TABLE II. $C^{14}O_2$ Production from Stearate-1-C^{14} by Liver Homogenates.*

Rat	Dietary supplement	$C^{14}O_2$ (counts/min.) Per mg N	Per liver of 100 g rat
a	None	121	14,750
b	"	212	25,800
c	Choline	835	70,000
d	"	1000	116,000
e	GDM	256	32,500
f	"	163	17,000

* Each value is avg of data obtained on duplicate flasks containing the same homogenate. Each flask contained, in a total volume of 6 ml of Ringer-phosphate without Ca and Mg (pH 7.4), 0.375 g of homogenized liver, 12 μmoles of adenosine triphosphate, 3 mg each of penicillin and dihydrostreptomycin and 1 μmole (= 1 μC) of stearate-1-C^{14}.

diet oxidized isotopic stearate at a much higher rate than the livers of the control rats. On the other hand, $C^{14}O_2$ produced by the livers of the animals receiving MTG or GDM was about the same as that produced by livers of rats on the unsupplemented diet.

It is now established that the transfer of the methyl group of methionine must be preceded by the conversion of the free amino acid into an active form, S-adenosylmethionine(5). It seems probable that transmethylation from the thiomethyl groups present in other compounds should require a similar activation of the sulfur to the "onium" level. The lack of specific enzymes for the conversion of the glucose derivatives which we have tested, into active forms might perhaps explain their inability to act as methyl donors in the organism.

Summary. Supplementation of a high fat, low-protein diet with two thiomethyl derivatives of glucose (glucose dimethylmercaptal and methyl-thioglucoside) did not prevent the fatty infiltration of the liver, and did not enhance the ability of the tissue to oxidize fatty acid added *in vitro*.

The continued interest and helpful suggestions of Dr. C. Artom are gratefully acknowledged.

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Free Electrophoresis of Lipoproteins. (22585)

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There are comparatively few reports on serum lipoprotein investigations by moving boundary electrophoresis(1,2), because of the difficulty inherent in the interpretation of the patterns due to the low density of the lipoproteins(3) and their possible transformations during dialysis. Paper electrophoresis (4,5) has yielded some information on these substances but this method too, has its shortcomings because of the adsorption of lipid or lipoproteins and their stains on the paper(6). In addition, differences have been found between moving boundary and paper electrophoresis for α_2-, β-, and γ-globulins(7).

In the present investigation, advantage is taken of the ultracentrifugal technic for isolation of the low density lipoproteins(8). The characteristics of the latter as revealed by moving boundary electrophoresis were studied and it is believed that this procedure can be used profitably in comparative lipoprotein investigations.

Methods. Blood (80 ml) was collected from each of 5 healthy male medical students,

TABLE I. Chemical Analysis of Fractions Obtained in Ultracentrifugal Separation.

	Total protein, g %	Phospholipid, mg %	Total cholesterol, mg %
Whole serum	6.85-7.21	194.5-245.0	224- 258
Lipoprotein concentrate	1.52-1.23	580 -720	920-1040
Lipoprotein-poor solution	3.13-3.92	135 -144	64- 67

was allowed to clot at room temperature for 2 hours, after which the serum was separated by centrifugation. A portion of this serum was used for electrophoretic analysis. The rest was diluted with an aqueous sodium chloride solution to give a final solution density of 1.0631 and centrifuged at 30,000 rpm (16°C) for 18 hours in a Spinco Model L ultracentrifuge. The top layers containing the low density lipoproteins were taken off and combined and the bottom fractions were also combined and saved. The former is designated below as the lipoprotein concentrate and the latter as lipoprotein-poor serum solu-

