

# Molecular Weight Determination of Protein-Dodecyl Sulfate Complexes by Gel Electrophoresis in a Discontinuous Buffer System

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## SUMMARY

This report describes methods and results obtained by combining the techniques of sodium dodecyl sulfate (SDS) gel electrophoresis and electrophoresis in discontinuous buffer systems. The SDS gel system utilizes a sulfate-borate discontinuity which stacks and unstacks protein-SDS complexes over a range of 2,300 to 320,000 daltons, providing high resolution fractionation. The properties of protein-SDS complexes are investigated by calculating retardation coefficients and apparent free mobilities from Ferguson plots. Apparent free mobilities are approximately constant, establishing a linear relationship between the logarithm of the relative mobility and the retardation coefficient. The retardation coefficient is shown both empirically and theoretically to be a uniform function of molecular weight of protein-SDS complexes over specified ranges, providing a rationale for determining molecular weight from plots of the negative logarithm of relative mobility against molecular weight.

This report describes an electrophoretic system capable of stacking and fractionating protein-dodecyl sulfate complexes over a range of 2,300 to 320,000 daltons. The system combines the advantages of electrophoresing protein-SDS<sup>1</sup> complexes pioneered by Shapiro, Viñuela, and Maizel (1) and the advantages of achieving thin starting zones by use of discontinuous buffers discovered by Ornstein (2) and Davis (3). This SDS discontinuous system was developed to fractionate plasma membrane proteins solubilized in SDS and does provide high resolution patterns of solubilized membranes, resolving over 40 discrete bands (4, 5).

In the course of accumulating data with this SDS system, it became apparent that the usual type of calibration curve used in SDS gel electrophoresis was only approximately linear and that the actual curves of log molecular weight ( $M$ ) versus relative mobility ( $R_F$ ) were sigmoidal in nature. It also became apparent that certain protein markers could not be fitted on linear

log  $M$  versus  $R_F$  plots unless 25% deviations were tolerated. It was not apparent whether this was due to the alkaline nature of the discontinuous system, the lower ionic strength, or the particular proteins used. In order to investigate the sources of these deviations, it became necessary to explore the theoretical foundations of determining molecular weights from relative mobilities in gels. This subject had been investigated for proteins, but at the time of this study only empirical correlations of molecular weight and relative mobility had been reported for protein-SDS complexes. Recently, however, Chrambach and Rodbard (6, 7) have outlined a theoretical treatment for this subject which is similar to the one presented here.

In this report I explore the relationship between relative mobility and molecular weight of protein-SDS complexes by calculating retardation coefficients and free mobilities from gels of differing acrylamide concentrations. The finding that protein-SDS complexes have nearly identical free mobilities provides a theoretical justification for calculating molecular weights from relative mobility values obtained at a single gel concentration. The assumptions behind these calculations illustrate the sources of error inherent in the method, and ways of minimizing these errors are suggested.

## MATERIALS AND METHODS

The principle used in obtaining a disc system to operate in SDS was to find a system capable of stacking and unstacking charged, highly mobile polymers. After trying several systems designed for nucleic acids, we discovered that borate-sulfate buffers gave excellent resolution. While attempting to optimize this system, we discovered that this system was one of the 4269 multiphasic buffer systems calculated from theory by Jovin, Dante, and Chrambach (8). The recipe given below comes from their computer output. The only modification consists of the addition of 0.1% SDS to the upper reservoir buffer. Upper reservoir buffer, pH 8.64, 0.04 M boric acid-0.041 M Tris-0.1% SDS; upper gel buffer, pH 6.1, 0.0267 M H<sub>2</sub>SO<sub>4</sub>-0.0541 M Tris (running pH 8.64); lower gel buffers, pH 5.77, 0.0954 N HCl-0.0954 M Tris, (running pH 8.64); pH 8.47, 0.0494 N HCl-0.1716 M Tris (running pH 9.05); pH 9.18, 0.0308 N HCl-0.4244 M Tris (running pH 9.50); pH 9.81, 0.0237 N HCl-1.3147 M Tris (running pH 10.01); and lower reservoir buffer, same as lower gel buffer.

The gels are described by the notation of Hjerten (9), in which the first numeral ( $T$ ) denotes the total weight of monomer

<sup>1</sup> The abbreviation used is: SDS, sodium dodecyl sulfate.

