THE NATURE OF THE DIETARY DEFICIENCIES OF RICE.[1]

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In former papers from this laboratory we have made clear the nature of the dietary deficiencies of the corn kernel and wheat kernel as the sole source of nutriment for growing animals.[2]

In the present communication we present experimental data showing the specific properties of polished and of unpolished rice as a food, and show the supplementary relationship between these and certain purified and naturally occurring foodstuffs. These studies, in addition to extending our knowledge concerning the dietary position of rice, have contributed to our understanding of the factors involved in normal nutrition, especially as regards the unknown accessory constituents of the diet which have received so much attention in recent years in connection with the "deficiency diseases," scurvy and beri-beri.

I. The Supplementary Relationship between Polished Rice and Purified Foodstuffs.

Gibson[3] found that a partial compensation of the deficient mineral salt content of a diet of polished rice did not prevent, though it seemed to delay, the onset of polyneuritis in fowls. This is in harmony with our results obtained with young rats fed polished rice with such salt additions (Lot 308, Chart 1) as,

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

[2] McCollum, E. V., *Jour. Biol. Chem.*, 1914, xix, 323. Hart, E. B., and McCollum, E. V., *ibid.*, 1914, xix, 373. McCollum, E. V., and Davis, M., *ibid.*, 1915, xxi, 179, 615.

[3] Gibson, R. B., *Philippine Jour. Sc.*, *Section B*, 1913, viii, 351.

from experience with other rations, we know eliminate the inorganic portion of the ration as a possible factor in the failure of these animals to grow. This point is illustrated by Lot 313, Chart 2, which received a ration derived from milk powder, butter fat, and agar-agar, to which inorganic additions were made which gave the entire food mixture an inorganic content closely similar to that of polished rice. Such a modification of this diet in no way interfered with the growth of young rats.

Commonly accepted standards regarding the protein requirement during growth would point to the possibility that the failure of young rats to grow on polished rice with its inorganic deficiencies corrected might perhaps be due to the low protein content of rice or to its proteins being of poor quality.

To test this point we fed Lot 316 (Chart 3) with polished rice, a salt mixture, together with 5 per cent of ash-free egg albumin prepared according to the procedure described by Taylor.[4] The adjustment of both the inorganic and protein portions of the food mixture produced no effect on the well-being of the animals. They failed to make any growth, and died within two months.

The possibility that polished rice carries some toxic principle which causes injury to the nervous system and thereby the symptoms of polyneuritis has been urged by Caspari and Moszkowski.[5] If this explanation is valid we might anticipate a noticeable improvement in rats when they receive a ration like Lot 316 (Chart 3), except that the quantity of polished rice was reduced nearly one-half, this being replaced by dextrin. The history of Lot 334 (Chart 4) shows that this modification of the diet had no beneficial effect.

The addition of the unknown accessory present in butter fat and certain other fats[6] to a diet of polished rice plus a salt mixture (Lot 317, Chart 5) shows clearly that it is not the lack of this substance which accounts for the failure to grow and maintain a

[4] Taylor, A. E., *University of California Publications, Pathology*, 1903-07, 1, 71.

[5] Caspari, W., and Moszkowski, M., *Berl. klin. Wchnschr.*, 1913, l, pt. ii, 1515.

[6] McCollum and Davis, *Jour. Biol. Chem.*, 1913, xv, 167; 1914, xix, 245; 1915, xxi, 179; *Proc. Soc. Exper. Biol. and Med.*, 1914, xi, 101. Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1913-14, xvi, 424.

good condition. There was no improvement whatever in animals eating rice, salts, and butter fat, over those similarly fed, but without the butter fat.

In harmony with the failure of purified egg albumin to supplement rice and salts (Lot 316, Chart 3) is the lack of benefit seen as the result of adding purified casein to rice, butter fat, and a salt mixture (Lot 329, Chart 6, Period 1). Here again, lowering the rice content of the ration by nearly one-half failed to benefit the animals, and speaks against the idea that rice is inherently toxic (Period 2). The addition of wheat embryo to the extent of 50 per cent of the food mixture (Period 3) led to prompt growth and prolonged life.

Raising the protein content markedly by the addition of casein to the extent of 13.4 per cent in a mixture of polished rice, casein, butter fat, and salts (Lot 324, Chart 7, Period 1) produced no noticeable effect in inducing growth. The appearance of these rats was very miserable. They were rough coated and emaciated. In Period 2, 5 per cent of lactose (Merck) was introduced into the ration, replacing a portion of the rice, with the result that a slow increase in body weight began which continued over a period of four months with some improvement in appearance.

Lot 340 (Chart 8) proved extremely interesting. The ration differed from Lot 329 (Chart 6) only in having 10 per cent of Merck's lactose replace an equivalent amount of polished rice. There was a marked improvement, and slow growth during two to three months, showing that lactose of the purity of the ordinary reagent still carries some substance having a marked influence in promoting growth in a diet of polished rice supplemented with purified protein, salts, and butter fat.

In Lot 309 (Chart 9), whose ration consisted of polished rice supplemented with purified foodstuffs (casein, butter fat, salts, dextrin) but contained 2.6 per cent of commercial lactose of unknown purity, the animals showed decided improvement over rats which received similar additions to rice, but without the lactose. One vigorous animal reached nearly half the normal adult size and maintained this weight until he was five months old. This result is without doubt to be explained by the fact that the lactose was not very pure. By including 10 per cent of Merck's lactose in the ration the vigorous rat just referred to was kept from losing weight until he was nine months old (Period 2).

Lot 382 (Chart 10) illustrates the fact that certain preparations of lactose are by no means as efficient as others in promoting growth when added to rations of polished rice supplemented with casein, salts, and butter fat. This preparation of Merck's lactose did not improve the well-being of the animals as have some other preparations. Sweet, Corson-White, and Saxon[7] in their studies on diet in relation to tumor growth, have called attention to similar differences in the dietary effects of different samples of lactose.

Lot 351 (Chart 11) obtained all its protein from 71.4 per cent of polished rice. It seems probable that the failure of 20 per cent of Merck's lactose to induce growth in this instance was the result, in part, of the low protein content of the diet.

The performance of Lot 355 (Chart 12) makes it clear that the effect produced by lactose in certain of the rations previously described, is not due to its containing the same accessory substance as is carried by butter fat. This lot was given a ration which contained 20 per cent of butter fat without any apparent benefit from this generous supply of this unknown factor. The experiments already described force us to accept the conclusion that *there are necessary for normal nutrition during growth two classes of unknown accessory substances, one soluble in fats and accompanying these in the process of isolation of fats from certain foodstuffs, and the other soluble in water, but apparently not in fats.* It will be shown later that the water-soluble accessory is also soluble in alcohol. The latter substance is present in milk and is removed from milk sugar only by thorough crystallization. Stepp has expressed the belief that there is more than a single class of unknown accessory substances necessary for prolonged maintenance of an animal, but he employed only solvents for the lipoids in preparing his foodstuffs.

The curves of Lot 326 (Chart 13) are offered in further support of our contention that milk sugar of the ordinary purity may be contaminated with sufficient of the water-soluble growth-promoting accessory to cause pronounced increase in body weight of animals whose ration is adequate except for this factor.

[7] Sweet, J. E., Corson-White, E. P., and Saxon, G. J., *Jour. Biol. Chem.*, 1915, xxi, 314.

The records of Lot 383 (Chart 14) are in marked contrast to Lots 329, 324, 355, and 326 (Charts 6, 7, 12, 13) all of which make it evident that *purified proteins, fats having the growth-promoting property, and salt mixtures of appropriate composition, cannot adequately supplement polished rice so as to produce a diet which will support growth.* These curves (Lot 383) reveal the fact that *unpolished rice* is so supplemented by additions of purified foodstuffs as to make a food mixture which supports normal growth. In this respect unpolished rice is like whole wheat, which we have previously shown[8] is completely supplemented by casein, salts, and butter fat, so as to produce normal growth, reproduction, and rearing of the young.

II. The Supplementary Relationship between Polished Rice and Certain Naturally Occurring Foodstuffs.

We have as yet made no experiments with isolated rice fats, but a consideration of the curves of Lots 396, 392, and 395, Charts 15, 16, and 17, indicates that the fats of rice do not contain much if any of the fat-soluble accessory essential for growth. Lot 396 (Chart 15) was fed a mixture of rice 58 grams, rice polishings 40 grams, and Ca lactate 2 grams. The curves show that rice polishings in amount sufficient to furnish about 5 per cent fat do not supply enough of this accessory to permit of any growth. From the results of feeding unpolished rice, Lot 383 (Chart 14), it is evident that rice polishings carry the water-soluble accessory; but without the addition of the fat-soluble accessory in the form of butter fat we have not secured growth on rations composed of mixtures of polished rice and rice polishings with appropriate salt additions. (Compare Charts 8 and 9, in the following paper.) Lot 392 (Chart 16) illustrates the fact that there is nothing appreciably toxic in rice polishings, even when fed alone with the addition of a small amount of calcium lactate. This ration carried about 12 per cent of rice fats, and since there was a slight increase in body weight in each case it would appear that there is a very small amount of the fat-soluble accessory present, but that the amount is inadequate.

[8] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 643.

The nearly normal rate of growth on a mixture of polished rice 82, rice polishings 10, butter fat 5, and a salt mixture 3 per cent (Lot 395, Chart 17) points again, especially in connection with Charts 15 and 16, to the belief that rice polishings, which carry most of the fat from the rice kernel, do not provide the fat-soluble accessory in amount essential for growth. Here again we have definite evidence that rice polishings contain the water-soluble accessory.

Excellent growth with reproduction has been obtained on a monotonous mixture of polished rice 80 and desiccated egg 20 per cent (Lot 311, Chart 18). This shows that the egg contains both the fat- and water-soluble accessories. This result is further confirmed by the fact that egg yolk alone will induce good growth in young rats.[9] Attention should be called to the fact that both whole egg and polished rice are among the natural foodstuffs carrying an excess of potential acidity in their mineral content.

Regarding the minimum amount of egg which must be added to polished rice in order to supply the two classes of accessory substances in amount sufficient for growth, little can be said at present. Chart 19, Lot 323, Period 1, shows that 5 per cent of desiccated egg is not a sufficient quantity for this purpose. (The low protein content of this ration was not the cause of failure to grow. See Chart 33, Lot 381, and Chart 20, Lot 337.) Since in Period 2 these rats received a ration (Ration 312) which carried a mineral content closely similar to polished rice, the inorganic factor is eliminated as a possible cause for failure of the rats to grow in Period 1.

That 5 per cent of desiccated egg does supply enough of the fat-soluble accessory is shown by the curves of Lot 337 (Chart 20) where the rice-egg mixture was supplemented by 10 grams of lactose per 100 of ration (Period 2). Growth was secured through the added amount of the water-soluble accessory contained in the lactose. In Lot 323 (Chart 19), therefore, the failure of 5 per cent of desiccated egg to induce growth was apparently due to its inadequate content of water-soluble accessory.

The failure of certain combinations of natural foodstuffs to induce growth because of a deficiency of one or both of the acces-

[9] McCollum, *Am. Jour. Physiol.*, 1909–10, xxv, 127.

sory substances is further illustrated by Lot 354 (Chart 21) which was fed a mixture of polished rice 82.4, wheat embryo 13.3 per cent, and a salt mixture 4.3 per cent. No appreciable amount of growth could be secured with this ration, the reason being that not sufficient fat-soluble accessory was present. The same ration with 5 per cent of butter fat replacing an equivalent amount of rice (Lot 339, Chart 31) induced normal growth and supported reproduction, and certain young from these rats are still thriving on this ration.

In a previous paper we have given evidence that the fat-soluble accessory is carried by wheat embryo.[10] This material contains about 10 per cent of oil. In Lot 369 (Chart 22) which was fed 30 per cent of wheat embryo with polished rice and salts there was furnished by the ration enough of the fat-soluble accessory to support growth for a time at somewhat below the normal rate.

Wheat embryo contains a high content of the water-soluble accessory. This is shown by the curves of Lot 377 (Chart 23). Even 2 per cent of wheat embryo with rice, salts, and butter fat induces a fair amount of growth (Period 1). The rate of growth was limited by the protein content and not by a lack of water-soluble accessory, since the addition of 5 per cent of casein in Period 2 led to much more rapid growth.[11]

Skim milk powder (Merrill-Soule) is also very rich in the water-soluble accessory, since in combination with polished rice 2 per cent of milk powder supplies enough of this substance for nearly normal growth (Lot 378, Chart 24).

When 4 per cent of wheat embryo is combined with polished rice, salts, and butter fat, Lot 360 (Chart 25), the condition and rate of growth of the rats were noticeably better than of those which received only 2 per cent of the embryo. This may reasonably be assigned to the slightly higher protein content of the ration of the lot which received 4 per cent of wheat embryo addition, rather than to the higher content of water-soluble accessory. Both these factors may, however, have operated to induce this result. The improvement of Lot 361 (Chart 26) which was fed 4 per cent milk powder with polished rice, salts, and butter fat (*i.e.*, received all their water-soluble accessory from 4 per cent of

[10] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 179.

