DIETARY REQUIREMENTS FOR REPRODUCTION.

II. THE EXISTENCE OF A SPECIFIC VITAMIN FOR REPRODUCTION.*

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(Received for publication, October 20, 1923.)

During 1922, at the time the experiments reported in this paper were nearing completion, Evans and Bishop announced in Science "The existence of a hitherto unrecognized dietary factor essential for reproduction." They state (1):

"The fact has been abundantly demonstrated that rats may be reared on a dietary regime consisting of 'purified' protein, fat and cabohydrate to which an appropriate salt mixture and adequate doses of the growth vitamines Fat Soluble A and Water Soluble B have been added. We have employed a ration of casein (18), cornstarch (54) and lard (15) to which butterfat (9) and salts (4) are added, the animals receiving separately and daily .4 gram each of dried whole yeast.

"Such animals are sterile. They are chiefly so in the first generation and wholly so in the next succeeding one..... ...

"Natural foodstuffs contain a substance, X, which prevents such a sterility or which cures the disorder occasioned by the purified dietary regime. We have thus been able to witness a comparatively sudden restoration of fertility to animals of proven sterility, and whose controls continued sterile, by the administration of fresh green leaves of lettuce. Even the dried leaves of alfalfa appear to possess a similar potency."

Several papers by the same authors (2, 3) have since appeared "On the relations between fertility and nutrition," showing their failures with reproduction on synthetic rations and the restoration of fertility accompanied by successful rearing of succeeding genera-

*Research paper No. 3, Journal Series, University of Arkansas.

A preliminary report of this paper has been presented before the Biochemical Division of the American Chemical Society at Milwaukee, September 13, 1923, under the title "Suggestive evidence for the existence of a specific vitamin for reproduction." The accumulation of more experimental data justified the omission of the words "suggestive evidence."

tions by the introduction of at least one natural foodstuff to their diets of purified food substances. They find this hitherto unrecognized dietary factor essential for reproduction to be present not only in green lettuce and dried alfalfa leaves, but also in wheat, oats, meat, and, to a lesser extent, in milk fat.

Evans and Bishop's work[1] is in harmony with the author's findings that lactalbumin, replacing casein in the ration, does not induce fertility; neither is fertility restored by increasing the casein content to 50 per cent.

Since there is no experimental evidence available, in terms of specific dietary units, in connection with the requirement of water-soluble B vitamin for the physiological function of reproduction as contrasted with growth, it may be inferred that the failure to secure success with reproduction on purified food substances may be due to an insufficient intake of that vitamin, rather than to the deficiency of an additional unidentified dietary factor. Nelson, Heller, and Fulmer state in a recent publication (5) that even upon 8 per cent air-dried yeast, which they found to be four times the amount necessary for normal growth to maturity, the rearing of young is not entirely successful, and that failure in reproduction cannot be ascribed solely to the level of vitamin B. These investigators, however, report success with yeast from the standpoint of reproduction when the level is increased to 30 to 45 per cent. Three generations have been obtained on these high yeast levels, although the young of the second and third generations have not grown normally. They ascribe the failure of Evans and Bishop to secure fertility, employing 25 per cent yeast, to the high fat content of their diets. Such an explanation is hardly reasonable since Evans and Bishop first begin to secure success in reproduction with milk fat when they increase it to 24 per cent of the ration. Since yeast has been shown to be abundant in the water-soluble B vitamin, why is it not possible that, when it is fed at such a high level as 30 per cent, it may introduce some of the as yet unrecognized syndrome that is essential for reproduction?

Is it possible that the failure in reproduction on synthetic diets may be ascribed to neglect to make proper provisions for the mineral element complex? That a sufficient amount of the inorganic elements is added when 3.7 per cent of a salt mixture is

[1] Evans and Bishop (4), p. 273.

employed in a ration is evident from the work of Nelson, Heller, and Fulmer. On the contrary, increasing their salt mixture 185 to 5 per cent, they find their animals without exception to be sterile, although they grow at the normal rate to maturity. Purified milk fat is certainly free from any significant amount of mineral elements, yet, keeping the amount of the salt mixture in their basal ration constant, but increasing the milk fat to 24 per cent, sterile females become pregnant and manifest a certain degree of success in rearing their young.

Evans and Bishop's findings[2] agree with those of Mattill and coworkers and with those of the author, that a preponderance of cod liver oil, in the presence of adequate concentrations of all the other recognized dietary essentials, does not produce fertility and success in rearing young.

The experimental data which convinced the author that there must exist an as yet unrecognized dietary factor essential for reproduction are summarized in the charts.