(acrylamide plus *N,N'*-methylenebisacrylamide) per 100 ml of solvent, and the second numeral (*C*) denotes the amount of *N,N'*-methylenebisacrylamide expressed as a percentage (w/w) of the total amount of monomer. Upper gels are all  $3.2 \times 6.25$ . Lower gels vary between  $5 \times 2$  and  $25 \times 0.1$ . The standard gel ( $11.1 \times 0.9$ ) is polymerized with 0.15% *N,N,N,N'*-tetramethylethylenediamine and 0.05% ammonium persulfate at 15°. Several methods insure flat reproducible surfaces. (a) *N,N,N,N'*-tetramethylethylenediamine and ammonium persulfate are made up fresh daily in aqueous solutions and are added to concentrated acrylamide and buffer stock solutions. (b) The amounts of *N,N,N,N'*-tetramethylethylenediamine and ammonium persulfate are the minimal amounts required to produce polymerization at 10 min. If either catalyst is present in excess, polymerization time will be increased and distortion of the surface will occur. (c) Isobutyl alcohol (30  $\mu$ l) is layered over the lower gel solution after filling tubes and is removed after polymerization prior to casting the upper gel.<sup>2</sup> (d) If gels 10 cm or longer are cast in narrow tubes (10  $\times$  0.5 cm), contraction of the volume on polymerization can cause a downward curvature of the upper surface. Substituting thin vinyl sheeting tied with dental floss or rubber bands for rubber stoppers allows the curvature to take place at the tube bottom thus minimizing the problem.

Gels 5 mm in diameter were run at 1.5 ma per tube at 25° in a jacketed reservoir with circulation of buffer around the gels by means of a magnetically driven stirring bar in the lower reservoir. With large samples (0.2 to 0.4 ml), current was reduced to 0.5 to 0.1 ma until the sample entered the upper gel.

Staining or destaining was done with Coomassie blue as described by Weber and Osborn (10), except that staining time was increased to 15 hours and the dye concentration was reduced to 0.007% to 0.07%.

Relative mobilities were calculated relative to the borate-sulfate front which stacked the marker dye bromphenol blue (Canalco, Rockville, Maryland) or a red impurity of high mobility in the high pH or high gel concentration runs. Dye fronts were marked by insertion of a section of stainless steel surgical wire.

Proteins used as markers are listed in Table I along with their sources and references for molecular weights. Individual protein concentrations generally ranged between 0.1 and 5  $\mu$ g per sample. When available, chromatographically purified proteins were purchased. Proteins, except as noted, were dissolved in 0.05 M Na<sub>2</sub>CO<sub>3</sub> and exposed to 8 mg of SDS per mg of protein for 1 min before the addition of 10% by volume  $\beta$ -mercaptoethanol. They were then dialyzed against upper gel buffer containing 0.1% SDS, 0.05% dithiothreitol, 2% sucrose, and a trace of bromphenol blue. When the dimers of  $\gamma$ -globulin and  $\beta$ -galactosidase were desired, these proteins were dissolved in 0.1% SDS without reduction or heating.

Experiments designed for the calculation of retardation coefficients utilized a constant ratio of bisacrylamide to acrylamide of 1:90.

#### RESULTS

*Log M Versus R<sub>F</sub> Plots*—The sulfate-borate system stacks protein-SDS complexes over a molecular weight range of 2,300 to 320,000, providing very sharp bands even when sample column heights of 2 cm are used. Under conditions of these experiments, 20 ng of protein in the 40,000 to 100,000 molecular weight range could be resolved by the Coomassie blue stain.

<sup>2</sup> A. Chrambach, personal communication.

TABLE I

Code	Protein	Mol wt	Reference for mol wt	Source
		daltons $\times 10^{-3}$		
1	$\gamma$ -Globulin dimer, rabbit	320	11	Hyland Labs <sup>a</sup>
2	$\beta$ -Galactosidase dimer	260	12	Worthington
3	Myosin	200	13	— <sup>b</sup>
4	$\beta$ -Galactosidase	130	10	Worthington
5	Phosphorylase <i>a</i>	94	10	Worthington
6	Bo ine serum albumin	68	10	Pentex
7	Catalase	60	10	Worthington
8	O albumin	43	10	Worthington
9	DNase I	31	14	Worthington
10	Carbonic anhydrase	29	10	Pentex
11	Chymotrypsinogen	25.7	15	Worthington
12	Trypsin-diisopropyl fluo rophosphate	23.3	15	Worthington
13	Cytochrome <i>c</i> dimer	23.4	15	Sigma
14	$\beta$ -Lactoglobulin	18.4	10	Pentex
15	Myoglobin	17.2	15	Pentex
16	Hemoglobin	15.5	15	Pentex
17	Chymotrypsin C chain	13.0	15	Worthington
18	Cytochrome <i>c</i>	11.7	15	Sigma
19	Chymotrypsin B chain	11.0	15	Worthington
20	Pancreatic trypsin in- hibitor	6.16	15	Mann
21	Glucagon	3.5	15	Sigma
22	Insulin B chain	3.3	15	Mann
23	Insulin A chain	2.3	15	Mann

<sup>a</sup> Los Angeles, California.

<sup>b</sup> Gift from Richard Vernick, Peter Bent Brigham Hospital, Boston, Massachusetts.

