leased from the pigment glands, some of it reacts with the reactive groups in the meal and is "bound." Under such circumstances the gossypol is detoxified. For the combination products described in this publication the gossypol is rendered more toxic by a process which maintains it in solution. A more thorough study of the reactions of gossypol with amino acids, proteins, and carbohydrates, and of the effect of storage and heat on such products would undoubtedly clarify the nature of this combination product and probably throw light on the reaction that takes place when cottonseed or cottonseed meats are cooked in order to detoxify the gossypol.

Summary. (1) Gossypol was combined with proteins, amino acids, and carbohydrate materials by a procedure involving mixing of the gossypol and other combining substances in alkaline solutions and subsequent lyophilization of the neutralized frozen solutions. (2) Crystalline gossypol when added to the aquarium bath had no visible effect upon goldfish. The water-soluble combination products of gossypol were toxic to fish in concentrations as small as one part of gossypol product to a hundred thousand parts of water. (3) Separated pigment glands were toxic to fish but were significantly less toxic than the water-soluble combination products of gossypol.

Received May 10, 1950. P.S.E.B.M., 1950, v74.

Lipotropic Effects of Liver Extract, Vitamin B_{12} and Choline.* (17997)

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It has been reported that crude liver extract exerts a lipotropic effect and will prevent fibrosis of the liver when administered to rats receiving a high fat diet(1). The lipotropic effect of the liver extract did not seem to be due to choline content. In fact, other supplements which did not give protection against dietary induced liver injury had a higher content of choline than was present in liver extract(2). In further investigations a vitamin B_{12} concentrate, prepared from liver, also exerted a definite effect in preventing accumulation of fat in the liver of rats(3). In the present study the relationship between dosage of liver extract and lipotropic effect was studied. Observations were also made on the possible lipotropic effect of combinations of choline, inositol and folic acid, which are present in small amounts in liver extract and vitamin B_{12} concentrate. Crystalline vitamin B_{12} alone and in combination with small amounts of choline was also observed for lipotropic activity.

Methods. Male Sprague-Dawley rats weighing between 120 and 150 g were used. They were fed the high-fat diet (51% lard) and the control diet (6% lard) recently described(1). These diets were fed for the periods listed in the tables. When the animals were placed on the synthetic diet, injections of the supplements were begun. All supplements were administered subcutaneously except in one experiment with liver extract as noted in Table I. At the end of the study sections were taken from the left lobe of the liver and stained with hematoxylin and eosin or with Sudan III for fat. The remainder of the liver was analyzed for total

* The authors wish to thank Eli Lilly and Co. for the liver extract, Merck and Co. for the crystalline vit. B_{12} and Mead Johnson and Co. for the Oleum Percomorphum.

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2. Drill, V. A., and Hall, C. A., *Am. J. Med. Sciences,* 1950, v219, 197.

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TABLE I.
Effect of Subcutaneous and Oral Liver Extract on Hepatic Changes of Rats Fed a High-Fat Diet.

No. of rats	Diet	Liver extr., cc 3x week, γ	Avg wt gain, g	Avg liver wt, g/100 g rat	No. animals with hepatic fatty change: 0	1+-2+	3+-4+	Avg liver fat, %
			Exp. 1—66 days					
4	Normal	—	161± 3.1	4.0±0.37	3	1	0	5.6±1.08
8	H.F.*	—	43± 9.7	8.5±0.62	0	0	8	24.8±0.59
4	”	1.0 (s.q.)	77± 4.7	4.4±0.46	2	1	1	8.4±1.27
			Exp. 2—150 days					
6	Normal	—	172± 9.3	3.9±0.12	3	3	0	7.5±0.35
8	H.F.	—	63± 7.7	6.8±0.55	0	1	7	14.6±1.52
6	”	1.0 (s.q.)	114±10.9	4.9±0.25	0	5	1	9.7±1.21
			Exp. 3—92 days					
12	Normal	—	135± 4.9	3.9±0.08	9	3	0	6.8±0.49
9	H.F.	1.0 (oral)	51± 7.7	4.8±0.22	1	7	1	11.4±1.32
10	”	.75 (s.q.)	71± 6.1	4.1±0.12	3	7	0	14.8±1.54
10	”	.5 ”	51± 6.5	5.5±0.41	1	8	1	16.3±1.30
10	”	.25 ”	72± 5.0	6.9±0.44	0	3	7	20.3±1.74
12	”	—	9± 6.3	8.7±0.58	0	3	9	18.7±1.39

