropathy occurred earlier in life in males than in females and was already present in a high percentage in males in the age group of 18-23 months, while in females a high percentage was found from the age of 30 months onward. The animals developed one or more of three forms of glomerulonephritis: membranous, proliferative and chronic, as described in detail by Snell & Stewart (1967). The membranous and chronic forms predominated in our series. Grossly, most kidneys were light brown

TABLE XLIV

RENAL LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=60)				Females (n=84)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	27	14	1	9	36	31	8
Glomerulo-nephropathy								
grade 1	1(6)*	-	-	-	6(67)	13(36)	2(6)	1(13)
grade 2	7(39)	8(30)	6(43)	-	3(33)	17(47)	17(55)	1(13)
grade 3	10(56)	19(70)	8(57)	1(100)	1(11)	6(17)	12(39)	5(63)
Nephritic scars	12(67)	12(44)	3(21)	1(100)	5(56)	14(39)	17(55)	5(63)
Renal cysts	1(6)	2(7)	-	-	1(11)	2(6)	2(6)	-
Hydronephrosis	1(6)	1(4)	2(14)	-	-	2(6)	-	-
Granulomatous inflammation	-	-	-	-	-	1(3)	-	-
Suppurative pyelitis	1(6)	1(4)	1(7)	-	-	1(3)	-	-
Cystic adenoma	-	-	1(7)	-	-	-	-	-
Adenocarcinoma	-	1(4)	-	-	-	-	1(3)	-

* number of animals with lesion (percentage)

and had a finely or coarsely granular surface. Histologically, the membranous form was characterized by thickening of the capillary basement membranes, expansion of the mesangial matrix and a mild increase in cellularity. The typical changes of the chronic form were segmental or total glomerular sclerosis, synechiae, tubular atrophy, cast formation and lymphoplasmacellular infiltration. The infiltrate was usually seen around blood vessels or sclerotic glomeruli. Foam cells in Bowman's spaces, cholesterol clefts in proximal convoluted tubules and tubular abscesses were sometimes seen and may have been secondarily related to the typical changes. Only in one case was a proliferative glomerulonephritis, which was characterized by hypercellularity of the glomerular tufts and Bowman's capsule, recognized. Hypercellularity appeared to be due to increased numbers of mesangial cells.

Van Noord et al. (1972) studied the renal lesions in Mastomys of the Rijswijk colony at the ultrastructural level. The earliest alterations were seen in 6-month-

old males and consisted of diffuse thickening of the basement membranes of the peripheral capillary loops with loss of the three-layered structure and the appearance of small nodules on the epithelial side of the membrane. At eight months of age, the basement membrane showed diffuse thickening of up to three to four times normal size and the visceral epithelial cells were enlarged. Many epithelial cells had bizarre nuclei. Foot processes were fused over long distances. In addition, the mesangial region was notably expanded and increased cellularity was observed. Collagen fibrils and lipofuscin granules were commonly observed in the mesangial matrix. From the age of 24 months on, the lesions were the same but still more prominent.

An immunofluorescence study using rabbit antimastomys immunoglobulins and rabbit antimouse βIC showed small amounts of immunoglobulins and complement in the mesangium of Mastomys older than 13 weeks. No increase in fluorescence was observed until the age of 12 months. From that age on, a clear increase in fluorescence in the mesangium was seen. No further increase in mesangial fluorescence occurred after the age of 18 months. It was sometimes possible to demonstrate immunoglobulins and complement along the peripheral loops in older animals (van Noord et al., 1972; van Pelt & Blankwater, 1972). The results of immunofluorescence and ultrastructural studies are suggestive for an immune complex glomerulonephritis in Mastomys. The complexes were present in the mesangium and beneath the endothelium.

Zurcher et al. (1980) compared a number of renal function parameters with the severity of renal lesions as diagnosed histologically in male Mastomys of the Rijswijk colony. A definite relation was found between decreased concentrating capacity (urine osmolality) and the severity of renal lesions, while no relation was found with plasma creatinine levels, creatinine clearance and urinary protein concentration.

Single or multiple nephritic scars were also commonly observed in male and female Mastomys and were found in all age groups. The scars appeared as sclerotic areas which were shrunken and depressed below the surface of the kidney. In general, the scars did not extend beyond the midcortical zone. The cause of these scars was not discovered. Besides hyalinization of small muscular arteries, no other vessel changes were observed nor were thrombi seen.

Other nonneoplastic renal lesions such as renal cysts, hydronephrosis, suppurative pyelitis and granulomatous inflammation were only occasionally encountered. The renal cysts were solitary structures occurring in three males and five females. They were present at the periphery of the kidney or deep in the renal parenchyma. Hydronephrosis was found in animals with spinal cord compression due to intervertebral disk herniation. Herniation may give rise to paralysis of the bladder and ultimately to hydronephrosis. Suppurative pyelitis was associated with suppurative inflammation of the accessory sex glands or hydronephrosis or both.

Granulomatous inflammation was seen in only one 25-month-old female, the cause of which could not be further identified by use of special stains. Furthermore, mineralization in the papilla and peri- or parapelvic mononuclear cell infiltration were common, but did not seem to be related to other renal lesions.

Three neoplastic lesions were found. A cystic papillary adenoma was diagnosed in a 30-month-old male. The tumor consisted of cystic spaces into which papillary projections extended. The projections had a fibrous connective tissue stroma covered with uniform epithelial cells. A tubular adenocarcinoma was diagnosed in a 20-month-old male and in a 31-month-old female. The pleomorphic epithelial cells formed nests or tubules which infiltrated into the surrounding normal parenchyma. Mitoses were numerous and necrosis was extensive.

Other types of renal tumors described in Mastomys but not observed in our series are a nephroblastoma (Jobard et al., 1974), a clear-cell adenoma (Soga, 1977b), a papillary clear-cell adenocarcinoma and a papillary adenocarcinoma (Snell & Stewart, 1967).

3.8.2 Urinary bladder

Suppurative cystitis associated with hyperplasia of the bladder epithelium and focal ulceration were the only lesions encountered in the bladder. Cystitis was found in four of the 55 males examined. These four animals varied in age from 21 to 30 months. The animals showing this lesion also had suppurative inflammation of the accessory sex glands. Focal ulceration was seen in only a 22-month-old male. No lesion was found in any of the females.

Obvious was the presence of submucosal aggregations of lymphoreticular cells in the urinary bladders of male and female Mastomys. These cellular aggregations are found not only in old Mastomys but also in young ones (Solleveld, unpublished observations). In most cases where a lymphoreticular tumor was diagnosed, the aggregations were increased in size and consisted of neoplastic cells. Infiltration into the muscularis was then a common finding.

3.9 Reproductive system

3.9.1 Testis and epididymis

In total, testes of 59 male Mastomys were examined histologically. The most common change in the testes of aged male Mastomys was unilateral or bilateral testicular atrophy. This was found in 44 of the 59 males examined (75%) and was most severe in animals of the two oldest age groups. Testicular atrophy was characterized by diminished or absent spermatogenesis, formation of multinucleated cells, calcification of the seminiferous tubules and sometimes interstitial edema. Dilatation of tubuli of the epididymis lined by irregular epithelial cells was seen in

the most severe cases of testicular atrophy. Suppurative orchitis was recognized in one male and suppurative epididymitis in six others. The suppurative inflammation in these organs was always seen in association with severe inflammation of the accessory sex glands. Only in four cases was a tumor of the male reproductive system diagnosed. These were a Leydig cell tumor in a 30-month-old male, two papillomas of the rete testis in animals of 30 and 31 months of age and a mesothelioma arising from the mesothelium of the tunica vaginalis. Tumors of the testes in Mastomys have been described in more detail by Snell & Hollander (1972).

3.9.2 Accessory sex glands

3.9.2.1 Seminal vesicle

The most common lesion seen in the seminal vesicles of aged male Mastomys was suppurative seminal vesiculitis. A mild to severe suppurative inflammation was found in 18 of the 49 cases (37%) and occurred predominantly in the 30-35 month age group. Moderate to severe papillary hyperplasia of the seminal vesicular epithelium was observed in three males with seminal vesiculitis. In a 23-month-old male, a small carcinosarcoma such as previously described by Snell & Hollander (1972) was found. Occasionally, focal cystic dilatation of the seminal vesicles and mild proliferative stromal reactions were seen, most likely resulting from inflammation.

3.9.2.2 Prostate gland

The lesions found in the prostate glands of male and female Mastomys generally corresponded to those observed in the seminal vesicles (Table XLV).

The incidence of suppurative prostatitis in males (36%) was five times higher than in females (7%) and was found predominantly in the age groups of 24-29 and 30-35 months. Grossly, the inflamed prostate gland was enlarged and had a brown-yellow appearance. Microscopically, the suppurative inflammation was often confined to the acini but was sometimes extensive and had given rise to large

TABLE XLV

PROSTATIC LESIONS IN AGED MALE AND FEMALE MASTOMYS

	Males (n=44)				Females (n=43)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	12	19	12	1	6	13	21	3
Suppurative prostatitis	3(25)*	8(42)	5(42)	-	-	1(8)	2(10)	-
Hyperplasia	1(8)	8(42)	1(8)	-	2(33)	7(54)	13(62)	1(33)
Adenocarcinoma	-	-	-	-	-	-	3(14)	1(33)

* number of animals with lesion (percentage)

abscesses. No attempts were made to determine the etiology by culturing for the presumed microorganism(s) responsible for this inflammation. Hyperplasia and metaplasia of the prostatic epithelium were occasionally found to accompany prostatitis. In addition, prostate gland acini were often distended with secretion in the aged male and female Mastomys. Corpora amylacea-like concrements were often prominent in prostates of both sexes and appeared to occur regardless of whether other lesions were present.

Hyperplasia and adenocarcinomas of the prostate gland were most frequently seen in females, as was also reported by Holland (1970) and Snell & Stewart (1965). The diagnosis adenoma was avoided, since criteria for distinguishing advanced hyperplastic changes from lesions which might be called adenoma could not be found. In prostatic hyperplasia, the organ is grossly enlarged without distortion of its shape. Microscopically, the lesions varied from a slight increase in papillary infoldings to large branching papillary structures (Fig.53) and the formation of glandular structures within acini (Fig.54). Hyperplasia was either focal or diffuse. Irregular appearance of epithelial lining cells was common but this was also seen in normal prostate glands of old Mastomys (Fig.52)

Adenocarcinoma was diagnosed in four of the 24 females older than 30 months. Glandular enlargement and an increase in consistency were the only gross findings. Microscopically, the tumors were found only within acini and involvement was restricted to a few acini. Cellular pleomorphism, mitoses and invasion of the fibrous tissue surrounding the acinar wall by tumor cells were evident in all four cases. The four tumors all showed a different pattern: glandular, tubular, cribriform (Fig.55) and solid.

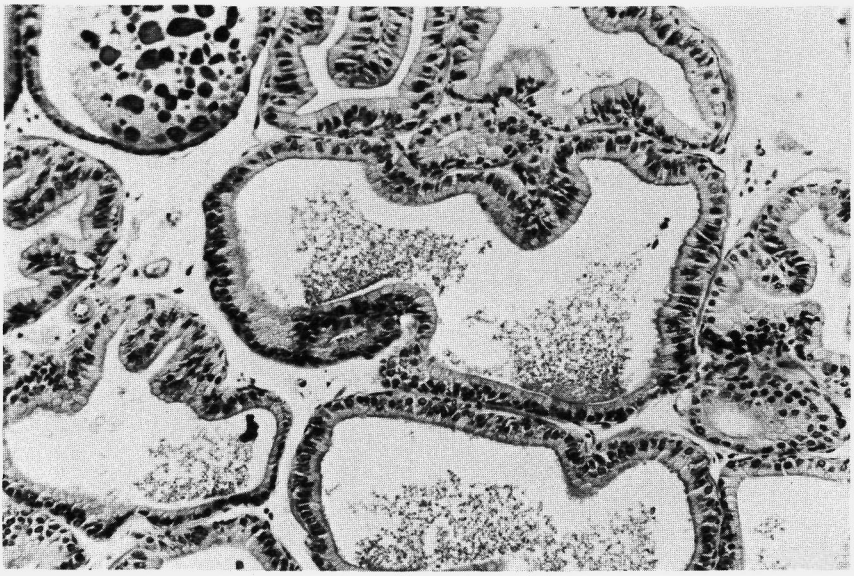

Fig. 52 Normal histological appearance of prostate gland of a 23-month-old female. HPS, x 140

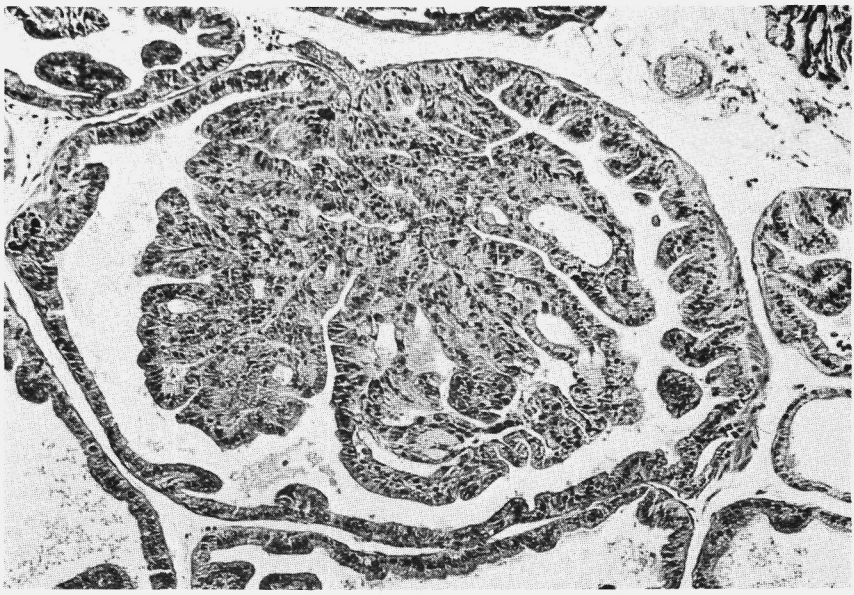

Fig. 53 Papillary hyperplasia of prostate gland of a 28-month-old female. HPS, x 87

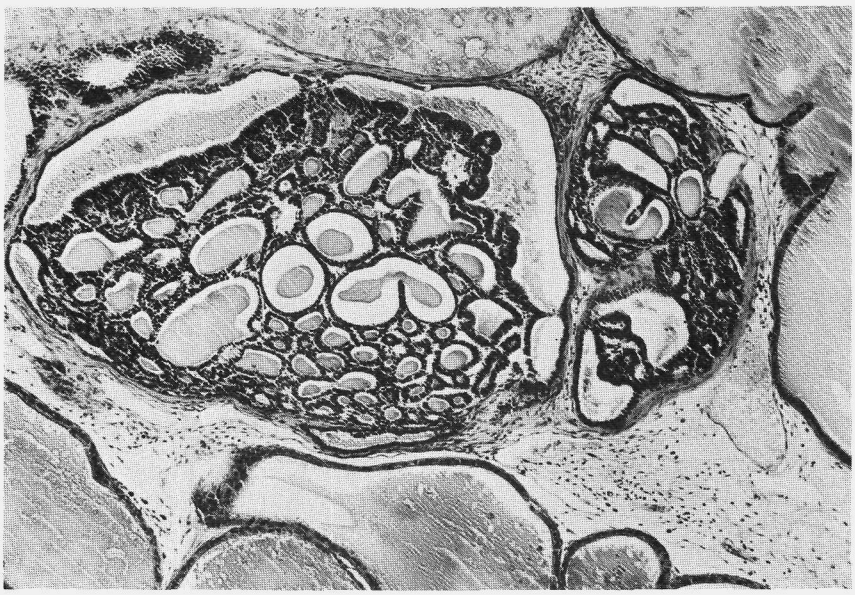

Fig. 54 Hyperplasia of prostate gland with formation of glandular structures within acini. Female, 34 months. HPS, x 64

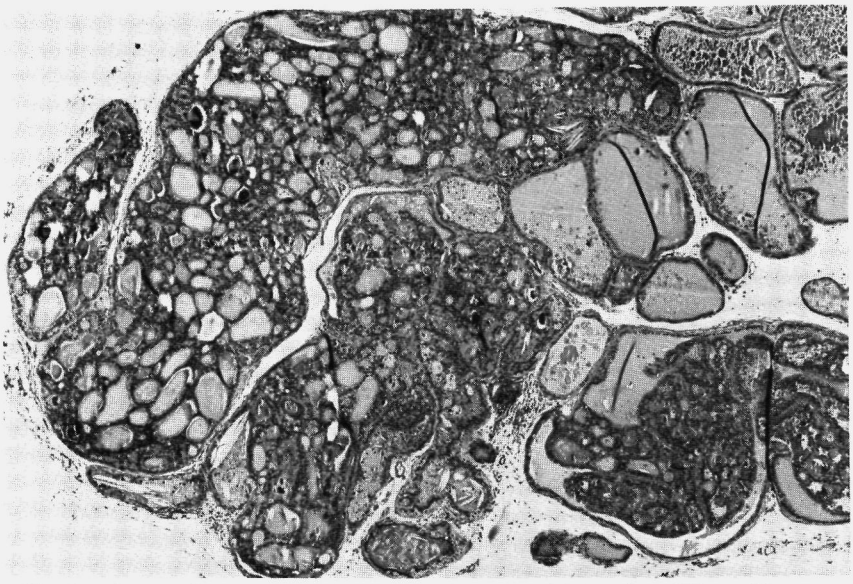

Fig. 55 Carcinoma of prostate gland of a 33-month-old female. HPS, x 21

3.9.3 Ovary

Seventy-three of the 80 females (91%) examined had atrophic ovaries. The atrophy was characterized by a marked reduction in the number of ova, Graafian follicles and corpora lutea and an increase in interstitial cells. Obvious was the extensive accumulation of yellow-brown pigment in the interstitial cells (Fig.56), which imparted a yellow color to the ovaries. There is still confusion over the nature of this pigment. It has been called ceroid by Deane & Fawcett (1952) and Lemon & Gubareva (1979) and lipofuscin by others (Burek, 1978; Crichton et al., 1978). These two pigments are indistinguishable on light microscopic examination *Porta & Hartroft, 1969; Wolman, 1975). Granulosa-theca cell proliferation, either* nodular or diffuse along the tunic, was also common, as were aggregations of lymphoreticular cells in the ovarian stroma. In cases where a lymphoreticular tumor was found, these aggregations were often greatly increased in size and consisted of neoplastic lymphoreticular cells.

