XLII. THE GROWTH OF RATS UPON DIETS OF ISOLATED FOOD SUBSTANCES.

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In a recent communication on the growth of rats upon artificial diets containing lactose, published in this journal, Drummond [1916] has discussed a number of problems with which we, as well as other investigators, have been concerned for several years. The facts reported are essentially in harmony with our own experience in the study of the growth of white rats fed upon mixtures of isolated food substances. Inasmuch as readers of Drummond's paper who have not carefully followed our own publications might easily gain the impression that our views are at variance with his conclusions as well as with those of some other workers in this field at the present time, we regard it as desirable to refer here to a few of the topics involved.

In order to appreciate some of the changes of opinion which have taken place as the result of more recent studies in feeding animals on isolated food mixtures, it should be pointed out that earlier we have recorded experiments showing successful growth for such considerable periods that it seemed reasonable, at that time, to conclude that the diets employed were fully adequate for prolonged nutrition. Subsequent and more extended experience demonstrated, however, that failure and decline of body weight inevitably ensued unless additional accessory substances were furnished in the diet. By the use of what we termed "protein-free milk," along with isolated proteins, starch, and fats in the form of lard, we usually succeeded in promoting the growth of albino rats for about eighty days; and when the failure on

these diets manifested itself, the decline was averted by the substitution of certain natural fats, notably butter fat, for a part of the lard. This agrees with the experience of other investigators in this field. The inevitable ultimate failure of all experiments in which a fat-soluble accessory component was missing was at first overlooked, owing to the fact that the earlier observations were made over too short a period of time to permit the nutritive decline to manifest itself. This statement applies to the results of the feeding experiments with fat-free food mixtures which we published in 1912 [Osborne and Mendel 1912]. We have not claimed, however, that the diets in these experiments were "*lipoid*"-free. We considered them as evidence of the possibility of very considerable constructive nutrition in the absence of the ordinary fats. Indeed, some time ago we attempted to avoid any misconception regarding our previous experience by the following statement: "In reviewing our earlier published experiments Funk has erroneously assumed that we secured completed growth with the diets in which the butter component was not yet employed. It is true that the increments in weight were in some cases very noteworthy; but in every instance cessation ultimately ensued before the completion of the normal progress of the growth or subsequent maintenance. We have never denied the necessity of a growth-promoting food accessory in accord with the claim of Hopkins; and recently we pointed out that the successful partial completion of growth, such as has been obtained in our experiments, may well have been due to a store of the essential compound in the body of the experimental animals at the beginning of the trials. It is by no means necessary to assume with Funk that small quantities of these accessory substances were inadvertently left in our food preparations owing to insufficient extraction with alcohol" [1913, 2, p. 430].

After our discovery that "protein-free milk" together with starch, butter fat, and an adequate protein furnishes a suitable ration for rats, we imitated the inorganic ingredients of the "protein-free milk" by a suitable combination of bases and acids,—our "artificial protein-free milk." When this was used in place of the natural product we obtained considerable growth in several instances; but in the great majority of cases little, if any, growth was made. McCollum and Davis [1915] also have reported not a few examples of very considerable growth upon similar rations consisting of isolated food substances and artificial salt mixtures. The possibility of obtaining adequate growth in this way is very slight; and it is always merely a question of time before a nutritive decline ensues unless a change is instituted in the ration,

whereby the lacking accessory substances are furnished. Whether the growth occasionally made on these purely artificial mixtures is caused by impurities attached to the lactose used—as has been intimated by Drummond—or whether certain animals are possessed of a sufficient store of nutrition-promoting “hormones” to enable them to utilise highly purified isolated food substances in a constructive way for some time, is debatable in view of the very small proportion of rats which make any material growth of such foods.

Our attitude was summarised in 1913 as follows: “Our own experiments, as well as those of the other investigators mentioned, make it clear that something further than a sufficient supply of energy-yielding food material is required to promote a normal growth. The animal cells need for their activities not only energy, but also suitable constructive material to replace the wear and tear therein. Furthermore the cells are concerned in the elaboration of a great diversity of complex and little understood substances such as enzymes, products of internal secretion, etc., which unquestionably play an indispensable rôle in life and may require either special antecedent products for their construction, chemical activators of some sort, or minute quantities of readily overlooked rarer elements and compounds. It is easy, yet futile at the present time, to develop detailed hypotheses respecting the almost innumerable possibilities involved. The greatest promise of success in discovering the food factors which determine successful growth lies in seeking them in some chemical constituents of such diets as have proved adequate to promote growth.