* H.F. = High fat diet.
Grading of fatty change:
0—None or only a rare large droplet.
1+—A few large droplets in each lobule.
2+—More than 1+, but half of lobule involved.
3+—Over half of lobule involved, but some cells fat free.
4+—Almost no fat free cells.

S.E. of the mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

TABLE II.
Effect of Crystalline Vitamin B_{12} on Hepatic Changes of Rats Fed a High-fat Diet.

No. of rats	Diet	Cryst. B_{12} 3x week, γ	Avg wt change, g	Avg liver wt, g/100 g rat	No. animals with hepatic fatty change: 0	1+-2+	3+-4+	Avg liver fat, %
			Exp. 1—29 days					
6	H.F.	—	+ 55± 6.1	8.1±0.47	0	0	6	31.5±1.18
4	”	0.2	+ 42± 3.3	5.8±0.52	1	1	2	21.4±2.97
			Exp. 2—30 days					
10	Normal	—	+ 77± 5.7	4.1±0.13	9	1	0	4.8±1.20
10	H.F.	—	+ 26± 5.2	4.7±0.20	0	8	2	17.5±1.49
10	”	1.0	+ 33± 4.1	5.6±0.36	1	2	7	14.1±1.77
			Exp. 3—64 days					
7	Normal	—	+151±10.2	3.6±0.25	5	2	0	8.4±1.05
8	H.F.	—	— 6± 5.7	8.1±0.74	0	2	6	26.7±1.83
10	”	1.0	+ 34± 4.2	8.6±0.53	0	1	9	26.2±2.54
10	”	4.0	+ 23± 6.9	8.4±0.49	0	3	7	24.6±0.83
			Exp. 4—30 days					
8	Normal	—	+113± 4.2	4.4±0.12	6	2	0	6.8±0.31
8	H.F.	—	+ 23± 5.5	8.1±0.39	0	0	8	28.8±1.03
10	”	2.5*	+ 19± 3.5	7.8±0.38	0	0	10	27.3±1.12

* Dose administered 6x week.

TABLE III.
Effect of Crystalline Vitamin B_{12} and Choline on Hepatic Changes of Rats Fed a High-Fat Diet for 30 Days.

No. of rats	Diet	Supplement administered 3x wk		Avg wt gain, g	Avg liver wt g/100 g rat	No. animals with hepatic fatty change			Avg liver fat, %
		Cryst. B_{12} γ	Choline, mg			0	1+-2+	3+-4+	
10	Normal	—	—	77±5.7	4.1±0.13	9	1	0	4.8±1.20
10	H.F.	—	—	26±5.2	4.7±0.20	0	8	2	17.5±1.49
9	,,	1	.5	40±4.0	5.7±0.39	0	5	4	19.0±1.53
10	,,	—	.5	18±6.2	6.4±0.37	0	4	6	26.6±2.01
10	,,	1	1.	47±5.0	5.6±0.34	0	5	5	23.5±2.26
9	,,	—	1.	24±4.6	5.7±0.39	0	5	4	23.4±1.83
10	,,	1	2.	40±4.2	5.4±0.42	0	7	3	18.9±2.24
10	,,	—	2.	20±4.5	6.9±0.39	0	3	7	26.1±2.38

fat by a modification of the method of Outhouse and Forbes(4).

Results. Crude liver extract. Crude liver extract, containing 1 U.S.P. unit of antianemic principle per cc was administered 3 times a week subcutaneously to rats receiving a high-fat diet for periods of 66 and 150 days (Table I). The liver extract exerted a lipotropic effect and fibrosis, which developed in 6 untreated rats fed the high-fat diet for 150 days, was also prevented by administration of liver extract, confirming results previously reported. The weight of the liver of the treated rats was also in the range of that observed in the normal control animals. Another group of 4 rats, not shown in Table I, received choline, 2 mg per rat 3 times a week subcutaneously, with the high-fat diet for 66 days. This is a greater amount of choline than is supplied by the liver extract. No significant effect of this amount of choline was obtained, the total liver fat averaging 19.0 ± 3.54%.

