THE RELATION OF GROWTH TO THE CHEMICAL CONSTITUENTS OF THE DIET.

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL,

With the Coöperation of Edna L. Ferry and Alfred J. Wakeman.

(From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Connecticut.)

(Received for publication June 21, 1913.)

Our earlier experiments, undertaken to determine the relative value of different purified proteins in nutrition, revealed the fact that *growth* depends on nutritive conditions which are distinct from those required for maintenance.[2] This fact has since been confirmed by the experimental work of others, notably McCollum,[3] and Hopkins.[4] Some of the viewpoints in respect thereto have been discussed by us elsewhere.[2] In addition to our earlier experience with rats, the dissimilarity in the nutritive requirements of maintenance and growth have more recently been clearly manifested in experiments on mice conducted by Dr. Ruth Wheeler in our laboratories.[5]

[1] The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Osborne and Mendel: Feeding Experiments with Isolated Food-Substances, Carnegie Institution of Washington, Publication 156, Parts I and II, 1911; The Rôle of Different Proteins in Nutrition and Growth, *Science*, xxxiv, pp. 722–732, 1911; Beobachtungen über Wachstum bei Fütterungsversuchen mit isolierten Nahrungssubstanzen, *Zeitschr. f. physiol. Chem.*, lxxx, pp. 307–370, 1912; The Rôle of Gliadin in Nutrition, this *Journal*, xii, pp. 473–510, 1912; Maintenance Experiments with Isolated Proteins, *Ibid.*, xiii, pp. 233–276.

[3] McCollum, E. V.: The Nature of the Repair Processes in Protein Metabolism, *Amer. Journ. of Physiol.*, xxix, pp. 215–237, 1911.

[4] Hopkins, F. G.: Feeding Experiments Illustrating the Importance of Accessory Factors in Normal Dietaries, *Journ. of Physiol.*, xliv, pp. 425–459, 1912.

[5] Wheeler, Ruth: *Journ. of Exp. Zoöl.*, 1913 (in press).

These well verified facts serve to raise the question as to what is the factor in the diet which is peculiarly essential for growth. 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.[6] The superiority of the latter foods, compared with any

[6] Osborne and Mendel: Feeding Experiments with Isolated Food-Substances, Carnegie Institution of Washington, Publication 156, 1911, Part II, p. 80.

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.[7] 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.[8] 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.

In our numerous experiments milk has proved to be an adequate food, both for growth and maintenance. Young rats fed solely upon the milk food which we have been accustomed to use[9] not only have grown from infancy to full maturity, but have also given birth to litters of normal young which in turn have thriven on diets precisely like that furnished to their parents, as illustrated by chart I in the appendix. One must conclude from these facts that the milk food contains all that is essential for *both* growth and maintenance.

We have imitated the gross composition of this highly successful milk food by preparing mixtures of purified protein, lard,

[7] Osborne and Mendel: Feeding Experiments with Isolated Food-Substances, Carnegie Institution of Washington, Publication 156, Part II, 1911; The Rôle of Different Proteins in Nutrition and Growth, *Science*, xxxiv, pp. 722–732, 1911; The Rôle of Gliadin in Nutrition, this *Journal*, xii, pp. 473–510, 1912.

[8] Osborne and Mendel: Maintenance Experiments with Isolated Proteins, this *Journal*, xiii, pp. 233–276, 1912. Successful feeding trials extending over six months with similar diets on mice have been conducted by Dr. Wheeler; *Journ. of Exp. Zoöl.*, 1913 (in press).

[9] The food is in the form of a paste consisting of milk powder, 60 per cent; starch, 12 per cent; lard, 28 per cent (see p. 318).

starch and "protein-free milk." Such foods have been singularly efficient in promoting growth of young rats. Individual animals vary in respect to their capacity to grow on this food, a few stopping after sixty days of growth, others continuing to grow for one hundred days or more. After normal growth stops, the animals may remain at constant weight for a few days, or grow very slowly, and then suddenly decline and die unless a change is made in the diet. Both interesting features of these experiments, namely, the excellent earlier growth and the ultimate failure, are exemplified in charts II and III, where they may be compared with the almost invariable complete success that attends the use of the milk food. The conclusion seems inevitable, therefore, that the "protein-free milk foods" are deficient in, or completely lack, something which milk contains and which is indispensable for perfect growth.