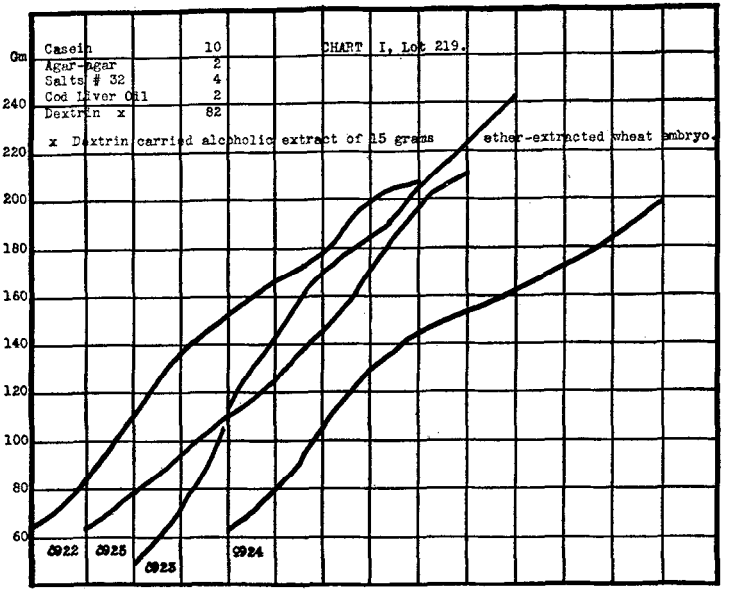

CHART I, LOT 219. This chart clearly shows that, employing an alcoholic extract of 15 gm. of ether-extracted wheat embryo as a source of water-soluble B vitamin, very good growth is obtained.

[2] Evans and Bishop (3), pp. 202–231; (4), pp. 233–273.

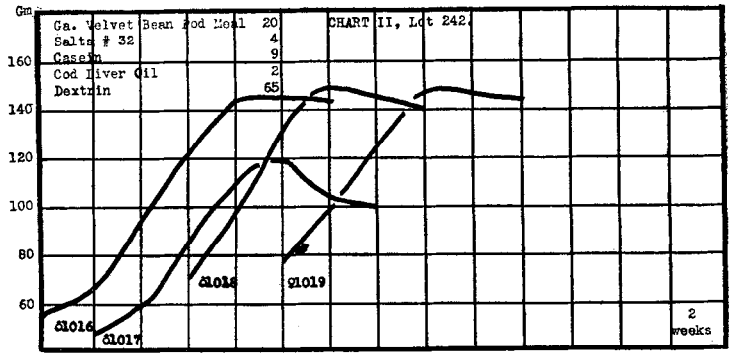

CHART II, LOT 242. 20 per cent of the Georgia velvet bean pod meal as a source of water-soluble B allows fairly good growth to take place during the first 6 to 8 weeks of experimentation, but the growth is followed by rapid decline in body weight.

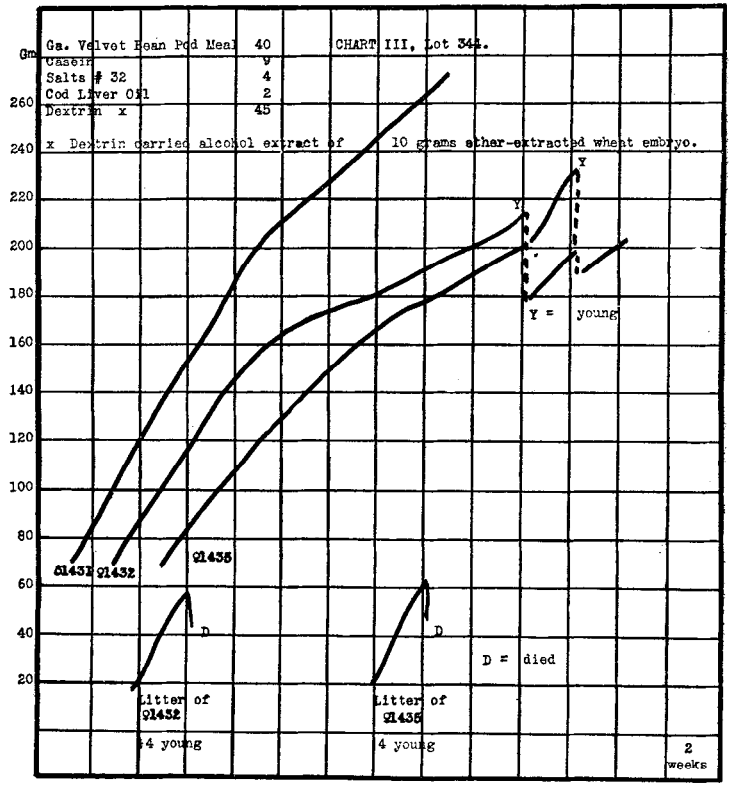

CHART III, LOT 344. This chart shows that fertility is secured when 40 per cent of the ration is composed of velvet bean pod meal, in which diet is

incorporated an alcoholic extract of 10 gm. of wheat embryo. Female 1432 had six young, and was allowed four, weighing 20 gm., to rear. The young reached a maximum weight of 56 gm., but on the 15th day there was a rapid decline of body weight followed by death. Female 1435 had five young, and was given four, weighing 20 gm., to rear. The young reached a maximum weight of 63 gm., but died on the 15th day.

