It would appear that A is presently heterogeneous with respect to amino acid composition and that B may be much less so. On the other hand, both Fractions A and B are not grossly heterogeneous with respect to molecular weight of the monomer kinetic unit, since both gave symmetrical, but broad, peaks on prolonged centrifugation in 6 M urea. More significantly, the statistics of Table I indicate that there was no curvature attributable to heterogeneity of the q_a *vs.* $(c_a - c^0)$ plots since the standard deviation of the slope is low.

Discussion

There may be some advantage in using the Archibald principle in various solvents on a material with a known large activity coefficient term to see if a correct value for the term can be determined. In cases where the correction is small or known, the use of higher density solvents to slow down the sedimentation may extend the usefulness of the Archibald principle to higher molecular weight classes which yield patterns too steep to measure accurately even at the lowest speeds and concentrations now practical.

The data of Fig. 2 are taken from the same experiments reported elsewhere[16] to show the variation of $(\partial c/\partial r)/rc$ with r and to locate the position of r_a from the common intersection of such curves. It was of interest to know whether such information also could be obtained from a q_a *vs.* $(c_a - c^0)$ plot, where the prime represents an incorrect choice of the position of r_a. Treating r_a as a variable, the data were analyzed to see what value minimized the quadratic term in the least squares polynominal. Actually, the very slight curvature reversed as r_a was varied by 0.1 mm. on either side of the geometrical value of r_a. This is interpreted to mean that the q_a *vs.* $(c_a - c^0)$ representation does not sacrifice the ability to determine r_a, if desired. Also, it indicates one cause of non-linearity in the plot. But this curvature is so slight as to be normally undetectable; hence the wrong choice of r_a will still introduce the same systematic error in M_{app} as it does in the $(\partial c/\partial r)/rc$ method—about 10%/0.1 mm. on the plate. On the other hand, polydispersity of molecular weight will yield a large curvature.[2] This has recently been discussed by Erlander and Foster.[18]

The values of M found here for the chromatographic histone Fractions appear significantly greater than those reported by Luck, *et al.*,[7] especially for histone fraction B. Perhaps less degradation is involved in the isolation.

Acknowledgments.—The authors wish to acknowledge with thanks the interest and encouragement of Drs. S. Moore and W. H. Stein.

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NEW YORK, N. Y.

[CONTRIBUTION FROM THE PLUM ISLAND ANIMAL DISEASE LABORATORY, ANIMAL DISEASE AND PARASITE RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE]

Partition by Zone Ultracentrifugation of the Two Complement-Fixing Particles in the Foot-and-Mouth Disease Virus System[1]

BY RODES TRAUTMAN, MILTON SAVAN AND SYDNEY S. BREESE, JR.

RECEIVED JANUARY 31, 1959

The sedimentation coefficient of the smaller complement-fixing (CF) particle of the foot-and-mouth disease virus system, type A, strain 119, in bovine-passed material was determined by assay of moving boundary fractions from a swinging bucket rotor to be $s_{20,w} = 14 \pm 1$ S. Zone preparative centrifugation of the velocity type was used to separate infectious fluids into a virus fraction and a smaller particle fraction. Tests of cross-contamination revealed that there was less than 5% of the original smaller particle material remaining in the virus fraction as determined by CF activity measurement. Correspondingly, there was less than 1% of the virus remaining in the smaller particle fraction as determined by infectivity measurement. Three different pooled samples of freshly harvested infectious vesicular fluid showed half of the CF activity to be in the virus fraction. The corresponding fractions from epithelial extracts showed only one fourth.

The foot-and-mouth disease virus system has been shown to consist of two particles which are regularly associated with the infection in animals and in tissue culture. The larger of the two is the infectious virus itself, which has a sedimentation coefficient of $s_{20,w} = 140 \pm 10$ S (s-rate).[2-5] Both particles exhibit complement-fixing (CF) activity. Recent information on the s-rate of the smaller particle and the partition of total CF particles has been reported by Bradish, *et al.*[6] They reported the s-rate to be 7.8 S and assigned 50–100% of the CF activity to this smaller particle in preparations obtained from infected guinea pig, mouse and bovine sources.

