THE ESSENTIAL FACTORS IN THE DIET DURING GROWTH.[1]

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In a previous paper[2] we described experiments in which we had attained growth approximating the normal rate, together with reproduction and rearing of a portion of the young, with rations made up of comparatively pure proteins, dextrin, lactose, butter fat, and a salt mixture from reagent bottles. We pointed out at that time that we suspected that the presence of lactose in our diets was the determining factor in inducing growth, although several samples showed a content of nitrogen ranging from only 0.020 to 0.034 per cent. We stated that we reserved our conclusion concerning the necessity of accessories other than those carried by butter fat and certain other fats, until we should obtain further evidence.

In a preceding paper[3] we have shown clearly that lactose of the purity of the ordinary reagent (Kahlbaum's and Merck's preparations) does in fact contain enough of the unknown water-soluble accessory essential for growth or prolonged maintenance, to promote growth at a fairly rapid rate when included in a diet of polished rice supplemented with casein, butter fat, and salts. Without the addition of lactose this ration does not support growth. In conformity with this observation we found that the ration previously employed in our "nutrition with purified foodstuffs," *viz.*, casein 18 per cent, lactose 20 per cent, dextrin 56.3 per cent, butter fat 5 per cent, agar-agar 2 per cent, and salts

[1] Published with the permission of the Director of the Wisconsin Experiment Station.

[2] McCollum, E. V., and Davis, M., *Jour. Biol. Chem.*, 1915, xx, 641.

[3] McCollum and Davis, *ibid.*, 1915, xxiii, 181.

3.7 per cent, that if the 20 per cent of lactose was replaced by dextrin no growth was secured.

During the last three years Osborne and Mendel have presented numerous curves of rats fed "fat-free" diets, which indicate that the essential fat-soluble accessory is apparently stored to some extent as a reserve material, so that growth can go on for a period of sixty to one hundred days in nearly a normal manner before the supply of this substance becomes exhausted. With this view that growth can proceed for such a period with diets carrying no fats our former published data are in accord. We are now led to doubt the truth of this assertion, for reasons which will appear later.

We were convinced by our experience in feeding polished rice[4] supplemented with purified casein, lactose, butter fat, and salt additions, that all preparations of lactose were not equally efficient in promoting growth. This ration, without the lactose, promoted growth in a satisfactory manner when minute amounts of water extract of boiled egg yolk or of water extract of wheat embryo (freed from protein), or the alcoholic extract of wheat embryo were added (see Charts 32 to 42). The results all pointed to the necessity in this diet, of an accessory substance which is soluble both in water and in alcohol, as well as the necessity of the fat-soluble accessory furnished by butter fat and certain other fats.

We were convinced from these observations that the employment of lactose of relatively high purity in rations made up of foodstuffs otherwise carefully purified, is open to serious objection and that its use in such rations has led to erroneous conclusions, since it must be of exceptional purity to render it free from the water-soluble accessory.

With rations composed of polished rice, supplemented with a liberal amount of water-soluble accessory, but lacking in the fat-soluble one[5] we have not been able to secure a preliminary period of growth such as the published curves of Osborne and Mendel[6] and our own indicate to be the regular performance of rats on diets which were supposed to be free from this fat-soluble accessory.

[4] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxiii, 181.

[5] See Lot 354, Chart 21, p. 211.

[6] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1912, xii, 81.

The "fat-free" diets of Osborne and Mendel as well as our own contained, it is true, practically no fats and seemingly insignificant amounts of lipoids of any character, but they contained either lactose or casein and in some cases both these constituents from milk and the marked difference in respect to the ability of the rats to grow during the first few weeks on these diets and their failure to do so when fed other rations known to carry but very little of the fat-soluble accessory, but carrying an abundance of the water-soluble one, strongly supports the belief that casein and milk sugar of supposed good quality still retain amounts of both classes of accessories, which are sufficient to exercise a pronounced effect on growth in young animals.

We therefore determined to examine the whole question of nutrition with highly purified foodstuffs in order to make certain whether the curves which we have presented in the past showing normal growth during periods of one to three months could be secured with rations which, in the present stage of our experience, we were convinced were entirely free from either the fat-soluble or water-soluble accessories. Our studies on polished rice had convinced us that if growth is to proceed at all both these accessories must be present.

