SPARING ACTION OF FAT ON THE ANTINEURITIC VITAMIN B.*†

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About 3 years ago, in an attempt to use more rigorously purified diets than had hitherto been employed in nutrition research, this laboratory began to employ dietary mixtures consisting essentially of casein and sucrose with the added salts and vitamins. Our experiments yielded some surprises which led us to wonder whether a new dietary factor was essential for life. Prominent in the phenomena brought to light was the favorable effect secured by the addition of fats to the diet. It was true that other substances (liver, lettuce, egg yolk) also conferred this improvement, substances which had in common the possession of considerable amounts of the antineuritic vitamin B. It was, in fact, possible to show that the favorable effect of these other substances was in proportion to their content in antineuritic vitamin B. Thus, though we thought we had employed an adequate amount of vitamin B,[1] this experience finally led us to the conviction that deficiency in the antineuritic vitamin B was an actual and main trouble with the sucrose-casein diet. This was confirmed by the

* Due to the confusion regarding the nomenclature of the water-soluble B vitamins, we have carefully refrained from the sole use of letters. For the heat-labile factor of yeast, we have kept the original nomenclature, the antineuritic vitamin B. For the heat-stable factor of yeast often referred to by various workers as P-P, F, and G, we have employed the term heat-stable water-soluble vitamin.

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[1] 700 mg. daily of a whole dried bakers' yeast (Fleischmann) had been employed and an increase to 1 gm. daily was without appreciable effect, so that we were led off the "scent" of a vitamin B deficiency.

TABLE I.

Composition of Diets Employed in This Study.

	No fat.			10 per cent fat.						50 per cent fat.	51 per cent fat.			54 per cent fat.
Diet No.	540	542	414	550	415	563	564	565	551	559	571	572	573	579
Casein (L-3)*	25.0	20.0	19.0	20.0	20.0	23.0	23.0	23.0	27.0	36.0	38.0	38.0	38.0	40.0
Autoclaved yeast.†		10.0	10.0	10.0	10.0	10.0	10.0	10.0		10.0	10.0	10.0	10.0	
Sugar (commercial)	75.0	70.0		59.0		56.0	56.0	56.0	59.0					
Corn-starch (dextrinized)‡			67.0		59.0									
Salts 185.§	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Lard				10.0	10.0				10.0	50.0				53.0
Crisco.‖						10.0					55.0			
Wesson oil.¶							10.0					55.0		
"Synthetic" Wesson oil								10.0					55.0	

All diets were supplemented with 2 drops of cod liver oil (Patch) daily.

* *Casein (L-3).*—Commercial casein (Golden State Milk Products Company), which had been precipitated from milk with hydrochloric acid, dried, and delivered to us in sacks, was extracted in wooden tubs with acidulated water (40 cc. of glacial acetic acid to 40 liters of filtered tap water). The acidulated water was siphoned off and replaced twice daily for 6 days. On the 7th day the casein was washed twice with distilled water replacing the acidulated tap water. The water was thoroughly drained from the casein and the casein washed once with 50 to 80 per cent alcohol and once with 85 to 95 per cent alcohol followed by one ether treatment. It was then spread in pans and the ether allowed to dissipate at room temperature under a fan.

† *Autoclaved Yeast.*—Whole dried yeast (bakers') was generously supplied us by the Fleischmann Company of New York. It was spread in pans to a depth of less than 1 inch and autoclaved for 5 hours at 18 to 20 pounds pressure. This yeast was added as the source of the thermostable water-soluble factor throughout the experiment. Abundant trials showed it to be almost entirely, if not absolutely, free from antineuritic vitamin B. A whole, dried brewers' yeast of high potency was used as the source of the thermolabile or antineuritic factor B. It was generously furnished by the Vitamin Food Company through Dr. Edward A. Rumley.

‡ Dextrinized corn-starch was prepared by pouring cold starch paste into boiling water, boiling for 5 minutes, spreading in thin layers in aluminum pans, and drying at 110–120°.

§ *Salt Mixture 185, after McCollum.*—Sodium chloride 51.0, crystals of magnesium sulfate 159.6, monobasic sodium phosphate 104.1, monobasic calcium phosphate 162.0, dibasic potassium phosphate 286.2, ferric citrate 35.4, and calcium lactate 390.0.

‖ Crisco, the well known name for a partially hydrogenated cottonseed oil. It is always hydrogenated to a melting point of about 38°.

¶ Wesson oil, the trade name for the cottonseed oil marketed for table use. It is a "winterized" oil, which has been chilled to remove the "stearine." The product is marketed by the Southern Cotton Oil Company, and represents a carefully standardized product which has been thoroughly refined and purified.

improvement invariably secured by the administration of all concentrated sources of antineuritic vitamin B such as wheat germ, brewers' yeast, rice polish extract, etc.

A novel problem is presented by the benefit due to fats, substances not hitherto thought to be seriously contaminated with the water-soluble vitamins. The preliminary data convinced us that fat was in some obscure way related to the body's needs in the antineuritic vitamin, and a note[2] was published to the effect that the presence of fats always decreased the amount of antineuritic vitamin B necessary for any definite amount of growth secured with a fat-free diet. We ventured to term this phenomenon "the sparing action of fat on the antineuritic vitamin."

Methods.

