Effect of Different Dietary Fats on Choline Requirement of Rats.* (22867)

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Channon and Wilkinson(1) studied the effect of various dietary fats on level of liver fat in rats fed on low choline diets. They concluded that an increase in quantity of long chain saturated fatty acids in the diet caused increased liver fat deposition. Hartroft(2) has cited a number of observations which support the conclusion of Channon and Wilkinson(1). Work in this laboratory(3) with choline-free diets further substantiated this conclusion and also demonstrated that fats containing long chain saturated fatty acids caused increased liver fat deposition when fed with 9% casein diet containing 0.15% of choline chloride.

In none of these studies has it been determined whether the long chain saturated fatty acids increase the amount of choline needed to reduce the level of liver fat. Best, Lucas, Ridout, and Patterson(4) have pointed out the importance of using dose response curves in studying lipotropic factors. Therefore, a study has been made of liver fat deposition in rats fed on diets containing either butter fat or corn oil with various levels of choline.

Methods. Male rats of Sprague-Dawley strain weighing 70 to 84 g were used in Exp. 1. In Exp. 2 and 3, male rats of same strain weighing 40 to 50 g were used. The rats were separated into similar groups of 6 according to weight and were housed in individual cages with raised screen floors. During experimental period of 2 weeks the rats were fed *ad libitum*. The diet in first experiment contained in per cent: casein, 8; gelatin, 12; salts IV(5), 4; cellulose,† 2; sucrose, 44; and fat (as specified in the Table), 30. Vitamins were added to give as mg/100 g of diet: calcium pantothenate, 2; niacin, 2.5; riboflavin, 0.5; thiamine hydrochloride, 0.5; pyridoxine, 0.25; inositol, 10; biotin, 0.01; folic acid, 0.02; and vit. B_{12}, 0.002. Two drops of halibut liver oil fortified with vit. E and K (6) were given orally each week. Diets used in Exp. 2 and 3 contained 9% of casein instead of 8% and did not contain cellulose. All alterations in diets were compensated by adjusting level of sucrose. Butter fat was prepared by melting butter and decanting the fat layer through cheese cloth. At the end of the 2 weeks experimental period, the rats were stunned by blow on head and decapitated. Livers were removed and stored at —4°C until levels of liver fat were determined. Liver fat was determined by ether extraction of dried and ground liver(7). Those rats from which kidney sections were made, were killed in same manner. The left kidney was immediately removed and a portion of the organ was fixed with Bouin's Solution. Paraffin sections were stained with hematoxylin and eosin.

Results. Growth and liver fat results for the 3 experiments are presented in Table I. In the first experiment the basal diet was fed without amino acid supplements. Seven levels of choline were fed with corn oil and with butter fat. When no choline was added to diets, butter fat promoted a higher level of liver fat than corn oil. Choline reduced the level of liver fat with both dietary fats. Liver fat values were within the normal range when 0.12% of choline chloride was included in diets containing corn oil and when 0.15% of choline chloride was included in diets containing butter fat.

The groups receiving 0.04% of choline chloride or greater, grew at about the same rate whether corn oil or butter fat was fed. However, with no choline or only 0.02% of choline chloride, groups receiving corn oil grew at a slower rate, while groups receiving butter fat grew as well as those receiving higher levels of choline.

The basal diet is low in methionine, cystine, and tryptophan. Since dietary methionine and cystine and rate of growth are

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† Solka-Floc, Brown Co., Berlin, N. H.

TABLE I. Liver Fat and Growth of Rats Fed Corn Oil or Butter Fat with Levels of Choline.

