STUDIES ON THE PHYSICAL AND CHEMICAL PROPERTIES OF THE VIRUS OF FOOT-AND-MOUTH DISEASE.

II. CATAPHORESIS AND FILTRATION.

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In the first¹ of this series of papers we described the strain of foot-and-mouth disease virus employed in our studies. We have also reported therein that guinea pigs were susceptible to the active agent in dilutions of 1:10,000,000, and that the virus was non-centrifugable. The indications pointed to a minute size of the incitant.

In the experiments to be presented in this article on cataphoresis and filtration, the findings regarding the minuteness of the incitant are confirmed and, furthermore, a relative delimitation of its size will be demonstrated. In addition, the electric charge carried by the virus will be revealed and phenomena dependent on the charge and on certain filtration reactions will be discussed.

Cataphoresis.

The electric charge carried by the active agent, as well as its isoelectric range, was a criterion by which different filtration tests could be interpreted. Its determination was useful not only to explain the remarkable resistance of the virus to certain chemicals to be described elsewhere, but also to compare the charge with that characterizing certain known microorganisms.

The main difficulty of cataphoresis experiments with this virus was the absence of a recognizable or visible particulate body. But this was overcome by adapting to our purposes the Vlès² apparatus.

¹ Olitsky, P. K., and Boëz, L., *J. Exp. Med.*, 1927, xlv, 673.

² We wish to express our gratitude to Professor Fred Vlès of the University of Strasbourg for his cordial cooperation and his invaluable assistance, not only in these experiments, but also in others on filtration.

Moreover, guinea pigs could be regularly infected with even minute quantities of the active agent, so that its attraction to one or another pole could be determined by the biological test.

The apparatus³ employed consists essentially of horizontal U-tubes, containing in the curved arms a stoppered ampulla, and in each long arm a stop-cock. These are connected to communicating cups by curved syphons which are joined to each other by a transverse tube carrying also a stop-cock. The latter allows a prior establishment of a hydrostatic equilibrium. The orifices of the curved syphons are plugged with moist cotton to prevent hydraulic disturbances. The tubes, fixed to a board, are placed in series of four (because four determinations at different hydrogen ion concentrations were made at one time). At either end of the series, union with the house current is made through a solution of cupric sulfate, and in the connecting cups through physiological saline solution (0.9 per cent at pH = 8). In the syphons of each tube of the series is placed the phosphate buffer⁴ at the hydrogen ion concentration at which the virus is tested. In the ampullæ is added the virus, diluted with phosphate buffer at different pH readings, thus filling completely the respective U-tubes to the stop-cocks.

In the following experiments the difference of potential at the limits of the series in an open circuit was 220 volts. A milliamperemeter was then placed in the circuit, which registered from 0.8 to 4.7 ma. for the four tubes, and the fall of potential for each tube was from 20 to 50 volts. The time of cataphoresis lasted from 20 to 70 minutes. We have found that the best result was obtained when the virus was filtered and diluted 1:1000 in phosphate buffer, at a milliamperage of 0.8, with a fall of potential of 20 to 30 volts and cataphoresis for 70 minutes.

This experiment, one of a series of ten, is illustrative:

Protocol 1.—24 hour aspirated lymph virus was diluted 1:1000 with phosphate buffer at pH = 7.5, and filtered through Berkefeld V candles. This material, on injection intradermally in the pads of guinea pigs, showed activity in each of graded dilutions from 1:2000 to 1:200,000.

The substance was then separated into four portions and each was adjusted to different pH readings by the colorimetric method. At the same time phosphate

³ Vlès, F., and de Coulon, A., *Arch. physiq. biol.*, 1924, iv, 43.

⁴ Phosphate buffer is made by adding 2.5 gm. potassium acid phosphate (KH_2PO_4) to a liter of distilled water. This is then adjusted to the desired pH by means of potassium hydroxide. It is of utmost importance to readjust the material just before use, for sterilization renders it acid.

buffer adjusted to similar degrees was prepared. At the termination of the cataphoresis experiment the following electrometric pH measures were obtained:

Tube	Buffer	Virus	Average buffer and virus
1	6.63	6.84	6.73
2	7.68	7.62	7.65
3	8.00	7.90	7.95
4	8.17	8.05	8.11

The cataphoresis was continued for 70 minutes with a fall in potential of 20 to 30 volts with a milliamperage of 0.8.