This ultimate inhibition of growth, and nutritive decline in our feeding trials with the mixtures of isolated food stuffs is clearly connected with the diet factors. Our milk food has invariably brought prompt recovery and continuation of normal growth (see charts II and III). Even brief periods of milk feeding suffice to replenish, or provide, or permit to develop, that non-protein factor in the lack of which, cessation of growth ensues. If rats are allowed to grow on our "protein-free milk" food for some time, but are given milk food for a short period before growth ceases, or even after the decline has begun, the return to the "protein-free milk" food may again be attended with a long period of successful growth until there sets in a second inhibition, or decline, which can likewise be averted, or repaired by further exhibition of milk food.

All the essential factors for growth must be present in the diet if normal growth is to occur and continue. Failure to grow may result from a variety of factors some of which, like a deficiency of protein, or carbohydrate, or inorganic salts, or an inappropriate type or mixture of these nutrients, are apparent.[10] What light does the experience thus far accumulated throw upon the nature of the essential substance, if there be such? Is it organic or inorganic, or both?

[10] See further our discussion of the subject in *Zeitschr. f. physiol. Chem.*, lxxx, pp. 307–370, 1912.

We have already noted that ultimately failure invariably ensues when rats furnished a diet containing an adequate protein and "protein-free milk" have made a considerable part of their natural growth at a normal rate. Since no such failure to grow is observed when young rats are fed with the milk food, and since also those that have ceased to grow on the "protein-free milk" diet or have declined are promptly restored to satisfactory conditions of growth by the use of milk, it is evident that the latter contains something which our "protein-free milk foods" lack. It seems probable that the missing substance is organic in nature; for the "protein-free milk" may be presumed to contain all of the inorganic constituents of the milk. Nevertheless, in view of the limitations of our knowledge regarding minute quantities of elements which may play an important part in nutrition, hasty generalizations in this direction are scarcely permissible.

In a recent paper[11] we gave charts showing very considerable growth, at a normal rate, of the young white rat when supplied with a diet consisting solely of purified starch, lard, protein, lactose and inorganic salts; or, in other words, with foods containing our so-called "artificial protein-free milk," the preparation of which is described in the communication referred to. We also reported normal growth for a relatively long time on a similar diet in which the fat was replaced by carbohydrate. The number of these experiments, while not large, was sufficient to show that growth can be made on such diets; and the fact that they were conducted at different times and with several batches of food made with chemicals of different origin, excluded the possibility of error due to any accidental incorporation in the food of substances other than those they were intended to contain. These results were so different from any we had previously obtained with purely artificial diets[12] that we at once proceeded to confirm them by new experiments conducted on a much larger scale.

The chemicals used in making the first lot of "artificial protein-free milk" (designated I) were ordinary laboratory preparations

[11] Osborne and Mendel: Beobachtungen über Wachstum bei Fütterungsversuchen mit isolierten Nahrungssubstanzen, *Zeitschr. f. physiol. Chem.*, lxxx, pp. 307–370, 1912.

[12] Osborne and Mendel: Growth and Maintenance on Purely Artificial Diets, *Proc. Soc. for Exp. Biol. and Med.*, ix, p. 72, 1912; Beobachtungen über Wachstum bei Fütterungsversuchen mit isolierten Nahrungssubstanzen, *Zeitschr. f. physiol. Chem.*, lxxx, p. 356, 1912.

of good quality. Later we used Kahlbaum's preparations, and with the foods containing the "artificial protein-free milk" thus made (which we designate II) in many cases we obtained growth quite comparable with that previously secured with foods of similar character, but containing the natural "protein-free milk." The outcome of all our growth experiments with the "artificial protein-free milk" food mixtures I and II are shown in chart IV. For the new experiments special care was taken to use chemicals of a high degree of purity, as ascertained by careful analyses. To our surprise the "artificial protein-free milk," III, made with these purer chemicals failed in every case but one to promote more than slight growth (see chart V). Since the only apparent difference in the conditions under which these later trials were conducted was the greater purity of the chemicals used, our attention was at once turned to those inorganic elements which have been found in animal tissues in traces, but of the need of which in the diet nothing has as yet been learned. Traces of such elements may have been present as impurities in the chemicals first employed.

We accordingly made another preparation of "artificial protein-free milk," IV, to which traces of iodine, manganese, fluorine, and aluminium were added. The composition of these different preparations of "artificial protein-free milk" is shown by the table on following page.

Without a guide as to the proper amount of these "traces" of inorganic elements to add, the quantities in IV were chosen arbitrarily. These might, therefore, be either too much, or too little, in respect to any one or all of the additions.

An inspection of chart V shows plainly that much better growth was secured with preparation IV than with III. The findings may be summarized by saying that with "artificial protein-free milk" mixtures we have, under certain conditions, obtained a very considerable growth which, nevertheless, in most instances has ceased sooner than that induced by the natural "protein-free milk." The latter food, however, also invariably fails sooner or later to satisfy the nutritive requirement for growth.

