Lipotropic Factors and the Fatty Liver Produced by Feeding Cholesterol

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The excessive deposition of liver lipids which is produced in rats by feeding diets containing cholesterol is inhibited by choline and betaine (Best & Ridout, 1933; Best, Channon & Ridout, 1934). Choline was shown to affect both glycerides and cholesteryl esters but deposition of the former was inhibited more than that of the latter. Many preventive and curative experiments were subsequently reported and the results were in general agreement with those of the earlier investigations.

In the early studies no evidence of any significant waning in the lipotropic effect of choline was observed with or without cholesterol in the diet (Best & Ridout, 1936). Himsworth & Glynn (1944), however, report that this occurs in cholesterol-fed rabbits under certain experimental conditions. Furthermore, it has been stated by McHenry & Patterson (1944) that inositol but not choline has a definite effect on cholesterol metabolism. It became evident, therefore, that further studies were necessary to determine (*a*) the relative lipotropic effects of choline and inositol, and (*b*) the duration of the action of these agents when fed separately and in combination.

These problems have been reinvestigated in cholesterol-fed rats observed for periods up to 16 weeks. The effectiveness of choline in preventing deposition of cholesteryl esters did not diminish during this period, while that of inositol, which initially was definitely less than that of choline, progressively decreased. Under certain conditions inositol exerted no lipotropic action.

EXPERIMENTAL

White rats of the Wistar strain, reared in our own colony, were used. A few days before an experiment was started the animals were placed in individual cages, with a false bottom of coarse wire screen, in order to accustom them to the environment. The groups in each experiment were then balanced as far as possible with respect to weight and sex.

Weighed amounts of fresh diet were given daily, in feed trays designed to minimize spilling, and the following morning the amount left over and the scatter were weighed. From these data the individual daily food consumptions were calculated. The average values are recorded in the Tables. The rats were 'group pair-fed' and cared for as described previously (Best, Lucas, Patterson & Ridout, 1946).

The composition of the basal diets is given in Table 1. The supplements used in the test diets are shown in Tables 2–4. Choline was incorporated as its chloride, in molecularly equivalent amount, e.g. 0·345% choline chloride was used in diets stated to contain 0·30% choline.

In Exp. 1 the test diets (see Table 2) were fed for periods of 3, 8 and 16 weeks in order to determine whether any

Table 1. *Percentage composition of basal diets*

Component	Content (%) in diet nos. 1	1A	2	2A	3	3A	3B	3C	3D
Casein	8	8	8	8	10	10	10	10	10
Gelatin	12	12	12	12	—	—	—	—	—
Sucrose	60	59·5	73	70	84	81	51	51	51
Salts	5	5	5	5	4	4	4	4	4
Cellu flour	2	2	2	2	2	2	2	2	2
Beef dripping	10	10	0	0	0	0	30	—	20
Corn oil	2	2	0	2	0	0	—	30	10
Vitamin powder*	1†	1†	‡	‡	0	1	1	1	1
C.L.O. concentrate§	0·010	0·010	0·010	0·010	0·015	0·015	0·015	0·015	0·015
Cholesterol	0	0·5	0	1	0	2	2	2	2

* The vitamin powder consisted of aneurin hydrochloride 500 mg., riboflavin 250 mg., pyridoxin 200 mg., calcium pantothenate 1000 mg. and nicotinic acid 1000 mg. made to 1000 g. with very finely powdered (100 mesh) sucrose. Rats eating 10 g. of food per day receive 50 μg. aneurin hydrochloride and proportional amounts of the other vitamins daily.

† Diets 1 and 1 A contained not only the usual B vitamins but also per 10 g. diet, 1·5 mg. vitamin E (α-tocopheryl acetate) and 50 μg. vitamin K (2-methyl 1, 4-naphthoquinone).

‡ The rats received daily subcutaneous injections of the following B vitamins in 0·5 ml. physiological saline; aneurin hydrochloride 50 μg., riboflavin 25 μg., pyridoxin 20 μg., calcium pantothenate 100 μg. and nicotinic acid 100 μg.

