generation did not appear to affect the pathogenicity of the strains for the rabbit, as judged by the length of the incubation period of the orchitis and by the subsequent development of generalized lesions.

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Further Evidence of Additional Substances Essential to Mammalian Nutrition

H. K. STIEBELING (Introduced by H. C. Sherman)

From the Department of Chemistry, Columbia University

This paper briefly reports some of the experimental evidence encountered in the course of experiments on vitamin G, which can best be interpreted by postulating the existence of more than the 6 vitamins generally recognized as factors in mammalian nutrition. The evidence included is of two types. The first type deals with the supplementary nature of 2 sets of vitamin G-containing products, and the second with the contrasting growth response of animals of different nutritional history, during the latter part of a vitamin G-test period.

In testing by the Bourquin and Sherman method[1] the vitamin G potency of laboratory preparations obtained in fractionation and concentration experiments, a supplementary effect on growth was observed when a mixture of 2 fractions of skim milk powder was fed, as contrasted with the results obtained upon feeding twice the quantity of either fraction by itself. Fig. 1 shows the effect upon gain curves of feeding daily, (a) a definite quantity ($2x$) of an extract made at room temperature from skim milk powder with 80% (by weight) alcohol; (b) a definite quantity ($2y$) of the residue left after extracting the milk powder as described in (a), and (c) a mixture ($x + y$) of half of each quantity of the products just described. During the first few weeks of the test period, the rate of gain permitted by the mixture of the 2 supplementary materials was between that permitted by either given separately, as would be expected if vitamin G were the sole growth-limiting factor. But during the latter part of the test period, the rate of growth permitted by the mixture exceeded that allowed by either of the supplements alone. These results can be explained by postu-

[1] Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 3501.

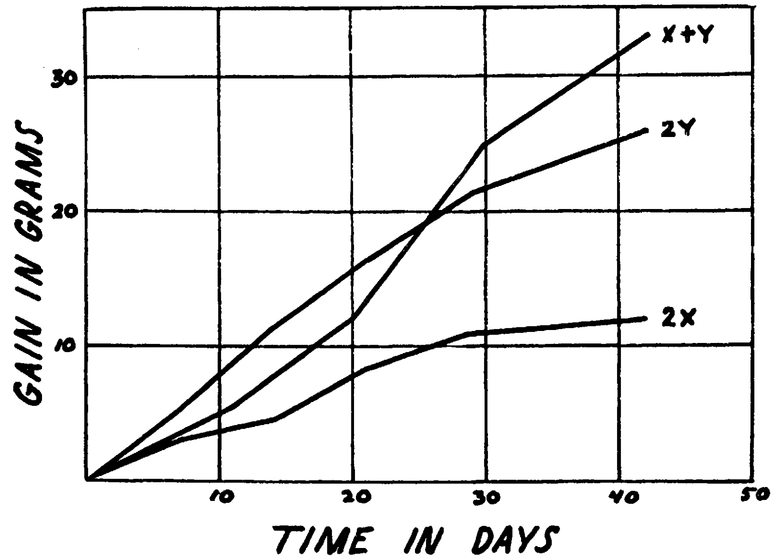

FIG. 1.

Gain curves of rats between the 6th and 12th weeks of their life, showing in vitamin G tests a supplementary effect of two products upon growth. X is an extract of skim milk powder made at room temperature with 80% alcohol; Y is the residue corresponding to X. Each curve represents averages of 3 or 4 experiments.

lating that the bodily requirement of some nutritive essential other than vitamin G (in the strict sense of the Bourquin method) was not furnished by the basal diet, and was unequally supplied by the 2 fractions of the milk powder, the residue containing relatively more of the "new" factor. This factor was furnished to the group of animals receiving the extract from a bodily store of the animals, until this reserve was depleted, whereupon growth practically ceased for lack of the "new" factor. On the other hand, the mixture of the 2 fractions contained enough of the "new" factor, or enough was furnished jointly by the bodily store and by the mixture fed, to permit continuous growth throughout the experimental period.

This view is confirmed by the data presented in Fig. 2. The gain curves there shown are of experimental animals which had received from one to 2 units of vitamin G between the 6th and 14th weeks of their life, the period usually included by a vitamin G test. At the end of this period the bodily store of the "new" factor was probably much depleted. Matched animals were then given (*a*) a definite quantity of an extract ($2x$) made from skim milk powder by treating it with 80% alcohol at room temperature; (*b*) a definite quantity of a filtrate ($2z$) left after treating with Lloyd's reagent