Values of relative mobility ( $R_F$ ) obtained with the sulfate-borate system in a gel ( $11.1 \times 0.9$ ) are plotted against molecular weight ( $M$ ) on a semilog scale (Fig. 1A). This relationship, first introduced by Shapiro, Viñuela, and Maizel (1), has been found to be empirically valid for at least 40 different protein-SDS complexes (10, 11). The results shown in Fig. 1A are similar to those reported in neutral continuous systems in that (a) a relationship exists between  $R_F$  and  $M$ , (b) the curve connecting the high  $M$  points is hyperbolic, and (c) the low  $M$  region is the region of maximum scatter from a smooth curve. These results differ from previously reported data in that in the low  $M$  region it is possible to fit points with two different straight lines. In the range of 70,000 to 15,000, a straight line can be drawn through the points for albumin and hemoglobin and nine markers lie on this line (deviations in  $M$  less than 5%). The points for DNase, chymotrypsinogen, cytochrome *c*, and chymotrypsin B chain all show deviations of more than 20% of  $M$  from this line. However, a steeper line will fit these points and four others. A shallow sigmoidal curve will provide the least deviation of points from a line. When data from 7, 9, 13, and 15% gels are plotted in the same manner, the results are similar, and the impression is gained that the true nature of the function is sigmoidal. This point, illustrated by Fig. 1B, will be considered in relation to Equation 12 in the discussion section.

*Ferguson Plots*—Values of the logarithm of relative mobility obtained at five different gel concentrations are plotted against the gel concentration ( $T$ ) for 10 different protein-SDS complexes

