14315

Lipotropic Action of Inositol.

J. C. FORBES.

From the Medical College of Virginia, Richmond, Virginia.

It has been shown by Gavin and McHenry[1] that the fatty livers which result from the administration of biotin to rats on a high carbohydrate-low protein diet are very resistant to the lipotropic action of choline but respond readily when inositol is given in addition. A recent publication by McHenry and his associates[2] shows that inositol is apparently inactive against the fatty livers which develop when thiamine is the only member of the B-vitamins fed, however, when riboflavin and pyridoxin, or these plus additional B vitamins are given in addition to thiamine, inositol is definitely lipotropic. Engel[3] also has shown that inositol exerts a lipotropic effect in the absence of biotin in the food. The results obtained in the present investigation are in good agreement with those of the above authors.

In order to control scattering of food, especially during the deficiency period, the food was made up as a concentrated aqueous solution containing insoluble material in suspension. This diet had the following composition:

Casein, vitamin free, Labco brand	120	g
Sucrose	1200	"
Salt mixture (Wesson)[4]	36	"
Cellu flour B*	12	"
Percomorph oil, 50%	0.5	cc
Water, distilled	960	"
Vitamin B-complex as given in text		

Experimental. White rats weighing 70-90 g were put in individual cages and fed daily 10-15 cc of the above diet, supplemented with 3 mg of thiamine chloride per 1200 g of sucrose. A 15 cc Folin and Wu pipet with a broken tip was used for measuring it. The amount given was so regulated that it was completely, or almost completely, consumed each day. After the animals were on this deficient diet for 3 weeks they were put on

TABLE I. Neutral Fat and Cholesterol Content of Liver, Body, and Skin.

Exp. No.	1			2			3			4			5			6			7		
Thiamine chloride	+																				
Riboflavin				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyridoxin chloride				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ca pantothenate				+	+	+	+	+	+				+	+	+	+	+	+	+	+	+
Niacin																					
Choline chloride, mg*																1	1	1	5	5	5
Inositol mg*		10	10	20	20	20	20	20	20	10	10	10	20	20	20	10	10	10	5	5	5
No. of rats	2	4	4	4	4	4	4	5	5	5	5	5	5	5	3	3	3	4	4	2	4
Daily avg gain wt, g	0.6	1.6	1.9	1.4	1.8	1.8	1.8	1.9	2.3	2.5	0.6	0.6	1.9	1.6	2.4	1.8	1.9	2.1			
Avg liver wt, g	4.75	5.25	5.18	6.76	5.66	5.32	5.26	5.40	5.20	5.50	5.00	6.05	5.10	4.73	4.98	5.88	4.62				
Liver neutral fat†	9.2	9.8	7.1	18.7	9.5	11.1	3.5	7.7	7.1	10.4	6.4	20.9	11.0	5.8	9.9	16.2	4.1				
Liver cholesterol	.45	.56	.45	.73	.45	.54	.33	.74	.48	.61	.54	.82	.73	.48	.55	.55	.56				
Body neutral fat†	5.5	7.7	8.3	6.2	8.5	6.6	6.7	9.8	9.8	6.0	6.3	8.8	8.7	9.4	8.5	8.9	9.9				
Body cholesterol†	.18	.16	.19	.15	.19	.18	.18	.22	.22	.19	.21	.17	.17	.16	.18	.17	.18				
Skin neutral fat†						14.8	15.2	21.3	21.3	13.2	14.7	17.0	15.6	20.4	17.2	17.0	21.3				
Skin cholesterol†						.21	.22	.18	.19	.27	.31	.17	.16	.14	.19	.18	.20				

* Mg per 10 g of sucrose in diet. † % wet weight. All animals in experiments 3, 4 and 5 received 0.5 γ of biotin per 10 g of sucrose in the diet during the supplemental feeding periods.

[1] Gavin, G., and McHenry, E. W., *J. Biol. Chem.*, 1941, **139**, 485.

[2] Gavin, G., Patterson, J. M., and McHenry, E. W., *J. Biol. Chem.*, 1943, **148**, 275.

[3] Engel, R. W., *J. Nutrition*, 1942, **24**, 175.

[4] Wesson, L. G., *Science*, 1932, **75**, 339.

* Obtained from Chicago Dietetic Supply House, Chicago.

additional supplements for a period of 9-11 days, following which the animals were sacrificed for lipid analyses. These supplements were always given at the following levels, riboflavin 9 mg, pyridoxine hydrochloride 6 mg, calcium pantothenate 21 mg, niacin 21 mg, and biotin 60 γ per 1200 g of sucrose. Choline chloride and inositol were administered as recorded in the accompanying table.

The animals were rapidly exsanguinated by decapitation at the end of the supplemental feeding period. Their livers were removed, weighed, and after grinding in a mortar, analyzed individually for neutral fat and cholesterol. The intestinal tract was removed and the animal skinned, after which the neutral fat and cholesterol content of both the bodies and skins from each group was collectively determined. The analytical procedures have been described previously.[5,6]

From the experimental results, which are recorded in Table I, it will be seen that the lipotropic action of inositol in the absence of biotin from the diet is confirmed. The effect, however, is much less than one would expect from choline under the same conditions. The effect of choline plus inositol is greater than of either alone but the data are not sufficient to show whether they act synergistically or not. The effect on liver cholesterol is, on the whole, less than on the neutral fat. The small amount of biotin used in experiments 3, 4 and 5 did not apparently influence the lipotropic action of the inositol, neither did it increase the lipid content of the livers in the absence of inositol. These results are contrary to those obtained by McHenry and his associates[1] but it is possible that if we had used larger amounts of biotin, choline-resistant inositol-sensitive fatty livers might have been obtained.

The neutral fat content of the body and skin of those animals receiving both choline and inositol is greater than that of the corresponding animals receiving these individually. Whether this is due to an increased food intake, as suggested by the greater rate of growth of these animals, or to a stimulating effect on fat synthesis, cannot be stated at this time as food intake was not sufficiently accurately controlled to justify definite conclusions.

Summary. The lipotropic action of inositol in the presence of other members of the vitamin B-complex has been confirmed. The effect of inositol plus choline is considerably greater than that of either alone.

[5] Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1940, **25**, 1157.

[6] Forbes, J. C., *J. Nutrition*, 1941, **22**, 359.

14316 P

Preparation of the Pituitary Corticotrophic Hormone.*

A. H. NEUFELD. (Introduced by J. B. Collip.)

From the Research Institute of Endocrinology, McGill University, Montreal, Canada.

The procedure used for the preparation of the corticotrophic hormone from whole and from dissected calf anterior pituitary tissue was based on methods recommended by Lyons, by Li, Simpson and Evans, and by Sayers, White and Long.[1-3] It has been our experience that most of the posterior lobe potency present in the starting material remains with the fraction showing corticotrophic activity. Attempts made to separate these factors by their solubilities at various pH and salt concentrations were without success. The same held true in utilizing dialysis

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[1] Lyons, W. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 645.

[2] Li, C. H., Simpson, M. E., and Evans, H. M., *Science*, 1942, **96**, 450.

[3] Sayers, G., White, A., and Long, C. N. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 199.