Many nonneoplastic lesions were seen in the ovaries, but all occurred with a low frequency (≦ 3%). These included bursal cysts, bursal hemorrhage, parovarian cysts, hemorrhagic follicles, hematocysts, calcium deposits and cholesterol granulomas arising in corpora lutea. A peculiar finding was smooth muscle hyperplasia in one ovary (Fig.57). The same type of lesion has been recently described in the ovaries of domestic ferrets (Cotchin, 1980).

Of the neoplastic lesions, seven granulosa-theca cell tumors (9%) and one luteoma were found in this series. The granulosa-theca cell tumors were spread equally over the age groups of 24-29 and 30-35 months and the luteoma was found in a 23-month-old female. Grossly, the granulosa-theca cell tumors had a yellowish-

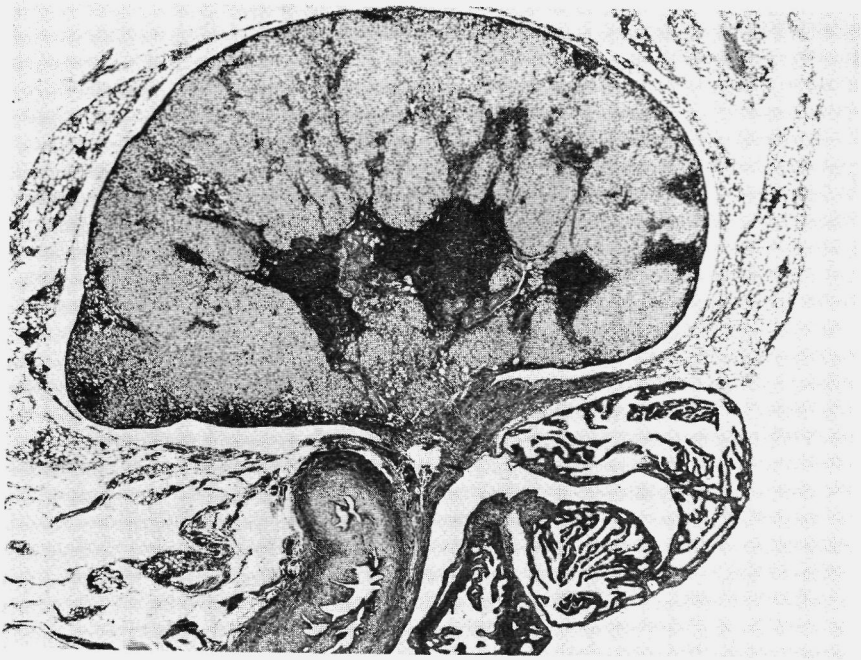

Fig. 56 Ovary of a 30-month-old female. Ovary consists almost entirely of pigment-laden cells. Note accumulations of lymphocytes in the center. HPS, x 29

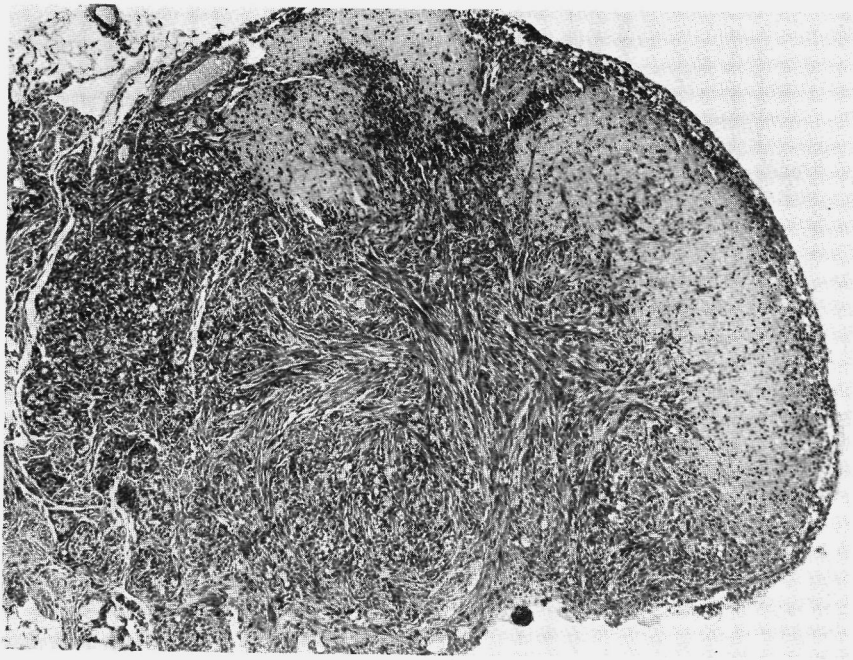

Fig. 57 Smooth muscle hyperplasia in the ovary of a 29-month-old female. HPS, x 54

white color and an irregular surface. They were unilateral, firm or cystic and usually measured 2-3 cm in diameter. Microscopically, they were primarily composed of granulosa cells. Most tumors showed a solid as well as a follicular pattern; in only one case was an adenomatous arrangement seen. The follicular areas often showed central liquefaction, sometimes resembling a normal Graafian follicle. Large cystic spaces filled with blood were present in some areas. Mitoses were numerous but metastases were not found. In two cases, tumor tissue was found along the uterine horn and invasive growth was seen in the uterine wall in one of these two cases. One granulosa-theca cell tumor was associated with marked hypertrophy of the uterine muscular wall and severe dilatation of the uterine lumen. Only slightly dilated uterine lumina and glands were observed in the other five cases. Hosoda (1977) found nine granulosa-theca cell tumors in 54 female Mastomys and also mentioned the relation between these tumors and hypertrophy of the uterine muscular wall. However, more obvious than the uterine changes was the marked hypertrophy of the smooth muscle component of the ovarian ligament at the site of the tumor in our series. Such a finding was not mentioned by Hosoda (1977). Snell & Stewart (1975) suggested that most of the granulosa-theca cell tumors in Mastomys must be nonfunctional, because mammary gland hyperplasia and neoplasia were exceedingly rare lesions. These changes were also not observed in our series (see Section 3.9.5) but the marked hypertrophy of smooth muscle found by Hosoda (1977) and in our material is suggestive for hormonal activity of these tumors; however, this has not been substantiated, since hormone levels in tumor tissue or serum were not determined.

The luteoma consisted of cells filled with vacuoles and was associated with a slightly dilated uterine lumen. Tumor cells of a mesothelioma arising in the abdominal cavity were found in the ovarian bursa of a 24-month-old female.

Besides granulosa-theca cell tumors, only two other primary ovarian tumors have been reported in Mastomys: a teratoma (Hollander & Higginson, 1971) and a hemangiosarcoma (Fujii & Sato, 1972).

3.9.4 Oviduct, uterus, cervix and vagina

Mineral deposition in the lumen was the only abnormality encountered in the oviduct. Fujii & Sato (1972) described a hemangioma and a leiomyoma of the Fallopian tube. Except for the uterine changes associated with granulosa-theca cell tumors, nonneoplastic lesions were relatively rare, just as were nonneoplastic lesions of the cervix and vagina. The lesions seen were suppurative endometritis, cervicitis and vaginitis, telangiectasia, hemorrhage, endometrial cysts, endometrial hyperplasia and stromal proliferation, hydrometra, squamous metaplasia of the endometrial epithelium and mucoid metaplasia of the vaginal epithelium.

Of the neoplastic lesions, endometrial stromal polyps were most common (Table XLVI). Grossly, the polyps were seen as local swellings of the uterine

horns. When opened, they appeared as polypoid masses protruding into the uterine lumen. Microscopically, they were composed of a fibrovascular stroma lined by endometrial epithelium.

TABLE XLVI

NEOPLASTIC LESIONS OF THE UTERUS, CERVIX AND VAGINA IN AGED FEMALE *MASTOMYS*

	Females (n=79)			
Age groups (months)	18-23	24-29	30-35	36-41
Number of *Mastomys*	9	34	29	7
Uterus: Endometrial stromal polyp	1(11)*	1(3)	5(17)	1(14)
Stromal sarcoma	-	1(3)	1(3)	-
Leiomyosarcoma	-	1(3)	-	-
Cervix/vagina: Squamous cell carcinoma	-	1(3)	-	-

* number of animals with lesion (percentage)

In addition to the polyps, a hemangioma and three sarcomas were found. One of the sarcomas was a well differentiated leiomyosarcoma observed in a 28-month-old female and two were diagnosed as stromal sarcomas and were found in a 24- and a 35-month-old female. Mitoses were numerous in these sarcomas but metastases were not found. A well-differentiated squamous cell carcinoma arising from the cervix or vagina was diagnosed in a 24-month-old female. Carcinoma cells were spread throughout the abdominal cavity. Other neoplastic lesions described in *Mastomys* were leiomyomas (Snell & Stewart, 1975) and two adenocarcinomas and one leiomyosarcoma (Hosoda, 1977) of the uterus.

3.9.5 Mammary gland

No mammary gland lesions were observed in our series and this supports the opinion of Snell & Stewart (1975) that mammary gland lesions occur rarely in *Mastomys*. A peculiar finding was the presence of keratinized squames in mammary gland ducts (Fig.58). The squames were seen in 19 of the 44 cases examined. It appeared that they were produced by the squamous epithelium of the nipple duct, in which they accumulated and gradually filled up the lactiferous sinus and ducts as illustrated in Fig.59.

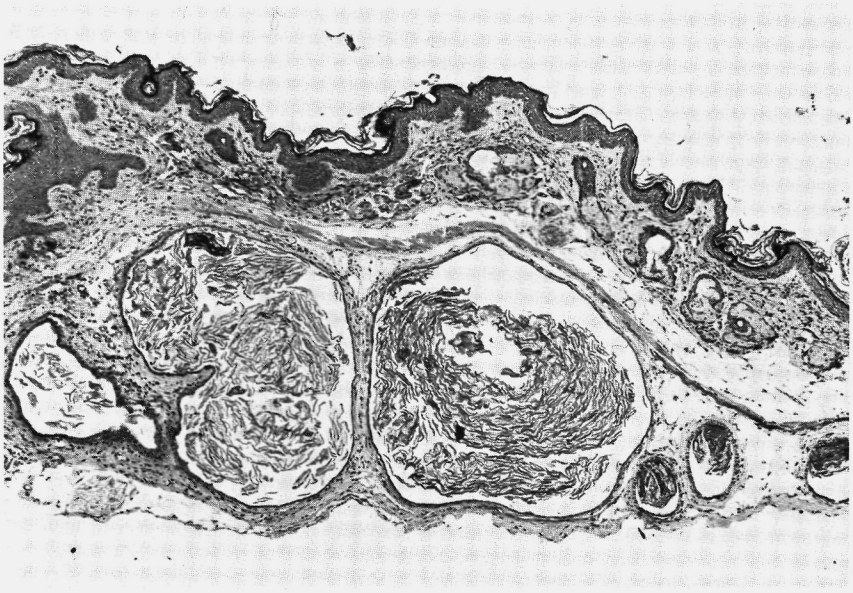

Fig. 58 Dilated mammary gland ducts containing keratinized squames in a 28-month-old female. HPS, x 54

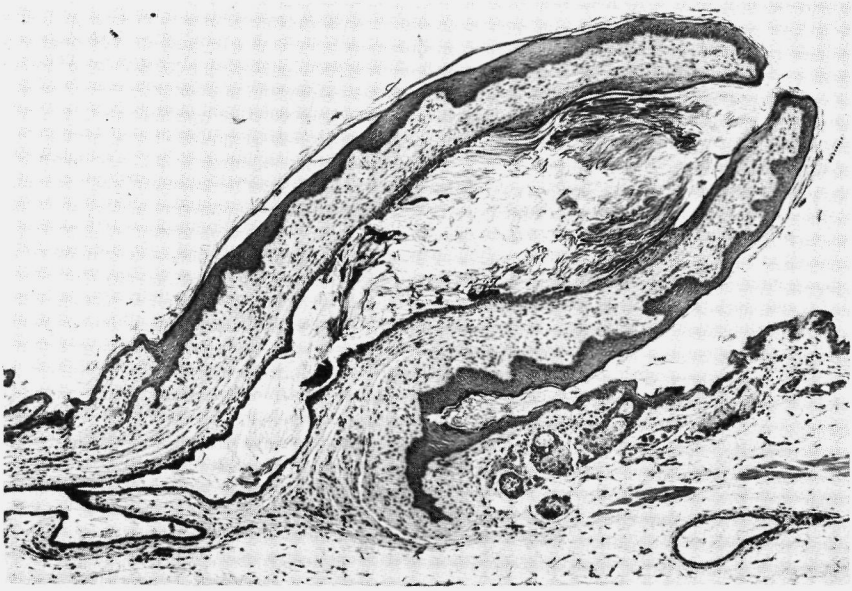

Fig. 59 Keratinized squames produced by the nipple duct epithelium. Female, 12 months. HPS, x 54

3.10 Nervous system

3.10.1 Brain

Only a few lesions were found in the brain (Table XLVII). The most common one was vacuolization of the white matter occurring with a high frequency in all age groups. It was most prominent in the cerebellum but was also seen in the other regions of the brain. The vacuoles were not accompanied by a cellular reaction and did not stain with routine and special stains.

TABLE XLVII

CHANGES IN THE BRAIN OF AGED MALE AND FEMALE *MASTOMYS*

	Males (n=46)				Females (n=69)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of *Mastomys*	14	20	11	1	9	24	29	7
Vacuolization	12(86)*	18(90)	10(91)	1(100)	8(89)	17(71)	28(97)	6(86)
Mononuclear cell infiltration in meninges	2(14)	-	-	-	1(11)	2(8)	-	2(29)

* number of animals with lesion (percentage)

Vacuolization is also a common finding in aged rats (Burek, 1978; Coleman et al., 1977; Garner et al., 1967) and mice (Garner et al., 1967). Burek (1978) and Coleman et al. (1977) found in rats an increase in incidence with age. Burek (1978) did not observe vacuoles in young rats. Nevertheless, he stated that vacuolization may be an artifact of the tissue processing procedure. The holes may indeed be artifacts but, since Burek (1978) did not find the changes in young rats and observed an increase in incidence with age, it seems more likely that they represent real changes in the white matter (e.g., demyelination), which result as empty spaces ultimately. The vacuoles may have contained a substance which was removed during tissue processing, again resulting in empty holes.