[11] See also Chart 4, *ibid.*, 1915, xxiii, 231.

skim milk powder), over Lot 378 (Chart 24) is probably to be similarly accounted for. Both in Lots 377 and 378 the protein content was somewhat too low to admit of growth at the maximum rate.

With certain mixtures of polished rice and wheat embryo, supplemented with salt additions and added fat-soluble accessory (in butter fat) very vigorous growth may take place. Lot 350 (Chart 27) which was fed 82.7 per cent of polished rice and 8 per cent of wheat embryo is an illustration. Since we have shown that the fat-soluble accessory is found in the plant kingdom,[12] it is evident that, employing the knowledge which we have at the present time, it should be possible to compound rations strictly of vegetable origin which will induce perfectly normal nutrition. Experiments in this direction will be reported on later.

In order to determine the distribution of the water-soluble accessory in the wheat kernel we tried feeding polished rice with 8 per cent of wheat bran plus salt and butter fat additions (Lot 357, Chart 28). We learned, however, that commercial wheat bran always contains a small amount of the embryo, and since there are still no data showing the efficiency of the proteins of the bran in supplementing the protein of polished rice for growth it is not possible to say definitely whether or not the water-soluble accessory is limited to the embryo. The results of studying the curative power of rice polishings in polyneuritis throw no light on the distribution of the curative agent in the seed, since in removing the bran layer of rice the exposed embryo of the seed is also removed. Rice polishings consist of a mixture of bran and embryo, and it is not improbable that the widespread view that it is the bran layer of rice which contains the curative power may be erroneous. It seems to us that the experience of investigators that doses of extract of rice polishings equivalent to 10 grams of the polishings are necessary to produce noticeable curative effect on fowls, points to the belief that the embryo portion of the polishings is the source of the active principle. Wheat bran 8 per cent is no more efficient than wheat embryo 4 per cent in promoting growth (Lots 357 and 360, Charts 28 and 25). Since 10 grams of wheat embryo added to 500 grams of a ration of rice, salts,

[12] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 179.

casein, and butter fat will induce good growth, it seems highly probable that the growth-promoting substance (water-soluble accessory) is concentrated in the embryo. We are studying this question further.

A high content of polished rice in the diet is not at all injurious to animals, provided the diet contains suitable supplementary additions. Lot 310 (Chart 29) received 85 per cent polished rice with 8 per cent milk powder, salts, and butter fat, and has grown and remained in good condition during eight months. The low protein content of this diet probably accounts for their failure to reach the normal adult size and to reproduce. This view is supported by the behavior of Lot 335 (Chart 30) in which the milk powder was added to the extent of 15 per cent, but the ration was otherwise like the preceding (Lot 310). Here growth and well-being have been good, as is attested by the rapid rate of growth and repeated reproduction, in one case to the third generation. The mortality of the young was somewhat high, a fact for which we have as yet no adequate explanation, but the ration is apparently adequate for growth in the second generation, some of the curves of which are shown (Chart 30). Satisfactory growth we have shown is no criterion that the ration will be adequate for reproduction. When the wheat embryo was increased to 13.3 per cent of the food mixture in a diet of rice 77.4, wheat embryo 13.3, butter fat 5, and salts 4.3 per cent (Lot 339, Chart 31), the addition of the water-soluble accessory and adequate protein supplementing was accomplished and nutrition was close to normal. When one considers that this ration, which carried only about 10 per cent of protein supported normal growth and repeated reproduction in these females, it must be admitted that wheat embryo in the proper amount supplements polished rice very satisfactorily.

III. The Supplementary Relationship Between Certain Extracts of Naturally Occurring Foodstuffs and Polished Rice.

Among all the naturally occurring foodstuffs, the yolk of a boiled egg yields, we believe, the smallest amount of water-soluble organic matter. The proteins are rendered insoluble by coagulation during heating, the fats are not soluble in appreciable degree in water, and any emulsified fats in the water extract are easily removed by ether. There are no appreciable amounts of carbohydrates. Since egg yolk is highly efficient in inducing growth it should on extraction with water yield the water-soluble accessory contaminated with some inorganic salts and but a slight amount of organic impurities. That such is the case is made clear by the curves of Lot 367 (Chart 32) which was fed Ration 324 (Chart 7), but in addition the water extract of 6.4 grams of dry boiled egg yolk per 100 grams of ration. These rats made vigorous growth with this addition, while the same ration without the extract produced no growth whatever. 200 grams of dry egg yolk extracted with 800 cc. of water in successive small portions yielded after removal of the emulsified fat only 4.5 grams of water-soluble substances, mostly inorganic salts. That this small amount of material rendered 3.12 kg. of ration efficient for growth shows how slight must be the quantity of active principle necessary for normal nutrition. The temporary fall in body weight of these rats after five weeks' feeding with the extract was caused by the employment at that point of an extract made from egg yolk which had undergone some bacterial decomposition owing to the steam having been turned off the drying oven for a time while the yolks were being dried. On substituting a new preparation of extract from untainted yolks growth was at once renewed.

The stimulating effect of water extract of boiled egg yolk on growth is again shown in the records of Lot 381 (Chart 33). In Period 1, during five weeks growth proceeded on a ration of rice 88, butter fat 5, and salts 3 per cent, together with 4 grams of dextrin upon which the water extract of 8 grams of dried boiled egg yolk had been evaporated. All the protein of this ration (5.8 per cent) was furnished by its content of 88 per cent of rice. In the second period the hot water extract of 5 grams wheat embryo per 100 of ration was employed to furnish the water-soluble accessory. With these extracts growth was continued during three months. There was added from wheat embryo 0.0368 gram of nitrogen per 100 grams of ration, or about 3.27 per cent of the total nitrogen content of the ration from this source. The preparation and description of the extracts of egg yolk and wheat embryo employed in this paper are described on pp. 193–194.

The ready solubility of the unknown accessory in water is shown by the extremely rapid growth of Lot 401 (Chart 36). This ration differed essentially from that of Lot 324 (Chart 7), which does not support growth, only in containing the water extract of 15.9 grams of wheat embryo per 100 of ration. The water extract added to this ration is much higher than is essential for growth. This is shown by the curves of Lot 385 (Chart 35) whose ration was identical with the preceding one, except that it contained the water extract of only 5 grams of wheat embryo per 100 grams of ration. Even here the growth is somewhat more rapid than the normal expectation.

The growth-promoting accessory hitherto referred to as the water-soluble accessory is soluble in alcohol as well. Lot 399 (Chart 38) illustrates in a striking manner the stimulating action on growth of a small amount of the material extracted from wheat embryo by boiling acidified 90 per cent alcohol. It should be remembered that this ration without water or alcohol extract additions does not support growth. The hot, acidified alcoholic extract of 10 grams of wheat embryo included in each 100 grams of ration, induced growth at a rate much faster than the normal. Only about 1.40 per cent of the nitrogen of the ration was added in the alcoholic extract.

Lot 400 (Chart 39) received a ration of the same character as the preceding, but with the hot acidified alcohol extract of 5 grams of wheat embryo per 100 grams of ration. The growth of these rats was distinctly slower than that of Lot 399 (Chart 38), but still somewhat faster than the normal expectation. Growth appears to be, within certain limits at least, dependent upon the amount of the accessory present.

95 per cent alcohol without the addition of acids readily extracts the water-soluble accessory from wheat embryo. This is shown by the curves of growth of Lot 386 (Chart 40) whose ration contained the alcoholic extract of 10 grams of wheat embryo per 100 of ration. 0.018 gram of nitrogen, equivalent to about 0.6 per cent of the total nitrogen of the diet, was in this ration derived from the wheat embryo extract. The addition of more nitrogen to this ration in the form of pure protein would have no effect in inducing growth.

Acetone likewise extracts to some extent from wheat embryo the active principle contained in the water and alcohol extracts.

Lot 387 (Chart 41) grew on a ration similar to the ones we have employed in the work with other extracts. Acetone is, however, not as good a solvent for the active principle as is water or alcohol. Our experience confirms the observation of Stepp,[13] that certain accessories essential for growth are soluble in some degree in acetone.

The studies of the dietary deficiencies of rice which we have described in this paper illustrate a method of procedure which yields valuable information of a kind which has not hitherto been available, concerning the supplementary relationship between rice and a number of the proximate constituents of foodstuffs, and between rice and certain naturally occurring food substances. Such knowledge when available for a wide variety of foodstuffs must, we believe, be of great value in the formulation of human dietaries which will promote health. Furthermore, it must produce far reaching economic improvement in the feeding of farm animals. When we see that a ration carrying as low as 10 per cent of protein (Lot 339, Chart 31) and this derived entirely from vegetable sources can serve to support vigorous growth when supplemented adequately by one of the growth-promoting fats and an inorganic salt mixture, we must realize the great possible saving in the cost of feeding animals when it becomes known just where the favorable combinations of protein lie. This combination of rice and wheat embryo fed without a fat of the growth-promoting group would have been pronounced a failure as a diet. There is presented here convincing evidence that the older practice of experimenting with combinations of natural foodstuffs is not searching enough in character to reveal any of the fundamental principles of nutrition or to lead to the acquisition of the kind of knowledge of the specific properties of our naturally occurring foods, which could lay the foundation of a system of feeding based upon scientific principles.

SUMMARY OF CONCLUSIONS.

1. Polished rice cannot be supplemented so as to produce a ration which will induce growth by the addition of purified protein, fats which possess the growth-promoting property, and salt additions.

[13] Stepp, W., *Ztschr. f. Biol.*, 1913, lxii, 405.

2. The inorganic content of polished rice has been closely imitated by suitable additions of salts and free mineral acids in a ration derived from milk powder and dextrin and in one from desiccated egg and dextrin, without causing any loss of growth-promoting power of the food mixture.

3. Polished rice does not exert a toxic effect on animals even when it constitutes as much as 80–90 per cent of the food mixture. Simple mixtures of rice and egg, rice and milk powder, rice and wheat embryo, carrying such a content of rice, have proven perfectly satisfactory for growth and for prolonged well-being.

4. The addition of quantities of wheat embryo or of milk powder as small as 2 per cent of the food mixture, consisting aside from these constituents, of polished rice, casein, salts, and butter fat, furnishes enough of an essential accessory to induce growth.

5. The essential accessory aside from that carried by butter fat is present in water and in alcoholic extracts of wheat embryo and of egg yolk.

6. The accessory substance which is soluble in water and in alcohol is stable to heat. Prolonged boiling does not injure it to a noticeable degree.

7. The amounts of water extract (freed from protein by coagulation) which we have found necessary to supply enough of the water-soluble accessory to induce normal growth, carry nitrogen equivalent to about 1.0 per cent of the total nitrogen of the ration. Amounts of alcoholic extract of wheat embryo carrying as little as 0.6 gram of solids, and 0.0095 gram N = 0.33 per cent of the total nitrogen of the ration suffice to induce normal growth.

8. The water-soluble accessory is not the same one as is furnished by butter fat. 20 per cent of butter fat addition does not induce any growth unless the other accessory is supplied.

9. Polished rice and salts, together with sufficient wheat embryo to supply liberal protein and water-soluble accessory additions, do not support growth. The fat-soluble accessory must likewise be supplied before growth can proceed.

Preparation of Extracts Employed in the Rations.

1. The Cold Water Extract of Wheat Embryo.—400 gm. of wheat embryo were stirred up with 4 liters of water and allowed to stand with occasional stirring for one hour. The solution was then separated from the solids left undissolved by centrifugation. The solution was then acidified with acetic acid and boiled to coagulate the proteins. The coagulum was filtered off on a paper pulp filter with suction, and evaporated on 200 gm. of dextrin. The dextrin was ground when dry. About 77 gm. of solids were extracted from 400 gm. of embryo.

2. The Hot Water Extract of Wheat Embryo.—The procedure differed from the preceding only in that the water was heated to boiling and the embryo slowly stirred in. Acetic acid was then added to induce coagulation of the proteins. The solution was allowed to cool, then centrifugated, the liquid filtered and evaporated on dextrin as in the case of the cold water extract. About 95 gm. of solids were in this manner extracted from 400 gm. of embryo.

3. 95 Per Cent Alcoholic Extract of Wheat Embryo.—Wheat embryo was extracted for eight hours with 95 per cent alcohol, in a continuous extraction apparatus. The alcohol together with sugars, fats, etc., in the flask, was then placed on dextrin and the solvent was evaporated. About 8 gm. of solids were extracted from each 100 gm. of wheat embryo.

4. Acid Alcoholic Extract of Wheat Embryo.—400 gm. of wheat embryo were treated with 800 cc. of 90 per cent alcohol and 10 cc. of concentrated HCl added. The alcohol was heated to incipient boiling, and filtered with suction on a pulp filter which had been washed with alcohol just before use. The filtrate was placed on 200 gm. of dextrin and neutralized with NaOH. The solvent was then evaporated off.