Guinea pigs were then injected intradermally in the pads with the material removed from the negative and positive poles of each of four differently adjusted specimens with these results:

$pH = 6.73$

Negative pole: 2 guinea pigs; both positive after 48 hours.

Positive pole: 2 guinea pigs; both negative.

$pH = 7.65$

Negative pole, 1:2000: 2 guinea pigs; both positive after 48 hours.

1:20,000: 2 guinea pigs; both positive after 48 hours.

Positive pole, 1:2000: 2 guinea pigs; both negative.

1:20,000: 2 guinea pigs; both negative.

$pH = 7.95$

Negative pole, 1:2000: 2 guinea pigs; both positive after 48 hours.

1:20,000: 2 guinea pigs; both positive after 48 hours.

Positive pole, 1:2000: 2 guinea pigs; both negative.

1:20,000: 2 guinea pigs; both negative.

$pH = 8.11$

Negative pole: 2 guinea pigs; both negative.

Positive pole: 2 guinea pigs; both positive after 48 hours.

Results of other tests at pH readings different from those mentioned showed:

$pH = 6.6$

Negative pole, 1:500: 2 guinea pigs; both positive after 48 hours.

Positive pole, 1:500: 2 guinea pigs; both negative.

$pH = 7.0$

Negative pole, 1:500: 2 guinea pigs; both positive after 48 hours.

Positive pole, 1:500: 2 guinea pigs; both negative.

$pH = 7.4$

Negative pole, 1:500 to 1:100,000: in all dilutions, all guinea pigs positive after 48 hours.

Positive pole, 1:500 to 1:100,000: in all dilutions, all guinea pigs negative.

$pH = 7.5$

Negative pole, 1:250 to 1:250,000: in all dilutions, all guinea pigs positive after 2 to 4 days.

Positive pole, 1:250 to 1:250,000: in all dilutions, all guinea pigs negative.

$pH = 8.0$

Negative pole, 1:150 to 1:30,000: in all dilutions, all guinea pigs positive after 48 to 72 hours.

Positive pole, 1:150 to 1:30,000: in all dilutions, all guinea pigs positive after 24 to 48 hours.

$pH = 8.5$

Negative pole, 1:500: 2 guinea pigs; both negative.

Positive pole, 1:500: 2 guinea pigs; both positive after 48 hours.

It appears, therefore, that the foot-and-mouth disease virus is capable of wandering in an electric field and then, if the active agent is at a hydrogen ion concentration below $pH = 8$, the direction of motion is toward the negative pole (electropositive); if above $pH = 8$, toward the positive pole (electronegative). At about $pH = 8$, there is a tendency to move in either direction (isoelectric range). Hence it may be stated that under ordinary conditions, the active agent has an electropositive charge, and that its isoelectric range is at $pH =$ about 8. It should be remembered that, in view of the absence of a visible particulate body, and the slight changes in hydrogen ion concentration during cataphoresis, this figure is relative; hence the term "range" is employed rather than the word "point."

It was noted that, as a rule, there was clouding at the pole opposite to that to which the virus was attracted. For example, if the fluid at the positive pole appeared hazy, and at the negative limpid, then the material at the negative pole contained the active agent. Whether the force of the electric current is sufficient to tear the virus from the electronegative protein particles to which it may be attached,⁵ we are unable to say, but these findings are suggestive. The possible objection that the virus wanders along with protein does not hold, for the virus moved in a different direction from the ordinarily electronegative proteins.

The fact that the virus has an unusually high isoelectric range and

⁵ In another section will be described the adsorption of the virus to large protein coagula.

is, under ordinary conditions, electropositive does not speak against its possible protein or living nature. For, although bacteria are generally electronegative in charge, spirochetes, as a genus, carry electropositive charges (with the exception of *Treponema pallidum*); so do trypanosomes, such as the species of *brucei*, *equiperdum*, *gambiense*, and *rhodesiense*. Among protein substances, fibrin and gliadin (at pH = 9.3) are known to possess a high isoelectric point. The outstanding fact is that the virus, in respect to its charge, is different from ordinary bacteria.