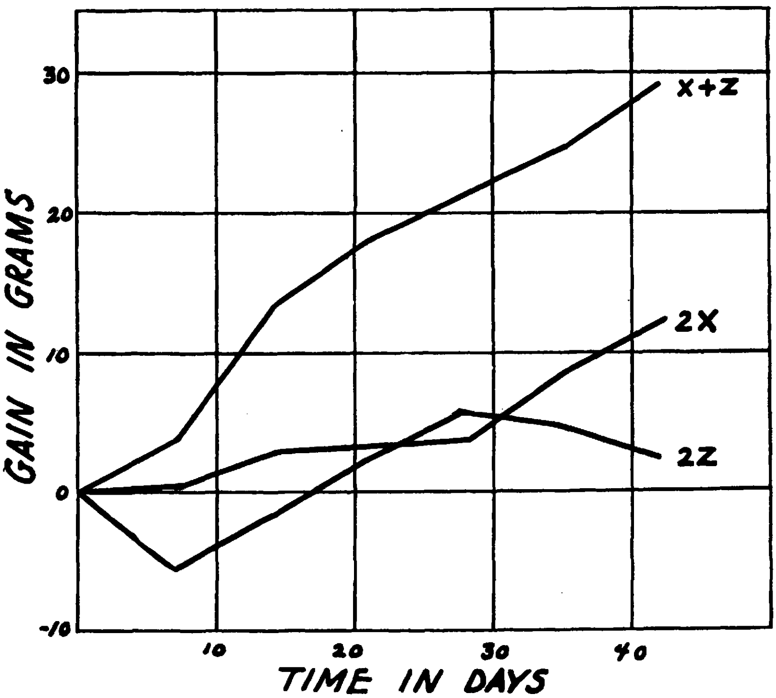

FIG. 2.

Gain curves of rats between their 14th and 20th week of life showing, in vitamin G tests, a supplementary effect of two products upon growth. X is an extract of skim milk powder made at room temperature with 80% alcohol; Z is the filtrate left after treating with Lloyd's reagent, an extract of skim milk powder made with boiling 95% alcohol. Each curve represents averages of 3 or 4 experiments.

an extract made from skim milk powder by means of boiling 95% alcohol; and (*c*) a mixture ($x + z$) of half of each of the quantities of products just described. One product was, apparently, a relatively good source of vitamin G, and a poor source of the "new" factor; the other, a relatively good source of the "new" factor and a poor source of vitamin G. The mixture promoted much better growth in these older rats than double the quantity of either product by itself.

The successful demonstration of supplementary effects on growth of these products was partially contingent on the use of experimental animals whose bodily store of the "new" factor was fairly low. The animals used in these experiments were reared by mothers receiving a diet consisting essentially of 1/6 whole milk powder and 5/6 ground whole wheat. But the attempt to use as vitamin G reagents, rats with this nutritional history, rather than those with a more optimal one, as is customary in most vitamin studies in this laboratory, also led to the discovery of a second type of evidence on the existence of "new" factors necessary to mammalian nutrition.

In the development of their method for the determination of vitamin G, Bourquin and Sherman[1] used as reagents, animals reared by mothers receiving a diet composed essentially of ⅓ whole milk powder and ⅔ ground whole wheat with or without the addition of raw lean beef. When the young with this nutritional history, depleted of their bodily reserves of vitamin G by a basal diet free from that vitamin, were given graduated portions of milk as vitamin G-containing food, they made a continuous and uniform growth response throughout an 8-week test period. No marked cessation of, or change in the rate of, growth was observed when the gains were averaged for large numbers of animals. But on using experimental animals with a less favorable nutritional history, employing otherwise the same experimental procedure, we found that only for the first few weeks of the test period did practically all of the animals respond in growth as did those of Bourquin, and only about one-third of more than 100 animals responded thus throughout an 8-week period. Instead of continuous and uniform growth, a flattening of the growth curve or a decline in weight was frequently observed after the 4th or 6th week of the experimental period. This result may undoubtedly be attributed to a depletion of the bodily store of some factor or factors other than those generally recognized as necessary to mammalian nutrition.

The main difference between the family diets on which the 2 groups of rats were reared consisted of the proportion of milk included. This and the fact that supplementary effects on growth in the course of vitamin G experiments may be observed with certain fractions of milk powder, when rats from family dietaries containing the less milk are used as reagents, indicate that milk is a fair source of the "new" factor. From the limited amount of evidence afforded by these experiments, it would appear that the "new" factor is less readily extracted than vitamin G from milk powder by alcohols 80% (by weight).

In the course of these and other experiments some evidence was also obtained of the existence of 2 other "new" factors, but the work of the author in these directions was discontinued because of transfer to a position at a distance from the laboratory. Evidence on the existence of accessory factors other than those generally recognized as necessary to mammalian nutrition has also been adduced by several other investigators. Palmer and Kennedy[2, 3, 4, 5], Salmon, Guerrant and Hays[6, 7], Hunt and his associates[8, 9], Chick and coworkers[10, 11, 12, 13], Coward and others[14, 15], Reader[16, 17, 18], Guha and Drummond[19, 20], Williams and Lewis[21], Halliday[22, 23], and others have found evidence of "new" factors or of factors "interfering" with the assay of the well-recognized factors. Only further research can determine whether the factors encountered in the work noted here are new in the sense of being different from factors already postulated by other investigators, or with which they are identical.

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