beef AHF fraction was as active as whole human plasma. This finding indicates that the improved prothrombin consumption induced by beef AHF fraction is a true improvement of clotting, and not a nonspecific action resulting in loss of prothrombin. Fig. 2 shows the paper electrophoretic patterns of the various bovine fractions. Both fraction I and acid globulin were mixtures of α_2 and β globulins. AHF fraction was virtually pure β_2 globulin. It is evident that AHF fraction represents a considerable gain in both AHF activity per mg of protein, and purity as compared to previous preparations.

Beef AHF fraction contained no demonstrable fibrinogen, as tested by the addition of purified thrombin (Parke Davis). Neither was there any demonstrable prothrombin, or proconvertin (method of Owren and Aas)(11). Traces of labile factor activity were found, tested by the method of Quick(12). PTC activity(13) was kindly assayed by Dr. Paul M. Aggeler. Although beef AHF fraction was able to shorten the clotting time of *PTC*-deficient blood, 25 fold concentrations did not elicit any improvement in prothrombin consumption. In the precipitated form, beef AHF fraction was stable for at least 3 months in deep freeze; but freezing solutions of the material caused precipitation of the protein with complete loss of AHF activity.

Summary. The anti-hemophilic factor (AHF) of human plasma is unstable to storage, and shows great loss with attempts at fractionation. Bovine AHF is stable to storage, and protein fractions can be obtained which show a good yield of AHF. Beef "AHF fraction" was prepared as an almost pure β_2 globulin, and had about 70 times the AHF activity of fresh human plasma per mg of protein.

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Particle Size of Soluble Antigen of Rabies Virus. (20632)

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A study of the size and the antigenicity of the complement fixing "soluble antigens" of viruses may throw considerable light on virus structure. Recently Hoyle, Reed and Astbury (1) have shown that influenza virus can be broken down by ether treatment into complement-fixing and haemagglutinating particles. They obtained pure preparations from allantoic fluid and by electronmicroscopy estimated the particle diameter to be 12 mμ.

We have obtained the soluble antigen of rabies virus from infected suckling mouse brains. The particle size could not be determined in the Spinco preparative ultracentrifuge using the angle head (Polson and Linder (2)) because the amount of sedimentation

during 2 hours at 30000 rpm was insufficient. We therefore used a technic of diffusion coupled with biological assay.

Method. For determination of the diffusion constants of pure substances which can be obtained in relatively large amounts the conventional optical method of Lamm is most suitable. When substances are difficult to purify a more satisfactory method is to investigate their diffusion constants by quantitative evaluation of their biological activities. A very useful diffusion technic for such measurements is that described by Polson(3,4). The diffusion apparatus consists of 6 multichambered diffusion cells clamped to a base provided with levelling screws. Sharp interfaces between medium and solution were formed by filling adjacent cells with medium and solution and rotating the upper section of the cells until the liquid in the segment above came into apposition with that in the segment below. This was done by watching the index lines on the proximal and distal sides of the cells. The diffusion experiments were conducted in a water thermostat kept at 20°C. After suitable intervals the material that diffused past the initial boundary was isolated from that in the distal portion by rotating the upper sections to their cut-off positions. The activities of the diffusates and original solution were determined by their appropriate methods.

The results of activity determinations of the diffusates are presented graphically by plotting the ratios of concentration in the diffusates C to that in the original solution C_o against the square root of t, the time in hours. It is a necessary requirement from the laws of diffusion that C/C_o should be a linear function of $\sqrt{t}$.

The diffusion constants were calculated from these data using the equation

$$D = \left(\frac{C}{C_o}\right)^2 \frac{H^2\pi}{t} \qquad 1$$

Where H is the height of the column of liquid above the original boundary, regarding the chambers in the diffusion cells as cylinders. The value of H in the present experiments has been 3 cm. Estimation of C_o: Since C_o enters into equation 1 as the second power it is of the utmost importance to have a value for C_o that differs as little as possible from the real value. To obtain a value for the activity that proximates the true figure, activity estimations were made on the original solution diluted initially to different degrees. The activities thus determined are then multiplied by the dilution factor and the average value of the different determinations then represents C_o.

