the Fisher-Hirschfelder-Taylor atomic models of the analogues. The greater effective volume of trichloromethyl over methyl tends to maintain the benzene rings in a more nearly rigid configuration by virtue of steric blocking of rotation of the benzene rings. Secondly, by virtue of an inductive effect through the resonating ring system, the chlorine atoms would tend to increase the activity of the p,p'-hydroxyl groups. This latter effect may not markedly influence activity, however, since the trichloromethyl group is not attached directly to the ring but is 'insulated' by one aliphatic carbon atom. Much more effective in increasing estrogenic potency would be the influence of the trichloromethyl group on the p,p'-hydroxyl hydrogens by inductive action through space via the field effect.

In conclusion it must be pointed out that the distance between the active hydrogens of DHDT can never achieve the optimum (14.5 A) and one would not anticipate activity approaching that of estradiol or diethyl stilbestrol.

Summary. 1. DDT and 2,2'-bis-(p-methoxyphenyl)-1,1,1-trichloroethane possess no estrogenic activity in ovariectomized rats in total doses up to 45 mg. 2. 2,2'-bis-(p-Hydroxyphenyl)-1,1,1-trichloroethane possesses seven times the estrogenic activity of its non-halogenated congener 2,2'-bis-(p-hydroxyphenyl)-ethane and this increase in potency may be related to field and steric effects exerted by the trichloromethyl group.

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Effect of Factors other than Choline on Liver Fat Deposition.* (19904)

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The effects of certain amino acids on the metabolic relationship between tryptophan and niacin have been studied extensively (1-6). Recently we were surprised to observe fat deposition in the livers of animals receiving similar diets to those used in the above studies. Since the basal ration contained 0.1% choline and since methionine failed to prevent the occurrence of fatty livers, it appeared that other factors in the ration were involved.

In this paper, we wish to present experiments which demonstrate certain effects of proteins, amino acids and carbohydrate on liver fat deposition.

Experimental. Three-week-old male Sprague-Dawley rats kept in individual cages

TABLE I. Basal Ration for Fat Deposition Studies.

Component	Level in diet (per 100 g ration)
Casein	9 g
L-cystine	.2
Sucrose or dextrin	81.9
Corn oil	5
Salts IV(7)	4
Thiamin • HCl	.2 mg
Riboflavin	.3
Pyridoxine • HCl	.25
Ca pantothenate	2
Choline chloride	100
Inositol	10
Biotin	.01
Folic acid	.02

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TABLE II. Effect of Various Supplements to Sucrose Basal Ration on Liver Fat and Growth Rate.

Supplement	No. of animals	50 mg DL-tryptophan	1.5 mg niacin	% fat in rat livers — Wet basis Avg	Wet basis Range	Dry basis Avg	Dry basis Range	Avg growth rate (g/wk) Avg	Avg growth rate (g/wk) Range
0	9			8.8	(4.4-13.3)	20.4	(12.7-28.4)	14.4	(10.4-20.8)
0	9	+		9.3	(6.6-15.9)	23	(13.5-37.8)	19.8	(15.2-26.4)
0	9		+	13.4	(9.5-19.9)	32.3	(24.4-41.7)	16.4	(8.8-20.8)
6% gelatin	6			2.9	(2.2- 3.8)	8.3	(6.2-10)	3.2	(1 - 6.6)
	6	+		4.8	(3 - 9.6)	11.9	(8.4-15.2)	25.6	(19.2-35)
	6		+	3.7	(3 - 4.4)	11.1	(8.9-14)	25.6	(14.2-38.8)
.16% threonine	9			3.2	(2.3- 4.7)	8.7	(6.5-10.1)	4.4	(.8- 7.6)
	9	+		6.6	(3.8-13.5)	17.5	(11.3-26.6)	23	(14.8-29)
	9		+	7.3	(5.1-15.1)	19.7	(15.1-35.5)	20	(12.2-35.2)
.31% threonine	6			2.4	(2.3- 2.5)	6.9	(6.4- 7.4)	3.2	(2.2- 4)
	6	+		6.2	(5.9- 6.9)	16.1	(15 -18.1)	22	(21.8-22.4)
	6		+	6.5	(5.7- 7.9)	17.2	(15 -19.9)	22.8	(19.2-26.2)