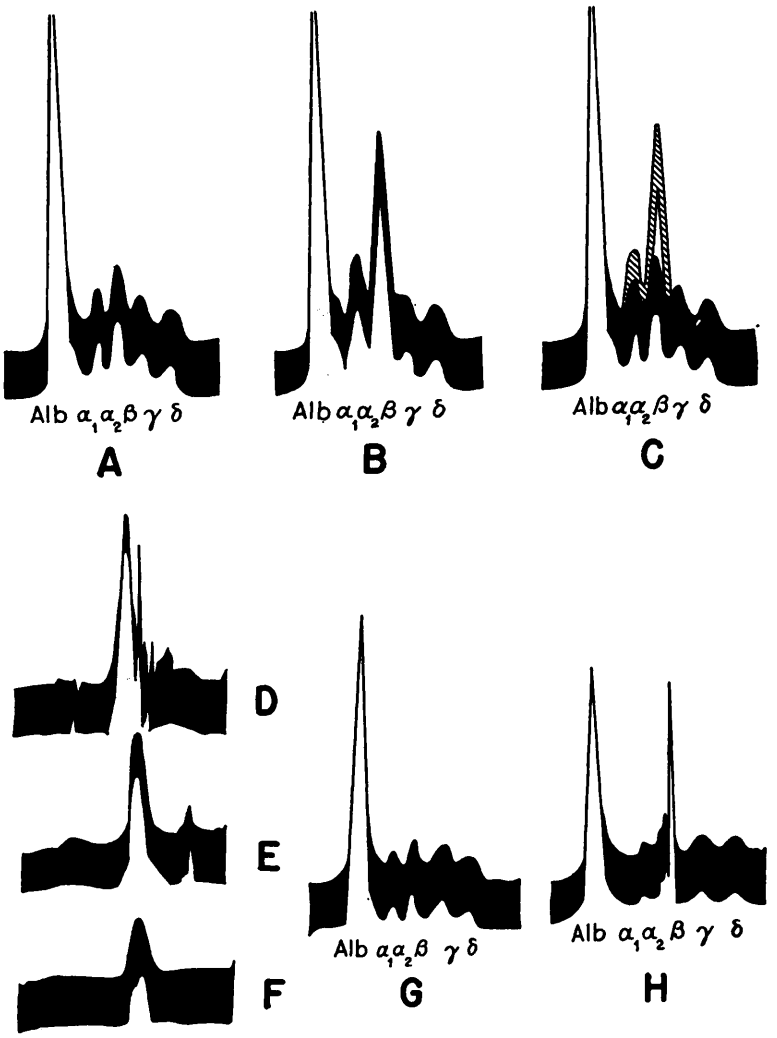

FIG. 1. Representative electrophoretic patterns obtained in these experiments. *A* is normal serum. *B* is normal serum plus 0.5 ml lipoprotein concentrate. *C* shows *B* superimposed on *A* (crosshatched area is effect of added lipoprotein). *D*, *E* and *F* are patterns obtained with solutions of lipoprotein concentrate 1.5, 1.0 and 0.5 ml, respectively, in a total volume of 3 ml. *G* is lipoprotein-poor serum and *H* shows effect of adding 0.5 ml of lipoprotein concentrate to *G*.

tion. Table I gives the analyses on the 3 fractions. For electrophoretic analysis, 1 ml of clear serum was diluted with 2 ml veronal buffer pH 8.6 and ionic strength 0.1. The same buffer was used for all fractions. The diluted serum was dialyzed for 30 hours against 450 ml of the buffer at 4°C. Analyses were made in the Perkin-Elmer electrophoresis apparatus model 38. The length of the runs was 4,200 sec. (1°C) and the patterns were enlarged and calculated from the areas under the curves. A representative pattern is shown in Fig. 1 A. Lipoprotein concentrates, 1.5, 1.0 and 0.5 ml were diluted to 3 ml with the buffer and dialyzed for 48 hours against the same buffer. Two changes of the buffer were made. Electrophoretic runs were carried out for 4,800 sec. (Fig. 1 D, E, F). Lipoproteins-poor solution was dialyzed against veronal buffer for 72 hours with 3 changes of the buffer. Runs were made for 4,200 sec. (Fig. 1 G). .1, .25, .40, .50, and 1.0 ml of lipoprotein concentrate was added to 1.0 ml of normal serum and these were diluted with 1.9, 1.75, 1.6, 1.5 and 1 ml respectively, of buffer. These were dialyzed twice against 450 ml of buffer for 48 hours. Electrophoretic runs were made for 4,200 sec. (Fig. 1 B and C)

Results. Fig. 1 D, E and F show the electrophoretic pattern of the lipoprotein concentrate at different dilutions. Addition of this material to normal serum results in an increase of the β-globulin and a concomitant decrease in albumen (Fig. 1 B, C and Fig. 2). This excess lipoprotein causes no major alteration or disturbance of the general pattern and the individual peaks are easily discernible. It might, therefore, be inferred that the pattern disturbance seen in lipemic serum (9, 10) is not due to the low density lipoprotein but to some other substances.