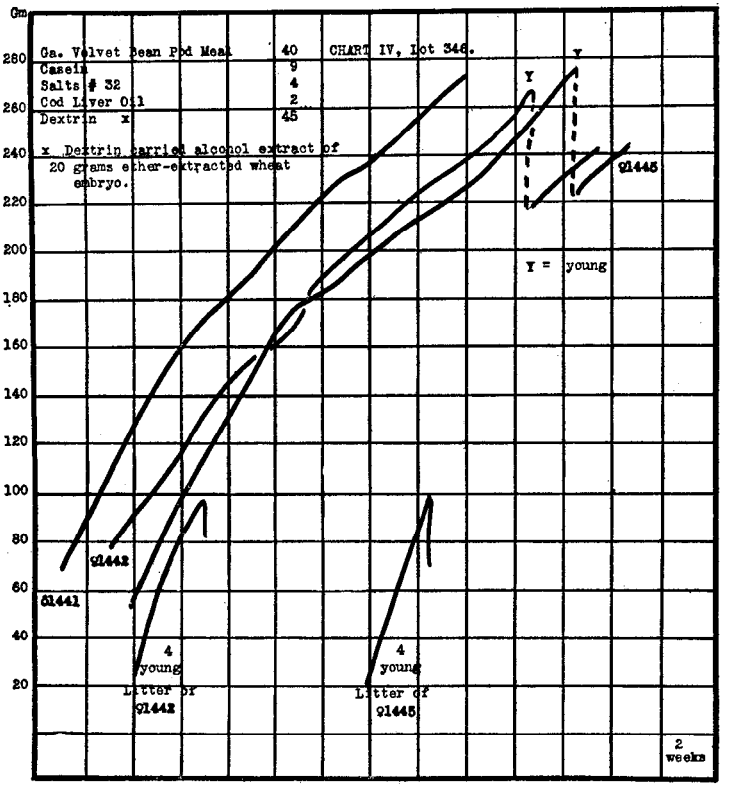

CHART IV, LOT 346. This chart clearly indicates the increase of success in rearing of young of two females, due to the increase of the water-soluble B vitamin from an alcoholic extract of 10 to 20 gm. of wheat embryo. Female 1442 had eleven young and reared four for 18 days to a maximum weight of 94 gm. On the 19th day the young began to lose weight rapidly, and rearing was abandoned. Female 1443 had five young, and four, weighing 24 gm. at birth, were reared to a maximum weight of 93 gm. in 18 days, after which period rapid decline in weight of young necessitated the discontinuance of further rearing.

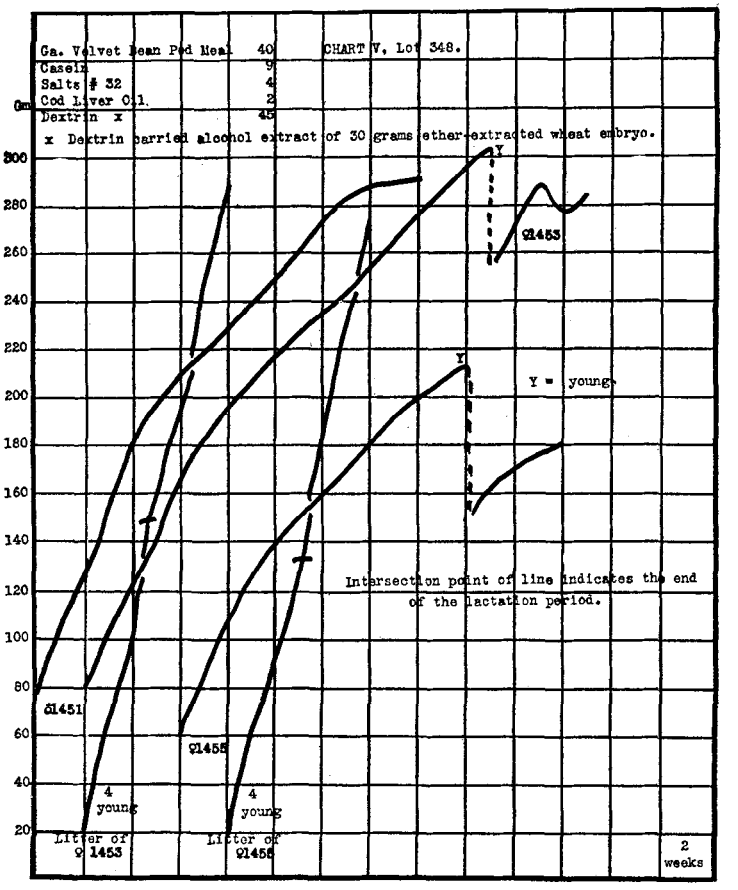

CHART V, LOT 348. Increasing the concentration of the water-soluble B vitamin to an alcoholic extract of 30 gm. of ether-extracted wheat embryo resulted in a further improvement in success of rearing of young. Female 1453 had ten young weighing 50 gm., and was given four, weighing 20 gm., to rear. At the end of 23 days, which marks the termination of the lactation period, the four young collectively weighed 150 gm., and at the end of 5 weeks, weighed 289 gm. Female 1455 had eleven young, weighing 50 gm., and was allowed four, weighing 19 gm., to rear. At the end of the lactation period the group reached a weight of 128 gm., and at the end of 5 weeks weighed 286 gm.