were fed *ad libitum* and were weighed at weekly intervals. The basal diet to which supplements were made at the expense of the carbohydrate is shown in Table I. This basal diet contained cystine in all cases except where indicated. The fat-soluble vitamins were administered orally in the form of 2 drops of halibut liver oil each week. The animals were sacrificed at the end of a 5-week period and the livers of the animals were removed and analyzed for fat content using essentially the procedure outlined by the Association of Official Agricultural Chemists(8). The livers were dried at 50°C in a vacuum oven for moisture determination, and the fat content was determined on duplicate samples of the dried livers. Samples were ground with sand and extracted with ether for 16 hours on Goldfisch extractors. After evaporation of the ether the fat samples were heated in a 100°C oven for 15 minutes, cooled in a desiccator, and weighed to constant values.

Results. The data in Table II show the effect of various supplements when added to sucrose rations containing 9% casein with 0.2% L cystine. When either 50 mg % DL tryptophan or 1.5 mg % niacin was added to this basal ration the growth rate increased but no protective effect upon liver fat deposition was noted. When 6% gelatin was added to the basal ration, however, a decided depression of liver fat together with a reduced growth rate occurred. Either tryptophan or niacin, added with the 6% gelatin supplement, stimulated growth over the controls while the liver fat was still significantly lowered. From this experiment, it appeared that the factors controlling growth and liver fat deposition were not the same. When 0.15% DL threonine was added to the basal ration, growth was inhibited as much as that caused by 6% gelatin and the liver fat was lowered to the same level. When the indicated amounts of either tryptophan or niacin were added to the threonine supplement, growth was increased markedly, but no depression of liver fat occurred. The growth depression was undoubtedly due to an amino acid imbalance(4). Either niacin or tryptophan alleviated this growth depression but an increase in liver fat still occurred.

Griffith and Nawrocki(9) have reported intensification of a choline deficiency when threonine was added with cystine to a low casein diet. The influence of threonine was believed to be due to a direct stimulation of growth or metabolism rather than an antagonistic action towards labile methyl. This seems to be the case since 0.1% choline was present in the basal ration under our conditions. The growth depression which we observed is probably due to a tryptophan-niacin deficiency, yet when either of these factors was added to bring about a growth response, fatty livers occurred. When the level of threonine was doubled as in the last experiment in Table II in the presence of either tryptophan or niacin, growth was restored and liver lipids were reduced, but not to the extent brought about by the gelatin supplement

TABLE III. Effect of Various Supplements to Dextrin Basal Ration on Liver Fat and Growth Rate.

Supplements	No. of animals	50 mg DL-tryptophan	1.5 mg niacin	% fat in rat livers — Wet basis Avg	Wet basis Range	Dry basis Avg	Dry basis Range	Avg growth rate (g/wk) Avg	Avg growth rate (g/wk) Range
0	6			4.9	(3.7-6)	13.9	(11.2-17.6)	21.8	(14 -26.8)
0	6	+		3.9	(3.2-5.2)	11.4	(9.4-14.7)	17.8	(9.2-26.2)
0	6		+	5.9	(5.3-6.5)	16.3	(14.2-18.5)	22.4	(16.2-26)
6% gelatin	6			3.1	(2.5-4.1)	9.3	(7.2-12.5)	7.6	(6 - 9)
	6	+		4.2	(3.5-4.7)	12.9	(11.2-14.1)	22.8	(14.8-30.4)
	6		+	3.2	(2.4-4.4)	9.6	(8 -12.2)	22	(12.8-32.6)
.18% threonine	3			3.9	(3.3-5)	8.9	(7.7-10.7)	11	(5.4-15.6)
+.24% phenyl-	3	+		3.1	(2.4-4.1)	8.9	(7.2-11.3)	31.4	(10.4-32.4)
alanine	3		+	4.5	(4.1-5.3)	13.5	(12.6-15.2)	23.6	(18.8-26)
2% acid-hydro-	3			4.3	(2.9-6.4)	12.8	(8.8-18.8)	15	(13.6-17.6)
lized casein+	3	+		5.1	(4 -5.8)	15.1	(12.2-17.6)	30	(27.6-33.8)
1.5% glycine	3		+	5.6	(5.4-5.7)	16.8	(16.5-17.1)	23.4	(19.2-27.8)

alone. It is clear that gelatin exerts an effect beyond the action of threonine.