McCollum and Davis,[13] whose significant experiments have con-

[13] McCollum, E. V. and Davis, M.: The Influence of the Composition and Amount of the Mineral Content of the Ration on Growth, Proc. of Amer. Soc. of Biol. Chem., this *Journal*, xiv, p. xl, 1913.

Composition of "artificial protein-free milk."

(Quantities used to make sufficient of the mixture to prepare 1 kgm. of food.)

	I, II,* III†	IV
	grams	*grams*
$CaCO_3$	13.48	13.48
$MgCO_3$	2.42	2.42
Na_2CO_3	14.04	3.42‡
K_2CO_3	14.13	14.13
H_3PO_4	10.32	10.32
HCl	12.75	5.34‡
H_2SO_4	0.92	0.92
Citric acid + H_2O	10.10	11.11§
FeCi.$1\frac{1}{2}H_2O$	0.634	0.634
KI		0.0020
$MnSO_4$		0.0079
NaF		0.0062
$K_2Al_2(SO_4)_2$		0.0024
Lactose	246.0	246.0

* Prepared from Kahlbaum chemicals.

† Prepared from specially analyzed chemicals.

‡ The slight differences in the amounts of these compounds used in salt mixture IV, in contrast with I, II, and III, are due to the fact that in the latter, allowance was made for the sodium chloride produced by the neutralizing process in the preparation of our "protein-free milk." This addendum was omitted in IV in order to make its composition conform still more closely to that of the milk salts as such.

§ The small variations in the amounts of citric acid added are due to the fact that they were inadvertently made to correspond with two different analyses reported in the literature.

firmed ours in showing the possibilities of very considerable growth on the "artificial" dietaries, likewise appear to have encountered this cessation of growth for they state:

> Rats grow normally during seventy-five to one hundred days on a ration consisting of pure casein, 18 per cent, dextrin, agar-agar and salt mixtures giving an inorganic content closely similar to either milk or egg yolk, and on certain other salt mixtures, in about the proportions found in milk and in eggs. With the same organic ration, fed with a salt mixture giving the ration an inorganic content closely similar to that of the wheat kernel, there is a complete suspension of growth. . . . . Normal growth has been secured during seventy days on a ration of casein, 34 per cent, dextrin, agar-agar and a salt mixture giving an inorganic content similar in composition and quantity to that of dry skim milk.

The trenchant fact that failures can be averted or repaired by the use of milk foods leads to the inquiry wherein our "protein-free milk" food differs from the conspicuously successful "milk food." The composition of three typical food mixtures is given for comparison.

	C		E		M
	per cent		*per cent*		*per cent*
Casein	18	Edestin	18	Milk powder*	60
Starch	29	Starch	26	Starch	12
Lard	25	Lard	28	Lard	28
Protein-free milk	28	Protein-free milk.	28		

* This product is the "Whole Milk Powder" supplied by the Merrell-Soule Company of Syracuse, N. Y. For the analysis see *Report of the Connecticut Agricultural Experiment Station,* Food and Drug Products, 1909, p. 238.

These contain, in every 100 grams:

	C	E	M
	grams	*grams*	*grams*
Protein	18.0	18.0	15.4
Lactose	23.8	23.8	22.3
Starch	29.0	26.0	12.0
Milk salts	4.2	4.2	3.6
Total fats	25.0	28.0	44.4
Lard	25.0	28.0	28.0
Butter	0.0	0.0	16.4
Moisture	0.0	0.0	2.3

To what can we attribute the difference in the relative efficiency of the foods in promoting growth? First, not to the proteins, although these are unlike; for such evidence as we have already secured makes it extremely improbable that they are responsible for the nutritive differences. Second, not to the carbohydrate and inorganic constituents; for these are essentially alike in all the food mixtures. Third, not to the effect of the heat applied in the production of the "protein-free milk" component of the foods; for the milk powder used by us has also been subjected to an equally high temperature. An inspection of the tables discloses the fact that the foods C and E lack all those components of milk which are separated in the process of centrifugation of milk, *i.e.*, the cream and likewise any cellular elements (mammary gland cells, leucocytes, bacteria, etc.) removed mechanically by the centrifugal process preliminary to the manufacture of our protein-free milk, or removed by subsequent filtration processes.