A systematic effort to study this phenomenon was now made. Litters of female rats 21 days old were selected and sisters distributed as far as possible to each of several dietary groups. All diets used carried the heat-stable, water-soluble factor free from antineuritic vitamin B, the heat-labile factor, as autoclaved yeast, so that we were working with only one factor as a variable. Throughout the course of the experiment the animals were maintained in groups of three in cages with wire mesh floors. The feeding boxes for administering supplementary food (yeast doses, etc.) were also equipped with wire mesh floors. The animals were watched daily for breakdown of the vaginal closing membrane and thereafter for estrous changes in the vaginal smear indicative of ovulation. (The small circles interrupting the growth curves in our charts indicate times of ovulation.) The diets employed are given in Table I. Special effort was made to keep the nutritive ratio approximately 1:3 so that even on the highest fat diets animals could not suffer from protein starvation.

Growth on Sucrose-Casein Diet and Improvement Due to Coincident Presence of Fat with Any Level of Antineuritic Vitamin B.

Fig. 1 shows the influence of three different fats added to the basal diet at levels of 10 and 50 per cent *in the absence of antineuritic vitamin B.* There is very little added growth due to

[2] Evans, H. M., and Lepkovsky, S., *Science*, **68**, 298 (1928).

10 per cent fat, but at the 50 per cent level of fat a very considerable growth takes place. *We may note that with 50 per cent of lard, animals have attained a weight of 120 to 160 gm. and are still alive and in fairly good condition at the end of 6 months.* With 51 per cent Crisco and Wesson oil results are not so uniform, but two animals in each group are still alive after being on the diet for $4\frac{1}{2}$ months. The growth in these groups is rather irregular, one animal in each group attaining a weight well over 100 gm. and another attaining a weight of close to 100 gm. One animal in each group died soon after the initiation of the experiment.

Fig. 2 represents the same oils at the same levels, the only difference being that 50 mg. of brewers' yeast were added as a low source of antineuritic vitamin B. In this series the increment of growth at the level of 10 per cent of fat is very evident over that shown by the animals in the group receiving no fat. It will be recalled from Fig. 1 that 10 per cent of fat made very little difference in the absence of antineuritic vitamin B, but it makes a considerable difference in the presence of 50 mg. of brewers' yeast. These animals are now 5 months of age and only recently have begun to decline in weight. At the high level of fat (50 to 51 per cent) the improvement is very much beyond that due to 10 per cent of fat in the case of all fats studied; these animals have only recently reached a plateau of growth at approximately 160 gm. of body weight.

Fig. 3 presents the performance at the same levels of fat with Crisco and Wesson oil when 200 mg. and 800 mg. respectively of brewers' yeast are added to supply the antineuritic vitamin B. In these cases it is seen that the maximum improvement due to fat is shown at the 10 per cent level.[3] The difference between the 200 and 800 mg. levels of yeast is shown only in the complete absence of fat. The data presented may be briefly summarized as follows: In the absence of antineuritic vitamin B it takes relatively more fat to bring into evidence the sparing action of fat.

[3] For some unknown reason the 50 per cent Wesson oil actually brings about a poorer performance than 10 per cent Wesson oil. We suspect that the ingestion of such a large amount of the oil exerted a deleterious effect, for there was also a slight diarrhea, which persisted during the 1st month of the experiment. There is no significant difference between the high and lower levels of Crisco.

Little sparing action is evident when 10 per cent of fat is added but with 50 per cent of fat the sparing action is marked. At the low level of antineuritic vitamin B, a marked effect of 10 per cent of fat is evident, further marked improvement taking place at its high level; whereas at the higher levels of yeast little (10 per cent) fat brings about a maximum effect.

Fig. 4 shows that fat acts in its capacity as a sparer of antineuritic vitamin B when dextrinized corn-starch is used as a source of energy in place of sucrose. In this figure it is clearly seen that superior growth is obtained when starch replaces sugar, but that if a portion of the starch is replaced with 10 per cent of fat there is further added growth. This is evident in the presence of 50 mg. of brewers' yeast as well as in the entire absence of yeast.

Is Fat Contaminated with the Antineuritic Vitamin B?

The question, of course, arises as to whether the sparing action of fat is due to the fat *per se* or to antineuritic vitamin B with which the fat may be contaminated. Attempts to extract antineuritic vitamin B from one of these fats were without success.

Wesson oil was dissolved in 5 times its volume of ether and shaken with 4 times its volume of 25 per cent alcohol containing 1 cc. of concentrated sulfuric acid per liter. This was repeated three times. The 25 per cent alcoholic extract was concentrated in a vacuum and neutralized and fed at a level equivalent to 5 gm. of the original fat daily to animals receiving sufficient of the thermostable water-soluble vitamin as autoclaved yeast, but only enough antineuritic vitamin B so that they plateaued at about 90 to 95 gm. in weight. No response was obtained.

Abundant experience in this laboratory under the same conditions has convinced us that should the extracts have contained any antineuritic vitamin B, an immediate response would have been obtained.

Another attempt was made by dissolving 500 cc. of Wesson oil in glacial acetic acid with enough ether added to bring about solution of the Wesson oil. The solution was thoroughly shaken with fullers' earth, filtered, and the earth fed to animals receiving a diet low in antineuritic vitamin B as the only limiting factor without results.

To the above filtered solution, water was carefully added until

the Wesson oil separated out. This fat-free acetic acid solution was shaken with fullers' earth, filtered, and the earth fed to animals on a diet low in antineuritic vitamin B without response.

Thus we were unable to shake out antineuritic vitamin B either from the glacial acetic acid solution containing the fat or from the solution after the fat had been separated out.

