XXI. MAINTENANCE OF A STANDARDISED BREED OF YOUNG RATS FOR WORK UPON FAT-SOLUBLE VITAMINS, WITH PARTICULAR REFERENCE TO THE ENDOWMENT OF THE OFFSPRING.

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IN an accompanying paper [Chick, 1926] one of the writers discusses some sources of error in the technique usually adopted in the investigations of fat-soluble vitamins by the biological method. The conclusion reached, based on the collective experience of the workers in this Institute over a period of several years, is that one, perhaps the most important, disturbing factor is the variation in the state of nutrition of the young rats employed in the tests; in other words variation in the reserves of these vitamins possessed by the young animals at the beginning of the experimental period.

In order to attain a reasonable degree of accuracy in the estimation of fat-soluble vitamins in different foodstuffs and the comparison of one such material with another, it is essential to have at one's command a standard young rat, which can be depended upon to react subsequently in a reasonably constant manner to the same type and degree of deficiency. Special care is needed in breeding such animals if this ideal is to be realised, and in the Lister Institute, as elsewhere, the workers in this field have found it necessary to supervise directly the breeding of the rats destined for their own use.

During several years' experience the greatest difficulty has been experienced in obtaining the constancy required for accurate work. At the present time, however, the difficulty appears to be overcome to a large extent and the following account of our methods is published here in the hope that it may prove useful to others who may be confronted with the same problems.

The amount and nature of the reserves with which the experimental animals are equipped at the beginning of the observation is more important in nutritional experiments of long duration than has been generally recognised. This is especially so when the subsequent work involves diets deficient in fat-soluble vitamins. For even very young animals possess reserve stores of these factors to a surprisingly variable degree, depending on the diet of the mother before and during pregnancy, and during the period of lactation.

Korenchevsky [1921, 1922] and Korenchevsky and Carr [1923, 1924, 1925] have shown that the effects produced upon young rats receiving diets deficient in fat-soluble vitamins will be widely divergent, in respect both to skeletal disorders and to the degree of growth in weight, according as the diet of the mother during pregnancy and lactation has been defective or generous in these vitamins as well as in the salts of calcium. When, for example, the mothers received cod-liver oil and extra calcium during lactation, the young rats after 30 days' independent existence on a diet deprived of all fat-soluble vitamins, were found to possess skeletons which might be regarded as normal [Korenchevsky and Carr, 1923]. Cramer [1922] drew attention to the remarkable difference in growth exhibited by two breeds of young rats after being maintained for several weeks upon diets devoid of fat-soluble vitamins. There was a difference between the diets on which the two groups had been reared; in one case the mothers had received a diet consisting mainly of cereals and in the other there had been additions of cod-liver oil. Hess, Weinstock and Tolstoi [1923] noticed that certain young rats bred outside the laboratory could maintain a normal condition of the skeleton after receiving a standard, low phosphorus, rickets-producing diet for several weeks. Here also the cause was traced to a superior diet enjoyed by the breeding stock. For, if the mothers at the end of pregnancy were brought into the laboratory and thereafter received the usual laboratory diet, the young rats proved to have the usual susceptibility, when placed later upon the experimental diets. These authors explain the differences, resulting from the use during lactation of diets richer and poorer respectively in anti-rachitic foods, as due to the food taken directly by the young during the last days of the period, when they are capable of feeding themselves [Hess and Weinstock, 1924, 1, 2]. Korenchevsky and Carr [1923], on the other hand, on the basis of experiments in which the mother alone had access to the special anti-rachitic food (cod-liver oil) provided showed that the protective substances can be transferred to the young with the mother's milk.

It is therefore clear that in breeding rats for experimental work upon fat-soluble vitamins, the diet, whilst possessing an abundance of other essential elements—protein of good quality, mineral salts and vitamins B and C—should contain a constant, but not excessive, amount of both vitamin A and vitamin D. For the above researches give abundant proof that young rats endowed with unduly large reserves of these substances will later on prove insensitive to the deficiencies in diet which are the object of study.

Steenbock and Nelson [1923] were the first to point out the necessity of considering the reserves of these two vitamins, A and D, separately (see also Chick, 1926]. The hypothesis of these workers is that the degree of growth subsequently manifested by the young when placed upon a diet deprived of *both* factors, is dependent jointly upon the reserves of *both*, either factor alone being unable to promote growth, though capable of maintaining weight for a time, in the absence of the other.