§ The cod-liver oil concentrate (Ayerst, McKenna & Harrison, Ltd. Montreal), contained 200,000 i.u. vitamin A and 50,000 i.u. vitamin D/g.

waning of the lipotropic effect of choline or inositol occurred during this period. In Exp. 2, of duration 8 weeks, a comparison was made of the relative effectiveness of choline and inositol, singly and in combination, when fed at several different levels, in diets with and without added cholesterol (Table 3). The rats in Exp. 3 were fed a ration devoid of B vitamins for a preliminary period of 3 weeks. Comparable groups of these animals were then fed the cholesterol-containing test diets (Table 4) for 1 week to examine the effect of the preliminary period of vitamin depletion (similar to that used by McHenry and his associates) upon the relative efficacy of the lipotropic factors.

The animals were stunned and the livers removed immediately. The liver lipids were extracted with hot absolute ethanol and analyzed as described by Best *et al.* 1946.

RESULTS

The data for total lipids (Tables 2–4) are presented as percentage of wet liver weight for ease of comparison with many values in the literature. The individual components of the liver lipids are reported as mg./liver. From these values and the other data in the Tables, each component may be calculated to either a wet weight or fat-free dry residue weight basis. For many purposes the latter method of expression is to be preferred. The magnitude of normal values for liver lipids expressed as percentage of dry, fat-free residue weight, was determined to serve as a base for comparison. Livers of ten rats (125–160 g.) on a stock diet (Master fox breeder cubes), analyzed individually, gave: total lipids, average 25·3 (range 20·1–31·6); phospholipids 11·8 (10·2–12·9); free cholesterol 0·87 (0·73–0·98); total cholesterol 1·02 (0·90–1·21); cholesteryl esters, calculated as oleate 0·26 (0·18–0·36); glyceride 12·4 (8·8–17·7). A summary of the more pertinent data, calculated on the basis of fat-free dry residue, is given in Table 5.

DISCUSSION

Exp. 1. The results (Tables 2 and 5) provide further evidence that the lipotropic action of choline is not evanescent. They do not support the claim that inositol is more effective than choline upon cholesteryl esters.

Total liver lipids of rats on the cholesterol-free ration (diet 1, groups 1, 5 and 9) continued to increase beyond the three-week period frequently used in previous studies. The value obtained at 8 weeks was, however, just as high as at 16 weeks. As the glycerides accumulated in the liver, cholesteryl esters tended to rise proportionately, whether dietary cholesterol was present or not (cf. also groups 2, 6 and 10, in which cholesterol was present in the diet). Comparison of these two sets of data shows that the amount of cholesterol in the diet affects the ratio in which cholesteryl esters and glycerides are deposited. A graphic representation

Table 2. *Effects of dietary supplements on the rate of deposition and composition of liver lipids*

(Average initial weight of rats 150 g. (range 125–175 g.).)

Basal diet no.	Group no.	Addition to diet: Substance	Addition to diet: %	Survivors/ Starters	Average food intake (g./day)	Weight change (%)	Liver: Average wet weight (g.)	Liver: Average fat-free dry residue weight (g.)	Liver: Total lipids (% wet liver wt.)	Composition of liver lipids (mg./liver): Total lipids	Phospholipids	Cholesterol: Free	Cholesterol: Total	Cholesterol: Ester*	Glyceride
Test diets fed for 3 weeks															
1	1	None	—	12/12	10·4	+ 2	10·4	1·748	27·19	2833	210	14·6	31·7	29	2579
1A	2	None	—	11/12	10·6	5	11·3	1·720	37·69	4248	248	20·9	147·7	214	3765
	3	Choline	0·3	10/12	11·5	12	8·5	1·779	8·38	712	178	16·5	89·6	123	394
	4	Inositol	0·3	11/12	10·9	3	11·1	1·651	30·35	3373	248	20·0	117·5	164	2941
Test diets fed for 8 weeks															
1	5	None	—	8/12	10·1	+ 3	13·3	1·837	37·50	4973	236	20·4	78·1	'97	4619
1A	6	None	—	9/12	10·6	9	16·2	2·075	37·59	6089	280	37·7	412·1	631	5141
	7	Choline	0·3	10/12	9·9	27	7·8	1·748	8·08	627	236	16·8	91·7	126	248
	8	Inositol	0·3	7/12	10·0	6	13·4	1·825	39·31	5246	270	33·6	273·9	405	4538
Test diets fed for 16 weeks															
1	9	None	—	10/12	10·0	+15	14·6	2·042	36·68	5420	247	23·5	78·5	93	5057
1A	10	None	—	9/12	10·0	8	16·7	2·051	38·66	6463	230	44·5	490·3	753	5436
	11	Choline	0·3	9/12	10·4	54	8·7	2·101	7·71	672	253	18·8	73·7	92	308
	12	Inositol	0·3	8/12	10·3	33	14·9	2·042	36·29	5418	246	34·4	400·7	617	4521

In this and subsequent Tables, the cholesteryl ester value has been expressed as cholesteryl oleate, obtained by multiplying the weight of bound cholesterol by the factor 1·684.