Experimental. Material. The Flury strain of rabies was adapted to 3- to 4-day-old suckling mice and carried through 20 generations. The mouse infectivity titre LD_{50} of a 10% emulsion in rabbit serum saline of different batches of infected brain material varied between 9 and 10 if the titrations were made on freshly harvested material. The complement fixing titre varied between 1/512 and 1/1024. For diffusion measurements 15 infected baby mouse brains were emulsified in 30 cc saline containing 10% inactivated rabbit serum and spun for 60 minutes at 2500 rpm in a refrigerated centrifuge. The supernatant fluid was dialysed against a large volume of M/15 phosphate buffer pH 8.2 for 48 hours in the cold. The brain extract was next spun for 60 minutes at 30000 rpm in the No.

TABLE I. C.F. Titres of the Diffusates Obtained from the Different Diffusion Cells.

Cell	Time of diffusion in hr	C.F. titre	Avg
A	1	$1/2$	$1/2$
B	13	$1/4$–$1/8$	$1/6$
C	20	$1/8$–$1/16$	$1/12$
D	36	$1/16$	$1/16$
E	61	$1/16$–$1/32$	$1/24$
F	84	$1/16$–$1/32$	$1/24$

TABLE II. Results of Titrations of Varying Dilutions of the Original Solution.

Dilution	Titre	Titre/Dilution
1	$1/384$	$1/384$
1.33	$1/256$	$1/341$
1.50	"	$1/384$
3	$1/96$	$1/288$
5	"	$1/480$
5	"	"
6	"	$1/576$
6	"	$1/384$
Avg $1/411$ $\therefore C_o = 411$		

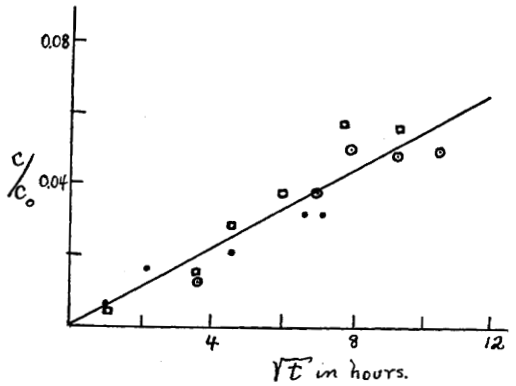

FIG. 1. Ratios of concentration of soluble antigen in diffusates to those in the original solution plotted against the square root of diffusion time in hours.

40 rotor of the Spinco preparative ultracentrifuge to remove all infective virus particles as well as any free lipoid or suspended brain material. Experiments with such extracts spun at different rotor velocities showed that after removal of the infective virus particles there was little further reduction in C.F. titre. The virus free supernatant fluid and the buffer were deaerated in a vacuum before placed in the diffusion cells.

Results. The results of one of the diffusion experiments are given in Tables I and II. These are titrations of the diffusates after different periods of diffusion and titrations of the original material diluted to different degrees respectively.

In Fig. 1 the ratios C/C_o from three different experiments are plotted against $\sqrt{t}$ where t is the time of diffusion in hours. It is quite evident that C/C_o is a linear function of $\sqrt{t}$ and that the straight line so obtained passes through the origin. The diffusion constant calculated from this line is 2.7×10^{-7} cm^2/sec.

Particle size of the C.F. antigen of rabies. Particle size calculations from the well known equation of Einstein