5. Cold Acetone Extract of Wheat Embryo.—300 gm. of wheat embryo were treated with 400 cc. of acetone and allowed to stand over night. The acetone was removed by pressure, filtered, and evaporated upon dextrin sufficient to prevent stickiness in the product. The hot acetone extract was similarly prepared except that the acetone was heated and removed while hot. About 7 gm. of solids were dissolved from 100 gm. of wheat embryo.

6. Water Extract of Boiled Egg Yolk.—200 gm. of dry boiled egg yolk were ground in a mortar with water (200 cc.) and then the solution was removed by filtration with suction. A paper pulp filter was employed. This was repeated four times. The solution contained some fat in emulsified form, which was removed by shaking with ether. The ether was mechanically separated, the solution again filtered, and evaporated on dextrin. 200 gm. of dry egg yolk yielded about 4.5 to 5 gm. of solids.

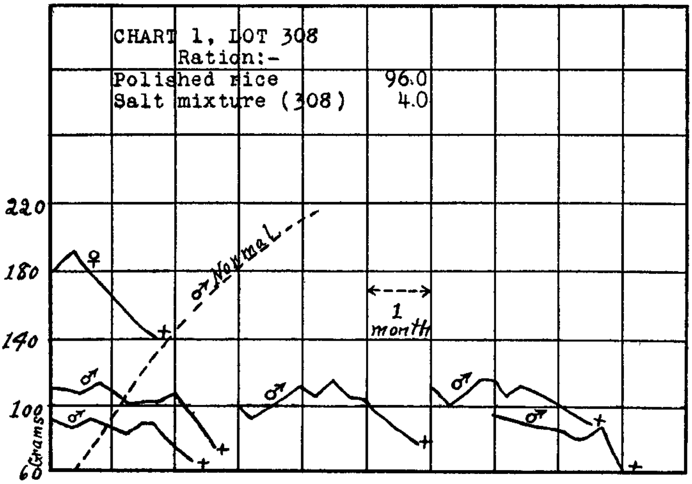

CHART 1. Lot 308. Relation between polished rice and purified foodstuffs. These curves illustrate the fact that polished rice cannot be supplemented so as to induce growth or prolonged maintenance by the addition of a suitable salt mixture alone. That failure of maintenance was not due to the low protein content of the ration, nor to the presence of proteins of a poor character in rice, is proven by the curves of Lot 381, Chart 33, which grew fairly well on a ration, the protein of which was derived solely from polished rice and was appreciably lower than in the ration here employed. We have successfully employed other rations for growth which contained mineral contents closely similar to the above ration (308).

Salt mixture 308:

	gm.
NaCl	5.00
K_2HPO_4	12.10
$CaH_4(PO_4)_2 + H_2O$	2.56
Ca lactate	29.44
Fe citrate	1.00

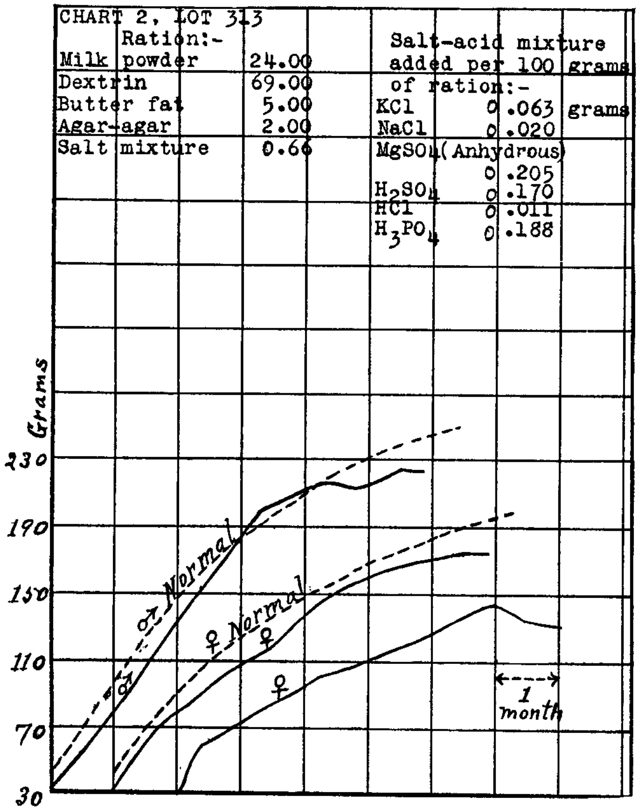

CHART 2. Lot 313. In this ration the mineral content was adjusted by salt and free mineral acid additions so as to approximate closely the mineral content of polished rice. The excellent growth curves make it clear that for growth the mineral content cannot be solely responsible for the failure of animals to grow on a diet of polished rice.

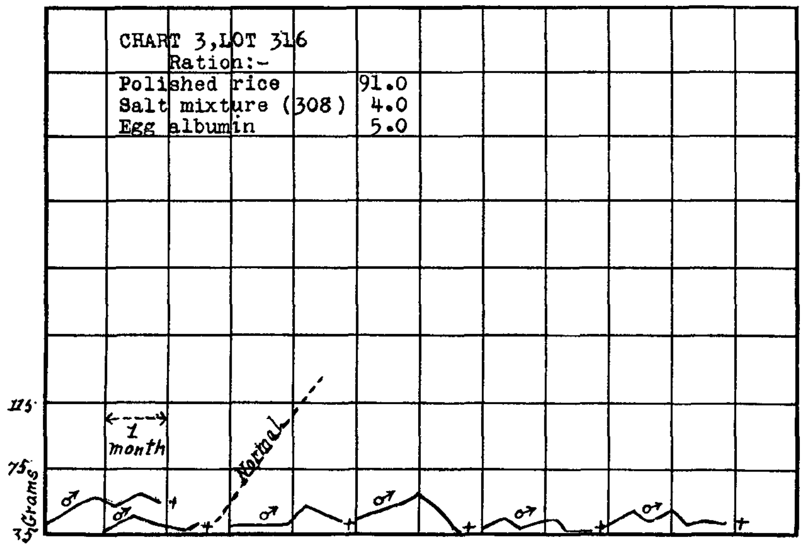

Chart 3. Lot 316. The above curves show that polished rice is not supplemented so as to induce growth or prolonged well-being by correcting the mineral content and the addition of 5 per cent of ash-free egg albumin. It is evident from these curves and Charts 1 and 2 that the deficiency of rice rests in something other than the inorganic or protein factor.

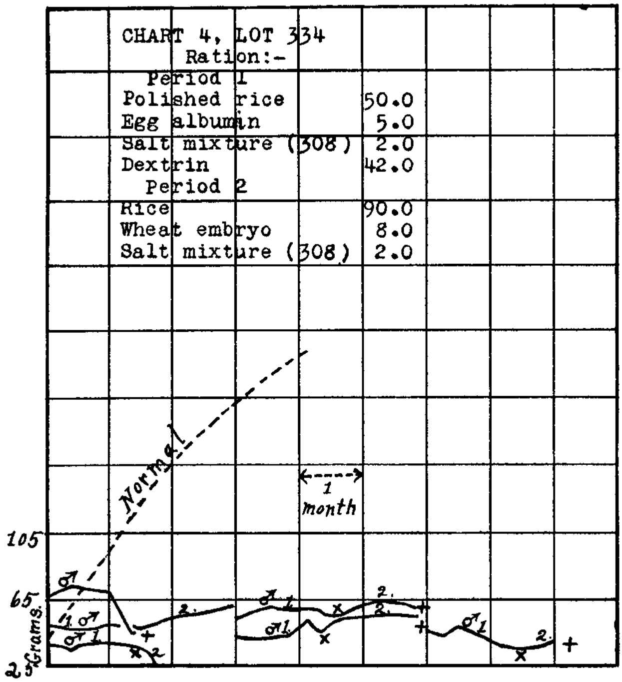

CHART 4. Lot 334. That there is not in rice an inherent toxicity responsible for their failure to grow on high levels is indicated by the performance of the rats whose curves are here shown. The rice was reduced to 50 per cent of the ration instead of 91 per cent as in Lot 316, Chart 3. The condition of the animals was not bettered by the change in the composition of the ration. (See also Lot 338, Chart 9 in the paper following this.)

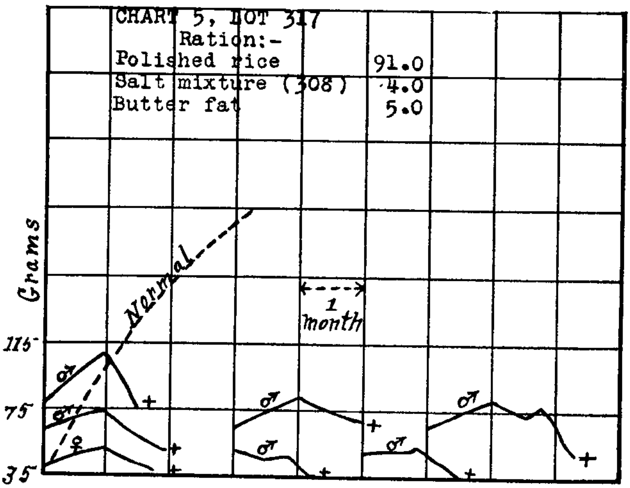

CHART 5. Lot 317. From the above curves it is evident that the failure of rats to grow or be long maintained on a diet of polished rice, a salt mixture, and butter fat, involves some factor other than the lack of the accessory of unknown nature found in certain fats, as butter fat, egg fat, kidney fat, corn, etc., but not in certain others. The protein carried by this ration is adequate for growth at a fairly good rate. (Compare Lot 381, Chart 33.) The mineral content is satisfactory (Lot 381), and the fat possesses the peculiar biological properties which promote growth, yet young rats cannot long maintain their body weight or well-being on this ration.

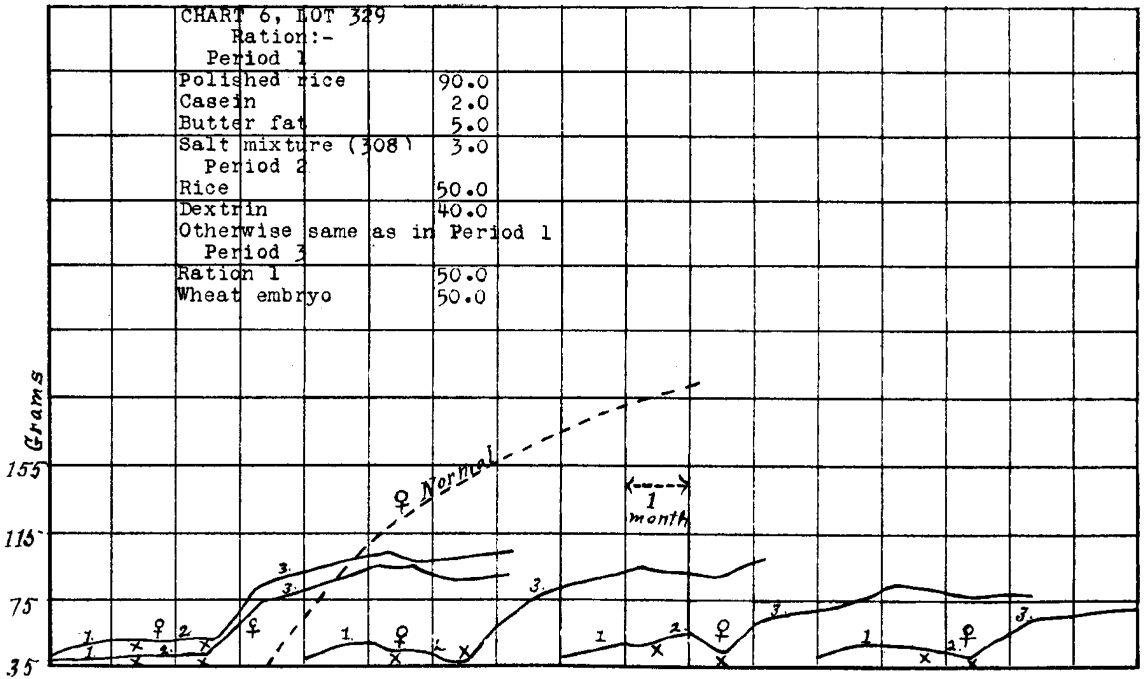

Chart 6. Lot 329. Period 1 illustrates the failure of nutrition of rats fed polished rice supplemented with purified foodstuffs. The inclusion of 2 per cent of casein in a ration closely similar to that of Lot 317 (Chart 5) does not lead to growth.

In Period 2 the reduction of the amount of rice to 50 per cent of the ration did not lead to improvement in the condition of the animals.

Period 3 illustrates the marked stimulus to growth exerted by combining wheat embryo with rice.