Table 3. *Lipotropic effects of choline and inositol with and without cholesterol*

Basal diet no.	Group no.	Addition to diet: Substance	Addition to diet: %	Survivors/ Starters	Average food intake (g./day)	Weight change (%)	Liver: Average wet weight (g.)	Liver: Average fat-free dry residue weight (g.)	Liver: Total lipids (% wet liver wt.)	Composition of liver lipids (mg./liver): Total lipids	Phospholipids	Cholesterol: Free	Cholesterol: Total	Cholesterol: Ester*	Glyceride
2*	13	None	—	21/22	11·5	0	10·5	1·584	31·24	3238	240	18·6	77·7	100	2879
	14	Choline	0·1	9/10	11·4	+14	7·9	1·792	8·30	659	266	13·8	26·1	21	359
	15	Choline	0·5	12/12	11·3	+ 9	7·0	1·613	8·26	582	273	15·0	24·6	16	278
	16	Inositol	0·1	9/10	10·6	+ 4	8·0	1·442	21·81	1744	228	14·8	38·9	41	1461
	17	Inositol	0·3	11/12	11·4	− 4	8·7	1·615	23·34	2084	264	17·6	41·6	40	1762
	18	Choline + inositol	0·1 + 0·1	9/10	11·5	+19	6·7	1·510	7·18	483	249	13·3	21·7	14	208
	19	Choline + inositol	0·1 + 0·3	10/10	10·9	+17	7·0	1·617	6·44	453	239	12·8	18·8	10	191
	20	Choline + inositol	0·5 + 0·3	11/12	11·7	+10	6·7	1·615	6·20	415	252	15·0	18·2	5	142
2A*	21	None	—	20/22	11·3	+ 4	12·1	1·591	39·71	4824	283	28·9	316·7	485	4027
	22	Choline	0·1	10/10	10·8	+26	7·0	1·493	15·00	1056	280	16·5	112·5	162	597
	23	Choline	0·5	11/12	11·4	+ 6	6·2	1·454	8·95	556	241	16·4	56·8	68	231
	24	Inositol	0·1	10/10	10·8	+18	11·2	1·356	34·86	3915	265	23·0	250·0	382	3245
	25	Inositol	0·3	11/12	11·1	+ 1	11·4	1·523	36·04	4109	275	26·4	231·8	346	3462
	26	Choline + inositol	0·1 + 0·1	10/10	11·2	+25	6·6	1·481	12·20	808	271	17·0	118·9	168	352
	27	Choline + inositol	0·1 + 0·3	9/10	10·5	+21	6·0	1·375	10·28	620	241	14·3	58·3	74	290
	28	Choline + inositol	0·5 + 0·3	11/12	11·9	+11	6·5	1·555	7·52	485	275	16·4	30·7	24	169

* Limited availability of animals necessitated doing the experiment in two sections with a time interval of about a month. The average weight of the 12 animals used in each group in the first part was 175 g. (range 140–220 g.) and of the 10 in the second part was 140 g. (range 80–200 g.). The difference in size of rats used in the two parts of the experiment did not affect appreciably the total liver lipids (the values in the two basal groups were: diet 2, 33·11 and 28·74, diet 2A, 38·83 and 40·78% of wet liver weight) or the composition of the lipids. The figures given in the Table for basal Groups 13 and 21 are weighted averages of the two sets of values.

of another example of this relationship has been published recently (Best *et al.* 1946).

When the basal diet contained cholesterol (groups 2, 6 and 10) the liver lipids appeared to reach a maximum within 3 weeks, if calculated as percentage of wet liver weight. If expressed as percentage of fat-free dry residue weight, however, it will be noted (Table 5) that the liver lipids continued to increase throughout the test period, the values at 3, 8 and 16 weeks being 247, 294 and 315%, respectively. This presumably means that, after 3 weeks, fat and water tend to be deposited in the same ratio. The increase in fat was due mainly to accumulation of glycerides with smaller increments due to cholesteryl esters.