The purpose of the work reported here was to apply zone centrifugation for the quantitative separation of the two sizes of antigens. Such a fractionation would enable direct measurement on crude biological fluids of the partition of the CF activity as well as provide the two antigens free from each other for subsequent experiments. Before investigating the zone method, it was necessary to redetermine the s-rate of the smaller CF particle since

(1) This material was presented before the Division of Biological Chemistry at the 134th National Meeting of the American Chemical Society, Sept. 1958.

(2) A. Randrup, "Physico-chemical and Immunological Studies on the Virus of Foot-and-Mouth Disease and Its Host Tissue Microsomes," E. Munksgaard, Copenhagen, 1957.

(3) H. L. Bachrach, *Am. J. Vet. Res.*, **13**, 13 (1952).

(4) G. Pyl, *Arch. Exp. Vet. Med. (Reims)*, **10**, (3), 358 (1956).

(5) S. S. Breese, Jr., R. Trautman and H. L. Bachrach, *Biochemica et Biophysica Acta*.

(6) C. J. Bradish, J. B. Brooksby, J. F. Dillon and M. Norambuena, *Proc. Roy. Soc. (London)*, **B140**, 107 (1952).

preliminary experiments failed to confirm the 7.8 *S* value. This was done by a non-optical moving boundary method, following the results of ultracentrifugation by the CF assay.

Experimental

Material.—These various biological fluids containing both foot-and-mouth disease virus, type A, strain 119 (FMDV-A119), and smaller CF particles were obtained from: (a) tissue culture (TC) harvest from infected bovine kidney monolayers, partially purified and concentrated as described elsewhere,[7] (b) fresh bovine tongue epithelium scraped from infected lesions and triturated in a mortar or in a blender with Veronal-buffered saline (VBS), *p*H 7.4, Γ/2 0.15 as a 10–20% suspension (in preliminary experiments epithelium was used which had been stored at −60° for 8–18 mo.), (c) bovine tongue vesicular fluid expressed 24 hr. postinoculation. Epithelium suspensions and vesicular fluid were clarified within 4 hr. of preparation by centrifugation selected to pellet all contaminants, including bacteria, with *s*-rate greater than 10 times that of the virus.

Complement-fixing Test.—A direct semi-quantitative CF test[8] modified slightly from that of Traub–Möhlmann[9] with an overnight fixation period at 4–8° was used to determine the highest dilution of antigen in a twofold dilution series capable of fixing 1.5 units of guinea pig complement. A constant amount of guinea pig serum containing specific antibody was used in all CF tests. Visual reading of the end-point was made, grading the level of fixation as 4, 3, 2, 1 or 0. Tubes with a value of 3 or 4 were considered as fixing complement; 0 as 100% hemolysis. The actual dilution of the *antigen* at the end-point was used as the concentration of the antigen in the sample. Starting material and subsequent fractions were assayed simultaneously so that per cent. recoveries could be calculated. This is the restrictive sense in which the abbreviation "CF activity" is used in this paper. From repeated determinations the error of this activity measurement was estimated to be ±25%.

Moving Boundary Ultracentrifugation.—The moving boundary technique was used to determine the *s*-rate of the smaller CF particle by centrifuging the boundary about $^2/_3$ of the length of the tube in a swinging bucket rotor (Spinco SW 39).[10] The antigenic solution throughout the tube was initially compartmented against radial convection due to collision with the walls during centrifugation and torque due to deceleration by using a slight density gradient[11] layered as follows: 1 ml. of the solution containing 50 mg. of sucrose was put into the bottom of the tube, then 2 ml. containing 50 mg. and finally 2 ml. without sucrose. The highest density was thus about 1.02 g./ml. Sampling was done by removing measured volumes of approximately 1-ml. fractions from the top with a Pasteur pipet of 1-mm. bore at the tip. The position of the bottom of each fraction was read from a scale alongside the tube aligned so that the bottom of the tube was at 10.0 cm., its true radius from the axis of rotation. The CF activity of each fraction was measured, and the data analyzed to locate precisely the boundary position.

