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Spontaneous Tumours in Rats and Mice

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Introduction

For those concerned with the aetiology of cancer it would be presumptuous to regard any tumour in any species of animal as "spontaneous". Perhaps a more appropriate title for the present dissertation would be "Tumours in animals not deliberately exposed to any known carcinogenic agent". The material presented here was derived from untreated animals, or animals treated with solvents only, in experiments concerned directly with the problem of carcinogenesis. In presenting this material I am conscious of the fact that detailed information of this type is of little value to persons working in different laboratories with different strains or sublines of animals. However, its presentation provides a basis for stressing certain historical and philosophical aspects of spontaneous tumour development in rats and mice.

Historical

It is vastly interesting, but also amusing, to look back half a century and to read descriptions of spontaneous tumours in mice by people such as Tyzzer (1909), Maud Slye and her colleagues (Slye, Holmes & Wells, 1914), Haaland (1911) and Murray (1908). Taking their cue from the way in which clinicians both then and now present case reports, they described separately the case history of each animal. Thus, Tyzzer's description of his first case begins "A brown female mouse, age 1 year $11\frac{1}{3}$ months . . . Attention had been directed to the mouse on account of great enlargement of the belly. A blood smear showed no notable increase in white cells. The animal was killed and the tissues fixed at once". Subsequent examination revealed that the animal had an unusual form of generalised lymphosarcoma and also a cystadenoma of the lung.

Unfortunately, not all the case records are so complete. Tyzzer (1909) begins the description of his second case with "An old brown mouse, sex not noted and age unknown".

These, of course, were the pioneering days, several years before cancer was first induced deliberately in experimental animals by coal tar. Even so, the mice studied by these early workers were, for the most part, born and bred within the laboratory. The fact that tumours arose in them so frequently led to a widespread suspicion that they were not cancers at all. Thus, Haaland (1911) found it necessary to give reasons for his view that "cancer in the mouse is essentially the same process as in man". In some circles today, the same suspicion persists, especially in relation to particular neoplasms such as the pulmonary adenoma of mice and to the fact that, as a result of inbreeding, certain strains have an exceptionally high incidence of specific neoplasms.

It seems to be an almost essential part of the mystique of cancer that it arises spontaneously, as it were, without any cause, that if it can be induced easily then probably it is not cancer, and that mechanisms involved in the induction of human cancer are quite different from those in laboratory animals.

It is, perhaps, not so difficult to see how this attitude has come about. Familial trends in, for example, mammary cancer, or leukaemia, are difficult or impossible to demonstrate in man, and yet high mammary tumour and high leukaemia strains of mice are readily available as laboratory tools. It is too easily forgotten that the strains of mice exhibiting these extraordinary propensities to develop particular types of cancer have been obtained as a result of closely supervised and selective inbreeding for between 20 and over 100 generations. We know very well that unfavourable traits are liable to appear as a result of relatively little inbreeding in man. Who can say what the situation with regard to cancer might be in a strain of man inbred to the extent of strict brother–sister mating for 20 or more generations, that is since the middle of the sixteenth century?

Heredity

Lynch (1926) found that the incidence in relation to age of spontaneous pulmonary tumours was different in two inbred strains of mice. She also observed that the progeny of parents in which pulmonary tumours were found were more likely to develop similar tumours than the progeny of pulmonary tumour-free parents. This observation led to detailed studies on the mechanism of genetic control of susceptibility of mice to lung tumours and to the conclusion that multiple genes are involved (Heston, 1948, Shimkin, 1955). A further conclusion was that the inherited susceptibility to lung tumours is not associated genetically with susceptibility to mammary tumours (Bittner, 1936) or hepatomas (Heston, Deringer, Hughes & Cornfield, 1952).

In fact, most of the inbred mouse strains with exceptionally high susceptibilities to pulmonary tumours, hepatomas, mammary tumours and lymphoma have been purposely bred for these characteristics. It would be interesting to know, therefore, the extent to which wild mice develop neoplasms.

The incidence of "spontaneous" tumours in wild mice

It is interesting to look back to the time before the laboratory animal became so different from the natural wild members of its species—before the days of intensive inbreeding—to see to what extent the occurrence of cancer resembled that in man. Unfortunately, although there are good records of the spectrum of tumours seen in man, it is difficult to get a clear picture of the incidence of different tumours in relation to the populations at risk at different ages. Andervont & Dunn (1962) wrote "A review of the literature and inquiries to other investigators revealed no publication concerning tumours in wild house mice", and proceeded to report the first systematic study of this kind. Ninety-eight out of a total of 225 wild house mice raised in captivity were found to have tumours at necropsy and these shared a total of 121 neoplasms. Of 107 mice examined between 2 and 24 months of age, 33% had tumours, and of 118 mice examined between 25 and 33 months, 53% were similarly affected. It is not clear to what extent neoplasia contributed to the cause of death in these animals, although it is reasonable to suppose it did so in between 30 and 40% of the mice with neoplasms, or 15–20% of the mice examined. This is of the same order as in man. Hoag (1963) has reviewed the whole subject of "spontaneous" neoplasia in mice.

The incidence of "spontaneous" tumours in wild and laboratory rats

Tumours are known to arise in wild rats (McCoy, 1909; Woolley & Wherry, 1911) but there is no report in the literature describing their incidence in relation to age. Such information is available only in the case of different strains of laboratory rat. Thompson, Huseby, Fox, Davis & Hunt (1961) reported on spontaneous tumour development in 125 Sprague-Dawley rats allowed to survive their entire lifespan; they saw 18 tumours in 43 males and 44 in 82 females. an overall tumour incidence of 41·6%. Almost half the tumours in females were of mammary gland origin. Only one case of generalized lymphocytic neoplasm was seen and this was in a female. Davis, Stevenson & Busch (1956) reported a tumour incidence of 57% in Sprague-Dawley rats, Crain (1958) one of approximately 25% in 786 Wistar rats, and Saxton, Sperling, Barnes & McCoy (1948) one of 45% in Osborne-Mendel rats. Much lower incidences have been recorded in Wistar rats by Ratcliffe (1940) and in stock rats by Bullock & Rohdenburg (1917) and Bullock & Curtis (1930).