Perivascular cuffing in the meninges was recognized in six animals: two males and four females. The etiology was understood in only one female which showed generalized lymphoid hyperplasia. One female showed diffuse plasma cell infiltration in the meninges; this animal showed an excessive number of plasma cells in the lymphoid tissues and other organs. Compression of the brain secondary to the presence of a pituitary tumor was found in two males and one female.

Obvious was the absence of brain tumors in our study. This is probably an indication that they are rare in Mastomys and is supported by literature data. Only two spontaneously occurring brain tumors have been reported: an angiomatous meningioma (Oettlé, 1955) and a papillary ependymoma (Rabson et al., 1962).

3.10.2 Spinal cord

As previously mentioned in this chapter under Musculoskeletal system, compression of the spinal cord with secondary degenerative changes due to intervertebral disk herniation occurred very frequently. The secondary degenerative changes consisted of demyelination and infiltration by gitter cells. Compression of the spinal cord occurred more frequently in males than in females and seemed to occur earlier in life in males than in females. In males, an incidence of 100% was found in the age group of 24-29 months, while the highest incidence in females was 52% and was found in the age group of 30-35 months (Table XLVIII)

TABLE XLVIII

INCIDENCE OF SPINAL CORD COMPRESSION SECONDARY TO INTERVERTEBRAL DISK HERNIATION IN AGED MALE AND FEMALE Mastomys

	Males (n=47)				Females (n=74)			
Age groups (months)	18-23	24-29	30-35	36-41	18-23	24-29	30-35	36-41
Number of Mastomys	18	21	8	-	8	31	29	6
Spinal cord compression	13(72)*	21(100)	6(75)	-	3(38)	10(32)	15(52)	2(33)

* number of animals with lesion (percentage)

4 Discussion

The main objective of this study was to make a survey of lesions occurring in Mastomys which may be attributed to autoimmunity. Autoimmune diseases are often related to the presence of autoantibodies. However, several such diseases are caused by delayed-type reactions (Sell, 1978). These reactions as well as some induced by autoantibodies are characterized by a mononuclear inflammatory cell response. Taking such an inflammatory reaction as a possible indication for autoimmune lesions in Mastomys, the following lesions must then be considered: lymphocytic or lymphoplasmacellular dermatitis and folliculitis, sialoadenitis, thyroiditis, myocarditis, myositis and possibly also lymphocytic infiltration of pancreatic islets. Since the thymus plays a central role in immune function and thymic hyperplasia

and neoplasia occur more frequently in human patients suffering from the autoimmune disease myasthenia gravis than in controls (Oosterhuis et al., 1976), it was of interest to investigate whether the above-mentioned lesions in Mastomys were related to the frequently occurring lymphoepithelial thymomas in this animal species. A relationship was found between thymomas, on the one hand, and myocarditis, myositis and thyroiditis on the other. However, the relationship for the latter lesion was found only in male Mastomys. This makes it questionable whether the two phenomena are causally associated, since a distinct relationship was found between thyroiditis and autoantibodies to colloid (Solleveld et al., 1978 and see Chapter VI, Section 4). Another lesion that might be indirectly related to autoimmunity and which was found in almost every aged Mastomys is immune complex glomerulonephritis (van Noord et al. 1972; van Pelt & Blankwater, 1972). The immune complex nature in Mastomys was evident from the presence of immunoglobulins and complement in the same location of the glomeruli. The type of antigen (or antigens) involved, however, is obscure. Most cases of glomerulonephritis (70%-80%) resulting from immunologic causes in humans have been found to be due to antibodies complexed with circulating antigens (Theofilopoulos & Dixon, 1980). These complexes may be deposited in the vessels and filtration system of the kidney (Wilson & Dixon, 1976). Investigations have shown that viral antigens cross-reacting with an antiserum to Rauscher murine leukemia virus can be found in the glomeruli of Mastomys. Attempts to elute antiviral antibodies from pooled kidneys and the detection of C-type particles in kidneys by electron microscopy were not successful (van Pelt et al., 1976). These findings led to the conclusion that virus production in Mastomys must be extremely low. Hence, it is conceivable that, due to the occurrence of a wide variety of neoplasms and autoantibodies in a rather high incidence, tumor antigen-antibody and/or autoantigen-antibody complexes play an important role in Mastomys renal disease. Such complexes may also be involved in the observed vascular lesions such as periarteritis nodosa and fibrinoid necrosis of small- and medium-sized muscular arteries. However, further investigations are needed to substantiate these hypotheses.

The abundance of plasma cells in the lymphoreticular organs, accumulations of lymphoreticular cells in a number of organs and lesions, including periarteritis nodosa, hyalinization and fibrinoid necrosis of small- and medium-sized arteries, glomerulonephritis and lymphoproliferative lesions as found in this study show similarities to Aleutian disease of mink (Porter et al., 1980). This disease is caused by a parvovirus. One must consider the possibility that a viral agent is the direct stimulus for changes and lesions in Mastomys and possibly also for autoantibody formation in these animals. With the exception of screening of Mastomys sera for a number of rodent viruses (van Zwieten et al., 1981), which only gave negative results (Solleveld, unpublished data), no search for other viruses has been made in our animals.

The second aim of the study was to make and record a survey of neoplastic and nonneoplastic lesions occurring in Mastomys. Most neoplastic lesions observed in this study had already been described. However, when comparing the various studies, marked differences in incidence were found for a number of lesions. Lymphoepithelial thymomas were found in 8% of the animals examined by Kurokawa et al. (1968) and in 24% of the cases of Stewart & Snell (1968), while an incidence of 40% was found in our study. The same applied to liver cell tumors, adrenocortical tumors and pituitary tumors. Liver cell adenomas were observed in 31% of the animals examined in our series, while Soga et al. (1969a) and Kanahara et al. (1972) found a frequency of 13.5%. Liver cell carcinomas were found in a lower percentage (5% in males and 14% in females) but was still higher than that reported by Hollander & Higginson (1971), namely 2.7% and 3.4%, respectively. Adrenocortical tumors were diagnosed in a 27% frequency in our series, while others found an incidence varying from 6.7% to 13.2% (Hollander & Higginson, 1971; Sato et al., 1974; Soga & Tazawa, 1977; Soga et al., 1969b). In addition, pituitary gland tumors were found three to four times more frequently in our study (10%) than in those of others (Fujii & Sato, 1972; Soga et al., 1969a). On the other hand, the incidence of gastric carcinoids was extremely low in our study (4%) when compared to others, in which the incidence varied between 12% and 68% (reviewed by Soga, 1977a). Further, no exocrine pancreatic tumors were seen in our material, while Hosoda et al. (1976) found such tumors in 19% of Mastomys older than two years. All of these differences in incidence are of a magnitude that cannot be ascribed simply to the small number of animals investigated. Therefore they must be the result of genetic selection procedures or environmental factors or both. Genetic selection is probably the most likely explanation since Oettlé (1961) found a decrease in gastric carcinoid tumors during the process of inbreeding under comparable circumstances.

As stated by Snell & Stewart (1975), Mastomys differ in their disease pattern from other rodents. Comparing neoplastic lesions of Mastomys with those generally found in mice and rats, Mastomys is more or less unique with respect to the development of lymphoepithelial thymomas, gastric carcinoids, parathyroid adenomas and prostatic tumors and the rare occurrence of brain, lung and mammary tumors. Of the nonneoplastic lesions, thymic, prostatic and parathyroid hyperplasia and generalized degenerative joint disease occur rarely in mice and rats. It must be noted here that there is one inbred subline of WAB rats that also develops thymomas in a high incidence (22.9%) (Hinsull & Bellamy, 1977) and that there are some mouse strains (STR/1N; STR/ORT and C57BL) which develop degenerative joint disease (reviewed by Zurcher et al., in press), although less severely and not in a generalized manner as do Mastomys. It has also been reported (Silberberg et al., 1979) that intervertebral disk degeneration and herniation is a common finding in aged sand rats (Psammomys obesus).

It was investigated whether the neoplastic and nonneoplastic lesions showed

an age-related pattern. This was seldom found. However, the incidence of most of the lesions was too low to observe such a relation. Nevertheless, the conclusion, particularly for those lesions that occurred frequently, must be that most changes in Mastomys develop before 18 months of age.

CHAPTER VI

ASSOCIATION OF AUTOANTIBODIES WITH PATHOLOGICAL CHANGES IN MASTOMYS OF THE RIJSWIJK COLONY

1 Introduction

An attempt is made in this chapter to relate the presence of autoantibodies to pathological changes in Mastomys of the Rijswijk colony. As described in Chapter IV, a considerable number of autoantibodies with different specificities has been found in animals of this colony. A survey of the spectrum of pathological changes of the same animals in which autoantibodies were determined was presented in Chapter V. The wide diversity of autoantibodies and lesions found in Mastomys induced us to restrict the number of autoantibody-lesion combinations to be studied. The selection was based primarily on the current knowledge of autoimmune disorders, especially as regards the relation between specific histopathological changes and the presence of autoantibodies in man and other species.

In view of the fact that the thymus plays a central role in immune function and that thymic lesions have been shown to occur more frequently in human patients suffering from myasthenia gravis than in controls (Oosterhuis et al., 1976), it seemed relevant to investigate whether the various thymic changes observed in Mastomys influenced autoantibody formation. As discussed in Chapter V, the question of whether thymomas and autoantibodies in Mastomys are related was raised, since no association was found between thymomas and thyroiditis in females, while a relationship was found between thyroiditis and autoantibodies to colloid in both males and females (Solleveld et al., 1978). It will be considered here whether differences in incidences of autoantibodies are found in animals with atrophy, hyperplasia and neoplasia of the thymus.

There is now evidence from human and animal studies to suggest that autoimmunity and lymphoproliferative lesions may be related events (Talal, 1977). Evidence for a relation is the frequent appearance of autoantibodies in patients with lymphoma or chronic lymphocytic leukemia and the occurrence of lymphadenopathy in patients with Sjögren's syndrome. On the other hand, it was also observed that some patients with Sjögren's syndrome developed undifferentiated malignant lymphomas which were associated with loss of autoantibodies (Talal, 1977). Similar findings were made in mice. Antinuclear antibodies disappeared or were less common in SJL/J, (NZBxNZW)F_1 hybrid, C57BL/6 and AKR/Cu mice following spontaneous or induced tumor development (Haran-Ghera et al., 1973; Peled et al., 1979; Walker & Bole, 1976). Based on these findings, it was of interest to determine the mean number of autoantibody specificities in Mastomys with lesions involving the lymphoreticular tissues and in those without such lesions.

It was also investigated whether any lesions in Mastomys were associated with autoantibody formation. According to Aho (1980), autoantibodies can be separated into three categories: those with direct pathogenic effects, those which exert their effect through immune complex formation and those of which the effects are still not understood. As described in Chapter V, lesions in Mastomys which may be the result of a direct pathogenic effect of autoantibodies are lymphocytic or lymphoplasmacellular dermatitis, sialoadenitis, thyroiditis, myocarditis, myositis and possibly also lymphocytic infiltration of pancreatic islets. Skin, salivary glands and pancreas were not used as substrates in the immunofluorescence test, so that only the lesions thyroiditis, myocarditis and myositis remain for investigating a possible correlation. It was determined whether a relationship existed between thyroiditis and the presence of autoantibodies to colloid and thyroid cytoplasm and similarly between autoantibodies to striated muscle and myocarditis and myositis.

Lesions in Mastomys which may be attributed to autoantigen-antibody complexes are glomerulonephritis and vascular lesions such as periarteritis nodosa and fibrinoid necrosis. No studies directly aimed at determining the role of immune complexes in the etiology of such lesions were performed. It was thought to be of value, however, to investigate whether certain autoantibodies occur more frequently in animals with moderate and severe glomerulonephritis than in those with mild lesions. The same was done in animals with vascular lesions. Moreover, it will be considered whether smooth muscle autoantibodies, which in man are not related to specific lesions and thus belong to the third category according to Aho (1980), are associated with the vascular lesions.

2 Thymic changes related to the incidence of autoantibodies in Mastomys

The following thymic changes were recognized histologically: atrophy, hyperplasia and thymoma (see Chapter V, Section 3.7.3). The incidences of autoantibodies were determined for each of these changes and for each sex. Since cytoplasmic staining of liver parenchymal cells, renal proximal tubular cells, thyroid follicular cells and all epithelial lining cells of the glandular stomach were highly correlated in males and females (Chapter IV, Section 3.1), only autoantibodies to cytoplasmic antigens of liver parenchymal cells were chosen for comparison. The results are listed in Table XLIX.

TABLE XLIX

THYMIC CHANGES RELATED TO THE INCIDENCE OF AUTOANTIBODIES IN AGED MALE AND FEMALE MASTOMYS

Sex	Autoantibodies to:		Thymic changes: Atrophy	Hyperplasia	Thymoma
Males	Liver:	nuclei	6/27 (22) *	3/10 (30)	7/23 (30)
		cytoplasm	8/27 (30)	4/10 (40)	17/23 (74)
		erythrocytes	1/27 (4)	0/10 (0)	0/23 (0)
	Thyroid:	colloid	1/27 (4)	0/10 (0)	4/23 (17)
	Kidney:	mesangium	1/27 (4)	2/10 (20)	2/23 (9)
		distal tubules	0/27 (0)	0/10 (0)	0/23 (0)
	Diaphragm:	intermyofibrillar type	3/27 (11)	0/10 (0)	7/23 (30)
		striated type	1/27 (4)	0/10 (0)	4/23 (17)
		zebra type	6/27 (22)	3/10 (30)	4/23 (17)
		mitochondrial type	0/27 (0)	0/10 (0)	1/23 (4)
		myasthenia gravis type	1/27 (4)	2/10 (20)	6/23 (26)
	Heart:	intermyofibrillar type	6/27 (22)	1/10 (10)	8/23 (35)
		striated type	5/27 (19)	0/10 (0)	11/23 (48)
		mitochondrial type	0/27 (0)	0/10 (0)	2/23 (9)
		myasthenia gravis type	7/27 (26)	5/10 (50)	6/23 (26)
	Stomach:	parietal cells	5/27 (19)	4/10 (40)	7/23 (30)
		smooth muscle	7/27 (26)	1/10 (10)	12/23 (52)
Females	Liver:	nuclei	9/43 (21)	0/7 (0)	4/35 (11)
		cytoplasm	13/43 (30)	3/7 (43)	17/35 (49)
		erythrocytes	4/43 (9)	0/7 (0)	2/35 (6)
	Thyroid:	colloid	8/43 (19)	2/7 (29)	4/35 (11)
	Kidney:	mesangium	3/43 (7)	0/7 (0)	3/35 (9)
		distal tubules	2/43 (5)	0/7 (0)	3/35 (9)
	Diaphragm:	intermyofibrillar type	5/43 (12)	1/7 (14)	11/35 (31)
		striated type	1/43 (2)	0/7 (0)	2/35 (6)
		zebra type	6/43 (14)	0/7 (0)	4/35 (11)
		mitochondrial type	0/43 (0)	0/7 (0)	1/35 (3)
		myasthenia gravis type	7/43 (16)	1/7 (14)	5/35 (14)
	Heart:	intermyofibrillar type	9/43 (21)	1/7 (14)	12/35 (34)
		striated type	4/43 (9)	0/7 (0)	4/35 (11)
		mitochondrial type	2/43 (5)	0/7 (0)	0/35 (0)
		myasthenia gravis type	10/43 (23)	1/7 (14)	6/35 (17)
	Stomach:	parietal cells	24/43 (56)	4/7 (57)	12/35 (34)
		smooth muscle	6/43 (14)	0/7 (0)	8/35 (23)

* number of animals with autoantibody / total number of animals with thymic change (percentage)

As appears from Table XLIX, no distinct differences were found for most autoantibodies when comparing the three groups. After statistical evaluation (2-tailed exact Fisher test), it appeared that autoantibodies to cytoplasmic antigens, skeletal muscle (myasthenia gravis type) and cardiac muscle (striated type) occur significantly more frequently in male Mastomys with a thymoma than in animals with atrophy of the thymus ($p < 0.05$). In females, only autoantibodies to skeletal muscle of the intermyofibrillar type were more common in animals with a thymoma than in those with thymic atrophy ($p < 0.05$). In most of these cases, also a significantly higher incidence of autoantibodies was found in animals with a thymoma than in animals with hyperplasia of the thymus. Furthermore, the mean number of autoantibody specificities in animals with thymic changes was determined (data not shown). It appeared that a significantly higher mean number of autoantibody specificities was found only in male Mastomys with thymomas ($p < 0.05$, with the Mann-Whitney test) when compared with male Mastomys with thymic atrophy.

