pyrogen, and production of hypercalcemia also had no effect. These observations confirm the inhibitory effect of potassium on release of pyrogen from leukocytes *in vitro*.

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Received September 3, 1964. P.S.E.B.M., 1965, v118.

3,5 Diiodothyroacetic Acid Mobilization of Rat Liver Lipid and Relative Toxicity of Oral Triac and Triprop. (29749)

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In earlier studies, a series of thyroxine-like compounds was tested for their relative effects on tissue cholesterol and lipid concentrations, growth and oxygen consumption rates in hypercholesterolemic rats(1,2). The purpose of these investigations was to find a thyroxine analogue(s) which would lower tissue cholesterol concentrations without significantly affecting other biological parameters. The animals were fed a 29% lard, 19% casein, 1% cholesterol purified diet, and thyroxine analogues were either fed or injected in an effort to prevent or reverse the condition of hypercholesterolemia. Of the compounds tested, 3,5 diiodothyroacetic acid (diac) appeared to show the greatest preferential effect on cholesterol and lipid concentrations without significantly affecting oxygen consumption and growth rates. Diac was more effective when fed in the diet than when injected intraperitoneally at a comparable dose level, and optimum dietary concentration of diac was found to be 5 mg%.

Additional studies have been made of the effects of diac on the mobilization of liver and plasma lipid concentrations. The total decrease in liver weight produced by diac feeding has been accounted for, and the conditions for optimum lipid mobilization have been determined. Two other thyroxine derivatives, 3,5,3′ triiodothyroacetic acid (triac) and 3,5,3′ triiodothyropropionic acid (triprop) were found to be more toxic than T_4 when fed in the diet although they have only 20 and 10% respectively the calorigenic activity of T_4 when injected subcutaneously.

Methods. It had been noted in previous experiments that the livers of animals receiving a high fat-cholesterol-cholic acid diet were enlarged, and that the liver size was reduced to control levels when diac was administered. It was proposed to determine the nature of the substances mobilized, and to assess the effects of the level of dietary fat and protein on the diac effect. A large group of weanling male rats was started on the high fat-cholesterol-cholic acid diet which was composed fundamentally of 19% casein, 26% lard, 1% cholesterol, 0.5% cholic acid and sucrose, vitamins and salts to make 100%. The actual composition of the diet has been described(2). The animals were housed by pairs in wire mesh bottom cages (8 animals per group) in a room maintained at a constant temperature of 25°C. Water and food were supplied *ad libitum*. At the end of 3 weeks, 8 animals were sacrificed and liver weights, plasma cholesterol, liver cholesterol, total liver lipid, liver total reducing sugar and liver water determinations were performed.

* This investigation was supported in part by Public Health Service Research Grant HE-04567, Nat. Heart Inst. and in part by a grant from Warner Lambert Research Inst., Morris Plains, N. J.

TABLE I. Effects of Diac and Different Dietary Lipid and Protein Levels on the Mobilization of Lipid Deposits.

Regimen	Body wt (g)	Liver wt (g)	% B.W. as liver	Plas chol (mg%)	Liver chol (mg%)	Liver lipid (g%)	Liver CHO (g%)	Liver water (g%)
3 week								
Chow	159 ± 2	7.6 ± .3	4.8 ± .2	71 ± 3.1	301 ± 21	9.7 ± .2	5.40 ± .31	70.1 ± .2
HF-C-CA	143 ± 4	8.1 ± .4	5.7 ± .1	200 ± 17	4785 ± 84	21.4 ± .7	5.51 ± .40	61.9 ± .6
6 week								
Chow	248	10.8	4.3	66	220	6.9	3.60	68.8
HF-C-CA	242	13.2	5.5	234	3913	27.4	2.42	55.8
LF	246	10.5	4.3	130	2320	11.2	3.61	67.2
LF-D	229	8.3	3.6	103	1840	8.3	.20	71.0
LFHP	270	11.7	4.3	113	901	9.1	3.55	68.7
LFHP-D	277	10.9	3.9	80	633	7.5	.21	71.3
HF	239	10.1	4.2	104	2130	16.9	2.66	67.8
HF-D	256	9.7	3.8	113	2045	11.4	1.32	69.0
HFHP	265	11.7	4.4	113	1294	12.6	2.29	66.4
HFHP-D	258	9.5	3.7	115	596	7.8	.98	71.8
8 week								
Chow	314 ± 4	11.8 ± .3	3.8 ± .1	70 ± 2.4	247 ± 16	7.5 ± .2	3.23 ± .38	71.1 ± .4
HF-C-CA	259 ± 4	14.6 ± .3	5.7 ± .2	260 ± 11.2	8288 ± 237	26.5 ± 1.8	2.44 ± .43	57.3 ± .9
LF	281 ± 5	10.3 ± .3	3.7 ± .1	109 ± 4.0	1047 ± 37	8.9 ± .5	2.96 ± .09	69.5 ± .9
LF-D	270 ± 6	10.1 ± .4	3.8 ± .2	105 ± 6.5	564 ± 69	7.7 ± 1.0	.11 ± .03	71.6 ± .8
LFHP	293 ± 6	10.3 ± .1	3.5 ± .5	88 ± 5.3	508 ± 87	7.7 ± .4	2.97 ± .03	70.1 ± .3
LFHP-D	323 ± 7	11.0 ± .3	3.4 ± .1	87 ± 5.2	417 ± 32	7.2 ± .4	.00 ± —	71.9 ± .5
HF	276 ± 10	12.2 ± .4	3.9 ± .1	107 ± 3.9	2304 ± 168	13.7 ± 1.1	2.17 ± .17	65.7 ± 1.1
HF-D	313 ± 6	9.6 ± .2	3.1 ± .1	117 ± 5.3	351 ± 17	6.7 ± .3	.70 ± .14	72.4 ± .4
HFHP	315 ± 5	9.9 ± .3	3.6 ± .1	102 ± 5.7	500 ± 140	10.7 ± 1.1	2.73 ± .20	66.7 ± .8
HFHP-D	325 ± 9	10.0 ± .2	3.3 ± .1	91 ± 4.0	273 ± 18	6.0 ± .2	.26 ± .04	72.4 ± .4

