acetic acid precipitate, it can be inferred that the mercury was bound to renal tissue proteins.

The results of the present study show that the intravenous administration of organically bound mercury is associated with a decrease in the renal cortical concentration of sulfhydryl radicals.

Summary. Hg^{203}-labeled mercaptomerin sodium, in a dosage of 14 to 30 mg of mercury/kg of body weight, was injected intravenously into normal rabbits immediately following a unilateral nephrectomy, and the remaining kidney was removed one hour later. The mean cortical and medullary concentrations of mercury, were, respectively, 2.53 and 0.77 mg/g of dry weight of tissue. The mean sulfhydryl concentration in the control cortices was 10.08 mg/g of dry tissue, expressed as l-cysteine; the mean value obtained one hour after administration of the tagged organic mercurial agent was 6.76 mg/g. The SH concentration of the cortex decreased in all 12 test animals following the injection of labeled mercaptomerin, and increased in the 2 uninjected control animals.

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Effect of Thyroxine and Related Compounds on Heparin-Activated Fatty Acid Liberating Enzyme.* (22052)

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Plasma from a human given intravenous sodium heparin contains an enzyme capable of hydrolyzing the triglycerides of egg lipoproteins and human S_f 20-400 lipoproteins, the latter both *in vitro* and *in vivo*, releasing fatty acids and increasing the S_f 4-20 lipoprotein level of the plasma(1-3). The lipoprotein transformations involve primarily the lipid components since the protein components of the S_f 4-20 and S_f 20-400 lipoproteins of normal humans seem to be of quantitatively identical amino acid composition(4). No definite evidence exists that the heparin-activated enzyme normally mediates lipoprotein metabolism, though Jeffries(5) has demonstrated the presence of a very similar, if not identical, enzyme in the rat following olive oil administration, and Korn(6) has obtained from rat heart an enzyme catalyzing the hydrolysis of the neutral fat of chylomicra. Though very much less susceptible to hydrolysis by the heparin enzyme, human S_f 4-20 lipoproteins can be significantly lowered in concentration by the administration of desiccated thyroid(7,8). For these reasons, and particularly if the heparin-activated enzyme is physiologically important (requiring for its activity no elements not normally present in the body), it seemed of interest to examine the effect of thyroxine and related compounds on the triglyceride-splitting enzyme.

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Materials and methods. Enzyme (preheparin serum or post-heparin plasma) and human or egg lipoprotein substrate were prepared as described previously(1). L-thyroxine (THRX, obtained from Smith, Kline, French Laboratories and from Sigma Chemical Co.), L-triiodothyronine (TRIT, obtained from Smith, Kline, French), D-diiodothyronine (DIIT, obtained from Wyeth Laboratories, Inc.), and related compounds were dissolved in a minimum volume of 0.1 N sodium hydroxide and added to serum or plasma, the pH being adjusted to 8.0 with 0.1 N hydrochloric acid and the ionic strength made identical in all cases with 0.1 N sodium chloride. The calci-

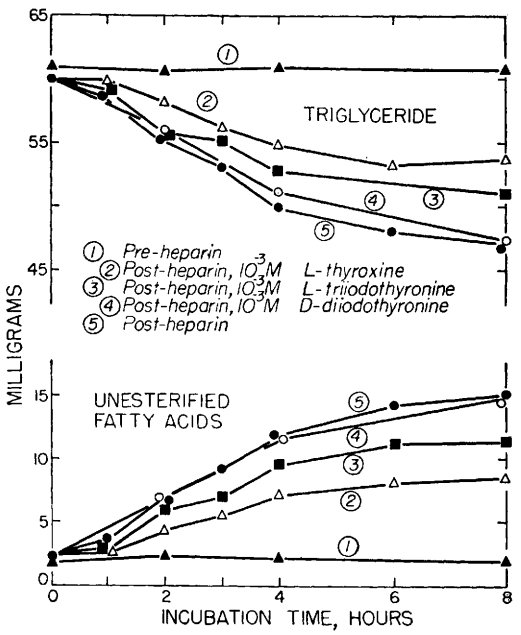

FIG. 1. Effect of thyroxine and related compounds on fatty acid liberation from egg lipoprotein triglycerides by post-heparin plasma.

um and magnesium experiments were carried out by adding the THRX-enzyme solution to substrate-cation solution and by adding THRX-enzyme-cation solution to substrate. In all experiments, after incubation of enzyme and inhibitor for 30 minutes at room temperature, the lipoprotein substrate was added and the contents incubated at 37.5°C. The pH remained constant in all experiments except for the 0.2-0.4 unit lowering brought about by the liberated fatty acid. At the end of the incubation, the contents were extracted with methanol-ethyl ether, the lipids separated chromatographically on silicic acid, and the fractions analyzed by infrared spectrophotometry(1).