The question as to the best method of preparing casein for such experiments arose. In the course of our work relating to the supplementary relationship between polished rice and other foodstuffs[7] we learned that prolonged heating even at temperatures of 90–100°C. could cause deterioration of the nutritive properties of milk, and by a systematic investigation we learned that the casein is the component of milk which suffers alteration during heating. For this reason we thought it unwise finally to extract our casein for a long period with boiling alcohol, as Funk and Macallum[8] have done in order to remove all traces of unknown accessory substances, since through this treatment the value of the casein may be decidedly reduced.

The method adopted was the following: Casein purified by twice repeated precipitation was washed, dried, and ground. It was then placed in a large jar having an outlet at the bottom which was closed with a plug of cheese-cloth loose enough to permit a slow passage of water through it. The jar was filled with

[7] McCollum and Davis, *Jour. Biol. Chem.*, xxiii, 247.

[8] Funk, C., and Macallum, A. B., *Ztschr. f. physiol. Chem.*, 1914, xcii, 17.

water acidified with acetic acid. When it had nearly all drained off the jar was again filled. The casein was frequently stirred to prevent its forming a compact mass. This washing was continued during seven or eight days, the last twenty-four hours' washing being with distilled water. The product thus obtained was dried and ground. It was very poor in ash, 10 gram samples yielding but a trace of calcium. By this treatment practically all the water-soluble constituents were dialyzed out of the granules.

With casein prepared in this way combined with dextrin,[9] butter fat, and salts we have been unable to obtain appreciable growth even during the first month. This is illustrated by Chart 4. The results are strikingly different from those obtained with casein purified only by reprecipitation, together with liberal amounts of lactose of fairly good quality. Such rations apparently carry adsorbed as impurities quantities of both classes of essential accessories which are easily detectable by the qualitative demonstration of growth in young animals.

We must, therefore, conclude with Stepp, Hopkins, Funk, and others[10] from the extensive data now available that certain at present unidentified substances aside from protein, carbohydrates, fats, and salts are indispensable for growth or prolonged maintenance, and furthermore that there is a class of such accessories soluble in fats and another soluble in water and alcohol.

From the data available in our records it seems highly probable that, while the amount of accessory substances of either of these classes which is required to induce growth is small, the evidence points to the belief that a certain quantity must be present before any growth can take place, and that above this amount growth seems to be in some measure proportional to the amount of accessories present.

It is obvious that in the study of the relative values of isolated proteins fed with mixtures of purified food substances comparable amounts of these two classes of accessories must be supplied. Otherwise no safe interpretation can be put upon the results.

[9] The dextrin was made from high grade corn-starch moistened with a 0.2 per cent solution of citric acid and heated four hours in an autoclave at 15 pounds' pressure. It was then dried in a current of air at about 70°C.

[10] Stepp, W., *Ztschr. f. Biol.*, 1912, lvii, 135; 1913, lxii, 405. Hopkins, F. G., *Jour. Physiol.*, 1912, xliv, 425. Funk, C., *Ztschr. f. physiol. Chem.*, 1913, lxxxviii, 352; 1914, xcii, 13.