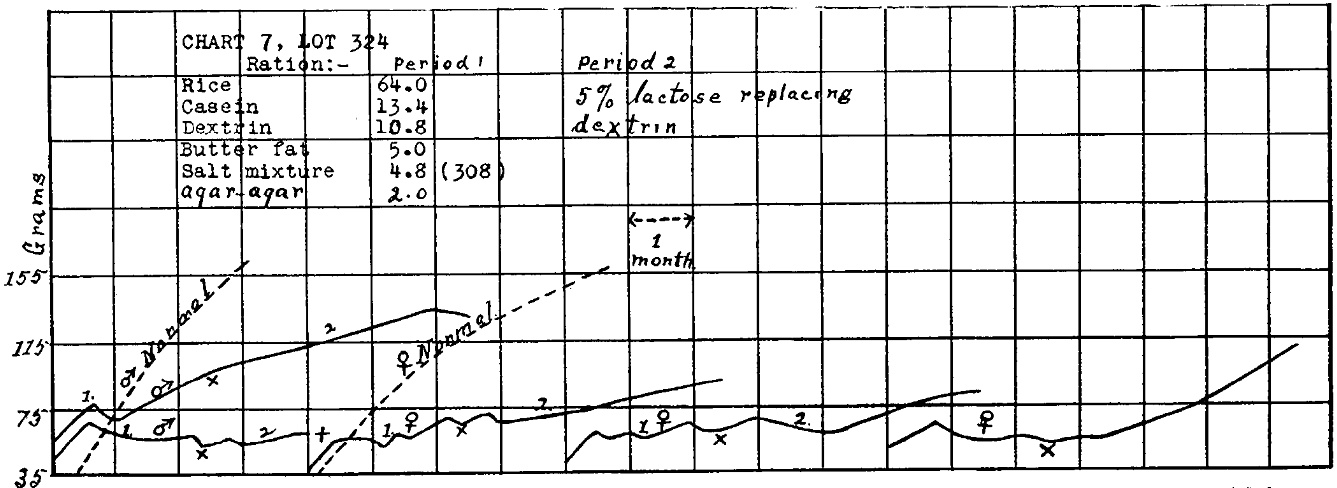

CHART 7. Lot 324. In Period 1 polished rice was supplemented with a liberal amount of purified casein, and with butter fat and suitable salt mixture additions, yet almost no growth resulted. A marked change in the rate of growth followed the introduction of 5 per cent of lactose (Merck's) into the ration in Period 2. Lactose of this purity still contains a growth-promoting accessory. (Compare with Lot 340, Chart 8.)

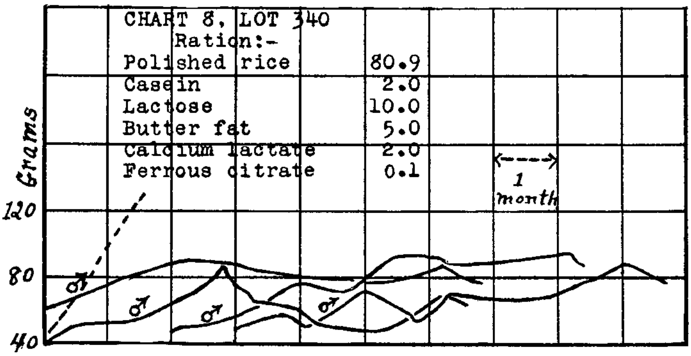

CHART 8. Lot 340. This ration differed essentially from that of Lot 329, Chart 6, only in containing 10 per cent of lactose replacing its equivalent of polished rice. The improvement in well-being and the slow growth during two or three months show that lactose of the purity of ordinary reagents (this lactose contained about 0.034 per cent N) still carried some substance having pronounced biological value in promoting growth and sustaining well-being.

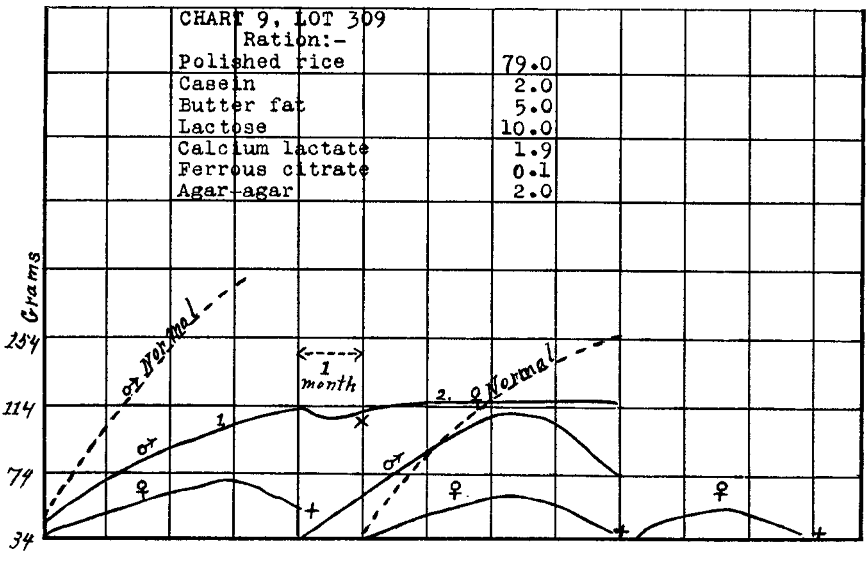

CHART 9. Lot 309. This group of rats manifested a marked improvement over those previously described, an exceptional individual reaching nearly half the normal adult size and maintaining this weight until he was nine months old. This improvement was apparently due to the lack of purity of the lactose which the ration contained. (Compare with Lots 324 and 340, Charts 7 and 8.)

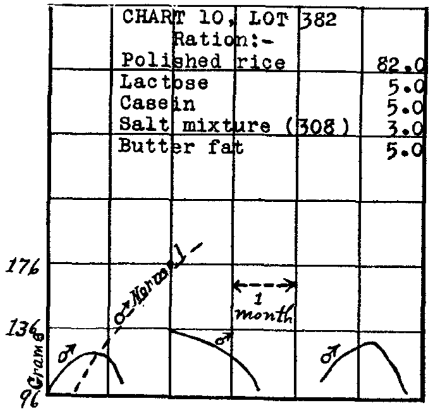

CHART 10. Lot 382. This ration is similar to Lot 329 (Chart 6) but carried more casein (5 per cent) and 5 per cent of Merck's lactose. There is no noticeable improvement as a result of these modifications of the diet. (Compare Lot 329, Chart 6.) These results indicate that lactose itself is unnecessary during growth. This is also borne out by feeding experiments with egg yolk alone on which good growth is attained. Egg yolk contains no lactose.

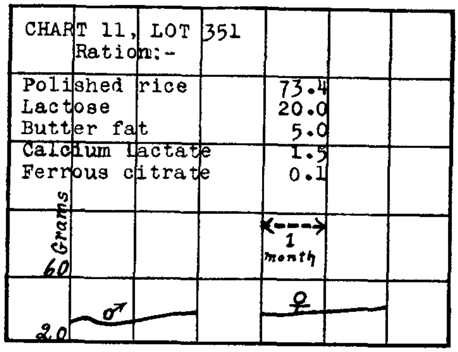

CHART 11. Lot 351. This lot which failed to increase their body weights on a ration of rice, salt mixture, butter fat, and 20 per cent of lactose, apparently did so because of the low protein content of the ration. (Compare with Lot 324, Chart 7.)

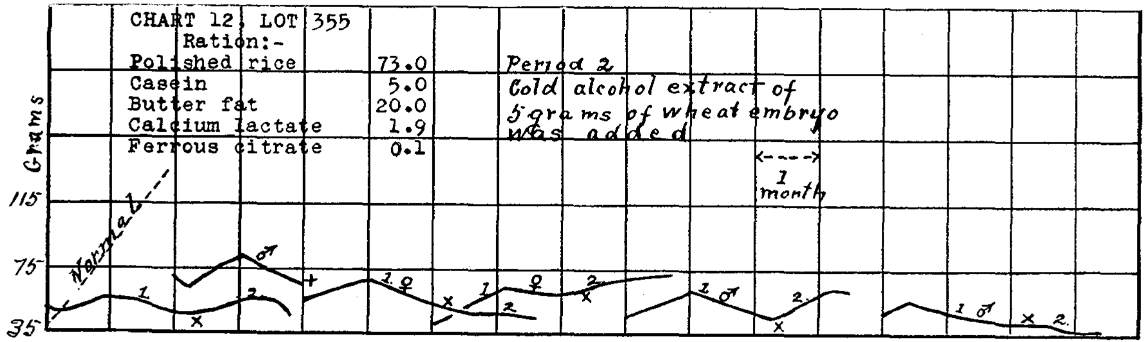

Chart 12. Lot 355. The curves here exhibited established the fact that the unknown accessory foodstuff carried by the butter fat is not the only one required by the growing animal. Increasing the butter fat content to 20 per cent in a ration made up of rice plus purified foodstuffs, does not induce growth. Another accessory which is present in small amounts in lactose of commercial purity is also required. This confirms the observation of Stepp[14] who found with mice that the addition of the ether extract alone to an ether-alcohol extracted ration did not prolong life.

[14] Stepp, *Ztschr. f. Biol.*, 1912, lvii, 151.

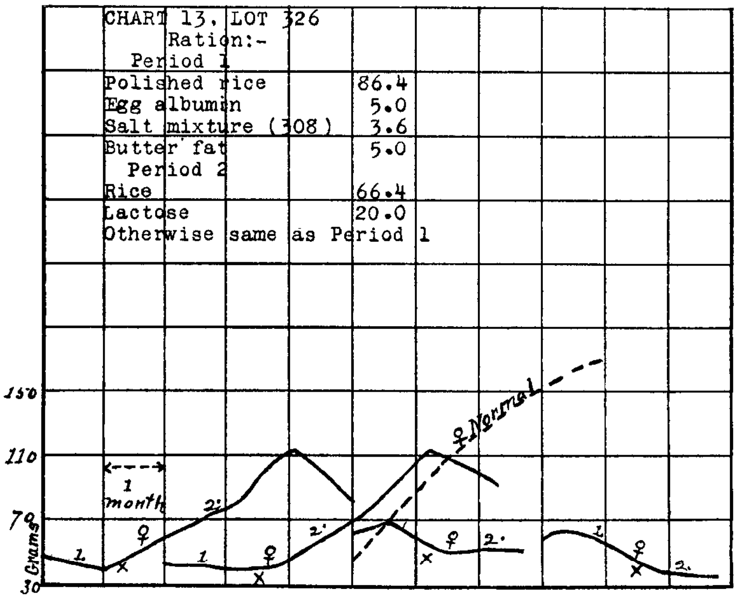

CHART 13. Lot 326. These curves support what was shown in Chart 12, Lot 355; *viz.*, that purified protein, fats, and salt mixtures cannot supplement polished rice so as to induce growth. Note the decided improvement in rate of growth in Period 2 when 20 per cent lactose was introduced into the ration. (Compare with Lots 340, Chart 8, 324, Chart 7.)

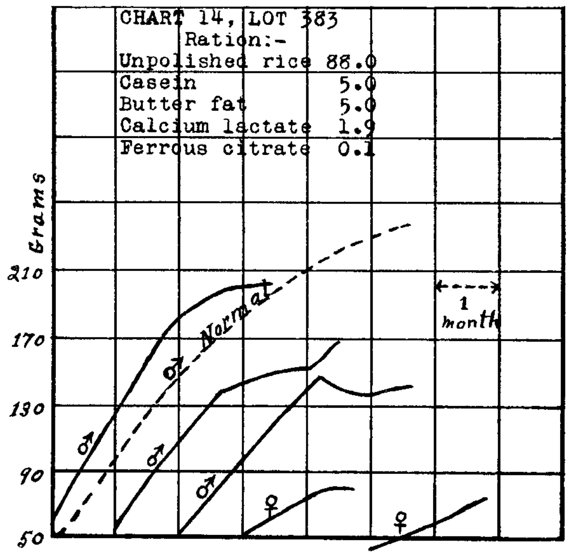

CHART 14. Lot 383. In marked contrast to Lots 326, 355, 324, and 329, Charts 13, 12, 7, and 6, all of which make it evident that purified proteins, fats, and salt mixtures cannot adequately supplement polished rice so as to induce growth, are the records here shown which reveal the fact that unpolished rice is adequately supplemented by such additions. Unpolished rice behaves in this respect like whole wheat which we have previously shown is made adequate for complete growth and normal reproduction and rearing of the young, by the addition of purified casein, a salt mixture, and butter fat.[15]

[15] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 615.

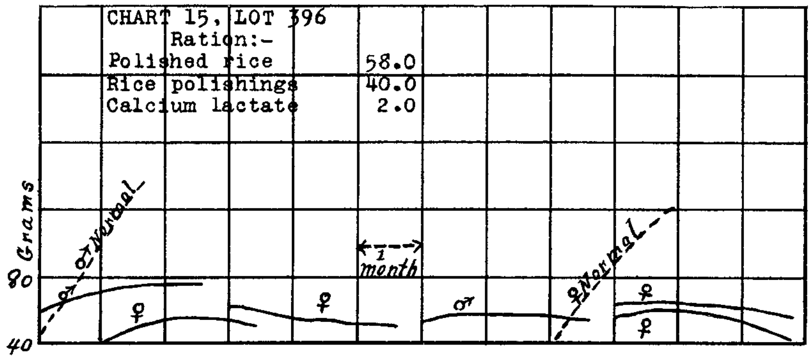

Chart 15. Lot 396. That rice polishings in amount sufficient to supply about 5 per cent of fat do not furnish the fat-soluble accessory, at least in adequate amount, is indicated by the curves of the rats of this lot. Rice polishings evidently supply the water-soluble accessory (Lot 383, Chart 14), but without the addition of the fat-soluble one no growth was attained with this ration. (Compare with Lots 392 and 395, Charts 16 and 17.)