On the other hand, removal of the low density lipoproteins results in a decrease in the β-globulin peak and to a smaller extent in the α_2- and γ-globulin peaks (Fig. 1 G). When lipoprotein concentrate is added to this solution it gives almost the same results as when it is added to whole serum (Fig. 1 H). The only difference is that the α_2-globulin does not change as it does with normal serum.

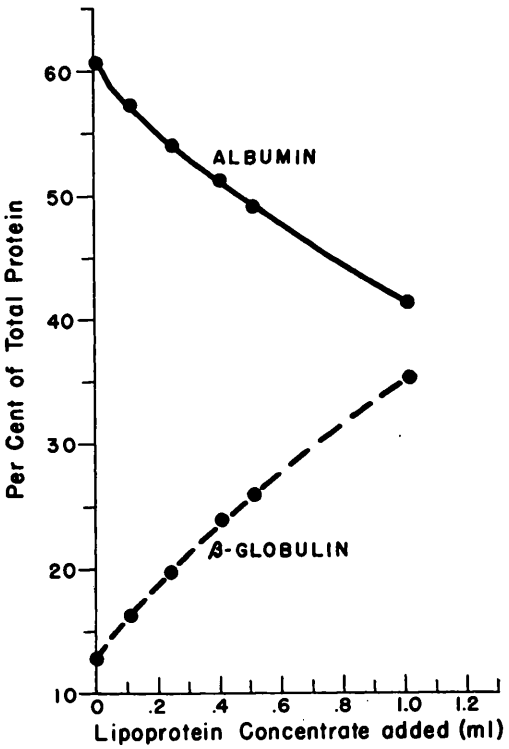

FIG. 2. Effect of adding lipoprotein concentrate to normal serum as calculated from electrophoretic patterns.

It is realized that lipoproteins produce various boundary artifacts in free electrophoresis; however, the technic here suggested can be used to study various changes in lipoproteins during metabolic studies. The effect of various agents on the electrophoretic properties can be observed on a macro scale, and clinical comparisons of lipoproteins under different conditions can be made.

Summary. Analyses by free electrophoresis have been made on the low density lipoproteins obtained by centrifugation of serum at a solution density of 1.063. At pH 8.6 the lipoproteins give a clear electrophoretic pattern showing concentration dependence, but no change in mobility with increase in concentration. Addition of this material to normal serum or lipoprotein-poor serum results primarily in an increase in the β-globulin peak. On the other hand, when the lipoproteins are removed from the serum a significant decrease in this peak is observed. This method is sug-

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Daily Changes in 5-Hydroxytryptamine Concentration in Mouse Brain.*† (22586)

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The status of our knowledge on 24-hour periodicity has been reviewed from neurophysiologic(1) and endocrinologic points of view(2). Almost certainly, 24-hour periodic body function involves a nervous-endocrine-metabolic sequence(3), and in this connection the behavior of neurohumors around the 24-hour time scale appears to be of interest. Earlier work from this laboratory has dealt with the effects of 5-hydroxy-tryptamine (5-HTA) upon the number of circulating eosinophils(4), and thus, perhaps, with the effects of this neurohumoral agent upon adrenal mechanisms of physiologic 24-hour periodicity. This report is concerned with the daily changes in the concentration in mouse brain of 5-HTA itself. From thorough reviews of the literature on 5-HTA, made by Erspamer (5,6), it would appear that this problem has not previously been considered.

Materials and methods. Seven series of determinations were made on ZBC‡ mice, male or female, 5 or 7 weeks of age. Purina Fox Chow and tap water were available to all of the mice since weaning and until sacrifice. For at least 7 days prior to sacrifice, the mice were singly housed in a room maintained at 78 ± 2°F, and illuminated by artificial light only. A clock controlled switch turned the lights on at 06:00 and off at 18:00.

In a given series, groups composed each of 25 mice were used during certain periods of day (specified under results). During any one period of day, individual mice were decapitated, a mid-line incision was then made through the skull with small, pointed scissors, the skull divided, and the entire brain was removed and weighed to the nearest milligram on a torsion balance. Immediately after weighing, the brain was transferred to a previously cooled mortar containing 95% acetone (20 ml/g, as suggested by Amin *et al.* (7)) and crushed with a pestle. The time elapsed between decapitation and the beginning of extraction did not exceed 1½ minutes.

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‡ ZBC mice are obtained by mating F_1 hybrid females of several stocks to a male of the Z or Zb stock.