In seeking for the "essential" accessory factor we have, therefore, been led first of all to supply the cream component, in the form of butter, to rats which have ceased to grow on the "protein-free milk" foods. Numerous experiments still in progress have resulted in restoring rats, which have declined on the "protein-free milk" dietaries, to a weight normal for their age, quite as rapidly as does the efficient milk food. Examples of such recoveries are presented in charts VI and VII. Chart VI shows the effect of replacing part of the lard in our "natural protein-free milk" foods with a corresponding quantity of unsalted butter. It will be observed that after the cessation of growth, or after decline in body weight, recovery and renewed growth take place with the same rapidity as when the animals receive the milk food (compare charts II and III). Chart VII furnishes similar examples of recovery of animals which had ceased to grow on diets containing "artificial protein-free milk" IV, when part of the lard of this diet likewise was replaced with butter. These results are the more striking in view of the less rapid and continued growth manifested by most of the animals fed with the "artificial protein-free milk" foods. The illustrations presented in these charts are representative of a large number of similar experiments which we have conducted. It would seem, therefore, as if a substance exerting a marked influence upon growth were present in butter, and that this is largely, if not wholly, removed in the preparation of our natural "protein-free milk." Whether or not the latter is wholly deficient in this substance cannot be determined as yet from any data which we possess. It is true that young rats are able to make very considerable growth when fed on the natural "protein-free milk" diet; but possibly this is accomplished at the expense of some reserve substance stored in the cells of the young animal. It is too early to draw inferences as to the effective substance supplied by the butter. The detailed study of these important questions is being continued by us.