A third attempt was made when 500 cc. of Wesson oil were dissolved in a mixture of alcohol and ether and shaken with clay. The clay was fed without results.

This attempt was repeated with lard. The lard was dissolved in about 6 times its volume of ether and thoroughly shaken out five times with water containing 1 cc. of glacial acetic acid per liter. The acidulated water was concentrated in a vacuum and mixed with our Diet 542 so that 1 gm. of the diet would carry extract equivalent to 1 gm. of lard. The residual lard was incorporated to form Diet 559, so that 1 gm. of diet would carry only 0.5 gm. of lard. Very little or no response was obtained with the extract, while the extracted fat caused an immediate and marked response in animals receiving a diet low in antineuritic vitamin B. Since our Diet 542 plus the lard extract carried extract equivalent to 100 per cent of lard, and since it brought about no marked response (while only one-half the concentration of the extracted lard brought about an immediate and marked response) it must be admitted that lard carries little if any antineuritic vitamin B as an impurity.

A more rigorous treatment of the oil was now instituted, a saponification, distillation, and reesterification.

Wesson oil was saponified by boiling 1500 gm. of the oil for about $\frac{1}{2}$ hour in 2000 cc. of 95 per cent alcohol in which 400 gm. of KOH were dissolved. The soap solution was acidified with sulfuric acid and the fatty acids were collected and washed with hot water until the washings no longer gave the test for the sulfate ion with barium chloride. Generally five to six washings were sufficient to accomplish this, although as many as ten washings were sometimes necessary.

The fatty acids were then distilled in a vacuum, which was obtained with a mercury-vapor pump, at a temperature of 185–205°. To the distilled fatty acids, redistilled glycerol was added, an excess of 50 per cent over that required by theory being used. These were placed in a flask in an oil bath, the temperature

of which was maintained at about 200°. Dry carbon dioxide was passed through the fatty acid-glycerol mixture to remove the water formed in the process of esterification. The esterification was carried out in this manner for 5 to 6 hours. Generally, 80 to 85 per cent of the fatty acids were esterified during this procedure.

The mixture was then subjected to heating at a temperature of 215–220° for about 4 hours in a high vacuum, obtained with a mercury-vapor pump.

In this manner it was possible to obtain an oil which contained only 2.5 to 4 per cent of free fatty acids as determined by titration and calculated as oleic acid. This oil no longer had the uniform consistency of the original cottonseed oil at room temperature, but a white solid layer always settled out.

In the above procedures there are at least four treatments, any one of which should have removed or destroyed antineuritic vitamin B. The four are, (1) solubility in hot acidulated water, (2) destruction with alkali, (3) loss by distillation—the antineuritic vitamin B not being known to be volatile, (4) very high temperatures employed for a period of from 8 to 10 hours.

As Fig. 5 shows, when no yeast is added as a source of antineuritic vitamin B, a pronounced sparing action is observed in the presence of our so called "synthetic" Wesson oil, which judging by the severity of the treatment it received should be entirely free from the antineuritic vitamin B. Essentially the same results are obtained as with the original Wesson oil. Little or no effect is seen at the 10 per cent level of the oil while a marked response takes place at the 51 per cent level. At this level the response is slightly inferior to that given by the untreated Wesson oil.

When a low level of the antineuritic vitamin B was fed in the form of 50 mg. of brewers' yeast, 10 per cent of the "synthetic" oil brought about a marked response, equal, if not superior, to that brought about by the untreated Wesson oil. A still greater improvement was brought about by raising the "synthetic" oil to 51 per cent. Little or no difference is observed between the two oils at the high level. In the presence of higher levels of the antineuritic vitamin B (200 and 800 mg. of brewers' yeast) the response is the same with the "synthetic" Wesson oil as with the

untreated Wesson oil, the maximum response occurring at the level of 10 per cent of these oils.[4]

It must, therefore, be considered as highly improbable that the sparing action of fat on the antineuritic vitamin B is due to the actual presence of this vitamin as an impurity in the fat. It would appear that this is a phenomenon displayed by fat *per se*. The physiological function which we must thus assign to fats is in the present state of our knowledge inexplicable.

Fat Does Not Appreciably Spare the Heat-Stable Water-Soluble Vitamin.

It became of interest to us to determine whether the sparing action of fat was also exerted on the heat-stable water-soluble factor in autoclaved yeast.

In this work (Fig. 6) adequate antineuritic vitamin B was supplied all groups by an 85 per cent alcoholic extract of rice bran.[5] One group of animals was given none of the heat-stable water-soluble factor in autoclaved yeast, one group 50 mg. daily of autoclaved yeast, and a third group twice this amount. Lard was the only fat used, and it was fed at levels of 10 and 50 per cent. As Fig. 6 indicates, there is no longer the very marked superiority of the groups receiving fat, so evident in the work in which the level of the antineuritic vitamin B was varied.

It must, however, be admitted that the groups receiving the highest level of fat were somewhat improved, both in growth and in the absence of gross symptoms of disease.[6] No effort was made

[4] As the figures show, at the higher levels of the two oils inferior growth was obtained. In both cases diarrhea accompanied this inferior growth but with greater severity in the animals receiving the "synthetic" Wesson oil.