Comparison of observations made in different laboratories

The incidence of neoplastic lesions found in a group of animals depends not only on factors such as sex, age and, what in the case of humans would be called, marital status, all of which were taken into account by Andervont & Dunn (1962), but also on two other variables. The first is the accuracy of diagnosis of lesions found at autopsy, and the second, the thoroughness of the post-mortem examination itself. It is relatively easy to overcome inaccuracy due to the first of these, but not so easy in the case of the second.

Routine autopsy

Any conscientious person with reasonable intelligence and the willingness and ability to describe clearly what he sees can quickly learn to carry out a post-mortem examination on a small rodent. Conscientiousness is particularly important when it comes to animals in the control groups in which interesting lesions may be few and far between. It is also vital when it comes to dealing with animals found dead and showing post-mortem degenerative changes.

Where tumour induction is the main interest and observation extends over the entire lifespan, animals acquire considerable value as the end of the experiment approaches. It is ludicrous to organize such experiments, especially in the case of mice, without arranging for them to be observed at least once each day, including Sundays. If a mouse dies on a Saturday it is likely to be useless for the purposes of accurate autopsy by Monday. In laboratories where animals are observed neither on Saturdays nor on Sundays it is common for little more than two thirds of the animals to be found suitable for autopsy.

In our department we have a standard post-mortem examination form suitable for both rats and mice (Figure 1). Each animal in every long-term experiment is examined *post-mortem*. Every graduate and every technician in the department is trained to carry out autopsies, and post-mortem degeneration is not readily accepted as an excuse for not doing so. The name of the person carrying out the autopsy is recorded on the form. A camera, set to a fixed focal length and filled with flash attachment is mounted in the post-mortem room, so that description can be supplemented by a visual record where necessary. Autopsies are numbered serially and the numbers transferred to the records pertaining to individual experiments. Between 4000 and 5000 autopsies are recorded in this way each year.

EXPERIMENTAL PATHOLOGY DEPT. POSTMORTEM No. HH127821

EXPERIMENTAL DATA

EXPERIMENT No.	ANIMAL IDENTIFICATION				
	Mouse or Rat No.	Sex	Strain	Litter No.	Date of Birth
A.P. EXPT	BOX 17 XM	♂	C.B.Stock		7.2.64

TREATMENT DATA UNTREATED CONTROL

Date of Death	Age at Death	How Killed?	Date of P.M.
28.8.65	569 days	Found dead	28.8.65

P.M. EXAMINATION CARRIED OUT BY B.C. Mitchley

Skin ✓

Subcutaneous ... 10x10mm nodule left flank

Parotid Glands ✓ Submaxillary glands ✓

Mammary Glands

Lymph Glands: Axillary ✓ Inguinal ✓ Cervical ✓

Mesenteric + Pararenal ✓ Lumbar and caudal +

Liver Multiple haemorrhagic foci in all lobes, slight enlargement

Spleen Enlarged Size 22x14x7mm

Pancreas Small nodule (10x10mm) ? tumour

Kidney L. Pale - finely granular

R. " " " "

Adrenals L. ✓ R. Slightly +

Oesophagus Wall thickened & slightly nodular

Stomach ✓ (fixed)

Small gut ✓

Caecum and Colon Gas ++

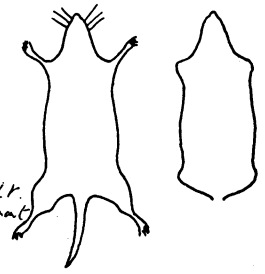
Rectum Gas ++

Bladder ✓

Brain ✓ (fixed)

Pituitary ✓

DORSAL VENTRAL



ORGANS AFTER FIXATION

Subcut nodule - fibrous throughout

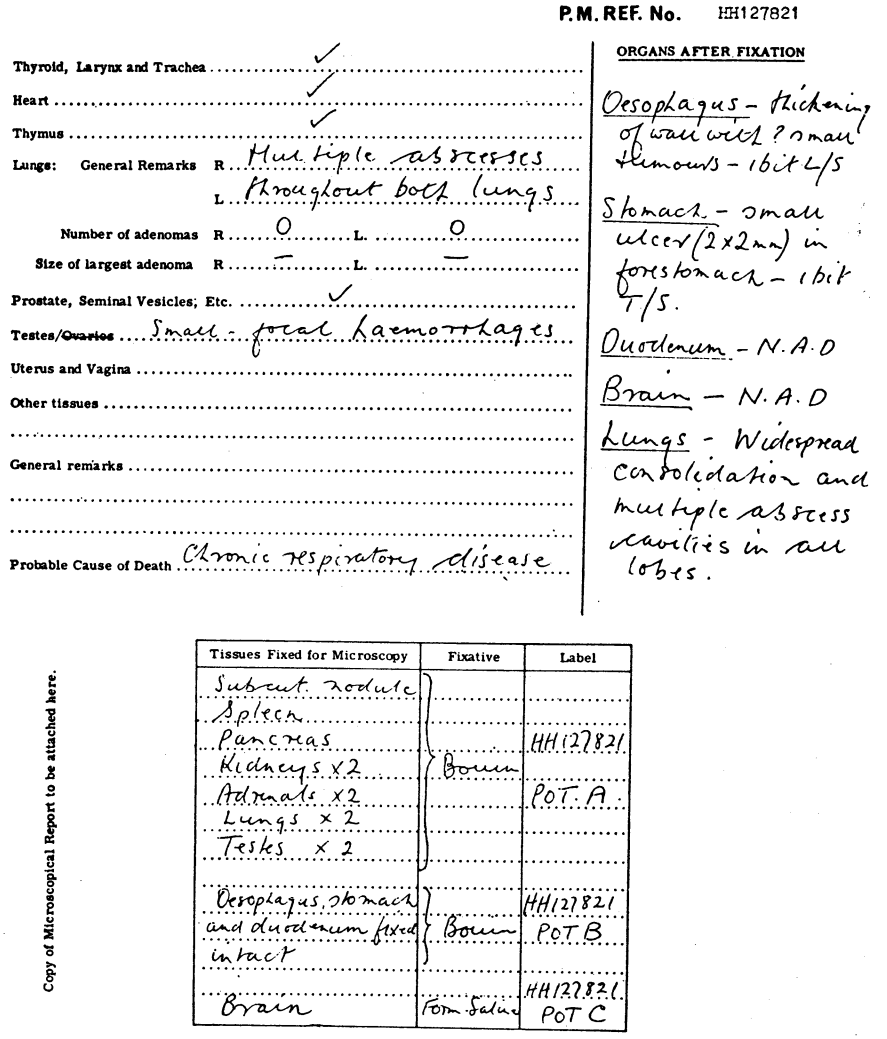
Liver - 2 bits

Kidneys - Both have multiple cysts in cortex - 1 bit of each

Adrenals - Central cystic space in right adrenal left - N.A.D

(Continued Overleaf)

