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THE PHYSICAL AND CHEMICAL PROPERTIES OF THE FILTRABLE VIRUSES

It is as a matter of convenience rather than because of any true taxonomic relation that the filtrable viruses are grouped together. Though they possess in common filter passing and possibly ultramicroscopic dimensions, there is little reason to believe that they are all of similar nature, or for that matter, that they are not. There is, however, the tendency to assume a similar physical and chemical nature for all the filtrable viruses merely because they have come to be associated in our thinking. But it is at least a tenable, and perhaps fruitful, hypothesis to consider the viruses as all of essentially the same nature, but the time is not yet when it can be advanced to a position beyond that of an hypothesis.

At the present time close definition of the various ultraviruses is not possible. Furthermore any discussion concerning their living or non-living character can not advance far in view of the lack of any close definition of life. But although the fundamental question as to the nature of the filtrable virus cannot yet be answered, it is always useful in a problem as complex as this to collect, correlate and appraise the scattered bits of evidence, for in this method new leads for investigation are apt to suggest themselves.

At the present time the various opinions entertained concerning the nature of the filtrable viruses tend to be allied with one of three hypotheses. It is of course possible that at least some of the ultraviruses

may be established as being as much outside the bounds of the scientific imagination of the present as were the enzymes in the earlier half of the nineteenth century. But as working hypotheses three general opinions hold the field at present.

1. The first is the theory entertained most actively by Handuroy[1] in France and by Rosenow in America, that some, if not all, of the ultramicroscopic viruses are a transformation of bacteria to a filtrable form.

This theory has been held in some quarters since the discovery of the first filterable virus (tobacco moisaic disease virus) by Iwanouski in 1892. It was given considerable impetus by Almquist's[2] discovery in 1911 that filterable forms were to be found in B. typhosus, and since that time numerous filterable forms of bacteria, protozoa and spirochetes have been reported.

The best positive evidence for the theory is the finding of streptococci in the brain tissue, culturally and histologically, in poliomyelitis by Rosenow and by Evans and Freeman[3] in epidemic encephalitis. The validity of this evidence is considered in detail in connection with bacteriology. It may simply be stated here that until these streptococci can be considered seriously as the causative agents, undoubted proof must be given that an isolated strain of such an "ultra-pleomorphic" organism has become infective in an ultramicroscopic form, a piece of evidence which is not yet forthcoming.

The strongest evidence against the theory is based on imunological grounds. Firstly, antipoliomyelitic serum, capable of neutralizing the virus, has no effect on the streptococci which Rosenow has isolated from poliomyelitic material.[4] One would expect, though not with absolute certainty, to be sure, that there would be some antigenic similarity between

the filtrable and non-filtrable form. Secondly, attenuated, or heated forms of the virus are no longer specifically antigenic, whereas the same is not true of the visible streptococcus, a fact which indicates a fundamental dissimilarity in their molecular stability, and consequently in composition.

2. The second theory regards the nature of the ultraviruses as a non-living cytolysin, initiating a specific autolytic processes in cells, and reproduced by the specific cells which degenerate because of its action.

Bauer in 1896[5] was probably the first to suggest that a filterable virus, namely the virus of infective chlorosis, was an inanimate infective agent. He suggested that the virus need not multiply in the ordinarily accepted sense of the term, but that it may be possible that some abnormal metabolic product of the cells of the diseased leaf may function as the virus and stimulate the formation of the same product on the part of the cells in other leaves. Twort was perhaps the first to suggest that some of the viruses of the animal diseases may be inanimate cytophages.

The theory is most actively held today by Bordet[6] and by Bronfenbrenner[7] in regard to bacteriophage and by Carrel[8] in regard to certain tumors of chickens.

There is much evidence in support of this theory which will be cited below in the discussion of the properties of the various viruses, but none of it conclusive, because of the lack of any precise definition of life.

3. The third theory regards the ultraviruses as living agents, possibly simpler, and, from an evolutionary point of view, previous to cell forms, capable of metabolic processes and multiplication in a suitable environment, and because of their relative simplicity, possessing properties in regard to viability, growth, etc., possibly somewhat different from those

usually expected of cellular forms.

Beijerinck[9] in 1899 voiced the opinion that the virus of tobacco mosaic disease was non-corpuscular, but being endowed with the power of reproduction it was living matter, or a contagium vivium fluidum. Though the idea of a non-corpuscular living body is not popular today, a considerable number, led by d'Herelle, believe that the bacteriophage and possibly other viruses are living bodies.

THE SPONTANEOUS GENERATION OF VARIOUS VIRUSES

Since spontaneous generation is a property no longer accorded living matter, any evidence to prove that a so-called filtrable virus may arise spontaneously, will tend to indicate an autolytic nature as against a living form. A considerable amount of such evidence has accumulated, although in no case yet reported is there an absolute freedom from the suspicion that the virus in question may have been lurking around somewhere.