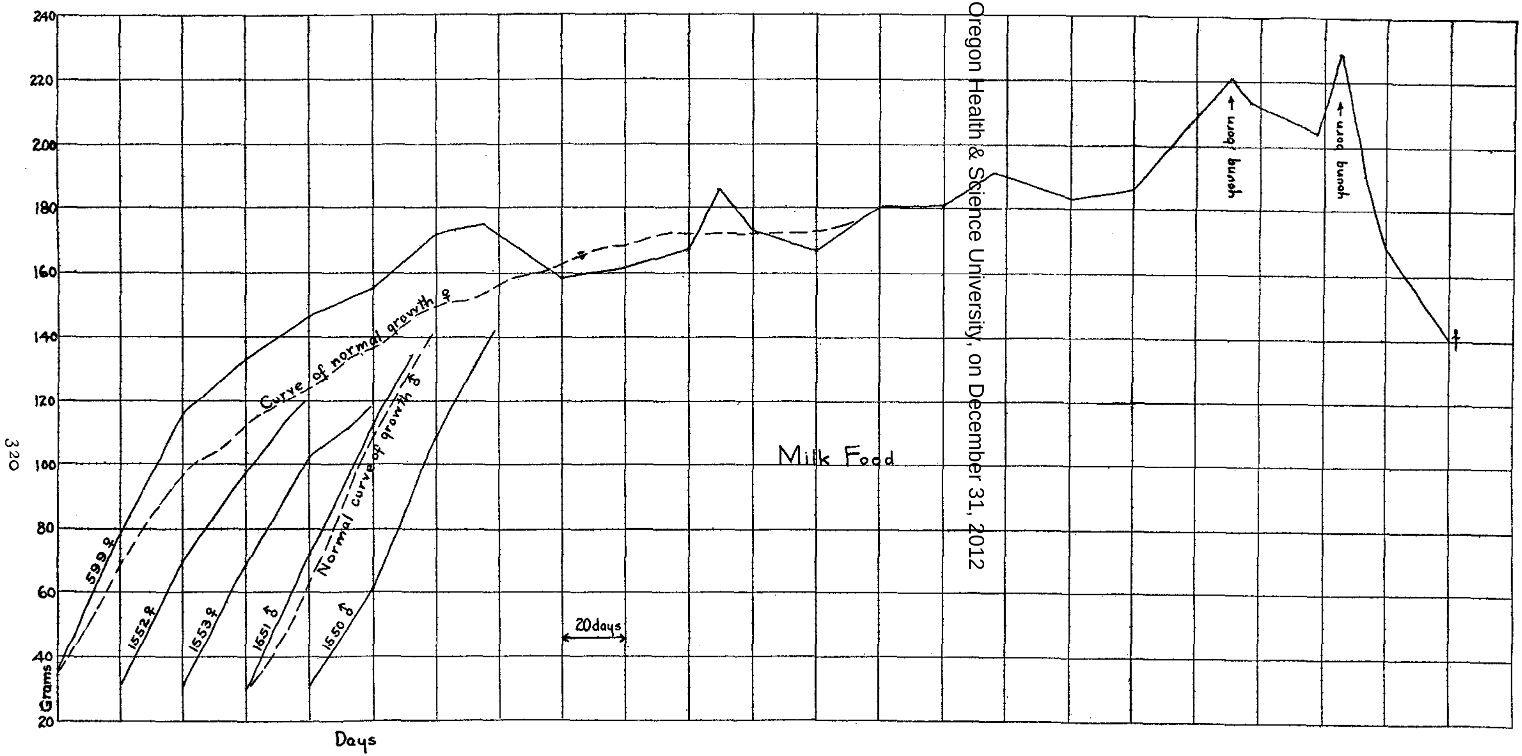

CHART I. Typical curves showing normal growth of white rats on our milk food. Rat 599 ♀, after 371 days of growth and maintenance, gave birth to two litters of young. Rats 1550, 1551, 1552 and 1553 are the young of a mother fed on the milk food from the age of 59 days for 126 days prior to their birth. The normal growth of her young, which in turn were fed from the time of weaning on the milk food, is here represented.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

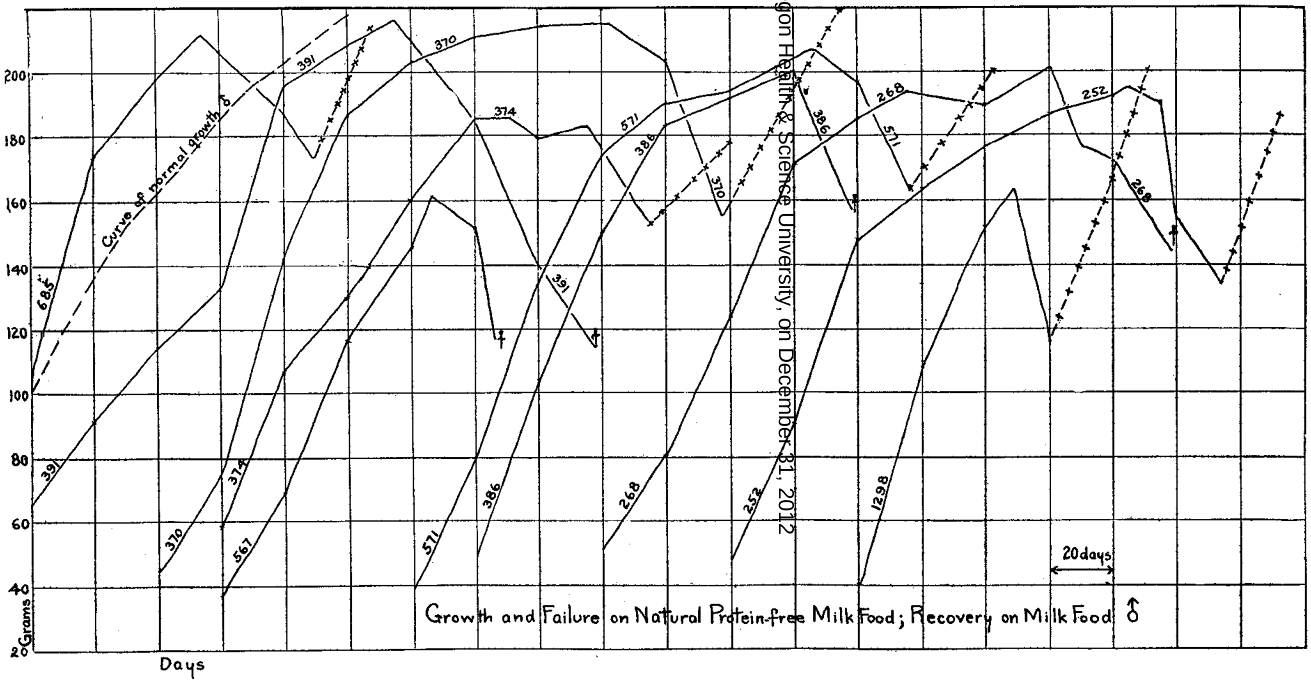

CHART II. Curves showing normal growth of *male* rats during many days, followed by cessation of growth, and decline on our "natural protein-free milk" foods. In every case where our milk food replaced the earlier mixture prompt recovery followed as indicated by the interrupted lines (x-x-x-x). The "protein-free milk" foods fed to the different rats contained various proteins as follows: casein, Rats 252, 268, 370, 386, 391; glutenin, Rat 374; lactalbumin, Rat 685; maize glutelin, Rat 567; ovovitellin, Rats 571, 1298.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