The extent of autopsy examination in fact varies. Ideally, one would like blood counts, marrow smears, and records of body and organ weights on all animals, but it has been necessary to compromise and to vary the completeness of post-mortem examination from experiment to experiment. Thus, only where gastro-intestinal lesions or bladder lesions are expected are these organs distended with fixative and ligated. When this is done, the organs in question are bisected the following day and the appearance of the fixed interiors recorded on the post-mortem form in spaces reserved for this purpose. Likewise, the contents of the cranium and the spinal cord are examined in some experiments only. This is a pity and probably accounts for the fact that we rarely encounter neoplasms of the central nervous system.



P.M. REF. No. HH127821

Thyroid, Larynx and Trachea ✓
Heart ✓
Thymus ✓
Lungs: General Remarks R Multiple abscesses
L Throughout both lungs
Number of adenomas R 0 L. 0
Size of largest adenoma R — L. —
Prostate, Seminal Vesicles; Etc. ✓
Testes/~~Ovaries~~ Small - focal haemorrhages
Uterus and Vagina
Other tissues
General remarks
Probable Cause of Death Chronic respiratory disease

ORGANS AFTER FIXATION

Oesophagus - thickening of wall with ? small tumours - 1 bit L/S
Stomach - small ulcer (2×2mm) in forestomach - 1 bit T/S.
Duodenum - N.A.D
Brain - N.A.D
Lungs - Widespread consolidation and multiple abscess cavities in all lobes.

Copy of Microscopical Report to be attached here.

Tissues Fixed for Microscopy	Fixative	Label
Subcut. nodule	Bouin	
Spleen		
Pancreas		HH127821
Kidneys x2		
Adrenals x2		POT A.
Lungs x 2		
Testes x 2		
Oesophagus, stomach and duodenum fixed intact	Bouin	HH127821 POT B
Brain	Form Saline	HH127821 POT C

FIG. 1. The two sides of the form used for routine autopsy in Department of Experimental Pathology, Chester Beatty Research Institute. Note space left for comments on tissues after fixation.

A most important aspect of the system we use is that wherever an organ is deliberately examined and no abnormality found, a record to this effect is made. Thus, when we do encounter something strange, we can immediately ascertain how many times we have looked for but not found a similar lesion.

Examination of individual organs at postmortem

In the mouse the careful external examination of each organ from every aspect is likely to reveal almost all neoplasms of any size, but small neoplasms, particularly centrally placed hepatomas, pulmonary adenomas, or adenomas of the renal cortex, may be missed. Nothing short of the microscopic examination of serial sections of all tissues could exclude

the possibility that a small neoplastic lesion is missed. Clearly this is impracticable, and it must be accepted that even the most thorough autopsy is incomplete. However, it is still important to set, and keep to, a standard. This should involve making a routine number of parallel slices into organs such as the kidney and liver for the purpose of examining the cut surfaces.

Microscopic examination

The diagnosis of neoplasia begins and sometimes ends at the time of autopsy. The uninitiated sometimes think that the microscope is capable of revealing all, and that the description of gross pathology is of little importance. From time to time I have been asked to give an opinion on slides without at the same time being given any autopsy details. This complete divorce between autopsy and histopathological assessment reduces the accuracy of diagnosis considerably. Neoplasia, particularly of the reticuloendothelial system, is not always easy to diagnose on the basis of histopathological criteria only. For instance, it may be essential to know which lymph nodes were enlarged or whether there was evidence of skin infection in the area drained by a particular node. Another danger is that sight of small lesions may be completely lost. A nodule of only 1 mm in diameter is easily lost as a result of slightly inaccurate trimming, embedding or orientation during cutting.

The above remarks serve, amongst other things, to describe the circumstances in which we have obtained the information recorded below on the incidence of neoplasms in some of our own strains of rats and mice.

Neoplasms in untreated or solvent-only treated Chester Beatty Stock mice

This strain, which varies in colour from black through brown and café au lait to albino, has been maintained by haphazard rather than strictly random mating for more than 25 yr. Mice suitable for mating are selected on the basis of robust health and size. A 6-wk-old male weighs 25 g and a 1-yr-old male between 50 and 100 g. Lifespan averages about 16 months in our laboratory, with a few animals living up to 2·5 yr. We rarely keep mice under observation beyond 2 yr. Tables 1 and 2 show the incidence of tumours of all sites seen in 289 male and 63 female stock mice kept without treatment or receiving treatment with solvents, such as arachis oil or water by injection only.

In both sexes, but more so in the females, the predominant neoplasm is recorded as localized or generalized lymphocytic neoplasm. Included under this heading are lymphosarcomas, lymphomas of all types and reticulum-cell sarcomas. After the seventh month of life more than a third of the females showed evidence of this type of neoplasm. In males, more than a quarter of those surviving beyond the twelfth month did so. By comparison, the incidence of lung tumours, hepatomas and mammary tumours was low. It was surprising to see skin tumours in these mice. In fact we saw three, all in males. Two were squamous carcinomas (Figs. 2 and 3) which arose close to the site of injection of arachis oil. The third was a basal-cell carcinoma of the facial skin (Fig. 4), also in an arachis oil-treated mouse. We presume that the adenocarcinoma which arose in the subcutaneous tissues of the lumbar region of a 10-month-old untreated male originated from a rudimentary mammary gland. The only other rather surprising finding was that of a transitional-cell carcinoma of the renal pelvis of a 15-month-old untreated male (Fig. 5).

Because, particularly, of the very high incidence of lymphocytic neoplasms, the chance of a mouse of either sex dying without neoplasia of any kind fell to about 50% by 18 months of age.