Charts III, IV, and V show the rôle water-soluble B vitamin plays in the matter of rearing of young, but also indicate that the fertility which may occur in the first place must be attributed to something contained in the velvet bean pod meal which is absent in rations composed of purified food substances. This matter will be elaborated on under the heading "Discussion."

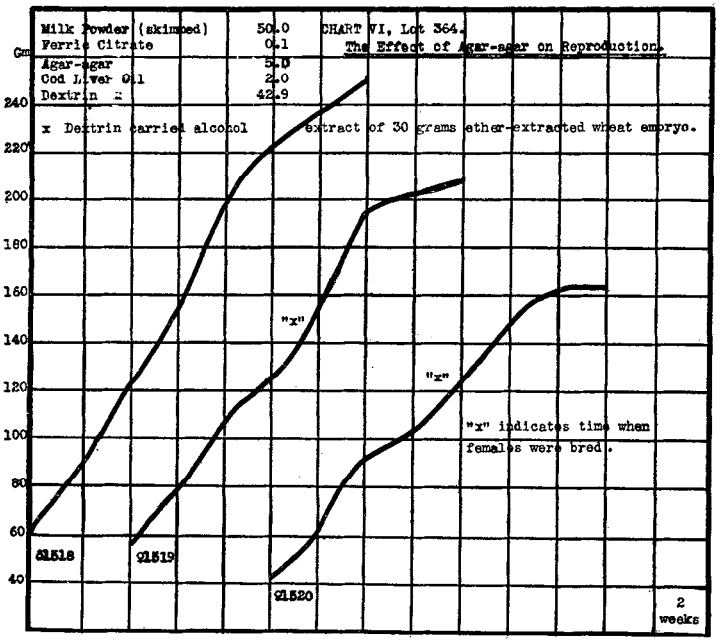

CHART VI, LOT 364. The presence of 5 per cent of agar-agar in a milk ration, fortified with an abundance of fat-soluble A and water-soluble B vitamins, does not induce fertility.

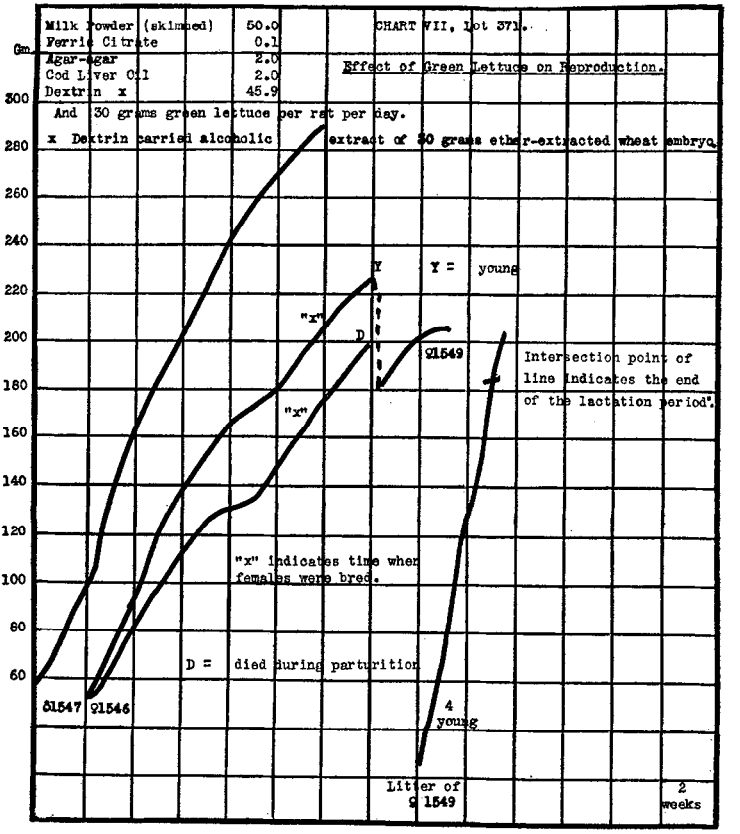

CHART VII, LOT 371. The addition of 30 gm. of fresh lettuce daily per rat produced fertility. Female 1549 gave birth to six young and was allowed four, weighing 24 gm., to rear. At the end of the lactation period they collectively weighed 180 gm., which may be considered as normal rearing. Female 1546, however, died during parturition, showing hemorrhage in womb.

