The mechanism and biological significance of the negative nitrogen balance after injury is not clear. It is generally assumed that the direct destruction of tissue during operation is not the responsible factor. Hormonal and enzymatic factors might be decisive. There are many similarities in the nitrogen metabolism in response to injury and the general "alarm" or "adaptation" syndrome(9). The adrenocortical hormone of the pituitary(10) and the 11-oxycorticoid(11) produced negative nitrogen balance. Apart from the decrease of the total mass of liver proteins, the concentration of protein enzymes(12,13) including D-amino acid oxidase(14) was found to be decreased in the liver of rats fed a low protein diet. A disturbance of the enzyme systems of the liver, especially those connected with the protein metabolism, may be at least in part responsible for the postoperative protein disequilibrium.

Summary. 1. Distribution of serum protein components was investigated by the use of paper strip electrophoresis in 45 patients undergoing surgery. 2. A significant decrease in the albumin and increase of α_1 and α_2 globulins occurred in one-third of the cases during the first 24 hours following operation. These changes were present in 80% of all cases during the following 4 days. Thereafter the serum components tended to return to their preoperative levels. Components with mobilities different from those found preoperatively occurred in 3 cases after operation. 3. Total serum proteins and the hematocrits did not show any significant variation in the early postoperative period.

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An Electrophoretic Method for Titration of Antisera and Its Application to Anti-Tumor Sera.* (20761)

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Coulter(1) reported that the electrophoretic mobility of washed sheep erythrocytes which had been sensitized with homologous antiserum was less than that of normal washed cells. Shortly thereafter De Kruif and Northrop(2) concluded from their observations on the behavior of *Bacillus typhosus* in the presence of its homologous antiserum that the antibody combines with the surface of the bacteria, producing a surface charge density dependent upon the proportion of the surface so combined. This specific effect of homologous antiserum has since been demonstrated for the

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erythrocytes of several species and for numerous species of bacteria. Shibley(3) further showed for a variety of bacterial species that the electrophoretic mobility continued to change with increasing concentration of antiserum with an apparent convergence upon a common value, shown to be that of denatured pseudoglobulin.

From consideration of these early data it appeared probable that an electrophoretic technic could be employed for the titration of antisera with respect to their combination with the cell surface, as distinct from any secondary reaction possibly following combination. Such a method should contribute to the fundamental study of antigen-antibody reactions as well as provide a practical method for the assay of antisera in which either no secondary reaction occurs or in which such a reaction cannot be suitably measured. The work presented here describes the development of such a method using cells of the Gardner Mouse lymphosarcoma (6-C3H-ED) and homologous antiserum for which no suitable *in vitro* assay method had been developed. Our objective has been the development of the simplest and most rapid method that would give a precision equivalent to that of a doubling dilution tube serological test.

Methods and materials. Tumor: The Gardner lymphosarcoma 6-C3H-ED was grown as an ascites tumor. The line was started by the intraperitoneal injection of 0.5 ml of 20% whole cell homogenate, and thereafter line transfers were made at seven-day intervals by the intraperitoneal injection of 0.5 ml of ascitic fluid. *Buffer:* The buffer used was M/15 sodium potassium phosphate buffer, pH 7.3, diluted with 9 vol. of a 5.4% glucose solution. The capacity of this buffer was sufficient to maintain the pH of the sample at pH 7.3 $\pm$ 0.2 throughout all the necessary manipulations. The advantages of this buffer in electrophoresis have been described elsewhere(4). *Antisera:* Antisera were prepared by the immunization of rabbits with either whole tumor cells from ascitic fluid (No. 4, No. 5b, and No. 6) or with a homogenate of lymphosarcoma tissue (No. 9 and No. 10). *Preparation of the samples for electrophoresis:* The ascitic fluid was centrifuged for 15 minutes at 2800 rpm in a small angle head machine. The resulting pellet was twice washed with buffer and was then made up to a concentration by volume of 0.2%. The pellet was uniformly suspended by forcing the suspension through a 21-gauge syringe needle. Approximately 90% of the cell concentration appeared as single cells. The concentration of erythrocytes was very low. Dilutions of antiserums were made by the addition of the required amount of serum to the buffered cell suspension.

It is obvious that variation among samples standardized in this manner will be high. However, for all cell concentrations suitable for microelectrophoresis (approximately 0.1% to 0.4%) the mobility was found to be independent of the cell concentration. Measurements of the electrophoretic mobility of tumor cells made at periods from 0-20 hours after the addition of antiserum failed to reveal any significant change with time. Therefore, the measurements were made 15-30 minutes after the addition of antiserum.

Electrophoresis apparatus and technics: The electrophoresis apparatus was that described by Hartman, Bateman, and Lauffer(4) with the cell oriented in the lateral position. This orientation, in combination with an eyepiece reticle having a rectangular grid, allows the measurement of the horizontal component of travel (the elecetrophoretic velocity) while the cells are sedimenting through a distance up to 500 μa. At currents from 0.25-0.50 ma. satisfactory data were obtained at all concentrations of antiserum as long as the tumor cell concentration was held within the range recommended above. The excursion times of 10 individual cells were measured, the electric field then reversed, and a similar set of measurements were made on 10 different cells. Measurements were made at the upper stationary level only.

Neutralization test: Antisera were doubly diluted from 1:2 to 1:64. To the serum dilution was added an equal volume of a 2% homogenate of lymphosarcoma tissue in saline. Normal C3H mice were then inoculated subcutaneously with 0.2 ml of the tumor-antiserum mixture in both the right and left groin regions.

