

tein is subjected to conditions which actually denature it. Although Anson and Mirsky consider hemoglobin a typical coagulable protein, they do not differentiate between coagulation and denaturation. We cannot agree with their point of view that the effects of acid on hemoglobin are the same as on other coagulable proteins. Besides a conceivable denaturation of the entire hemoglobin molecule, acids exert other effects such as the splitting of hemoglobin into hematin and globin and the denaturation of the globin which are characteristic of hemoglobin and are entirely absent when dealing with the simple proteins. We feel that insolubility at the iso-electric point cannot be used as a criterion for denaturation of hemoglobin because of the complex transformations which this particular protein undergoes upon treatment with acids. Such a physical property, though valuable in the case of simple proteins like ovalbumin, is of little significance in the case of hemoglobin because of a lack of definite information on solubility and other relationships between the various components of the reaction mixtures.

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Some Observations on the Growth of Rats on "Fat-Free" and Fat-Containing Diets.

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Recently Burr and Burr¹ and McAmis, Anderson, and Mendel² have shown that the growth of rats on a diet from which all true fat has been excluded is distinctly inferior to that of rats on a diet which contains some fat, even though the fat used is devoid of vitamin A. Furthermore the health of the animals raised on the fat-free diet also suffers. Burr and Burr¹ believe that the total deprivation of food fat leads to the development of a deficiency disease, the symptoms of which are impaired growth, scaliness of the feet and tail, excessive production of dandruff, with ultimate hematuria and albuminuria, loss in weight, and premature death. In a later paper³ Burr and Burr state that fat is an essential constituent of the diet

¹ Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, lxxxii, 345.

² McAmis, A. J., Anderson, W. E., and Mendel, L. B., *J. Biol. Chem.*, 1929, lxxxii, 247.

³ Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1930, lxxxvi, 571.

because of the inability of rats to synthesize sufficient quantities of the more highly unsaturated fatty acids, specifically linoleic acid, for the maintenance of the normal functioning of the tissue lipids.

In the present paper the author wishes to present a few observations which would seem to have a significant bearing on the question of the rôle of fat in animal nutrition, especially with respect to the theory that linoleic acid and other highly unsaturated fatty acids are essential constituents of the diet. For the most part these observations are purely incidental, having been made on rats raised on various diets in connection with a study of phospholipid metabolism.

In the earlier phase of the work, all rats on experimental diets were raised individually in cages constructed throughout of No. 2 mesh wire screen and raised on 2-inch legs. Without exception, the rats which were being fed on the "fat-free" diet* developed a peculiar scaly condition of the tail similar in every respect to that described and illustrated by Burr and Burr.

However, since the need for animals raised on the "fat-free" diet exceeded the supply of false-bottom cages, it was decided to raise groups of rats in the ordinary stock cages. This necessitated a change in the method of feeding. Heretofore the extracted yeast and the Oscodal† had been fed separately from the main ration but it seemed advisable to mix the vitamin supplements and the basic ration if the animals were to be raised in groups. Accordingly the "fat-free" diet which has been fed to animals in the stock cages contains 2.5 gm. of yeast and 0.025 gm. of Oscodal for each 100 calories in the basic ration.

In view of the unailing occurrence of scaliness on the tails and feet of rats fed essentially the same diet in the false-bottom cages, it was quite unexpected to find that the rats in the stock cages remained quite normal in appearance. To date between 45 and 50 rats have been raised on the "fat-free" diet in the stock cages and in not a single instance has there been any sign of scaliness or of any other abnormality in appearance. However, the rate of growth has been noticeably slower than that of rats on the stock diet of kitchen scraps. Most of the animals were killed when they reached a body weight of about 150 gm. so that it is impossible to say whether or not these animals would have continued in apparent good health

* The basic "fat-free" diet is identical in composition to No. 550 of Burr and Burr.¹ The casein is extracted in a continuous extractor with boiling alcohol, the yeast with ether in a Soxhlet apparatus. By analysis of the ingredients the mixed "fat-free" diet contains about 0.25% of fatty acids.