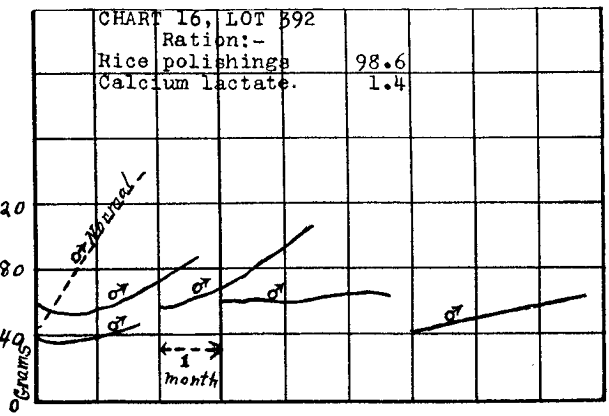

Chart 16. Lot 392. These curves indicate that rice polishings with calcium lactate are not toxic to young rats. The failure of Lot 396, Chart 14, to grow on a ration containing 40 per cent of this constituent cannot, therefore, be attributed to any injurious effect of rice polishings. (Compare Lot 396, Chart 15, and 395, Chart 17.)

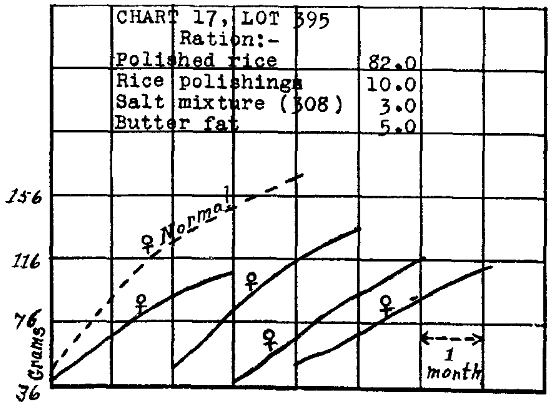

CHART 17. Lot 395. These curves demonstrate the power of rice polishings to supplement rice, butter fat, and salts, and make a ration on which good growth is attained. It seems evident that the fats of rice do not carry the fat-soluble accessory, at least in appreciable amounts. (Compare with Lot 396, Chart 15, and 392, Chart 16.)

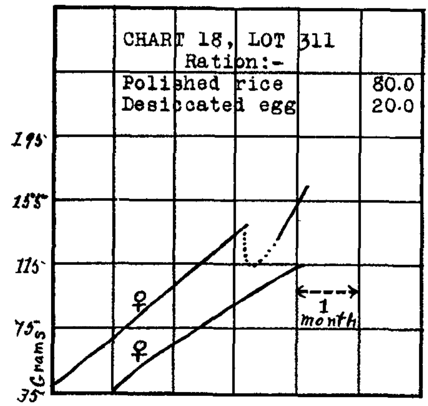

CHART 18. Lot 311. Illustrating the good growth of rats confined to a mixture of polished rice and desiccated egg. The young produced by one of these females were eaten by the mother. Our experience with reproduction on other rations has shown that rations adequate for growth are not necessarily so for reproduction and rearing of the young.[16] In discussing the curves reported in this paper we reserve all conclusions respecting the adequacy of the rations for reproduction.

[16] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 615.

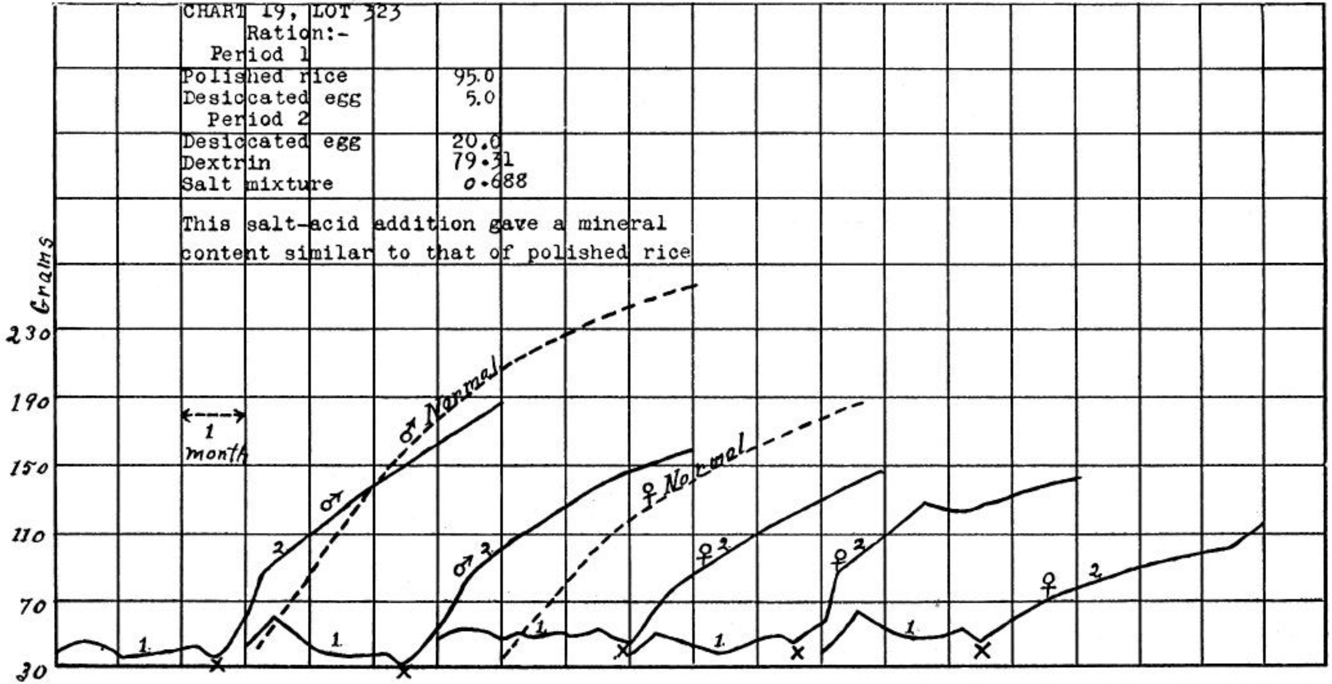

CHART 19. Lot 323. It is evident from these curves, Period 1, that 5 per cent of desiccated egg,[17] is not sufficient to supply the water- and fat-soluble accessories in the amounts necessary for growth. A comparison of these records with Lot 381, Chart 33, eliminates the protein element as the limiting factor (compare with Lot 337, Chart 20), and in Period 2 (Ration 312) of this chart the ration carried a mineral content closely similar to that of polished rice. Since good growth was observed in Period 2 it is evident that the mineral content was not the limiting factor. Salt acid mixture:

	gm.		gm.
NaCl	0.049	$CaCl_2$	0.049
$MgSO_4$ (anhydrous)	1.760	H_2SO_4	0.008
$KHSO_4$	0.200	H_3PO_4	0.206

[17] The desiccated egg employed was of high quality and was obtained from the National Bakers' Egg Co., Sioux City, Iowa.

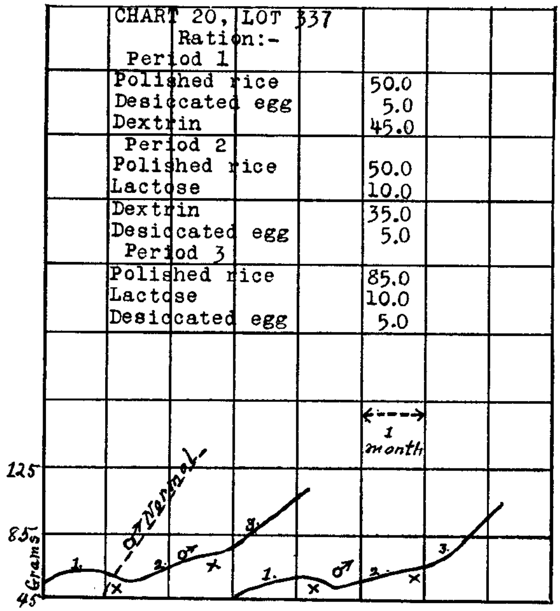

CHART 20. Lot 337. These curves illustrate in a convincing manner that lactose of fairly high purity (N content 0.034 per cent) carries the water-soluble accessory essential for growth. (Compare Lots 340, 309, Charts 8, 9.) No growth is secured in Period 1, while in Period 2 in which 10 gm. of dextrin are replaced by 10 gm. of lactose growth at a slow rate is observed. In Period 3 when the content of rice was increased to 85 per cent and thereby the protein content raised, the growth rate becomes about normal.

Lot 323 (Chart 19) shows that growth in this period is not due merely to the increased protein content, but primarily to the accessory added in the desiccated egg.

In Period 2 the amount of accessories was adequate—probably near the minimal limit—but the protein content limited growth. These curves show further, that 5 per cent of desiccated egg supplies enough fat-soluble accessory for growth, since lactose contains none of this constituent. More than 5 per cent of desiccated egg is necessary to supply enough water-soluble accessory for growth. In regard to the relative amounts of the water-soluble accessory in milk powder and wheat embryo compare with Lots 377 and 378, Charts 23, 24.

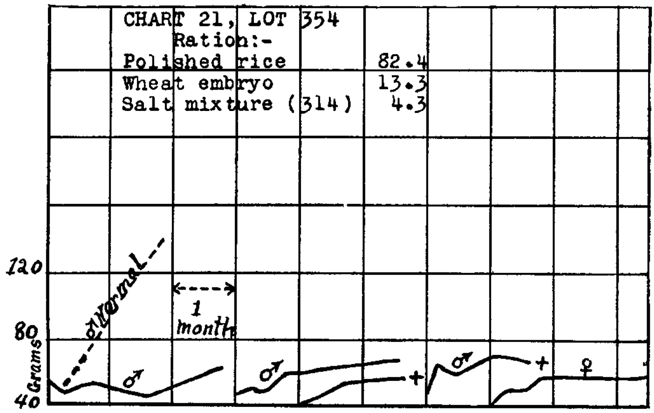

CHART 21. Lot 354. Wheat embryo to the amount of 13.3 per cent fails to provide an adequate amount of one of the necessary accessories for growth in this ration. Reference to Charts 31, 23, Lots 339, 377, reveals the fact that it is the fat-soluble accessory which is not present in this ration (Lot 354) in adequate amount.

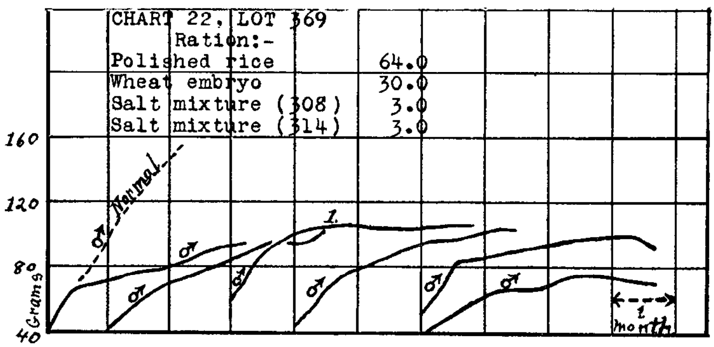

CHART 22. Lot 369. When wheat embryo is present to the extent of 30 per cent in a mixture of polished rice and wheat embryo, the latter supplies enough of the fat-soluble accessory to promote growth for a time. (Compare with Lot 354, Chart 21.)

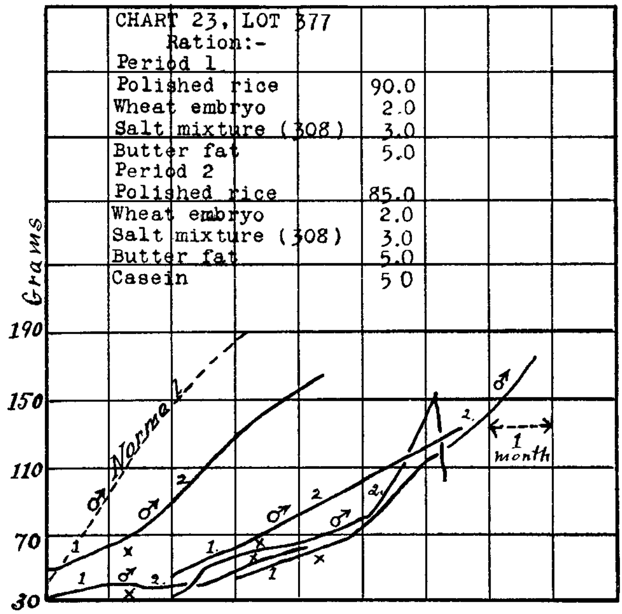

CHART 23. Lot 377. These curves show clearly that wheat embryo to the extent of only 2 per cent of the food mixture suffices to supply enough of the water-soluble accessory to enable growth to proceed at nearly the normal rate. (Compare these curves with Lot 378, Chart 24. See also Chart 9 in the following paper.) In Period 2 the rate of growth was accelerated somewhat by raising the protein content through the addition of 5 per cent of casein.