Exp. No.	Supplement	Choline chloride, %	Butter fat		Corn oil	
			Liver fat, % dry liver*	Gain, g/wk*	Liver fat, % dry liver*	Gain, g/wk*
1	None	.00	67.4 ± 2.0	13.2 ± 2.5	51.0 ± 2.8	6.9 ± 2.6
		.02	58.1 ± 4.2	13.4 ± 2.2	42.0 ± 4.4	8.3 ± 3.2
		.04	50.5 ± 3.5	12.2 ± 2.5	28.7 ± 2.0	10.4 ± 3.0
		.08	31.2 ± 5.5	13.4 ± 2.0	21.7 ± 1.7	11.0 ± 2.2
		.12	20.8 ± 1.0	13.8 ± 2.5	16.3 ± 1.7	11.0 ± 1.0
		.15	16.6 ± .5	12.2 ± 3.8	17.7 ± .7	13.6 ± 2.0
		.30	14.0 ± .4	8.4 ± 2.6	15.7 ± 1.4	10.3 ± 2.2
2	None	.00	61.8 ± 1.7	11.6 ± 1.9	47.6 ± 2.7	6.2 ± 1.6
		.04	52.1 ± 3.1	13.8 ± 1.0	36.4 ± 2.2	14.2 ± 1.1
		.12	18.7 ± 1.0	17.3 ± 1.2	16.5 ± 1.4	14.8 ± 1.4
	Meth + trypt†	.00	62.7 ± .3	26.1 ± 1.3	51.9 ± 2.3	19.0 ± 2.6
		.04	46.8 ± 2.2	27.1 ± 1.3	31.3 ± 2.5	19.6 ± 1.7
		.12	29.8 ± 1.0	28.3 ± 1.8	18.2 ± 1.2	22.7 ± 1.7
		.15**	21.6 ± .6	21.1 ± .5	18.0 ± 1.1	21.3 ± .5
		.30**	22.1 ± 1.1	19.3 ± .4	15.9 ± 1.1	18.3 ± 1.6
	Cyst‡	.00	62.0 ± 2.9	8.9 ± 1.9‖	63.4 ± 4.0	10.9 ± 2.8¶
		.04	55.4 ± 4.5	18.5 ± 1.2	46.3 ± 4.0	15.0 ± 2.5
		.12	30.0 ± 3.8	13.1 ± .9	11.9 ± 1.3	15.3 ± 1.6
		.15**	17.4 ± 1.1	9.5 ± .8	13.7 ± 1.3	9.8 ± .7
		.30**	15.8 ± .4	11.0 ± .9	12.4 ± 1.0	9.9 ± .9
3	Meth + trypt + cyst§	.00	62.3 ± 1.3	19.8 ± 1.1	51.5 ± 1.5	15.2 ± 1.2
		.04	38.1 ± 5.7	18.9 ± 1.0	30.5 ± 2.7	19.9 ± 2.1
		.12	28.0 ± 1.9	19.2 ± .8	16.6 ± 1.6	17.2 ± 1.4
		.30	21.5 ± 1.4	17.7 ± 1.1	16.0 ± .8	19.5 ± 1.6

* The mean ± stand. error of mean for 6 rats. † .3% DL-methionine + .1% DL-tryptophan. ‡ .3% L-cystine. § .3% DL-methionine + .1% DL-tryptophan + .3% L-cystine. ‖ Two rats died before end of exp. ¶ Three rats died before end of exp. ** These groups were not run at same time as other groups in Exp. 2 but with Exp. 3.

known to affect liver fat deposition, the plan of Exp. 1 was repeated in Exp. 2 with supplements of cystine alone, or methionine and tryptophan together, added to basal diet. The level of casein in basal diet was increased to 9% and cellulose was omitted. Groups receiving 0.15% and 0.30% of choline chloride in Exp. 2 were run at the same time as Exp. 3 and not simultaneously with the other groups in Exp. 2.

When no supplement was added to the diet, liver fat results were similar to those in Exp. 1. The group which received butter fat without choline, grew at a more rapid rate than the group which received corn oil without choline, as had been observed in Exp. 1.

Supplements of methionine and tryptophan caused marked growth response. Liver fat values within the normal range were obtained in the group receiving 0.12% of choline chloride with corn oil and in the group receiving 0.15% of choline chloride with butter fat. Groups receiving 0.12%, 0.04%, and no choline chloride with butter fat, grew more rapidly than similar groups receiving corn oil. Groups which received either 0.15% or 0.30% of choline chloride were not run at the same time and there was no difference in growth between those receiving butter fat and those receiving corn oil.

The cystine supplement alone did not increase growth rate of rats. With no choline added to the diet containing cystine, 3 rats died in the group receiving corn oil and 2 died in the group receiving butter fat. These deaths occurred on 9th and 10th days of experiment. Growth rates and levels of liver fat could be calculated only for surviving rats in each group and therefore may not be representative. This is probably the reason that the group receiving butter fat did not appear to grow more rapidly than the group receiving corn oil, for only 3 rats which were growing at the most rapid rate survived in this group. Liver fat values for groups which received cystine, corn oil and no choline or

0.04% of choline chloride were higher than those for groups which received similar diets without the amino acid supplement. There was no effect of cystine on the level of liver fat in groups receiving butter fat with these two levels of choline chloride. However, cystine caused an increase in level of liver fat in the group receiving butter fat and 0.12% of choline chloride. There was no difference in level of liver fat between groups which received corn oil and those which received butter fat when diet contained no choline and cystine. In this case liver fat values for both groups were very high. With 0.04% of choline chloride, butter fat promoted a significantly higher level of liver fat than corn oil. Also levels of liver fat within the normal range were obtained with the same levels of choline as in Exp. 1 (corn oil, 0.12% of choline chloride and butter fat, 0.15% of choline chloride).

