but must be increased about 10-fold.

Exposure to atomized histamine induces a severe and frequently fatal bronchoconstriction in the guinea pig. It was found that hetramine can prevent the fatal bronchoconstriction so produced by protecting the animals with varying doses prior to exposure to a histamine atmosphere. The chamber into which atomized histamine is introduced was constructed in accordance with the methods described by Loew, *et al.*[7] It was found that vapor from 5 cc of a 0.125% solution of histamine was almost uniformly fatal to unprotected animals. This mortality could be significantly and increasingly reduced with doses of 0.3 mg/kg to 2.5 mg/kg of hetramine administered intraperitoneally. The data on this experiment are shown in Table III.

The antianaphylactic effect of hetramine was determined by sensitizing guinea pigs through intraperitoneal injection of 0.25 cc normal horse serum 14 days prior to shocking doses of 1.0 cc horse serum administered intracardially. Control animals uniformly succumbed to the shocking dose with typical symptoms of fatal anaphylactic shock. Pretreatment with intraperitoneal doses of hetramine up to 3.5 mg/kg were ineffective under our conditions. When doses of 5 mg/kg were administered 20 minutes before the shocking dose of horse serum, 50% of the guinea pigs survived and 6.0 mg/kg reduced the severity of the reaction still further, 7 out of 8 animals surviving the shocking dose. The data are shown in Table IV.

The antihistamine effect of hetramine as demonstrated by the above laboratory experiments would indicate that this drug may have a beneficial effect on some of the varied symptoms associated with hypersensitivity and physical allergies. Clinical trials are in progress to determine the effect of this compound in a variety of conditions such as, vasomotor rhinitis, hay fever, urticaria, bronchial asthma, cold allergy and other physical allergies, serum reactions and contact dermatitis.

15534

On Breeding "Wild" House Mice in the Laboratory.

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The white laboratory mouse may well be regarded as a classical example of the domestication of an animal at the hand of man. The consequences of that domestication have provided laboratory experimenters with a small docile animal the usefulness of which has been demonstrated so often as to be an established tradition. However, another consequence of that domestication has been the emergence of many genotypes distinguishable one from another by a variety of characters. This multiplicity of genotypes is a forceful reminder of the variation inherent in the species, and when, for example, a given diet produces a certain result in a certain laboratory strain of mice, we next may be expected to inquire whether (a) other genotypes will react similarly, or (b) all genotypes will do so, or (c) whether the wild species will do so. In many ways an investigation of the wild type would seem to have the greatest interest and meaning. This necessitates a laboratory supply of the wild genotype obtained either by trapping the numbers required, or by breeding them in the laboratory. For many purposes the latter method is the more preferable.

Recent investigations in the nutritional aspects of experimental epidemiology[1] have raised this question of host genotypes among

* With the technical assistance of Mr. S. A. Greenhalgh.

[1] Schneider, H. A., and Webster, L. T., *J. Exp. Med.*, 1945, **81**, 359.

laboratory mice feeding on different diets. In order to extend the experiments to include the wild genotype of *Mus musculus domesticus* we attempted to establish a laboratory colony of wild house mice. The difficulties encountered, the fact that others[2] have commented on their inability to breed wild house mice in the laboratory, and the absence of published accounts on the breeding of wild house mice have prompted us to report here the measures found necessary to establish a breeding colony with mice trapped "in the wild."

Foundation Stock. The wild house mice† which formed the base of our laboratory stock were trapped in spring-door traps in the fall of 1943. Every effort was made to insure that the mice trapped were not hybridized with domestic stocks which may have escaped from laboratories or dealers. An attempt was made, too, to secure representatives from different localities in and near New York City. Thus mice were trapped in a rural community (East Northport, Long Island), in New York City apartments, in suburban Woodside, Valley Stream, and in Long Island City, Long Island. All of these mice, and all subsequent additions to a total of 35, (17 males, 18 females) were subjected to a period of quarantine of 2 weeks before being admitted to the colony. During the quarantine period each mouse was subjected to 4 successive stool cultures testing for the presence of *Salmonella enteritidis* and *Salmonella typhimurium*. All of the 35 mice examined proved to be free of these pathogenic species and in the 2½ years elapsed since the inception of the wild mouse colony no mouse typhoid disease has made its appearance. Indeed, the mice have remained healthy throughout with no signs of overt disease among them. Of the 35 mice of the original foundation stock 6 (3 males and 3 females) are still alive more than 2 years later. As far as can be judged deaths have occurred mainly through fighting, and, less often, due to what can only be described as senility. No grossly visible tumors have been observed.