In the following three rations single additions of natural foodstuffs, in the form of polished rice, rolled oats, and yellow corn, were made to milk protein diets, on which previous failure in reproduction was reported. The success in fertility and rearing of young is indicated in Charts VIII, IX, X, and XI.

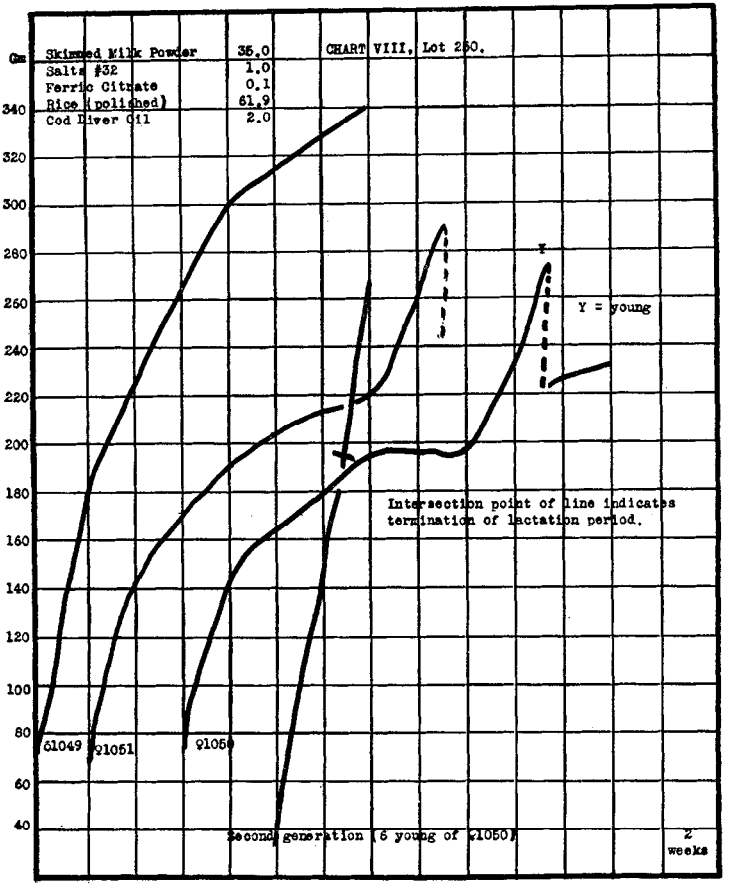

CHART VIII, LOT 250. Since polished rice is reported to have only traces of water-soluble B vitamin, the 35 per cent of skimmed milk powder furnished practically all of that complex in this ration. Female 1051 gave birth to three young which were disposed of on the 3rd day. Female 1050 gave birth to six young which were successfully reared, and on the 15th day weighed 152 gm. On the 21st day one of the young died, the five remaining young then weighing 192 gm. On the 23rd day another of the young died, leaving four, which collectively weighed 192 gm. on the 24th day. On the 31st day after birth the remaining four young collectively weighed 263 gm. The remarkable result in this experment is that on such a low concentration of water-soluble B fertility was secured with two females and successful rearing of four young at a normal rate.