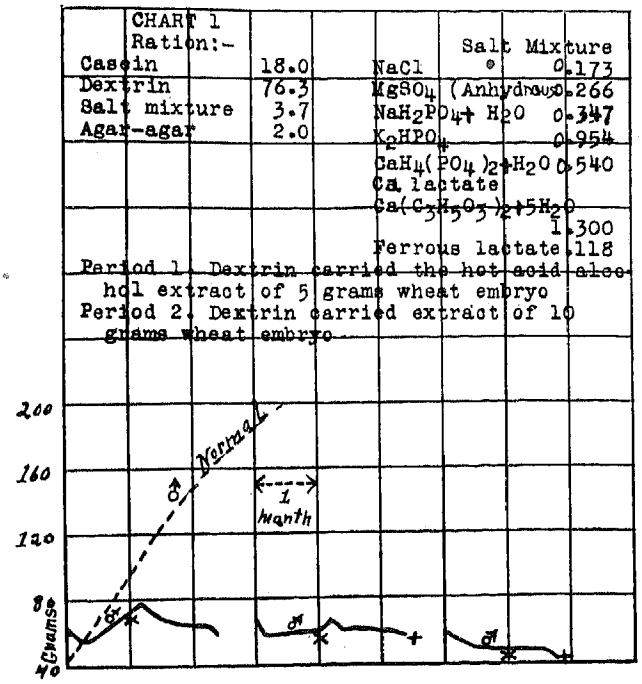

CHART 1. The curves shown in this chart are typical of the behavior of rats fed highly purified casein, dextrin, and a salt mixture. The ration was free from the fat-soluble accessory essential for growth, but a liberal amount of the water- and alcohol-soluble accessory was provided in the form of an acid alcoholic extract of wheat embryo. Growth could not proceed on this ration. Both the water-soluble and the fat-soluble accessories must be present before growth can take place. Casein and lactose of ordinary purity cannot be employed as purified foodstuffs. Growth during a period of a few weeks on diets of isolated foodstuffs is an indication that both classes of accessories are retained in the lactose and casein as impurities. A high degree of purity must be attained in order to eliminate these substances.

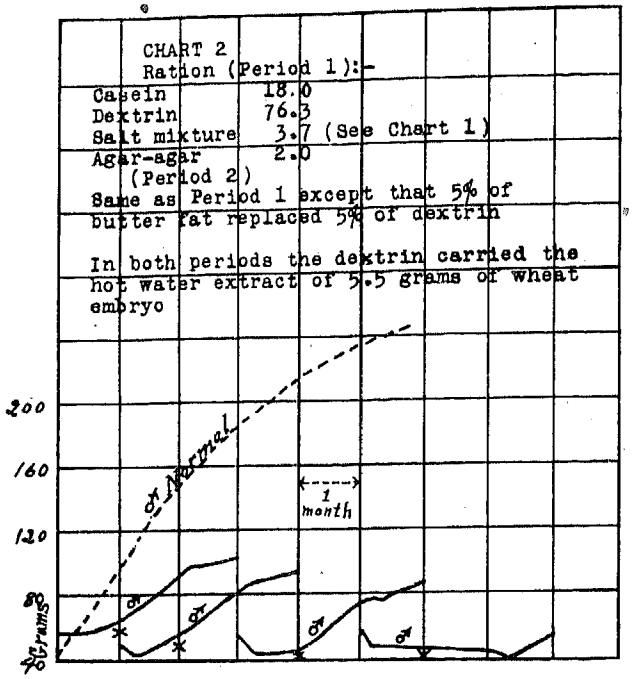

CHART 2. Illustrating the usual performance of young rats with respect to growth when fed a mixture of casein, dextrin, and salts, the casein being of exceptional purity. In Period 1 there was added sufficient water-soluble accessory in the form of water extract of wheat embryo (freed from protein by coagulation) to support growth. Growth did not take place, however, because there was no fat-soluble accessory present in the diet. In Period 2 when butter fat was included in the ration growth at once began.

