The rate of migration of the lipids associated with the albumin, $alpha_1$-globulin area was not apparently affected by the temperature variations. It will be seen from the results shown in Table I that a fair amount of the cholesterol normally associated with the beta-globulin area moved considerably faster at 35°C than at 5°C. The amount of cholesterol remaining around the starting point was usually less at the higher temperature than at 5°C. This was especially evident in serums from normal subjects with normal cholesterol concentrations. The Sudan Black-B staining material in nearly all cases agreed quite well with the cholesterol distribution.

Discussion. The cholesterol distribution which we obtained at 5°C is in reasonably good agreement with that obtained by Boyd (3) using a temperature of 1-12°C. The deterioration of resolution of the $alpha_2$- and beta-globulins which Boyd obtained at higher temperatures was evident in only a few of our cases when electrophoresis was conducted at 35°C, and even in these cases the difference was not marked. The fact that the temperature differences did not apparently affect the rate of migration of the cholesterol-containing lipids found in the albumin, $alpha_1$-globulin area is probably a reflection of their high protein content and great stability as contrasted with the beta-lipoproteins.

Summary. Paper electrophoretic studies showed that the rate of migration of beta-lipoproteins was considerably less at 5°C than at summer room temperature or at 35°C. No effect of temperature was evident in the case of the lipoproteins migrating with the albumin, $alpha_1$-globulin. The rate of movement of the main serum proteins was apparently the same at the different temperatures, but the resolution of the $alpha_2$- and beta-globulins was sometimes less distinct at the higher temperature. The results presented show the importance of using relatively constant temperatures when comparative paper electrophoretic studies are carried out, especially of beta-lipoproteins.

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Effect of Hg^{203}-Labeled Mercaptomerin on Renal Sulfhydryl Concentration in Normal Rabbits.* (22051)

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When labeled organic mercurials are injected intravenously into normal animals, the radioactivity is localized in the kidney(1,2). The purposes of the present study were to measure the concentration of mercury in the kidneys of normal rabbits following the administration of large doses of the tagged mercurial, and to determine the effect of this agent on the renal concentration of sulfhydryl compounds.

Material and methods. Mercaptomerin sodium‡ was labeled with Hg^{203} to contain 40 μc of Hg^{203} per mg of mercury; each ml of the stock solution contained 40 mg of mercury in 140 mg of mercaptomerin sodium. Fourteen young rabbits were used. Each of 12 animals was anesthetized with ether and a unilateral nephrectomy was performed. The desired

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Hg^{203} supplied by Oak Ridge National Lab., Oak Ridge, Tenn., on allocation from U. S. Atomic Energy Commission.

‡ Hg^{203}-labeled mercaptomerin sodium was synthesized by the Wyeth Institute of Applied Biochemistry.

TABLE I. Distribution of Hg^{203}-Labeled Mercaptomerin Sodium in Rabbit Kidneys and Its Effect on the Sulfhydryl Content.

I.V. dose of mercaptomerin (mg Hg/kg)	Conc. of mercury (mg/g dry wt tissue)		Serum Hg conc. (μg/ml)	Conc. of sulfhydryl (mg/g dry wt tissue)	
	Cortex	Medulla		Control kidney cortex	Test kidney cortex
17.9	1.20	.20	78.5	12.9	8.6
16.7	1.90	.70	46.0	7.8	5.4
19.6	1.90	.70	42.9	10.7	8.4
20.9	2.40	1.40	85.7	12.5	5.7
22.0	2.60	.80	—	10.8	6.4
32.5	2.60	.30	—	8.6	7.8
14.8	2.70	.50	—	9.2	7.5
26.9	2.70	.90	74.0	9.3	5.8
20.9	2.80	—	—	10.1	5.3
14.3	3.10	1.50	—	11.0	7.1
30.2	3.10	.90	96.5	8.4	6.4
16.8	3.40	.60	32.5	9.6	6.7
0	0	0	0	11.3	12.2
0	0	0	0	8.3	12.2
Mean values	2.53	.77		10.08	6.76

dose of tagged mercaptomerin (ranging from 14 to 30 mg of mercury per kilo of body weight) was then immediately injected into a marginal vein of the ear, and ether anesthesia was continued for another hour. Exactly one hr after injection of the mercurial, the animal was killed and the remaining kidney was removed. Two animals were used as controls and were treated in the same manner as the test animals, except that the injection of the mercurial was omitted. The cortex and medulla were separated by gross dissection. Samples from each, weighing approximately 1 g, were weighed and homogenized to a 5% suspension (weight to volume) in a phosphate buffer at pH 7.5 in the Elvejhem-Potter glass homogenizer. Three 1-ml aliquots of each of the 5% homogenates were analyzed for sulfhydryl content by the method of Anson (3). The SH concentrations found in the tissues have been expressed in terms of the 1-cysteine equivalent per g dry weight of tissue. Another 1-ml aliquot of each of the 5% homogenates was placed in a test tube of known weight and was assayed for radioactivity content. The test tubes were then dried at 110°C until constant weights were reached. The precipitates obtained after the addition of trichloracetic acid were decanted free of supernatant fluid, and were also assayed for radioactivity content.

Results. Table I. The concentration of mercury in the cortex varied between 1.2 and 3.4 mg/g of tissue (dry weight), with a mean value of 2.53 mg/g. These figures are considerably higher than the values found in the medulla, which ranged from 0.2 to 1.5 mg/g, with a mean of 0.77 mg/g. From 90 to 98% of the radioactivity found in the homogenates was recovered in the trichloracetic acid precipitate; the mean value was 94%.

The sulfhydryl concentrations in the renal cortices removed before injection of the mercurial ranged between 7.8 and 12.9 mg, expressed as 1-cysteine/g dry weight of tissue, with a mean value of 10.08 mg/g. In all 12 instances in which labeled mercaptomerin was administered intravenously, the sulfhydryl concentration in the test kidney (5.3-8.6 mg/g, with a mean value of 6.76 mg/g) was lower than that in the control kidney. In the 2 control animals not given mercaptomerin, however, the SH values increased during the period of anesthesia. The results of the SH determinations on the renal medulla were not reliable, due to technical difficulties, and have not been reported.

Comment. That Hg^{203} has a predilection for the renal cortex is evident from the fact that cortical concentrations of mercury were, on the average, 3.3 times greater than those in the medulla. This distribution would be expected if the mercurial had localized in the proximal tubules, as others have found in histochemical studies(4). Since most of the radioactivity was recovered in the trichlor-

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-