Breeding trials. The initial attempts to breed wild house mice were conducted with 8 captured females and 14 captured males. With the exception of 1 female, described below, all attempts met with failure for a period of 9 months extending from October 1943 to July 1944. There would be little gain now to list the details of these failures except to state that a variety of conditions were tried involving various sizes of caging and varying periods of illumination ranging from complete darkness to a light-day of 16 hours such as Baker and Ranson[4] found necessary for the breeding of the wild field mouse (*Microtus agrestis*). In addition various beddings of wood shavings and peat moss were tried, as well as several stock diets all of which were satisfactory in supporting the prolific breeding of laboratory mouse stocks in adjacent cages in the same room at the same time.

During this period of breeding failure a single female, pregnant on admission to the laboratory on 10/11/43, was observed to deliver a litter of 3 mice on the following day. These she consumed. Subsequently this single female, during the time of breeding failure of 7 other captured females, gave birth to 3 litters following mating with captured males. The females among these litters, however, failed to breed with captured males even when 8 weeks of age and with perforate vagina.

Anoestrous of Wild Females. The failure of captured house mice to breed was traced to the failure of the females to achieve oestrus. Captured males were potent at all times and crossing with laboratory mouse females was easily done. The reciprocal cross failed, however, although tried on numerous occasions. Further evidence of the oestrous failure of the captured wild females was obtained by daily vaginal smear examination of

[2] Andervont, H. B., and Bryan, W. R., *J. Nat. Cancer Inst.*, 1944, **5**, 143.

† The house mice of the northern United States are not truly "wild" types, but commensals (*Mus musculus domesticus*). Under favorable conditions these will adopt feral habits. The true wild type, from which *M. m. domesticus* was derived, is *M. m. wagneri*, the wild mouse of Russian Turkestan (Schwarz and Schwarz[3]).

[3] Schwarz, E., and Schwarz, H. K., *J. Mammalogy*, 1943, **24**, 59.

[4] Baker, J. R., and Ranson, R. M., *Proc. Roy. Soc. London*, B, 1932, **110**, 313; *ibid.*, B, 1932, **112**, 39; *ibid.*, B, 1933, **113**, 486.

7 of them for a period of 30 days. No evidence of oestrus was observed during this period. A variety of fresh green foods and a mixture of grass seeds fed as dietary supplements failed to bring the mice into oestrus.

Breeding Achieved. In May, 1944, we received a gift of 4 male and 4 female wild house mice from Dr. L. V. Heilbrunn of the University of Pennsylvania, at Philadelphia. (These mice were subsequently incorporated into our foundation stock). Dr. Heilbrunn informed us that these mice were the progeny of some wild house mice bred in his laboratory as pets. Comparison of the conditions under which these mice bred and our own failed indicated that, apart from dietary and bedding differences, the Philadelphia mice, in addition, had access to an exercise wheel. Breeding success in our own laboratory for all of our captured females was suddenly and finally achieved in July, 1944, following the introduction of exercise wheels. From that time forward we have been able to breed wild house mice at will and have prepared about 1,000 mice for experimental purposes. In this connection it is interesting to note that in their studies on the factors affecting the breeding of the field mouse *(Microtus agrestis)*, Baker and Ranson[4] recorded that their cages included a "revolving wire wheel" and that "the mice made frequent use of the wire wheels." With such cages these investigators have shown clearly that the breeding of *Microtus* proceeds best in an environment which supplies a daily period of incandescent illumination of 15 hours and that shortening the light day to 9 hours almost prevents reproduction. It is the female that is chiefly affected. At the moment our own experiments, conducted with a light day of 16 hours, do not permit a decision whether for *Mus. musculus* the effect of light is direct, or indirect through its effect on activity. However, it is a fact that in our experiments a 16-hour-light day, by itself, did not result in successful breeding. Experiments are now in progress to test the effect of activity with and without light.