The inclusion of 0·3% choline (groups 3, 7 and 11) in the diet kept the total liver lipids at a low level (about 8% of wet liver weight, which is only slightly above normal) throughout the whole 16-week period.* Inositol fed at the same level (groups 4, 8 and 12) was never nearly as effective as choline. At 3 weeks choline had reduced total lipids from 247 to 40% of fat-free dry residue weight, while inositol had brought the value down only to 204%, and at 16 weeks the influence of inositol was even less (Table 5).

Free cholesterol showed a small and possibly insignificant rise in the livers of the animals on the cholesterol-free ration (diet 1, groups 1, 5 and 9) as the experiment was prolonged (Table 5). The presence of 0·5% cholesterol (diet 1A, groups 2, 6 and 10) caused a slightly greater and probably significant increase in the free sterol. Chanutin & Ludewig (1933) have also noted increases in free cholesterol after feeding cholesterol under somewhat different conditions. Choline tended to restore the level of free cholesterol in the liver to normal (Table 5), while inositol had a negligible effect.

The cholesterol intake (0·5%), while considerably smaller than in some previous experiments, was still greatly in excess of the amount contained in the normal diet of rats. While cholesteryl esters were not reduced to normal values by 0·3% choline there was a marked and progressive reduction,

* Himsworth & Glynn (1944) noted an evanescent effect of 4 mg. choline daily in rats fed a diet containing 50% lard. Data to be published shortly indicate that this amount of choline is insufficient to maintain liver fat at low levels under these conditions.

Table 4. *Effect of preliminary depletion of B vitamins on lipotropic activity*

(Average initial weight of rats at beginning of test period 65 g. (range 55–85 g.).)

Basal diet no.	Group no.	Addition to diet: Substance	Addition to diet: %	Survivors/ Starters	Average food intake (g./day)	Weight change (%)	Liver: Average wet weight (g.)	Liver: Average fat-free dry residue weight (g.)	Liver: Total lipids (% wet liver wt.)	Composition of liver lipids (mg./liver): Total lipids	Phospholipids	Cholesterol: Free	Cholesterol: Total	Cholesterol: Ester*	Glyceride
3	29	None (Vit. B depletion)	—	6/6	7·2	−13	4·9	1·226	4·83	238	151	7·9	8·2	1	79
3A	30	None	—	12/12	10·2	+18	8·1	1·760	20·93	1701	199	15·4	50·0	58	1428
	31	Choline	0·5	12/12	10·2	+18	6·2	1·308	12·63	787	178	13·6	35·0	36	559
	32	Inositol	0·5	12/12	10·2	+20	6·3	1·280	15·78	998	178	12·5	36·5	41	766
3B	33	None	—	9/10	5·4	+ 5	6·1	0·833	32·43	1971	128	13·7	83·3	117	1712
	34	Choline	0·5	9/10	5·8	+18	4·5	0·952	10·95	490	123	11·4	30·7	33	324
	35	Inositol	0·5	9/10	5·3	+ 8	5·4	0·785	28·33	1523	117	12·6	76·4	107	1286
	36	Choline + inositol	0·5 + 0·5	10/10	5·9	+18	4·8	1·042	7·28	350	116	11·9	20·3	14	209
3C	37	None	—	8/10	5·0	+ 9	6·8	1·013	24·47	1670	131	12·3	80·7	115	1411
	38	Choline	0·5	9/10	5·3	+21	5·1	1·086	16·40	769	163	13·5	34·7	36	557
	39	Inositol	0·5	8/10	5·3	+13	6·2	0·912	30·96	1931	118	13·8	87·2	124	1675
	40	Choline + inositol	0·5 + 0·5	8/10	6·0	+22	5·1	1·133	8·71	449	118	10·9	35·9	42	278
3D	41	None	—	11/12	7·2	+ 6	7·4	1·076	35·96	2663	184	16·4	97·0	136	2327
	42	Choline	0·5	12/12	7·1	+ 7	4·6	1·092	8·32	386	173	12·0	43·1	53	148
	43	Inositol	0·5	12/12	7·0	+ 7	6·4	1·051	31·61	2088	173	14·8	106·3	154	1745

compared with the controls, throughout the 16-week period; at 3 weeks 0·3 % inositol was not as effective in reducing steryl esters as was choline and its effectiveness diminished as the experiment was prolonged.

The constancy of the phospholipid values (12·0, 11·3, 12·0, 12·0 % fat-free, dry residue weight for Groups 9, 10, 11 and 12, respectively) is remarkable in view of the large changes in glyceride and cholesteryl esters.