All values reported represent the arithmetic mean ± standard deviation for groups of 8 rats.
HF-C-CA = high fat (26% lard) diet containing 1% cholesterol and .5% cholic acid.
LF = low fat (5% lard) diet. Sucrose was added at expense of fat.
HF = high fat (26% lard) diet.
HP = high protein (30% casein), in combination with either LF or HF diets. Casein was added at expense of sucrose.
D = 5 mg% 3,5 diiodothyroacetic acid (diac) added to respective diets.

The procedures employed for determination of cholesterol and total lipid have been described(2). The water content of the livers was determined by drying a weighed sample of liver at 100°C to a constant dry weight. The liver content of total reducing sugar was measured by the Somogyi(3) procedure. A sample of liver was hydrolyzed in 1 N HCl in a boiling water bath for 3 hours. Following neutralization and precipitation of protein with $BaSO_4$, an aliquot of the supernatant was assayed for total reducing sugar by measuring the reduction of arseno-molybdic acid.

Following the 3-week period on the high fat-cholesterol-cholic acid diet, the remaining animals were switched to the regime shown in Tables I and II, and were sacrificed at the end of either 3 or 5 weeks. Chow and HF-C-CA control groups were also included for comparative purposes. The low fat diets (LF) fed to certain of these animals contained 5% lard, and the high protein diets (HP) contained 30% casein. Sucrose was used to balance out the composition of the diets, and no attempt was made to keep the diets isocaloric.

Results. Effect of diac and various levels of dietary protein and lipid on reversal of hyperlipemia. The data in Table I show that the livers of the HF-C-CA group which was sacrificed at 3 weeks were larger than those of chow control animals and averaged 5.7% of body weight *vs* 4.8% for the chow fed animals. Plasma and liver cholesterol and liver total lipid concentrations were markedly increased (Table I) whereas the concentration of liver reducing sugar remained approximately the same and liver water was reduced somewhat. The increase in liver size was due

TABLE II. Effects of Diac and Different Dietary Lipid and Protein Levels on Liver Composition.

Regimen	Total liver lipid (g)	Total liver CHO (g)	Total liver water (g)	Lipid + CHO + water (g)	% liver wt acct for
3 week					
Chow	.74	.41	5.32	6.47	85
HF-C-CA	1.73	.44	5.01	7.18	87
6 week					
Chow	.75	.39	7.42	8.55	79
HF-C-CA	3.62	.32	7.38	11.32	86
LF	1.18	.38	7.08	8.64	82
LF-D	.69	.02	5.91	6.62	80
LFHP	1.06	.41	8.02	9.49	81
LFHP-D	.81	.02	7.74	8.57	79
HF	1.70	.27	6.83	8.80	87
HF-D	1.00	.13	6.67	7.80	81
HFHP	1.46	.27	7.74	9.47	81
HFHP-D	.74	.09	6.84	8.56	89
8 week					
Chow	.89	.38	8.43	9.70	82
HF-C-CA	3.90	.36	8.42	12.68	86
LF	.93	.31	7.19	8.43	86
LF-D	.74	.01	7.29	8.04	79
LFHP	.79	.30	7.18	8.27	81
LFHP-D	.80	—	7.94	8.88	—
HF	1.68	.27	8.03	9.98	82
HF-D	.65	.07	6.98	7.70	80
HF-HP	1.06	.27	6.60	7.93	80
HFHP-D	.65	.03	7.70	8.39	79