Interesting was the association between autoantibodies to skeletal muscle of the myasthenia gravis type and thymomas in male Mastomys. Such an association can also be found in human patients suffering from myasthenia gravis. Despite the fact that autoantibodies to skeletal muscle of the myasthenia gravis type in man give more information on the presence or absence of a thymoma than of myasthenia gravis (Feltkamp, 1975), it seemed appropriate to perform a pilot study on the occurrence of this disease in Mastomys, since no animal model that spontaneously develops this disease is available. This study is described in Chapter VII.

3 Lesions involving the lymphoreticular tissues related to the incidence of autoantibodies in Mastomys

The lesions which were included in this investigation and which have been discussed in Chapter V are lymphoid hyperplasia, atypical lymphoid hyperplasia, different types of malignant lymphomas (poorly differentiated lymphocytic, immunoblastic and heterogeneous types), histiocytic sarcoma and granulocytic leukemia. The number of autoantibodies is expressed as the mean number of autoantibody specificities per animal and per lesion. Mastomys without such proliferative lesions served as controls. A total of 12 different autoantibodies were taken into account (see Chapter IV, Section 2.6). The mean values for male and female Mastomys are tabulated in Table L.

The mean number of autoantibody specificities in animals with lymphoid hyperplasia and with atypical lymphoid hyperplasia was higher in males and lower in females when compared to the male and female control animals. The differences, however, were not statistically significant. The situation seems to be clearer in animals bearing a tumor. Since only two males had a lymphoreticular tumor and tumors involving the lymphoreticular tissues were found in 19 females, only the females were chosen for a comparison. The females with a tumor had a significantly

lower mean number of autoantibody specificities ($p < 0.05$, with the Mann-Whitney test) than the control animals. It appeared that the severity of the lesion was correlated with the number of autoantibodies. Females in which the lymphoreticular tissues were entirely replaced by tumor cells did not have autoantibodies. Such a finding was made in eight of the 19 females. Two of these eight females had a malignant lymphoma, immunoblastic type, four females a malignant lymphoma, heterogeneous type, one female a histiocytic sarcoma and one female had granulocytic leukemia. On the other hand, eleven females in which only a part of the lymphoreticular tissue was replaced by tumor cells had at least an equal number of autoantibodies per animal as did the controls.

TABLE L

LYMPHOPROLIFERATIVE LESIONS AND MEAN NUMBER OF AUTOANTIBODY SPECIFICITIES PER ANIMAL

Lymphoproliferative lesions	Mean number of autoantibodies per animal (range)			
	Males	Number of animals	Females	Number of animals
Lymphoid hyperplasia	2.9 (1-5)	8	2.3 (1-4)	12
Atypical lymphoid hyperplasia	3.0 (2;4)	2	2.3 (0-4)	8
ML*, lymphocytic poorly differentiated	4.0	1	-	-
ML, immunoblastic type	-	-	1.3 (0-4)	6
ML, heterogeneous type	2.0	1	1.9 (0-5)	9
Histiocytic sarcoma	-	-	2.0 (0-4)	3
Granulocytic leukemia	-	-	0	1
Controls	2.5 (0-6)	48	2.6 (0-6)	46

* ML = malignant lymphoma

4 Thyroiditis related to autoantibodies to colloid and thyroid cytoplasm

Thyroid glands of 57 males and 77 females were evaluated histopathologically. Five of the 57 males and 14 of the 77 females had circulating autoantibodies to colloid and 22 of the males and 33 of the females had autoantibodies to thyroid cytoplasm. Thyroiditis was diagnosed in 6 males and 18 females. The relationship between thyroiditis and these autoantibodies is indicated in Table LI.

TABLE LI

RELATIONSHIP BETWEEN THYROIDITIS AND AUTOANTIBODIES TO COLLOID AND THYROID CYTOPLASM IN AGED MALE AND FEMALE MASTOMYS

Sex	Animals with (+) and without (-) thyroiditis	Autoantibodies to: Colloid		Thyroid cytoplasm	
		+	-	+	-
Males	+ n= 6	5	1	1	5
	- n=51	0	51	21	30
Females	+ n=18	14	4	5	13
	- n=59	0	59	28	31

The large number of animals with autoantibodies to thyroid cytoplasm when compared with the number with thyroiditis had already suggested that an association between the lesions and these autoantibodies could not be expected. This was proved statistically by performing the 2-tailed exact Fisher test. However, thyroiditis and autoantibodies to colloid were correlated in both sexes ($p < 0.001$).

In two males and eleven females which had autoantibodies to colloid, the titers of these autoantibodies were determined. This offered the possibility to explore whether a relationship existed between the severity of thyroiditis and autoantibody titers to colloid. The severity was graded according to the system used by Kite et al. (1969) (see Chapter V, Section 3.6.2). Animals with grade 1 and grade 2 lesions had mean titers of 320, those with grade 3 a mean titer of 900 and those with grade 4 a mean titer of 400. This means that animals in which 50% to 75% of the thyroid gland was affected had the highest autoantibody titer. It is necessary to interpret these data with caution, since the number of animals involved was small.

5 Myositis and myocarditis related to autoantibodies to cardiac and skeletal muscle

Lymphocytic myositis was diagnosed in four males and eight females and lymphocytic myocarditis in nine males and nineteen females. Three males and seven females showed both lesions, one male and one female showed myositis only and six males and twelve females myocarditis only. These lesions were associated with autoantibodies to cardiac and skeletal muscle. Since the incidences of the various types of autoantibodies were not similar for the two substrates and the zebra type can be detected only in skeletal muscle, the incidences for the two substrates are listed separately. As a small number of animals showed these lesions and only minor differences in incidence of autoantibodies were found among animals with myositis, myocarditis or both, no distinction was made among the three groups. The results are listed in Table LII and compared with those for animals without these lesions. After statistical evaluation it appeared that none of the autoanti-

bodies was correlated with myositis and/or myocarditis.

TABLE LII

MYOSITIS AND MYOCARDITIS RELATED TO DIFFERENT SPECIFICITIES OF AUTOANTIBODIES TO CARDIAC (C) AND SKELETAL (S) MUSCLE

Sex	Number of animals with (+) and without (-) lesion	Intermyofibrillar type		Striated type		Zebra type	Myasthenia gravis type	
		C	S	C	S	S	C	S
Males	+ n=10	5*	4	2	1	2	3	3
	- n=50	10	6	3	14	11	15	6
Females	+ n=20	8	7	0	1	0	5	4
	- n=65	14	10	8	2	10	12	9

* number of animals

6 Severity of glomerulonephritis related to the incidence of autoantibodies

The severity of glomerulonephritis was graded according to a modification of a grading system used by Zurcher et al. (1980) (Chapter V, Section 3.8.1). Three grades were distinguished: 1+ for mild lesions, 2+ for moderate and 3+ for severe lesions. For each grade, the incidence of autoantibodies was determined; the results are shown in Table LIII.

In males, the incidence of autoantibodies could be compared only between animals with grade 2 and grade 3 lesions, since only one male had a grade 1 lesion. In females, all three groups could be compared. As appears from Table LIII, only minor differences were generally found between the two groups in males and the three groups in females. A statistically significant difference was only found for antibodies to mesangial antigens, which occurred more frequently in female Mastomys with grade 3 lesions ($p < 0.05$, with the 2-tailed exact Fisher test).

TABLE LIII

SEVERITY OF GLOMERULONEPHRITIS RELATED TO THE INCIDENCE OF AUTOANTIBODIES IN AGED MALE AND FEMALE MASTOMYS

Sex	Autoantibodies to:		Severity of glomerulonephritis 1	2 *	3
Males	Liver:	nuclei	-	7/21 (33)	9/38 (24)
		cytoplasm	-	9/21 (43)	20/38 (53)
		erythrocytes	-	0/21 (0)	1/38 (3)
	Thyroid:	colloid	-	1/21 (5)	4/38 (11)
	Kidney:	mesangium	-	1/21 (5)	4/38 (11)
		distal tubules	-	0/21 (0)	0/38 (0)
	Diaphragm:	intermyofibrillar type	-	2/21 (10)	8/38 (21)
		striated type	-	1/21 (5)	4/38 (11)
		zebra type	-	5/21 (24)	8/38 (21)
		mitochondrial type	-	0/21 (0)	1/38 (3)
		myasthenia gravis type	-	4/21 (19)	5/38 (13)
	Heart:	intermyofibrillar type	-	5/21 (24)	10/38 (26)
		striated type	-	7/21 (33)	9/38 (24)
		mitochondrial type	-	0/21 (0)	2/38 (5)
		myasthenia gravis type	-	8/21 (38)	10/38 (26)
	Stomach:	parietal cells	-	8/21 (38)	8/38 (21)
		smooth muscle	1/1 (100)	6/21 (29)	13/38 (34)
Females	Liver:	nuclei	3/22 (14)	5/38 (13)	5/24 (21)
		cytoplasm	10/22 (45)	16/38 (42)	7/24 (29)
		erythrocytes	0/22 (0)	1/38 (3)	5/24 (21)
	Thyroid:	colloid	2/22 (9)	7/38 (18)	5/24 (21)
	Kidney:	mesangium	1/22 (5)	0/38 (0)	5/24 (21)
		distal tubules	0/22 (0)	4/38 (11)	1/24 (4)
	Diaphragm:	intermyofibrillar type	2/22 (9)	12/38 (32)	3/24 (13)
		striated type	1/22 (5)	1/38 (3)	1/24 (4)
		zebra type	4/22 (18)	3/38 (8)	3/24 (13)
		mitochondrial type	1/22 (5)	0/38 (0)	0/24 (0)
		myasthenia gravis type	5/22 (23)	2/38 (5)	5/24 (21)
	Heart:	intermyofibrillar type	6/22 (27)	11/38 (29)	5/24 (21)
		striated type	1/22 (5)	6/38 (16)	1/24 (4)
		mitochondrial type	1/22 (5)	1/38 (3)	0/24 (0)
		myasthenia gravis type	5/22 (23)	5/38 (13)	6/24 (25)
	Stomach:	parietal cells	10/22 (45)	15/38 (39)	14/24 (58)
		smooth muscle	5/22 (23)	5/38 (13)	4/24 (17)

* number of animals with autoantibody / total number of animals with glomerulonephritis (percentage)

7 Vascular lesions related to the incidence of autoantibodies

Seven males and eleven females showed periarteritis nodosa or fibrinoid necrosis of the media of medium- and small-sized arteries. The incidence of autoantibodies was compared between animals showing such a lesion and those without these lesions. The data are shown in Table LIV.

TABLE LIV

COMPARISON OF INCIDENCE OF AUTOANTIBODIES BETWEEN ANIMALS WITH (+) AND WITHOUT (-) VASCULAR LESIONS IN AGED MALE AND FEMALE MASTOMYS

Autoantibodies to:		Males		Females	
		-	+	-	+
Liver:	nuclei	13/53 (25)*	3/7 (43)	8/74 (11)	5/11 (45)
	cytoplasm	25/53 (47)	4/7 (57)	31/74 (42)	2/11 (18)
	erythrocytes	1/53 (2)	0/7 (0)	6/74 (8)	0/11 (0)
Thyroid:	colloid	4/53 (8)	1/7 (14)	11/74 (15)	3/11 (27)
Kidney:	mesangium	5/53 (9)	0/7 (0)	3/74 (4)	3/11 (27)
	distal tubules	0/53 (0)	0/7 (0)	3/74 (4)	2/11 (18)
Diaphragm:	intermyofibrillar type	10/53 (19)	0/7 (0)	13/74 (18)	4/11 (36)
	striated type	4/53 (8)	1/7 (14)	1/74 (1)	2/11 (18)
	zebra type	12/53 (23)	1/7 (14)	9/74 (12)	1/11 (9)
	mitochondrial type	1/53 (2)	0/7 (0)	1/74 (1)	0/11 (0)
	myasthenia gravis type	7/53 (13)	2/7 (29)	13/74 (18)	0/11 (0)
Heart:	intermyofibrillar type	13/53 (25)	2/7 (29)	18/74 (24)	4/11 (36)
	striated type	12/53 (23)	4/7 (57)	7/74 (9)	1/11 (9)
	mitochondrial type	1/53 (2)	1/7 (14)	2/74 (3)	0/11 (0)
	myasthenia gravis type	18/53 (34)	0/7 (0)	16/74 (22)	1/11 (9)
Stomach:	parietal cells	15/53 (28)	1/7 (14)	36/74 (49)	4/11 (36)
	smooth muscle	19/53 (36)	1/7 (14)	12/74 (16)	2/11 (18)

* number of animals with autoantibody / total number of animals with or without vascular lesions (percentage)

No significant differences (2-tailed exact Fisher test) were observed in males, while autoantibodies to nuclear antigens ($p < 0.05$), mesangial antigens ($p < 0.05$) and skeletal muscle of the striated type ($p < 0.05$) were found in a higher incidence in female Mastomys with vascular lesions. It also appears from Table LIV that autoantibodies to smooth muscle were not associated with vascular lesions.

8. Discussion

In this chapter, it was considered whether certain selected pathological changes could be related to specific autoantibodies or more to autoantibody formation in general. It was first investigated whether there was a relation between one of the thymic changes and autoantibody formation. In male Mastomys, autoantibodies to cytoplasmic antigens, skeletal muscle (myasthenia gravis type) and cardiac muscle (striated type) were positively associated with thymomas. In females, this was the case only with autoantibodies to skeletal muscle of the intermyofibrillar type. A significant difference was found in the mean number of autoantibody specificities in male Mastomys with thymoma when compared with males with thymic atrophy. Particularly, the four autoantibody specificities which were found in a significantly higher incidence in the males with thymomas accounted for the higher mean number of autoantibodies in these animals. The significance of this finding is not yet clear. The nonuniform findings in males and females and the fact that antinuclear antibodies were found as early as two months of age (Solleveld, unpublished data), while thymomas occur late in life (Snell & Stewart, 1975), make the existence of a direct relationship between thymomas and autoantibody formation doubtful. A similar situation is found in man. It has been suggested that a number of systemic diseases is associated with thymomas, especially lupus erythematosus, rheumatoid arthritis, myasthenia gravis, polymyositis and myocarditis (Goldstein & Mackay, 1969). Gray (1979), however, stated that the incidence of associated syndromes varies considerably in different series and appears to be a reflection of patient selection. His conclusion was that, with the exception of myasthenia gravis, the exact relationship between thymomas and the various syndromes remains unclear. An association between myasthenia gravis and thymomas has been recognized (Oosterhuis et al., 1976). Rennert (1979) more or less came to the same conclusion. He stated that the concurrence of thymomas with various diseases has been studied too infrequently to allow any conclusions about the significance of these correlations.

The association between thymomas and polymyositis and myocarditis as mentioned by Goldstein & Mackay (1969) was also observed in Mastomys. The latter two lesions were not associated with one of the autoantibodies to striated muscle. Thymomas, in turn, were related with some antistriated muscle autoantibody specificities. This illustrates the complex situation of cause and effect relationships in Mastomys, which is probably also true for man.

It was also considered whether hyperplasia and neoplasia of the lymphoreticular system (excluding the thymus) influenced autoantibody formation. No significant difference was found between animals showing lymphoreticular hyperplasia and those without this lesion.The situation was clearer in animals with a tumor. The extent of involvement of the lymphoreticular tissues determined the mean number of autoantibodies per animal. Animals in which the lymphoreticular

tissues were entirely replaced by tumor cells did not have autoantibodies, while in those in which most of the lymphoreticular tissues were still intact, no significant difference was found in the mean number of autoantibodies per animal when compared to control animals. Several hypotheses have been advanced to explain the disappearance of autoantibodies following spontaneous or induced tumor occurrence (Peled et al., 1979). For example, it has been hypothesized that the proliferating nonneoplastic cells may absorb certain types of autoantibodies thereby accounting for the absence of autoantibodies. Another hypothesis is that the impairment of the immune system of tumor-bearing individuals, which may result by various mechanisms, is responsible for the absence of autoantibodies. The findings in Mastomys indicate that destruction of the normal architecture of the lymphoreticular tissues and replacement of the normal cell population by tumor cells per se may explain the absence of autoantibodies.