Gratia,[10] Bordet and Ciuca[11] have shown that by starting with a culture of bacteria, devoid from bacteriophageal properties, these can be made to appear by various artificial means, such as an unfavorable temperature, treatments with immune serum, the mere process of filtration through porcelain filters, etc.

Iannovics,[12] impressed with the occasional encephalitis-like consequences following months or longer after gunshot wounds of the skull, drew the conclusion that traumatic areas in the brain might lead to an absorption of dead tissue which secondarily acted on the surrounding traumatised areas. He experimentally produced similar trauma in rats by con-

tusions of the skull and the injection of suspensions of the brain of rats, and claims that in these animals a disease developed not unlike the slow, encephalitis-like conditions following the injuries of the skull in man.

Zinsser[13] suggests that such a conception might also explain in an entirely new way the supposed development of encephalitis from the injection of bacteria into the brain, which has been interpreted by those who claim to have observed it as a mutation of the bacteria to a filtrable form.

Carrel[8] produced a tumor in chickens by injecting embryonic pulp at the same time with solutions of indol, arsenic and tar, and this tumor was transmissible with filtrates. He concludes, therefore, that this filtrable incitant of fowl sarcoma is not a living agent, which conclusion, though it is hasty, has the merit of being the most simple explanation of the experimental facts.

Andrewes and Miller[14] inoculated the testicles of rabbits with the fresh blood of apparently normal rabbits. Every 5th. day the animals were sacrificed and the testicles inoculated into another pair of rabbits. In six series, histological evidence of infection appeared in the fourth to the seventh generations with typical nuclear inclusions. Microscopical evidence appeared two generations later. The virus was immunologically identical with two similar viruses produced on previous occasions, once by Rivers and Tillett while trying to transmit varicella to rabbit testicles, and another time by Andrewes while injecting rabbit testicles with blood from rheumatic fever patients. This work, as indeed all other work bearing on the question is open to two interpretations; a lytic non-living principle active on the testicular cells may have been developed, or a living

virus, frequently present in rabbit blood may have intruded into the injury, for injury naturally plays an important part in the infectiousness of viruses that exhibit pronounced cytotropic properties, for injury makes it possible for the viruses to come in contact with susceptible types of cells.

CELL SPECIFICITY OF THE VIRUSES

A great many of the viruses show a specificity for definite tissues or cells which is so remarkably constant as to suggest that they are intimately connected with the cell as autolytic products rather than living parasites. This specificity is still more precise in that young cells only may be an effective media for the multiplication of the virus.

Though we know that a small group or the most highly parasitized bacteria, as for example the gonococcus, require, or thrive best, in media containing protein but little changed from the state in which it exists in the human or animal body, no organism of microscopic dimensions is quite as specific in its requirements as are many of the viruses. A possible exception to this statement is the various rickettsia bodies most of which have an intracellular stage. But there appears to be little or no cell specificity in their requirements. The Rickettsia prowazek; is found quite generally throughout the body and also in various cells of the louse. Most of the viruses, on the other hand, are highly tissue specific. Not only may they be highly central nervous system specific, as are the viruses of rabies, poliomyelitis and encephalitis, but they show a distinct specificity for certain regions of the central nervous system. In encephalitis the pons

medulla, optic thalmus and substantia nigra are more especially involved, whereas in poliomyelitis the mesencephalon is less affected than the cord, and this regardless of where the virus is inoculated.

The bacteriophage show a remarkable specificity not only for a single species but very often for single strains. The virus of vaccinia, rabies, poliomyelitis, foot and mouth disease, epidemic encephalitis, small pox, herpes, and others, show a marked ectodermal preference which has led Levaditi[15] to call them "Les Ectodermoses". But in many of these cases, probably all if they were tried, the testicle, which is of mesodermal origin, appears to be as good a medium for the multiplication of the viruses as the ectodermal tissues. Douglas, Smith, and Price,[15 a] developed a strain of vaccinia virus which produced macroscopic lesions in all the visceral organs excepting the kidneys. Goodpasture and Teague[17] report that on a few occasions they have apparently been able to infect the spleen(endodermal) and striate muscles (mesodermal) with herpes virus. But though the testicle is a medium for the cultivation of these viruses, Hirano has shown that in the case of vaccinia virus at least, the virus becomes more virulent when cultivated in the brain than when it is cultivated in the testes.[18] The testicle and the ectodermal tissues, the skin and brain are peculiar among the tissues of the body for the large quantity of phosphatids which they contain, and it is barely possible that it is by virtue of the lecithins that these organs are better media than the other tissues which contain some lecithin to be sure, but in smaller amounts.