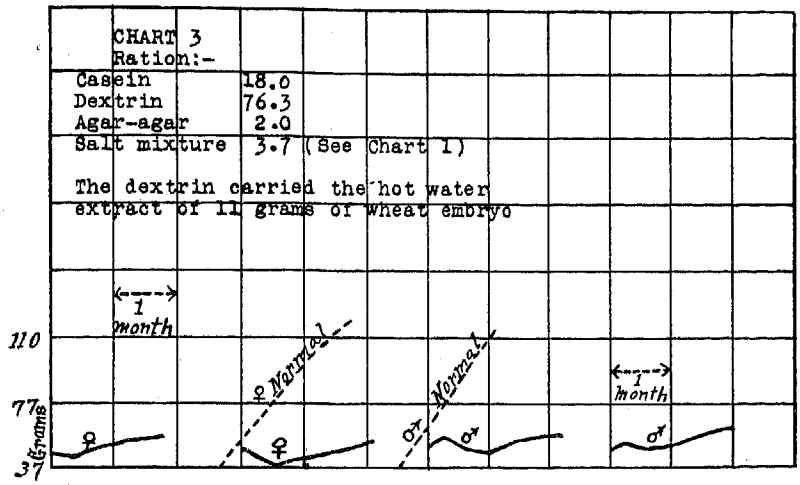

CHART 3. These curves emphasize the absolute necessity of having both classes of accessories present in the diet before growth can take place. These rats received double the amount of water-soluble accessory given to those of Lot 2, but this high intake, in the absence of the fat-soluble accessory, did not lead to growth. A comparison of Lot 355 (Chart 12, page 204) which ration contained a large amount of the fat-soluble accessory but was free from the water-soluble one shows a similar behavior.

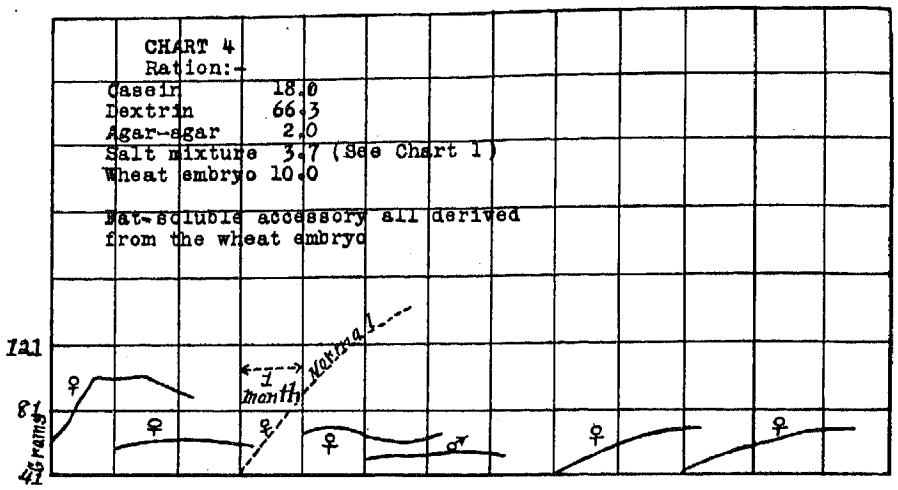

CHART 4. This ration consisting of casein (which was free from dietary accessories), dextrin, and salts, to which 10 per cent of wheat embryo was added, promotes a little growth in vigorous animals. The wheat embryo furnishes an abundance of the water-soluble accessory and a small but insufficient amount of fat-soluble accessory. Even 2 per cent of wheat embryo supplies the water-soluble accessory in amount sufficient for growth. Yet without a higher content of the fat-soluble one very little growth can be made. This ration further confirms our view that in our earlier curves and in those of Osborne and Mendel with similar rations where pronounced growth during two months or more was observed on diets containing lactose and casein, the growth was due to the fact that these components of the ration still carried small amounts of the essential growth accessories.

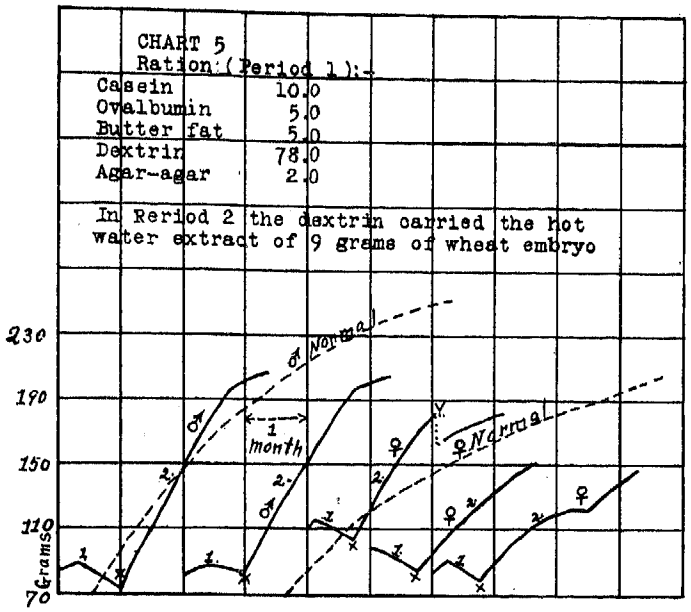

CHART 5. This ration carried adequate fat-soluble accessory with its content of butter fat, but in Period 1 was free from water-soluble accessory. No growth could take place. In Period 2 water-soluble accessory was likewise supplied in the form of water extract of wheat embryo (freed from protein by coagulation with heat). Growth proceeded at once at a rapid rate. The evidence all points to the necessity of both classes of accessories in the diet if appreciable growth is to ensue.