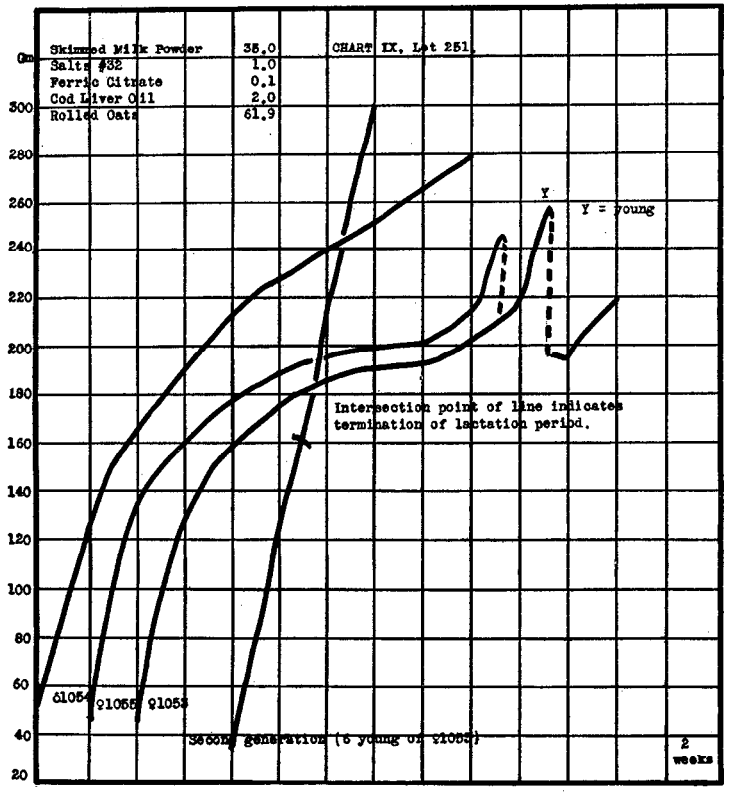

CHART IX, LOT 251. The natural foodstuff incorporated in this ration is rolled oats. Fertility with two females was secured and considerable success was obtained in rearing of young with one. Female 1055 gave birth to six young which were disposed of on the 2nd day. Female 1053 gave birth to six young, weighing 30 gm. At the end of the lactation period they weighed 160 gm.; on the 33rd day, 216 gm.; and on the 41st day, 300 gm.

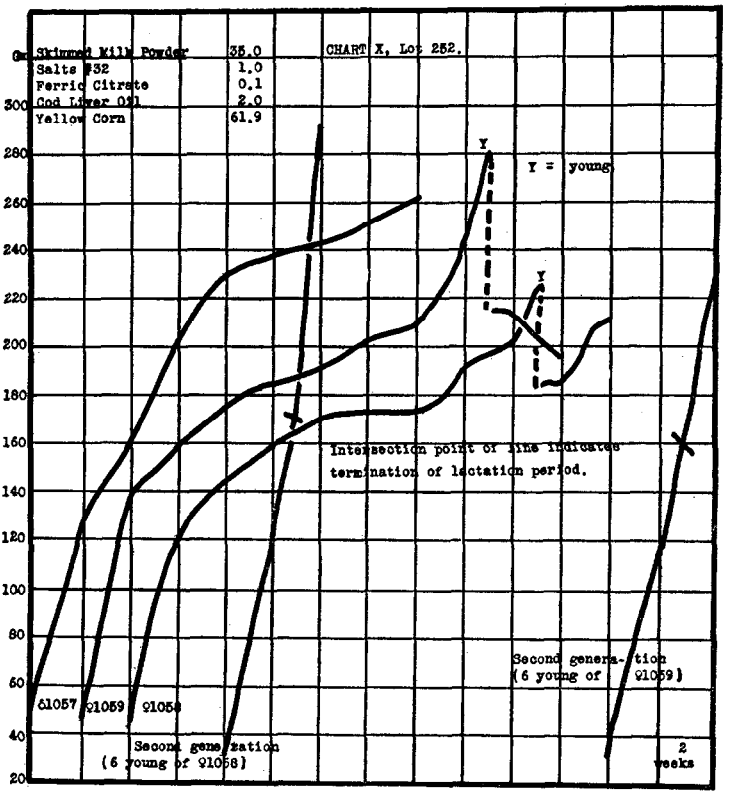

CHART X, LOT 252. Yellow corn is the seed introduced in this diet. Female 1058 gave birth to six young, weighing 30 gm., which, at the end of the lactation period, weighed 172 gm., and at the end of 31 days, 290 gm. Female 1059 gave birth fo six young, weighing 33 gm., which, at the termination of the lactation period, weighed 160 gm., and at the end of 31 days, 232 gm.

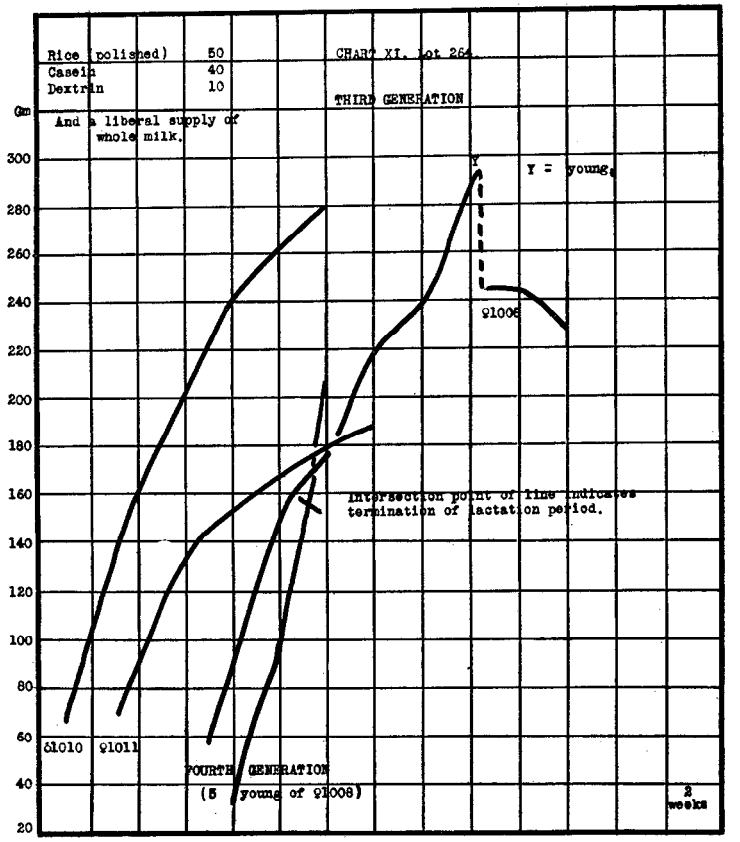

CHART XI, LOT 264. Although it has been shown that fortifying milk diets with protein, in the presence of liberal amounts of digestible carbohydrates, produces no success in reproduction, the addition of polished rice to such a dietary regime resulted in four successful generations.