Lecithin offers a further analogy with the viruses which is interesting and possibly not without significance. The lecithins in the normal nerve tissue hold in check the autolysins of the nerve cells, and of

the various cells in the body the nerve cells escape autolysis longer than do others after death.[19] But lecithin, it is know, is itself quite readily changed into a lytic agent by hydrolysis with a weak acid or alkali.[20] Flexner and Noguchi showed that cobra venom united with some element of the blood to produce a hemolysin, and this element Kyes[21] later showed to be lecithin. If we carry the analogy with the viruses to a conclusion we can illustrate in a plausible manner how multiplication of an infectious agent may hypothetically occur without itself being capable of true reproduction, by suggesting that the viruses may be derived from certain lecithin split products formed reciprocally by the acids liberated on cell autolysis, a cycle which recurs as long as both lecithin and cell are present.

The phospholipins or phosphatids, of which class lecithin is a member, are fundamental elements in all protoplasm, and Matthews believes that the number of different phosphatids is a great as the number of functionally different cells. For this reason, and also because any single phophatid has a molecule so complex that it can be split in a number of specific ways, there are a great variety of possible phosphatid split products. The high degree of cell specificity which the viruses show may therefore easily be explained on this hypothesis.

Furthermore there are certain elements in the pathology of various of the virus diseases which suggest that the phosphatids may be fundamentally involved. These factors will be discussed below in relation to the pathology of the virus diseases.

If such a phosphatid-cell cycle, or any similar chemical interrelation were responsible for the production of the viruses, it would be expected that the brain and cord, and to a lesser extent other tissues, would become infective after they had antolyged sufficiently following death. The

work of Andrewes, Rivers and Tillett, and Iannovics, (cited above) indicating that a spontaneous, specific virus arose following tissue injury, suggests that such an expectation might possibly be fulfilled. It would not be difficult to carry investigation farther along this line.

THE ANTIGENIC PROPERTIES OF THE VIRUSES

Following the recovery from the virus diseases the animal is more or less permanently immune, although Abramson and Geiber[22] found that monkeys which recovered from poliomyelitis could be re-infected a year and a half later.

The existence of an acquired immunity to experimental reinfection is usually accompanied by a virucidal effect of the serum. There appears to be but a single exception to this rule and that is in the case of contagious epitheliomia in the fowl, in which Burnet, Lipschutz, Sanfelice and Mantenfel[23] showed that no virucidal substances were demonstrated in the serum of recovered animals.

The important observation has repeatedly been made in connection with various viruses that immunity in virus diseases may be linked with the presence in the body or the living virus, and that an animal once infected continues to carry the virus indefinitely in spite of a refractory state to reinfection from without. Sanfelice[24] found the virus of contagious epithelioma in fowls present three months after recovery with the animal still refractory to reinoculation. Both deKoch and Theiler[25] report the persistent specific infectious nature of the blood of horses that have recovered from pernicious anemia. Cole and Kuttner[25] have shown that the "salivary gland virus" of guinea pigs can be obtained at will from immune animals, and

Olitsky and Long[26] have demonstrated the same thing in the case of vaccinia virus. The existence of active virus in a serum containing virucidal properties is difficult to interpret but a recent ray of light has been thrown on this puzzle by Todd,[27] and Andrewes[28] and Long and Olitsky[29] by demonstrating the in vitro dissociation of a vaccine virus-immune serum mixture. A mixture of virus and specific virucidual serum, inocuous when injected into a susceptible animal becomes disease producing after dilution with appropriate amounts of Ringer's solution. Andrewes[30] working with the vaccinia virus of Douglas, Smith and Price[16] which produced general visceral lesions, found that doses of immune serum did not protect against the local eruptions but did protect against generalization of the lesions. The immune serum in successful in preventing generalization only if inoculated before the virus. He thus suggested that when the virus has attached itself to a cell or has entered it, immune serum has no effect. Smith,[31] continuing the work of Andrewes, showed that vaccina virus inoculated into the circulation of a rabbit is rapidly fixed by some formed element in the blood, and this formed element be showed to be white blood cells, although the platelets have not been definitely excluded. The virus may appear in the white blood cells at the same time that immune bodies are present in the plasma. This particular riddle of the filterable viruses seems therefore to have been answered.

Another interesting peculiarity in the antigenic properties of the viruses is the fact that only the active virus possesses specific antigenic properties. The viruses are rather easily destroyed, their attenuation has not as yet proven possible and all attempts to immunize with an altered virus have proven ineffective. Heating most viruses to fifty-five degrees C. or even less for a short time inactivates them and renders them non-antigenic. Occasional successes have been reported in immunization by

virus rendered inactive with various chemical agents such as chloroform and formalin. But these reports have been interpreted by Duran-Reynolds[32] in the case of vaccine virus, and by Olitsky and Long[33] in the case of foot and mouth disease virus as being due to the fact that some of the virus remained alive, protected from the action of the chemicals by the proteins of the virus emulsion. (Olitsky and Boez)[34]