The determination of the charge, however, was of greatest importance in interpreting the results of ordinary and "molecular" filtration experiments, and therefore gave indirectly some indication of the relative dimensions of the causal agent.

Filtration.

The filters employed in the following experiments comprised the Seitz' asbestos discs, Berkefeld V and N candles, Chamberland's L 1, L 2, L 3, L 5 (F), L 7 (B), L 9, L 11, and L 13 bougies, various thicknesses of collodion membranes, and, finally, Bechhold's ultra-filter prepared with different percentages of acetic collodion.

It is important to note that the foot-and-mouth disease virus, as employed in the following tests, may contain the incitant in a free state, as well as adsorbed to particles. That the virus can be free and that there is a lack of complete adsorption to a number of different substances has been observed by several investigators (Dahmen, Gins, Bedson, Burbury and Maitland, and Stockman and Minett). Furthermore, there is practically no loss in activity of the virus after filtration through coarse and fine filters. In addition, filtrations proceed in a definite, regular manner. For these reasons, and from the results of cataphoresis experiments showing a separation of the virus from protein particles, one cannot regard the whole of the incitant as adsorbed to minute particles of the same size.

Experience with the Seitz' filter showed that the virus filtered easily through one disc of asbestos. We have not employed this method for study of filtration phenomena, but merely to purify the

virus from contaminating microorganisms. For this purpose, the Seitz' filter is quite effective.⁶

In regard to the other methods of filtration, a general principle was involved in all cases, namely, all the filters carry an electronegative charge. As the virus has an electropositive charge, considerable adsorption occurs during the passage of the active agent through the candles, bougies, or membranes. In some filters in which the pore size is minutely small, and the charge strongly negative, complete adsorption takes place with the consequence that the filtrates contain no virus.

Filtration through Berkefeld V and N Candles.—The material for these tests consisted of aspirated lymph, or of ground infected pads suspended in phosphate buffer at pH = 7.5 or 7.6. In any event, preliminary filtration through one layer of filter paper was resorted to. This was done to prevent clogging of the small candles with particles of tissue, fibrin, or cells.

As tests of the charge and relative pore size of these filters, there were passed through them suspensions containing particles of either charge and of different sizes. Electropositive micellæ, such as methylene blue, basic fuchsin, and night blue regularly failed to pass, but electronegative particles, such as acid eosin and Prussian blue, readily traversed the candles. Hence these filters carried an electronegative charge.

It was therefore necessary, in determining the size of particles capable of passing through the V and N filters, to eliminate adsorption due to filtering oppositely charged substances. Accordingly, collargol, the mean diameter of its micellæ being 20 m μ and colloidal arsenic trisulfide, 100 m μ , were tested. Both passed rapidly through V, and definitely, but less speedily, through N candles. Both substances have an electronegative charge. Hence these Berkefeld filters, with the adsorption action counteracted, allow the passage of particles of at least 100 m μ in diameter. This agrees in part with the determination of the size of the intergranular spaces by Bechhold's formula,

⁶ For more extensive studies on this type of filter, the reports of the investigators of the British Committee should be consulted. References are cited in the first paper of this series.

as given by Mudd.⁷ The latter found the diameter of the V type to be $0.38\ \mu$ ($380\ m\mu$), and of the N type, $0.45\ \mu$ ($450\ m\mu$).

In respect to the virus of foot-and-mouth disease, a standard substance for comparison should be one with a positive charge, and of a definite known size. For this purpose, pure, crystallized hemoglobin in solution, and therefore molecular, was employed. Its isoelectric range is at $pH = 6.75$, and the size of its molecule, as computed by Bechhold,⁸ is $3.6\ m\mu$ in diameter. Suspended in phosphate buffer (employed also in suspensions of the virus) at $pH = 6$, hemoglobin is electropositive, and at $pH = 7.5$, electronegative. In the former condition this substance passes through V and N candles; in an electronegative condition, the passage is more free and completely unobstructed. Since the virus with an electropositive charge also passes regularly through both these filters, showing at times activity in at least a 1:2,000,000 dilution of the filtrate, the conclusion derived from these observations is that the active agent is at least larger than the molecule of hemoglobin. This has been confirmed by our experiences with filtrations through Chamberland bougies.