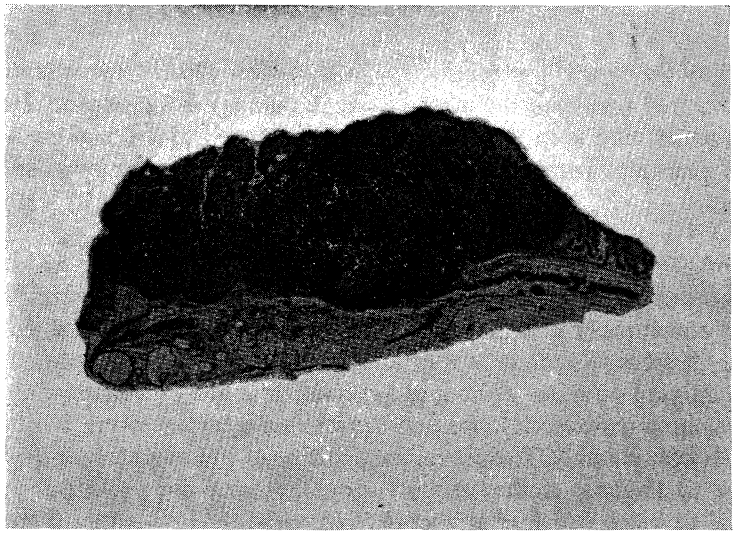


FIG. 2. Squamous-cell carcinoma of skin in a 13-month-old male mouse of the Chester Beatty Stock strain. The tumour developed in the right axilla not far from the site of subcutaneous injection of arachis oil given once weekly for 50 wk. Penetration of the panniculus muscle is clearly visible. Haematoxylin and eosin × 4.

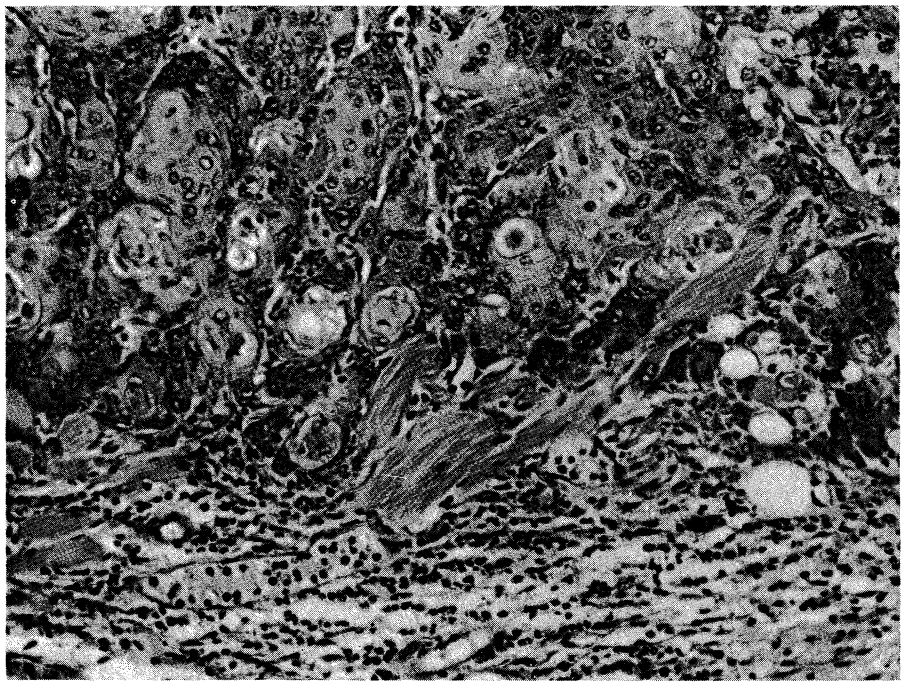


FIG. 3. Enlargement of tumour shown in Fig. 2 which demonstrates muscle penetration and slight inflammatory response. Haematoxylin and eosin × 204.

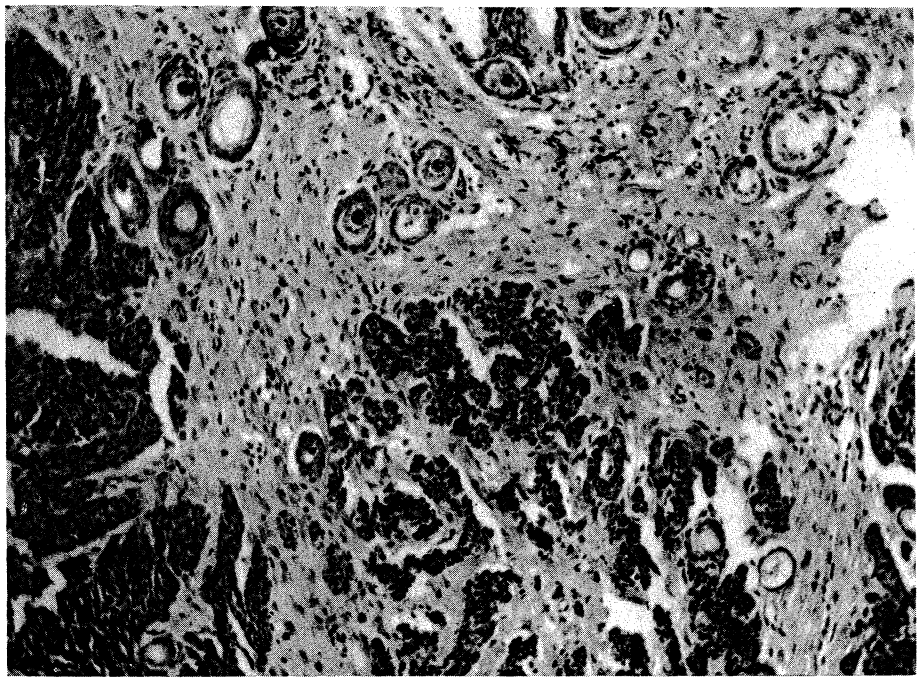


FIG. 4. Basal-cell carcinoma of neck skin in an 11-month-old male mouse of the Chester Beatty Stock strain given subcutaneous injections of 0·2 ml distilled water into the right flank once weekly for 1 yr. Haematoxylin and eosin × 128.

