CCIII. AN EFFECT OF CHOLINE ON THE VITAMIN B₁-SPARING ACTION OF FATS

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(Received 2 July 1937)

EVANS AND LEPKOVSKY [1928; 1932] demonstrated that dietary fats exercise a sparing action on the requirements for vitamin B₁. This was found to be the case not only with respect to growth but in preventing the development of polyneuritis. These observations have been confirmed so far as growth is concerned by Guerrant & Dutcher [1934]. Several laboratories have failed to confirm the results and among the negative opinions those of Gregory & Drummond [1932] and of Sure & Buchanan [1935] may be particularly cited. The latter found that diets containing up to 50 % of fat failed to show a sparing action on the need for vitamin B₁. Recently Salmon & Goodman [1937] have confirmed the results of Evans & Lepkovsky not only with regard to growth but also in the delay and alleviation of polyneuritis. They found that the sparing action of fats was dependent upon the length of the carbon chain of the fatty acid.

Evans & Lepkovsky and all those who have been mentioned, used brewer's yeast as a source of vitamin B₂. This material contains large quantities of choline, which has been shown by Best and his associates [1935; 1936] to be an essential factor in fat metabolism, and which also has, with vitamin B₁, a complementary effect in increasing the weights of young rats [McHenry, 1935]. It seemed desirable to study a possible interrelationship between vitamin B₁, choline and fat in influencing weight changes and to determine whether dietary choline was a factor in the vitamin B₁-sparing action of fats.

Methods

Young male albino rats of the Wistar strain, reared in the Connaught Laboratories' colony, have been used. The initial weights of all rats were approximately 70 g. The animals were studied in groups of ten, and fifteen such groups have been employed. They were kept in individual wire-mesh cages. Food was provided *ad lib.* in aluminium dishes and daily records of food consumption were kept. Weights were measured on alternate days. The animals were maintained on experimental diets for 4 weeks.

In the diets there was a constant proportion (15 %) of protein. This is probably too low to secure good growth but was kept so because of the belief that protein, or at least casein, may exercise a lipotropic effect. Casein (British Drug Houses, vitamin-free) was the main source of protein, and dried egg white was used as a source of protein and to supply vitamin B₂. Varying proportions of cane sugar (78, 68, 52, 36, 20 %) furnished carbohydrate and inversely varying proportions of Crisco (0, 10, 26, 42, 58 %) supplied fat. A salt mixture and agar were included. Vitamins A and D were given in the form of a cod liver oil concentrate which was dissolved in ether and mixed with the diet. The mixture was subsequently air-dried to remove ether. When choline was given, required quantities of a solution of choline chloride were mixed into each day's rations. A concentrate of vitamin B₁, practically free of choline, and kindly furnished without charge

by Merck Inc., was employed in the series in which the vitamin was fed. The basal diet is not claimed to be complete but was especially chosen to contain as little choline as possible. The composition of the diets used was as follows:

	%
Casein	10
Egg white (dried and powdered)	5
Salts (McCollum's 185)	5
Agar	2
Cane sugar	78, 68, 52, 36 or 20
Crisco	0, 10, 26, 42 or 58

Results

Three series of five groups each were used and each group contained 10 rats. The first series was given only the basal diet without vitamin B₁ or choline. In the second, choline was supplied and the amount of choline varied with the amount of fat in the diet. The following quantities of choline were fed:

Fat in diet %	Amount of choline fed mg./rat/day
0	5
10	5
26	15
42	20
58	30

In the third series each rat received 4 international units of vitamin B₁ per day.

Fig. 1. Weight curves, series I. Rats given neither choline nor vitamin B₁

Figs. 1-3 record the changes in weight in each series. Each point is the average for a group of 10 rats. For convenience the total changes in weight for each group from the beginning to the end of experimental feeding have been calculated and are given in Table I.

Fig. 2. Weight curves, series II. Rats given choline, but not vitamin B₁.

Fig. 3. Weight curves, series III. Rats given vitamin B₁, but not choline.

Table I

Fat in diet %	Average total changes in weight (g.)		
	Series I No vitamin B ₁ , no choline	Series II Choline, no vitamin B ₁	Series III Vitamin B ₁ , no choline
0	- 18	- 3	+ 22
10	- 11	+ 2	+ 29
26	- 3	+ 1	+ 29
42	0	+ 12	+ 16
58	+ 1	+ 7	+ 5

DISCUSSION

In Series I animals without dietary fat were approximately at the initial weight at the end of the first week and thereafter the weights markedly declined. The weights of the other groups receiving different amounts of fat increased till the 10th day and then declined. After 4 weeks the weights of those groups receiving 26, 42 and 58 % fat were approximately the same, were slightly above the initial weight and were appreciably greater than the weights of the groups receiving no fat or 10 % fat. In no case can it be said that there was good growth, but the supply of 26 % or more fat in the diet did prevent a definite decline in weight below the initial level. In this sense the results of Evans and Lepkovsky may be said to have been confirmed.