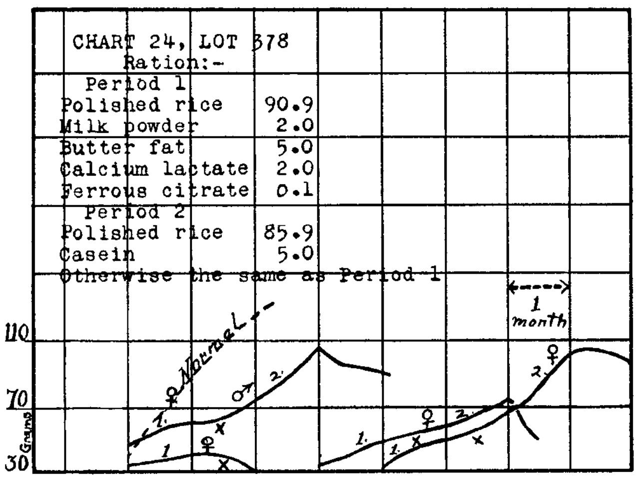

CHART 24. Lot 378. 2 per cent of skim milk powder with rice suffices to supply enough of the water-soluble accessory to induce growth at nearly the normal rate.

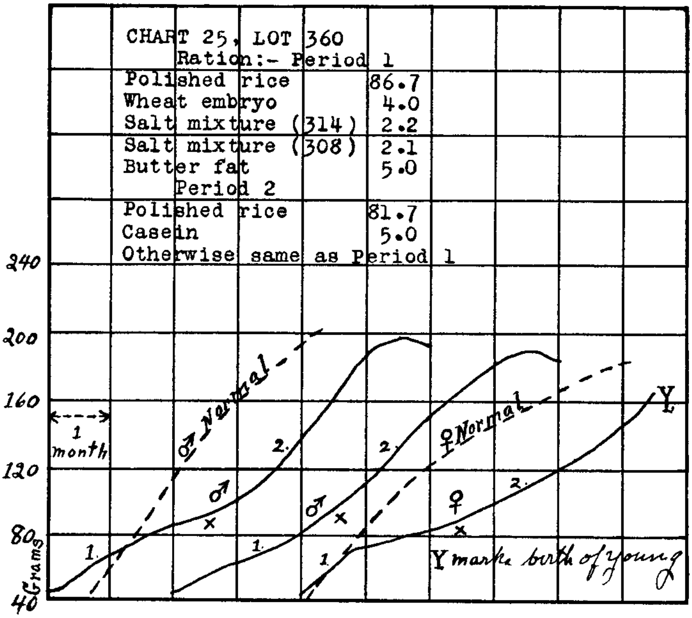

CHART 25. Lot 360. The condition with respect to growth of these rats which received their water-soluble accessory supply from 4 gm. of wheat embryo is noticeably better than those of Lot 377 which received only 2 per cent of this constituent. This may reasonably be assigned to the slightly higher protein content of the ration of Lot 360 derived from the additional 2 gm. of wheat embryo. (See also Lots 369, 377, Charts 22, 23.) Salt mixture 314:

	gm.
NaCl	1.067
K citrate	0.205
K_2HPO_4	3.016
$CaCl_2$	0.386
$CaSO_4.2H_2O$	0.381
Ca lactate	5.553
Fe citrate	0.1000

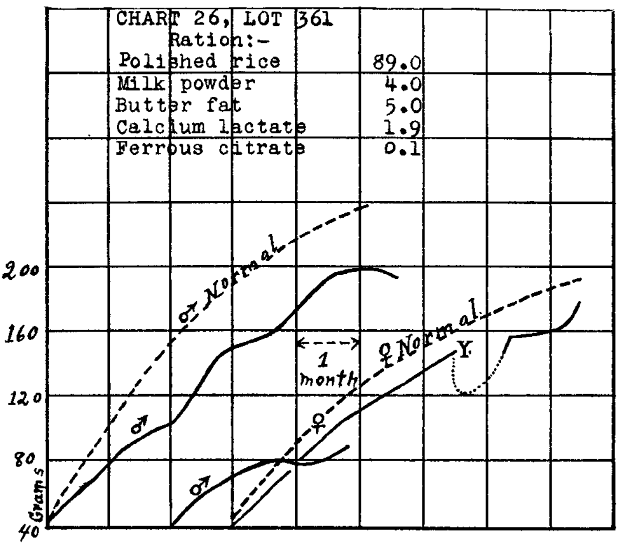

CHART 26. Lot 361. The noticeably better growth of these rats receiving 4 per cent of skim milk powder with rice, butter fat, and salts as compared with Lot 378 which received 2 per cent, may be reasonably assigned in great measure at least to the added content of protein. 2 per cent of skim milk powder contains enough of the water-soluble accessory to support growth at a fairly rapid rate.

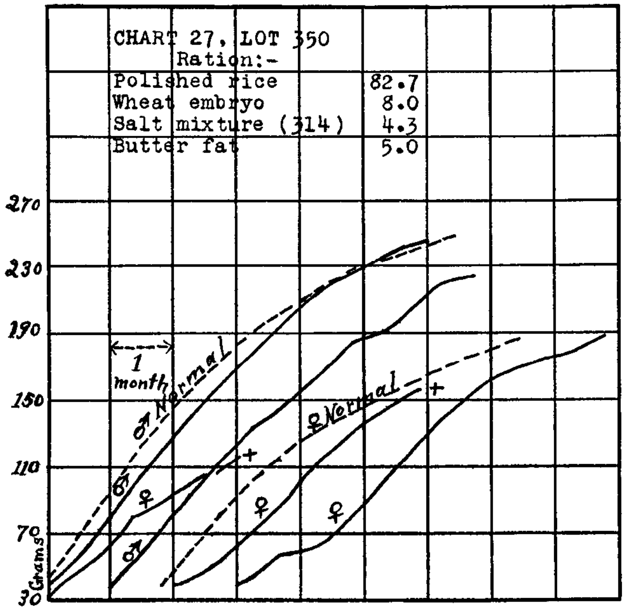

CHART 27. Lot 350. This chart illustrates how vigorous may be the growth of animals deriving their ration from polished rice, wheat embryo, butter fat, and a salt mixture. Since we have elsewhere shown[18] that the fat-soluble accessory essential for growth is present in corn and in wheat embryo, it is apparent that with suitable combinations entirely satisfactory growth is to be expected from certain rations derived from vegetable sources exclusively.

[18] McCollum and Davis, *Jour. Biol. Chem.*, 1915, xxi, 179.

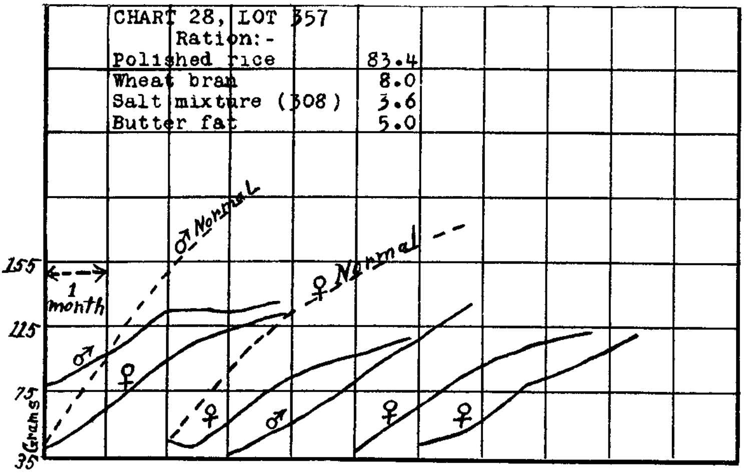

CHART 28. Lot 357. This ration was designed to show whether the water-soluble accessory so necessary to normal nutrition is present in the bran of wheat. The stimulus to growth in this lot was noticeably less than in Lot 350, Chart 27, which was given the same amount of wheat embryo as the bran content of the present ration. We have subsequently learned that in the milling process some embryo always passes into the bran. It is therefore possible that the effects here observed are in great part due to the small embryo content and not to the presence of the unknown accessory in the outer layer of the wheat kernel. We are investigating this matter further.[19]

[19] An inspection of polished rice reveals the fact that in the process of polishing not only is the bran layer removed, but the embryo, which is easily detachable, as well. The great richness of wheat embryo in this water-soluble accessory, and its apparent absence from that portion of the wheat kernel which makes up bolted flour, exclusive feeding of which, according to Little (Little, J. M., *Jour. Am. Med. Assn.*, 1912, lviii, 2029), produces symptoms typical of beri-beri, lead us to suspect that the curative effects of rice polishings and of extracts of the same owe this property to the presence of the embryo rather than to the bran layer. This subject is receiving further attention.

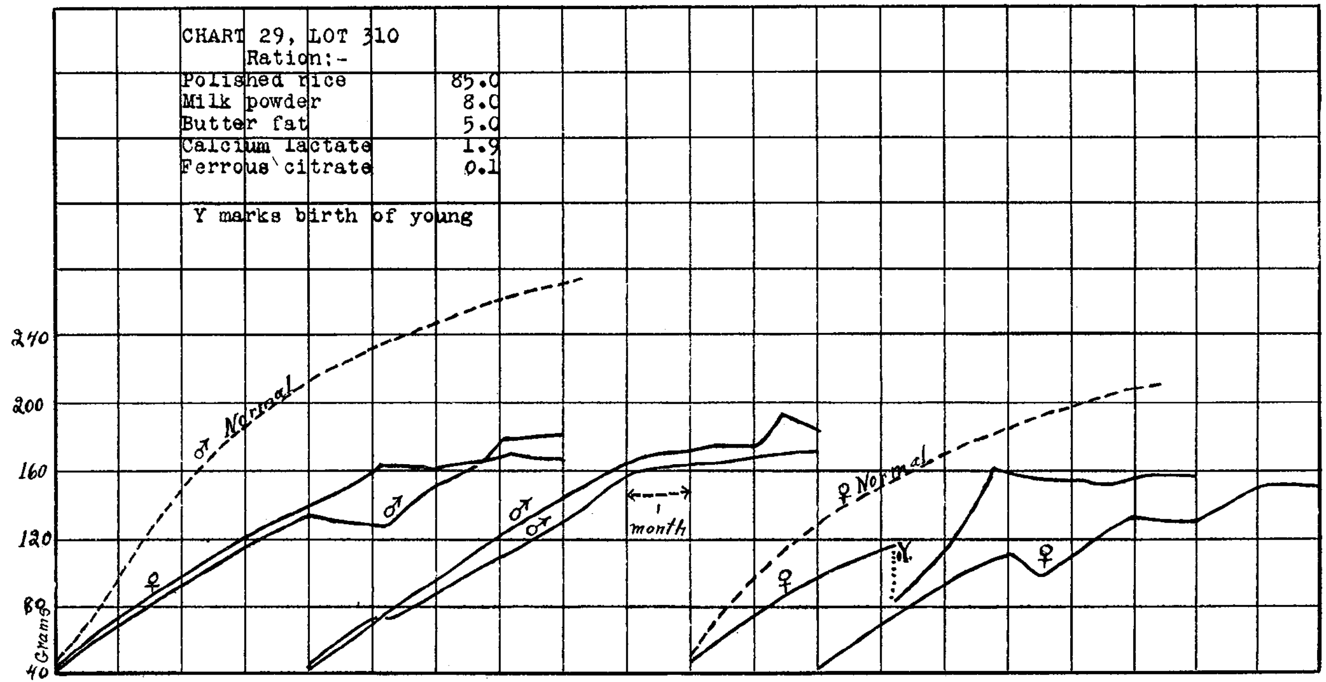

CHART 29. Lot 310. A high content of polished rice in a monotonous diet is not necessarily detrimental to a growing animal. The failure of these rats to reach normal size and support reproduction may well be ascribed to the low protein content of the ration (about 8 per cent).