[5] Fresh rice bran was supplied us gratis by Rosenberg Brothers and Company of San Francisco. 1 kilo of bran was shaken several times daily for a period of at least 48 hours with 4 liters of 85 per cent alcohol. It was filtered and concentrated in a vacuum. The total extract generally obtained was about $\frac{3}{4}$ of the original volume used. We, therefore, considered the extract as derived from or equivalent to $\frac{3}{4}$ of the rice bran used. The equivalent of 8 gm. of rice bran was fed daily to supply the antineuritic vitamin B (Extract 8 FB).

[6] The symptoms appearing in our animals on a diet low in the heat-stable water-soluble vitamin can be described as follows: The eyelids are at

to determine whether such improvement as was noted with 50 per cent of lard, was due to vitamin impurity in the lard. The reader will note a truly remarkable variation in the behavior of individual animals in every group, a variation, which save for the phenomenon of "refection," is without parallel in any dietary experiments known to us. A repetition of this work has yielded essentially the same results. As a whole, therefore, our work indicates that, contrary to the situation with the antineuritic vitamin B, there is little sparing action of fat on heat-stable water-soluble vitamin.

DISCUSSION.

So far as we know, no definite physiological function has been assigned to fats. In general, investigators have looked upon fats as contributing only in the energy they supplied to the diet. The data presented leave little doubt that fats as such exert an important function in the metabolism of the animal other than supplying energy. Fats in common with substances carrying antineuritic vitamin B are able to delay or avert entirely the appearance of symptoms known as beriberi or polyneuritis. This is most marked on diets in which sugar forms the source of energy, but it is also

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first swollen, then the hair around the lids is lost, presenting bare rings surrounding the eyes, the condition described as "spectacles." This bareness spreads from a circumocular zone until at times almost the whole face is free from hair. The skin is thin and red. Very often sores are noted around the mouth. We have not observed lesions under the tongue as have been described. Diarrhea often accompanies the deficiency. Very often bloody vibrissæ are noted. The bloody vibrissæ are present where no lesions are seen about the mouth to offer a possible source of the blood, though it must be remembered that slight lesions are often present on the paws, and blood on vibrissæ may be due to the animal's attempts to clean itself. We have also had occasion to observe animals with the classical bilateral lesions on the back and sides, though this is not very common. Lesions on the nose are very common; these are sometimes accompanied by scaliness, though this is not always the case. Since scaliness has been so frequently described as characteristic of the deficiency, due to absence of the heat-stable water-soluble factor present in autoclaved yeast, it might be mentioned that we have observed it in animals in which there was an abundance of autoclaved yeast and that the sign is thus hardly pathognomonic of this deficiency. The symptoms did not supervene in all groups, nor in all animals in each group.

evident when starch is the source of energy. The activity of fats has been observed in this laboratory regardless of the level of antineuritic vitamin B extracts fed. To be sure, the sparing activity of fats is most marked when low levels of antineuritic vitamin B are fed. Whether fat no longer exerts a beneficent influence when antineuritic vitamin B has been supplied in sufficiently large amounts, must be left as an open question. The answer to this question is of great importance, for only then shall we know whether fat acts as a sparer of antineuritic vitamin B only or has other special and unknown uses in purified diets. All we can say here at present is that we have as yet found no case where fat did not improve the diet slightly after we had supposedly satisfied the antineuritic vitamin B requirements with unusually high levels of this vitamin. This question is at present under investigation.

Recent work on refection by Fridericia and others has brought to the fore the rôle that organisms may play in the digestive tract of animals and their importance in all vitamin B work. We have been led to wonder whether or not fats may be acting indirectly through their beneficial effect on the microorganisms in the intestinal tract of the rat. The question must be left open. We must, however, point out that the data here shown present such a quantitative picture of the activity of fat as to differ markedly from refection, for there is little uniformity or quantitative aspect to refection, nor has refection so far as we are aware been shown possible on diets such as we have employed. The data on the influence of fats on the course of the deficiency due to the absence of the heat-stable water-soluble vitamin strongly suggest that the two vitamins differ fundamentally in their relation to the presence of fat in the diet.

All natural fats studied by us have shown the sparing action on the antineuritic vitamin B and we have used butter, corn oil, coconut oil, and walnut oil besides those previously specifically mentioned. The influence of physical and chemical properties of the fats (melting point, degree of saturation, etc.) on their ability to act in the way herein described is being investigated.

Components of Diets Used in Experiments Represented in Figs 1 to 3.

No Fat.

Diet 542.	
Casein (L-3)	20
Autoclaved yeast	10
Sugar	70
Salts	4

10 Per Cent Fat.

Diet 550.		Diet 563.		Diet 564.	
Casein (L-3)	20	Casein (L-3)	23	Casein (L-3)	23
Autoclaved yeast	10	Autoclaved yeast	10	Autoclaved yeast	10
Sugar	59	Sugar	56	Sugar	56
Salts	4	Salts	4	Salts	4
Lard	10	Crisco	10	Wesson oil	10

50 to 51 Per Cent Fat.

Diet 559.		Diet 571.		Diet 572.	
Casein (L-3)	36	Casein (L-3)	38	Casein (L-3)	36
Autoclaved yeast	10	Autoclaved yeast	10	Autoclaved yeast	10
Salts	4	Salts	4	Salts	4
Lard	50	Crisco	55	Wesson oil	55

All diets were supplemented with 2 drops of cod liver oil daily.

Components of Diets Used in Experiments Represented in Fig. 4.