In this ration the nitrogen added in the form of the hot water extract of wheat embryo amounted to 0.0657 gm. per 100 gm. of ration (= 2.31 per cent of the total N in the ration). This is only about one-third as much nitrogen of unknown form as is added by Osborne and Mendel to their rations, otherwise consisting of purified foodstuffs, in the 28.3 per cent of protein-free milk containing 0.76 per cent of nitrogen.

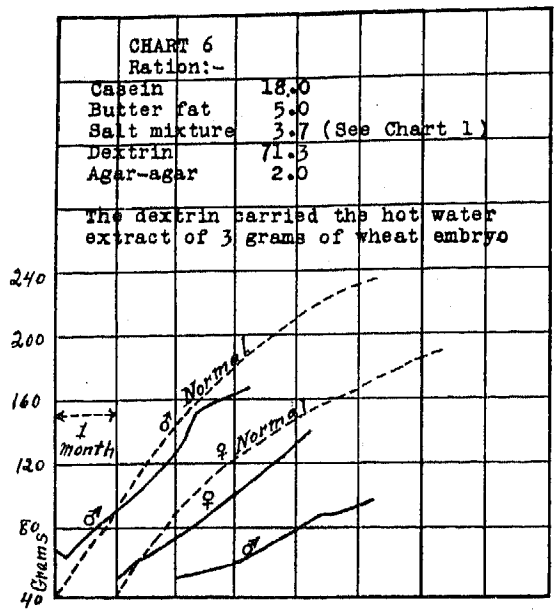

CHART 6. Shows a fair rate of growth during three months on a ration in which all the water-soluble accessory was derived from the hot water extract of 3 gm. of wheat embryo per 100 of ration. 0.77 per cent of the total nitrogen of the ration was in the unknown forms present in the wheat embryo extract. This appears to supply the accessory in amount somewhat below the optimum.

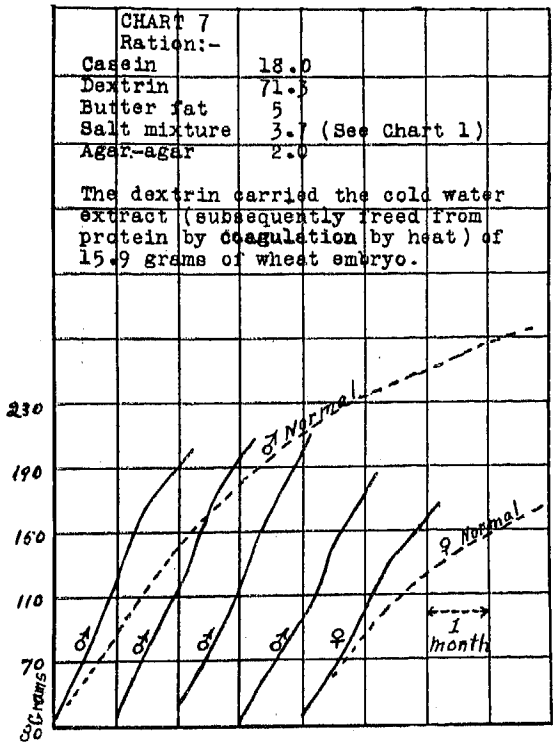

CHART 7. In the ration of these rats all the water-soluble accessory was furnished by the cold water extract of 15.9 gm. of wheat embryo per 100 gm. of ration, the fat-soluble one as butter fat. (The water extract was subsequently acidified and boiled to remove the protein.) 4.05 per cent of the total nitrogen of the ration was furnished by the embryo extract. Extremely rapid growth resulted from this addition, while without it no growth would have taken place. (See Chart 5, Period 1.) These rats appear to be in perfect nutritive condition.

