inhibitor for the 18-day period of this study. A partial reversal of this growth inhibitory activity of cortisone may be accomplished by the simultaneous administration of beef amniotic fluid.

I wish to thank Ovia Walker, Angela Ramos, and Tom Dillon for their technical assistance.

1. Howes, E. L., *Surgery,* 1950, v28, 177.
2. Spain, D. M., and Malomut, M., *Science,* 1950, v112, 335.
3. Montgomery, P., O'B., and Green, C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 657.
4. Fischer, A., *Nature,* 1930, v144, 113.
5. ———, *Chem. Products,* 1940, v3, 79.
6. ———, *Acta Physical Scand.,* 1942, v4, 207.
7. Herman, E., *Histochemical and Cytological Techniques,* 22nd edition, Department of Anatomy, Harvard Medical School.

Received September 12, 1955. P.S.E.B.M., 1955, v90.

Effect of Temperature Variations on Paper Electrophoretic Migration of Serum Lipoproteins.* (22050)

J. C. FORBES AND P. C. TAYLOR.
Department of Biochemistry, Medical College of Virginia, Richmond.

In a study on filter paper electrophoresis of serum proteins it became quite obvious that temperature changes markedly affected the migration of the serum lipoproteins even though the migration of the main serum proteins was affected to only a negligible extent. In order to determine what variations extremes in laboratory temperature might cause, we have carried out a study on the cholesterol distribution along filter paper strips following electrophoresis at 5°C versus summer room temperature and 35°C. The summer room temperature, though not known exactly, was probably around 26°C to 30°C most of the time.

Methods. A Kelab paper electrophoresis apparatus was used with Whatman #3 filter paper strips (4.6 x 35 cm) and a barbital-sodium acetate buffer of pH 8.6 and ionic strength .05. Three strips of filter paper separated from one another by plastic strips were placed between the glass plates of each of the 2 compartments, thus making a total of 6 strips for each run. To each of the strips at a line 12 cm from the cathode end of the strip a total of 0.05 ml of serum was applied evenly across the strip except for a margin of 2 mm at each edge. The duration of the electrophoresis was 15 hours. A current of 1.2 milli-amps per strip was employed consistently and the potential was approximately 210V. At the end of the electrophoretic procedure all strips were removed and processed as follows: a) 2 strips were oven dried at 100°C for about 45 minutes then stained with bromphenol blue for protein after the method of Köiw *et al.*(1); b) one strip was air dried then stained with Sudan Black-B for lipids; and c) the other 3 strips were kept from drying by suspending them in a 1000 ml glass cylinder containing water at the bottom and covered with an inverted petri dish to reduce evaporation. As soon as the position of the various proteins along the dried strips was determined, which seldom exceeded 90 minutes from the time the strips were removed from the electrophoretic apparatus, the wet strips were cut as indicated below and the sections transferred to 25 ml glass stoppered Erlenmeyer flasks. Nine sections were employed: 1) the albumin area, which also contained the alpha$_1$-globulin; 2) the area between the albumin, alpha$_1$-globulin and the alpha$_2$-globulin; 3) the alpha$_2$-globulin; 4) a narrow strip between the alpha$_2$- and beta-globulins; 5) the beta-globulin; 6) a strip between the beta-globulin and 5 mm from the starting point; 7) the area from 5 mm of the starting point to 5 mm on the other side of the starting point

* Grateful acknowledgement is made to the Reynolds Metals Co. for research grant and to Lederle Laboratories for summer research fellowship to PCT.

TABLE I. Effect of Temperature Variations on the Electrophoresis of Serum Lipoproteins.