The Complete Breeding Technic. The technic now followed in our laboratory for the breeding of wild house mice is as follows:

Environment. A single room (18′ x 15′) is devoted to the colony. A temperature of 75°F is maintained by thermostatic control, except for summer temperatures in excess of this. Temperature rises up to 85°F are tolerated without any change in the breeding performance, but temperatures in excess of 90°F result in breeding failure. Some preliminary experiments indicated that this failure was attributable to the males, probably due to the recognized effect of excessive heat on sperm viability. The animal room is lighted from 5 a.m. to 9 p.m. (16 hours) by 2-200 watt incandescent lamps controlled by a time-clock. All daylight is excluded.

Caging. The mice are bred, 1 male to 5 females, in galvanized iron boxes 9″ x 12″ x 9″ high with galvanized mesh covers through which the water bottle tubes are inserted. Centered and 3½″ from the top edge of the box the exercise wheel-drums are suspended on steel axles extending through the sides of the box. These exercise drums are lightly constructed of copper mesh for ease of turning and are 5″ in diameter and width. Four spokes permit the mice to enter the drum easily even while it is in motion, which is often. Revolution counters attached to these exercise drums indicated that in a 24-hour period a single pair of mice, male and female, will complete 40 to 60 thousand revolutions. At this rate, by free choice the mice have run approximately 10 to 15 miles per day. When a female is observed to be pregnant she is transferred to an individual galvanized box, 7″ x 10″ x 5″, to await her litter. The young are weaned at 4 weeks of age.

Bedding. Autoclaved pine shavings are used for bedding. The shavings must be of a size large enough so that a loose mass is formed on the floor of the cage into which the mice can penetrate and hide. This is important from the standpoint of mating behavior, for if smaller sized shavings were used which readily pack down and form a firm mass so that the mice were unable to hide in it easily, then, upon mating 5 females with 1 male it was observed that the females not in heat at a given time would attack the male and often kill him upon his attempting to mate with a female which was ready to accept him. No litters were ever obtained under such conditions even if the male sur-

TABLE I.
Effects of Peat Moss and Exercise on the Breeding of Wild House Mice on a Diet of Whole Wheat Flour, Dried Whole Milk, and Salt.
(16-hr light-day.)

Diet No.	Diet description	Exercise drums	Within 60 days after mating: No. of females bred	No. of litters	Pregnancies
					%
100	Whole wheat, dried whole milk, NaCl	0	16	2	13
100	" " " " " "	+	13	11	85
259	Diet 100 plus 10% peat moss	0	15	6	40
259	" " " " " "	+	13	13	100

vived. This behavior problem could be solved either by mating the mice in single pairs, in which case the physical nature of the bedding was unimportant, or, preferably, by providing the shavings bedding in a state which allowed the mating pair to hide. Peat moss will serve as well as shavings.

Diet. At the time of the first breeding success in July 1944, following the introduction of exercise drums, all of the captured mice on hand were on a stock diet of ⅔ whole wheat, ⅓ dried whole milk and 1% NaCl (Diet 100). The bedding was peat moss. It was found that equally good results could be obtained by using pine shavings as bedding and adding 10% ground peat moss to the diet (Diet 259). This satisfactory, if unorthodox, diet served well in bringing up the numbers of the breeding stock at the same time that progeny were taken in large numbers for other experiments. At the present we are able to breed these wild mice very well on Diet 100 without the addition of peat moss. A recent experiment to test the effects of peat moss in the diet, as well as demonstrating the rôle of the exercise drums, is summarized in Table I. The females mated in this experiment were assembled into the experimental groups by dividing litter mates. There is thus no reason to attribute the obvious differences in breeding performance due to the presence or absence of exercise drums to genetic differences. The exercise effect is statistically significant; for Diet 100, $P<0.001$; for Diet 259, $P<0.01$. The effect of peat moss in improving the breeding performance, while suggestive, is not demonstrated with the same statistical adequacy ($P>0.1$).