Another aspect of this investigation was concerned with a possible relationship between thyroiditis and autoantibodies to colloid and thyroid cytoplasm. Such a relationship was found only for thyroiditis and autoantibodies to colloid. The uniform pattern of colloid staining observed in Mastomys corresponds to the pattern seen in humans with autoantibodies to the so-called second colloid antigen. The second colloid antigen is a noniodinated protein of colloid and is unrelated to thyroglobulin (Pinchera et al., 1980). These autoantibodies in man are also associated with thyroiditis, as are autoantibodies to thyroid microsomal antigens. Autoantibodies to thyroid cytoplasm in Mastomys and those to thyroid microsomal antigens in man differ from each other, as will be explained below; this may explain the finding that thyroiditis and autoantibodies to thyroid cytoplasm were not related in Mastomys. Autoantibodies to thyroid microsomal antigens in man are organ- and species-specific. In Mastomys, autoantibodies to cytoplasmic antigens were observed in various organs, among which was the thyroid gland. These autoantibodies were highly correlated with each other and this excludes organ-specificity of these autoantibodies in Mastomys. They are also not species-specific, since rat organs were used as substrates in our test system. Moreover, the immunofluorescence staining pattern is quite different in Mastomys and man. In Mastomys, a diffuse granular staining of the cytoplasm is seen, while only the apical zone of the cytoplasm is stained in man. (Pinchera et al., 1980). These data indicate that the autoantibodies to cytoplasmic antigens in Mastomys are not directed to microsomal antigens. Further, the anticolloid antibody titers were compared with the severity of thyroiditis. Animals with grade 3 lesions (which means that 50% to 75% of the thyroid gland was affected) had the highest titers. This finding agrees with the observations made in BUF rats, which serve as a model for autoimmune thyroiditis. Rats with severe thyroiditis had lower titers than those with intermediate infiltration (Noble et al., 1976). Possible explanations could be that animals with severe disease have less antigen available for maintaining high levels of antibody formation, the lower levels of antibody in severely diseased animals reflect

the formation of circulating immune complexes or a combination of these factors.

It was also investigated whether certain autoantibodies occurred in a higher or lower frequency in animals with severe glomerulonephritis when compared to animals with mild to moderate renal lesions. The same was done for animals with and without vascular lesions. This investigation was performed to gain an impression of whether some autoantibodies may be involved in lesions which are or which may be caused by immune complexes. The basis for this investigation was the fact that nuclear antigen-antibody and thyroglobulin antigen-antibody complexes can be found in, e.g., kidneys and vessel walls in animals and man (Dixon, 1979; Jordan et al., 1978; O'Regan et al., 1976; Ploth et al., 1978; Theofilopoulos & Dixon, 1980). Renal and vascular lesions were chosen for the comparison, since investigations have demonstrated that immune complexes were most likely responsible for the renal lesions (van Noord et al., 1972; van Pelt & Blankwater, 1972) and that the vascular lesions in Mastomys resemble more or less the vascular lesions observed in the autoimmune mouse strains (Accinni & Dixon, 1979) in which it is known that these lesions are the result of immune complex deposition. Regarding the renal lesions, only antibodies to mesangial antibodies were found in a higher incidence in females with severe (grade 3) lesions. No significant difference was found between males with and without vascular lesions, while autoantibodies to nuclear antigens, mesangial antigens and skeletal muscle of the striated type occurred in a higher incidence in female Mastomys with vascular lesions when compared with females without these lesions. Despite some significant differences in females, the importance of these findings remains unclear. Most autoantibodies which were found in a higher incidence have not been found to be involved in immune complex depositions. If these autoantibodies should play a role, then it would be reasonable to expect a similar correlation between the occurrence of certain autoantibodies and renal and vascular lesions in male Mastomys. Finally, smooth muscle autoantibodies could not be related to vascular lesions.

In summary, to the extent that it has been investigated, only one lesion in Mastomys was related with autoantibodies. Thyroiditis was associated with autoantibodies to thyroid colloid. In addition, an inverse relationship was found between tumors involving the lymphoreticular tissues and the presence of autoantibodies.

CHAPTER VII

DOES MYASTHENIA GRAVIS OCCUR IN MASTOMYS OF THE RIJSWIJK COLONY ?

1 Introduction

Myasthenia gravis (MG) is a spontaneously occurring autoimmune neuromuscular disorder of man (reviewed by Drachman, 1978a,b) which is characterized by muscle fatigability that increases with exertion and improves with rest. This symptom results from a defect in neuromuscular transmission, namely, a reduction in the number of functional acetylcholine receptors (AChRs) at the neuromuscular junction, as demonstrated by reduced α-bungarotoxin (α-BuTx) binding (Fambrough et al., 1973). Antibodies and complement are thought to be responsible for this reduction in funtional AChRs. Recently, Sahashi et al. (1980) provided evidence for an antibody-dependent, complement-mediated injury of the postsynaptic membrane. The occurrence of complement-mediated membrane injury may account for the fewer junctional folds and wider synaptic clefts in the postsynaptic membrane of MG patients (Engel & Santa, 1971) and for a reduction in functional AChRs. However, antigenic modulation of the receptor by anti-AChR antibodies in the absence of complement may also contribute to a deficiency of the receptor (reviewed by Lindstrom, 1979 and Vincent, 1980).

MG in humans is associated not only with anti-AChR antibody (~85%) (Lindstrom & Lambert, 1978) but also with antistriated muscle antibody (~40%) of the so-called myasthenia gravis type (Feltkamp, 1975) and thymomas (~10%) (Feltkamp et al., 1974). A reduction in miniature endplate potential amplitude which is most likely due to a reduced number of active AChRs can also be found in human MG patients (Ito et al., 1978).

Mastomys of the Rijswijk colony develop certain features characteristic of MG in humans, as was discussed in Chapter VI. Both antistriated muscle antibody of the MG type and thymomas are found with a relatively high frequency in Mastomys. On the other hand, however, typical clinical signs of MG have not been observed (Solleveld et al., 1978; Stewart & Snell, 1968) and tests for neuromuscular function have not been reported in Mastomys.

Some important questions such as what triggers the autoimmune response to AChR and whether factors other than antibodies to AChR are involved in the pathogenesis of MG cannot be solved in animal models in which MG is induced experimentally. It was considered worthwhile therefore to further investigate Mastomys with regard to this disease entity. We have for this purpose searched for serum anti-AChR antibody and investigated muscle AChR in a well-defined group of Mastomys, including animals with and without thymomas and with and without antistriated muscle antibody.*

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* Study performed in collaboration with Dr.A.Vincent from the Department of Neurological Science, Royal Free Hospital, School of Medicine, London, England.

2 Materials and Methods

2.1 Animals

Nine male and ten female Mastomys varying in age from 20 to 36 months were used in this study. Details on housing and maintenance have been described in Chapter III, Section 4.2.1.

Five animals (3 males and 2 females) were killed at between 25 and 27 months of age. The diaphragms of these animals were used for the determination of specific motor endplate binding and the muscles of the limbs for determining the AChR contents. Fourteen others (6 males and 8 females) were allowed to complete their life spans and were killed when moribund. Their sera were assayed for anti-AChR antibody and for antibodies against striated muscle, nuclear factors, smooth muscle, gastric parietal cells and thyroid colloid. For these latter determinations, the indirect immunofluorescent technique using cryostat sections of rat heart, diaphragm, liver, stomach and thyroid as substrates was employed (see Chapter IV, Sections 2.3, 2.4 and 2.5). Sera giving an antistriational staining pattern corresponding to that found in human patients with MG (Feltkamp, 1975) in either heart or diaphragm or in both tissue types were scored as positive for antistriated muscle antibody (Fig.20d). The presence or absence of a thymoma was based on histopathological examination (Chapter V).

2.2 Sera

Blood samples were collected by orbital sinus puncture before the animals were killed. Serum was separated and stored at -20°C. The serum samples were used undiluted for the determination of anti-AChR antibodies and diluted 1:10 for determination of the other autoantibodies.

2.3 Anti-AChR antibody assay

Fourteen serum samples from six males and eight females varying in age between 20 and 36 months were screened for the presence of anti-AChR antibodies. Muscle extracts from 4 two-month-old Mastomys were isotope-labelled with excess alpha-bungarotoxin (^{125}I-α-BuTx). The concentration of α-BuTx binding sites was 0.5 pmoles.ml^{-1}. One hundred to 200 µl of ^{125}I-α-BuTx labelled extracts were incubated for 2 hours at room temperature with 10-20 µl of Mastomys sera. Precipitating antimastomys IgG was not available in a sufficient quantity to precipitate large amounts of mastomys IgG. Therefore, precipitation of AChR-IgG complexes was accomplished by addition of polyethylene glycol (PEG, 6000 M.W.; Sigma®). PEG at various concentrations precipitates serum proteins, including IgG (Polson et al., 1964). The optimal concentration for IgG precipitation is 8-10%. It was found (Vincent, unpublished results) that 8% PEG is sufficient to precipitate >65% of

AChR-anti-AChR complexes formed in excess AChR and this method has been used to detect low levels of antibody in human patients with MG. After incubation of labelled muscle extracts with Mastomys sera, 16% PEG was added to give a final concentration of 8% PEG. The tubes were left overnight at 4°C and spun for 10 minutes in an Eppendorf 5310 microfuge. The pellets were washed briefly in 20 mM phosphate made up in 0.1 per cent Triton X-100 (PTX buffer) and counted for 1 minute. Nonspecific precipitation of ^{125}I-α-BuTx was estimated by testing serum against extract which had been preincubated in nonradioactive α-BuTx. Nonspecific precipitation of ^{125}I-α-BuTx-AChR was determined by using 8% PEG in the presence of 10-20 µl normal human serum as the control. For comparison, 12 serum samples of human MG patients with and without thymoma or antistriated muscle antibody were also tested by using Mastomys muscle extract. Anti-AChR antibody was present in all 12 MG patients.

2.4 Determination of the number of ^{125}I-α-bungarotoxin binding sites per endplate

The diaphragms were removed from 5 Mastomys (3 males and 2 females) of between 25 and 27 months of age and incubated in Krebs' solution with ^{125}I-α-BuTx at a concentration of 1 µg.ml^{-1} for four hours as described elsewhere (Green, et al., 1975). The diaphragms were then washed overnight in Krebs' solution at 4°C and fixed in 2 per cent glutaraldehyde. Endplates were located by acetylcholinesterase staining and the diaphragms were divided into strips. The strips were weighed and measured. Endplate regions and nonendplate regions were counted in a gamma counter. Nonendplate radioactivity was subtracted from endplate reactivity. The results were expressed as endplate specific α-BuTx binding sites per mg weight of muscle fibres 1 cm in length.

2.5 Determination of AChR content of muscle extract

The limbs, including bone, from the same five Mastomys mentioned above were homogenized in 5 volumes of 100 mM phosphate, pH 7.4, and spun at 20,000 g for 30 minutes. The pellet was resuspended in an equal volume of 20 mM phosphate, pH 7.4, in 2 per cent Triton X-100. After extraction for 2 hours at room temperature, the homogenate was again centrifuged at 20,000 g for 1 hour. The supernatant was assayed for Triton-extracted AChR as previously described for human muscle (Newsom-Davis et al., 1978). Aliquots (100 µl) were taken and ^{125}I-α-BuTx added in excess (0.05 µg.ml^{-1}). After overnight incubation at 4°C, 25 µl was diluted to 225 µl with PTX buffer and applied to Whatman DE 81 filter disks. The disks were washed with PTX buffer and counted. Control incubation was done by preincubating muscle extract in nonlabelled α-BuTx before addition of ^{125}I-α-BuTx.

TABLE LV

ANTIACETYLCHOLINE RECEPTOR ANTIBODY IN MASTOMYS COMPARED WITH HUMAN MYASTHENIA GRAVIS PATIENTS AND HUMAN CONTROLS

Thymoma	Antistriated muscle antibody	Mean ± S.E.M. serum Anti-AChR titer in nmols.l^{-1} (Mastomys α-BuTx binding sites precipitated) Mastomys (number)	Human MG (number)*	Human controls (number)**
+	+	0.23 ± 0.03 (5)	11.4 ± 10.1 (3)	-
+	-	0.20 ± 0.02 (3)		-
-	+	0.15 ± 0.03 (4)	0.69 ± 0.84 (3)	-
-	-	0.19 ± 0.01 (2)	5.3 ± 6.7 (6)	0.25 ± 0.01 (3)

* Human α-BuTx binding sites precipitated 2.0-80.0 nmols.l^{-1}
** Human α-Butx binding sites precipitated 0.17±0.02 nmols.l^{-1}

TABLE LVI

ACETYLCHOLINE RECEPTORS IN MASTOMYS

Sex	Age in months	Thymoma	Antistriated muscle antibody	Specific Endplate ^{125}I-α-BuTx binding (x 10^7 sites/1 mg diaphragm strip)	AChR content of muscle extract (pmoles α-BuTx binding sites/g)	% of AChR in muscle extract precipitated by 8% PEG
Male	25	+	+	60.3	0.92	10%
Male	27	-	+	59.5	1.24	5%
Male	25	-	+	52.1	0.96	10%
Female	25	-	-	60.0	1.08	11%
Female	25	-	-	61.7	1.28	6%
Human control muscle		-	-	n.d.*	1.50	10%

* not determined

3 Results

The anti-AChR antibody levels determined in 14 Mastomys, 12 human MG patients and 3 human controls when sera were tested against Mastomys muscle extract are given in Table LV. There was no evidence of anti-AChR antibody in any of the 14 Mastomys, 9 of which were positive for antistriated muscle antibody, although Mastomys AChR was precipitated by human MG sera. The serum anti-AChR antibody titers in Mastomys determined by using Mastomys muscle extract correspond with those found in human negative control sera tested on Mastomys and human muscle extract.

The data on the number of α-BuTx binding sites at the motor endplate and in the muscle extracts are summarized in Table LVI. Only small differences were found among the 5 animals. In addition, no appreciable amount of the extracted AChR had IgG attached as determined by precipitation of α-BuTx labelled AChR by polyethylene glycol in the presence of normal human serum as carrier.

The occurrence of autoantibodies other than antistriated muscle and anti-AChR antibodies was also compared among the 14 Mastomys, 9 of which were positive and 5 negative for antistriated muscle antibody. No significant differences were found between the two small groups (Table LVII), although antinuclear and antiparietal antibodies were observed more frequently in animals having antistriated muscle antibody than in those not having them.

TABLE LVII

OTHER AUTOANTIBODIES FOUND IN MASTOMYS
WITH (+) AND WITHOUT (-) ANTISTRIATED MUSCLE ANTIBODY

Antibodies to:	Antistriated muscle antibody			
	+	(%)	-	(%)
nuclear antigens	3/9	(33)	1/5	(20)
smooth muscle	2/9	(22)	1/5	(20)
gastric parietal cells	6/9	(66)	2/5	(40)
thyroid colloid	2/9	(22)	1/5	(20)

4 Discussion

No significant titer of anti-AChR antibody was found in Mastomys, not even in animals having both thymoma and antistriated muscle antibody.

The small differences found in the number of α-BuTx binding sites at the motor endplate and in the muscle extracts may be regarded as being of no relevance if compared to human data. Fambrough et al. (1973) found that the α-BuTx binding sites at the motor endplate in patients with MG was reduced to 30% of control values and this was later confirmed by Ito et al. (1978).

The negative results which we obtained are disappointing, since there is a need for an animal model which spontaneously develops myasthenia gravis at a

relatively high frequency. Such a model is lacking at present. However, the results are not entirely surprising when one compares the incidence of thymoma and autoantibody to striated muscle in man and Mastomys. Although the incidence of antistriated muscle antibody in humans is about 0.2% in control populations (Feltkamp et al., 1974), the incidence in cases with thymoma is much higher than would occur by chance (about 30%, Oosterhuis et al., 1976). There is a highly significant association between the two phenomena. In addition, many patients with both thymoma and antistriated muscle antibody also have MG (Oosterhuis et al., 1976) and a high level of anti-AChR antibody (Vincent & Newsom-Davis, 1979). Such an association was not found in Mastomys. Of 145 aged Mastomys, 58 had a thymoma (40%) and 12 showed antistriated muscle antibody of the pattern found in human MG (21%). Seventy Mastomys had thymic atrophy, of which 17 had antistriated muscle antibody (24%). This suggests that thymomas and antistriated muscle antibody are unrelated in this animal species. Furthermore, no significant differences were found for antibodies directed against nuclear antigens, smooth muscle, gastric parietal cells and thyroid colloid between animals with and without antistriated muscle antibody in our study, while an increased incidence of antibodies to thyroid, gastric parietal cells and nuclei can be found in human patients with MG if compared to controls (Feltkamp, 1975).

Our findings seem to justify the conclusion that it is very unlikely that myasthenia gravis occurs with any frequency in Mastomys.