This theory, for which there is much experimental support, has not only an important bearing upon the methods usually employed in work with fat-soluble vitamins, dealt with in detail in the preceding paper, but has also focussed attention upon the importance of the relative amount of vitamins A and D in the diet upon which the experimental rats are reared. Steenbock and Nelson [1923] called attention to the fact that young rats placed upon diets deficient in fat-soluble vitamins may cease to grow several weeks before the onset of the ophthalmia which is the characteristic sign of vitamin A deficiency. From this circumstance they conclude that it is usual for such young rats to possess more vitamin A in reserve than anti-rachitic vitamin D. Although in our experience the first signs of ophthalmia have usually been detected before cessation of growth in weight, we agree with the conclusion of Steenbock and Nelson, and in the light of later knowledge we realise that the more unexpected and unexplained irregularities have occurred when we have unintentionally enriched the breeding diet with vitamin D, as provided, for example, in summer cow's milk or egg-yolk. Steenbock and Black [1925] encountered similar disturbing effects when unwisely submitting their breeding stock to irradiation with ultra-violet light as a therapeutic measure when the young were not thriving in a normal manner.

General management of the breeding stock.

We have worked hitherto, almost exclusively, with the black and white pied variety of the Norwegian rat, but the albino type has been found equally suitable by many workers, notably by Donaldson [1924] at the Wistar Institute. Our original stock was obtained from the department of physiology at King's College for Women by the courtesy of Dr Gladys Hartwell. Although inbreeding is our custom, it has been our habit about once a year to obtain some young rats from outside to refresh the stock, and these have usually been obtained from Dr Hartwell.

The rats are kept in a laboratory with north aspect and the temperature maintained between 65° and 70°. Breeding is not permitted before the female rats are about four months old. After that time about 4 to 8 females are placed with 1 to 2 bucks in large zinc wire cages of dimensions 20 inches by 30 inches by 30 inches high. The cages have loose wire bottoms and rest upon large trays filled with peat. The rats are provided with a bedding of wood wool, which is changed every day, whilst the cages and trays are scoured thoroughly twice weekly.

Pregnant females are removed from the general cage 7 to 10 days before the birth of the young and are kept separately in cages of dimensions 20 inches by 10 inches by 7 inches high. Here the young family remain together with the mother for 21 days which is the usual interval before weaning. The mother is then removed and enjoys 2 weeks' rest before breeding is again permitted. As a rule not more than the first three litters from any female are used; our experience in this matter coincides with that of the Wistar

Institute that fourth and subsequent litters are too small and irregular for this type of work. We have found the greatest number of large and healthy litters to be born from April to September. In our experience the months of October, November and December, especially the last two, have proved bad for breeding. Few litters are born, these are frequently feeble and the mothers appear to lack a sufficient milk supply. After the turn of the year things gradually improve as spring approaches. Excluding the month of November the average number of young rats reared in the first and second litters cast by the females of our breeding stock is between 8 and 9, a figure considerably higher than that given by Donaldson [1924], viz. 6 to 7 per litter for first and second litters of the tame albino, and 5 to 6 per litter for the wild Norwegian rat.

Diet.

The diet we have found satisfactory consists of cow's milk, brown or white bread, or rice or oats or other whole cereals and seeds, with fresh raw vegetables, carrot, turnip, cabbage or spinach. Marmite is mixed with the food every day to provide an abundance of vitamin B and raw lean meat is given about twice a week. Within the above choice of foods the ration is varied daily so that the animals get plenty of change. This we have found a necessary measure to encourage a good appetite and an adequate intake of food, which is particularly necessary in the case of the female rats from which litters are expected. During the last few days of pregnancy and during the period of lactation the mother receives only milk, cereals and raw vegetables, with marmite added. Lean meat has occasionally been given when a tendency has been suspected to eat the young, which may occur at the times of the year which are unfavourable for breeding.