Filtration through Chamberland Bougies.—The Chamberland bougies of all types are similar to the Berkefeld candles in that they are charged electronegatively. For, electropositive particles, such as methylene blue, basic fuchsin, and night blue dye do not pass through their walls, but the electronegative micellæ of acid eosin, Prussian blue, collargol, and colloidal arsenic trisulfide do. In regard to hemoglobin, at $pH = 6$, with an electropositive charge, this substance passes these filters only slightly—the first few drops of filtrate are clear but after a time traces of hemoglobin are revealed by spectroscopic examinations. At $pH = 7.5$, with an electronegative charge, hemoglobin traverses freely all the types of Chamberland filters. In other words, as an electropositively charged molecule, hemoglobin first satisfies the avidity of the electronegative material of the bougie and later its minute size enables it to pass through the barrier.

A similar experience was obtained with the electropositive virus. With bougies of graded series to L 5, the active agent traversed their

⁷ Mudd, S., *J. Bact.*, 1923, viii, 459.

⁸ Bechhold, H., *Z. physik. Chem.*, 1908, lxiv, 328.

walls. But with more dense types, the passage became more difficult, until with the L 11 type,⁹ the filtrates were free from virus. For example, five trials with five different new L 7 type bougies yielded in only one case a positive filtrate; three filtrations through three different, new L 9 type bougies, resulted in one positive; and seven tests with seven different L 11 type bougies, six of which were new, the seventh having been used more than once, failed in all instances. In these experiments the virus was suspended in phosphate buffer at pH = 7.5 (electropositive). Diluted in similar material at pH = 8.5, the virus, then in an electronegative state, passed freely through these bougies.

The pore size of the L 11 type of Chamberland filter has been determined after Bechhold's⁸ air pressure, revised method, as 110 to 150 m μ . It appears, therefore, that the foot-and-mouth disease virus can, by elimination of adsorption due to opposite electric charges, traverse these small spaces. On the other hand, if adsorption due to opposite charges is unhindered, the virus fails to pass. But, in the latter relation, hemoglobin under similar conditions can traverse this filter. Hence the active agent is larger than the hemoglobin molecule of 3.6 m μ diameter. Experiments with filtration through collodion membranes, to be described immediately, confirm this relative measurement.

Filtration through Collodion Membranes.—For a determination which might give a measurement of the virus between narrower limits, and for a more extended study of the filtration phenomena, a still tighter filter was employed, namely, the collodion membrane.

Collodion membranes are negatively charged. Indeed, the individual particles of pure collodion remain so charged, or become even more strongly negative, in the presence of either acid or alkali, as measured by the cataphoretic potential difference between these particles and water (Loeb¹⁰).

The collodion membranes, usually in the form of small sacs, were

⁹ Several trials with a number of the L 13 type bougie, supposed to be the tightest of all of the series, resulted in irregular filtrations of titrating reagents. Hence work with this type was discontinued.

¹⁰ Loeb, J., *Proteins and the theory of colloidal behavior*, New York and London, 2nd edition, 1924, 79 ff.

prepared with French Codex Collodion (5 per cent). In all 68 sacs were made with different proportions and conditions of collodion, so that eighteen different experiments were performed. The concentration of collodion varied from 5 per cent to 12 per cent (the 12 per cent corresponding to 15 seconds reading on the apparatus devised by Gates,¹¹ for measuring its relative viscosity). From one to three layers were used, and dipping in 95 per cent alcohol was resorted to in some instances to increase the permeability of the sacs. Negative pressure from 10 to 25 cm. of mercury was applied. The virus comprised 24 hour aspirated lymph, diluted 1:50 or 1:100 in phosphate buffer at pH = 7.5 or 7.6 to pH = 8.5 which in all tests, as measured by control inoculations in guinea pigs, was quite active. Six experiments were also made with sacs prepared after the manner of Levaditi, Nicolau, and Galloway,¹² who used three layers of 5 per cent collodion (Codex), without alcohol immersion.