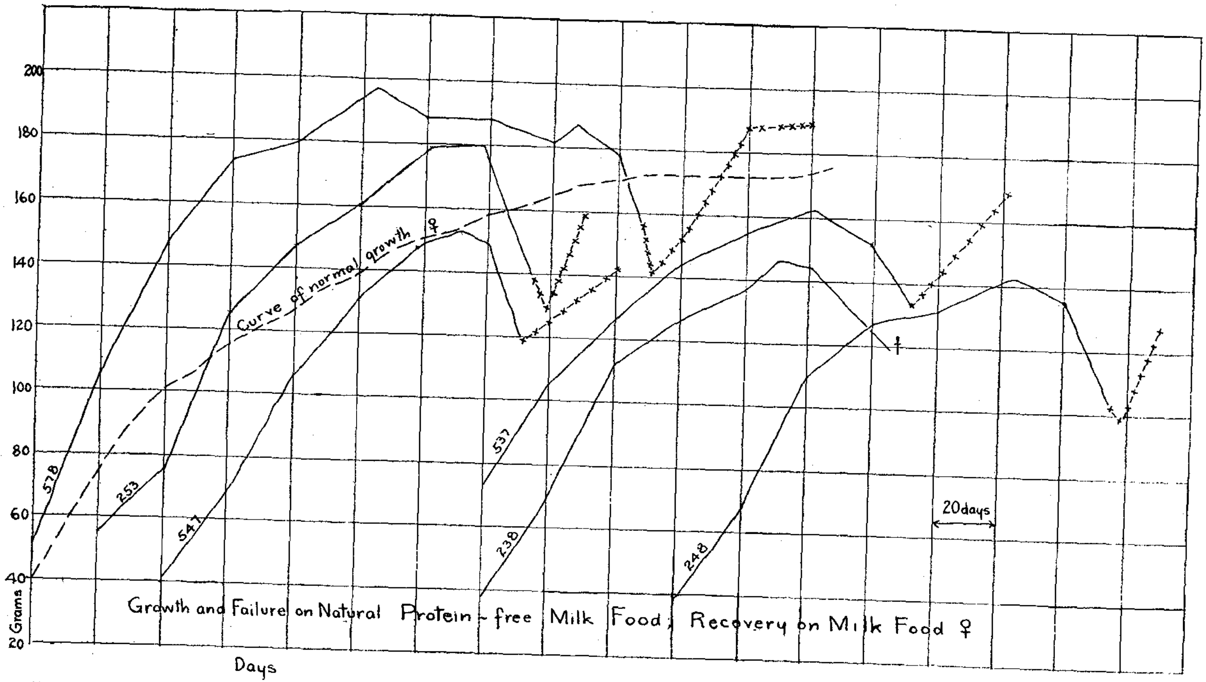

Chart III. Curves showing normal growth of *female* rats during many days, followed by cessation of growth, and decline on our "natural protein-free milk" foods. In every case where our milk food replaced the earlier mixture, prompt recovery followed as indicated by the interrupted lines (x-x-x-x). The "protein-free milk" foods fed to the different rats contained various proteins as follows: casein, Rat 238; edestin, Rats 248, 253; maize glutelin, Rat 547; ovovitellin, Rat 578; squash-seed globulin, Rat 537.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