Diet 542.		Diet 414.		Diet 415.	
Casein (L-3)	20	Casein (L-3)	19	Casein (L-3)	20
Autoclaved yeast	10	Autoclaved yeast	10	Autoclaved yeast	10
Sugar	70	Corn-starch	67	Corn-starch	59
Salts	4	Salts	4	Salts	4
				Lard	10

All diets were supplemented with 2 drops of cod liver oil daily.

Components of Diets Used in Experiments Represented in Figs. 5 and 6.

No Fat.

Diet 540.		Diet 542.	
Casein (L-3)	25	Casein (L-3)	20
Sugar	75	Autoclaved yeast	10
Salts	4	Sugar	70
		Salts	4

10 Per Cent Fat.

Diet 551.		Diet 564.		Diet 565.	
Casein (L-3)	27	Casein (L-3)	20	Casein (L-3)	27
Sugar	59	Autoclaved yeast	10	Autoclaved yeast	10
Salts	4	Sugar	56	Sugar	56
Lard	10	Salts	4	Salts	4
		Wesson oil	10	"Synthetic" Wesson oil	10

51 to 54 Per Cent Fat.

Diet 572.		Diet 573.		Diet 579.	
Casein (L-3)	38	Casein (L-3)	38	Casein (L-3)	40
Autoclaved yeast	10	Autoclaved yeast	10	Salts	4
Salts	4	Salts	4	Lard	53
Wesson oil	55	"Synthetic" Wesson oil	55		

All diets were supplemented with 2 drops of cod liver oil daily. Diets 540, 551, and 579 were supplemented with 1 cc. daily of an alcoholic extract of rice bran (Extract 8 FB).

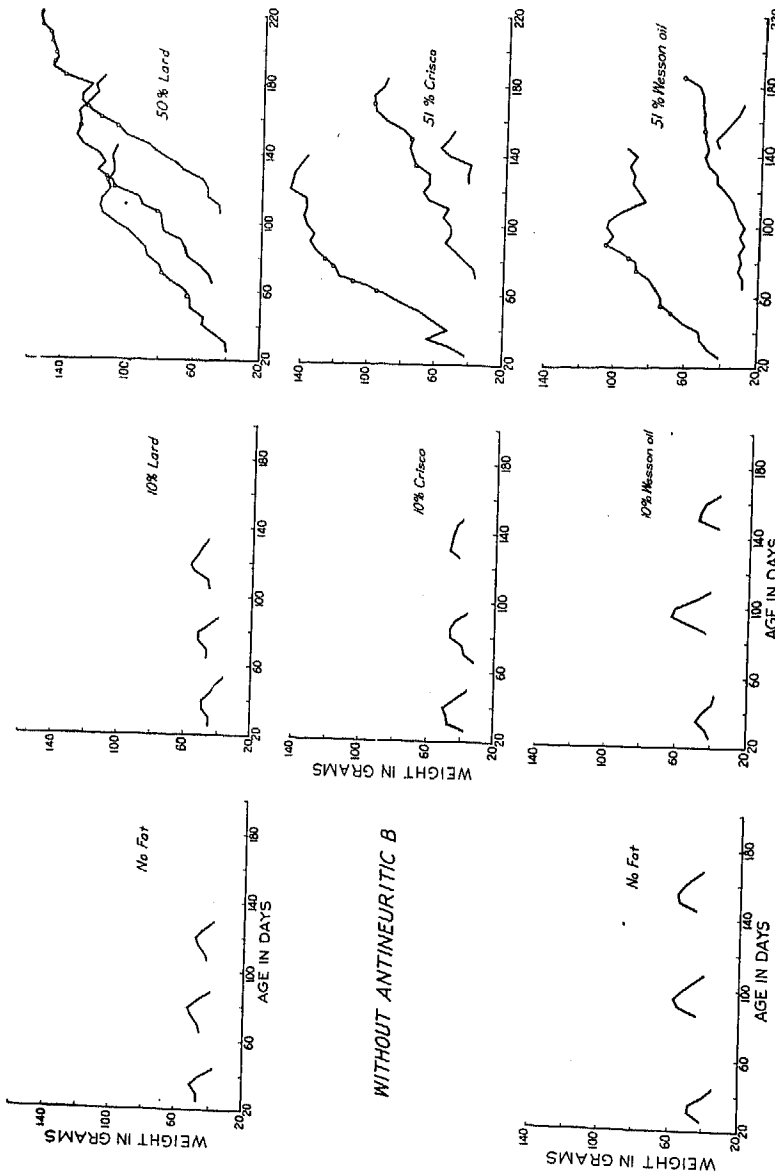

FIG. 1. Growth of animals when no antineuritic vitamin B was added to diets containing no fat (Diet 542), 10 per cent fat (Diets 550, 563, 564), or higher levels of fat (Diets 559, 571, 572).