CHAPTER VIII

CONCLUDING REMARKS

This monograph deals with divergent aspects of the animal species Praomys (Mastomys) natalensis. Most of these have been extensively discussed in the various chapters. Since the emphasis of the study was on autoimmune phenomena, this chapter will primarily deal with general considerations concerning the value of Mastomys as a model for autoimmunity in man. For a critical evaluation, it is necessary to compare certain immunopathological findings in Mastomys with those described for the current animal models in this area of research.

As was known from previous studies (reviewed by Soga & Sato, 1977) and confirmed again by this one, Mastomys develops a wide variety of neoplastic and nonneoplastic lesions with age. Several authors (Snell & Stewart, 1969b; Solleveld, 1978; Stewart & Snell, 1968) had suggested that certain pathological changes observed in Mastomys may have an autoimmune basis. These included lymphocytic or lymphoplasmacellular thyroiditis, myositis, myocarditis and sialoadenitis, often accompanied by lymphoepithelial thymomas. As appears from this study, lesions such as lymphocytic dermatitis and lymphocytic infiltration of pancreatic islets may be added. Some evidence for the presence of autoantibodies in this species was provided by Strauss et al. (1968), who demonstrated serum globulin reactivity against striated muscle in all of the 14 aged Mastomys tested and by van Pelt & Blankwater (1972), who found antinuclear factor (ANF)-positive sera in approximately 14% of the males and 21% of the females older than one year. These findings prompted us to conduct a more extensive search for autoantibodies in aged Mastomys and to relate the presence of autoantibodies to the pathological changes found in the same group of animals. This investigation has demonstrated that Mastomys develops a wide variety of autoantibodies with age. On this basis, it seems to be an attractive model for studying autoimmunity. However, to justify its use in this area of research, Mastomys should offer additional advantages over the already extensively studied mouse strains which have proved to be helpful in investigating some aspects of autoimmunity in man. To reach a conclusion, it is necessary to briefly discuss the similarities and differences between Mastomys and the mouse models, especially with regard to the most common pathological findings and the types of autoantibodies. The so-called autoimmune-prone mouse strains are the NZB, (NZBxNZW)F_1 hybrid (B/W), BxSB/Mp and MRL/1. The NZB mouse and the B/W hybrid have been traditionally considered to be models for human autoimmune disease, particularly systemic lupus erythematosus (SLE) and autoimmune hemolytic anemia (Talal & Steinberg, 1974). Murphy & Roths (1978) have recently developed new inbred mouse strains which show an early onset of autoimmunity and lymphoproliferative changes controlled by defined genetic factors. Among these are

TABLE LVIII

PRINCIPAL PATHOLOGICAL CHANGES IN SEVERAL MOUSE STRAINS AND MASTOMYS

	Lymphoreticular system	Urogenital system	Cardiovascular system	Miscellaneous	References
NZB/Lac	thymus: atrophy lymph nodes: many plasma cells; lymphoreticular hyperplasia and neoplasia spleen: extensive erythropoiesis and hemosiderosis; lymphoreticular hyperplasia and neoplasia	kidney: chronic glomerulonephritis; genital tract: purulent inflammation	heart: atrial thrombi; focal ischemic necrosis vessels: hyalinization and fibrinoid necrosis of small arteries	ischemic liver necrosis; gastrointestinal tract ulceration; cystic endometrial hyperplasia; dystrophic calcinosis; lymphoplasmacellular infiltrates in various organs	Blankwater, 1978a Zurcher et al., in press van Zwieten et al., 1981
(NZBxNZW)F1	thymus: atrophy lymph nodes: normal, or mild lymphoreticular hyperplasia and lymphoreticular neoplasia spleen: extensive erythropoiesis and hemosiderosis; lymphoreticular hyperplasia and neoplasia	kidney: chronic membranous glomerulonephritis	heart: myocardial infarcts vessels: fibrinoid necrosis of small- and medium-sized arteries	lymphoplasmacellular infiltrates in various organs	Accini & Dixon,1979 Andrews et al.,1978 Haustein et al.,1973 Helyer & Howie, 1963 Hicks, 1966 Kelly & Winkelstein, 1980 Talal, 1976

MRL/1	thymus: atrophy lymph nodes: extreme hyperplasia spleen: increase of lymphoid compartment	kidney: subacute proliferative glomerulonephritis	heart: myocardial infarcts vessels: fibrinoid necrosis of small- and medium-sized arteries; acute and/or necrotizing polyarteritis	mononuclear vascular infiltrates in various organs; acute to chronic (peri-) arthritis	Accinni & Dixon,1979 Andrews et al., 1978 Murphy & Roths, 1978 Theofilopoulos et al. 1980
BxSB/Mp	thymus: atrophy lymph nodes: moderate hyperplasia spleen: moderate increase of lymphoid compartment; greatly increased erythropoiesis	kidney: in females acute to subacute exudative and proliferative glomerulonephritis; in males: chronic glomerulonephritis	heart: myocardial infarcts vessels: fibrinoid necrosis of small- and medium-sized arteries		Accinni et al., 1980 Andrews et al., 1978 Murphy & Roths, 1978
Mastomys	thymus: hyperplasia and neoplasia lymph nodes and spleen: many plasma cells lymphoreticular hyperplasia and neoplasia	kidney: membranous, proliferative and chronic glomerulonephritis genital tract: purulent inflammation	heart: myocardial degeneration and fibrosis, lymphocytic myocarditis vessels: hyalinization and fibrinoid necrosis of medium- and small-sized muscular arteries; periarteritis nodosa	a wide variety of neoplastic and non-neoplastic lesions, including lymphoplasmacellular inflammation of various organs	Kurokawa et al.,1968 Snell & Stewart, 1967, 1969b Stewart & Snell,1968 and Chapter V of this monograph

the BxSB/Mp strain, a recombinant inbred strain derived from a cross between a C57BL/6 female and an SB/LE male and which is characterized by moderate lymphadenopathy, hemolytic anemia and immune complex glomerulonephritis. Another strain is the MRL/1 which develops massive generalized lymph node enlargement due primarily to the proliferation of a Thy-1 positive cell population (Andrews et al., 1978), fulminant renal disease and arthritis in the hindlegs and feet which has many of the histological characteristics of human rheumatoid arthritis. The principal pathological changes in Mastomys and the mouse models are given in Table LVIII. Only a few lesions, particularly those which may have an immunological basis will be discussed.

The findings in the lymphoid organs in the mouse models as well as in Mastomys point primarily to an underlying dysfunction of the immune system. In addition to lymphoplasmacellular infiltrates in various organs and the development of lymphoreticular hyperplasia and neoplasia, the NZB mouse and Mastomys show a high proportion of plasma cells in the spleen, lymph nodes and Peyer's patches. Lymph node enlargement also occurs in the other mouse strains and varies considerably among them (Andrews et al., 1978), ranging from normal to two or three times normal size in B/W mice, 10 to 20 times normal size in BxSB/Mp males and up to 100 times normal size in MRL/1 mice. In the largest nodes of about 33% of BxSB/Mp males, a diffuse loss of lymphocytes with fibrosis of the stroma was found and, in 30 to 50% of MRL/1 mice with the largest nodes, extensive hemorrhage and necrosis were observed (Andrews et al., 1978). In contrast to the mouse strains, Mastomys of the Rijswijk colony develops thymic hyperplasia and neoplasia in a high percentage of cases (52%) with age.

Regarding renal lesions, the mouse models develop a lupus-like immune complex glomerulonephritis. After a light microscopic study of renal lesions in Mastomys, Snell & Stewart (1967) concluded that the pathological features of the glomerulonephritis in this animal species resembled those found in NZB mice and their hybrids as well as the nephropathy of human SLE. This resemblance was not so clear in the present work; e.g., the characteristic wire loop lesions were not found in Mastomys. Our findings are supported by an electron microscopic study of renal lesions of the same stock of Mastomys (van Noord et al., 1972) in which many dissimilarities between the renal lesions of NZB mice and Mastomys were noted. Van Noord et al. (1972) concluded that Mastomys spontaneously develops a peculiar type of immune complex glomerulonephritis.

Cardiac and vascular lesions were apparent in both animal species, particularly in the mouse models. Atrial or auricular thrombi associated with focal ischemic necrosis of cardiac muscles and hyalinization and fibrinoid necrosis of small muscular arteries were often seen in NZB mice (Blankwater, 1978a; Zurcher et al., in press; van Zwieten et al., 1981). In the B/W, BxSB/Mp and MRL/1 mice, vascular lesions were most frequently seen in the medium and small coronary arteries and arterioles (Accinni & Dixon, 1979). They were focal and segmental in

distribution and characterized by deposits of amorphous eosinophilic or PAS-positive material in the vessel walls. In addition, acute and chronic polyarteritis was seen in MRL/1 mice. Clear-cut myocardial infarcts were seen in all three mouse strains. In Mastomys, the major cardiac lesions were myocardial degeneration and fibrosis and lymphocytic myocarditis. A small proportion of the animals also had vascular lesions such as periarteritis nodosa, fibrinoid necrosis and hyalinization of medium- and small-sized arteries.

Regarding the other pathological changes, the variety of neoplastic and non-neoplastic lesions in Mastomys is much wider than in the mouse models. Obvious lesions in Mastomys are gastric carcinoid tumors, parathyroid hyperplasia and adenoma associated with fibrous osteodystrophy and degenerative osteoarthropathy. It must be mentioned that the incidence of gastric carcinoid tumors is low in animals of the Rijswijk colony (4%), but can be as high as 68% in other colonies (reviewed by Soga, 1977a).The much wider variety of lesions occurring in Mastomys than in the so-called autoimmune-prone mouse strains makes Mastomys of much more interest for aging research than those models, since "multiple pathology" is one of the hallmarks of aging. The more limited variety of lesions occurring in the mouse models may be attributed to their relatively short life span, which is determined mainly by the development of vascular lesions, hemolytic anemia or renal lesions.

The types of autoantibodies found in the mouse models and Mastomys are shown in Table LIX. From this survey, it appears that Mastomys probably develops a much wider variety of autoantibodies with age than do the mouse models.

Immune complexes have been found in kidney and vessel walls of the mouse models (Accinni & Dixon, 1979), in ovarian follicles of female B/W, MRL/1 and BxSB/Mp mice (Accinni et al., 1980) and in the skin of NZB and B/W mice (Blomjous & Feltkamp-Vroom, 1972; Gilliam et al., 1975; van Meter et al., 1978; Pertschuk et al., 1976; Sommer et al., 1975). The glomeruli of these mice have been shown to contain ds-DNA (Lambert & Dixon, 1968), ss-DNA (Seegal et al., 1969), the retroviral antigen gp 70 (Andrews et al., 1978), IgG antibodies to these antigens and the third component of complement (C3) (Andrews et al., 1978; Lambert & Dixon, 1968; Seegal et al., 1969; Theofilopoulos et al., 1980). Similar observations were made in ovarian follicles (Accinni et al., 1980). Immunoglobulin G, C3 and retroviral gp 70 were found in the vessel walls (Accinni & Dixon, 1979) and IgG, IgM and C3 at the epidermal-dermal junction of NZB and B/W mice (van Meter et al., 1978). In Mastomys, retroviral antigens were also found in the kidneys, suggesting the presence of viral antigen-antibody complexes (van Pelt et al., 1976). Attempts to elute antiviral antibodies from pooled kidneys and the detection of C-type particles in kidneys by electron microscopy were not successful. These findings led to the conclusion that virus production in Mastomys must be extremely low, which is in contrast to the situation in all mouse models. Hence, it is more conceivable that other immune complexes such as autoantigen-antibody

TABLE LIX

VARIETY OF AUTOANTIBODIES OCCURRING IN MASTOMYS AND SEVERAL MOUSE STRAINS

	NZB	B/W	BxSB/Mp	MRL/1	MASTOMYS
Autoantibodies to:					
nuclear antigens	+	+	+	+	+
erythrocytes	+	+	+	-	+
thymocytes	+	+	+	±	+*
cytoplasmic antigens**	-	-	-	-	+
colloid**	-	-	-	-	+
skeletal muscle**	-	-	-	-	+
smooth muscle**	-	-	-	-	+
gastric parietal cells**	-	-	-	-	+

* A preliminary investigation has shown that two of seven aged Mastomys of the Rijswijk colony had thymocytotoxic antibodies (van Pelt, unpublished data).

** It has been assumed that these autoantibodies are absent in the mouse strains, although literature data do not specifically indicate whether determinations for the presence of these autoantibodies have been made; therefore, they are indicated here as being negative.

complexes and/or tumor antigen-antibody complexes may play an important role in the pathogenesis of glomerulonephritis. Zurcher (1972) investigated whether renal lesions were more severe in animals bearing tumors than in those without. He found that animals with tumors had more severe renal lesions than those without, so that the involvement of tumor antigen-antibody complexes must be seriously considered. In the present work, no studies on determining the presence of immune complexes in Mastomys were performed , but it seemed of value to investigate whether certain autoantibodies occurred more frequently in animals with severely affected kidneys than in those with mild and moderate lesions. The same was done for animals with and without vascular lesions. No significant differences were found in male Mastomys with regard to the severity of renal lesions and between animals with and without vascular lesions. In females, the incidences of some autoantibodies were significantly increased, particularly in those showing vascular lesions. Since the findings in males and females were not in agreement, no hypothesis regarding a role of autoantibody in these lesions can be formulated. Studies to recover and identify the antigens involved are necessary .

From this survey, it appears that Mastomys and the mouse strains described above have much in common. However, Mastomys is distinguished from the mouse models by developing thymic hyperplasia and neoplasia, lesions such as thyroiditis, myositis, myocarditis, etc., and probably by a much wider variety of autoanti-

bodies. It was of importance to know whether these lesions could be related to autoantibodies. Since skin, salivary glands and pancreas were not used as substrates in the immunofluorescence tests, lesions such as lymphocytic dermatitis, sialoadenitis and lymphocytic infiltration of pancreatic islets could not be related to autoantibodies. The latter lesion was also described by Kolb et al. (1980) in 80% of NZB mice, in about 50% of MRL and B/W mice, and in less than 20% of BxSB mice. The authors reported that they were unable to find autoantibodies to islet cells in the sera of NZB mice.

Our study has shown that even though a significant difference was found in the mean number of autoantibody specificities in male Mastomys with thymoma when compared with males with thymic atrophy, the existence of a direct relationship between thymic changes and autoantibody formation is doubtful. This conclusion was based on the dissimilarity between males and females with regard to thymoma and autoantibody formation and the fact that antinuclear antibodies can be found as early as two months of age (Solleveld, unpublished data), while thymomas occur late in life (Snell & Stewart, 1975). It is, of course, self-evident that this does not exclude disturbances in thymic function at a very young age. The frequent occurrence of thymomas and autoantibodies to striated muscle (myasthenia gravis type) bears some resemblance to the situation in patients with myasthenia gravis (Feltkamp, 1975). However, this is only a superficial resemblance, as no clinical myasthenia-like disease has been observed in Mastomys. Moreover, no autoantibodies to acetylcholine receptors were found in this study; therefore, it is very unlikely that myasthenia gravis occurs with any frequency in Mastomys.

As far as could be observed, the other autoantibodies also seemed not to be related to overt clinical disease. In this respect, Mastomys resembles aged man, in whom a variety of autoantibodies can be found, and only a few of which appear to be associated with diseases (Aho, 1980; Walford, 1980). Antibodies commonly associated with clinical disease in man are, e.g., antiparietal and antierythrocyte antibodies. Antiparietal cell antibodies are associated with inflammation and atrophy of the gastric fundal mucosa (Type A gastritis) (Strickland & Mackay, 1973) and antierythrocyte antibodies with hemolytic anemia (Dacie & Worlledge, 1975). These two types of autoantibodies were also found in Mastomys but were not associated with clinical disease or with histological lesions, which suggests that they are possibly directed to antigens other than those involved in man. A lesion observed in Mastomys which was positively associated with autoantibodies but not with overt clinical disease was thyroiditis. This lesion could be related to the presence of autoantibodies to colloid. A uniform staining pattern of colloid was observed in Mastomys, so that it is most likely that the autoantibodies are directed to the so-called second colloid antigen (Pinchera et al., 1980). Autoantibodies of this type in man are also associated with thyroiditis. Other autoantibodies in Mastomys, such as autoantibodies to mitochondria, smooth muscle and cytoplasmic antigens, were not associated with lesions or diseases. The significance of these autoantibodies in man is also still unclear (Aho, 1980).