Results. Effect of THRX and related compounds on fatty acid release. The data of Fig. 1 and of Table I indicate that THRX and TRIT inhibit the heparin-activated enzyme, the former compound being about 3 times more effective than the latter at equimolar concentrations. DIIT, L-tyrosine, and sodium iodide do not inhibit. Calcium or magnesium ion (3×10^{-3}M) or heparin (9×10^{-4} M, a concentration at least 400-fold that present in plasma after administration of 100 mg heparin) do not reverse the inhibition. In titrations carried out by the method of Alper(9), 3.9 ml 0.01 N sodium hydroxide were required to titrate the butyric acid liberated from tributyrin by post-heparin plasma, 3.7 ml that liberated by pre-heparin serum, 2.6 ml that liberated by post-heparin plasma 1×10^{-3} M in THRX, and 3.3 ml that liberated by post-heparin plasma 3×10^{-4} M in THRX. All the titrations were carried out in triplicate, with an uncertainty of 5-10%.

Effect of oral administration of THRX on fatty acid release and lipoprotein transformations. Before THRX administration, and after 10 mg intravenous heparin each day, the plasma of a normal individual at intervals of 2 days liberated 14.1, 13.4, and 13.9 mg fatty acid from 65 mg egg lipoprotein triglyceride prepared from different eggs. After daily oral administration of THRX (Smith, Kline, French tablets) for 8 days, 0.6 mg the first 2 and 1.0 mg thereafter, and following injection of 10 mg heparin at 2 times, the second 10 hours after the first, the plasma liberated 10.5 and 10.8 mg fatty acid, respectively from 66 mg lipoprotein triglyceride. During THRX consumption, the protein-bound iodine value rose from 5.6 to 14.9 μg/100 ml, corresponding at most to a THRX level of 2×10^{-6} M. At the conclusion of the experiment, THRX added to the plasma to a final concentration of 1×10^{-4} M was as effective in inhibiting fatty acid release from egg lipoprotein substrate as THRX added to a final concentration of 1×10^{-3} M had been before oral THRX consumption, 12.0 (10^{-3} THRX) and 12.5% (10^{-4} THRX) of the substrate triglyceride being hydrolyzed by THRX-containing, post-heparin plasma and 19.8% by the non-THRX-containing, pre-THRX administration post-heparin plasma.

The fasting standard S_f 0-20, 20-100, and 100-400 lipoprotein levels of the subject before THRX administration were 263, 54, and 21 mg%, respectively; after 10 mg intravenous heparin (Lederle) and withdrawal of the blood 10 minutes later, these values had become 288, 7, and 0 mg%. At the conclusion of THRX administration, these levels had become 129, 51, and 19 mg%, respectively; 10 mg intravenous heparin changed these levels to 144,

TABLE I. Liberation of Fatty Acid from Lipoprotein Triglycerides after Incubation with Pre-heparin Serum or Post-heparin Plasma and Various Compounds at 37.5°C for 4 Hours.

Incubation constituents	mg fatty acid increase*	$100 \times \frac{\text{mg fatty acid increase}}{\text{mg triglyceride in substrate}}$
(1) 2 ml S_f 20-400 lipoprotein (20-400 lp)	14.0	23.5
(2) 20-400 lp, 0.02 mg s. h.	.4	.6
(3) *Idem*	.5	.7
(1) " , 1×10^{-3} M L-thyroxine	7.6	12.7
(1) " , 3×10^{-4} M *Idem*	10.0	16.7
(1) " , 1×10^{-4} M "	12.0	20.1
(1) " , 1×10^{-3} M " , 9×10^{-4} M heparin	7.8	13.1
(2) " , 1×10^{-3} M "	.3	.5
(1) " , 1×10^{-3} M L-triiodothyronine	10.8	18.1
(1) " , 3×10^{-4} M *Idem*	12.3	20.6
(1) " , 1×10^{-3} M D-diiodothyronine	13.6	22.8
(1) " , 1×10^{-3} M L-tyrosine	13.7	22.9
(1) " , 1×10^{-3} M Sodium iodide	13.7	22.9
(2) " , 1×10^{-3} M L-triiodothyronine	.3	.5
(1) 2 ml egg lipoprotein (elp)	15.2	16.1
(1) elp, 1×10^{-3} M L-triiodothyronine	12.2	12.9
(1) " , 1×10^{-3} M L-thyroxine (THRX)	8.9	9.5
(2) " , 1×10^{-3} M THRX	.5	.6
(1) " , *Idem* , 3×10^{-3} M $CaCl_2$†	9.6	10.2
(1) " , " , 3×10^{-3} M $CaCl_2$‡	9.0	9.5
(1) " , " , 3×10^{-3} M $MgCl_2$†	9.3	9.8
(1) " , " , 3×10^{-3} M $MgCl_2$‡	9.3	9.8
(1) " , 3×10^{-3} M $CaCl_2$	16.0	16.9
(1) " , 3×10^{-3} M $MgCl_2$	15.5	16.4
(2) " , 3×10^{-3} M $CaCl_2$	.7	.8
(1) " , 1×10^{-3} M disodium versenate,§ pH 8.0	14.0	14.7
(1) " , 1×10^{-3} M sodium citrate, pH 8.0	13.9	14.6
(1) " , 1×10^{-2} M *Idem*	9.8	10.4

