XII. NOTE ON XEROPHTHALMIA IN RATS.

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AMONG the diseases which have been attributed to a deficiency in the fat-soluble *A* accessory substance is a condition of the eyes now described as xerophthalmia. McCollum, Simmonds and Pitz [1918] indeed speak of this relation in very definite terms. "Xerophthalmia and polyneuritis are abundantly demonstrated to have their origin in the lack of a sufficient amount of the fat-soluble *A* and water-soluble *B* respectively in the diet." That eye symptoms in rats may occur as the result of deficiencies in the diet seems to be common experience. It is very important, however, to distinguish between symptoms due directly to a specific dietetic error and any that may be due to infection, encouraged, may be, by diminished resistance due to the diet.

My own experience seems to point to the fact that so far as xerophthalmia is concerned the latter relation is the true one. In considering this question I have taken absence of normal growth as evidence of a deficient ration.

It is desirable at the outset to view the origin and growth of the idea that xerophthalmia is due to the absence of some essential nutritive factor and later of the view that fat-soluble *A* is the pivot on which the matter turns.

Osborne and Mendel [1914, 1] in writing of their protein-free milk state that the addition of butter fat to this substance avoids failure in growth and "an infectious eye disease." They repeat this statement [1914, 2, 1915] when, dealing with the question of separating the essential fat factor, mention is again made of the fact that animals fed with an inefficient fat fraction developed purulent eyes. McCollum and Simmonds [1917] cite eye disease as a possible deficiency disease due to absence of the fat factor, and the writers quote cases of children developing it on fat-deficient diets. As previously mentioned [1918] they definitely state that xerophthalmia is brought about by absence of the fat factor. Osborne and Mendel [1918] make a further allusion to the condition stating that on a fat deficient diet the symptoms occurred, and were cured by the addition of liver oil to the food. In a recent paper by Harden and Zilva [1918] eye symptoms are again taken as indicative of the absence of fat-soluble *A*.

In January, 1913, a series of experiments was carried out in this laboratory solely with a view to clearing up this matter of eye disease, which had made its appearance amongst animals then being fed by Professor Hopkins on synthetic diets in which the mineral supply was Osborne and Mendel's artificial

salt mixture. The results of these experiments have never been published, and it is with Professor Hopkins' permission that I allude to them here. He found that animals fed on extracted starch, extracted caseinogen, sugar, salts, and lard developed an ulcerative condition of the cornea with conjunctivitis within 14 days from the commencement of the experiment, identical with that present in animals fed, in previous experiments, on a similar artificial diet, though these earlier rats had been fed with a different end in view. In the more extreme cases there was complete dissolution of the eyeball with rupture of the cornea. Many of the animals died, but one or two survived and on being given 3 cc. milk as an addendum to the diet, the eye symptoms cleared up. This led to the assumption that the disease was due to some deficiency in the diet which the 3 cc. of milk supplied. In 1915 Dr Ginsaburo Totani, at the suggestion of Professor Hopkins, undertook some experiments based upon these previous findings with the intention of making a thorough histological study of the eye lesions. The experiments were not done in the same building as were the earlier ones, but other conditions were precisely similar. The weight of the animals fell as usual, but no eye disease at all was obtained with purified artificial diets identical with those previously used, and after many weeks the experiment was given up.

Save in the case of the experiments done by Hopkins I have no detailed information as to the similarity of the symptoms observed by the different workers, nor as to the rapidity with which the xerophthalmia develops. In Hopkins' 1913 experiments, as already stated, it appeared as early as the fourteenth day. It is perhaps legitimate to assume that in the other cases cessation of growth, or initial absence of growth, marked the point at which the condition of the eyes became pathological, and that on resumption of growth the eye disease abated and ultimately cleared up. This, at any rate, seems to be the correct conclusion to draw from the evidence available. My own experience in the matter which has led me to the view already expressed is the following:

Since January, 1918, to the present date I have had approximately 500 young rats on varying experimental diets. To all these diets has been added water-soluble *B* in the form of a fat-free alcoholic extract of a yeast preparation. About 50 per cent. of the animals have received a supply of fat-soluble *A*, the remaining 50 per cent. having been on diets where fat-soluble *A* has been either deficient or absent. As regards this latter statement a diet has been considered "deficient" in fat-soluble *A* when young rats, receiving a proper supply of the water-soluble accessory substance, have failed to grow after, and often long before, the eighth week of the experiment. They have been supposed completely lacking in the factor when lard has been the source of fat supply. The basal diet of all foods fed has consisted of extracted caseinogen, unextracted starch, sugar and salts. The stock animals from which the experimental animals have been selected have been fed on bread and milk and oats, with the addition for the last four months of various vegetables.

During the whole of the period five cases only of xerophthalmia have occurred. Two of these were rats which were being fed on margarine with which was incorporated an unknown quantity of egg yolk as the source of fat-soluble *A*; one animal was on basal diet plus lard plus 2 cc. of milk per day; another was being given fatty acids in place of fat, and the remaining rat was a stock animal which had never been on any diet save the normal stock food.

The two first-mentioned animals were given butter as an addendum to their diet when the disease was well established, and their eyes were treated daily with boracic lotion. Both animals became cured within a week. Their growth curve was poor before the addition and good after. The animal having 2 cc. milk daily did not clear up at all and ultimately died; the one on fatty acids as its source of fat was fed with an addendum of butter and began to get better after a week. (This animal is still under observation and is not completely cured yet.) The rat on stock diet was a considerable time recovering—some five or six weeks—but did so ultimately. Thus of the five cases,

Two occurred on a diet where fat-soluble *A* was supplied, if to a limited extent.

One occurred on a diet where fat-soluble *A* was presumably present in sufficient quantity.

One occurred on a diet where fat-soluble *A* was probably absent.

One occurred on an ample stock diet.

Again,

Two animals were growing moderately well.

One animal lost in weight.

One animal neither declined nor increased in weight.

Each experiment lasted some three or four months and in each experiment there were at least eight other animals on the respective diets, showing no symptoms at all. Xerophthalmia on this evidence would appear to have no direct relation to diet, since these five animals were on different types of food; and to have little relation to growth.

It may be objected that the basal diet was not entirely free from fat-soluble *A* or that the fats used contained a sufficiency of the essential factor to prevent the onset of the disease. In view of the fact that practically all the animals on fat-soluble *A* deficient diets, though having an abundant supply of water-soluble *B*, failed ultimately to grow normally, and in many cases to grow at all, the former factor, if there at all, must, according to current views, have been present only in negligible quantities.

In addition to the above experience there remains that of Totani, who was using more highly purified foods and always lard as his source of fat. I consider that the discrepancy between Hopkins' and Totani's results, in which the animals were upon a diet absolutely identical in each case, is due to there having been the infection present in the former case and not in the latter, and that the freedom of my own animals from this disease is owing

(1) to the fact that no trouble has been spared to keep the animals under the very cleanest and healthiest conditions, and

(2) that care has always been taken to treat very promptly with boracic lotion any cases of slightly sore-looking eyes.

As regards the clearing up of the symptoms, which the quoted workers have observed on the addition of fat-soluble *A* it is surely probable that, as in so many cases of obstinate infection, general improvement of health brings about a cure of the local condition. I feel convinced that with avoidance of initial infection, experimental animals can be kept almost entirely free from this so-called deficiency disease, whether fat-soluble *A* be present or absent, and that it is dangerous to draw conclusions as to the fat-soluble *A* content of any diet from the appearance of xerophthalmia.

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