“In attempting to ascertain what constitutes an adequate diet, by feeding experiments with isolated substances, we have found that our purely artificial foods—mixtures of isolated proteins, fats, carbohydrates and inorganic salts—sooner or later fail to maintain mature animals. In view of this such dietaries may naturally be expected to fail to maintain the less resistant young during their adolescent period. It is true that in several instances we have succeeded in keeping grown rats in health and in apparent nutritive equilibrium on purely artificial food mixtures over periods far longer than the experience of our predecessors had led us to expect. But the outcome has never been satisfactory in the sense of extending over what may be considered as the larger portion of the life span of an adult animal. Successful maintenance has been secured only when the animals were fed, in part at least, with foods containing our ‘protein-free milk,’ the preparation and composition of which has been detailed elsewhere. The superiority of the

latter foods, compared with any purely artificial food mixture in repairing the depleted body weight of animals that have begun to decline on the artificial salt mixtures tested, is beyond question. Instances of immediate recovery following the replacement of the inorganic constituents of the dietaries and part of the carbohydrates by the 'protein-free milk' have been published, and might be duplicated in great numbers from our protocols. Even greater success is manifested in maintenance experiments in which the 'protein-free milk' alone furnished the inorganic constituents of the dietary during long periods of time. The superiority of the 'protein-free milk' foods over the diets containing artificial salt mixtures in the maintenance experiments also is unquestioned. Wherein the difference lies is not yet apparent. We have already pointed out that the efficiency of this adjuvant to the energy-yielding nutrients is not attributable to the minute trace of milk protein present. The fact of the greater efficiency of the natural milk product suggests that some constituent present in milk is essential for prolonged maintenance" [1913, 1, p. 312].

As a result of the circumstances here recorded we were led, like McCollum and Davis, to investigate the fat-soluble components (McCollum's fat-soluble A) that promote growth in the presence of other suitable dietary ingredients.

Similarly one of us has recently stated [Mendel 1915]: "It is not unlikely—to speak conservatively—that there are at least two 'determinants' in the nutrition of growth. One of these is furnished in our 'protein-free milk' which insures proper maintenance even in the absence of growth. When this was fed we have maintained rats without growth for very long periods. Without this 'determinant' (as, for example, in diets of isolated food substances containing artificial substitutes for 'natural' protein-free milk) the special components of butter fat or cod-liver oil or egg fat induce only limited gains at best. Another 'determinant' is furnished by these natural fats (or fractions more recently prepared therefrom by Osborne and myself or the saponification product of McCollum and Davis). Either of the determinants may become 'curative'; both are essential for growth when the body's store of them (if such there be) becomes depleted. It is too early to attempt a tenable conclusion."

We believe the foregoing statements published by us some time ago should make it apparent that, like Drummond, we have concluded "before complete growth can occur in a young animal, the diet, besides being adequate as regards its proteins, carbohydrates, fats and salts, must contain both fat-soluble and water-soluble accessory substances" [Drummond 1916, p. 102].

Drummond has stated [1916, p. 93]: "Osborne and Mendel have always been careful to utilise, throughout their extensive researches, proteins of a very high degree of purity, and yet they have gone to the other extreme in continuing to use in their diets 'protein-free milk.' Reference has already been made to the criticisms which have been directed against the use of this complex substance, as a source of lactose and salts." In this connection the reader should be reminded that our primary object was the study of the relative nutritive value of certain proteins which can be undertaken only when *all* the other (non-protein) essentials of the diet are available. The use of "protein-free milk," to which exception has been made¹ above, has proved valuable in that it has offered an unusually good source for the water-soluble "accessory," "vitamine," "determinant," or food "hormone" which, along with suitable fat-soluble accessory, has enabled us to conduct experiments, which otherwise would have been impossible, on the relative value of certain proteins and amino-acids¹. For our *studies of the rôle of proteins in nutrition* it has been essential that all necessary food "hormones" should be included in the diet. Consequently if the lactose used actually contained traces of "vitamines," this would offer no disadvantage for the conduct of such experiments. Obviously this does not apply to the use of such a product for investigations of the nature of the food hormones or vitamins.

¹ The possible significance of the small quantity of protein present in our preparation of "protein-free milk" has not been overlooked. [See Osborne and Mendel, 1915.]

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