Dosage of liver extract and lipotropic effect. Animals receiving the high-fat diet received various doses of liver extract subcutaneously 3 times a week (Table I). As the dose was decreased the amount of fat in the liver rose progressively in each group. Similarly, the average liver weight in grams per 100 g of rat weight increased and more animals showed an increase in liver fat histologically. Thus, one cc of crude liver extract administered subcutaneously 3 times a week represents approximately the minimum effective dose of this material under the conditions of these experiments. One group in this study received one cc of liver extract 3 times a week orally. The oral administration of the liver extract showed a definite lipotropic effect, as evidenced by a decreased total liver fat, reduction in liver weight, and prevention of histological fatty changes (Table I). However, the oral administration of the liver extract is slightly less effective than the same dose of this material administered subcutaneously.

Supplements of choline, inositol and folic acid. The liver extract, and also the vitamin B_{12} concentrate previously reported(3), con-

4. Outhouse, E. L., and Forbes, J. C., *J. Lab. and Clin. Med.*, 1939, v25, 1157.

tain small amounts of choline and folic acid. The concentration of inositol in these supplements is unknown but is quite small. To test the possibility that a combination of small amounts of choline, inositol and folic acid might exert a lipotropic effect, these supplements were administered in combination to rats receiving a high-fat diet. Ten rats received the high-fat diet plus the subcutaneous injection 3 times a week of choline 1 mg, inositol 1 mg, and folic acid 2 μg. Another group of 9 rats received these same supplements in half of the above concentrations. The animals were autopsied on the 92nd day of this study and liver fat of these treated animals was high (20.1% $\pm$ 0.58%) and not significantly different from that found in untreated high-fat controls animals (18.7% $\pm$ 1.39%). Similarly these supplements failed to prevent the histological fatty change in the liver or the increase in liver weight that is obtained when a high-fat diet is fed.

Crystalline vit. B_{12}. Since crystalline vitamin B_{12} is present in liver extract and vitamin B_{12} concentrate, the possible lipotropic effect of this substance was studied. In an initial study, 0.2 μg of vitamin B_{12} appeared to have a slight lipotropic activity. However, this effect probably represents a variation among the rats, as on further study with higher doses of vitamin B_{12} lipotropic activity could not be demonstrated. This was true even when 2.5 μg of crystalline vitamin B_{12} was administered subcutaneously 6 times a week (Table II). Crystalline vitamin B_{12} failed to prevent the fatty change as seen histologically, the increase in liver fat determined chemically, and the increase in liver weight.

Combination of crystalline vit. B_{12} and choline. Inasmuch as vitamin B_{12} has been demonstrated to exert a sparing effect on choline as measured by the growth of the chick(5,7) or the prevention of renal hemorrhagic necrosis in rats(6,7), it was important to test such a combination for lipotropic activity. Choline was administered with and without crystalline vitamin B_{12} in the doses in which it is present in liver extract and vitamin B_{12} concentrate. The vitamin B_{12} dose was kept constant at 1 μg 3 times a week. At each dose level the supplements of choline alone or in combination with crystalline vitamin B_{12} did not decrease the amount of liver fat as determined chemically below that obtained in untreated animals receiving the high-fat diet. These combinations of vitamin B_{12} and choline were also without effect in preventing the histological changes in the liver. The animals receiving both choline and crystalline B_{12} did, however, gain on the average 20 g more in weight than the animals receiving the choline alone (Table III).

Discussion. The lipotropic effect of liver extract was confirmed. One cc of crude liver extract subcutaneously 3 times a week represents about the minimum effective lipotropic dose of this material, under the conditions of this study. As the dose is lowered below this level the protective effect progressively decreases. Liver extract is also effective orally and one cc 3 times a week is only slightly less effective than the subcutaneously administered material. Liver extract also prevented fibrosis, confirming earlier reports(1,2). Recently it has also been noted to be of value in the treatment of experimental dietary cirrhosis in rats(8). In the same study the authors noted that crude liver extract administered orally seemed to enhance the effect of casein and methionine supplements.