The data indicate that Mastomys resembles man and the mouse models in certain respects. The difference between Mastomys and the mouse models can be summarized as follows: the autoimmune-prone mouse strains serve primarily as models for specific autoimmune disease, e.g., NZB mice for hemolytic anemia, B/W, BxSB/Mp and MRL/1 for lupus erythematosus and the latter possibly also for rheumatoid arthritis, while Mastomys lends itself more to being a model for the study of general mechanisms underlying autoimmunity. The development of Mastomys as a model for a specific autoimmune disease appears to be of limited value, because the presence of various other antibodies may interfere with the interpretation of results obtained.

The immunologic abnormality underlying autoimmunity in the murine strains is not yet elucidated. Talal et al. (1979) stated that enhanced T cell activity in MRL/1 mice specifically promotes autoantibody formation, while a deficiency of suppressor T cells is associated with autoimmunity in NZB mice. On the other hand, Theofilopoulos et al. (1980), who performed a comparative immunologic analysis of the murine strains with autoimmune manifestations, reached other conclusions. They pointed out that an enhanced helper T cell activity in MRL/1 mice may not necessarily be a prerequisite for the development of autoimmunity, since autoimmune disease also develops in the congenic MRL/1 substrain which does not show enhanced activity of T helper cells. Regarding the functional defect in T suppressor cells in NZB mice, Theofilopoulos et al. (1980) found that both antigen-specific and antigen-nonspecific suppressor T cells were within normal limits in NZB, B/W, BxSB/Mp and MRL/1 shortly before the clinical onset of overt disease. The only common factor they noted in these murine strains was B cell hyperactivity which appeared early in life in NZB and B/W and somewhat later in life in BxSB/Mp and MRL/1 mice. However, it is not known whether this activity is responsible for subsequent specific autoantibody production. This is illustrated by the results of a recent study (Pisetsky et al., 1980) in which peak anti-Sm antibody responses of MRL/1 mice did not differ appreciably from those of MRL/1 mice, while B cell activation is not apparent in the latter strain. Theofilopoulos et al. (1980) concluded from their studies and those of others that a given autoimmune condition may result from one of several different defects that differ from one individual to another. Therefore, it seems of interest to investigate in what way age-related functional changes in the immune system are related to autoimmunity in Mastomys. Moreover, since Mastomys develops with age a wide variety of autoantibodies mimicking in certain respects the human situation, it offers the possibility to evaluate the significance of autoantibodies occurring in aged man. A question of considerable importance is whether autoantibodies in apparently healthy aged individuals reflect subclinal disease in all cases or whether they are present only transiently (Hawkins et al., 1979). By performing longitudinal autoantibody determinations coupled with serial killing experiments in Mastomys, an insight may be gained into whether certain autoantibodies occur transiently or persist and accumulate progressively with age. Moreover, it can be investigated whether auto-

antibody formation and the time of appearance of the autoantibodies can be influenced experimentally, e.g., by thymectomy, castration, dietary manipulation, etc. Although some of these investigations can now be performed, of prime importance are studies directed at obtaining basic immunology data, since almost no information is available on immunological parameters in Mastomys. These baseline data are essential before further progress is possible. Some information can be obtained by employing reagents used for the mouse, since there is a distinct cross-reactivity between antisera raised against mouse immunoglobulins and Mastomys immunoglobulins. It is quite clear, however, that much basic work needs to be done before the true value of Mastomys as a model for aging research is realized.

SUMMARY

The study presented in this monograph was primarily concerned with the occurrence of pathological changes and autoantibody formation in aged Praomys (Mastomys) natalensis. Since this is a relatively unknown laboratory animal a part of the monograph is devoted to general information on Mastomys and to some characteristics of animals of the Rijswijk colony which were used in this study.

The introductory chapter (Chapter I) deals with the phenomenon of aging. Aging was defined as a time dependent process in which the body can no longer cope with environmental factors and change as easily as it could initially. The fundamental aspects of the aging process are the same in all mammals. Rodents are most often used as models for obtaining basic information on the aging process in man, primarily because: a) they have relatively short life spans; b) well-established inbred strains are available; c) they can be housed and handled under well-defined conditions; and d) their costs of production are relatively low as compared to larger laboratory animals.

Previous studies have demonstrated that Mastomys develops a wide variety of neoplastic and nonneoplastic lesions with age, some of which are suggestive for autoimmune disorders. These findings made this animal species of interest for aging research, since cancer and autoimmune phenomena are also common in the aged human population. The finding of a number of lesions which were suggestive for autoimmune disorders made it of interest to further study Mastomys with regard to these conditions.

Chapter II gives general information on Mastomys. It belongs to the family Muridae and is intermediate in size between Mus and Rattus. It is distinguishable from Mus and Rattus in some anatomical features. These are: a) the presence of a well-developed prostate in female Mastomys; b) the absence of preputial and clitoral glands in males and females, respectively; c) the presence of 14 to 18 mammary glands, hence the origin of its common name, the multimammate mouse; and d) the presence of an os penis with a cordate anterior process instead of a broad one as in the rat or a lanceolate one as in the mouse. Like the rat and unlike the mouse, Mastomys lacks a gall bladder.

The taxonomic position of Mastomys has been the subject of discussion since early in the century and is primarily concerned with the question of whether Mastomys merits recognition as a genus or a subgenus. A recent study in which cranial characters were analyzed strongly indicates that Mastomys must be considered a genus, but studies on characters other than only cranial ones are needed to reach a definite conclusion. Moreover, the discussion is not limited to the generic status alone; it also revolves around the position of Mastomys at the species level. Two chromosome forms have been found, namely, animals with 32 and

36 chromosomes, which even occur sympatrically. There is enough evidence now to regard the two chromosome forms as two separate species. Such a decision will also have implications for the species name. The type locality of Praomys (Mastomys) natalensis is Natal. Up to now, only animals with 32 chromosomes have been found in that area of South Africa. If no animals with 36 chromosomes are found in this area, the 32 chromosome form should then be given the species name natalensis and the 36 chromosome one would have to revert to the earlier species name, coucha. In the wild, Mastomys causes extensive damage to crops and also plays a role as vector of at least two zoonotic diseases. These are plague and Lassa fever. Both diseases are discussed briefly, as is the role of Mastomys in them.

Mastomys was introduced into the laboratory in 1939 and was used as a test animal for plague-suspected material and for the evaluation of plague vaccines. In the fifties, it appeared that Mastomys spontaneously developed a high incidence of stomach cancer. This stimulated the interest of research workers in the area of cancer research. About the same time, it was discovered that Mastomys was an excellent laboratory animal for protozoological and helminthological research. These findings have led to a world-wide distribution of this animal species. The origin and interrelationship of the various laboratory colonies, all consisting of animals with 36 chromosomes, are presented in this chapter. Since the introduction of this animal species as a laboratory animal, hematological and clinical biochemical data have become available and are reviewed. The hematology data and serum protein values do not differ much from those of the mouse and the rat. Serum urea nitrogen and creatinine concentrations are a factor of 2 or more higher than those reported for the mouse and the rat. The serum GPT and GOT levels are lower in Mastomys than in the mouse and the rat and the LDH levels higher in Mastomys. Chapter II ends with a summary of literature data on the use of Mastomys in various areas of biomedical research.

Chapter III deals with a number of unrelated topics, all concerned with Mastomys of the Rijswijk colony. These include the following: a) a TG- and C-banding chromosome analysis; b) breeding data of random bred and inbred Mastomys; c) the extent of inbreeding; d) growth data of random bred and inbred Mastomys; and e) survival data of random bred Mastomys.

TG- and C-banding chromosome analysis of our laboratory-bred animals showed only minor differences when compared with the data from such studies performed in animals from another laboratory colony which probably originated from the same stock as did the Rijswijk colony and with those for wild caught Mastomys from Zimbabwe.

The breeding data showed that the major problem in establishing and maintaining an inbred as well as random bred Mastomys colony was the occurrence of a large number of preweaning deaths. The high loss was due to cannibalism by the mothers. The first days after birth appeared to be the most critical period. Selection for animals not having this undesirable trait is necessary as is selection

of animal caretakers. Nervous or noisy persons were found not to be suitable for this job; they excited the animals too much and this resulted in increased cannibalism. Moreover, this study indicated that females not becoming pregnant within 60 days after introduction to the male should be culled from the breeding colony and that females should not be allowed to produce more than four litters. As criteria for the extent of inbreeding, serum transferrin phenotypes were investigated and skin transplantations were performed in animals from the random bred and inbred colonies. Clearly different transferrin phenotypes (M, KM, and kM) were found in the animals of the random bred colony, whereas only one phenotype (K) was identified in the inbred animals. Skin grafting from random bred to random bred animals, from random bred to inbred animals and vice versa resulted in rejection within 10 to 12 days. However, skin grafting between inbred animals gave different results. Some animals showed a permanent take of the graft, while others rejected it in approximately 10 to 12 days. These data indicate that the breeding program applied to the random bred animals was successful and that to the inbred colony only partly so, in spite of 30 generations of brother-sister mating.

No significant differences were observed in mean body weights between animals of the random bred and inbred colonies. At one year of age the males had reached a weight of 80 grams and the females 50 to 60 grams. Regarding survival data, an obvious difference was found in the 50% survival age between males housed 5 per cage and those housed individually. Males housed individually lived significantly longer than those housed 5 per cage. This difference can be explained by the aggressive behavior of the males when housed together. The 50% survival age for animals housed 5 per cage was 20 months for males and 27 months for females and the maximum ages were 35 and 38 months, respectively. The 50% survival age for males housed individually was between 23 and 24 months.

In Chapter IV, the results of an investigation on the occurrence of autoantibodies in a separate population of aged Mastomys are described. Sera of a total of 145 Mastomys were tested; included were 60 males and 85 females. The animals varied in age from 18 to 39 months. The sera were screened by an indirect fluorescent antibody technique against sections of various rat organs. A broad spectrum of autoantibodies were found, including autoantibodies to nuclear antigens, cytoplasmic antigens, erythrocytes, thyroid colloid, mesangial antigens, renal distal tubules, striated muscle, gastric parietal cells and smooth muscle. On comparing antinuclear antibody reactivity in various tissues, it appeared that the frequency of occurrence depended on the substrate used. It was highest when kidney was used as substrate and lowest when liver and gastric mucosa were used. The exact time of appearance of the various autoantibodies could not be determined in this study, but most developed before 20 months of age; some were already present at 7 months of age. Autoantibody titers showed a wide range among individual animals but were generally high. The calculated mean titer of the various autoantibodies ranged from 34 to > 1600. The majority of them were of the

IgM class. Only those to thyroid colloid and to striated muscle of the myasthenia gravis type were of the IgG class. Autoantibodies to erythrocytes and to striated muscle of the intermyofibrillar type were of both the IgG and IgM classes. The number of autoantibody specificities in males and females varied from 0 to 6; however, animals with 3 different autoantibody types were most common.

A histopathological survey of the same group of animals in which autoantibodies were determined is given in Chapter V. The most frequently observed neoplastic lesions were lymphoepithelial thymomas, hepatocellular neoplasms, adrenocortical tumors, parathyroid adenomas and endometrial stromal polyps. The most common nonneoplastic lesions were fibromyxomatous changes of cardiac valves, degenerative joint disease, ovarian atrophy, vacuolization of brain tissue, moderate to severe glomerulonephropathy, myocardial degeneration and fibrosis, testicular atrophy, spinal cord compression, hyperkeratosis of the squamous part of the stomach, nephritic scars, seminal vesiculitis and prostatic hyperplasia. Lesions which were of interest in terms of autoantibody formation were lymphoepithelial thymomas, lymphocytic or lymphoplasmacellular dermatitis, sialoadenitis, thyroiditis, myositis, myocarditis and lymphocytic infiltration of pancreatic islets. Those which may have been the result of immune complex formation involving autoantigens included glomerulonephritis, periarteritis nodosa and fibrinoid necrosis of medium- and small-sized muscular arteries.

In Chapter VI, an attempt is made to relate the presence of autoantibodies to pathological changes. Such a wide variety of autoantibodies and lesions was found that only a selected number of autoantibody-lesion combinations was investigated. It appeared that the existence of a direct relationship between one of the thymic changes (atrophy, hyperplasia and neoplasia) and autoantibody formation was doubtful. An association was found between autoantibodies and tumors involving the lymphoreticular tissues. The extent of involvement of the lymphoreticular tissues appeared to influence the mean number of autoantibodies per animal. Animals in which the lymphoreticular tissues were entirely replaced by tumor cells did not have autoantibodies.

Only thyroiditis could be related to autoantibodies. An association between lesions such as dermatitis, sialoadenitis and lymphocytic infiltration of pancreatic islets and the presence of autoantibodies could not be determined, since skin, salivary glands and pancreas were not used as substrates in the immunofluorescence test. Thyroiditis was associated with autoantibodies to thyroid colloid. The occurrence of autoantibodies to striated muscle of the myasthenia gravis type and thymomas in Mastomys shows a certain resemblance to the situation in human patients suffering from myasthenia gravis. On the other hand, however, typical clinical signs of this disease have not been observed and tests for neuromuscular function in Mastomys have not been reported. Since no animal model that spontaneously develops this disease is available, it seemed worthwhile to study Mastomys in more detail in this regard (Chapter VII). Antiacetylcholine receptor antibody titers were determined in animals with and without thymomas and with and without

autoantibodies to striated muscle of the myasthenia gravis type. No significant differences in autoantibody titers were found among the different groups; therefore, it is very unlikely that myasthenia gravis occurs with any frequency in Mastomys.

The final chapter (Chapter VIII) deals primarily with general considerations concerning the value of Mastomys as a model for autoimmunity in man. This necessitated a comparison between Mastomys and several mouse models for autoimmunity. The principal pathological changes and the types of autoantibodies occurring in Mastomys and these mouse strains are discussed. It is concluded that the mouse strains are likely to be of more value for specific autoimmune diseases and that Mastomys is more suited for studying general phenomena underlying autoimmunity.

SAMENVATTING

Het in deze monografie beschreven onderzoek heeft voornamelijk betrekking op spontaan voorkomende pathologische veranderingen en autoantilichamen bij Praomys (Mastomys) natalensis. Daar deze diersoort betrekkelijk onbekend is als proefdier is een deel van deze monografie gewijd aan algemene informatie over deze diersoort alsmede aan enkele kenmerken van Mastomys van de kolonie uit Rijswijk. Dit laatste omdat het beschreven onderzoek heeft plaatsgevonden bij dieren van deze kolonie.

Het inleidende hoofdstuk (Hoofdstuk I) handelt over veroudering in algemene zin. Veroudering werd gedefinieerd als een tijdsafhankelijk proces dat uiteindelijk resulteert in een minder adekwaat reageren van het lichaam op prikkels van buitenaf dan waartoe het aanvankelijk in staat was. De fundamentele aspekten van veroudering zijn hetzelfde in alle zoogdieren. Dit heeft geleid tot dierexperimenteel onderzoek om meer inzicht te krijgen in de basale mechanismen die ten grondslag liggen aan het verouderingsproces bij de mens. Knaagdieren worden het meest gebruikt en wel omdat a) ze een betrekkelijk korte levensduur hebben, b) inteeltstammen ter beschikking staan, c) ze gehouden kunnen worden onder gestandaardiseerde omstandigheden en d) ze betrekkelijk goedkoop zijn.

Onderzoekingen hebben uitgewezen dat Mastomys spontaan een grote variëteit aan tumoreuze en niet-tumoreuze afwijkingen ontwikkelt, waarvan enkele in verband gebracht zouden kunnen worden met autoimmuniteit. Aangezien kanker en autoimmuniteit frekwent voorkomen bij de oude mens leek Mastomys een geschikt proefdier te zijn voor verouderingsonderzoek. Het voorkomen van een aantal afwijkingen bij Mastomys, die zouden kunnen passen bij een immunologische reaktie tegen eigen lichaamskomponenten, was aanleiding deze diersoort nader te bestuderen met betrekking tot dit aspekt.

Hoofdstuk II is geheel gewijd aan algemene informatie over Mastomys. Mastomys behoort tot de familie Muridae en ligt wat grootte betreft tussen de muis en de rat in. Mastomys verschilt van de muis en de rat in enkele anatomische kenmerken, zoals a) de aanwezigheid van een goed ontwikkelde prostaat bij het vrouwtje, b) de afwezigheid van preputiaal- en clitoraalklieren bij respektievelijk het mannetje en het vrouwtje, c) de aanwezigheid van 14 tot 18 melkklieren, vandaar de veel gebruikte benaming "multimammate mouse" (veeltepelmuis) en d) de aanwezigheid van een hartvormig os penis in plaats van een breed of lancetvormig os penis zoals wordt gevonden bij respektievelijk de rat en de muis. Evenals de rat, maar in tegenstelling tot de muis, heeft Mastomys geen galblaas.