No. of subjects	Avg % of total cholesterol/section with stand. dev.							Temp., °C.	Total chol.,* mg %
	Albumin to $alpha_2$	$Alpha_2$	$Alpha_2$ to beta	Beta	Beta to +5 mm	+5 mm to −5 mm	Gamma		
9	19.2±4.2 (12.7-27.4)	10.2±7.4 (2.5-23.3)	4.6±4.6 (0-12.6)	40.6±5.6 (34.3-48.7)	21.6±7.9 (8.2-31.9)	3.1±1.7 (0-6.1)	.6±1.0 (0-3.0)	35†	(175-272)
	18.2±3.6 (13.3-25.1)	3.3±1.4 (.9-5.8)	.2±.6 (0-1.9)	34.6±15.8 (6.5-55.6)	34.2±9.9 (24.5-50.7)	8.1±4.6 (2.3-17.0)	1.1±1.1 (0-4.1)	5	
4	11.1±1.6 (8.8-12.7)	18.4±14.5 (7.9-39.8)	8.3±3.3 (5.3-12.1)	43.2±8.4 (33.5-53.7)	14.4±2.0 (12.1-16.9)	3.7±3.1 (0-7.6)	.9±1.3 (0-2.7)	35†	(331-346)
	12.8±2.4 (9.4-15.1)	4.7±2.9 (2.3-8.8)	1.8±2.2 (0-4.4)	54.5±4.6 (51.5-61.3)	17.3±4.8 (10.4-21.5)	7.1±1.5 (5.7-9.0)	1.7±1.6 (0-3.2)	5	
1	4.9	2.0	6.8	24.3	41.2	19.2	1.3	35	529
	6.4	1.2	3.0	31.4	36.0	21.0	1.0	5	
1	6.5	32.4	3.8	24.4	21.5	9.2	2.1	35	674
	5.8	6.8	6.1	44.0	22.8	12.4	2.0	5	
Approx. width of strip (mm)	45	20	5	22	17	10	10	(blank = 10 mm)	

* Values below 300 mg % are considered normal for total cholesterol; total concentration determined by method of Forbes(2). (Range.)

† 2 in each of these were run at summer room temp. instead of 35°C.

which usually included some of the gamma-globulin; 8) the gamma-globulin; and 9) a portion 10 mm wide towards the end of the strip to serve as a blank. Approximately 17 ml of a 1:1 mixture of ethyl alcohol and ethyl ether were added to each flask, then stoppered, shaken and set aside overnight. On the following day the flasks were immersed to a depth of about 1 inch for 4 hours in a water bath at 63-65°C. Occasionally the solutions boiled slightly at first but sufficient pressure was never developed to blow the stoppers out. Most of the ether had escaped by the end of the period, at which time the flasks were removed from the bath. The solution remaining in each flask was transferred to correspondingly labelled test tubes and the paper and flask rinsed twice with about 3 ml of the alcohol-ether mixture. The tubes were then put in a water bath not above 63°C and the contents evaporated to dryness with the aid of a current of air. Each tube was removed from the bath as soon as dry.

Cholesterol determinations were made on each set of 9 tubes as follows: 2 ml of chloroform and 0.8 ml of acetic anhydride were added to each tube which was then shaken and put in a water bath at 22°C for 10 minutes; 1 drop of concentrated sulfuric acid was added, the tubes shaken, replaced in the water bath and set aside in the dark for 20 minutes for development of the color. The light absorption was read in a Klett-Summerson photoelectric colorimeter using micro cuvettes and light filter #42. A cholesterol standard was run simultaneously. Separate blanks were run for the standard and unknown solutions.

Some experimental results are shown in Table I. Since there was little cholesterol in section 2) the results thereof are combined with section 1). The values in the Table represent the average of 3 strips for each serum. The agreement between strips was quite good and the average of the sum of the 3 strips was usually in close agreement with the total cholesterol. The results on the various subjects with cholesterol below 300 mg per cent have been combined as are also those with values between 300 and 400 mg.

Although the rate of migration of the main serum proteins, as indicated by the bromphenol blue stain, was not significantly affected by the temperature variations employed, the rate of migration of the beta-lipoproteins was quite definitely affected as shown by both the Sudan Black-B staining and the cholesterol distribution. The temperature effect was especially marked in normal sera with normal cholesterol concentration.

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.

1. Köiw, E., Wallenius, G., and Gronwall, A., *Scand. J. Clin. and Lab. Invest.*, 1952, v4, 47.

2. Forbes, J. C., *J. Lab. and Clin. Med.*, 1931, v16, 520.

3. Boyd, G. S., *Biochem. J.*, 1954, v58, 680.

Received September 12, 1955. P.S.E.B.M., 1955, v90.

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

JAMES P. DEMETRY AND JERRY K. AIKAWA.†
Department of Medicine, University of Colorado School of Medicine, Denver.

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

* This study supported in part by grants-in-aid from American Heart Assn. and Wyeth Lab., and by contract with U. S. Atomic Energy Commission.

† Established Investigator of the American Heart Assn.

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.