In Table III similar data are given when dextrin rather than sucrose was the carbohydrate. The controls on the dextrin basal showed a higher growth rate even without additions of niacin or tryptophan. At the same time it can be noted that the deposition of liver fat was not as severe as in the sucrose group. The addition of either tryptophan or niacin in the indicated quantities failed to promote increased growth over the controls. When 6% gelatin was added to the basal diet, a drop in liver lipids and growth rate was observed. Supplements of either tryptophan or niacin together with gelatin completely overcame the apparent growth antagonism precipitated by the gelatin and maintained the liver fat at low levels. When 0.18% DL threonine and 0.24% DL phenylalanine were supplemented to the basal ration, a similar effect was noted. The addition of either tryptophan or niacin restored the growth without affecting the liver fat. In the final experiment in Table III, 2% acid-hydrolyzed casein and 1.5% glycine were added together to the basal ration. The growth was depressed but liver fat was only slightly altered if at all with respect to the controls. Tryptophan or niacin increased growth and liver fat deposition slightly. The dextrin basal rations supported growth rates of control animals which were much greater than the sucrose controls as has been previously observed in this laboratory by Teply and coworkers(10). On the other hand, it can be observed that the lipid contents of the livers of the dextrin controls were lower. Gelatin exerted a greater effect when sucrose was the carbohydrate.

McHenry(11) cited the roles of biotin, thiamin and carbohydrate in the production of fatty livers. More recently Harper and Katayama(12) have studied the utilization of sucrose or corn starch for growth when added to low casein diets. They showed that corn starch supported better growth than sucrose in a 9% casein basal ration supplemented with methionine. This was also true when methionine was not added to the basal diet. One can surmise that the differences between the 2 carbohydrates may be in the rate of passage of the diet through the gut. A greater availability of the amino acids in the casein might be suspected if the ration passed through the intestine more slowly. The observations by Mulford and Griffith(13), and Salmon(14) that cystine supplementation aggravates renal damage and fatty infiltration of the livers of rats receiving 8% casein diets deficient in choline; and Griffith and Nawrocki(9) that further intensification of the choline deficiency occurred when threonine was added with cystine, prompted us to carry out a third set of experiments which are reported in Table IV. We decided to omit cystine from the basal ration and test the effects of certain protein and amino acid supplements. The most striking effect of the cystine omission may be seen in the control group where the growth was about 50% lower

TABLE IV. Effect of Increasing Protein and Single Amino Acids Supplemented to Sucrose Basal Ration Without Cystine.

Supplement, %	No. of animals	% fat of dry liver		Avg growth rate (g/wk)	
		Avg	Range	Avg	Range
0	6	29	(13.9-35.2)	7.6	(5.8-11.6)
3 Casein	6	11.5	(6.9-17)	15.6	(12.6-18.8)
6	6	10.2	(7 -15.2)	22.6	(16.2-24.8)
3 Gelatin	6	17.2	(9.7-25.8)	10.4	(8.8-12.2)
6	6	13.9	(9 -18.4)	10	(6.2-13.8)
.3 L Proline	6	23.6	(14.8-35.8)	6.6	(1.8- 9.2)
.6	6	24.5	(17 -25.4)	7.2	(4.8-10.4)
.6 DL Alanine	6	23.1	(15.6-28.1)	5.6	(4.2- 8.4)
.3 DL Methionine	3	26.7	(23.6-29.6)	14.9	(10 -17.6)