† The nonsaponifiable matter of cod liver oil, kindly supplied by the H. A. Metz Laboratories, Inc., through the courtesy of Dr. H. E. Dubin.

throughout the normal life cycle. However, one group of rats, now 4 months old, is still quite normal in appearance, although the average weight is sub-normal.

There seemed to be only two factors which could be responsible for the absence of scaliness from the rats raised on the "fat-free" diet in stock cages: one, the mixing of the yeast and the Oscodal with the basic ration; and the other, the fact that in the stock cages the animals were bedded with paper and had access to their feces. In order to see which explanation was correct the following experiment was begun: Nine littermate rats were divided into two groups. One group was placed in the false-bottom cages constructed throughout of No. 2 mesh wire screen; the other group was placed in similar cages over the bottoms of which window screening (No. 16 mesh) had been placed in order to retain the feces. Of each group, 3 rats were placed together in a cage, the others being kept in individual cages. All were fed on the same "fat-free" diet in which the yeast and Oscodal were mixed with the basic ration. These rats are now 23 weeks old and have been on the diet for 19 weeks. All of the rats in the cages with the No. 2 mesh wire bottoms have developed well marked scaliness of the tail and, in some instances, node-like constrictions which eventually caused a small piece of the tail to blacken and drop off. On the other hand, none of the 4 rats which have had access to their feces has developed any scaliness of the tail.

Now, although there is an unmistakable difference in the appearance of these two groups of rats, there has been no essential difference in their growth, all being definitely subnormal in weight. The poor growth of this particular group of rats is not entirely due to the absence of fat from the diet since rats fed on the same diet but raised in stock cages have grown very much better.

The question is whether or not the substance which is present in the feces (possibly in the bacteria) of animals on a "fat-free" diet is a highly unsaturated fatty acid, the consumption of which is responsible for the absence of scaliness from the rats raised in the stock cages. While the author has not been concerned with an intimate investigation of the phenomenon, certain incidental evidence has been obtained which is difficult to harmonize with such an explanation.

It has already been shown⁴ that the constituent fatty acids of the phospholipids in the tissues of rats raised on a "fat-free" diet have a low degree of unsaturation as compared with those of stock rats or of rats fed on a diet containing olive oil. Recent work has con-

⁴ Sinclair, R. G., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, xxvi, 793.

firmed this fact. Furthermore it has been found (unpublished experiments) that small amounts of cod liver oil added to the "fat-free" diet (60 mg. daily or 1% by weight) increase the iodine numbers of the phospholipid fatty acids from the level of 100, characteristic of the "fat-free" diet, to about 125, while 1% by weight of lard gives an I.N. of 115. With this fact in mind, one should expect to find a higher I.N. in the phospholipid fatty acids in rats raised in stock cages and therefore normal in appearance, than in rats raised in false-bottom cages and showing marked scaliness of the tail, if the protective action of the feces is due to a highly unsaturated fatty acid. Values for the I.N. of the phospholipid fatty acids of rats raised on the "fat-free" diet are as follows: rats showing marked scaliness 99, 99, 104; rats quite normal in appearance 100, 104, 100, 105. Since there is no difference it seems hardly likely that the rats raised in the stock cages have consumed with their feces appreciable quantities of unsaturated fatty acids.

Furthermore it has been found that rats raised in false-bottom cages and fed on the "fat-free" diet to which cod liver oil has been added (even to the extent of 10% by weight) develop marked scaliness of the tail. The scaliness in this case is certainly not due to a low degree of unsaturation in the tissue lipids since the latter are quite highly unsaturated. Lard, on the other hand, seems to be effective in preventing the scaliness when present to the extent of 1% of the diet.

While the evidence available at present seems to be rather against the probability that the failure of rats fed on a "fat-free" diet to develop scaliness if they are raised in stock cages is due to the consumption with the feces of appreciable quantities of a highly unsaturated fatty acid, the final solution of the problem will come only from direct experimentation. Since the author does not intend to undertake further investigation of this problem, it has seemed advisable to place the above observations on record.