COMPARISON OF THE LIPOTROPIC EFFECTS OF CHOLINE, INOSITOL, AND LIPOCAIC IN RATS

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In a preliminary communication (1) we reported that inositol would prevent the development of that type of fatty liver, characterized by a high content of cholesterol, which is produced in rats by the administration of biotin. The lipotropic action of inositol has been studied with other types of fatty livers and has been compared with the effects of two other lipotropic agents, choline and lipocaic.

Methods

Rats have been employed as test animals. The strain, age, and care have been previously described (2). To diminish the stores of B vitamins and of fat, the animals were fed Diet 1 for 3 weeks. During the 4th week the diet was varied as indicated for each series, the composition of the diets being given in Table I. Vitamin supplements were administered during the 4th week in the quantities previously used (4); the amounts of choline, lipocaic, and inositol used in each series are given in Tables II and III. Estimations of total crude fatty acids and of cholesterol were made by methods previously reported (2, 4). Results of these determinations are given as averages for groups of ten animals.

EXPERIMENTAL

Series A—This series was planned to compare the effects of choline, lipocaic, and inositol upon fatty livers caused by biotin. Diet 1 was fed throughout the experiment. During the supplemental period all animals received thiamine, riboflavin, pyridoxine, nicotinic acid, and calcium pantothenate; choline, biotin, inositol, and lipocaic were administered as indicated in Table II.

Series B—Since the results of Series A had indicated that inositol was concerned with cholesterol metabolism, it seemed advisable to test its effect upon rats fed cholesterol in a fat-free diet. Diet 1 was given during the depletion period and Diet 2 during the 4th week with thiamine, riboflavin, pyridoxine, nicotinic acid, and calcium pantothenate supplied to all animals. In those groups which received choline, an increased amount (30 mg. per rat per day) was administered, since Best and Ridout (5) had

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shown that large doses of choline would partially prevent the production of fatty livers in rats fed cholesterol.

TABLE I

Composition of Diets

Constituent	Diet 1	Diet 2	Diet 3
Casein, Labco, vitamin-free	10	10	10
Agar	2	2	2
Salt mixture (Steenbock-Nelson Salts 40 (3))	4	4	4
Sucrose	84	82	52
Cholesterol	0	2	2
Corn oil (Mazola)	0	0	30
Cod liver oil concentrate (Ayerst, McKenna, and Harrison)	0.015	0.015	0.015

TABLE II

Averages for Groups of Ten Rats Obtained during 4th Week of Experiment

Series	Basal diet No.	Special supplements	Crude fatty acids		Cholesterol	
			Liver	Body	Liver	Body
			per cent	*per cent*	*mg.*	*mg.*
A	1	None	15.3	5.6	19	95
		10 mg. choline	6.7	5.7	12	102
		5 γ biotin, 10 mg. choline	15.6	5.9	37	93
		5 " " 10 " " 200 mg. lipocaic	3.7	7.8	4	121
		5 " " 10 " " 2 mg. inositol	6.3	6.8	13	102
B	2	None	24.6	6.5	69	243
		30 mg. choline	9.1	7.3	37	279
		300 " lipocaic	13.8	6.0	36	254
		10 " inositol	13.3	6.9	27	298
		30 " choline, 300 mg. lipocaic	7.3	6.1	30	225
		30 " " 10 " inositol	3.9	7.1	18	264
C	3	None	26.0	9.5	72	205
		30 mg. choline	11.7	14.9	93	316
		300 " lipocaic	26.0	10.9	100	276
		10 " inositol	17.1	10.6	56	264
		30 " choline, 300 mg. lipocaic	8.7	13.6	71	215
		30 " " 10 " inositol	9.1	14.1	72	281
D	1	25 γ thiamine	10.2	4.3	15	142
		25 " " 10 mg. choline	3.1	4.2	8	132
		25 " " 10 " inositol	9.2	4.9	13	164
		25 " " 10 " choline, 10 mg. inositol	2.9	4.2	7	138

Series C—In this case a high fat diet (No. 3) was used during the supplemental period, so that a comparison could be made with Series B, in

which a diet practically devoid of fat was employed. Otherwise, the treatment of the animals was the same as in Series B.

Series D—It has been reported previously from this laboratory that choline will prevent fatty livers caused by thiamine (6). This series deals with the effect of inositol upon this type of fatty liver. Diet 1 was used throughout the experiment; thiamine was the only B vitamin supplement.