The difference between the antigenic properties of the ultraviruses and the usual bacterial cell is further widened by the work of Schultz, Bullock and Lawrence[35] and Schultz, Bullock and Brewer,[36] who found no evidence of specific complement fixation nor of specific precipitins, but only virucidal antibodies in vaccine virus or rabies virus immune serum, after antibody adsorption had been done with the tissue proteins which virus preparations necessarily certain. The precipitins which Gordon and others produced against vaccinia virus were undoubtedly produced against the tissue proteins in the virus solution. Because the virucidal antibody is neither a precipitin nor a sensitizer in the sense that it fixes complement, Schultz suggests that vaccinia and rabies viruses belong to Zinsser's first class of antigens, or monovalent antigens along with exotoxins, and snake venom. The American Commission[34] on foot and mouth disease similarly failed in demonstrating complement fixing antibodies or precipitins in immune serum, and this was confirmed also by Stockman and Minett.[37]

RESISTANCE TO CHEMICALS OF THE FILTRABLE VIRUSES

It has been thought that the resistance to chemicals by most of the ultraviruses was a compelling indication of their non-living nature. Abe[38] reported that the virus of foot and mouth disease when precipitated

with 75% alcohol and dried remained active for three days. Stockman and Minett[37] reported that the virus of foot and mouth disease remained alive at least twenty days in 10% alcohol, for three days in 50% alcohol, in pure acetone twenty but not thirty minutes, that ten drops of chloroform or ether to 5 cc of a 1:1000 dilution of the virus did not inactivate the virus for twenty-seven days. Almost identical chemical resistance has been shown for the virus of tobacco mosaic by Duggar,[39] Glaser[40] found that the virus producing sacbrood in bees remained active in 2% carbolic acid for three weeks. Abrahamson and Gerber[22] have found that poliomyelitic virus is not inactivated by 0.5% formaldehyde after four hours.

Clark and Schindler have shown that poliomyelitic virus has been found to be as active after remaining two months in a saturated salt solution as it was originally. Other similar instances of the chemical resistance of the viruses have been reported. However, Olitsky and Boez[34] have placed a new interpretation on this supposed chemical resistance. They have shown that this resistance operates only under limited conditions. This is due to the fact that most chemicals coagulate the proteins of the medium in which the virus is usually suspended. As a result of the coagulation, the positive charge of the virus and its minute size, the virus is held within the large coagula and is kept from direct contact with the chemicals. If advantage is taken of the periodic phenomenon attending coagulation, or if coagulation is prevented, as in the case of alcohol, by adding a trace of sodium hydroxide, the virus is then acted upon directly by the antiseptics. Under these conditions, the virus is as sensitive to destruction by the reagents as is the living staphylococcus. Reagents which do not form coagula, such as sodium hydroxide and antiformin, kill the virus extremely readily.

It is probable that the apparent resistance of tobacco mosaic virus, poliomyelitic virus, and other viruses, to chemicals is likewise due to the protecting coagulation of the proteins in the media.

McKinley, Fisher and Holden[41] found that ultra-violet light at a distance of one foot for forty minutes destroyed equally readily B. coli phage, herpes virus, and Levaditi's encephalitis virus; while Olitsky and Gates[42] report a similar sensitivity to ultra-violet light for staphylococcus aureus and vesicular stomatitis. Olitsky suggests that since the absorption of specific energies is one index of chemical character, the parallel sensitivity of Staphylococcus aureus and the various viruses to ultra-violet light indicates that the substance of the virus is similar in character and chemical constitution to bacterial protoplasm.

It is certain that on the basis of the resistance to chemicals the ultraviruses do not appear to differ markedly from bacteria.

THE SIZE OF THE ULTRAVIRUSES

The probable very small size of the ultraviruses is often adduced as evidence that their structure cannot be complex enough to admit of their having the organization which is expected of even the simplest living protoplasm. It is obvious that the methods used to determine their size is only roughly indicative. Methods of filtration are subject to a great many variable factors; the electrical change on the virus, the electrical charge on the filter, the adsorption of the virus by aggregates of protein or by cell detritus, the amount of protein or other substances in the virus emulsion, the temperature at which the filtration is conducted, the amount of negative or positive pressure employed, the duration of filtration, varia-

tions in individual filters, and other factors, must all be carefully controlled, but are seldom mentioned in the results of all virus measurements. No virus has been obtained in a pure state, therefore it is impossible to say that virus alone is being filtered rather than virus attached to aggregates of protein. However, all these factors tend to bring question upon the lower, rather than the higher, limit of the size of the viruses, and the probability is that the viruses are smaller and not larger, than usually reported.