Tiselius[12] has expressed the mass transport in a sector cell in such a way that the sedimentation coefficient can be calculated from the depletion of the material above a permeable partition. Ray and Deason[13] have given the derivation for a cylindrical tube under "non-convective" sedimentation. It was desired here to make the calculation of *s* in two steps, the first to determine the boundary position and the second, to convert this displacement from the meniscus, with the time and speed data, to an *s*-rate. To this end, the mass transport expression[12,13] has been solved for $\bar{r}$, the location of the boundary. This yields directly

$$e^{-\omega^2 s t} = r_a/\bar{r} = 1 - (r_p - r_a)(1 - Q)/r_p \qquad (1)$$

in which Q is the fraction of the initial material recovered between the meniscus at r_a and the partition level at r_p in the concentration plateau region of the tube ahead of the boundary. An alternate derivation of equation 1 which does not use *s* explicitly, nor depend upon its constancy can be made from the expression for the concentration in the plateau as $r_a c^0/\bar{r}$. The quantity of material present between r_a and r_p is equal to that calculated as though the plateau concentration extended back from r_p to $\bar{r}$. Then Q can be expressed directly as

$$Q = \frac{(r_a c^0/\bar{r})(r_p - \bar{r})}{c^0(r_p - r_a)}$$

from which equation 1 follows.

It is not known, *a priori*, in the non-optical applications which fraction will extend into the plateau. So the assay results on each fraction are included with those preceding to make a series of two compartment cells with a variable partition level r, the bottom of each successive fraction. Equation 1 can be applied to calculate an "apparent boundary position," with the purpose of determining the constant value $\bar{r}$, which will be obtained when the fractionation is in the plateau. To this end, equation 1 is generalized by replacing $\bar{r}$ by r^*, the apparent boundary position, and Q by q as

$$r^* \equiv \frac{r_a}{r_a/r + q(1 - r_a/r)} \qquad (3)$$

now

$$q \equiv \sum_{i=1}^{n} c_i \Delta v_i / c^0 \sum_{i=1}^{n} \Delta v_i \qquad (4)$$

is the fraction of the initial material recovered between the meniscus r_a and the partition level r, and c^0 and c_i are the concentrations of the initial solution and the ith fraction when the volume between r_a and r is divided into n not necessarily equal fractions of volume Δv_i.

The amount of error introduced into r^* by the error in the assay can be determined by differentiating equations 3 and 4

$$-\mathrm{d}r^* = (r^{*2}/r_a)q(1 - r_a/r)(\mathrm{d}c/c) \qquad (5)$$

Here the error $(\mathrm{d}q/q)$ is assumed constant and equal to $(\mathrm{d}c/c)$, the error in the assay. Equation 5 shows that $\mathrm{d}r^*$ depends upon q and is zero for the cut at r, which represents an upper compartment just emptied, *i.e.*, $\Sigma\, c_i \Delta v = 0$, even though the assay error itself is large.

After using the observed CF titers of the various fractions to locate the boundary position, $\bar{r}$, the *s*-rate in Svedbergs was calculated from

$$s = \frac{10^{13}}{\omega^2 t} \ln\left(\frac{\bar{r}}{r_a}\right) \qquad (6)$$

where r_a is the radius at the meniscus, ω is the angular velocity, and t is the equivalent centrifugation time, which is the time at full speed plus $^1/_3$ of the duration of acceleration and deceleration.

Zone Centrifugation.—The velocity type of zone centrifugation[14–16] was used to separate the virus from the smaller CF particle. A 4.5-ml. sucrose gradient (0 to 200 g./l.) without antigenic solution was used, giving a maximum density of 1.07 g./ml. The initial gradient was made by twirling a saw-toothed wire to smooth out the two sharp boundaries between three solutions of different density carefully layered in the tube by pipet. These solutions were, first, 1 ml. of 200 g./l. sucrose in the buffer, second, 2.5 ml. at 100 g./l., and finally, 0.5 ml. of buffer alone. It was found that this gradient would support an initial zone not exceeding 4% protein; hence starting materials were diluted with VBS when necessary. In preliminary experiments where 1-ml. initial samples were used, the three layers for the gradient had volumes of 1, 2 and 1 ml. In most experiments 0.5 ml. of antigenic solution was layered carefully on top. The rotor was immediately placed in the ultracentrifuge (Spinco model E), and slow acceleration

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started as soon as the chamber was closed. Five 1-ml. fractions were taken from the top at the completion of the centrifugation, using a Pasteur pipet. The speed and equivalent time were selected so that the virus zone moved through about 3 ml. The upper two 1-ml. fractions were considered the "smaller-particle fraction," and the lower 3 ml., the "virus fraction."