The combination of choline, inositol and folic acid that was used did not exert any lipotropic effect. The dosage of these materials was in the range of that supplied by the liver extract used in these studies and by the vitamin B_{12} concentrate used in an earlier report. Thus the lipotropic effect of liver extract or vitamin B_{12} concentrate is not due to a combination of the small amounts of choline, inositol and folic acid present in these materials. Crystalline vitamin B_{12} alone was also without lipotropic effect (Table II). György and Rose(9) have noted that crystal-

5. Schaefer, A. E., Salmon, W. D., and Strength, D. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 202.

6. Schaefer, A. E., Salmon, W. D., and Strength, D. R., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 193.

7. Schaefer, A. E., Salmon, W. D., Strength, D. R.,

8. György, P., and Goldblatt, H., *J. Exp. Med.*, 1949, v90, 73.

line vitamin B_{12} is without lipotropic activity in rats fed a high-fat diet. They did, however, obtain a partial lipotropic effect of crystalline vitamin B_{12} when administered to rats receiving a low-fat-low-protein diet, although the effect was less than that obtained with methionine. A combination of methionine and vitamin B_{12} did not further prevent the fatty changes in the liver.

A sub-optimal amount of choline, equal to or greater than that present in liver extract and vitamin B_{12} concentrate, was also without lipotropic effect. Crystalline vitamin B_{12} in combination with choline did not exert any sparing effect on the choline as judged by the failure to prevent an increase in liver fat (Table III). Thus, although vitamin B_{12} has a choline sparing effect on the growth of chicks or in the prevention of hemorrhagic renal necrosis in rats(5,6,7), it is without sparing action in preventing hepatic injury in rats receiving a high-fat diet with casein as the source of protein. As the lipotropic activity of liver extract or vitamin B_{12} concentrate is not due to the factors discussed above, the effect of these materials must be due to other known or unknown agents which influence the synthesis or transport of methyl groups. The possible effects of combinations of crystalline vitamin B_{12} and sub-optimal amounts of folic acid, with or without choline, are being studied.

Summary. With the diet employed, one cc of crude liver extract administered subcutaneously 3 times a week represents the approximate minimal effective dose of this material for lipotropic activity. The same dose of liver extract administered orally also has lipotropic activity but slightly less than that obtained on subcutaneous administration. The lipotropic effect of crude liver extract or vitamin B_{12} concentrate is not due to the small amounts of choline, inositol and folic acid present in these materials. Crystalline vitamin B_{12} alone is without lipotropic effect. Small amounts of choline, alone or in combination with crystalline vitamin B_{12}, also failed to prevent fatty changes in the liver.

9. György, P., and Rose, C. S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 372.

Received May 12, 1950. P.S.E.B.M., 1950, v74.

Effect of B Complex Vitamins on Liver and Heart Glycogen Levels of Hyperthyroid Rats. (17998)

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The importance of some of the members of the B-complex vitamins in partially counteracting the growth-retarding effect of experimental hyperthyroidism in immature rats is generally recognized. When a high B-complex diet is further supplemented with dried liver or a water-insoluble residue of liver, animals grow at a nearly normal rate in spite of the hyperthyroid condition(1-5). Since hyperthyroidism usually leads to a marked reduction in the concentration of glycogen in the heart and liver, it was decided to determine whether these supplements which so favorably influence the growth rate of the immature animal, would also reduce the effect of hyperthyroidism on the liver and heart glycogen concentrations.

Method. Young albino rats averaging 55 g were put into individual cages and fed the various diets shown in Table I. Food intake was unrestricted but a daily record of food

1. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 500.

2. Ershoff, B. H., *Arch. Biochem.*, 1947, v15, 365.

3. Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition*, 1947, v34, 431.

4. Ershoff, B. H., PROC. SOC. EXP. BIOL., AND MED., 1949, v71, 209.

5. Ershoff, E. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 459.