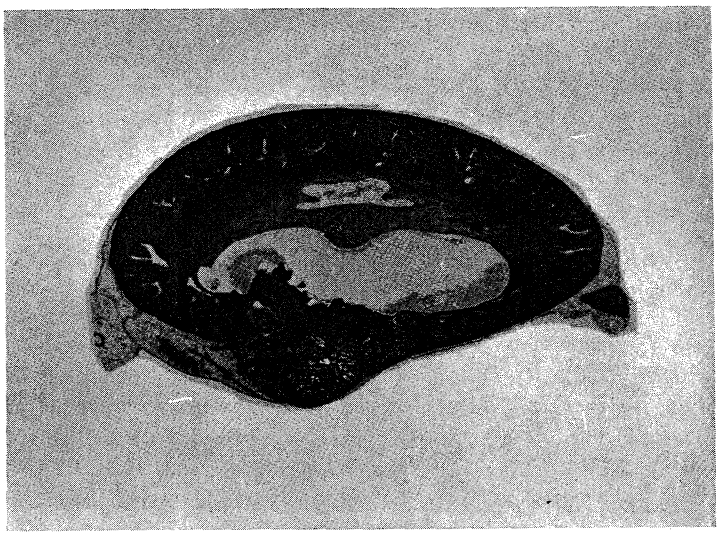


FIG. 5. Transitional cell carcinoma of the renal pelvis and hydronephrosis in an untreated 16-month-old male mouse of the Chester Beatty Stock strain. Haematoxylin and eosin × 4.

FIG. 6. Localized lymphosarcoma in the wall of the caecum of an untreated 14-month-old male rat of the Chester Beatty Stock strain. Haematoxylin and eosin × 4·5.

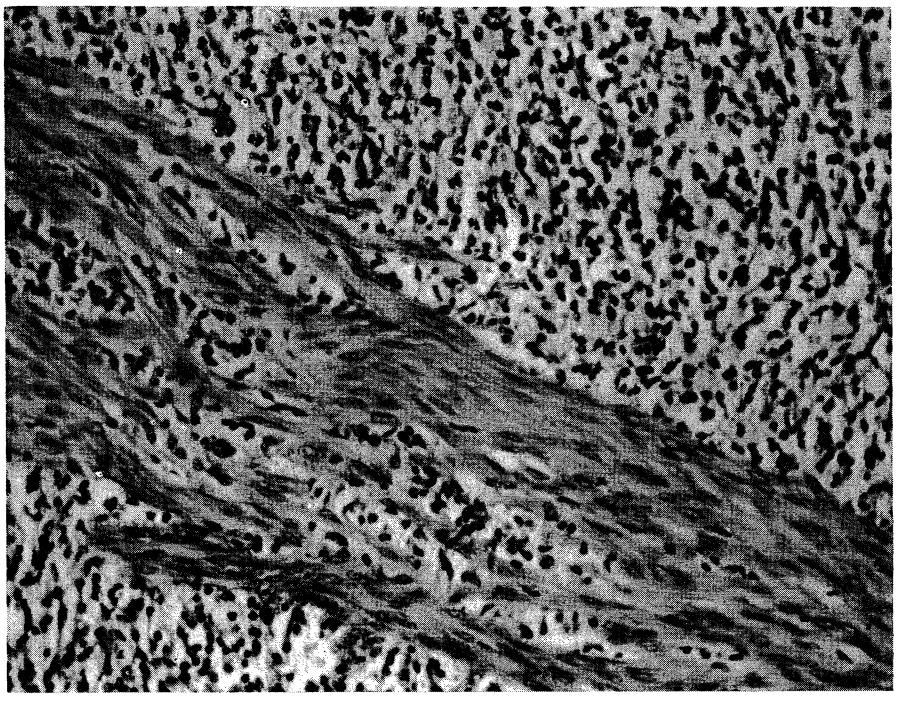


FIG. 7. Enlargement of tumour shown in Fig. 6 which demonstrates infiltration of tumour through muscle wall. Haematoxylin and eosin × 204.

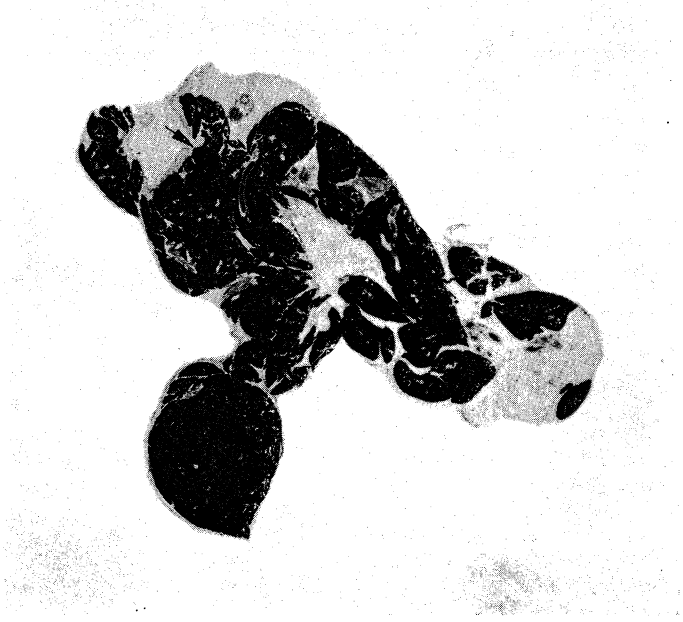


FIG. 8. Pancreas from a 24-month-old untreated male rat of the Chester Beatty Stock strain. Two adenomatous nodules are visible, the smaller being indicated by an arrow. Haematoxylin and eosin × 6.

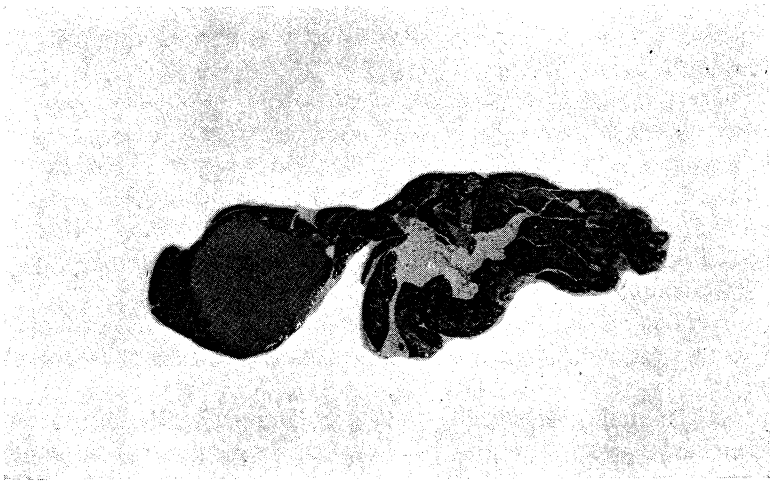


FIG. 9. Islet-cell adenoma of pancreas in a 26-month-old rat in which a piece of Ivalon sponge was implanted, subcutaneously, at the age of 2 months. The occurrence of the tumour is probably not related to the treatment given. Haematoxylin and eosin × 4.