Infectivity.—Infectivity assays in bovine kidney tissue culture (TC) were made by both TC ID_{50} and plaque-counting methods.[17,18] The plaque method gave the highest precision of assay when TC-passaged virus was employed, but the ID_{50} assay was preferred for bovine-passaged virus, since plaques were not formed in the usual manner. Very limited use was made of mouse titration,[19] injecting 0.05 ml. intraperitoneally into 8–12-day-old suckling mice and reading at 8–10 days postinoculation.

Results

Sedimentation Coefficient of Smaller CF Particle.—Figure 1 shows the plot of ($r^* \pm dr^*$) as a function of r for a sample prepared from fresh

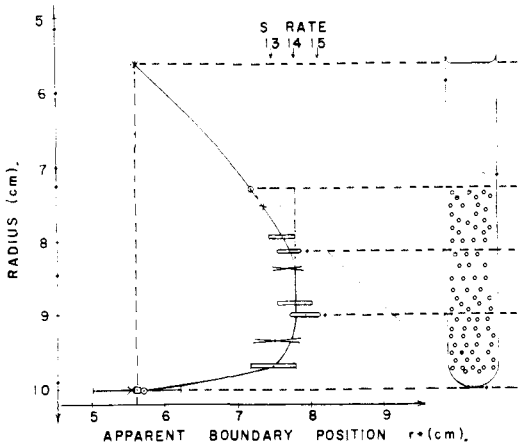

Fig. 1.—Moving boundary ultracentrifugation of smaller particle from FMDV-A119 system followed by CF activity. True boundary position is at 7.8 cm.; plateau of concentration extends from 8.3 to 9.5 cm.; ellipses, crosses and rectangles represent 3 separate tubes fractionated at different levels. The horizontal extent of the point represents an error of 25% in the relative assay; 35,600 r.p.m., 350 min., 17.5° in SW 39 rotor; epithelium suspension *ca.* 10%.

bovine tongue epithelium freed of virus by centrifugation. A plateau is evident between 8.3 and 9.5 cm., with the corresponding value of $\bar{r} = 7.78$ from the abscissa scale. In equation 6 this yields $s = 11.3\ S$. Correcting by 21.5% (6.5% for temperature from 17.5 to 20.0° and 15% estimated for the average density and viscosity effect of the sucrose, VBS, and 5 g./100 ml. slower sedimenting proteins) yields $s_{20,w} = 14\ S$. Since the range of level for which the line at r^* could be placed corresponds to about $\pm 0.5\ S$, and since the temperature is known only within 0.5–1°, the limits are about ± 1 for this value of 14 S. Table I summarizes all the moving boundary experiments performed on the smaller CF antigen. The early runs were used to bracket the s-rate and to ascertain the conditions of speed and time so that the boundary moved about $^2/_3$ of the length of the tube, as in Fig. 1. No significant effect on the s-rate due to storage was revealed by these data, but more studies on fresher material would be desirable, since the method yields the weight average s-rate of all the CF antigens present.

TABLE I

SEDIMENTATION COEFFICIENT OF SMALLER CF ANTIGEN IN FMDV-A119 BOVINE SYSTEM BY MOVING BOUNDARY PREPARATIVE ULTRACENTRIFUGATION

Experiment no.	Material[a]	Storage −60°	Time 4° (days)	CF recovery (%)	$s_{20,w}$ (Svedberg)
1	602 epith.	15 mo.	7	120	<72
2	602 epith.	15 mo.	8	46	>12
3	656 epith.	15 mo.	0	80	<72
4	602 epith.	15 mo.	1	83	>11
5	602 epith.	15 mo.	34	..	<83
6	602 epith.	15 mo.	30	61	10 ± 5
7	602 epith.	15 mo.	34	71	13 ± 5
8	513 epith.	18 mo.	1	57	16 ± 5
9[b]	Pool 1 epith.	6 days	25	104	14 ± 1
10	Pool 2 ves.	0 day	30	100	13 ± 2
11	Pool 3 ves.	0 day	60	104	13 ± 2

[a] Number is the ear tag when individual animal was used; epith. = epithelium; ves. = vesicular fluid. [b] Depicted in detail in Fig. 1.