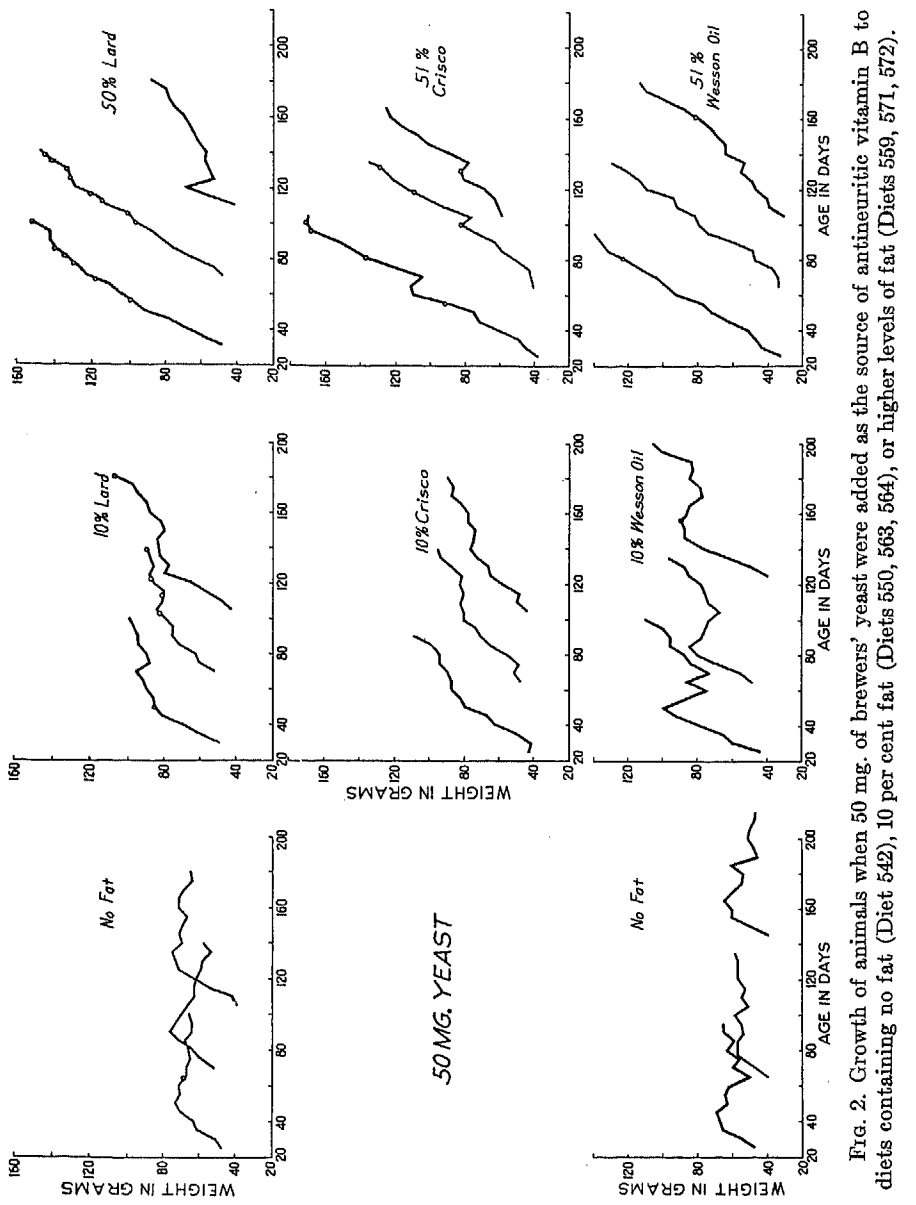

FIG. 2. Growth of animals when 50 mg. of brewers' yeast were added as the source of antineuritic vitamin B to diets containing no fat (Diet 542), 10 per cent fat (Diets 550, 563, 564), or higher levels of fat (Diets 559, 571, 572).