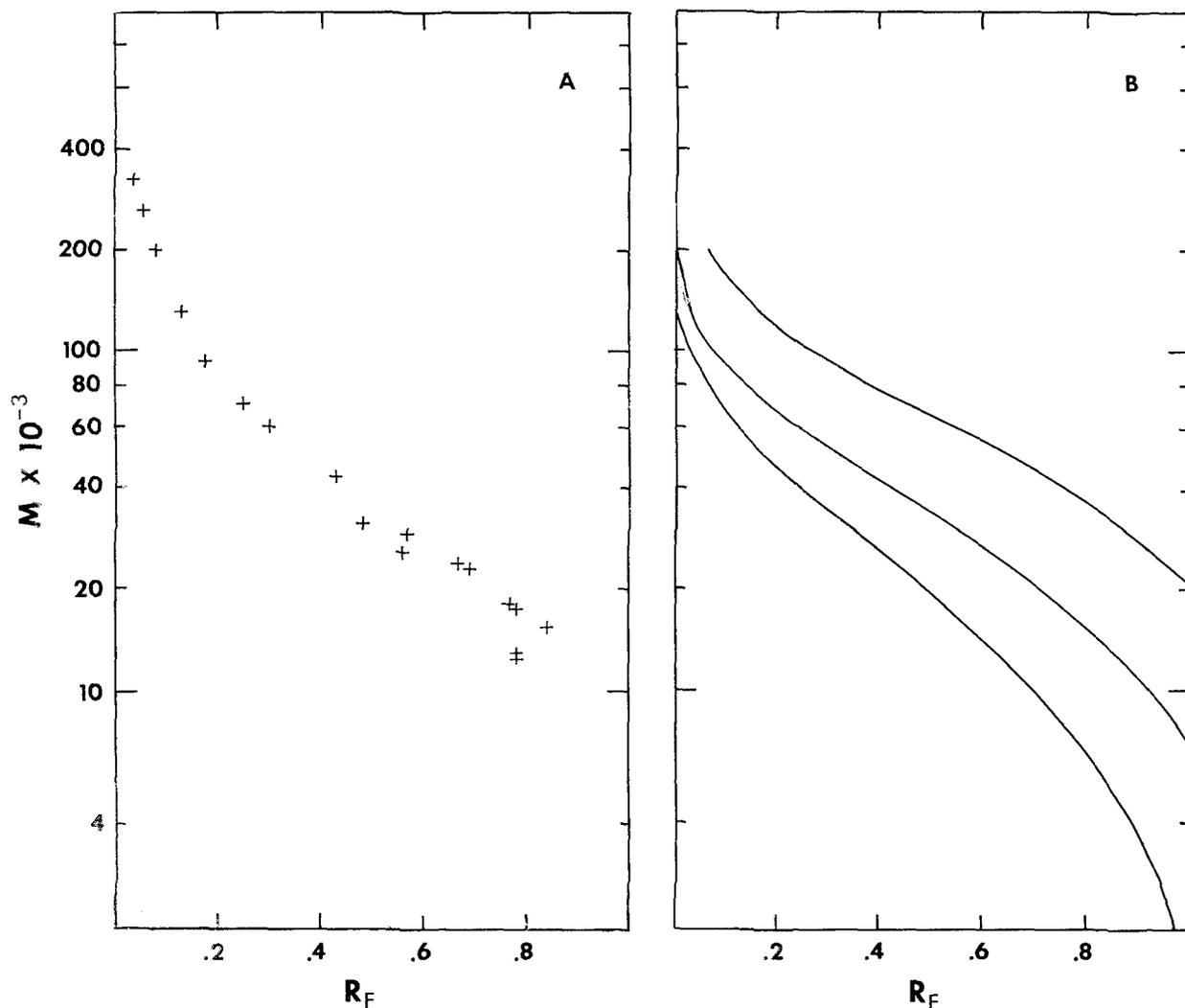


FIG. 1. *A*, molecular weight ( $M$ ) on a semilog scale is plotted versus relative mobility ( $R_F$ ) for a variety of protein-SDS complexes subjected to electrophoresis at pH 9.5 on gel ( $11.1 \times 0.9$ ). In the upper molecular weight region the curve is hyperbolic while in the lower regions the scatter obscures the nature of the relationship. In *B*, theoretical curves are constructed for  $R_F$

and  $M$  on the same scale from values of  $R_F$  computed from Equation 1 by assuming a constant value of free mobility,  $100 \log 100 Y_0 = 230$ , and a linear dependency of  $K_R$  on  $M$  (see Table II). Curves are: top,  $T = 7$ ; middle,  $T = 11$ ; bottom,  $T = 15$ .  $C = 0.9$  for each.

in Fig. 2. This type of plot, first described by Ferguson (16) for starch gels, has been shown by Hedrick and Smith (17) to adequately describe the behavior of 17 globular proteins on acrylamide gels varying in  $M$  from 45,000 to 500,000.

The protein-SDS complexes display two properties not seen with proteins. Complexes of  $M$  greater than 60,000 do not show a linear relationship between  $\log R_F$  and gel concentration. In addition, the molecular weight at which nonlinearity appears is dependent on gel concentration, high gel concentrations augmenting the effect.

The most interesting feature on the Ferguson plot is the fact that the values for the Y intercept are nearly identical for the 10 different complexes. The Y intercept value represents the relative mobility at zero gel concentration, and for proteins carrying different charges, different free mobilities are observed (17). Although it has been previously shown that proteins in SDS bind a constant amount of SDS per unit weight of protein (18, 19), this fact alone would not lead to free mobilities independent of  $M$ . In order to have free mobilities independent of

$M$ , the ratio of the effective charge to frictional coefficient must be independent of  $M$ . In other words, the complex must behave under free electrophoresis as a free draining structure so that the mobility of a large molecule is identical with the mobility of a segment or smaller molecule (20). It is interesting to note that the free electrophoretic mobility of DNA, both native and denatured, is independent of  $M$  between one-quarter million and 130 million (20).

Although the Y intercepts are nearly identical in Fig. 2, the variations are larger than that due to measuring error ( $0.02 RM$  for  $R_F = 0.5$ ). In Fig. 3, the slope of the lines connecting points ( $\log R_F$  and  $T$ ) for identical proteins (retardation coefficient) is plotted against  $\log R_F$  to determine whether any trend with molecular weight can be detected. (Plots are done only in regions where  $\log R_F$  versus  $T$  plots are linear.) There is no apparent trend with molecular weight. However, it is interesting that the points for DNase and hemoglobin show a significant deviation from the line. These deviations must be ascribed to an apparent free mobility different from the average free mobility.

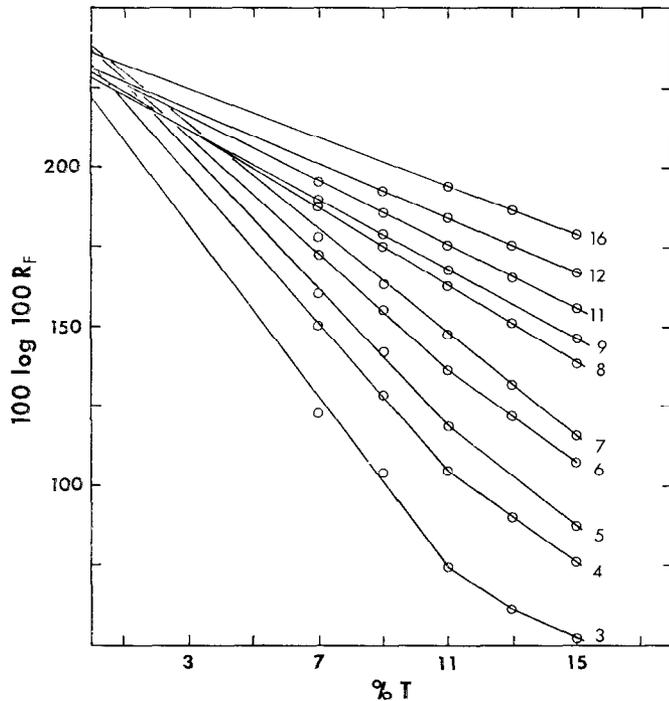


FIG. 2. The logarithm of the relative mobility for 10 protein-SDS complexes is plotted versus the gel monomer concentration ( $\% T$ ) and the plots are extrapolated to  $T = 0$ . All of the complexes have a nearly identical free mobility. Note that the highest molecular weight complexes show deviations from linear plots at high  $T$ . Numbers at right refer to protein code in Table I.