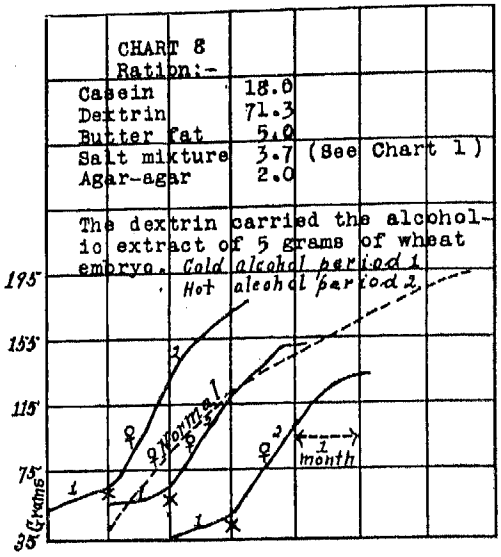

CHART 8. These curves show slight growth on a diet in which the water-soluble accessory was furnished by the cold alcoholic (05 per cent) extract of 5 gm. of wheat embryo per 100 gm. of ration (Period 1) and by a hot alcoholic extract of the same quantity in Period 2. This amount of accessory is adequate for vigorous growth. In this ration the hot alcoholic extract of wheat embryo supplied but 0.0095 gm. of nitrogen per 100 gm. of ration = 0.33 per cent of the entire nitrogen content of the diet.

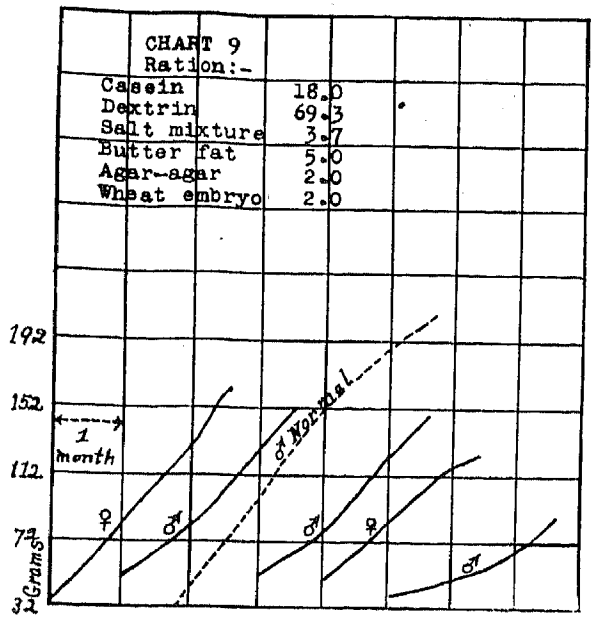

CHART 9. Showing that 2 gm. of wheat embryo per 100 gm. of ration furnish sufficient water-soluble accessory to induce vigorous growth with a diet which is otherwise satisfactory. Wheat embryo contains relatively much of this accessory and relatively little of the fat-soluble one. Similar results have been described by us in nutrition experiments with polished rice which lacks both accessories. The wheat embryo employed contained 5.1 per cent N. The nitrogen from this source was accordingly 3.57 per cent of the total in the diet.