DISCUSSION.

That the seed of the Georgia velvet bean is very deficient in water-soluble B vitamin has been demonstrated by Sure and Read (6). Unpublished work of J. W. Read on the biological analysis of Georgia velvet bean pod meal shows that 40 per cent of the pod meal as a source of water-soluble B allows as much growth to take place as that secured by the addition of an alcoholic extract of

15 gm. of wheat embryo. 20 per cent, however, of velvet bean pod meal as a source of that vitamin permits some growth during the first 6 to 10 weeks, but this is followed by rapid decline of body weight. A ration containing 40 per cent velvet bean pod meal and an alcoholic solution of 10 gm. of wheat embryo would be equivalent to a diet containing an alcoholic extract of 25 gm. of wheat embryo. On such a ration fertility was always secured, yet no success of rearing young resulted on a ration containing alcoholic extracts of 40 gm. of wheat embryo in the presence of such an excellent concentration of amino-acids as that furnished by the proteins, casein, lactalbumin, and gelatin, and the amino-acids, cystine, tyrosine, and tryptophane. (See Chart II of the preceding paper.) The inference made from such experiments is that the fertility and partial success of rearing of young on the velvet bean pod meal rations must be attributed to a dietary factor other than water-soluble B. Since the mineral elements, fat-soluble A, and the antirachitic factor were amply provided for in those rations (the rat being able to synthesize the water-soluble C vitamin (7)) the success of reproduction must be ascribed to a new unidentified factor which influences reproduction.

Lot 250, Chart VIII, discloses an interesting fact. In this ration no addition of water-soluble B vitamin was made, and polished rice, which is reported as being practically absent in that complex was the natural foodstuff introduced; therefore, the only source of that vitamin is the 35 per cent of skimmed milk powder. That 24 per cent of skimmed milk powder allows considerable growth to take place has been shown by McCollum and Davis in their paper, "The influence of the plane of protein intake on growth" (8), so it is not at all surprizing that the 35 per cent of skimmed milk powder in this ration furnished considerable of the water-soluble B complex. The addition of an alcoholic extract of 30 gm. of wheat embryo, however, to a 50 per cent skimmed milk powder ration, in the presence of an abundance of all the other dietary factors, produced no fertility (see Chart VI, Lot 239, preceding paper). Therefore, the success with fertility and partial success in rearing of young on this ration must be ascribed to some new factor in polished rice that is essential for reproduction.

In the preceding paper experimental evidence has been presented showing the inability to secure fertility by improving the protein

moiety of the milk diets both as to quantity and quality of amino-acids; yet, on a ration containing polished rice, 50, casein (commercial), 40, dextrin, 10, and a liberal supply of whole milk, four successful generations were secured, although the only foreign material added was the polished rice. The fourth generation is growing normally just as the former three generations did. I repeat and emphasize then that polished rice must contain some as yet unrecognized dietary factor that is essential for reproduction.

As shown in Charts IX and X, rolled oats and yellow corn play a similar rôle to polished rice in reproduction.

Effect of Agar-Agar on Reproduction.

Recently Mitchell (9) reported that she secured success with reproduction by incorporating 5 per cent of purified agar-agar in the ration, replacing an equivalent amount of starch. The data indicated in Chart VI do not bear out her contention. The ration contained protein of excellent quality, a suitable salt mixture derived from milk, iron citrate to make up for the deficiency of iron in milk, a liberal supply of fat-soluble A, the antirachitic factor, and water-soluble B. 5 per cent of agar-agar was introduced in the diet, yet no fertility resulted.

Effect of Green Lettuce on Reproduction.

Green lettuce, as claimed by Evans and Bishop, produces a pronounced effect on reproduction. It is unfortunate that more animals were not employed in this experiment. One female became pregnant but died during parturition, but the other reared four young, during the lactation period, at a normal rate.

While it is true that an insufficient number of females were employed in the experiments which had the addition of a single natural foodstuff, the data presented clearly show that fertility always resulted and that in each ration at least one mother manifested a significant degree of success in rearing her young, which seldom happened in any of the experiments in which synthetic diets of purified food stubstances were employed.

