These data suggest that the L.E. serum factor has an affinity for nuclear nucleoprotein and that deoxyribonucleic acid is involved in the bond. The fact that the L.E. serum factor is a γ-globulin that appears to react with antiserum to normal γ-globulin suggests that the factor may be an antibody. This possibility merits further investigation, with particular reference to the question of whether or not the L.E. factor could be an autoantibody to nucleoprotein or deoxyribonucleic acid.

Note added in proof: Recently we have learned that George Friou of West Haven, Conn., has applied the fluorescent antibody technique to the study of properties of L.E. serum and has obtained results similar to those reported here.

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Interface Enrichment of Methylene Blue by Fatty Acids with Microanalytic Applications

One of us (G.M.) has observed that thionine dyes are attracted by fatty acids to the interface between alkaline water and water-immiscible fatty-acid solvents. This attraction provides the basis for a convenient new method of measuring very small amounts of certain long-chain fatty acids. In particular, palmitic, oleic, stearic, elaidic, and linoleic acids, separately or in mixtures, have been shown to cause the basic dye, methylene blue, to become enriched at the interface of a two-phase system consisting of a heptane mixture (Skellysolve D) plus methylene blue in ammonia water. The loss of dye from the aqueous bulk phase is a logarithmic function of the amount of such fatty acids in the system. Thus, total concentration of the afore-mentioned fatty acids may be determined by application of this method.

To determine the fatty acids, a solution of 500 μg of methylene blue chloride (minimum assay, 98.5 percent) in 100 ml of 0.6*M* NH_4OH was prepared within an hour before use. Fatty acid working standard solutions containing 100 mμ mole of reagent-grade acid per milliliter of Skellysolve D solution were prepared and kept in glass-stoppered bottles (stoppers were wetted with glycerol to prevent evaporation) away from light. Standard curves for the fatty acids usually were made with mixtures simulating natural fats. Final volumes of 2 ml for the hydrocarbon phase were used in the determinations. Typical standard curves were made by pipetting 0, 20, 40, 80, 120, and 160 millimicroequivalents of the acids into sulfuric acid-dichromate cleaned Coleman spectrophotometer cuvettes (19 by 105 mm round). Into each tube, 6-ml volumes of the ammonia-methylene blue solution were added. All tubes were capped with Saran wrap covered stoppers and shaken 100 times vigorously in a rack. Immediately after this, the cuvettes were placed in a size 2, model V International centrifuge, and the power was adjusted to bring the centrifuge up to 1600 rev/min in 60 seconds; the centrifuge was then slowly braked to a stop. The cuvettes were carefully removed to prevent disturbance of the interface. The optical density was read immediately in a Coleman universal spectrophotometer at 665 mμ. The running of a standard curve with each set of unknowns, and uniform standardization of the procedure, and the maintenance of a constant ambient temperature were found to be very critical factors because this system is not in equilibrium when readings are made.

Table 1 shows typical optical density values observed. These were obtained using a mixture of fatty acids similar to lard (*1*). The precision of the standard curve is indicated by the function showing percentage standard error of duplicate analyses. This function was obtained from the formula

$$\frac{\sqrt{\dfrac{\Sigma(x_1 - x_2)^2}{2}}}{\dfrac{n-1}{\bar{x}}} \times 100 = \text{percentage standard error of duplicate analyses.}$$

In this formula,

$$\frac{x_1 - x_2}{2}$$

is the range in millimicroequivalents around the mean of duplicate values, $\bar{x}$; the number of sets of duplicates is denoted by n.

Preliminary studies have shown that this procedure can be used for the measurement of unesterified long-chain fatty acids in plasma (0.2 to 0.5 ml of sample is needed) and of total saturated and unsaturated long-chain fatty acids after alkaline hydrolysis of a 0.1-ml sample.

Furthermore, a check of this method was made on ethanolic KOH saponified samples of lard and sunflower seed oil. The amount of fatty acids found by titration of 4-g fat samples checked within 3 percent of the values obtained using the methylene blue interface enrichment method on aliquots of the lard and sunflower seed oil soaps.

Table 1. Typical optical density values obtained in the determination of total long-chain fatty acids with percentage standard error for duplicate readings. The mixture contained palmitic, stearic, oleic, and linoleic acids in the molar ratios of 5/3/9/1.

Total fatty acid (mμmole)	Optical density	Percentage of standard error for duplicate readings
10	1.030	18.3
20	0.960	4.6
40	0.810	3.6
80	0.525	3.1
120	0.320	6.6
160	0.245	11.2
200	0.220	8.4

It should be stated that caprylic and lauric acids do not cause any observable concentrations of methylene blue at the interface. Moreover, linolenic acid and myristic acids only weakly retain methylene blue at the interface in these procedures. However, iodinated linolenic acid behaves in the same manner as palmitic, oleic, stearic, elaidic, and linoleic acids. Nevertheless, it is evident that this method may be applied for assay of nearly all the long-chain fatty acids in mammalian blood and tissue lipids and in vegetable lipids, except butter and some vegetable fats.

This new method is not only more sensitive but also more practicable than previously available methods for measuring long-chain fatty acids: the materials and equipment required are within the scope of any basically equipped chemistry laboratory.

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Successive Reversal of a Position Habit in an Invertebrate

The performance of white rats on a series of discrimination reversals based on spatial cues (*1*) has been extensively investigated. These studies show that after a very brief period of negative transfer there occurs a gradual reduction in the number of errors with each succeeding reversal. This interreversal im-