Table 1. *Incidence of neoplasms in 289 male stock mice of CB strain, untreated or treated with solvents only, during 1961–1965*

Age in months	0–6	7–12	13–18	19–24
No. of mice dying	61	82	122	24
No. examined *postmortem*	47 (77%)	75 (91%)	111 (91%)	20 (83%)
No. with localized or generalized lymphocytic neoplasm	2 (4%)	8 (11%)	31 (28%)	5 (25%)
No. with lung tumours	3	2	12	3
No. with hepatomas	0	4	10	4
No. with skin tumours	0	1	3	0
No. with other tumours	0	1*	1†	0
Percentage with multiple neoplasms	0	3	6	10
Percentage without neoplasm	89	81	55	50

*Adenocarcinoma in subcutaneous tissues in lumbar region
†Transitional cell carcinoma of renal pelvis

Table 2. *Incidence of neoplasms in 63 female stock mice of CB strain, untreated or treated with solvents only, during 1961–1965*

Age in months	0–6	7–12	13–18	19–24
No. of mice dying	16	19	26	2
No. examined *postmortem*	13 (81%)	16 (84%)	24 (92%)	2
No. with localized or generalized lymphocytic neoplasm	0	6 (32%)	10 (38%)	1
No. with lung tumours	0	1	4	0
No. with hepatomas	0	0	0	0
No. with skin tumours	0	0	0	0
No. with other tumours	0	0	1*	0
Percentage with multiple neoplasms	0	0	4	–
Percentage without neoplasm	100	56	42	–

*Mammary adenocarcinoma

Neoplasms in untreated or solvent-only treated Chester Beatty Stock male rats

The Chester Beatty Stock rat is albino and was derived originally from the Wistar strain. The strain has been maintained for over 25 yr by haphazard mating of rats selected because of large size and robust health. Rats aged 4 wk already weigh 100 g and adults reach weights of between 600 and 1000 g. In the case of female rats we have no presentable data. In 64 control male animals (Table 3) observed for up to 24 months of age, 54 showed no evidence of neoplasia at death, 4 had localized or generalized lymphocytic neoplasm (Figs. 6 and 7), 2 showed exocrine adenomatosis of the pancreas (*vide infra*), 2 had papillomas arising in the renal pelvis, and 1 had a hepatoma. Cannibalism prevented postmortem examination in one case.

Mammary tumours, particularly fibroadenomas, are common in females and it is our impression that generalized lymphocytic neoplasia occurs more frequently in them than in males.

In our male animals observed for up to 2 yr the total incidence of neoplasia was 14%, but, as explained above, for purposes of comparison with observations in other laboratories, this figure has little meaning.

The histopathological appearances of all the types of "spontaneous" tumours seen by us in both rats and mice have been well described elsewhere, with one exception. We have been unable to find any description in the literature of exocrine adenomatosis of the pancreas in rats.

Table 3. *Incidence of neoplasms in 64 male stock rats of CB strain, untreated or treated with solvents only, during 1961–1965*

Age in months	0–6	7–12	13–18	19–24
No. of rats dying	1	6	19	38
No. examined *postmortem*	1	6	18	38
No. with localized or generalized lymphocytic neoplasm	0	0	1	3
No. with exocrine adenomatosis of the pancreas	0	0	0	2
No. with papilloma of renal pelvis	0	0	1	1
No. with hepatoma	0	0	1	0
No. dying without neoplasm	1	6	15	32

Exocrine adenomatosis and other neoplasms of the pancreas in Chester Beatty Stock rats

Altogether, we have encountered exocrine adenomatosis in rats three times, two of them in males. One of these cases has been reported elsewhere (Roe *et al.* 1964). In all three cases the lesion appeared to be of multifocal origin and was found at autopsy after the 20th month of life. The two males had received no treatment but the female had been given tetryl (picrylnitromethylamine), approximately 2 mg/day, 6 days/wk, in the drinking water, from the age of 2 months. This treatment gave rise to no tumours or other lesions elsewhere in the body, and it is therefore considered unlikely that it was responsible for the pancreatic lesion recorded here. Figure 8 illustrates these tumours.

We have searched our records for other evidence of pancreatic tumours in Chester Beatty Stock rats killed between March 1964 and January 1965. During that period 285 rats of either sex were autopsied and found to have normal pancreases. One case of Islet-cell adenoma (Fig. 9) was seen. The only treatment received by this animal was the implantation of a piece of Ivalon (cross-linked polyvinyl alcohol) sponge into the subcutaneous tissues of the right flank. Such treatment commonly induces sarcomas at the site of implantation (Dukes & Mitchley, 1962) but has not, so far, been shown to induce tumours elsewhere in the body. The adenoma was therefore considered to be an incidental finding. Islet-cell tumours have, of course, been recorded by other workers (e.g. Thompson *et al.* 1961).

Our search also revealed one exocrine adenocarcinoma of the pancreas. This arose in a male rat injected once-weekly with 0·5 mg lead phosphate dissolved in distilled water. Injections began at the age of two months and continued for 8 months. Such treatment commonly gives rise to cortical adenomas of the kidney, but the induction of tumours at other sites has not been reported (Boyland, Dukes, Grover & Mitchley, 1962). Microscopic examination of the lesion strongly suggested that the tumour arose secondarily out of a background of exocrine adenomatosis.

There are, of course, many investigations we should like to make on these tumours. In particular we should like to see whether they are transmissible by cell-free passage. However, further investigation is difficult because of the low incidence and because the tumours are not in evidence until half-way through autopsy.

Finally, to complete the picture, we have seen metastatic deposits in the pancreas from kidney tumours induced by lead in two rats.

