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Further observations on the breeding and rearing of BALB/C nude (nu-nu) mice under normal laboratory conditions

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Summary An account is given of the breeding and rearing of BALB/C (nu-nu) mice under normal laboratory conditions, in a research institute in Thailand. Starting with twenty pairs of mice in 1980 over 4,000 have now been successfully bred and reared in this unit: on reaching adult age (30 days), the mortality rate is nil.

A detailed description is given of the housing unit, cages, bedding, diet, animal husbandry and neonatal management. The approach used is demanding in terms of professional time and the need for constant attention to detail—but it is successful.

Our experience is in striking contrast to published evidence on the absolute need for specific pathogen-free conditions for this animal model.

Introduction

In previous publications we described our preliminary findings on the rearing of BALB/C nude (nu-nu) mice without recourse to specific pathogen-free conditions[1] and our experience in the inoculation of the hind footpads with *Mycobacterium leprae* of human origin.[2] Since that time, we have continued to breed and rear nude mice in this institute under similar, normal laboratory conditions with considerable success. The colony of healthy nude mice now exceeds 4,000 and this paper gives a detailed account of the principles and techniques developed here since 1980.

Materials and Methods

In 1979, Professor K Kohsaka of Department of Leprology, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, sent 20 pairs of BALB/C mice to this Institute, of which 20 were nu-nu males and 20 nu/+ females. From these we developed the present colony which now numbers over 4,000.

GENETIC CONSTITUTION

The animals are in every way identical to those used by Kohsaka for his previous studies in Japan,[3–6]

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and by other research workers from different parts of the world.[7–12] We have not encountered congenital deformities, unexpected hair or eye colour, or obvious evidence of tumours in any of the animals produced here since 1980. Although detailed genetic typing has not been done, it appears that the original stock and progeny are identical with BALB/C nude (nu-nu) mice used in other parts of the world.

CAGES

The mice are kept in standard plastic boxes 17 × 25 × 12 cm, with metal grid covering. The boxes are lined with coarse wood shavings obtained from a local saw-mill; the composition is variable, but teak and plywood, among others, are most common. Throughout the period of study, bedding has been changed twice-weekly.

FOOD

We use a standard laboratory mouse 'pellet' supplied by an agency in Bangkok, which contains crude protein (fish origin) 24%; crude fat 3%; fibre 4%; ash 8·5%; calcium 1·6%; phosphorus 1·0%; nitrogen-free extract 4·8% and water 12·0%. Detailed studies of intake have not been carried out, but we estimate that each adult consumes approximately 4 g daily; perhaps more during lactation and certainly less with progressive ageing.

ROOM SIZE

Cages are kept on wooden shelves in rooms measuring 7 × 5 × 3 m (for breeding) and 5 × 5 × 3 m (for rearing) both of which have narrow windows placed high up in the walls so that there is never any possibility that nude mice will be exposed to direct sunlight (which produces phototoxic dermatitis in this model).

NUMBER OF MICE PER CUBIC METRE OF ROOM SPACE

The number of mice accommodated in a room of the dimensions given above has increased steadily through the years, but in the last 12 months it has averaged 17. Figures above this produce a foul atmosphere from decomposition of urine and the production of ammonia; the influence of overcrowding on mice inoculated with *M. leprae* of human origin is clearly adverse.

AIR-CONDITIONING, TEMPERATURE, HUMIDITY

Although not absolutely essential, we have installed a single air-conditioning machine (capacity 18,000 BTU, Climatrol, window type) in 1980. This was due mainly in order to deal with foul-smelling air due to putrefaction of urine and ammonia formation, which often reached unacceptable levels for laboratory staff. It was, however, soon discerned that its introduction increased sexual activity in the mice and the number of progeny per litter: see Results and Discussion. The environmental temperature in this area ranges 21°C–39°C throughout the year with a mean of 30°C. The humidity ranged 55%–75%, with a mean of 65%. Air-conditioning reduces these figures in the mouse room to 23–28°C and 45%–60% respectively.