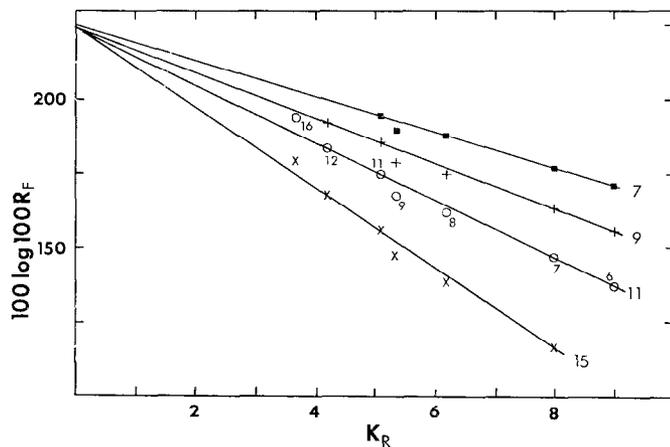


FIG. 3. Values of the retardation coefficient ( $K_R$ ) computed from the slopes in Fig. 2 are plotted versus  $\log R_F$  at four concentrations of  $T$  (numbers at right). Points are given for seven proteins (code next to circles). Points 16 (hemoglobin) and 9 (DNase) show significant deviations from the lines. The other points fall on a line giving a linear relationship between  $K_R$  and  $\log R_F$ .

It is possible that certain complexes exhibit more complicated interactions with the gel, such as adsorption, resulting in non-linear Ferguson plots as the gel concentration approaches zero. This situation would lead to an apparent free mobility different from the average free mobility when all measurements are made at high gel concentrations.

**Effects of Varying pH**—In Fig. 4, the relationship between  $-\log R_F$  and  $M$  for gels ( $11.1 \times 0.9$ ) at varying pH are shown. Increasing the pH results in ionizing more trailing ion and increas-

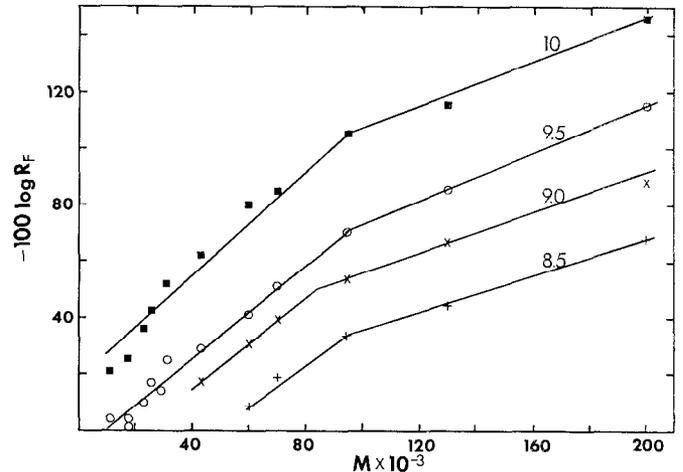


FIG. 4. Molecular weight is plotted versus the negative logarithm of the relative mobility for various protein-SDS complexes in gels ( $11.1 \times 0.9$ ) run at running pHs between 8.5 and 10 (see "Materials and Methods"). As the pH is lowered, more and more proteins travel within the stack in the separation gel. The influence of pH on  $Y_0$  has not been investigated but the relationship between some points on this plot appear significantly different for the pH 9.5 points versus the pH 10 points.

ing the apparent velocity of the stack (2, 8). Consequently, some proteins traveling within the stack at low pH are excluded from the stack at high pH. In a gel ( $11.1 \times 0.9$ ) at pH 8.5 (the running pH of the stacking gel), subunits having  $M < 50,000$  are in the stack, although at pH 10,  $M$  of 5,000 and above are excluded from the stack.

#### DISCUSSION

The results of this study of SDS gel electrophoresis show that the empirical relationship observed between molecular weight and relative mobility in neutral continuous systems is also observed in alkaline discontinuous systems. In addition, these results show that the apparent free relative mobilities ( $Y_0$ ) of protein-SDS complexes are nearly constant. When data obtained with discontinuous systems are plotted in the usual manner,  $\log M$  versus  $R_F$ , considerable scatter is observed in the low molecular weight regions. In an attempt to understand the causes of this scatter, it became necessary to investigate the theoretical foundation for obtaining molecular weights from a relative mobility value on a single gel. Previously, it had been shown that molecular weights of proteins could be estimated if relative mobilities were obtained at more than one gel concentration by use of the Ferguson equation (16).

$$\log R_F = -K_R T + \log Y_0 \quad (1)$$

where  $R_F$  is the relative mobility at gel concentration  $T$ ,  $Y_0$  is the relative mobility at zero gel concentration (the apparent free relative mobility), and  $K_R$  is the retardation coefficient and is a function of molecular size and the percentage of cross-linking. If the percentage of cross-linking is held constant,  $K_R$  can be determined from values of  $R_F$  taken at various values of  $T$ . For 17 globular proteins,  $K_R$  is directly proportional to  $M$  (17).