Series II, in which choline was supplied, presents a somewhat different picture. Even in the group receiving no fat there was not the marked decline seen in Series I, and in all five groups there was fairly good growth during the first week. After 4 weeks the group receiving 42 % fat was above the other groups in weight and this would lead to the opinion that, under the conditions of the experiment with choline supplied, there was an optimum amount of fat. The diets used in this series more nearly resemble those used by Evans and Lepkovsky. In so far as improvement in weight is concerned an appreciable amount of fat is an advantage when vitamin B₁ is deficient, especially when choline is present in the diet. While fat-rich diets prevent the marked loss of weight of rats receiving a vitamin B₁-deficient diet, the results are not as satisfactory as when the vitamin is supplied.

Guerrant & Dutcher [1934] reported that satisfactory growth responses, when rats were receiving a restricted intake of vitamin B₁, occurred when the fat content of the diet ranged from 15 to 20 %. In Series III rats receiving 10 and 26 % fat had the same weight after 4 weeks and this was greater than the weights of groups receiving 0, 42 and 58 %. In fact the weight of the last group was definitely low, but not less than the initial weight. It is quite apparent that there is an optimum range for dietary fat when vitamin B₁ is fed, and that this range is below the optimum found in the previous series.

Examination of records of individual food consumption has shown that it is difficult to correlate these values with changes in weight, except that in Series I and II food intake declines after the first 2 weeks, when body stores of vitamin B₁ are presumably exhausted. To economize in space these individual records are not included in this communication but Table II shows the average total consumption for each group in the three series.

Two points of interest may be deduced from Table II. In each series the food intake is lessened when the percentage of fat is increased in the diet, but not to a sufficient extent to make the food consumption isocaloric. For example, group 1 of Series II ate 173 g. with a caloric value of 700, while group 4 in the

Table II

Fat in diet %	Average total food consumption (g.)		
	Series I No vitamin B ₁ , no choline	Series II Choline, no vitamin B ₁	Series III Vitamin B ₁ , no choline
0	170	173	238
10	177	168	237
26	172	143	235
42	159	136	196
58	156	124	160

same series ate 136 g., with a value of 830 calories. Between these two groups there was a difference in weight at the end of the experiment of 15 g. in favour of group 4. Within each series the same is true and this difference in energy intake may explain the difference in weights and the "vitamin-sparing effect of fat". In contrasting Series I and II, however, the differences in weight are not due to variations in food intake, either expressed as total weight or as total calories. Group 4 of Series I was 12 g. lighter than the similar group of Series II, yet the former consumed more food of the same caloric value. It seems likely that the increased weight in Series II was due to an alteration in the utilization of fat brought about by choline.

The effect of choline which has been indicated above, may be explained by recalling that choline prevents excess deposition of fat in the liver when diets high in fat are fed [Best *et al.* 1936]. In all probability a picture different from that seen in Series III would be secured if choline and vitamin B₁ were both furnished. It has been previously shown that, at a level of 30 % dietary fat, the weights of young rats are increased by giving both factors. Since it has also been shown [McHenry, 1937] that vitamin B₁ markedly increases liver fat when choline is deficient in the diet the almost negligible growth at high fat levels when the vitamin only is given may be due to the production of fatty livers and consequent interference with metabolism. This condition would have been prevented by a supply of choline. It should be pointed out that the presence of choline in the diet in experiments of this type is a factor which should not be overlooked. Salmon & Goodman [1937] found that an increase in the amount of autoclaved yeast augmented the weight effects of fat-rich diets and concluded that this was due to undestroyed traces of vitamin B₁. Yeast is an excellent source of choline and the beneficial effects of larger amounts of yeast may be due to its choline content.

SUMMARY

1. The increase in weight of young rats is influenced by the amounts of vitamin B₁, choline and fat in the diet and the effects of these three dietary factors are probably interrelated.
2. When a diet deficient in vitamin B₁ is fed the inclusion of more than 26 % fat prevents a serious loss in weight. The effect of the fat is augmented by the addition of choline to the diet. Under the latter conditions, the optimum amount of dietary fat has been found to be about 40 %.
3. When vitamin B₁ is supplied and choline is deficient in the diet the amount of dietary fat optimum for weight increase ranges between 10 and 26 %.

Appreciation is expressed to Miss Lillian Barber for valuable technical assistance. Sincere thanks are extended to Dr J. G. FitzGerald and Dr C. H. Best for their helpful interest.

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