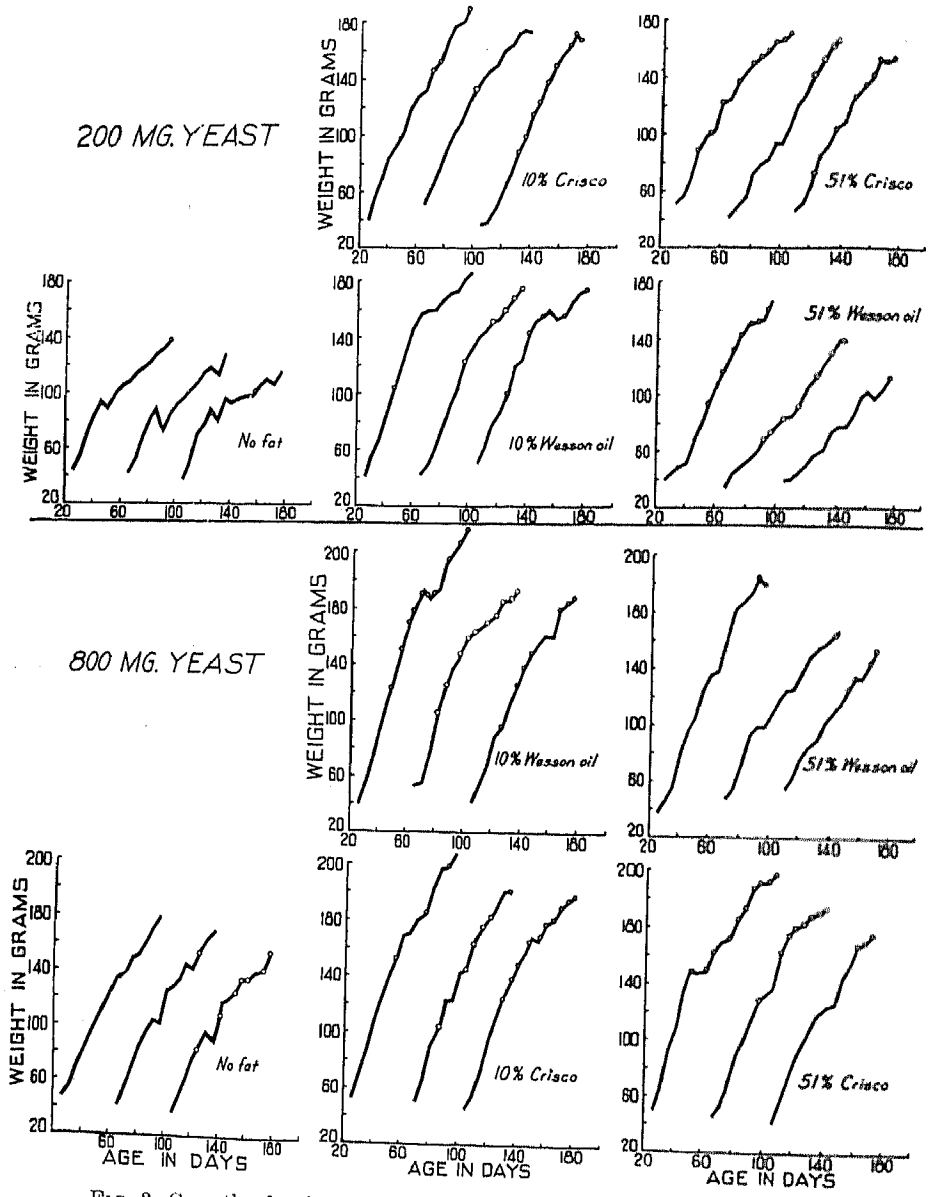

FIG. 3. Growth of animals when higher levels of antineuritic vitamin B (200 mg. and 800 mg. of brewers' yeast) were added to diets containing no fat (Diet 542), 10 per cent fat (Diets 563, 564) and 51 per cent fat (Diets 571, 572).

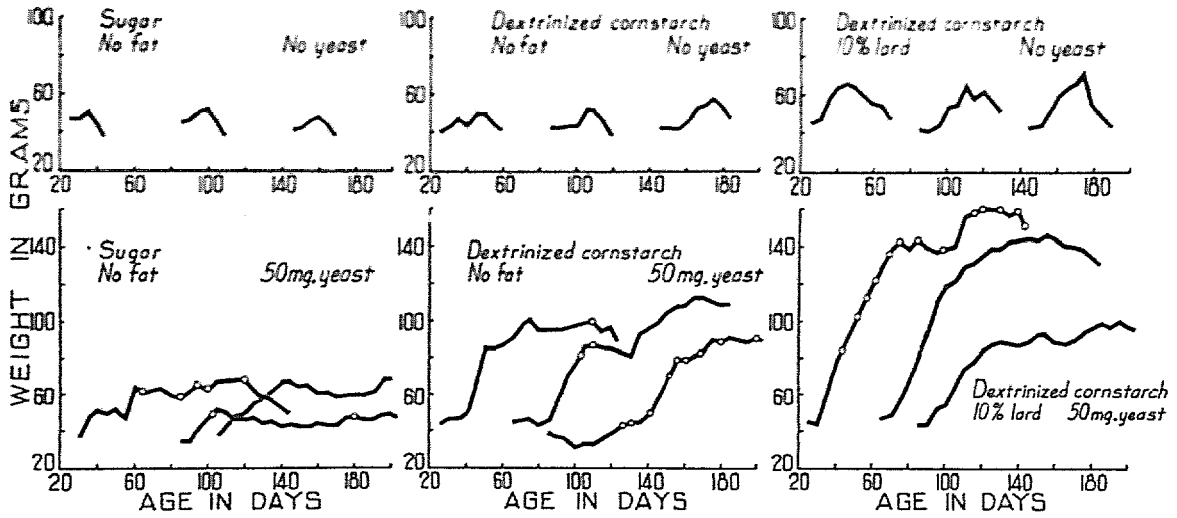

FIG. 4. Growth of animals on diets in which the sugar (Diet 542) had been replaced by dextrinized corn-starch (Diet 414). The diet with corn-starch gave superior growth. The addition of fat to this diet (Diet 415) further improved the growth, illustrating the sparing action of fat in the presence of this carbohydrate. These differences were most marked when 50 mg. of brewers' yeast were added as the source of antineuritic vitamin B.