To summarize the results of these experiments: We found that the collodion membranes prepared in the manner stated are not suitable for the filtration of the virus of foot-and-mouth disease. For more than half of the number of membranes showed macroscopic holes before put to test. In the remaining ones, we could not convince ourselves that microscopic holes were absent. In the absence of standardization of the membrane, it was necessary that each sac be tested separately by titration with known substances of definite charge and size. Otherwise the results were valueless. Membranes which we thought hole-proof and at the same time made so as to withstand a negative pressure of from 10 to 25 cm. mercury (without this negative pressure, filtration was impossible), failed, as a rule, to allow passage of methylene blue, eosin, collargol, hemoglobin, etc.—the materials employed for titration of the Berkefeld V and N and all types of Chamberland filters. They did allow the passage of peroxidase (which we found to be more easily filterable than blue litmus), of blue litmus, the size of which is computed by Bechhold⁸ to be 1.8 $m\mu$ in diameter, and of crystalloids. The crystalloids which traversed the membranes were, in order of molecular weights from

¹¹ Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 25.

¹² Levaditi, C., Nicolau, S., and Galloway, I.-A., *Compt. rend. Acad.*, 1926, clxxxii, 247.

strychnine, molecular weight, 397; acid quinine sulfate, to basic quinine sulfate, molecular weight, 890.¹³ The virus, on the other hand, failed to pass through these membranes.

It may therefore be concluded that collodion membranes of the types mentioned are permeable to colloids containing particles in size of the order of blue litmus, and to crystalloids, but not to the virus carrying positive or negative charges. Furthermore, in the absence of standardized membranes, they are unsuitable for titration experiments. We have, however, confirmed the observation of Stockman and Minett¹⁴ that the virus does not traverse two layers of 5 per cent collodion. One conclusion may be derived from these experiments: the active agent is not comparable to crystalloids in respect to filtration through collodion membranes.

It was then found necessary to seek a more permeable membrane, by which one could delimit the size of the virus more precisely and, at the same time, be free from the variables in the methods already mentioned. For these purposes we found the Bechhold ultrafilter membranes quite satisfactory.

*Filtration through the Bechhold Ultrafilter Membranes.*¹⁵—The prepared Bechhold discs are made by impregnating in a vacuum Schleicher and Schüll filter paper with different concentrations, from $1\frac{1}{2}$ to $7\frac{1}{2}$ per cent, of acetic collodion. By this method unevenness of the layer and microscopic holes are eliminated; the membrane is strong enough to withstand the necessary negative pressure and a concentration of as little as $1\frac{1}{2}$ per cent of the collodion can be used with, as a rule, unequivocal results.

We adapted these discs to our purposes by cutting each membrane to a circumference corresponding to that of the lower part of a Seitz (Manteufel,¹⁶ E. K. model) filter. The membrane was placed over the wire gauze support and a smooth, well fitting, firm, rubber washer

¹³ Strychnine was tested for its presence in the filtrates by inoculation of frogs; quinine, by opalescence, in a strong beam of light, after acidulation with sulfuric acid.

¹⁴ Stockman, S., and Minett, F. C., *J. Comp. Path. and Therap.*, 1926, xxxix, 1.

¹⁵ The membranes are manufactured by Schleicher and Schüll, Düren, Rhineland.

¹⁶ Manteufel, P., *Centr. Bakt., I. Abt., Orig.*, 1924, xciii, suppl., 259.

was superposed. The cup end was then tightly screwed in and the mechanism was tested for leakage at the negative pressure employed in each test.

The Bechhold disc is electronegative. To eliminate the adsorption of substances oppositely charged, both the virus and the test materials for titration were employed in a condition of the same, that is, negative charge. Hence, for virus, we prepared 24 hour old lymph as-

TABLE I.
Filtration through Bechhold's Ultrafilter Membranes.