These results, which show that  $\log Y_0$  is a constant, establish a linear relationship between  $\log R_F$  and  $K_R$  at any given  $T$  (Fig. 3). Once the average value of  $\log Y_0$  has been determined for a given buffer system and percentage of cross-linking, the retardation coefficient can be calculated from a single  $\log R_F$  value at

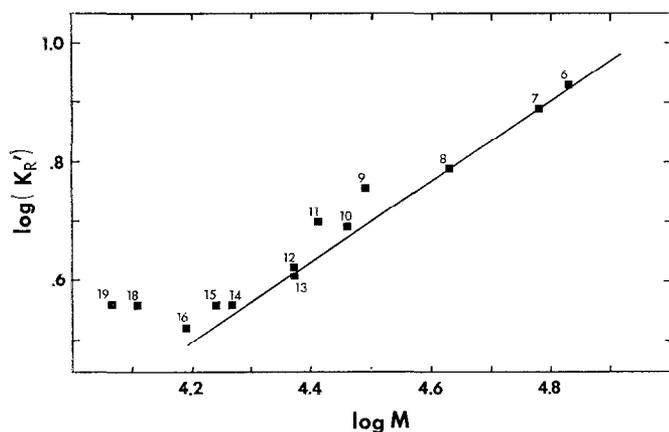


FIG. 5. The logarithm of the retardation coefficient computed from  $\log R_F$  and an average value of  $\log Y_0 (K_{R'})$  is plotted versus the logarithm of molecular weight for gel ( $11.1 \times 0.9$ ) at pH 9.5. The linear relationship holds between 18,000 and 70,000 daltons. Points 9 (DNase) and 11 (chymotrypsinogen) show large deviations from the line.

any single value of  $T$ . Retardation coefficients calculated in this manner are represented  $K_{R'}$ .  $K_{R'}$  will differ from  $K_R$  to the extent that the particular apparent free mobility differs from the average free mobility.

In order to determine molecular weight from  $K_{R'}$ , it is only necessary to determine that there exists a uniform dependence of  $M$  on  $K_{R'}$ . The theory of gel electrophoresis developed by Ogston (21) and Morris (22) and recently extended by Rodbard and Chrombach (23) provides a theoretical basis for the dependence of  $K_R$  on effective molecular radius. These workers consider a gel to be made up of spaces or pores and that for any molecule of effective radius,  $R_e$ , a fractional volume,  $f$ , of spaces is available. This model interconnects both gel filtration, where the distribution coefficient  $K_{av}$  is equivalent to  $f$ , and gel electrophoresis, where

$$f = \frac{R_F}{Y_0} \quad (2)$$

The dependence of  $f$  on  $R_e$  is exponential, with the exponent  $a$  being determined by assumptions involving pore geometry or the type of distribution of spaces.

$$\frac{R_F}{Y_0} = \exp^{-k_1(R_e+r)^a} \quad (3)$$

The most likely exponent is considered to be 2, and data consistent with this value have been reported for acrylamide in gel filtration (24) and gel electrophoresis (23). However, other relationships have not been excluded, and the nature of the data is such that many different dependencies can be fit.  $k_1$  is a constant including gel fiber length per unit of volume, and  $r$  is the fiber radius, which has a value of 5 Å for 1% cross-linking (24). Taking logarithms

$$\log R_F - \log Y_0 = -k_2(R_e + r)^a \quad (4)$$

and

$$k_2 = \frac{k_1}{2.303} \quad (5)$$

and by substitution from Equation 1,

$$K_R T = k_2(R_e + r)^a \quad (6)$$

Since  $T$  is proportional to  $k_2$  (24), this term may be eliminated by division.

$$K_R = k_3(R_e + r)^a \quad (7)$$

and where  $R_e > r$

$$K_R \approx k_4(R_e)^a \quad (8)$$

Reynolds and Tanford (18) and Fish, Reynolds, and Tanford (19) have shown that the hydrodynamic properties of protein-SDS complexes determined from viscosometric and gel filtration data are a unique function of molecular chain length. Their data are expressed in terms of the Stokes radius,  $R_s$ .

$$R_s = k_5(M)^b \quad (9)$$

By assuming that  $R_s$  can be substituted for  $R_e$ ,

$$K_R \approx k_4[k_5(M)^b]^a \quad (10)$$

$$\log K_R \approx ab \log M + \log k_6 \quad (11)$$

$$k_6 = k_4(k_5)^a$$

Equation 11 shows that plots of  $\log K_R$  or  $\log K_{R'}$  against  $\log M$  will be linear with a slope  $ab$  proportional to the relationship between  $R_s$  and  $M$ . Deviations from linearity will occur when the assumptions underlying Equation 11 break down, for example, when the condition  $R_e > r$  is not satisfied. When  $a = 2$ , deviations in  $ab$  greater than 10% do not occur until  $R_e \leq 15$  Å or  $R_e \leq 25$  Å for  $a = 1$ . The decrease in slope in Figs. 5 and 6 below  $M$  of 15,000 could be due to the influence of the  $r$  term. Equation 11 provides a way of relating relative mobility data to molecular weight by means of a plot which is linear under a set of known assumptions.