For the foot and mouth disease virus Olitsky and Boez[43] estimate a size of from 20 to 100 mμ, probably nearer the former. For the virus of chicken plague, a number of different investigations, Andriewsky,[44] Doerr and Pick,[45] and Berger[46] have all found that the size lies within the narrow limits of from 2.3 to 2.5 mu. Zinsser and Tang[47] estimate the size of Herpes virus as being not over 35 mu. The size of the tobacco mosaic virus is placed by Duggar, Harrer and Armstrong[48] as being approximately 30 mu. The dimensions of the bacteriophage is usually placed at about 20-35 mu (Praausnitz,[49] Beimond,[50] Angerer,[51] Bechhold[52]) but Eliara and Suarez[53] place it below 5 mu, while Stassano and Beaufort[54] estimate it as being below 1 mu.

The size of molecules of crystallized proteins has not been estimated within very narrow limits, and if it is difficult to determine the size of molecules of relatively pure crystalline substances there is little hope at present of ascertaining the size of viruses which have not been obtained in a pure form. Bechhold[55] states that an aggregate of 50 albumin molecules is from 4 to 10 mμ in diameter, but according to du Nony,[56] however, 1 molecule of egg albumen is 4.1 mu in diameter. Vles[57] estimates that a dimaeter of 50 mu is 2500 times the diameter of the hemoglobin molecule, while 30 mu may contain 360 protein molecules, and 90 mu may contain

9000 protein molecules. It is idle therefore, to pretend to know the lower limit in size of living bodies.

Attempts have been made to estimate the size of the viruses by throwing them down in high speed centrifugation or by standing. V. Prowazek[58] showed in the case of vaccine virus that the upper layer became less infective than the lower layer after prolonged standing. He though this evidence of the corpuscular nature of the virus, but without a creful comparative titration of the virus the possibility is not precluded that the virus in the upper layer degenerates more readily because of aerobic conditions. Remlinger and Landsteiner[59] found that the viruses of rabies and chicken plague were present in greater concentration in lower than in the upper part of the tube after centrifugalizing emulsions for a long time and at high speed. The obvious criticism here is that the virus may have been carried down in protein particles. McKinney found that certain of the plant disease viruses could be thrown down with a super-centrifuge of the design by Birge and Juday. Olitsky and Boez[60] found that the virus of foot and mouth disease has not thrown down in two hours at 3000 revolutions per minute.

The difficulty in estimating the size of a virus by centrifugation is admirably set forth by Duclaux[61] who shows that although theoretically with a centrifugal force 40,000 times the gravitational force of a particle, a particle of 10 mu will settle only 1 cm. in four hours, but that practically the whirling current and the convection currents produced by the heat of the moving parts operate to prevent deposition.

Probably the most valuable indication of the relative size of the viruses is that afforded by the work of Levaditi and Nicolai[62] who found that collodian membranes which more or less completely retain albumins, complement, hemolytic amboceptor, trypsin, tetanus and diphtheria toxins, allowed bac-

teriophage and the viruses of vaccinia, herpes, encephalitis and rabies to pass.

A method of determining the size of viruses which is ingenuous is that devised by Glaser and Cowdry.[63] Using the ultra-microscope of Siedentopf and Zsigmondy, capable of detecting reflections of particles as small as 10-15 mu, they found that normal insect blood contained as many such bodies as blood from insects infected by certain of the polyhedral viruses, and concluded from this evidence that the size of the viruses must lie below 10-15 mu.

THE RESPIRATION OF THE VIRUSES

Another piece of evidence, also inconclusive, often cited to indicate the non-living nature of the virus is the work done by Bronfenbrenner and Reichert[64] on the respiration of several of the viruses. By a device sufficiently delicate to detect the production of 1 cm. of CO_2 by fifteen million staphylococci in less than ten minutes, and considerably more sensitive when allowed to work over a period of days, they failed to detect any respiration of the bacteriophage nor of rabies or herpes viruses. It may be, however, that the viruses may respire at such a slow rate that its detection is beyond the limits of the sensitiveness of the method.

CULTIVATION

The fact that the viruses have not been cultivated in the absence of living cells indicates nothing either way as to their possible living or non-living nature.

THE ISOELECTRIC POINT OF THE ULTRAVIRUSES

Data on but two viruses are available concerning their isoelectric points. Olitsky and Long[65] report that vaccinia virus is electronegative within the ranges of physiologic Ph. This fact would place its isolectric point in a Ph below 6.9, and in this respect it is similar to the bacteria and the usual body proteins. The virus of foot and mouth disease, however,[60] is electropositive below Ph = 8.0. It can thus be separated from the usual media proteins by cataphoresis, although no serious attempt has yet been made to try to get it in a state free from media proteins. This high isoelectronegative point is shared by a few proteins such as fibrin and gliadin, but by none of the normally present proteins of the body. No bacterium is know having an isoelectric point above Ph. 6.9. But among the Spirochetes, brucei, equiperdum, gambiense, and rhodesiense are electropositive.