Exp. 2. The results of this experiment (Table 3), in which choline and inositol were fed singly and in combination, at several different levels, for 8 weeks confirm and extend the above-mentioned findings. The first comparisons were made using fat-free test diets (diet 2, groups 13–20) and a similar study was made with diets containing 2 % corn oil (Mazola) plus 1 % cholesterol (diet 2A, groups 21–28).

On the fat-free diets choline fed at the 0·1 % level was almost as effective on all the lipid components as when 0·5 % was provided (compare groups 14 and 15, Table 5). In the presence of cholesterol (groups 22 and 23) the smaller dose of choline was inferior to the larger. The latter brought the liver glycerides nearly to normal and caused an 85 % reduction in cholesteryl esters. Therefore even large amounts of dietary cholesterol (1 %) do not produce a condition as unfavourable for the action of choline as one might be led to expect from statements in the literature (McHenry & Patterson, 1944).

Inositol at 0·1 % dietary level exerted only a very limited lipotropic effect in the rats on the fat-free diet (Group 16) and even less in the presence of the corn oil plus cholesterol (Group 24). Three times as large an intake of inositol had no significantly greater effect on the glycerides and produced only a slightly greater decrease in cholesteryl esters.

In Tables 3 and 5 the effects of various combinations of choline and inositol are also presented. Inositol (0·1 %) added to the diet free from fat and cholesterol (diet 2) did not augment the effect of 0·1 % choline on total lipids or glycerides (compare groups 14 and 18, Table 5). The slight decrease in cholesteryl esters is of questionable significance. When the amount of inositol was increased to 0·3 % a slight but definite effect on both glycerides and steryl esters was noted (groups 14 and 19). Addition of 0·3 % of inositol to a diet already containing 0·5 % choline brought both glycerides and cholesteryl esters within the normal range (groups 15 and 20).

In diets containing fat and cholesterol (groups 21–28), the combined effects of choline and inositol in the amounts mentioned above were qualitatively

Table 5. *Composition of liver lipids*

Basa diet	Group no.	Supplement	%	Duration of exp. (days)	Liver: Total lipid	Liver: Free cholesterol	Liver: Cholesteryl ester	Liver: Glyceride
					% fat-free, dry residue weight			
1	1	None	—	21	162	0·83	1·7	148
1A	2	None	—	21	247	1·21	12·4	219
	3	Choline	0·3	21	40	0·93	6·9	22
	4	Inositol	0·3	21	204	1·21	9·9	178
1	5	None	—	56	271	1·10	5·3	252
1A	6	None	—	56	294	1·82	30·4	248
	7	Choline	0·3	56	36	0·96	7·2	14
	8	Inositol	0·3	56	288	1·84	22·2	249
1	9	None	—	112	265	1·15	4·5	247
1A	10	None	—	112	315	2·17	36·7	265
	11	Choline	0·3	112	32	0·90	4·4	15
	12	Inositol	0·3	112	265	1·69	30·2	221
2	13	None	—	56	218	1·17	6·3	195
	14	Choline	0·1	56	52	0·78	1·2	35
	15	Choline	0·5	56	36	0·94	1·0	17
	16	Inositol	0·1	56	168	1·02	2·8	148
	17	Inositol	0·3	56	137	1·18	2·7	115
	18	Choline 0·1 + inositol	0·1	56	53	0·88	0·9	35
	19	Choline 0·1 + inositol	0·3	56	40	0·79	0·6	24
	20	Choline 0·5 + inositol	0·3	56	26	0·93	0·3	9
2A	21	None	—	56	301	1·82	30·6	252
	22	Choline	0·1	56	71	1·11	10·8	40
	23	Choline	0·5	56	38	1·13	4·7	16
	24	Inositol	0·1	56	289	1·68	28·2	239
	25	Inositol	0·3	56	270	1·73	22·7	227
	26	Chloine 0·1 + inositol	0·1	56	55	1·15	11·4	24
	27	Choline 0·1 + inositol	0·3	56	45	1·04	5·4	21
	28	Choline 0·5 + inositol	0·3	56	31	1·05	1·6	11
3	29	None (Vit. B depletion)	—	21	19	0·65	0·05	6
3A	30	None	—	7	97	0·88	3·3	81
	31	Choline	0·5	7	60	1·04	2·8	43
	32	Inositol	0·5	7	78	0·98	3·2	60
3B	33	None	—	7	237	1·64	14·1	205
	34	Choline	0·5	7	52	1·20	3·4	34
	35	Inositol	0·5	7	194	1·60	13·7	164
	36	Choline 0·5 + inositol	0·5	7	34	1·14	1·4	20
3C	37	None	—	7	165	1·21	11·4	139
	38	Choline	0·5	7	71	1·25	3·3	51
	39	Inositol	0·5	7	212	1·51	13·6	184
	40	Choline 0·5 + inositol	0·5	7	40	0·97	3·7	25
3D	41	None	—	7	248	1·52	12·6	216
	42	Choline	0·5	7	35	1·10	4·8	14
	43	Inositol	0·5	7	199	1·40	14·6	166