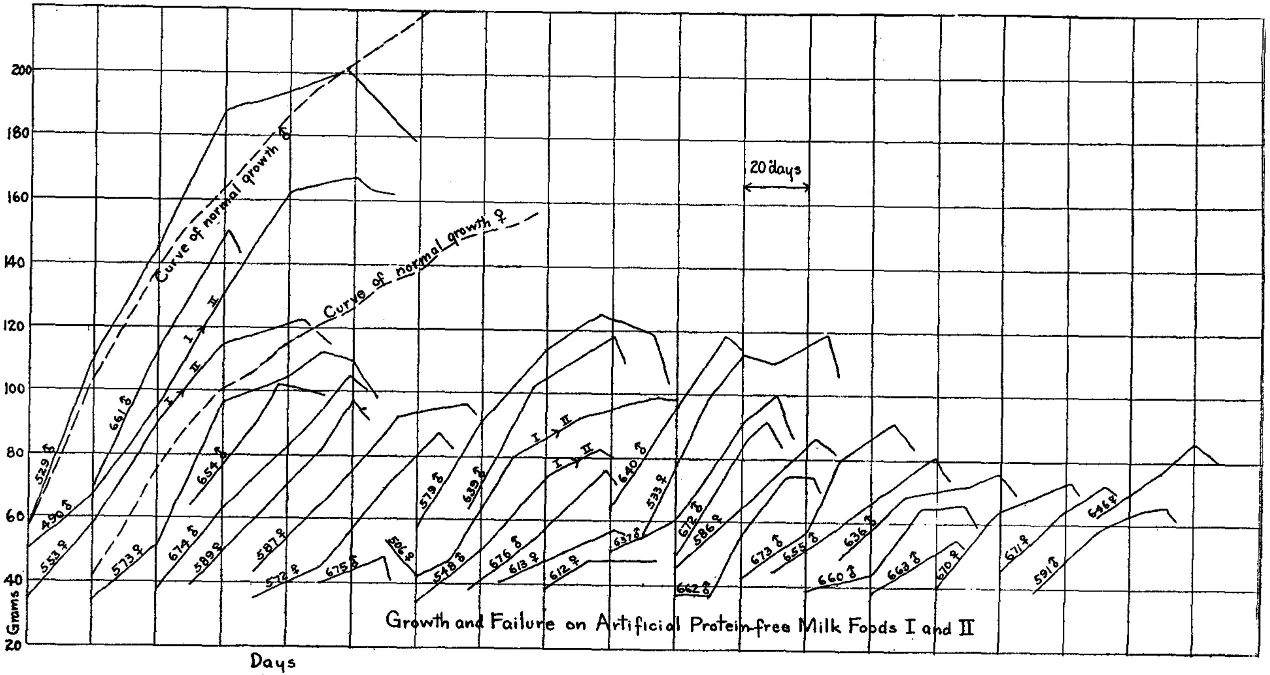

CHART IV. Curves of growth of all our rats fed on foods supplying the inorganic constituents in the form of "artificial protein-free milk" I and II. See page 317. Except where otherwise indicated in the chart, mixture II was used. It will be noted that in some cases growth was made at a rate comparable with that promoted by the "natural protein-free milk" as shown in charts II and III. In many cases the rats have more than doubled their weight. In general growth ceased sooner than when "natural protein-free milk" is fed.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

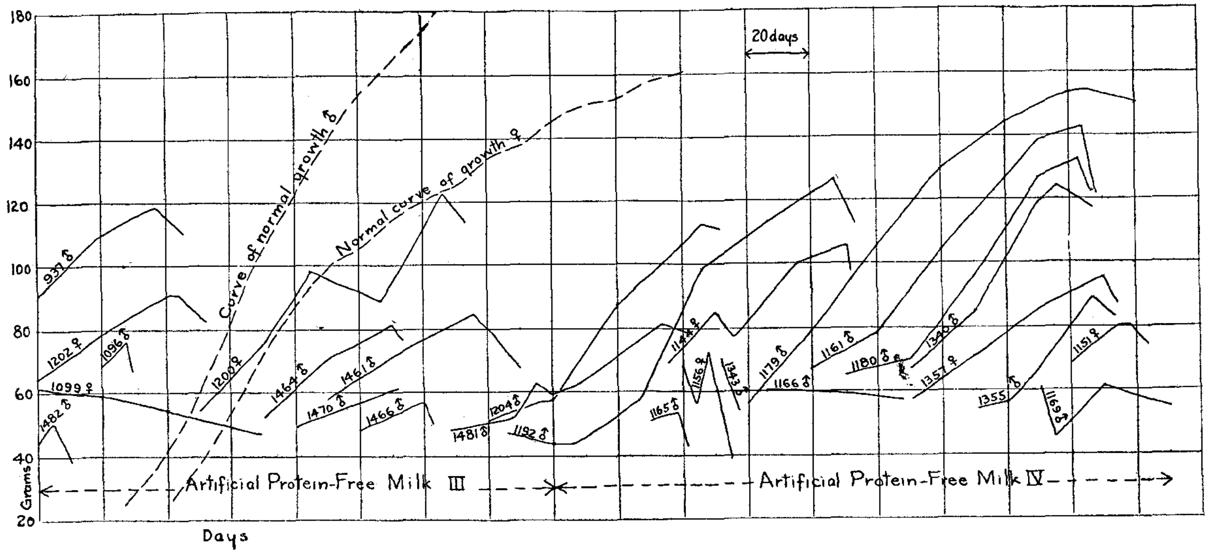

CHART V. Curves of growth of all our rats fed on foods supplying the inorganic constituents in the form of "artificial protein-free milk" III and IV. See page 317. Note the very slight growth made on III compared with that made on I, II (chart IV), and IV. The essential difference between III and IV is that the latter contained traces of Mn, Al, I and F.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