DISCUSSION

In recent years large inroads have been made into the territory of what were once regarded as spontaneous tumours of laboratory animals. I refer particularly to the effects of breeding mice in creosoted wooden boxes, to the demonstration of the vertical transmission of murine leukaemia viruses, to the occasional spontaneous occurrence of tumours attributable to polyoma virus, and to the now recognized carcinogenicity of aflatoxin. The demonstration by Gel'shtein (1961) that the incidence of liver tumours, lung tumours and leukaemia may be affected by exposure of the parents, or even of the grandparents, to a carcinogenic agent such as *o*-aminoazotoluene also merits attention, though no clear explanation of the phenomenon has been put forward.

Creosote

Rous (1956) reported that hairless mice of the Crew strain raised in boxes impregnated with a commercial wood preservative developed multiple skin tumours, sebaceous adenomas, papillomas and carcinomas. Animals of the same strain not exposed to the wood preservative developed no skin tumours. Direct application of the wood preservative to the skin induced tumours. About the time of the report of Rous (1956) marked differences in the results obtained in two different laboratories in tests using croton oil were giving rise to concern. Boutwell, Bosch & Rusch (1957) were obtaining high incidences of skin tumours in mice treated with croton oil only, whereas Roe (1956) obtained a much lower and later incidence of tumours in similar experiments. Shubik, Spencer & Della Porta (1957) reported that the mice obtained from the same source as those used by Boutwell and his colleagues developed many "spontaneous" skin tumours. Boutwell & Bosch (1958) ascertained that the mice used in their previous experiments had in fact been reared in wooden cages and that the commercial breeder who supplied the mice used creosote to preserve the wood of these cages. When animals of the same strain were bred in metal cages "spontaneous" skin tumours no longer appeared, and the response of the skin to croton oil was closely similar to that previously reported by Roe (1956). Subsequently, Roe, Bosch & Boutwell (1958) found that the incidence of "spontaneous" lung tumours was also exceptionally high in mice bred in creosote-treated wooden boxes: 138 mice obtained from the dealer before he stopped using creosoted boxes showed an average of 5·8 adenomas per mouse at ages of between 6 and 8 months. Progeny from the same mice reared in metal cages developed less than 0·5 adenomas per mouse by the same age. In this case the recognition and elimination of an environmental hazard led to more than a tenfold reduction in the incidence of what was previously regarded as a "spontaneous" tumour. It is difficult to believe that further reductions could not be effected by even closer environmental control.

The effect of type of cage on "spontaneous" tumour development

Finkel & Scribner (1955) reported that CF1 female mice housed in groups of ten in methyl methacrylate cages developed more "spontaneous" tumours than comparable animals housed 15 per cage in stainless steel cages. The former, consistently throughout the experiment, had a higher average body weight than the latter, but at no time was the difference great or statistically significant. Apart from some early deaths from acute respiratory or intestinal infections, survival was closely similar in the two groups. The excessive tumour incidence in the animals housed in plastic cages was due to higher

incidences of reticuloses of all types and of mammary tumours. The incidence of pulmonary tumours and of all other types of tumours was similar in the two groups.

It is interesting to speculate as to the reason for differences seen by Finkel & Scribner (1955). Firstly, there is the possibility that the plastics is carcinogenic. Secondly, the difference in heat conductivity between metal and plastics may necessitate more physical activity on the part of the mice in the metal cages in order to maintain body temperature. Mühlbock (1951) has shown that mammary tumour incidence may be reduced by physical exercise. The same author showed, as had Andervont (1944) previously, that mammary tumours increase in incidence as the number of mice per cage is reduced. He suggested that these effects may be mediated through the anterior pituitary. If this is so, it is not surprising that the incidence of reticular neoplasms moved in the same direction since there is evidence, including our own, that the female is more susceptible than the male to this type of tumour.

Sterilization of bedding with ethylene oxide

Reyniers, Sacksteder & Ashburn (1964) reported effects in germ-free mice which they considered were probably attributable to exposure to bedding treated with ethylene oxide. The mice were of the inbred 101-GF strain, and the animals were exposed to ethylene oxide-treated bedding for a period of 150 days. A large number of the males died from intra-abdominal or intrathoracic haemorrhage. Few females died in this way but 90% of those surviving to between 400 and 900 days developed tumours of various types. Lymphocytic neoplasms, mammary tumours, uterine and ovarian tumours, osteogenic sarcomas, subcutaneous sarcomas and lung tumours were all common. There were no strictly comparable control mice not exposed to treated bedding, but the authors point out that before its introduction the incidence of neoplasms was very much lower.

Incidental exposure to chemical carcinogens and carcinogenic contaminants

Animals in laboratories situated in urban areas will, of course, be exposed to carcinogens in the general atmosphere, unless special air-purification procedures are in use. Even in air-conditioned animal rooms carcinogens in tobacco smoke or in the pyrolysis products of overheated organic materials from neighbouring chemical laboratories may reach animals. In addition there is the possibility, especially where cancer research is carried out, that cages are contaminated with traces of carcinogens used in previous experiments. This may be the explanation of the findings of Gel'shtein (1961) referred to above. We have found that the injection of 0·06 μg of 9,10-dimethyl-1,2-benzanthracene into a newborn mouse is sufficient to raise significantly the incidence of lung tumours found at *postmortem* 40 or more wk later. O'Gara, Kelly & Mantel (1962) found that as little as 0·003 μg of 1,2,5,6-dibenzanthracene injected into newborn mice induced sarcomata at the injection site. These amounts serve to illustrate that even very slight contamination with a potent carcinogen may not be without effect.

Hormonal influences

In both mice and rats certain types of malignant lymphoma tend to be more conspicuous in females than in males. It is, moreover, well established that the administration of oestrogens may enhance the development of this and many other types of neoplasm (Marois, 1964). The incidence of "spontaneous" neoplasms, therefore, could well be influenced by the presence of oestrogenic materials in the diet, i.e., natural or synthetic

oestrogens from dietary components of animal origin, or plant materials which possess oestrogenic activity. Lemonde (1964) reported that the development of spontaneous lymphoma and consequent death were significantly delayed in female AK mice that had been pregnant compared with virgins. The occurrence of pregnancy did not, on the other hand, change the incidence of lymphoma.

Nutrition

Basically little has been added to our knowledge of nutrition in relation to cancer since the excellent review of the subject by Tannenbaum & Silverstone (1953). Their main conclusions were as follows:

(1) Dietary restriction of calories reduces both tumour incidence and the growth of tumours already present. This applies equally to "spontaneous" tumours and to carcinogen-induced tumours.