essentially to an increase in total lipid (Table II). A continuation of HF-C-CA diet feeding produced further lipid infiltration of the liver. Although the liver size was reduced in all groups of animals switched from the high fat-cholesterol-cholic acid diet, those animals receiving 5 mg% diac in the diet had a smaller percent of body weight as liver. The protein content of the diet appeared to be important in determining the rate of plasma cholesterol and liver cholesterol and total liver lipid mobilization under all conditions employed. The low fat diet did not significantly affect the rate of plasma cholesterol mobilization, but low fat diet groups did show a more rapid rate of liver cholesterol and lipid reduction. Diac administration tended to erase the differences due to the fat content of the diet, and produced the lowest liver cholesterol and lipid values obtained. Diac administration also reduced the liver total reducing sugar concentration to very low levels in low fat diet animals and to a somewhat lesser extent in animals receiving high fat diets. Dietary fat appeared to exert a partial sparing action on the liver glycogen content when measured in animals receiving diac. It would also appear from the data presented in Table I that the water content of the liver was inversely proportional to the liver total lipid concentration. Therefore, the combination of factors needed to produce an optimal rate of reversal of elevated plasma and liver lipid levels would appear to include the administration of a high protein diet containing 5 mg% diac. The level of lipid in the diet does not appear to be very important when diac is included in the regimen, but a low fat diet is beneficial in the absence of diac.

Comparative toxicities and lipid lowering activities of several thyroxine analogues. It had been observed in preliminary experiments that 3,5,3′ triiodothyroacetic acid (triac) and 3,5,3′ triiodothyropropionic acid (triprop) were toxic when fed in the high fat-cholesterol (HF-C) diet at a level of 5 mg%, whereas 5 mg% thyroxine (T_4) was not toxic at this concentration. This observation appeared unusual since triac and triprop are usually considered to have approximately 20 and 10% respectively the calorigenic activity of thyroxine. The following 2 experiments were performed to determine the relative calorigenic activities, toxicities and lipid lowering capacities of T_4, T_3, triac, triprop and diac. For these experiments, groups of 8 weanling rats were placed on the regime shown in Table III, and at 18 through 21 days, the metabolic rates of each animal were determined by a procedure described previously(1,2). The animals were then sacrificed by decapitation and the tissues collected for cholesterol and lipid analysis.

It can be seen from Table III that 2.5 mg% triprop and 1 mg% triac were essentially as toxic as 0.5 mg% T_3, and that up to 5 mg% T_4 did not produce deaths in this time period. Because of the relatively few dose levels employed and the rather large standard errors obtained, it is not feasible to quantitate the relative calorigenic activities of the different analogues, but qualitatively it is apparent that triprop and triac are more active than T_4 when the compounds are administered orally in the high fat-cholesterol

TABLE III. Relative Effects of Various Concentrations of T_4, T_3, Triac, Triprop.

Regimen	Avg 18-day wt gain (g)	Avg liters $O_2/M^2/hr$	Avg plasma cholesterol (mg %)	Avg liver cholesterol (mg %)	Avg liver lipid (g %)
Exp 1					
Chow	132 ± 11	7.6 ± .5	76 ± 8	308 ± 31	6.5 ± .6
HF-C	108 ± 8	7.3 ± .6	180 ± 16	2117 ± 230	13.2 ± .9
HF-C + .1 mg% T_4	112 ± 12	—	162 ± 12	2129 ± 121	13.9 ± 1.1
HF-C + .25 " "	112 ± 8	—	126 ± 19	1910 ± 411	11.6 ± 1.3
HF-C + .5 " "	97 ± 10	7.0 ± .8	125 ± 14	965 ± 244	9.4 ± .9
HF-C + 1.0 " "	93 ± 10	8.0 ± .8	155 ± 10	632 ± 179	7.4 ± 1.0
HF-C + 5.0 " "	80 ± 9	10.5 ± 1.2	171 ± 15	537 ± 116	7.7 ± .6
HF-C + .1 mg% triprop	92 ± 11	7.6 ± .8	125 ± 8	1326 ± 162	8.8 ± .8
HF-C + .5 " "	89 ± 10	9.9 ± .8	154 ± 10	526 ± 85	6.4 ± .6
HF-C + 1.0 " "	73 ± 9	11.8 ± 1.0	202 ± 21	480 ± 140	7.0 ± 1.0
HF-C + 2.5 " " *	—	—	—	—	—
Exp 2					
Chow	118 ± 10	—	77 ± 9	278 ± 36	
HF-C	97 ± 9	6.3 ± .6	136 ± 14	1848 ± 216	
HF-C + 5.0 mg% diac	87 ± 9	—	116 ± 10	532 ± 112	
HF-C + .05 mg% triac	89 ± 9	7.9 ± .8	118 ± 11	310 ± 98	
HF-C + .2 " "	93 ± 10	8.8 ± .8	145 ± 11	424 ± 106	
HF-C + .5 " "	58 ± 10	9.9 ± 1.0	165 ± 17	565 ± 128	
HF-C + 1.0 " " *	—	—	—	—	
HF-C + 2.5 " " *	—	—	—	—	
HF-C + .05 mg% T_3	68 ± 7	7.0 ± .6	116 ± 15	565 ± 76	
HF-C + .2 " "	41 ± 8	11.1 ± 1.2	149 ± 18	535 ± 101	
HF-C + .5 " " *	—	—	—	—	
HF-C + 1.0 " " *	—	—	—	—	