similar to those described in the preceding paragraph. The level of steryl esters in the basal group (21) was increased five-fold by feeding cholesterol. A profound decrease was produced by 0·1% choline. This effect was not augmented by 0·1% inositol but was by 0·3%. The level of steryl esters was lowered still further when choline 0·5% and inositol 0·3% were supplied (group 28) but the value remained above normal. Thus the synergism of choline and inositol can be clearly demonstrated

at certain dosage levels, but here again there is no evidence of any preferential effect of inositol on cholesteryl esters.

Exp. 3. This experiment was designed to compare the relative effectiveness of choline and inositol after a preliminary three-week period of depletion of B-vitamins (the procedure followed by McHenry and his associates). The influence of dietary fat upon the liver lipids of cholesterol-fed animals was also determined. Diet 3A contained 2% cholesterol without any fat, 3B contained an essentially saturated fat (beef dripping 30%), 3C contained the same amount of an unsaturated oil (Mazola) and 3D contained a mixture of these two fats (beef dripping 20%, Mazola 10%).

During the depletion period (diet 3), the food consumption fell from about 8 g. per day to about 3 g. The animals lost weight and several died. The survivors were divided into comparable groups to which the test diets were fed for 1 week only.

In every case choline was more effective than inositol in reducing total liver lipids and cholesteryl esters. The presence of fat in the basal diets (3B, 3C and 3D) increased the total liver lipids greatly. It appears to have caused a slight rise in the free cholesterol (Table 5) and about a four-fold increase in the steryl esters (compare group 30 with 33, 37, 41). The nature of the dietary fat influenced the deposition of glycerides in rats on the basal diets and, to a much smaller extent, that of steryl esters (compare groups 33 and 37). The slightly lower cholesteryl ester value in the rats receiving the corn oil (group 37) as compared with those on beef dripping (group 33) is probably a reflexion of the lower glyceride content of the livers of the 'corn-oil group'. It would appear from the data for groups 34 and 38 that choline is somewhat more effective in reducing glycerides in animals on beef dripping than it is when corn oil is the dietary fat. Inositol produced a small decrease in glycerides in rats on the beef dripping, but seemed to increase the deposition of glycerides in the 'corn-oil group'. This effect, which requires further study, has been noted previously (Beveridge & Lucas, 1945; Handler, 1946). The synergistic effect of choline and inositol was again evident.

When the two fats were present (Basal Diet 3D) inositol had some effect on glycerides, in contrast to the lack of effect on the 'corn-oil diet' (3C) (cf. groups 37, 39 with 41, 43). However, there was no lipotropic effect of inositol on steryl esters. In the presence of beef dripping there appears to be a partial removal of the blockage of the lipotropic effect of inositol which is observed in the diet containing corn oil alone. An almost completely saturated fat-fraction from beef dripping and a highly unsaturated one from corn oil, have been prepared and their influence upon the lipotropic activity of inositol is being studied.

SUMMARY

1. No waning of the lipotropic effect of choline was observed in rats fed a diet containing 0·5% cholesterol for 16 weeks.

2. Inositol, fed at the same level as choline, was never nearly as active in decreasing glycerides or cholesteryl esters and its effectiveness diminished as the experiment was prolonged.

3. The synergistic lipotropic effect of choline plus inositol was clearly demonstrated in cholesterol-fed rats but inositol did not exert any preferential effect on cholesteryl esters.

4. In experiments, similar to those of McHenry & Patterson (1944) and of Handler (1946) in which the rats were given a preliminary period of deficiency of vitamin B_1, choline was uniformly more effective than inositol in decreasing liver glycerides and cholesteryl esters in cholesterol-fed rats receiving diets with or without fat. These results agree with those reported by Handler (1946).

5. Further evidence that the nature of the dietary fat affects the lipotropic action of inositol has been presented and briefly discussed.

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