It may be stated at this point that an analogy between the viruses and some of the spirochetes is to be found in other directions than their isoelectric points. (2) Many of the spirochetes pass filters rather readily;[66] (3) in many spirochetal infections a refractory state to reinoculation exists simultaneously with the presence of the organism in the body; (4) the pathology of many of the spirochetal infections rather closely resembles that of many of the virus diseases.

THE PATHOLOGICAL PICTURE OF ULTRAVIRUS LESIONS

In the large number of diseases produced by ultraviruses in the central nervous system, among which are rabies, epidemic encephalitis, and

poliomyelitis, Zinsser[67] believes that the pathologic changes are inconsistent with the assumption of a bacterial process. The histologic picture in his opinion more strongly resembles that seen in the cytolysis resulting from toxic absorptions, as in lead poison, tetanus, botulism and other diseases in which toxins produced elsewhere in the body have an affinity for nerve tissue. The cell reaction is quite largely lymphocytic and mononuclear, while the polymorphonuclears play a part only early in the disease. There is a perivascular lymphocytic infiltration somewhat resembling that in syphilis excepting that it is not so pronounced. Zinsser states,[67] "Bacteria cannot arouse a pathologic reaction of this kind, certainly not in acute cases and probably not even in the most chronic cases." It is to be remembered that in typhoid fever the cell reaction is largely a lymphocytic one, while in typhus fever the histological picture in the central nervous system closely resembles that of poliomyelitis and encephalitis.

In poliomyelitis the nerve cell destruction, so marked in the anterior horns is secondary, apparently, to the hemorrhage and edema which is due probably to both increased fluid affinity of the tissues and injury to the capillary walls.[68] The capillaries outside the central nervous system are but slightly if at all affected, and conseauently it would seem that the injurious effect is associated with the multiplication of the virus in the stroma. From another angle also it would seem that the blood vessel damage is secondary to conditions produced by the presence or multiplication of the virus in the surrounding stroma. Many neurotropic virus diseases, such as poliomyelitis, are produced experimentally only with great difficulty by injecting the virus into the blood stream, but more easily by the nasal route in which Flexner Clark[69] have shown that the virus pro-

ceeds by direct extension along the nerve trunks to forebrain. Since the nerve cells are not primarily affected, there is perhaps, some element in the central nervous tissue stroma specifically associated with the progression and multiplication of the virus. It has already been suggested, from a different line of reasoning, that this factor might be the phosphatids. The phosphatids play an important role in cell and tissue metabolism by virtue of the facility with which they are oxidized and reduced. The oxidized phosphatids have a greatly increased affinity for water, which is lost on reduction. Amoss[68] showed that the edema in poliomyelitis was not due to increased osmotic pressure, since it was not decreased by the injection of hypertonic solution. It is therefor due either to increased colloidal affinity, after the theory of Martin Fisher or to an oxidation of the phosphatids. Such an oxidation is conceivably the initial process in the splitting of the lecithin according to the hypothesis outlined above.

It is a bit difficult to understand how, the virus, if it is a lytic principle produced by the autolyzing cell alone, according to the conception of Bronfenbrenner, Bordet, Doerr and others, should induce a vascular injury before it acted upon the nerve cell, since such lytic principles are certainly highly specific.

On of the most striking attributes of many of the diseases caused by the filtrable viruses is the development of large and conspicuous "inclusion bodies" within the cells. There is much divergence of opinion regarding the interpretation of these inclusion bodies, some workers being inclined to regard them as the actual etiologic agents while others view them as reaction products produced by the cell in response to injury caused by infective agencies which are ultra-microscopic. We cannot discuss the

problem very fully here. Some of them, perhaps all of them, are certainly high in lipoid content, and this lipoid is not neutral fat.[70] Goodposture[71] suggests that the mitrochondrial filaments of the axis-cylinders and of the cell body are involved in the formation of both the vacuolated negri bodies and lyssa bodies. Matthews[72] believes that the mitrochondrial bodies are probably phosphatids.

Woodruff and Good posture[73] have recently been able to isolate the inclusion bodies of fowl-pox by digesting the cell with a 1% solution of trypsin. A fatty capule protects the inclusion body from digestion. Inoculation with a single inclusion body caused epithelial pox, whereas a control of the surrounding cellular fluid (digested with trypsin) did not. The obvious objection is that tryptic digestion may have destroyed the virus in the surrounding.

II

THE HISTORY OF ANTERIOR POLIOMYELITIS

Heine[74] in 1840 recognized as a clinical entity the disease now known as acute anterior poliomyelitis, and observed that the spinal cord was the seat of the lesion. An American physician, Underwood of Philadelphia, had called attention to paralytic sequellae of illness in children as early as 1793.[75] Following Heine's description, Colmer[76] in 1843, Vogt[77] in 1858, and Duchenne[77] in 1864, described the disease in adults. The first good clinical description was that of Medin[78] in 1890, and following his report, the clinical entity came to be known as the Heine-Medin Disease.