(2) High fat diets enhance the development of some, but not all, types of tumour, and high protein diets similarly affect some types of tumour.

(3) In general, diets deficient in vitamins inhibit tumour development, but riboflavin deficiency enhances liver-tumour induction by azo dyes. It is not certain whether riboflavin deficiency affects the incidence of the "spontaneous" development of liver tumours.

Carcinogens in food

It is only during the past few years that we have known of the carcinogenic activity of the aflatoxins. These substances, produced during the growth of the mould *Aspergillus flavus*, have been found in a wide variety of cereals, such as those used in the compounding of feed for laboratory animals. A high incidence of liver tumours in rats has been attributed to aflatoxin in the groundnut meal moiety of a feed intended for guinea pigs and prepared according to the standardized formula for MRC Diet 18 (Schoental, 1961). Even though groundnut meal is not now ordinarily included in rations intended for rats and mice, it is likely that smaller or larger amounts of aflatoxin from other cereal sources are present in compounded diets from time to time.

Viruses

The Bittner agent, as a causative factor in mammary gland carcinogenesis in mice, is well known. This agent is normally passed from mother to daughter in the milk. Fostering from the moment of birth breaks the passage sequence and strains of mice free from the agent may be derived in this way. There is, however, some recent evidence that the agent may be transmitted by the male in the spermatic fluid. In any event, although removal of the agent from a high mammary tumour-bearing strain, such as BALB/c does not entirely abolish tumour incidence there is a drastic reduction in the incidence of mammary tumours.

Non-mammary-agent and mammary-agent-induced tumours show some general differences in the frequency of various histological types, but in no individual case is it possible on ordinary histological grounds to be sure whether the agent is involved in the aetiology or not. We have seen on rare occasions adenocarcinomas in the subcutaneous tissue of male mice. Whether these originated in rudimentary mammary glands or skin adnexa is uncertain.

Several mouse leukaemia viruses have now been found and their propagation by vertical

transmission in both mice and rats is an established fact (Gross, 1951; Salaman & Harvey, 1962). Obviously, factors such as methods of breeding are likely to affect the incidence of "spontaneous" lymphocytic neoplasm, and where experiments involve fairly small groups of animals, differences in the immediate geneology of the individual mice in various treatment groups may give rise to dramatic differences in the incidence of this type of tumour. This leads me to the view that randomization of animals between experimental groups is, if anything, more important and not less important when inbred rather than random-bred animals are used.

Spontaneous tumours due to polyoma virus in mice

When mice, born of parents uninfected with polyoma virus and carrying no antibodies to it, are injected during the first few days of life with the virus a wide variety of tumours develop. A multifocal undifferentiated tumour of the parotid gland is the neoplasm most frequently seen. Mammary gland tumours (which we saw in males as well as females), eyelid tumours, subcutaneous sarcomas, undifferentiated tumours of the renal medulla and osteogenic sarcomas were also common in our experiments of this kind (Salaman, 1959; Rowson, Roe, Ball & Salaman, 1961).

During the past few years we have been conducting experiments involving the introduction of chemical carcinogens and other agents into newborn mice. During the course of this work we have used several different strains of mice. In one of these strains, the '101' strain, we have seen 16 cases of unilateral (10) or bilateral (6) parotid gland tumours. The tumours occurred equally in the two sexes and were seen in mice injected only with aqueous gelatine, which was the vehicle for the introduction of carcinogen. In 11 cases tumours arose during the first 6 months of life, the first at the end of the fourth month. The last tumour to arise did so at 9 months.

The histological appearance of these tumours was identical to that of tumours attributable to the injection of polyoma virus, and we have now confirmed that in all of the animals with parotid gland tumours which were examined for antibodies to polyoma virus, high titres were present (Dr. G. Negroni, personal communication).

The occurrence of these tumours is intriguing because their spontaneous appearance has, to our knowledge, been recorded only once before. Law (1957) reported the occurrence spontaneously of 5 parotid gland neoplasms. All arose at or before the 6th month of life in C3H strain mice.

We then realised something which should have been apparent from the start. For the purposes of injecting newborn mice with different substances, a single syringe and needle were set aside for each substance. All mice injected with the same substance at the same dose level were injected with the same syringe and needle. Thus, if the needle became infected with polyoma virus early in the procedure, mice subsequently injected might become infected. This is probably the explanation of the high incidence of parotid gland tumours seen in this experiment.

However, this is not the full story. Miss Diana Noakes, at the Chesterford Park Laboratories of Fisons Pest Control, has also been engaged in studies using newborn '101' strain mice. She has seen unilateral and bilateral parotid gland tumours in 13 mice (bilateral in 8 mice). Slow-growing eyelid tumours typical of polyoma infection were present in 6 of the 13 mice. Particularly interesting in this case was the fact that some of the mice with bilateral parotid gland tumours and antibodies to polyoma virus in high titre had received no treatment at any time.

It is concluded firstly, that sterility is important when newborn animals are to be injected, and secondly, that polyoma-induced tumours may arise spontaneously on rare occasions.

Spontaneous tumours and the germ-free state

Pollard & Teah (1963) reported the occurrence of 19 mammary tumours, one subcutaneous fibrosarcoma and three lymphosarcomas in germ-free Wistar rats (21 females, 2 males). They saw no tumours in germ-free Fischer strain or Sprague-Dawley rats, but pointed out that further observation of these strains was necessary before a zero incidence could be accepted as established. The authors suggested that these findings indicate either that the "germ-free" Wistar rats are infected with an oncogenic virus or that such agents are not necessarily involved in the process. They were not successful in isolating a virus from any of the tumours. The same authors state that spontaneous tumours have not been noted in germ-free mice of the Swiss-Webster, CFW, ICR, C3H and BALB/c strains, though lung adenomas and local sarcomas have been induced in germ-free rats and mice of many strains.

Conclusions

Clearly the subject "Spontaneous tumours in rats and mice" is not what it seems. Many so-called spontaneous tumours are without doubt due to exposure of animals to environmental factors. In some cases such exposure can be prevented, in other cases it is difficult to see how to prevent it. The difficulty of controlling some of these factors, especially the viruses, underlines the need for strictly contemporary control groups and for the strict randomization of animals at the start of experiments.

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