SELECTION OF PAIRS FOR BREEDING

We select strong, healthy-looking mice only, aged 8 weeks for breeding. The male is BALB/C nu-nu (nude) and the female BALB/C nu/+ (white, hairy). The female nu-nu have only rudimentary,

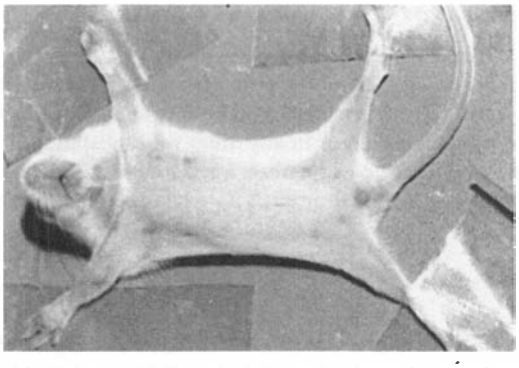

Figure 1. Spots of nipples are clearly observed at 12 days of age in female white mouse.

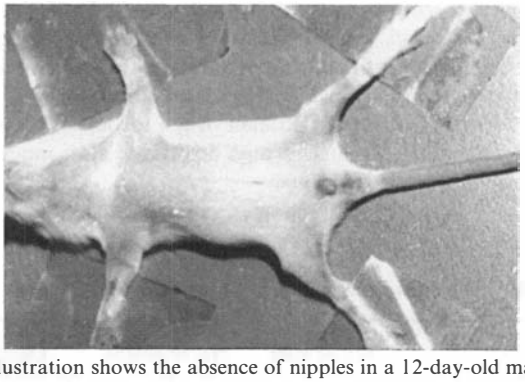

Figure 2. This illustration shows the absence of nipples in a 12-day-old male white mouse.

almost invisible nipples (and do not lactate); they breed normally but are thus unable to suckle the babies. The pairs are housed in separate cages, with two changes of wood shavings each week.

NEONATAL MANAGEMENT

From the litters, which range from 3 to 13 in number, with an average of 8, we redistribute nude (nu-nu) mice at 7 days in groups of five only to one (nu/+) mother, taking care that those from the real mother and the 'surrogate' mother were all born on the same day. This is done because white mice are stronger than nude mice; they displace the nude mice from the nipple and gain weight at their expense. At the same time (7 days) we group 5–10 white mice with one mother. When they are 11–12 days old it is possible to decide on the sex of the white mice by inspecting the abdomen for nipples (Figure 1); they are by this time obvious in the female and absent in the male (Figure 2). We then take out from the colony all white male mice, since they are of no breeding value to the further maintenance of the stock.

ADULTHOOD AND LIFE-SPAN

By 30 days the mice are fully developed adults and we separate them from the parents. They live on average for 70–75 weeks, but after about 50 weeks some of them lose weight, become slow in their movements and develop the well recognized 'hump-back' spinal curvature.

GENERAL ASPECTS OF HUSBANDRY AND STOCK MAINTENANCE

Attention to the technical details described above is essential on a daily basis. We record the number and condition of all mice born every day of the year. It is essential to start each breeding batch with healthy robust parents and it is our practice to take out and kill baby mice which seem weak, abnormally small in the neonatal period or in any other way substandard. Careful attention to the milk supply from lactation is vital; the white mice, aged 7 days in groups of 5–10 with one mother take less milk in the first few days, but soon pick up when the males are removed at 11–12 days.

Results

Using the methodology and techniques described above, we usually lay down 180 pairs of adult mice for breeding in 180 separate cages at any one time. From there, in due time, we obtain about 100 nude mice per month. With the continuing use of adults (30 days and over) for experimental purposes in this unit, together with the natural death rate at 70–75 weeks, we are able to keep the total number of mice in the room under control. We have no evidence that overcrowding is hazardous for young nude mice, or for young adults, though it should obviously be avoided if only to avoid air pollution by foul-smelling urine and ammonia. In the case of more mature adults inoculated in the footpad with *M. leprae* of human origin for leprosy studies, we have, however, strong evidence that overcrowding produces a high death rate after inoculation.

Table 1. Nude mice monthly death rate (room = 5 × 5 × 3 m).

Month	Year	No. of mice per month	No. of deaths per month	Deaths per month (%)
1	1983	1468	151	10·28
2	1983	1454	147	10·11
3	1983	1476	154	10·50
4	1983	1468	162	11·30
5	1983	1484	158	10·64
6	1983	1492	154	10·32
7	1983	1496	167	11·16
8	1983	1532	177	11·55
9	1983	1502	161	10·71
10	1983	1543	172	11·14
11	1983	1569	195	12·42
12	1983	1608	198	11·75
1	1984	1632	204	12·50
2	1984	1462	164	11·21
3	1984	1402	145	10·34
4	1984	1367	137	10·02
5	1984	1304	112	8·58
6	1984	1322	104	7·86
7	1984	1311	97	7·39
8	1984	1253	95	7·58
9	1984	1216	87	7·15
10	1984	1208	81	6·70
11	1984	1137	61	5·36
12	1984	1125	48	4·26

Table 2. Nude mice death rate after inoculating and rearing together (room of 5 × 5 × 3 m).