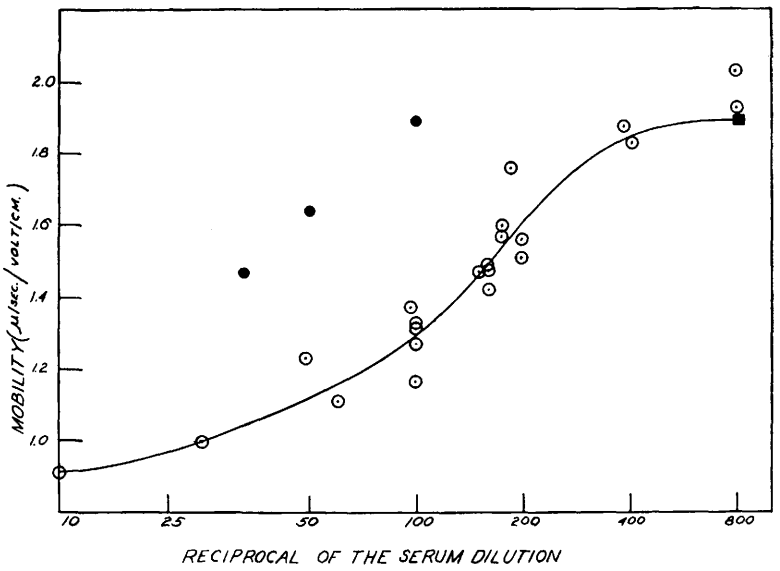

FIG. 1. Electrophoretic mobility at 25°C of lymphosarcoma cells in the presence of antiserum diluted with M/150 phosphate buffer containing 4.85% glucose, pH 7.3. All migration was toward the anode. ⊙ homologous antiserum. ● normal rabbit serum. ■ avg value in buffer only.

Discussion. It is obvious that, given an antiserum of sufficient antibody content, the cell mobility versus antiserum dilution curve will, at high dilution, approach asymptotically the mobility in buffer alone and, at low dilution, the mobility representing the combination of antibody with every available surface receptor. The development of an electrophoretic titration method is possible only if these maximum and minimum mobility values are well separated and if some portion of the curve between them has a slope sufficiently steep for practical experimental determinations. Therefore the first step is the determination of the curve using a potent antiserum so that the minimum mobility value can be measured directly or determined by reasonable extrapolation. Such a curve is given in Fig. 1. It is to be noted that at high concentrations of normal rabbit serum the mobility of the cells is also depressed.

Such curves serve to define several characteristics of the antigen-antibody system. From a comparison of such curves obtained for related systems it may be possible to detect more subtle differences between the systems than is possible using conventional titration procedures. Such detailed comparison will not be attempted at this time. If, however, it is assumed that various antisera differ only with respect to the concentration of antibody or antibodies present and not in their relative proportions, any characteristic property of the titration curve may be selected as the endpoint. The characteristic property which we have chosen is the concentration of the antiserum which produces a given mobility. For maximum sensitivity, this "endpoint" should be selected in the neighborhood of the inflection point of the curve. Applying this criterion to the data given in Fig. 1, the "electrophoretic titer" of sera prepared against whole cells or whole cell homogenates is defined as the reciprocal of the serum dilution which will cause homologous cells to assume an electrophoretic mobility of 1.5 μ/sec/volt/cm.

The assay of an antiserum of the type which the endpoint has been determined can be done very rapidly. As the excursion time corresponding to the endpoint under the given electrophoretic conditions is known, the change accompanying successive additions of antiserum can be followed by only a few observations until the endpoint is near. The titer can

TABLE I. Assay of Anti-lymphosarcoma Sera by Electrophoretic Method and Neutralization Test.

Antiserum No.	Neutralization test: Serum dilution 1/2	1/4	1/8	1/16	1/32	1/64	Electrophoretic titer
Normal*	19/30	16/30	17/30	26/30	30/30		<50
4	2/6†	0/6	2/6	6/6	5/6	—	50
6	0/6	0/6	0/6	3/6	6/6	—	100
5b	0/6	0/6	0/6	0/6	4/6	—	200, 210
10	0/6	0/6	0/6	3/6	4/6	4/6	250
9	0/6	1/4	0/6	0/6	1/4	4/6	335, 335

* Summary of assay of 5 normal rabbit sera.

† $\frac{\text{No. of tumor takes}}{\text{No. of sites inoculated}}$.

be estimated from accurate determinations of the mobility of a few different dilutions in the neighborhood of the endpoint.

The available antisera which had been prepared against whole cells or whole cell homogenates were assayed. Table I summarizes the promising correlation between the "electrophoretic titer" and animal protection as demonstrated by the neutralization test(5).

It is obvious from the results of the two completely independent assays of antiserum No. 5b and antiserum No. 9 as well as from consideration of the titration curve that the electrophoretic titration is considerably more precise than our original criterion of acceptability.

Summary. 1. An electrophoretic technic is described which permits the assay of antisera for their power to combine with the cell surface, as distinguished from any reaction subsequent to combination. 2. This technic has been applied to cells of the Gardner lymphosarcoma 6-C3H-ED. 3. Preliminary studies with regard to the correlation of the electrophoretic titer and animal protection are promising.

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Peripheral Assimilation of Fructose in Man.* (20762)

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Uptake of fructose by hepatic cells has been demonstrated by a number of investigators(1-3). On the other hand, the ability of extrahepatic tissue, and specifically of muscle, to utilize fructose as such has been

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