* All animals died within the 3-week experimental period.

diet. Little reduction in plasma cholesterol values resulted from administration of any of the compounds, but liver cholesterol and liver lipid values were markedly affected by the inclusion of sufficient concentrations of these compounds. It is also difficult to draw a quantitative comparison between the relative liver cholesterol lowering activities, but as little as 0.5 mg% triprop, 0.05 mg% triac and 0.05 mg% T_3 were as effective as 1-5 mg% T_4 and 5 mg% diac. In all cases, it appears that the optimum dose for preventing the elevation of liver cholesterol and lipid values lies just below the dose level needed to stimulate metabolic rate.

Discussion. It would appear that the enlarged livers seen in rats receiving the high fat-cholesterol-cholic acid diet were primarily due to an increase in lipid content. When animals were switched from this regimen to other diets not containing added cholesterol and cholic acid, the livers were reduced in size by the loss of lipid. Diac feeding on top of a high protein diet greatly accelerated the rate of mobilization of liver lipid, although glycogen stores were also markedly reduced. An increase in dietary protein from 19% to 30% casein produced a faster rate of liver lipid mobilization both in presence and absence of diac. The low fat diet also produced faster mobilization of liver lipid, except that diac feeding tended to eliminate the preferential effect of the low fat diet. Therefore, the protein content of the diet appeared to be important for the mobilization of liver lipid depots by a thyroxine analogue such as diac, whereas the lipid content appeared to be of much less importance.

It is particularly interesting that a higher level of dietary fat (26% lard) partially spared the depletion of liver glycogen by diac, and that higher dietary protein (30% casein) had no affect. This observation coincides with the findings of others(4-6) that an increased fat content in the diet affords some degree of an antithyrotoxic response. Greenburg(7) has presented evidence to indicate that linoleic acid might be the active antithyrotoxic principle.

Both the route of administration and nature of the diet employed may have considerable bearing on the relative calorigenic and

tissue cholesterol and lipid lowering activities of various thyroxine analogues. Triac and triprop have been found to be 20% and 10% as calorigenically active as L-T_4 when injected subcutaneously into rats receiving a chow diet(8), yet in our experiments, they were actually more active than L-T_4 in elevating metabolic rate and mobilizing liver lipid depots. In fact, triac and triprop were toxic at dietary dose levels of 1 and 2.5 mg% respectively, whereas T_4 did not kill any animals at dose levels as high as 5 mg%. This greater toxicity of dietary triac and triprop may be due to the fact that they are more calorigenically active than dietary T_4, and not to any real difference in the toxicities of the two groups of compounds. Kroc *et al*(9), using the same chow diet and experimental conditions employed in their earlier injection studies, have found that oral T_4 is approximately 12% as active as injected T_4 whereas oral triprop is more than twice as active calorigenically as injected triprop. It is now known that certain natural materials such as liver, cottonseed meal and hemoglobin, which are present in chow diets, contain an antithyrotoxic factor(s) that partially blocks the calorigenic response of dietary thyroxine and other analogues such as triac and triprop(10, 11). The quantitative relationships between the antithyrotoxic factor and T_4 have been worked out, and it has been found that the antithyrotoxic factor interferes with the absorption of T_4 from the gastrointestinal tract (12). However, the same degree of quantitation between the antithyrotoxic activity and effects on absorption have not been worked out for triac and triprop. It is possible that the naturally occurring antithyrotoxic factors present in chow diet may suppress the activity of T_4 more than that of triac and triprop. In our experiments, the toxicity of triac and triprop was observed in rats receiving the high fat-cholesterol (HF-C) diet. It is believed that cholesterol will reduce the magnitude of the calorigenic response obtained with dietary thyroxine(13), and it has been observed that dietary cholesterol will increase the survival time of rats fed toxic doses of T_4(14,15). As indicated previously, high levels of dietary fat are also known to exert some antithyrotoxic activity. It is possible that both cholesterol and fat feeding may exert a greater protective effect against large doses of T_4 than against comparable doses of triac and triprop. Therefore, both the route of administration and the nature of the diet with respect to the presence or absence of certain antithyrotoxic materials may be important in governing the relative activities of thyroxine analogues. It would appear that activities determined by one route of administration on a particular diet are not extrapolable to activities obtained by another route of administration on still another diet.