than similar controls in Table II where cystine was included. It is likely that the sulfur amino acids were limiting for growth in the 9% casein rations(4). Larger amounts of casein added to the basal diet increased growth and decreased liver fat. This, however, was not true in the case of gelatin. Increasing levels of gelatin failed to stimulate growth proportionately to the level added to the diet, but did decrease liver fat proportionately. L proline was chosen as an amino acid supplement because of its high concentration in gelatin and DL alanine was chosen because it showed a slight effect upon the reduction of liver lipids in preliminary trials. The supplements of 0.3% L proline and 0.6% DL alanine are comparable and each decreases the liver fat slightly while the growth in either group is similar to the controls. This observation strengthens the earlier conclusion that those factors controlling the liver fat deposition are not necessarily the same as those controlling growth. To show this more clearly, 0.3% DL methionine was added to the basal ration which resulted in a restoration in the growth equivalent to that exerted by cystine in the control group presented in Table II. While the addition of methionine to the basal ration without cystine increased growth 100% over the controls, it exerted no effect upon the liver fat deposition. It appears that 0.3% DL methionine improved growth without antagonizing liver fat deposition as is often the case when added cystine is present(9,13-15).

The fatty liver which we have obtained under our conditions seems to be one which may be influenced by at least 3 factors: protein, amino acids and carbohydrate. There has been much conjecture as McHenry(11) has pointed out, upon the role of the pancreas in preventing fatty livers. Some observations suggest the essential factor in the pancreas to be the hormone, lipocaic, while other workers claim to have ruled out the hormone action and assert the idea that the proteolytic enzymes from pancreatic juice are necessary for the complete digestion of dietary protein which then reduces fat deposition by some mechanism; perhaps one which involves one or more amino acids specifically. It seems likely from our data that, under our conditions, carbohydrate may effect liver fat and growth by controlling the rate of absorption of the necessary amino acids from the gut by affecting the rate of passage of the diet through the gut or affecting the microorganisms for production of necessary vitamins, or both. Whole protein, such as casein or gelatin, apparently supplies necessary amino acids for controlling liver fat deposition. While certain individual amino acids exert a slight effect on the reduction of liver lipids, it appears that combinations of these amino acids are necessary to bring about a greater reduction in liver fat deposition. (Harper, Monson, Elvehjem work in progress.)

Summary. A number of factors under our experimental conditions influence fat deposition in the liver: the availability of niacin to the animal; the type of carbohydrate used in the ration and type and level of protein used. Individual amino acids and mixtures of amino acids which support optimal growth conditions do not produce minimum levels of fat

in the liver.

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Fecal Electrolytes and Nitrogen During Cortisone or ACTH Therapy.*† (19905)

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In view of the established effects of cortisone, ACTH, and DOCA on the excretion of certain body constituents through the kidney and the skin(1-6), the possible influence of two of these agents on the composition of stools has been studied in a series of patients. Compound E, 50 to 1000 mg per day, or ACTH, 10 to 100 mg per day administered for 3 to 50 or more days, did not produce any characteristic alteration in the output of electrolytes and nitrogen. Collection of control data to establish this fact has incidentally provided information about the amounts of sodium, potassium, chloride and nitrogen excreted in feces during maintenance of a miscellaneous group of subjects on certain types of milk diets.

Materials and methods. Stools have been collected from hospitalized children and adults while on one of the following dietary regimens supplemented by vitamins and iron: a) essentially complete sodium restriction, *i.e.*, low-sodium milk fortified with carbohydrate and milk protein, providing an adequate nitrogen and caloric intake, or b) a whole milk diet similarly modified. Patients with gastrointestinal disorders were excluded, but the available clinical material made it impossible to limit the study to any one category of patients with respect to age or disease. This study is therefore based upon analyses of stools from a miscelleneous group of children and adults ill with rheumatic fever, diabetes mellitus, rheumatoid arthritis, leukemia, muscular disorders, *et cetera*. The daily intake of formula usually exceeded two liters but the seriously ill patients at times took less than the prescribed amount.

The *output* of sodium, potassium, chloride and of nitrogen in formed stools has been measured in control studies as well as during periods of therapy with cortisone or ACTH. Water was taken as desired. The beginning and end of a stool collection period, usually

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