The first pathological researches were made by Cornil in 1863, while Prevost and Vulpian[79] first described the degeneration of the anterior horn cells with chronic interstitial changes in the surrounding tissue. Further light was thrown on the pathology by Lockhart, Clarke, Charcot, Joffroy, Roger, Turner, Fredrick Taylor and others.[77]

The infectious nature of the disease was not adequately determined until Wickman[80] in 1905 adduced evidence of its communicability based on a survey of 1200 cases in Sweden. In 1909 Landsteiner and Popper[81] in Vienna first produced acute poliomyelitis in monkeys by the intraperitoneal injection of infectious material from human cases. Shortly afterwards this success was duplicated by Strauss[82] and by Flexner and Lewis in New York.[83] The latter, together with Leiner and Von Wiesner[84] in Vienna and Landsteiner

and Levaditi[85] in Paris, succeeded in passing the disease through a series of monkeys.

THE BACTERIOLOGY OF POLIOMYELITIS

Despite the fact that the infectious nature of the disease had been amply demonstrated by 1910, lack of success continued to be the outstanding feature in all attempts to isolate the causative microorganism. Flexner and Lewis,[86] in 1909, found that the disease producing factor, or "virus" as it has been called, would pass through a Berkefeld filter.

In the twenty years that have passed since the disease was first experimentally produced, a number of different organisms have been presented as the causative agent, but for only two of these organisms has the evidence been at all enduring. The first was that reported by Flexner and Noguchi,[87] the second a streptococcus sponsored by Rosenow,[88] Nuzum[89] and Mathers.[90]

The globoid body does not receive the acceptance today which it enjoyed in the enthusiastic moments following its discovery. Most of its former proponents are more than willing to reconsider the evidence. But the streptococcus, for which the case is decidedly weaker, is still being pushed forward by Rosenow, who insists, righteously but too boldly, that negative evidence is never perfect.

The Globoid Body: In ascitic fluid containing fresh rabbit kidney, Flexner and Noguchi[91] succeeded, after many failures, in growing a minute anaerobic globoid body from poliomyelitic tissues. The evidence which was offered for the globoid body as the causative agent of poliomyelitis may

be briefly summarized.

1. Monkeys came down with typical experimental poliomyelitis after inoculation of pure cultures of the globoid body intracerebrally, though the possibility of having carried the virus over along with the globoid body was not definitely excluded.

2. Cultures of the globoid body in a single instance proved pathogenic to the eighteenth generation, the dilution of the original inoculum being twenty-four to the seventeenth power which is far beyond the limits of infectivity of the original material.

3. Koch's postulates have apparently been fulfilled: the organism has been found in human poliomyelitic tissue,[92,93] and has not been found in other conditions; it can be cultivated therefrom, and when injected produces experimental poliomyelitis from the lesions of which it can again be recovered.

The evidence which indicates that the globoid body is not the causative agent in poliomyelitis is this:

1. Inoculation of cultures of the globoid body fails more often than not to induce the disease, indicating that possibly a second factor is involved.[94]

2. The globoid bodies have not been universally present in poliomyelitic tissue, and usually have not been found in highly infective filtrates.

3. A Moss[95] failed to find any immunological relationship between the globoid body and the true virus, although this work has neither been confirmed nor refuted.

<u>The Streptococci</u>: The case for the streptococcal etiology of poliomyelitis is certainly less well established than that for the globoid body. The evidence upon which the case is essentially this:

1. Streptococci have been cultivated from poliomyelitis tissue by a number of workers, particularly by Mathers,[90] Rosenow, Towne, and Wheeler, and Nuzum and Herzog,[89] and from the spinal fluid of both human and experimental cases by Rosenow.

2. Rosenow[96] believes he has developed an immune horse serum with his poliomyelitic streptococcus which is effective in the treatment of epidemic poliomyelitis.

3. Rosenow[97] has occasionally found streptococci in the brain of a monkey dying with poliomyelitis.

The claim for the streptococcus must first successfully meet the following criticisms before it can be entertained very strongly as the causative agent of poliomyelitis:

1. Bull[98] and Rosenow himself have repeatedly failed in inducing any symptoms of poliomyelitis in monkeys inoculated with the so called poliomyelitic streptococcus.

2. Rosenow has never tried the effect of his streptococcus immune serum on monkeys inoculated with active poliomyelitic virus, and the clinical evidence is open to too many variables to establish specificity for the streptococcus. Bull[98] on the other hand has failed to find the streptococcus anti serum effective in the treatment of the experimental disease, nor would it neutralize the virus.

3. Olitsky and Gates[99] in an unusually well controlled experiment showed that one can get streptococci from normal brain in numbers proportional to the care in removal and preparation, and that different streptococci from the same poliomyelitic brain were antigenically dissimilar.