Substance	Charge	Size of particle	Per cent acetic collodion in membrane																	
			7½ per cent		6 per cent		4½ per cent		3 per cent				1½ per cent							
			Number of membranes employed	Results	Number of membranes employed	Results	Number of membranes employed	Results	Number of membranes employed	Results in each case. Membrane No.	1	2	3	4	Number of membranes employed	Results in each case. Membrane No.	1	2	3	4
Virus	Neg.	X	4	—	4	—	4	—	4	+	—	—	—	4	+	+	+	+		
Blue litmus	Neg.	1.8mμ	4	+	4	+	4	+	4	+	+	+	+	4	+	+	+	+		
Hemoglobin	Neg.	3.6mμ	4	—	4	—	4	—	4	+	+	+	—	4	+	+	+	+		
1 per cent Collargol	Neg.	20mμ	4	—	4	—	4	—	4	+	—	—	—	4	+	+	+	+		
Colloidal arsenic trisulfide	Neg.	100mμ	4	—	4	—	4	—	4	—	—	—	—	4	+	—	—	—		

- indicates the non-filterability and + the filterability of the substance tested.

With the 4½ to 7½ per cent collodion membranes, the results were uniform. With the 1½ and 3 per cent membranes, there were slight variations, so that the tests with each are tabulated.

pirated from fresh, unbroken vesicles, diluted to 1:100 in phosphate buffer at pH = 8.5. The substances for titration consisted of blue litmus and hemoglobin, each suspended in the phosphate buffer at the same hydrogen ion concentration. The collargol and the colloidal arsenic trisulfide, both electronegative, were prepared fresh after the directions of Ostwald.¹⁷ As a test for the charge of the membrane

¹⁷ Ostwald, W., *Manipulations de chimie colloïdale*; translated by Veillinger, E., from the 4th German edition, Paris, 1924.

and its ability to adsorb an oppositely charged substance, a suspension of electropositive night blue dye (*bleu de nuit*) was used. Complete adsorption of this dye occurred in every case.

In Table I are summarized the experiments made with membranes of different permeability, that is, of different thicknesses, containing from $1\frac{1}{2}$ to $7\frac{1}{2}$ per cent acetic collodion. The virus under electronegative conditions is compared with the electronegative test substances, blue litmus, hemoglobin, collargol, and colloidal arsenic trisulfide. These varied in size from $1.8\text{ m}\mu$ to $100\text{ m}\mu$ in diameter. The negative pressure required for each type of membrane differed with the thickness of the layer. For example, with the $7\frac{1}{2}$ per cent membrane, -30 cm. mercury pressure was employed; with the 6 per cent, -10 cm. ; with the $4\frac{1}{2}$ per cent, from -5 to -10 cm. ; with the 3 per cent, -5 cm. ; with the $1\frac{1}{2}$ per cent, -5 cm. In all cases the filtrates were tested for the presence of virus by intradermal inoculation of the hairless pads of guinea pigs; and for the titrating substances by colorimetric, spectroscopic, and chemical tests. It may be stated here that the virus taken from the apparatus, before filtration and at $\text{pH} = 8.5$, was injected intradermally in three to four guinea pigs in each instance. After 24 hours all animals showed the typical experimental disease.¹⁸

An examination of the tabulated, summarized results reveals that, in general, there are only a few irregularities in filtration through the single layer Bechhold membranes—indeed, fewer than were expected. But in view of the fact that each membrane was titrated and its porosity delimited, the results of “molecular” filtrations appear to be conclusive. Furthermore, the selective passage of the titrating substances which we have employed is identical to that reported by Bechhold.⁸

Only the minute particles of blue litmus could traverse the $4\frac{1}{2}$ to $7\frac{1}{2}$ per cent membranes and then quite regularly. The virus uniformly failed to pass. The virus did, however, traverse one of four membranes of the 3 per cent type. In that instance collargol also passed. In the three others of this series, two allowed the passage of hemo-

¹⁸ In these, as in all filtrations, only from 5 to 10 cc. of material was filtered, and the time regulated. Results therefore cannot be ascribed to employment of large quantities, filtered over a long period.

globin and one the blue litmus only. On the other hand, the virus filtered uniformly through all membranes of the $1\frac{1}{2}$ per cent type. In these the porosity was of such an order of magnitude that in all collargol traversed the membranes as well, and in one case, the colloidal arsenic trisulfide.