When cystine, methionine, and tryptophan were included in the diet in Exp. 3, liver fat results were, in general, the same as when methionine and tryptophan were included in Exp. 2. All groups in this series grew at about the same rate except for the group which received corn oil and no choline, which grew at a slower rate.

Histological studies were made of sections from kidneys of 3 rats from each group in Exp. 2 which received either no choline or 0.12% of choline chloride. Rats which had greatest growth rate, smallest growth rate, and growth rate closest to the mean in each group were selected for histological study.

Kidney sections were examined for signs of kidney damage as described by Christensen(8) and Hartroft(9). Hyalin casts in tubules and areas of necrosis of tubules were observed in each group which did not receive choline. No hemorrhages were observed in sections studied. Great variation in extent of kidney damage within groups and the small number of animals which were studied from each group, made it difficult to determine which group had the most severe kidney damage. However, a positive correlation was observed between rate of growth of rats and extent of kidney damage. Rats which grew at poorest rates had very few normal tubules; and, while rats that grew at intermediate rates showed considerable damage, they also had a number of normal tubules remaining. Rats that grew at the most rapid rate had very little kidney damage. This suggests that growth depressions that occurred when choline was omitted from diets were an indication of extent of kidney damage. These growth depressions were much greater when corn oil was fed than when butter fat was fed.

Discussion. If the "requirement" for choline is taken as that level of choline which is necessary to reduce level of liver fat to a minimum, results of this study indicate that rats fed on diets containing 30% of butter fat have a higher "requirement" for choline than those receiving 30% of corn oil. The "requirement" with corn oil diet was not greater than 0.12% of choline chloride and was about 0.15% of choline chloride with butter fat diet. These values were not altered when the diets were supplemented with certain amino acids although growth rates and levels of liver fat were changed in some cases.

Supplements of cystine caused increases in level of liver fat in rats fed on diets low in choline and sulfur amino acids(10). It has been suggested that increased liver fat deposition is a result of improved rate of growth caused by cystine supplement(11). In this study the cystine supplement caused increases in level of liver fat of groups which received corn oil with either no choline or 0.04% of choline chloride. With butter fat diets the cystine supplements had no effect on level of liver fat except with 0.12% choline chloride where there was an increase. Since there were no growth responses to cystine supplements, the increases in liver fat can not be explained on this basis. No conclusion concerning the effect of methionine can be drawn from these experiments.

Choline deficiency causes a characteristic kidney damage in the rat. Gyorgy and Goldblatt(12) observed that kidney damage in rats receiving no dietary choline was more severe when the diet contained hydrogenated vegetable oil than when it contained lard. If the ratio of saturated to unsaturated fatty acids in the fat is the important factor in kidney damage the corn oil used in this study

would be expected to have the same effect as hydrogenated vegetable oil used by Gyorgy and Goldblatt and the butter fat to have the same effect as lard that they used. This was found to be true in the effects of these fats on liver fat deposition(3).

Summary. 1. Rats fed on diet containing butter fat required a higher level of choline to reduce the level of liver fat to within the normal range, than did rats fed the same level of corn oil. This "requirement" for choline, as measured by liver fat, was not greater than 0.12% of choline chloride with diet containing 30% of corn oil and about 0.15% of choline chloride with diet containing 30% of butter fat. These values were not affected by supplements of cystine, or methionine and tryptophan or all 3 amino acids. 2. When no choline was fed, rats receiving butter fat grew at a more rapid rate than those receiving corn oil. It is suggested that the reduced rate of growth of rats fed corn oil was caused by kidney damage.

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A Technic for Cross-Transfusion of Blood in Embryonic Chicks and Its Effect upon Hatchability.* (22868)

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Although several methods for intravenous injection of chick embryos are described in the literature(1-5), none were used for purposes which required the chick to hatch after injection or withdrawal of blood. In the course of our studies on the homograft problem, it became necessary to cross-transfuse blood between 2 embryonic chicks for subsequent testing of skin graft compatibility after hatching. Trials of presently known methods for intravenous injection of chick embryos did not lead to significant survival through hatching. Most of recent technics consist essentially of locating the vessel to be injected by candling, cutting a window in the shell, putting a drop of mineral oil on the shell membrane to make the vessel visible, injecting or withdrawing blood with a 26-27 gauge needle on a syringe, withdrawing the needle, and sealing. Although this procedure seems simple, we have encountered two principal difficulties. Firstly, it is difficult to hold the needle in a small vessel for any length of time —especially if considerable amounts of blood must be withdrawn. In an attempt to solve this problem, Goldwasser and Shelesnyak(5) constructed an U-shaped egg clamp and an elaborate syringe carrier which was movable in 3 dimensions by gears. The advantage of such a stable apparatus, however, is offset by its complexity and cost of construction. The second and most important difficulty is con-

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