Group	No. of mice per group	Total No. of mice in the room	12 months		15 months		18 months	
			death	%	death	%	death	%
1	80	1454–1608	19	23·75	24	30·0	33	41·25
2	120	1454–1608	31	25·83	43	35·83	56	46·66
3	40	1402–1632	9	22·5	13	32·5	17	42·2
4	80	1304–1632	21	26·25	27	33·75	34	42·5
5	100	1253–1632	25	25·0	34	34·0	48	48·0
6	90	1208–1632	21	23·33	27	30·0	38	42·22
7	100	1137–1632	13	13·00	18	18·0	27	27·0
8	40	1024–1311	3	7·50	5	12·5	6	15·0
9	80	984–1024	6	7·50	6	7·5	8	10·0
10	80	973–984	4	5·0	4	5·0	5	6·25

As noted above, the parents are selected for health and vigour. We also take out and kill baby mice (at any stage before 30 days) if they are obviously underweight, weak, damaged by trauma, or in any other way substandard. Those attaining adulthood at 30 days are thus well fed and robust. In the 7 years of this study, we have had very few deaths between the ages of 35 days and 70 weeks.

Discussion

The high success rate using the approach described above is in marked contrast to published work which emphasizes the absolute need to maintain nude mice under specific pathogen-free conditions at all times. In Nepal, Samuel[13] reported excellent results in breeding and rearing in 1984. To our knowledge, this is the only reported instance of success in normal laboratory conditions other than our own. Our ability to breed and rear nu-nu mice under the normal conditions described above is difficult to explain. It is possible that the original genetic stock was in some or other particularly robust and that the local temperature, humidity and diet are all favourable. It is however our belief that these factors are of relatively small significance compared with the vitally important matter of meticulous day-to-day husbandry of the colony, including the careful selection of healthy mice for breeding purposes.

Acknowledgments

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NEWS AND NOTES

WHO Symposium on Ocular Leprosy: London, September 1987

A symposium on ocular leprosy took place at the International Centre for Eye Health, Institute of Ophthalmology, London, 21–23 September 1987. It was organized by Professor Gordon Johnson, Director of the Department of Preventive Ophthalmology, and Dr Paul Courtright of the Proctor Foundation, San Francisco and was funded by the WHO through its Programmes for Leprosy and the Prevention of Blindness with contributions from LEPRA and Dutch and German Leprosy Missions.

The meeting, which took the form of a workshop, was attended by 15 ophthalmologists with a special interest in ocular leprosy—Dr J Anderson (London), Dr M Brand (USA), Dr F Brandt (Germany), Dr A Cherinet (Ethiopia), Mr T ffytche (London), Dr M Hogeweg (Holland), Professor G Johnson (London), Mr M Kerr-Muir (London), Professor P Lamba (India), Dr G Lim (Philippines), Dr B Ostler (USA), Dr R Pokhrel (Nepal), Dr N Suryawanshi (India), Dr K Waddel (Uganda) and Dr G Warren (Thailand). Dr M Jacob (India), pathologist, and Dr P Courtright, epidemiologist, both specializing in ocular leprosy, were also present.

The WHO was represented by Dr K Nordeen, Chief, Leprosy, WHO, Dr R Pararajasegaram, Regional Advisor to WHO (SEARO) and Dr B Thylefors, Programme Manager of Prevention of Blindness, WHO.

The meeting covered a wide range of topics relating to ocular leprosy including its global distribution, the present state of knowledge of its clinical and pathological manifestations and its prevention and management in the field. The training needs for all levels of personal working in leprosy were discussed and a policy for integration of ocular leprosy management in National Leprosy and Blindness Prevention programmes was formulated.

A report of the meeting will be published by the WHO and it is intended that its recommendations will be presented to the 13th International Leprosy Congress at the Hague in 1988.

(Contributed by Mr T J ffytche, Consultant Ophthalmic Surgeon, St Thomas' Hospital, London SE1 7EH.)