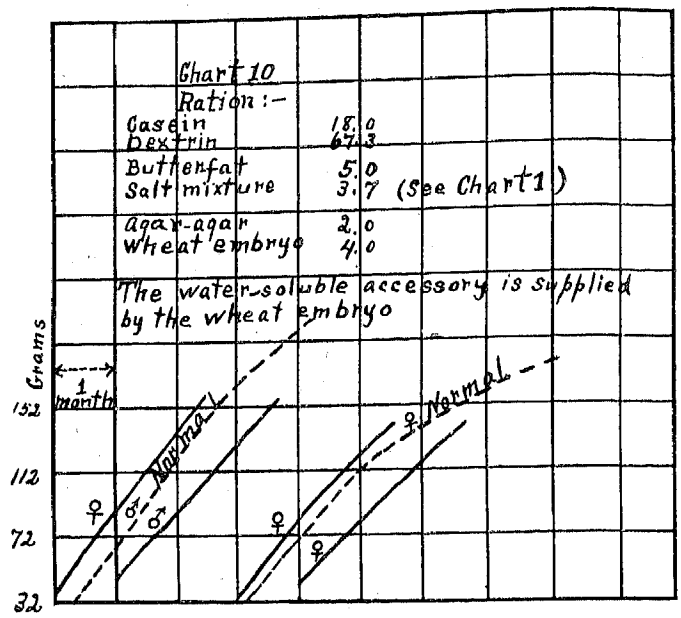

CHART 10. The ration of the rats whose curves are here shown was like that of Chart 8, except that it contained 4 per cent of wheat embryo. It did not produce growth at any more rapid rate than did the preceding one which contained but 2 per cent. It seems evident that 2 per cent of wheat embryo must supply enough of the water-soluble accessory to support growth at the normal rate.

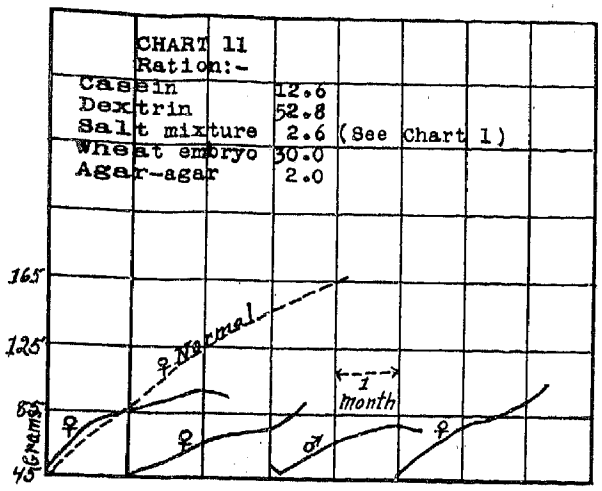

CHART 11. This chart shows that even 30 per cent of wheat embryo carrying fat equivalent to 3 per cent of the food mixture, although it supplies a certain amount, does not furnish enough of the fat-soluble accessory to enable growth to take place at the normal rate. (Compare with Chart 4.) That the rate of growth is within certain limits determined by the amounts of the accessories present is strongly supported by data which we have presented in the preceding paper (page 227).

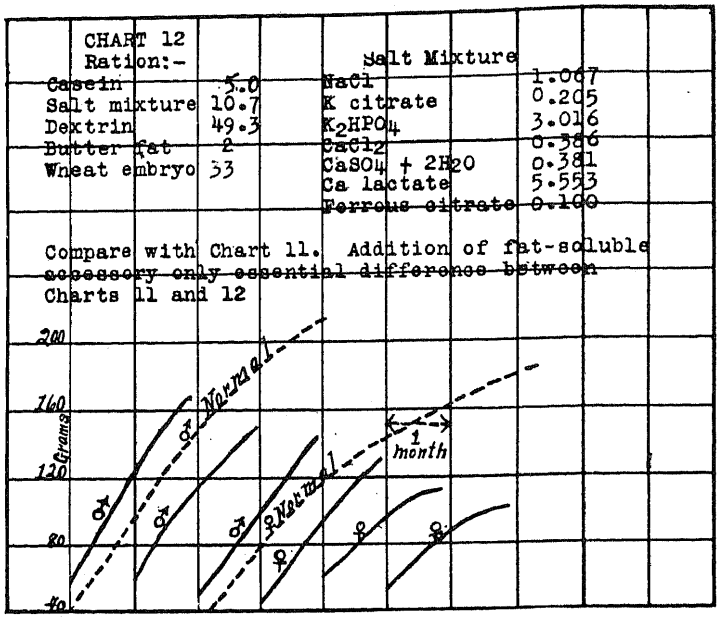

CHART 12. Lot 405. These rats received a ration entirely comparable to those of Chart 11, but the ration carried 2 per cent of butter fat. The excellent growth of these rats as compared with those in Chart 11 whose fat-soluble accessory was derived from 30 per cent of the wheat embryo, proves that it is in this particular respect that the ration of Chart 11 was deficient.