One may conclude, therefore, that the order of magnitude of the active agent of foot-and-mouth disease, after elimination of interference by adsorption due to unlike charge, is, in relative terms, correspondingly between that of collargol and of colloidal arsenic trisulfide particles. In other words, the size of the virus is relatively between 20 and 100 $m\mu$.¹⁹

These tests have not only served to delimit the size of the active agent, but also serve to refute the notion that the virus is a fluid or a *contagium vivum fluidum*, after the meaning of Beijerinck,²⁰ of which more will be said later. Furthermore, the same comparative ease with which the virus and collargol particles filtered through the $1\frac{1}{2}$ per cent membranes and the difficulty with which colloidal arsenic trisulfide traversed similar discs indicate that the size of the active agent may be nearer the minimal reading than the maximal. The minuteness of size may explain the invisibility of the virus; the difficulty of its artificial cultivation and its resistance to alcohol and other chemicals, to be described in another paper; its non-centrifugability; and its activity in very high dilutions. For particles of these relative dimensions are governed by different physical laws than those influencing larger structures.²¹ Finally, the question arises as to whether these measurements preclude the notion that the active agent is living. In the absence of a standard for comparison, we may compare the virus to bacteria in respect to their chief function, the enzymatic, and their composition, which is mainly protein. We have determined (*vide supra*) that peroxidase can pass freely through spaces which do not permit the passage of litmus, the particles of which are

¹⁹ These tests supply a basis of only a relative calculation. Hence it was decided, especially in view of the definite limitation of time for experimental work in Europe, to forego further trials in an attempt to narrow the range of size to closer approximations.

²⁰ Beijerinck, M. W., *Centr. Bakt.*, 2. Abt., 1899, v, 27.

²¹ Vlès, F., *Rev. scient.*, 1921, lix, 294.

estimated at $1.8 \text{ m}\mu$ in size (Bechhold). On the other hand, assuming the virus to be spherical and of a diameter of $50 \text{ m}\mu$, it should be more than 2500 times the volume ($V = \frac{4}{3}\pi r^3$) of the protein molecule hemoglobin. Furthermore, Vlès²² has calculated that spherical bodies of the order of magnitude of $30 \text{ m}\mu$ may contain 360 protein molecules, and those of $90 \text{ m}\mu$, 9000.

SUMMARY AND CONCLUSIONS.

Cataphoresis experiments show that the virus of foot-and-mouth disease carries, under ordinary conditions, an electropositive charge. Its isoelectric range is at the high point of $\text{pH} = \text{about } 8$. Although cultivable bacteria are, as a rule, electronegative, yet certain protozoa, such as trypanosomes and spirochetes are also electropositive. In respect to charge, then, the virus is different from ordinary bacteria, but there is nothing in this finding to indicate an inanimate character of the incitant. A knowledge of the charge, however, aids in the interpretation of certain filtration phenomena, and indirectly in delimiting the size of the virus. In addition, it serves to explain its remarkable resistance to certain chemicals—a subject to be dealt with in the next paper of this series. Finally, cataphoresis indicates the possible separation of the virus from protein particles.

Filtration experiments were made with different types of filters: Seitz, Berkefeld V and N, and Chamberland, of practically all sizes, collodion membranes, and Bechhold's ultrafilter. The results confirm the electropositive charge of the virus, as well as the minuteness of its size. Filtration was effected through the Seitz, Berkefeld, and Chamberland filters. In regard to the latter, the active agent passed through the L 11 only when its charge was shifted to negative: under ordinary conditions, carrying an electropositive charge, it failed to traverse this more dense wall, and was completely adsorbed in the oppositely charged barrier. Filtrations through electronegative collodion membranes, prepared in different ways and of varying thicknesses, resulted, as a rule, in failure, unless the thinnest and most permeable membranes were employed. But in these, the complication of microscopic holes was to be considered. Hence this method

²² Personal communication.

was regarded as impracticable. Success, however, was obtained with Bechhold's ultrafilter membranes of the most permeable type, and with these it was possible to measure relatively, by a system of "molecular" filtrations, the size of the incitant. This was found to be, in relation to other particles of like charge, between 20 and 100 $m\mu$ in diameter.

The filtration phenomena of the foot-and-mouth disease virus can be accounted for on the basis of the minute size of the particles of the incitant carrying an electropositive charge, and no evidence can be deduced therefrom that the virus is of a fluid character. For the relative size of the particles is constant and the invariability of the limits of measurement contradicts the notion that the incitant may be a "solute" varying in dimensions in different "solvents."