Series E—As Series D had indicated that inositol had no appreciable effect upon thiamine fatty livers, its action when administered with several other B vitamins was investigated. Diet 1 was employed during all 4 weeks; the supplements used and the results obtained are given in Table III.

TABLE III

Effect of Inositol in Prevention of Fatty Livers with Various B Vitamins

Series E on basal Diet 1.

Special supplements										
Thiamine		+	+	+	+	+	+	+	+	+
Riboflavin		+	+	+	+	+	+	+	+	+
Pyridoxine		+	+	+	+	+	+	+	+	+
Nicotinic acid						+	+	+	+	+
Pantothenic acid				+	+			+	+	+
Inositol, 10 mg.			+		+		+		+	
Choline, 10 "										+
Crude fatty acids										
Liver, %	2.9	20.4	12.2	22.9	18.5	15.7	9.4	25.9	16.5	8.4
Body, %	2.0	5.4	5.9	6.4	6.7	5.1	4.9	6.8	7.3	6.8

DISCUSSION

While choline has little effect in preventing biotin fatty livers, as has been reported previously (4), both lipocaic and inositol have definite effects in preventing increases in both fatty acids and total cholesterol in the liver. It should be noted that the dosage of inositol used in Series A was small; in similar experiments amounts of 5 to 10 mg. were found to be as effective as 200 mg. of the lipocaic preparation.

When fatty livers are produced by feeding a fat-free diet containing cholesterol, choline, lipocaic, and inositol all have lipotropic action, not only with regard to fatty acids but also with regard to cholesterol. Under these conditions lipocaic or inositol is more effective when fed with choline than when supplied alone; this is particularly true in the case of inositol.

In Series A and B an increase in fat was obtained by synthesis. In Series C a comparison was made with animals receiving a high fat diet, and fatty livers were produced by feeding cholesterol. While choline and inositol showed lipotropic action, lipocaic appeared to be entirely ineffective.

This observation was confirmed in three other experiments. The lipocaic, which was kindly supplied by Dr. Lester Dragstedt, was a sample of material that had been effective in depancreatized dogs; its potency for rats was proved by the prevention of biotin fatty livers.

While inositol is without obvious effect on thiamine fatty livers, the addition of other B vitamins as supplements enabled inositol to exert appreciable lipotropic action. The dosages of choline and inositol were the same in both Series D and E. It is noteworthy that neither supplement maintained liver fat at a normal level in Series E. Apparently the addition of other B vitamins diminishes the effect of choline but makes possible a response with inositol.

It has been pointed out previously from these laboratories (7) that there are several different types of fatty livers. We have little information as to how these livers differ in composition, but they can be distinguished in two ways: causative agents, and response to lipotropic factors. In describing the action of a lipotropic substance it is essential, in the light of present knowledge, to give the method of production of the particular fatty liver used. Reports of inactivity of lipocaic in rats were due to failure to use a suitable type of fatty liver. Even choline has no appreciable effect in preventing biotin fatty livers.

A previous, preliminary report by two of us (1) stated that either inositol or lipocaic could be used for the prevention of biotin fatty livers. The impression could have been secured easily from this report that lipocaic owed its activity to inositol; at that time this possibility seemed very likely to us. The data now reported show that lipocaic is ineffective with one type of fatty liver in rats, while inositol is active. This observation provides a means of distinguishing between inositol and lipocaic as lipotropic agents and it appears that lipocaic may contain a factor other than choline (or choline precursors) or inositol. However, all lipocaic preparations examined in these laboratories contained appreciable amounts of inositol. It may be that the inositol is present in a compound from which it is set free only under certain conditions. Available evidence is not sufficient to permit a decision as to whether lipocaic owes its activity to inositol or to an unknown constituent.

SUMMARY

A comparison of the lipotropic effects of choline, lipocaic, and inositol has been made with various types of fatty livers caused by diet in rats. Choline is effective for thiamine fatty livers, and partially effective with cholesterol fatty livers, but shows little activity with biotin fatty livers. Against this last type both inositol and lipocaic are active. Lipocaic apparently differs from inositol in being ineffective against fatty livers caused

by feeding cholesterol with a high fat diet. Inositol shows no activity with thiamine fatty livers; the addition of other B vitamins permits inositol to be lipotropic.

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