Movement of Virus in Zone Centrifugation.—Figure 2 shows the distribution of the infectivity of TC virus following zone centrifugation. The virus moved as a zone, with 73% total recovery of infectivity. Similar results were obtained in three

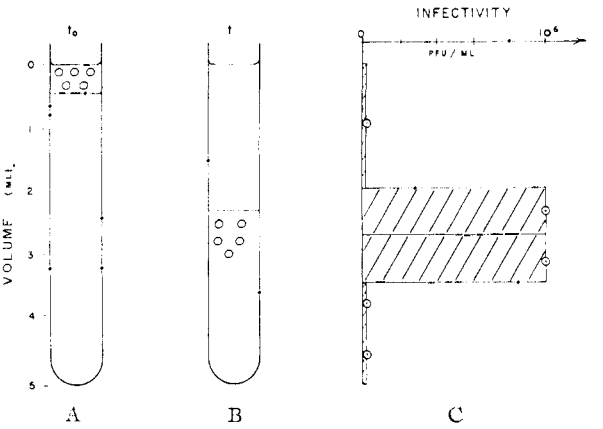

Fig. 2.—Zone centrifugation of TC FMDV-A119: (A) t_0 represents the initial loading of tube; (B) t, the estimated final position of the virus from the infectivity results in (C); PFU is plaque-forming units, 25,980 r.p.m., 59 min., 21° in SW 39 rotor.

of four experiments performed with slight variations in speed and time. From the knowledge that the s-rate of the smaller particles is $^1/_{10}$ that of the virus, it can be estimated that in moving the virus through 3 ml., the smaller particle will be moved through 0.3 ml. Thus the smaller particle should be in the top 2 ml., and the virus in the bottom 3 ml. as indicated in Fig. 3A. The CF titer of TC material was too low to study the movement of the smaller particle. For this, the animal-source material was used, employing speed and time conditions found suitable for the TC material.

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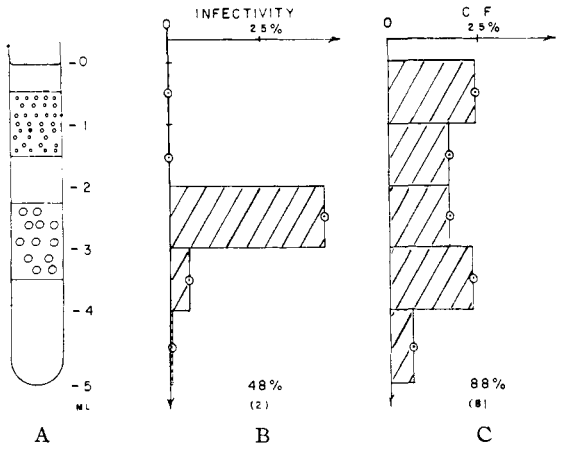

Fig. 3.—Separation by zone centrifugation of virus from smaller particle in FMDV-A119 bovine vesicular fluid. Numbers in parentheses indicate experiments averaged, with average total recovery immediately above; activity scales are in % recovered, 25,980 r.p.m., 64 min., 20-21° in SW 39 rotor. Schematic in (A) is drawn from the infectivity and CF activity results in (B) and (C), respectively.

Partition of CF Activity and Infectivity in Epithelium and Vesicular Fluid.—Table II contains the results of zone centrifugation applied to extracts from bovine tongue epithelium and bovine vesicular fluid. Individual data for complement fixation and infectivity from each of five 1-ml. fractions are given, as well as the sums of fractions 1 and 2 for the smaller particles and of fractions 3, 4 and 5 for the virus. The epithelium columns show that 69% of the CF activity and 0.8% of the infectivity were in the smaller-particle fraction. The corresponding partitions for vesicular fluid were 42 and 0.02%. In four preliminary experiments on epithelium frozen 8–18 months, the average partition of CF activity was 82% for the smaller particle and $\leq 9\%$ for the virus.