De taxonomische positie van Mastomys, een genus of subgenus, staat reeds vanaf het begin van deze eeuw ter diskussie. De resultaten van een recent uitgevoerd onderzoek betrekking hebbend op een aantal schedelkenmerken, wijzen duidelijk in de richting van een genus, maar er dienen ook nog andere kenmerken onderzocht te worden voordat men tot een definitieve standpuntsbepaling kan

komen. Een diskussie speelt zich ook af rond de soortnaam. Twee chromosoomvormen zijn namelijk bekend: dieren met 32 en 36 chromosomen. Beide vormen komen zowel gescheiden levend, als wel samenlevend voor. Er lijken voldoende aanwijzingen te zijn om ze als twee aparte soorten te beschouwen, hetgeen implikaties heeft voor de naamgeving. De soortnaam natalensis is afgeleid van de streek "Natal" en geeft het type vindplaats aan. In deze streek van Zuid Afrika is tot op heden alleen de 32 chromosoomvorm gevonden. Indien in de toekomst in dit gebied geen dieren met 36 chromosomen worden gevonden zal de 32 chromosoomvorm natalensis dienen te heten en dan zal de 36 chromosoomvorm de reeds eerder gebruikte soortnaam coucha moeten krijgen.

In het wild brengt Mastomys grote schade toe aan gewassen en speelt ook een rol als overbrenger van ziekten zoals pest en Lassakoorts naar de mens. Beide ziekten worden kort besproken, evenals de rol die Mastomys speelt bij de overbrenging van deze ziekten.

In 1939 werd Mastomys geintroduceerd in het laboratorium. Aanvankelijk diende dit dier, i.v.m. zijn grote gevoeligheid voor de pestbacil, als testdier voor onderzoek van mogelijk besmet materiaal en voor het uittesten van de werking van vaccins tegen de pest. In de vijftiger jaren ontdekte men dat deze diersoort spontaan een hoge incidentie aan maagtumoren ontwikkelde, waarmede de interesse van kankeronderzoekers voor dit proefdier was gewekt. Ongeveer tegelijkertijd bleek ook dat Mastomys een uiterst bruikbaar proefdier was voor experimentele protozoaire en vermineuze infekties. Deze bevindingen tezamen hebben geleid tot een wereldwijde verspreiding van Mastomys. De herkomst en de onderlinge verwantschap van de belangrijkste laboratoriumkolonies, alle bestaande uit dieren met 36 chromosomen, zijn weergegeven in dit hoofdstuk. Na de introduktie als proefdier zijn zowel hematologische als klinisch biochemische waarden bekend geworden. De hematologische en de serumeiwitwaarden komen min of meer overeen met die van de muis en de rat. Serum ureum en creatininewaarden blijken minstens een faktor 2 hoger te zijn dan in de muis en de rat. Wat de enzymwaarden betreft zijn de sGPT en sGOT waarden lager en de LDH waarden hoger in Mastomys dan in de muis en de rat. Hoofdstuk II eindigt met een opsomming van literatuurgegevens over het gebruik van Mastomys in verschillende biomedische disciplines.

In Hoofdstuk III worden een aantal geheel verschillende kenmerken van Mastomys van de kolonie uit Rijswijk weergegeven. Het betreft a) een TG- en C-banding chromosoom analyse, b) fokgegevens van wilde kweek en inteelt Mastomys, c) de mate van inteelt van zowel wilde kweek als inteelt dieren, d) gewichtgegevens van wilde kweek en inteelt dieren en e) overlevingsgegevens van wilde kweek Mastomys.

De TG- en C-banding chromosoomanalyses gaven alleen maar kleine verschillen te zien in vergelijking met overeenkomstige onderzoekingen uitgevoerd bij dieren uit een andere laboratoriumkolonie, die waarschijnijk dezelfde oorsprong heeft als de kolonie uit Rijswijk en bij in het wild gevangen dieren uit Zimbabwe.

De fokgegevens van zowel de wilde kweek als inteelt Mastomys kolonie wezen uit dat een hoog speenverlies het belangrijkste probleem was voor het opzetten en handhaven van een fokkolonie. Dit hoge speenverlies bleek het gevolg te zijn van kannibalisme van de moederdieren, dat vooral werd waargenomen tijdens de eerste dagen na de geboorte. Selektie op deze ongewenste eigenschap is nog steeds noodzakelijk evenals selektie op dierverzorgers c.q. dierverzorgsters die met deze dieren omgaan. Nerveuze of lawaaierige personen bleken ongeschikt te zijn voor de verzorging van Mastomys omdat zij opwinding teweegbrachten onder de dieren, hetgeen zich direkt manifesteerde in kannisbalisme. Bovendien bleek dat vrouwtjes die niet drachtig werden binnen 60 dagen en vrouwtjes die vier nesten geproduceerd hadden, beter uit de fok konden worden genomen. Om een inzicht te verkrijgen in de mate van inteelt werden serumtransferrine fenotypes bepaald alsmede huidtransplantaties uitgevoerd. Verschillende transferrine fenotypes werden gevonden in dieren van de wilde kweek kolonie (M, KM en kM) en maar één (K) in dieren van de inteelt kolonie. Huidtransplantaties uitgevoerd tussen wilde kweek dieren en tussen wilde kweek en inteelt dieren resulteerden steeds in afstoting in 10 tot 12 dagen. De resultaten van huidtransplantaties tussen inteeltdieren waren echter wisselend. Sommige dieren toonden een blijvende "take", terwijl andere het transplantaat in 10 à 12 dagen afstootten. Deze gegevens duiden erop dat het fokprogramma voor dieren van de wilde kweek kolonie met sukses was uitgevoerd maar voor de inteelt dieren maar gedeeltelijk suksesvol was geweest, ondanks 30 generaties van broer x zuster kruisingen.

Geen opvallende verschillen werden waargenomen tussen de gemiddelde lichaamsgewichten van wilde kweek en inteelt dieren. De mannetjes hadden na een jaar een gewicht bereikt van 80 gram en het gewicht van de vrouwtjes lag op deze leeftijd tussen de 50 en 60 gram. Betreffende de overlevingsgegevens werd een opvallend verschil gevonden tussen mannetjes gehuisvest in groepen van vijf en individueel gehuisveste mannetjes. Individueel gehuisveste mannetjes leefden langer dan die, welke gehuisvest waren in groepen. Dit verschil werd toegeschreven aan het agressieve gedrag van de mannetjes dat het duidelijkst tot uiting kwam als ze met geslachtsgenoten werden gehuisvest. De 50% overlevingsleeftijd voor dieren gehuisvest 5 per kooi bedroeg voor mannetjes 20 maanden en voor vrouwtjes 27 maanden en de maximum leeftijden waren respektievelijk 35 en 38 maanden. De 50% overlevingsleeftijd voor individueel gehuisveste mannetjes lag tussen de 23 en 24 maanden.

In Hoofdstuk IV zijn de resultaten weergegeven van een onderzoek naar het voorkomen van autoantilichamen in Mastomys. In totaal werden 145 sera van Mastomys onderzocht, die afkomstig waren van 60 mannetjes en 85 vrouwtjes. De dieren varieerden in leeftijd van 18 tot 39 maanden. Voor het aantonen van autoantilichamen werd een indirekte immunofluorescentietechniek gebruikt waarbij ratteorganen dienden als substraat. Een breed spektrum aan autoantilichamen werd gevonden. Het betrof autoantilichamen tegen kernantigenen, cytoplasmatische anti-

genen, erythrocyten, schildklier colloid, antigenen voorkomend in het mesangium van de nier, distale tubuli van de nier, dwarsgestreept spierweefsel, parietale cellen van de maag en gladde spier. De antilichaamreaktiviteit tegen kernantigenen bij gebruik van verschillende substraten werd onderling vergeleken. De hoogste reaktiviteit werd gevonden als nierweefsel werd gebruikt als substraat en de laagste als lever en maag werden gebruikt.

Het juiste tijdstip waarop de autoantilichamen ontstonden kon niet worden vastgesteld in dit onderzoek. De meeste autoantilichamen waren reeds vóór de leeftijd van 20 maanden aanwezig en sommige zelfs al op een leeftijd van 7 maanden. De autoantilichaamtiters waren over het algemeen hoog en varieerden van 34 tot > 1600. De meeste autoantilichamen behoorden tot de IgM klasse. Autoantilichamen die waren gericht tegen schildklier colloid en tegen dwarsgestreept spierweefsel van het zogenaamde myasthene type, behoorden tot de IgG klasse. Autoantilichamen tegen erythrocyten en tegen dwarsgestreept spierweefsel van het intermyofibrillaire type behoorden zowel tot de IgG als IgM klassen. Het aantal autoantilichaamspecificiteiten in mannelijke en vrouwelijke Mastomys varieerden van 0 tot 6, doch het meest frekwent werden 3 verschillende typen autoantilichamen per dier gevonden.

In Hoofdstuk V is een overzicht gegeven van de histopathologie van dezelfde groep dieren waarin ook autoantilichamen werden bepaald. De meest frekwent voorkomende tumoreuze aandoeningen waren lymfoepitheliale thymomen, leverceltumoren, bijnierschorstumoren, bijschildklieradenomen en poliepen in de uterus. De meest voorkomende niet-tumoreuze aandoeningen waren fibromyxomateuze veranderingen van de hartkleppen, degeneratieve gewrichtsafwijkingen, atrofie van de ovaria, vakuolisatie van hersenweefsel, middelmatige tot ernstige glomerulonefropathie, degeneratie en fibrose van de hartspier, atrofie van de testikels, kompressie van het ruggemerg, hyperkeratose van de voormaag, littekens in de nier, ontsteking van de zaadblaas en hyperplasie van de prostaat. Afwijkingen welke in verband gebracht zouden kunnen worden met autoantilichaamvorming waren lymfoepitheliale thymomen, lymfocytaire of lymfoplasmacellulaire ontstekingen van huid, speekselklier, schildklier, skeletspier, hartspier en lymfocytaire infiltratie van eilandjes van Langerhans. Afwijkingen welke het gevolg zouden kunnen zijn van immuunkomplex vorming waarbij autoantigenen een rol zouden kunnen spelen, waren glomerulonefritis, periarteritis nodosa en fibrinoide nekrose van middelgrote en kleine arteriën.

In Hoofdstuk VI is getracht een verband te leggen tussen autoantilichamen en pathologische veranderingen. Gezien het voorkomen van een grote diversiteit aan autoantilichamen en afwijkingen werd een selektief onderzoek uitgevoerd. Geen direkt verband werd gevonden tussen het voorkomen van thymusveranderingen (atrofie, hyperplasie en thymoom) en autoantilichamen. Een duidelijk negatieve korrelatie werd gevonden tussen autoantilichamen en tumoren, voorkomend in het lymfoid weefsel. Het gemiddeld aantal autoantilichaamspecificiteiten bleek afhankelijk te zijn van de graad van aantasting van het lymforeticulaire weefsel. Dieren

waarbij het normale lymforeticulaire weefsel geheel vervangen was door tumorweefsel bleken geen autoantilichamen te hebben.

Alleen een direkt verband werd gevonden tussen thyroiditis en autoantilichamen. Een dergelijk verband kon niet worden vastgesteld voor de afwijkingen: dermatitis, sialoadenitis en lymfocytaire infiltratie van eilandjes van Langerhans, daar huid, speekselklier en pankreas niet werden gebruikt als substraat in de immunofluorescentietest. Thyroiditis was geassocieerd met autoantilichamen tegen thyroid colloid. Het voorkomen van autoantilichamen tegen dwarsgestreept spierweefsel van het myasthene type en thymomen toonde enige gelijkenis met hetgeen wordt gevonden bij mensen die lijden aan myasthenia gravis. Klinische verschijnselen kenmerkend voor deze aandoening werden niet waargenomen in Mastomys doch neuromuskulaire testen werden niet uitgevoerd. Daar er echter geen diermodel bestaat dat spontaan deze ziekte ontwikkelt, leek het de moeite waard een onderzoek in te stellen naar het al of niet voorkomen van myasthenia gravis bij Mastomys (Hoofdstuk VII). Onderzocht werd of antilichamen tegen acetylcholine receptoren voorkwamen bij dieren met en zonder thymomen en met en zonder autoantilichamen tegen dwarsgestreept spierweefsel van het myasthene type. Geen noemenswaardige verschillen werden gevonden tussen de verschillende groepen zodat de konklusie luidde dat het onwaarschijnlijk is dat myasthenia gravis bij Mastomys spontaan voorkomt.

Het laatste hoofdstuk (Hoofdstuk VIII) is voornamelijk gewijd aan de waarde die aan Mastomys moet worden toegekend als model voor de bestudering van autoimmuniteit bij de mens. Hiervoor was het noodzakelijk Mastomys te vergelijken met reeds bestaande "muismodellen" op dit gebied. Aandacht werd besteed aan de belangrijkste pathologische veranderingen en autoantilichamen voorkomend bij Mastomys en bij deze muizestammen. Hieruit kwam naar voren dat de muizestammen met name van waarde blijken te zijn voor de bestudering van specifieke autoimmuunziekte(n) en Mastomys meer geschikt lijkt te zijn voor de bestudering van de algemene aspekten die aan autoimmuniteit ten grondslag liggen.

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APPENDIX

A partial listing of addresses of laboratories and institutes maintaining Mastomys colonies

Agouti-colored Mastomys colonies

Japan

College of Biomedical Technology
Niigata University
Asahimachidori 1
Niigata City 951

Contact person: Dr.J. Soga

The Research Institute for Tuberculosis, Leprosy and Cancer
Department of Oncology
Tohoku University
4-1 Seiyomachi
Sendai 980

Contact person: Dr. H. Sato

Institute of Medical Science
Tokyo University
Tokyo 108

Contact person: Dr. K. Suzuki

Aichi Cancer Center Research Institute
Laboratory of Pathology
Tashiro-cho
Chikusa-ku
Nagoya 464

Contact person: Dr. S. Hosoda

The Netherlands

Institute for Experimental Gerontology TNO
P.O. Box 5815
2280 HV Rijswijk

Contact person: Dr. C.F. Hollander

United Kingdom

London School of Hygiene and Tropical Medicine
Keppel Street (Gower Street)
London WC1E 7HT

Contact person: Mr. M.D. Shefki

United States of America

National Institutes of Health
Division of Research Services
Building 14 A, Room 102
Bethesda, Maryland 20205

Contact person: Dr. C. Hansen

Northwestern University
The Medical School
Ward Memorial Building
303 E. Chicago Ave.
Chicago, Illinois 60611

Contact person: Dr. J.M. Holland

Chamois-colored Mastomys colonies

Federal Republic of Germany

Institute for Parasitology and Parasitic Diseases of Animals
Justus-Liebig University
Rudolph-Buchheim-Strasse 2 — GRA-Giessen strain
D-6300 Lahn-Giessen 1

Contact person: Dr. H. Zahner

Republic of South Africa

Cancer Research Institute
University of Durban-Westville — Y- and Z-strains
Private Bag X 54001
Durban 4000

Contact person: Dr. J.D. Randeria

CURRICULUM VITAE

Na het behalen van het MULO diploma in 1961 volgde indiensttreding bij het Radiobiologisch Instituut TNO te Rijswijk als assistent van het hoofd van de biotechnische afdeling. In 1963 werd het diploma chemisch analist I behaald en vervolgens het diploma zoölogisch analist I en II in respectievelijk 1964 en 1965. Van oktober 1965 tot maart 1967 werd het dienstverband onderbroken voor het vervullen van de militaire dienstplicht. In juli 1970 werd het staatsexamen HBS-B afgelegd, waarna in september de studie in de diergeneeskunde werd aangevangen. In juni 1972 werd het kandidaatsexamen behaald, in juni 1974 het doctoraal examen en in oktober 1976 "cum laude" het dierenartsexamen. Op 1 januari 1976 vond aanstelling plaats tot wetenschappelijk medewerker aan het Instituut voor Experimentele Gerontologie TNO (Directeur Prof.Dr.C.F.Hollander) te Rijswijk, alwaar het hier beschreven onderzoek heeft plaatsgevonden. Het proefschrift vormt de afsluiting van de opleiding in de proefdierpathologie.