Summary. Maximal mobilization of previously elevated liver cholesterol and total lipid depots was achieved by feeding 5 mg% 3,5 diodothyroacetic acid (diac) in a 30% casein diet. An increase from 19% to 30% casein enhanced the lipid mobilization rate, but reduction of the fat content from 26% to 5% lard had little effect when diac was given. Plasma cholesterol concentrations were reduced slightly by diac feeding, but did not reach those levels seen in chow controls. Triac and triprop were found to be more calorigenically active and more toxic than thyroxine when fed in a high fat-cholesterol diet whereas they are known to be only 20% and 10% as active as thyroxine when injected subcutaneously into rats on a chow diet. Both route of administration and composition of the diet with respect to the existence of naturally occurring antithyrotoxic factors may be important in determining the relative calorigenic and cholesterol reducing activities of various thyroxine analogues.

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Received July 1, 1964. P.S.E.B.M., 1965, v118.

Reduced Liver Glucose-6-Phosphatase in Human Leukemia.* (29750)

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During experiments concerned with rat liver glucose-6-phosphatase it became of interest to study human liver glucose-6-phosphatase. Specimens of human liver obtained during post-mortem examinations were homogenized and assayed immediately. It was noted that each of the livers from children with acute lymphatic leukemia was very deficient in this enzyme activity. Some of the mechanisms responsible for this reduction in activity were investigated and form the basis of this report.

Methods. At time of autopsy the patient's age, diagnosis, drug therapy, and hours since death were recorded. A 10 g specimen of liver was placed immediately in 0.12 M KCl at 0°C, and was kept at 0°C during subsequent steps. A 10% homogenate was prepared using a glass-teflon homogenizer at 1000 rpm for 2 to 3 minutes. The homogenate was centrifuged at 600 × *g* for 10 minutes and the residue was discarded. The supernatant was centrifuged in a Servall refrigerated centrifuge at 37000 × *g* for 30 minutes and the residue was suspended in 0.12 M KCl at a concentration 5 to 10 mg protein per ml. It had been previously demonstrated that rat liver glucose-6-phosphatase activity was purified 3-fold in this residue, when compared to liver homogenate. The activity of the 37000 × *g* residue was determined by the method of Swanson(1), at pH 6.5 in malate buffer. Protein was determined by Lowry's(2) method.

The effects of protein synthesis inhibitors on enzyme activity were determined in two ways. First, 6-mercaptopurine, methotrexate (4-amino-N-methylpteroyl glutamic acid), and cortisol were added during the assay of enzyme activity. Second, 6-mercaptopurine (1.0 mg/150 g rat/day), and methotrexate (0.1 mg/150 g rat/day) were fed in 2 groups of rats with their regular Purina Laboratory Chow diet, and other rats received 100 μg actinomycin D by intraperitoneal injection daily for 4 days. After several days the rats were all weighed, their livers were weighed, and liver homogenates were assayed for glucose-6-phosphatase activity.

Results. Table I summarizes the human liver glucose-6-phosphatase data. It can be seen that enzyme activity was not clearly related to age, hours elapsing between death and post-mortem examination or drug therapy. The livers were generally larger than the normal weight for the patients' ages. Enzyme activity is expressed in μg P/mg protein/hour, and since the reduction of enzyme activity is extreme (4- to 10-fold) it is not possible to account for this reduction by dilution of normal tissue by leukemic cells. The patient with neuroblastoma had diffuse liver metastases and a normal enzyme activity.

It was noted that 6-mercaptopurine (0.1 mg/ml), methotrexate (0.02 mg/ml) and cortisone (50 μg%) in the reaction mixture

* This investigation was supported by U.S.P.H.S. Grant AM-04235.