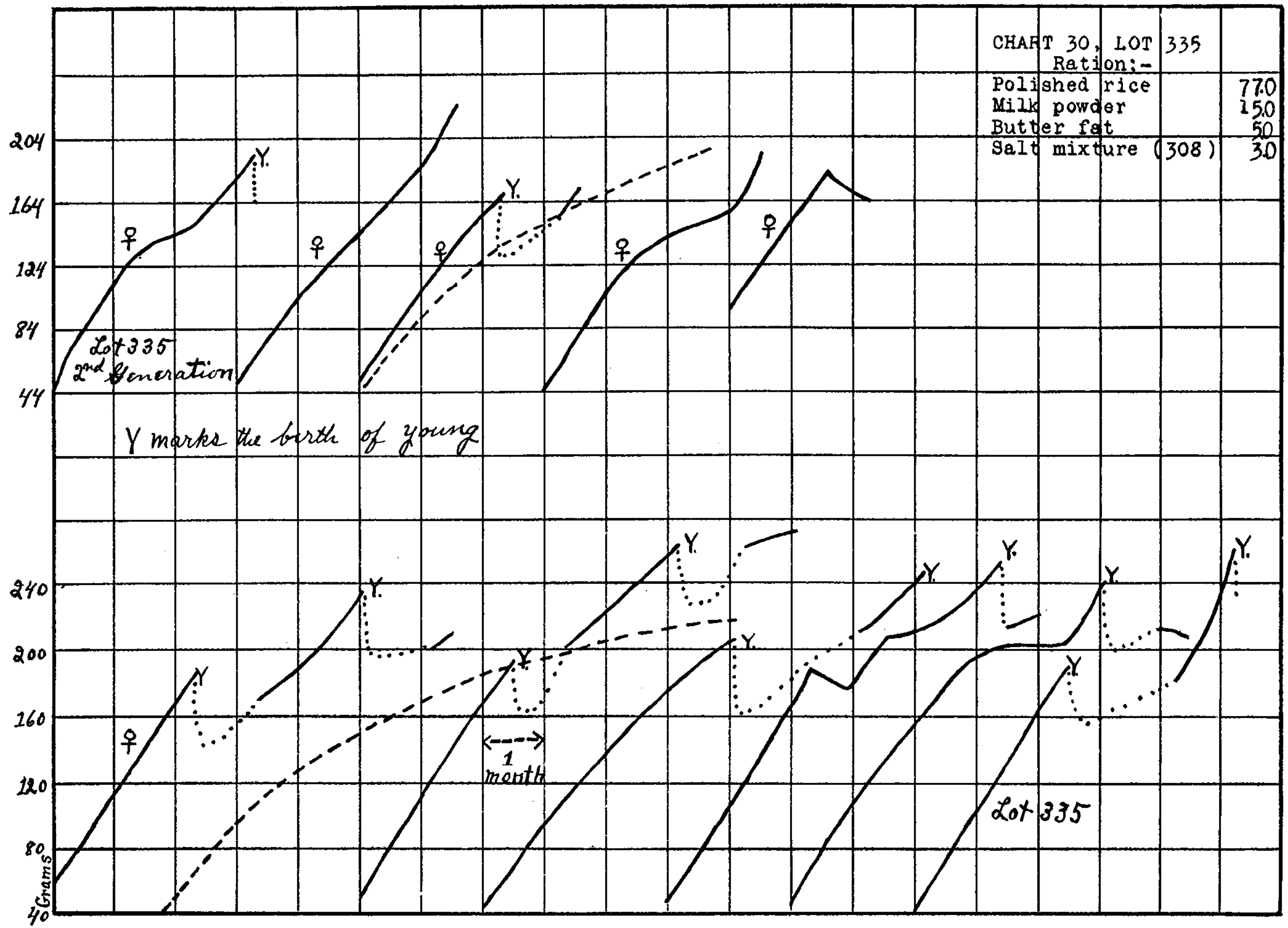

CHART 30. Lot 335. These curves illustrate the fact that a high content of polished rice in the diet is not prejudicial to the well-being of animals, provided it is properly supplemented. This ration not only induces excellent growth, but supports reproduction and rearing of the young. This ration contained only about 11 per cent of protein. The second generation is apparently nearly normal in its power of reproduction.

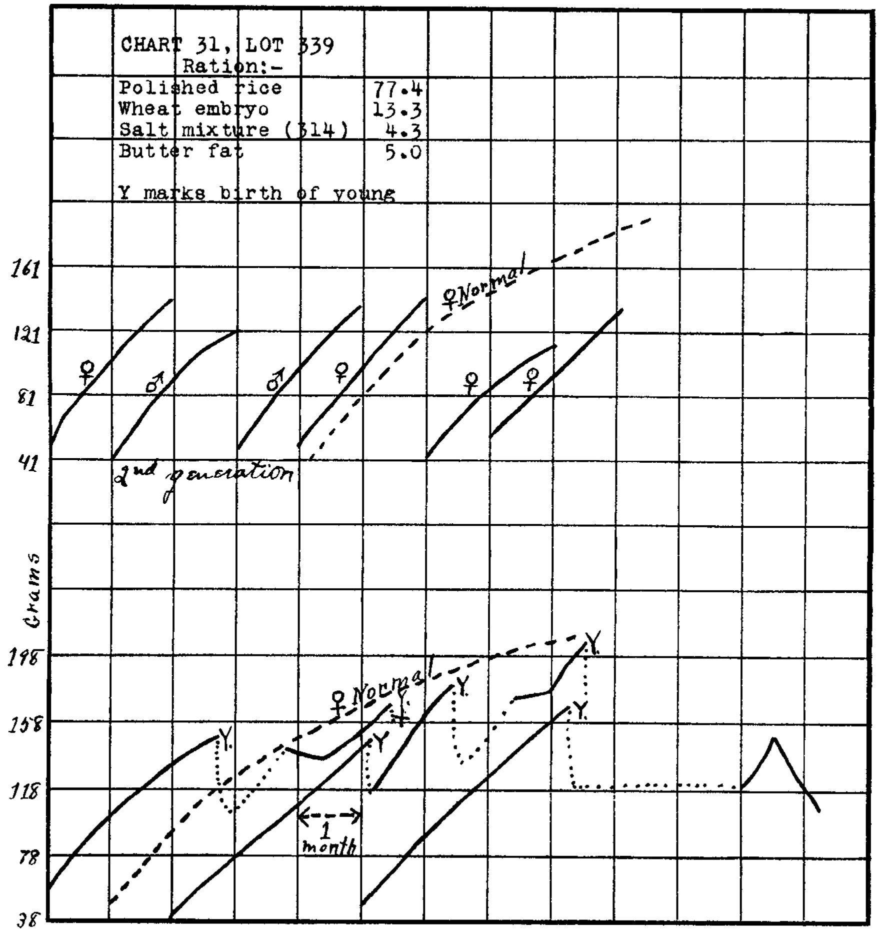

CHART 31. Lot 339. These curves show how with the addition of more fat-soluble accessory in the form of butter fat, 13.4 per cent of wheat embryo supplements polished rice in a manner so as to support nutrition closely approximating normal. When one considers that this ration contained only about 9 per cent of protein it is certainly remarkable that from three females five litters of young were produced in the first seven months of their lives. The mortality of these young was high, but one litter whose curves are shown is making good progress on the mother's diet at the present time.

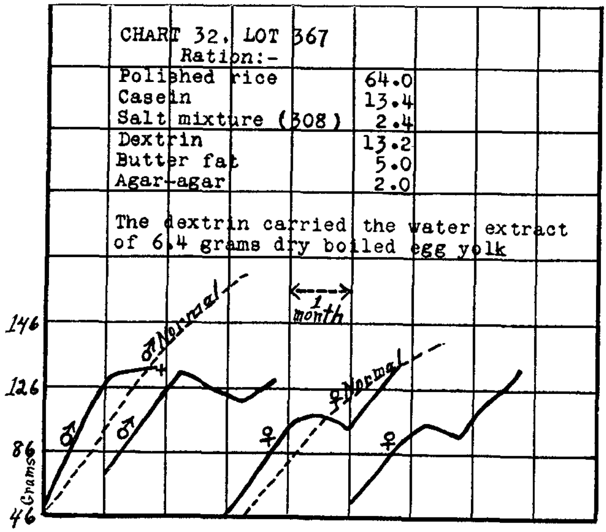

CHART 32. Lot 367. These curves show the depression in growth, due to a preparation of water extract made from slightly decomposed egg yolk. The recovery, as well as the initial growth, was on a preparation from better material.

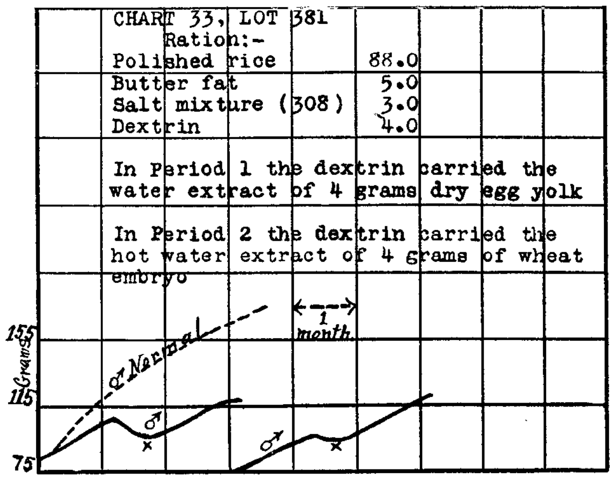

Chart 33. Lot 381. Without the addition of the accessory in the form of water extract, of egg yolk, or wheat embryo, no growth is possible on this ration. (Compare with Lot 317, Chart 5.) The drop in the curves is the result of the use of a preparation of extract made from egg yolk which had undergone putrefaction during drying. The same depressing effect of this preparation was observed with other lots of rats on other rations. In some of these recovery and renewed growth followed changing to another preparation of extract of egg yolk prepared from eggs which were of good quality. (Compare Lot 367, Chart 32.)

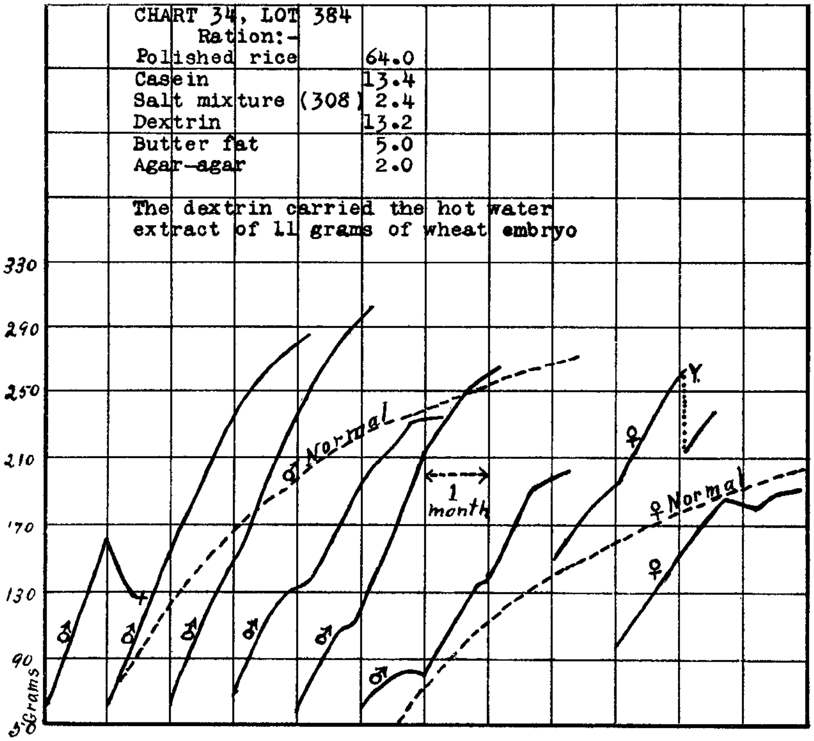

CHART 34. Lot 384. These curves illustrate the remarkable stimulating effect of water extract of wheat embryo, when added to a ration which without such addition was wholly unsatisfactory for growth. (Compare Lot 324, Chart 7.) The hot water extract was freed from protein by acidifying, boiling, and subsequent filtration.

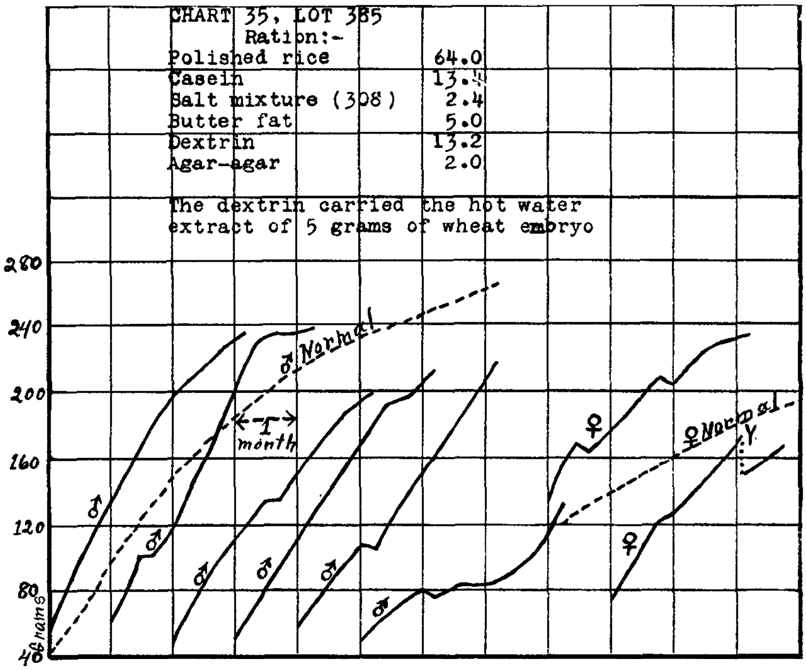

CHART 35. Lot 385. Illustrating the efficiency of the addition of the hot water extract of 5 grams of wheat embryo in promoting growth. (Compare with Charts 34 to 36.)