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi r\eta} \qquad 2$$

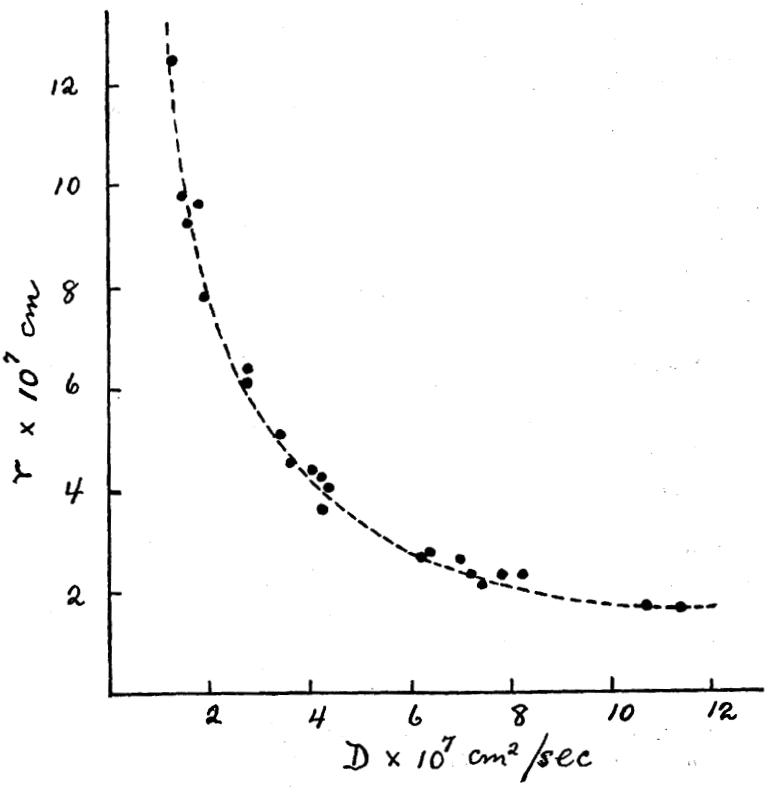

FIG. 2. Curve showing the relationship between diffusion constant and particle radius as calculated from the molecular weights of the globular proteins.

where R is the gas constant, T the absolute temperature, N Avogadro's number, r the radius of the particle and η the viscosity of the medium. This gives the diameter of the hydrated particle which will be appreciably higher than the unhydrated particle. To bring the size, as calculated from diffusion measurements, in line with that measured from electronmicrographs of the C.F. antigen of influenza the diffusion constant of the rabies C.F. antigen was interpolated into a curve relating the diffusion constants with particle radii. These are calculated from the molecular weights of the different proteins in their dehydrated states (Polson)(5). This curve is shown in Fig. 2. When the diffusion constant of the C.F. antigen of rabies is interpolated into this curve a particle radius of 6.2×10^{-7} cm is obtained. This gives a value for the diameter of the particle of 12.4 mμ.

Ultracentrifugation of the rabies soluble antigen. From ultracentrifugation experiments in which the swinging bucket type of rotor of the Spinco ultracentrifuge was used a sedimentation constant of approximately 25 Svedberg units was found. This value corresponded very well with that calculated for a protein particle of 12 mμ diameter. These centrifugation experiments, however, cannot be regarded as final as it was difficult to maintain a constant rotor temperature at velocities >30,000 rpm, and the calculations of the sedimentation constant had to be made on runs at lower speeds where only small boundary displacements took place, but during which the rotor temperature remained constant at 7°C.

Summary. 1. A particle size of 12 mμ has been determined by a method of diffusion coupled with biological assay for the soluble antigen of rabies extracted from infected suckling mouse brains. This value agrees with that calculated from the approximate sedimentation constant calculated from the sedimentation rate in the swinging bucket rotor of the Spinco ultracentrifuge. 2. No conclusions regarding the homogeneity of the soluble antigen can be drawn at this stage.

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Survival of Dogs with Bilateral Phrenectomy and Extensive Transection of Intercostal Nerves. (20633)

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According to Brown and Satinsky(1) dogs invariably die after destruction of both phrenic nerves. Partly on this basis they claimed functional restoration of the diaphragm by uniting the vagus in the thorax with the distal end of a severed phrenic nerve. After a suitable interval following such an anastomosis, dogs tolerated section of the remaining phrenic nerve and fluoroscopic studies seemed to indicate that the vago-phrenic anastomosis was responsible for rhythmic contraction of the diaphragm. On the other hand, there are numerous reports antedating that of Brown and Satinsky claiming a high degree of tolerance to section of both phrenic nerves in man and other mammals, including the dog. Bilateral phrenectomy in man without untoward results has been reported by Greene(2), Angle (3), Campbell(4), and according to Lemon (5) by many others. The effects of destroy-