When the values of Fish *et al.* (19) for  $k_5$  and  $b$  are used with our  $K_{R'}$  data in Equation 11, we calculate the value of  $a$  as 1. As mentioned previously, the preferred theoretical value is 2.

If it is assumed that  $a = 2$ , then  $b = 0.34$  and  $R_s$  varies as  $M^{0.34}$  rather than as  $M^{0.73}$ , as determined by Reynolds and Tanford (18). This would mean that the effective radius in gel electrophoresis is less than the effective radius in gel filtration. When the data of Hedrick and Smith (17) for globular proteins ( $R_s$  varies as  $M^{0.32}$ ) are plotted according to Equation 11,  $ab = 0.5$ , and again assuming  $a = 2$ , the effective radius is less than the Stokes radius.

Although the values for  $a$  and  $b$  in Equation 11 are in doubt for gel electrophoresis, the equation is useful in that alterations in the relationship of  $R_e$  on  $M$  will be apparent by a change in slope. Such a change occurs below 15,000 daltons and above 70,000 daltons. The decrease at 15,000 daltons was also noted for protein-SDS complexes in gel filtration (19) and may be due to the change in geometry from a prolate ellipsoid to a sphere in this region. The effect of the  $r$  term will also tend to decrease the slope in this region. Above 70,000 (Fig. 7), the slope decreases, indicating a decrease in effective radius. Fisher and Dingman (25) have presented data on electrophoretic behavior of nucleic acids in gels by means of  $\log K_R$  versus  $\log M$  plots. Rod-shaped nucleic acids<sup>3</sup> show a smaller slope  $ab$  than random coil nucleic acids of the same  $M$ . In addition, the  $R_F$  of rod-

<sup>3</sup> Both the charge density and the major and minor axes of protein-SDS complexes given by Reynolds and Tanford (18) are quite similar to the same parameters of double stranded nucleic acids of equivalent mass.

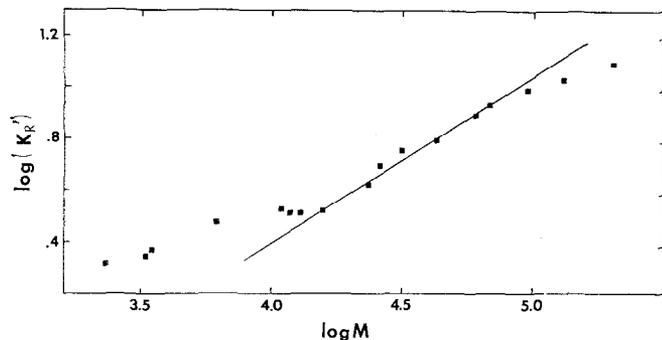


FIG. 6. Same plot as Fig. 5 except done on a gel ( $15 \times 0.9$ ) covering a range from 2,300 to 200,000 daltons. From 18,000 to 70,000 daltons the slope is 0.68 and decreases below and above this region.

shaped nucleic acids increased with increasing voltage gradient. These authors speculate that the highly asymmetrical rod-shaped molecules may be capable of orienting in a manner which minimizes their frictional resistance to the gel (25).

If  $K_R$  varied as  $M^{1.0}$  instead of  $M^{0.68}$  as found,  $k_6 M$  (see Equation 11) could be substituted directly into Equation 1, giving

$$-\log R_F \simeq k_7 M - \log Y_0 \quad (12)$$

If within the region of constant dependency of  $K_R'$  on  $M$  any two markers of known  $M$  have their  $R_F$  determined, the constants  $k_7$  and  $\log Y_0$  are fixed, and  $M$  can be determined directly from values of  $-\log R_F$  for any unknown. This type of plot is shown in Fig. 7. The standard error is  $\pm 3000 M$ . Comparing Figs. 5 and 7 shows that it is not necessary to have the exact relationship of  $K_R'$  on  $M$  for estimating  $M$ . Fig. 5 shows chymotrypsinogen and DNase to be anomalous points. However, this fact is not as obvious from the plot in Fig. 7. The commonly used plot,  $\log M$  versus  $R_F$ , is not as useful since it is sigmoidal in shape when there is a constant dependency of  $K_R$  on  $M$  and when  $Y_0$  is a constant (see Fig. 1B).