It will be noted that on Diet 100, in the absence of an exercise drum, there were 2 pregnancies out of 16 mice bred. These 2 mice, in the absence of peat moss and exercise, probably came into oestrus by virtue of a genetic constitution which permitted this event in the absence of the environmental factors found necessary for the great number of wild house mice represented here. Such genetic variation would serve as the basis of selection of stocks independent of the environmental factors we have described as necessary, and by such means, to extend the argument, laboratory stocks may very well have been derived.

Breeding Plan. In order to maintain genetic heterogeneity the wild mouse colony has been bred in a manner so that closely related mice never form breeding pairs, but instead are crossed within the limits of the stock as much as possible. It is true, of course, that various kinds of automatic selection are going on which are slowly changing the statistical character of the wild genotype first assembled in the colony. Thus, those females which remain sterile under the conditions of the laboratory, in spite of the exercise drums, cannot contribute to the genetic composition of the succeeding generation. Such sterile genotypes will slowly be diminished in frequency. Using the per cent of pregnancies begun during the first 30 days following mating as an index of breeding performance, during the period of January 1945 to June 1946, such pregnancies rose from

56.8% to 70.2%. The average number of mice weaned per female rose in the same period from 3.69 to 4.08.

Handling of Wild Mice. All of the mice seen thus far in our colony have been as active and agile as the captured foundation stock. Special measures are necessary for handling them, for it is impossible to open a cage without having many escape. The simplest practical expedient is to perform all manipulations of the mice in a large box 18 to 20″ deep with smooth sides. The escaping mice are thus confined to the larger box and may be recaught. With a little practice all of the ordinary manipulations of a mouse colony may be carried out quite easily.

Summary. The establishment of a breeding colony of wild house mice has been described. The inclusion of exercise drums in the breeding cages was found to be necessary.

15535 P

Effect of Oxygen on *P. lophurae* Infected Ducks.*

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It has been suggested that anoxia is an important factor in the mechanism of death in acute *P. falciparum* infection in man,[1] *P. knowlsi* infection in monkeys[2] and *P. lophurae* infection in ducks.[3] Furthermore it has been shown that ducks with malaria die sooner when placed in a decompression chamber than control birds kept at normal atmospheric pressure.[4] With the rapid destruction of red cells by the parasites the time may come during this disease when there is an insufficient number of red cells to carry a sufficient quantity of oxygen to supply the tissues of the host. The frequent intravenous injection of large quantities of duck red blood cells into birds that are moribund from malaria definitely prolongs their life.[5] In this study young ducks infected with *P. lophurae* have been placed in a chamber and given oxygen to determine its effect on the course of the infection.

In one experiment 10 ducks 15 days of age were inoculated intravenously with *P. lophurae* and put into the oxygen chamber. The oxygen within the tank was kept at approximately 50% concentration for 6 days at which time the amount of oxygen was increased to 75-85% for one day. Twenty similarly infected ducks were kept in batteries in the animal room. The time that death occurred in these 2 groups of ducks is shown in Experiment 1, Table I. A second group of 25 ducks 18 days of age were inoculated intravenously with *P. lophurae* infected blood. On the 4th day of the infection 10 of these were put into the oxygen chamber. The concentration of oxygen was kept at approximately 50% for 18 hours and then increased to 75% and ultimately to 85% during the following 24 hours. The 15 ducks in the control group were kept in the battery in the animal room. The time at which these birds died is shown in Experiment 2, Table I. In the third experiment 20 ducks 18 days of age were put into the oxygen chamber on the 4th day of

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