The data of this communication also bring out incidentally the rôle played by water-soluble B vitamin in reproduction. Increasing the level of the concentration of that vitamin from an

alcoholic extract of 10 to 30 gm. of ether-extracted wheat embryo, litters of four young of the mothers on those rations have been reared from a maximum weight of 60 to 150 gm. during the lactation period. On the highest level of water-soluble B intake the young were able to help themselves to food 3 weeks after birth and grew up to be large rats weighing over 70 gm. each at the age of 6 weeks.

Stability of the Reproductive Dietary Complex.

The Georgia velvet bean pod meal, with which significant success in reproduction[3] was secured on the higher planes of water-soluble B vitamin intake, was autoclaved for 1½ hours at 15 to 18 pounds pressure, because it was previously found that the velvet bean seed is toxic to rats when fed in the raw condition (6). It would, therefore, seem that this new dietary complex which plays such a significant rôle in reproduction is relatively thermostable.

Nomenclature of the Reproductive Factor.

It is now generally conceded that fat-soluble A signifies the antixerophthalmic vitamin, water-soluble B represents the anti-beri-beri and growth-promoting complex, and that water-soluble C indicates the antiscorbutic vitamin.

[3] In this connection the author would like to correct an erroneous conclusion drawn with respect to the rôle of cystine in reproduction in a previous publication (10). The data presented were of a preliminary character and a number of experiments were conducted to secure more evidence on this point. The technique then adopted was to add cystine during the second lactation period of the females, and note the degree of success of rearing of young, during that period when cystine was incorporated in the diets, as compared with that secured during the first lactation period when cystine was absent in the rations, and it seemed at that time that a small but definite improvement in rearing of young was apparent, which was attributed to the addition of cystine. Further attempts along that line do not bear out such results, and attention is called to the danger in employing such technique. A female rat may be more vigorous when it gets a little older and manifest some improvement in rearing its second litter without any dietary changes and no significance should be assigned to small differences in the degree of success of rearing young. The only safe method is to use controls, make specific additions to other experiments, and note the effect of such changes on fertility and rearing of young during the first lactation period, or several lactation periods if possible.

In 1921, Funk and Dubin (11) reported the separation from vitamin B of a substance which stimulates the growth of yeast and streptococcus, which they provisionally call "D." At the present time there is considerable controversy as to whether there actually exists a substance necessary for the growth of yeast. The work of Nelson, Fulmer, and associates seems to indicate that it is possible to grow yeast on a medium wholly synthetic in origin (12). MacDonald (13) maintains that the growth-stimulating substance for yeast which Funk and Dubin have provisionally named "D" cannot logically be placed in the classification of the vitamins, and is not an indispensable nutrient principle for yeast, since it is synthesized during the slow proliferation of yeast cells in a medium of purified nutrients.

In 1922 McCollum, Simmonds, Becker, and Shipley (14) produced evidence showing that oxidation destroys the fat-soluble A vitamin in cod liver oil without destroying another substance which plays an important rôle in bone growth. Cocoanut oil was shown to be lacking in fat-soluble A, since it will neither prevent nor cure xerophthalmia. This oil, on the other hand, contains a substance which stimulates the deposition of calcium salts in rickets in a manner similar to cod liver oil.

Since the experimental evidence of McCollum and coworkers on the separation of the antirachitic from the antixerophthalmic vitamin is clear and convincing, it is proposed that the term "D" be employed to represent the antirachitic factor, and since the term "vitamins" has hitherto been used to designate substances the chemical nature of which has not yet been disclosed, but which have specific physiological functions, the author feels that he is not premature in applying the term vitamin to this dietary complex essential for reproduction. Therefore, if the evidence presented by the author and that published by Evans and Bishop on the new dietary complex essential for reproduction is accepted as fully convincing, it is suggested that this factor be termed vitamin "E,"[4] instead of substance "X," as proposed by Evans and Bishop.

[4] It is, of course, not impossible that this dietary complex is a mineral, the physiological rôle of which has not yet been investigated.

SUMMARY.

1. From experiments initiated 4½ years ago and recently completed, the results of which are embodied in this and the preceding paper, I conclude that, in addition to the antixerophthalmic, antirachitic, antiberi-beri, and antiscorbutic vitamins,[5] there exists another hitherto unrecognized vitamin, that is essential for reproduction, which becomes evident only in breeding experiments where rations composed of purified food substances are employed.

2. This reproductive vitamin has been found to occur in Georgia velvet bean pod meal, polished rice, yellow corn, and rolled oats.

3. If the term "D" be accepted for the designation of the antirachitic factor, it is proposed that the term "E" be adopted to represent this new dietary factor that influences reproduction.

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[5] In autoclaving the velvet bean pod meal for 1½ hours at 15 to 18 lbs. pressure the antiscorbutic vitamin certainly must have been destroyed, and still, with such treated pod meal, fertility and a significant success in rearing of young were possible. From such results it would seem that failure in reproduction should not be ascribed to water-soluble C, or the antiscorbutic vitamin.