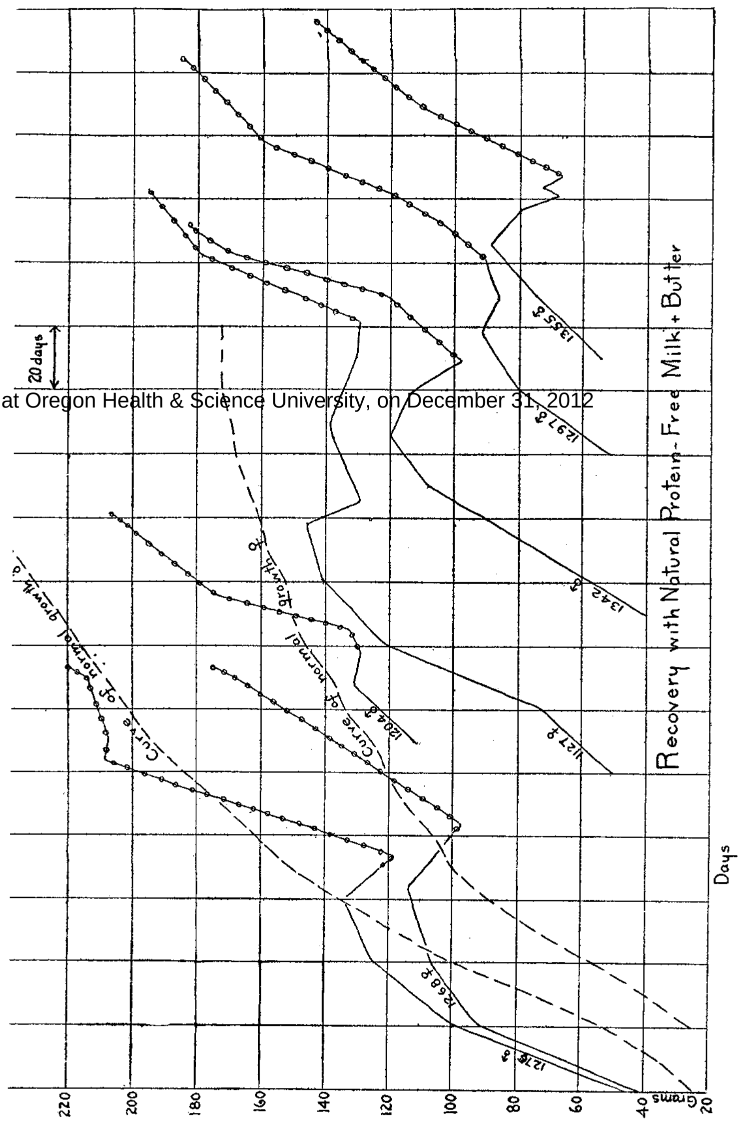

CHART VI. Curves of the body weight of rats which have ceased to grow or have declined on foods containing the natural "protein-free milk," and have recovered when part of the lard of the diet was replaced by a corresponding quantity of unsalted *butter* as indicated by the interupted lines (o-o-o-o-o). The proteins furnished in the different experiments were as follows: casein, Rats 1204, 1268, 1276, 1297, 1342; edestin, Rats, 1127, 1355; ovalbumin, Rats 1268, 1276.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.

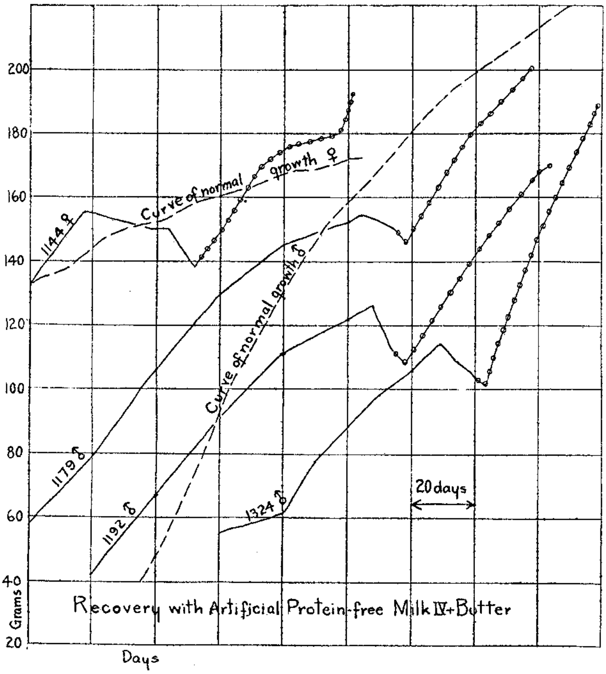

CHART VII. Curves of the body weight of rats which have ceased to grow or have declined on foods containing the "artificial protein-free milk" IV, and have recovered when part of the lard of the diet was replaced by a corresponding quantity of unsalted *butter* as indicated by the interrupted lines (o-o-o-o-o-o-o). The proteins furnished in the different experiments were as follows: casein, Rats 1144, 1192, 1324; edestin, Rat 1179; lactalbumin, Rat 1144.

The ordinates represent grams of body weight, as indicated. The divisions of the abscissa represent 20-day periods.