A schematic representation of zone separation of the two sizes of particles is shown in Fig. 3A. The infectivity and CF assays on the vesicular fluid used for Table II are indicated in Figs. B and C, respectively, which are consistent with the particle distribution proposed in Fig. 3A.

TABLE II

PARTITION OF CF ACTIVITY AND INFECTIVITY BETWEEN THE SMALLER PARTICLE AND THE VIRUS IN FMDV-A119 BOVINE SYSTEM BY ZONE PREPARATIVE ULTRACENTRIFUGATION

	Recovery, %			
	CF activity		Infectivity	
Fraction[a]	Epithelium[b] %	Vesicular fluid,[c] %	Epithelium,[b] %	Vesicular fluid,[c] %
1	50	25	0.1	0.01
2	19	17	0.7	0.01
3	11	17	65	43
4	10	23	≤ 98	5
5	3	6	≤ 16	0.5
Small (1 and 2)	69	42	0.8	0.02
Virus (3, 4 and 5)	24	46	≤ 179	49
Total	93	88	≤ 180	49

[a] Five 1-ml. fractions numbered from the top down. [b] Three experiments on 2 pools averaged for CF and 3 for infectivity, TC ID_{50} method, 5–10% suspension of epithelium. [c] Eight experiments on 3 pools averaged for CF and 2 for infectivity, 1 TC ID_{50} and 1 mouse method, 1:4 dilution of vesicular fluid.

Cross-contamination.—To interpret the above as partition between virus and smaller particles, it is assumed that all of the virus leaves the upper 2 ml. and that none of the smaller particle material advances into the bottom 3-ml. virus fraction. However, perfect fractionation is unlikely, and so with two assays for two particles there are four measurements of cross-contamination. With the FMDV system, where only the virus is infective, there are but three: (a) the percentage of virus infectivity that contaminates the smaller-particle fraction, (b) the percentage of smaller-particle CF activity that contaminates the virus fraction, and (c) the percentage of virus CF activity that contaminates the smaller particle fraction. These three will now be considered separately.

The percentage of infectivity contaminating the smaller particle fractions (no. 1 and 2) is shown in Table II. The average is about 0.4%, with the highest value in any of the five experiments being 2%. This cross-contamination could not be calculated from the average infectivity recovered in the virus fraction since it is 130%, with a range from 13 to 260%.

The smaller particle contaminating the virus fraction was studied in two ways. Both are special cases, for neither represents the actual zone centrifugation with the two particles present. The first way, with no centrifugation and standing the same length of time as the normal centrifugation experiment, measured the amount of the CF activity of the initial zone that moved into the lower 3-ml. virus fraction. Fig. 4A shows the initial loading of the tube. In Fig. 4B is the average of four experiments. The initial zone broadened considerably, but only 2% of the CF activity was found in the bottom 3 ml. (individually, 0, 0, 1 and 6%). One of the samples used was also infectious. Less than 0.003% of this infectivity was detected in the lower region, with a total recovery of 40%. The second

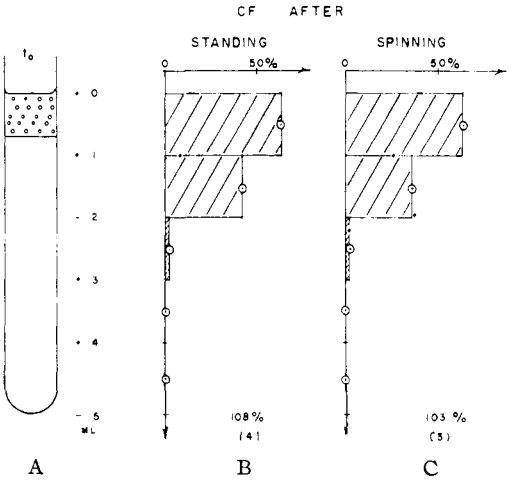

Fig. 4.—Contamination of virus region with smaller particle: (A) t_0 represents the initial loading of tube; CF activity scale is in % recovered in the fractions shown after (B) standing and (C) centrifugation under conditions for Fig. 3.