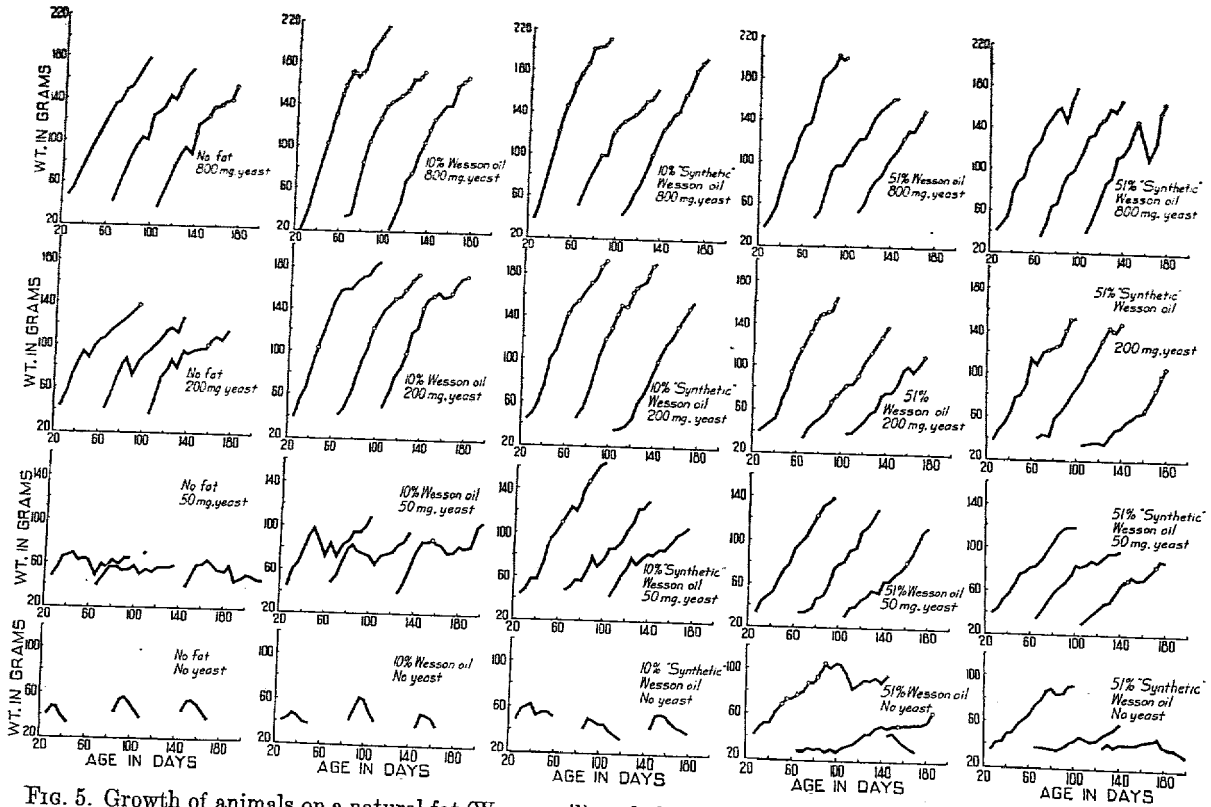

FIG. 5. Growth of animals on a natural fat (Wesson oil) and the same fat subjected to saponification, distillation, and reesterification ("synthetic" Wesson oil). Four levels of antineuritic vitamin B were added to the diets, one group receiving no yeast, a second group 50 mg., a third group 200 mg., and the fourth 800 mg. of brewers' yeast. Diets: no fat (Diet 542), 10 per cent Wesson oil (Diet 564), 10 per cent "synthetic" Wesson oil (Diet 565), 51 per cent Wesson oil (Diet 572), 51 per cent "synthetic" Wesson oil (Diet 573).

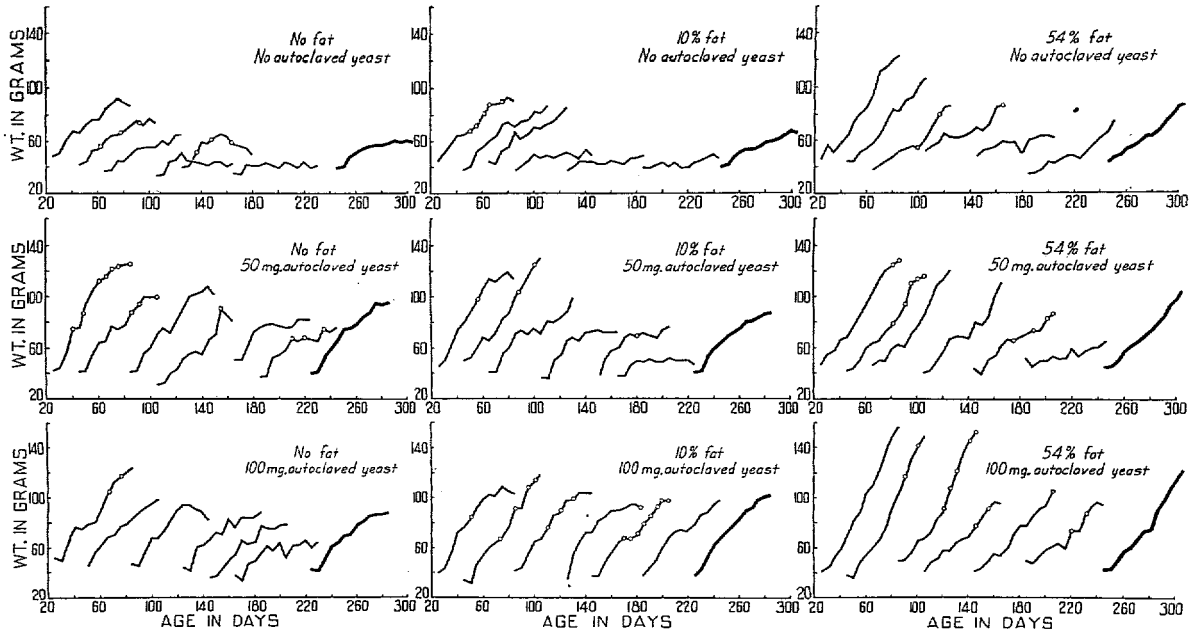

FIG. 6. Growth of animals upon diets containing inadequate amounts of the thermostable vitamin in the form of autoclaved yeast—one group receiving no yeast, a second 50 mg., a third 100 mg. of autoclaved yeast. The animals of all groups received daily an adequate amount of the antineuritic vitamin B in the form of 1 cc. of an alcoholic extract of rice polishings (Extract 8 FB). The diets had no fat (Diet 540), 10 per cent fat (Diet 551) and 54 per cent fat (Diet 579). The heavy curve in each instance is a composite for the group of six.