* The fatty acids produced came from the triglycerides, with no significant phospholipid or cholesterol ester hydrolysis to fatty acids. The values given represent averages of at least 4 determinations, with a reproducibility of 5-10%.

† Cation added to enzyme before enzyme addition to substrate.

‡ " " " substrate " " " " " " .

§ Disodium versenate = disodium salt of ethylene diamine tetracetic acid dihydrate.

(1) = 3 ml post-heparin plasma; (2) = 3 ml pre-heparin serum, s. h. = sodium heparin and present in all incubations with (2); (3) = 3 ml pre-heparin plasma, obtained by drawing blood into a heparinized syringe.

21, and 6 mg%. All the experiments on fatty acid release and lipoprotein transformation ability were repeated with two volunteers given 3 grains of desiccated thyroid (Armour) daily for 3 days (until complaint of unpleasantness); at most, a 5-10% inhibition was observed though the S_f 0-20 lipoprotein level had been reduced 15%.

Discussion. The approximate 3-fold superiority of THRX to TRIT in inhibiting fatty acid release is in conflict with the usual relative effectiveness of these compounds in biological systems, the latter being at least as effective and quite often 2 to 10 times more effective in mediating reactions(10). In view of the antagonism between the iodinated amino acids and magnesium (THRX binding the cation more strongly than does TRIT (11)), and in view of the role of calcium and magnesium cations, albumin(12-14), or lipoproteins(15) as fatty acid acceptors and facilitators of triglyceride hydrolysis, the experiments with calcium, magnesium and THRX were performed. Failure after cation addition to reverse significantly the thyroxine-mediated inhibition argues against a calcium or magnesium ion-amino acid complex as the cause of the inhibition. In addition, the data in Table I indicate that the potential cation-complexing agents, citrate and versene (10^{-3} M) are much less effective than the iodinated amino acids (10^{-3} M) as inhibitors of fatty acid release. Since heparin addition does not reverse inhibition, the amino acids probably

do not function by heparin removal.

Many workers(16-21) have presented data that there appears to be an α-globulin component in human serum with a binding mechanism so specific for THRX that TRIT fits less well and DIIT is excluded. If the heparin-activated enzyme is of such nature that THRX is bound to it more strongly than TRIT, or if the concentration of the latter available for inhibition is reduced by combination with non-enzymic plasma proteins, then the latter amino acid could be less inhibitory than the former. Our kinetic studies are not of sufficient accuracy to indicate amino acid-enzyme or amino acid-substrate binding. Inhibition of both tributyrin and lipoprotein triglyceride hydrolysis may be a reflection of non-specific esteratic enzyme site binding. The relatively high concentrations of THRX needed for inhibition in our experiments and the failure to observe an increase in S_f 20-400 lipoprotein levels accompanying the decrease in S_f 4-20 levels in patients given desiccated thyroid(7) —the increase to be expected if the thyroid is a natural antagonist of the heparin enzyme and if the enzyme itself is physiological—make it premature at present to associate the observed thyroxine effects with physiological thyroid function. Cairns and Constantinides (22), using amounts of THRX far greater (0.2 mg/rat/day for 8 days) than those used by us, found that THRX inhibited the "lipemia-clearing activity" induced by heparin injection, and that thyroidectomy alone failed to accelerate the activity, concluding that the THRX effect was not physiological.

Summary. 1. L-thyroxine and L-triiodothyronine inhibit the heparin-catalyzed release of fatty acid from lipoprotein triglycerides, the former inhibiting significantly at 3×10^{-4} M and being about 3-fold more effective than the latter at equimolar concentrations. 2. The inhibition does not result from removal of calcium or magnesium ions or heparin.

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