From the foregoing discussion it can be seen that the assumptions involved when  $M$  is calculated from a value of  $R_F$  in SDS gel electrophoresis are (a) a constant value of  $Y_0$  for marker and unknown protein-SDS complexes and (b) a constant dependency of  $R_e$  on  $M$  within some range of  $M$ . These assumptions are imposed by the use of the marker proteins used to form the calibration line when  $M$  is plotted as some function of  $R_F$ . If the markers used have significantly different values of  $Y_0$  than the unknown protein-SDS complexes, errors in  $M$  will result. Both DNase and hemoglobin are examples of this type of error. Each shows deviations in plots of  $\log R_F$  against  $K_R'$  and deviations in  $\log R_F$  versus  $M$  plots. That the latter deviations are due to the incorrect estimate of  $Y_0$  is seen by the fact that the deviations are not present when  $K_R$  rather than  $K_R'$  is plotted against  $M$  (see Table II). Therefore, the Ferguson plot provides a means of detecting this type of error. Similarly, if the markers used to form the calibration line have a different dependency of  $M$  on  $R_e$  than the unknowns, errors will result. Therefore, extrapolations into ranges not covered by markers should be avoided, and multiple markers should be used in transition regions. The deviation of chymotrypsinogen in  $-\log R_F$  versus  $M$  plots appears to reflect the second type of error. The deviation in  $M$  is +15% in a  $K_R$  versus  $M$  plot and +17% in a  $-\log R_F$  versus  $M$  plot. Cytochrome *c* and chymotrypsin C chain lie in a region of altered dependency of  $R_e$  on  $M$ . An explanation for the scat-

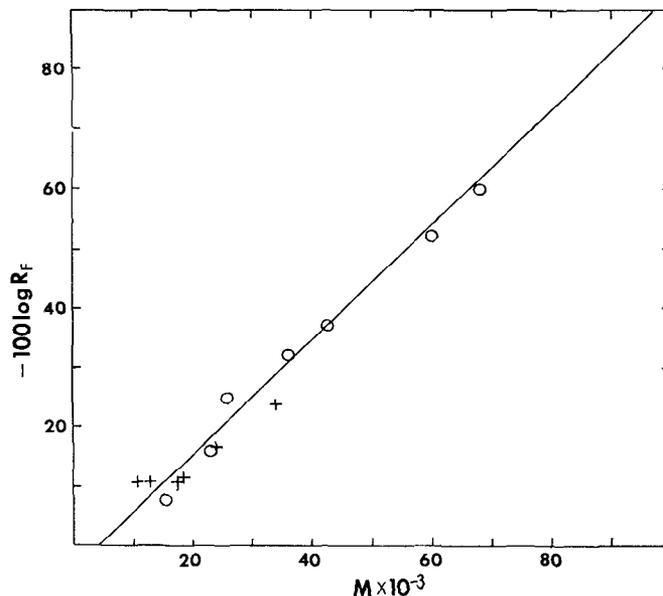


FIG. 7. The negative logarithm of relative mobility is plotted versus molecular weight. Circles represent proteins whose  $K_R$  have been determined. Lines fitted to all points or circles alone are essentially identical although the standard error drops from 3500 to 3000 when all points are used.

TABLE II

Least squares fit of  $K_R$  versus  $M$  data and  $-\log R_F$  versus  $M$  data of form  $y = mx + b$

For each case, the known value of  $M$  has been set equal to  $y$ , and the standard error of estimate of  $y$  on  $x$  ( $S_{y,x}$ ) has been calculated. When  $K_R = x$ , then  $b = -21.42$ ,  $m = 10.03$ , and  $S_{y,x} = 2.2$ . When  $(-100 \log R_F) = x$ , then  $b = 4.180$ ,  $m = 1.033$ , and  $S_{y,x} = 3.5$ .  $M$  and  $y$  values have been divided by 1000.

Protein	$M$	$m(K_R) + b$	Percentage of deviation of $M^a$	$m(-100 \log R_F) + b$	Percentage of deviation of $M^b$
Bovine serum albumin . . . . .	68	68.9	+1	66.2	-3
Catalase . . . . .	60	58.8	-2	57.9	-4
Ovalbumin . . . . .	43	40.6	-6	42.4	-1
DNase . . . . .	31	32.3	+4	37.2	+18
Chymotrypsinogen . . . . .	25.7	29.6	+15	30.0	+17
Trypsin-diisopropyl fluorophosphate . . . . .	23.4	20.5	-12	20.7	-12
Hemoglobin . . . . .	15.5	15.7	+1	12.0	-22

<sup>a</sup> Average deviation 5.8%.

<sup>b</sup> Average deviation 10.8%.

ter of the data is thus provided. Whether or not the conditions of the alkaline buffer system or specific effects of the borate ion aggravate these problems as compared to neutral systems remains to be seen. Gel electrophoresis of protein-SDS complexes with the use of other discontinuous buffer systems is being investigated and those studies may answer some of these questions (6, 26).

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