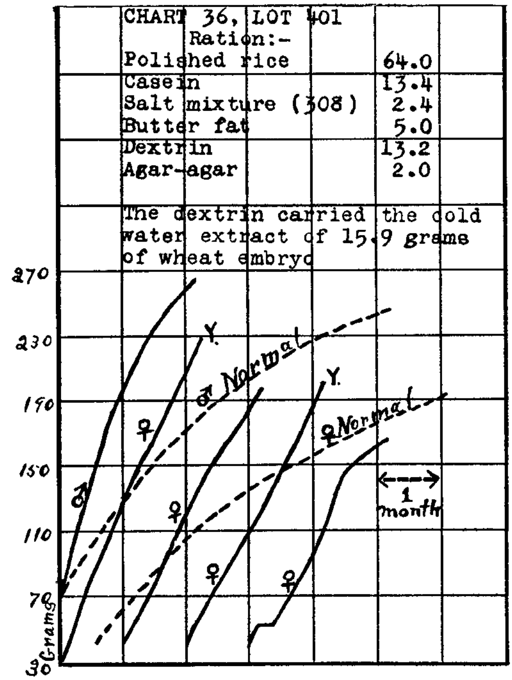

CHART 36. Lot 401. The extremely rapid growth of the rats whose curves are shown in Chart 36 indicates the ready solubility in cold water, of an unknown dietary accessory present in wheat embryo. This substance is stable toward heat, for the water extracts were subsequently acidified and boiled to coagulate the proteins. To each 100 gm. of ration were added the extract of 15.9 gm. of wheat embryo.

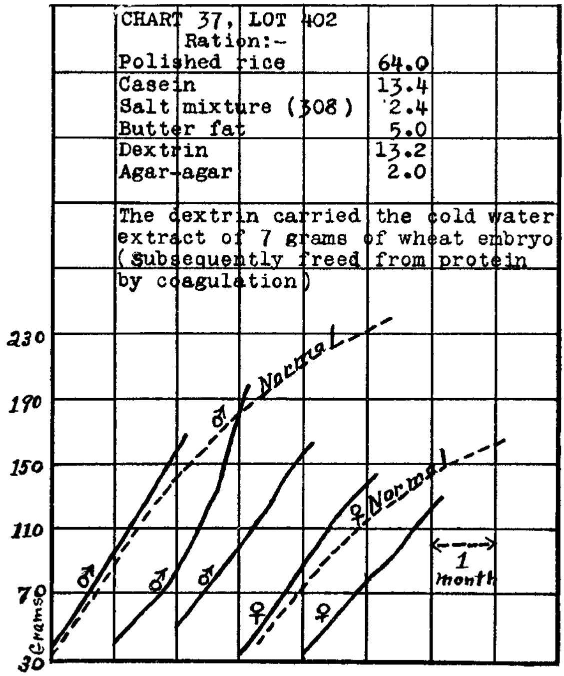

CHART 37. Lot 402. These curves show growth somewhat more rapid than the normal expectation, but not so rapid as in the rats in Lot 401, Chart 36. These rats received the same ration as Lot 401, but with the cold water extract of only 7 gm. of wheat embryo per 100 of ration.

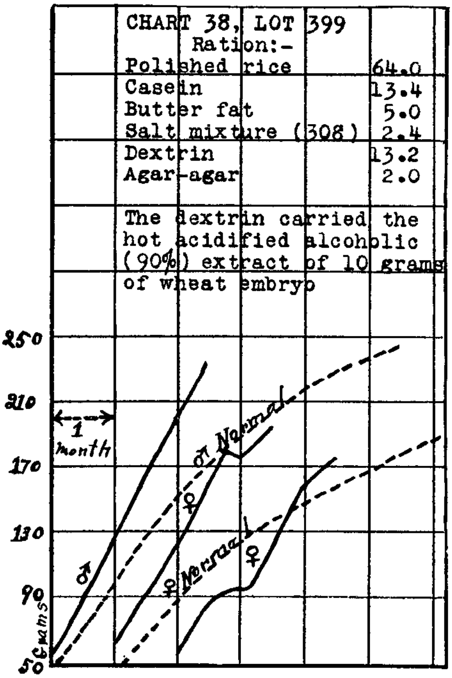

CHART 38. Lot 399. These curves illustrate in a striking manner the stimulating action on growth of a small amount of the material extracted from wheat embryo by hot acidified alcohol. This ration without the addition of an unknown accessory soluble in water and in alcohol does not support growth. (Compare Lot 324.) The extract obtained by boiling 10 gm. of wheat embryo with hot acidified alcohol was added to each 100 gm. of ration with the result that growth proceeded much faster than the normal rate.

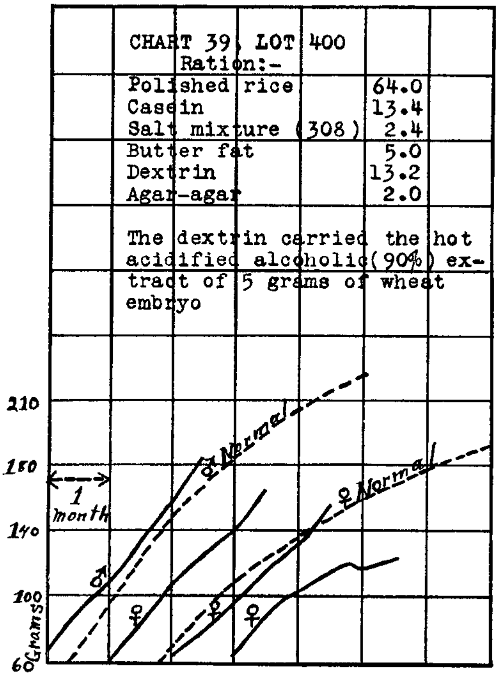

Chart 39. Lot 400. These curves should be compared with Lot 399, Chart 38. The rats in this lot received the hot alcoholic extract of only 5 gm. of wheat embryo per 100 of ration, and their rate of growth was distinctly slower. While very small amounts of the water- and alcohol-soluble accessory necessary for growth may suffice, it is evident from these curves that growth, at least within certain limits, is dependent on the amount present.

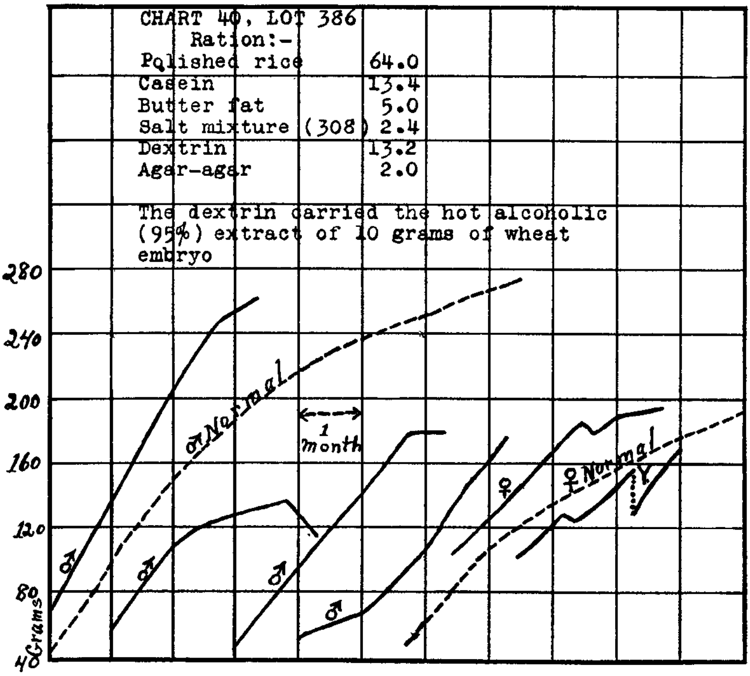

CHART 40.. Lot 386. These curves should be compared with Lots 399 and 400. They received the plain alcoholic (95 per cent) extract of 10 gm. of wheat embryo per 100 of ration. The ration without the addition of the unknown accessory soluble in water and in alcohol would not have supported growth. (Compare Lot 324, Chart 7.)

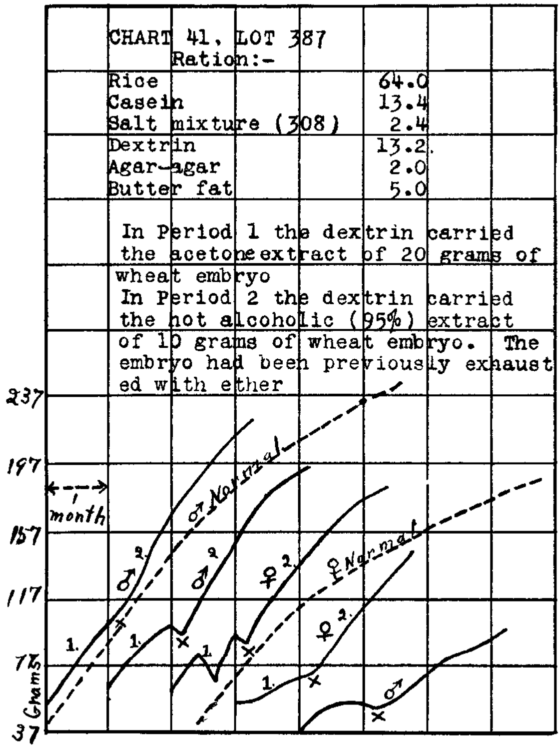

CHART 41. Lot 387. These curves show plainly that the unknown accessory essential for growth is soluble to some extent in acetone, for the addition of a hot acetone extract of 20 gm. of wheat embryo to 100 gm. of a ration which would not itself support growth, induced growth at a good rate during five weeks. The behavior of these animals led us to believe that they were growing on about the minimum amount possible, which assumption is strengthened by the response with more rapid growth, to the substitution of an alcoholic extract of half as much wheat embryo, for the acetone extract. Previous to the alcoholic extraction the wheat embryo had been exhausted with ether in a continuous extraction apparatus. The alcoholic extract (also by continuous extraction) was in no degree less potent in promoting growth than was alcoholic extract from unextracted wheat embryo. It is evident, therefore, that this accessory (water- and alcohol-soluble) is not soluble in ether.

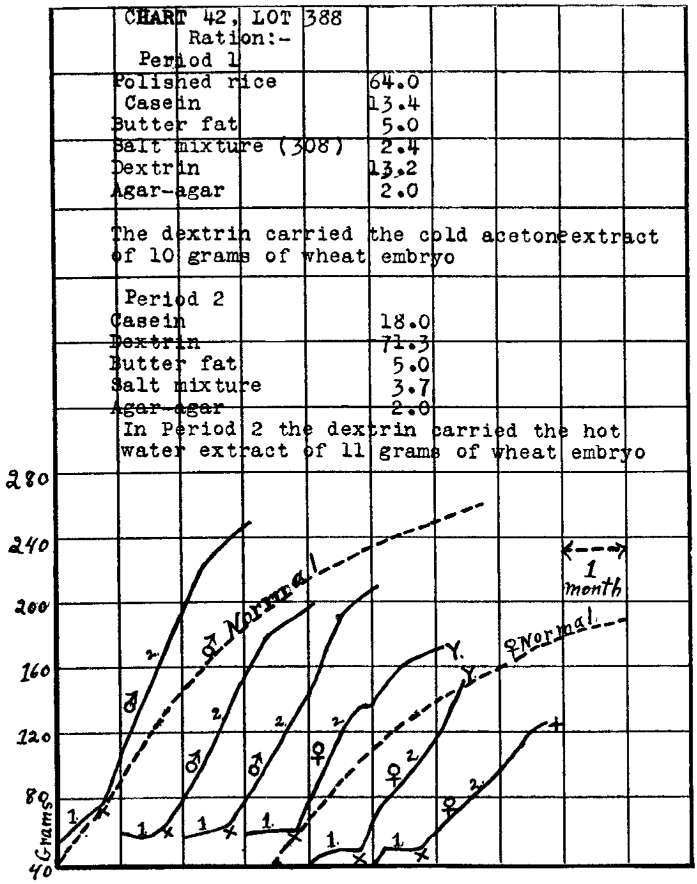

CHART 42. Lot 388. These curves should be compared with Lot 387, Chart 41. These rats received the acetone extract of 10 gm. of wheat embryo per 100 of ration (Period 1) and its influence in promoting growth was slight. In Period 2 the ration was made up of purified foodstuffs, and was one which without the addition of this accessory, would not support growth. The rats responded at once with excellent growth on the ration when the hot water extract of 11 gm. of wheat embryo per 100 of ration was added.