Although this breeding diet was maintained constant throughout the year, it had been noticed that the young rats born in winter and spring were markedly inferior to those born in summer and autumn. This was shown by a lessened capacity to grow and produce a well calcified skeleton, when placed on diets devoid of fat-soluble vitamins. After investigating many other possible sources of this irregularity, the cause was at length traced to the diet of the breeding stock and found to consist in the variations in the nutritive value of the daily milk ration at different seasons of the year. Luce [1924] and Boas and Chick [1924] have demonstrated the superiority as regards vitamins A and D of summer, "pasture fed" cow's milk compared with that obtained from cows kept in stalls and receiving dry fodder (hay, cereals and roots) in winter. The summer pasture feeding seems to affect the value of the ordinary milk supplied in London from about May to October or November, depending on the season; the lowest vitamin content occurs in February or March.

In order to avoid the irregularity due to this variation, it was determined to abandon the use of fresh cow's milk and to achieve constancy by substituting

a reconstituted dried milk, prepared in quantity from the ordinary milk supply of early spring. Winter rather than summer milk was chosen as it was considered desirable to avoid any possibility of excessive reserves in the young rats, which would render them insensitive to deficiencies of diet encountered later on. Accordingly, in March 1925, a supply of fresh milk sufficient to last over the summer and autumn was kindly dried for us by Glaxo Ltd. At first this reconstituted milk was supplied indiscriminately to the whole breeding stock, but, after making the change, the general nutritive condition was found to be lowered dangerously near the margin at which the young could be reared successfully. Fresh milk was therefore restored to the diet of the general stock and the dried winter milk reserved for mothers during the last days of pregnancy and the period of lactation. It is also fed to the young during the interval elapsing after weaning and before being used for experiment. This arrangement has proved successful in securing a reasonable degree of constancy in the young rats as regards their reserve supply of fat-soluble vitamins [see also Chick, 1926, Fig. 1 and Table V].

As is mentioned above, the months of November and December offer special difficulties in the breeding of rats, for some unknown reason. In our need for young litters at all times of the year we are probably guilty of forcing the rats somewhat in the matter of breeding, and at the less favourable seasons it is necessary to tempt the appetite of the mother with changes of food if the young are to be reared successfully. A ration of fresh milk is occasionally given at discretion and extra lean meat.

It is probable that the seasonal irregularity noticed previously in our breed was due to variation in the supply of anti-rachitic vitamin D rather than of vitamin A. The stock diet, consisting of cow's milk, whole cereals and fresh green vegetables, is moderately rich in the latter vitamin, even in winter, when the supply of the anti-rachitic vitamin D will be very low.

This view was confirmed in an interesting, if somewhat disastrous, manner in the autumn of 1924. Large quantities of fresh egg-white were being used in the Institute in connection with other researches, and in the interests of economy the yolks were used in the diet of the rat breeding stock and in some cases added to the ration of lactating mothers whose litters did not appear to be thriving. The effect of adding a substance so high in anti-rachitic value [Hess, 1923; Casparis, Shipley and Kramer, 1923; Hess and Weinstock 1924, 1, 2] was not contemplated and the results, though admirable from the standpoint of the mother and the young litters, proved disastrous from the point of view of the subsequent experiments. The young rats, placed at 50 g. weight upon McCollum's No. 3143 rickets-producing diet [McCollum, Simmonds, Shipley and Park, 1921], showed only a slight or moderate degree of rickets after 4 weeks upon the diet, in place of the usual severe type [see Chick, 1926, Table VI]. The calcium content of the bones was also at an unusually high level and varied from 12 to 17 % (reckoned on the dry weight of the bones) instead of the usual 9 to 13 % [see also Luce, 1924].

The experiments had been arranged to determine the anti-rachitic value of samples of milk, which were added to the diet in a range of daily doses, but they gave unreliable results and could not be used in comparison with the results of other tests in which the experimental animals had been less richly endowed with anti-rachitic vitamin and had reacted to the defective diet in a more normal manner. This marked enrichment of the breeding diet with vitamin D appeared to affect the reserves possessed even by the young rats born several weeks after its use had been discontinued.

SUMMARY.

1. A description is given of the diet and general management found satisfactory in breeding rats for nutritional work upon fat-soluble vitamins.

2. The diet used, cereals, fresh vegetables and milk, is moderately rich in vitamin A but poorer in vitamin D, except in summer, when fresh pasture milk contains a much increased amount of this vitamin. This circumstance has been the cause of a disturbing amount of variation in the nutritive condition of the young at different seasons of the year.

3. The expedient of replacing fresh milk in the diet of the pregnant and lactating mothers by dried milk prepared from the winter milk supply has proved successful in lessening the degree of irregularity among the young.

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