way of determining how far the smaller particles advance into the virus region was done by centrifuging an essentially virus-free preparation and determining the CF activity in the lower region of the tube. Such a sample was obtained as follows: The supernatant fluid from a moving boundary ultracentrifugation, which pelleted most of the virus, was subjected to zone centrifugation to separate out only the smaller-particle fraction. The results of three experiments, shown in Fig. 4C, were almost identical to Fig. 4B, showing that the centrifugation which was designed to move the smaller particle through 0.3 ml. did not cause any more broadening of the initial zone than that which occurred by standing and subsequent handling. However, 0, 3 and 4% of the initial CF activity was found below the upper 2-ml. fraction. The over-all average of these three experiments and the four used for Fig. 4B shows 2% (range 0–6%) cross-contamination of the smaller CF particle into the virus fraction.

In experiments to demonstrate the amount of virus CF activity contaminating the smaller particle fraction, zone centrifugation of virus, free from the smaller particle, was followed by CF assay. This should indicate how much CF activity fails to leave the smaller-particle fraction. It was found to be difficult to obtain such a preparation, for the virus breaks down into smaller particles with handling.[8,20–22] Since only small percentages of cross-contamination are to be measured, virus degradation during the dialysis necessary to remove sucrose was great enough to preclude establishing contamination limits.

Discussion

These experiments show that the major 14 *S* and 140 *S* particles can be separated in one centrifugation but do not establish that there are only two particle types in the FMDV system. The presence of other types might be ascertained by recycling the intermediate fractions from the first zone ultracentrifugation. Persistence of the activity at some level other than that corresponding to the 14 *S* and 140 *S* zones would indicate such a component. Verification could be obtained by measurement of its *s*-rate by the moving boundary technique as illustrated in Fig. 1.

A distinction must be made between the percentage of a component that contaminates another fraction and the percentage of an observed activity that is due to a contaminant. Thus, if 5% of the smaller particle is in the virus region and the virus represents only 5% of the original activity, then half of the activity in the virus fraction is due to the smaller particle. When zone centrifugation for the preparation of antigens free from each other is applied, an additional zone centrifugation of each major fraction from the first cycle presumably will reduce the contamination even further.

In explanation of the differences between the *s*-rate found here for the smaller CF particle and that reported by Bradish, *et al.*,[6] the limit of error of both results should be considered. Bradish, *et al.*,[6] derived the expression for the error in the *s*-rate due to an error in the assay, when using the two-compartment formula. Their expression is slightly different from that which follows from the dr^* of equation 5 but indicates that the accuracy depends upon the upper compartment's being very nearly emptied. In their five experiments the ratio of c/c^0 was between 0.39 and 0.63, with an average of 0.52. The corresponding averages of r_a and r were 71.2 and 87.8 mm., respectively. These data give an error, ds/s, of $\pm 27\%$ using an assumed error of 25% in the assay (their $n = 1.25$). Thus, their reported value of 7.8 *S* can be no better than ± 2.1 *S* by their own argument; however this does not completely resolve the difference. There is, of course, the possibility that there is a real difference between virus strains, since their work was on type O, or that they were dealing with a breakdown product of the smaller particle itself.

GREENPORT, NEW YORK

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[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES, HARVARD UNIVERSITY]

The Association of Divalent Cations with Glutathione[1]

BY R. BRUCE MARTIN AND JOHN T. EDSALL

RECEIVED FEBRUARY 19, 1959

Glutathione may combine with metallic ions, at two loci: either like an α-amino acid, by chelation through the amino and carboxylate groups of the glutamyl residue, or through binding at the cysteinyl sulfur. The interactions of several divalent metallic ions with glutathione and S-methylglutathione have been determined by titration. Since the sulfur atom is blocked in the S-methyl derivative, comparison of the data permits estimation of the relative importance of binding at the two loci in glutathione. For divalent Zn, Ni, Co and Mn it is estimated that 80, 10, 30 and 15%, respectively, of the bound metallic ion is attached at the sulfur atom.

In this paper we are concerned with the binding of certain divalent metal ions to glutathione; they may be attached either to the amino and carboxyl groups of the glutamyl residue or to the sulfhydryl group of the cysteinyl residue. We have studied binding by titration with base, with and without added metal ions. To formulate the relations involved, we consider first the acid–base equilibria involving the amino and sulfhydryl groups

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