Rosenow's contention, which is nothing more than a bold hypothesis, is that the streptococcus which he has isolated from poliomyelitic tissues

occurs in its virulent state in an ultramicroscopic form. It is a far cry from the evidence at hand to such a premise, and logic forces one to doubt it strongly until such a time as it is proven beyond question that the streptococcus does become virulent in an ultramicroscopic form and produce poliomyelitis.

III

THE CONCENTRATION OF THE VIRUS OF POLIOMYELITIS

In the summer of 1928, Dr. P. F. Clark began a series of investigations at the University of Chicago concerning the concentration of the virus of poliomyelitis. These investigations have been continued at the University of Wisconsin during the fall and winter of 1928-1929.

Among the numerous possibilities contingent upon such a concentration, that which suggested itself most strongly was the possibility that by concentrating the virus to a small fraction of its original volume any visible organisms present in the virus would appear in considerable numbers in stained smears. Some light might consequently be thrown upon the nature of the causative agent.

Several methods of concentration presented themselves, among them the methods which have been utilized in the purification of toxins, antitoxins etc. But the two methods which, by their comparative simplicity, offered themselves as a starting point in the investigation were the two following:

1. To concentrate the virus in vacus at a temperature well below its thermal death point, and at a speed fast enough to minimize any destruction attendant upon standing.

2. To concentrate the virus by possibly salting it out of solution with the various proteins of the filtrate.

A third method which was held in the offing should the first two fail, was to concentrate the virus by cataphoresis. In case the virus of poliomyelitis were electropositive at a Ph at which the usual proteins are electronegative, as is the virus of foot and mouth disease, this method would have the additional advantage of concentrating the virus free of media proteins. But as yet this method has not been utilized.

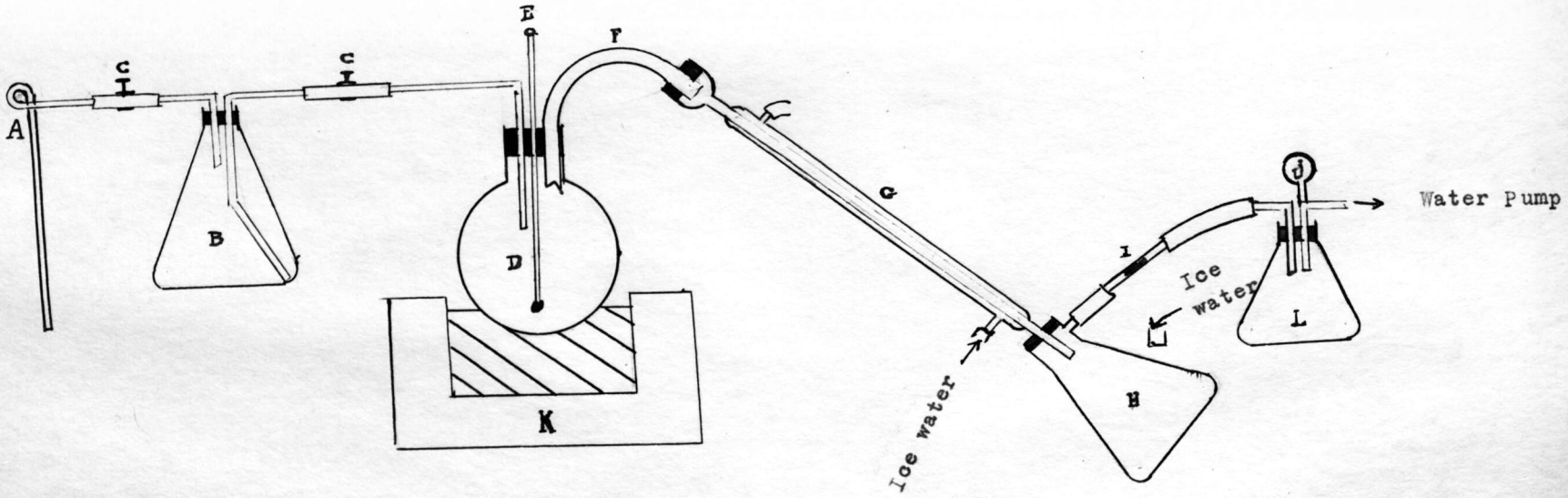
After investigation of the first two methods had been in progress sometime, another possible method was suggested by Seiberts[100] work on the concentration of tuberculin. She reduces the water content by pressure filtration through alundum cups impregnated with soluble gun-cotton. As yet this method has not been utilized for poliomyelitis virus, but preparations for its use are in progress.

After a few preliminary trials and errors, concentration of the virus in vacuo has proven successful. Furthermore, the peculiar properties of this concentrate have stimulated other lines of investigation concerning the possible nature, physical and chemical, of poliomyelitis and other viruses.

The concentration of the virus by salting processes has not yet been drawn to a conclusion, but already significant results have been obtained.

THE CONCENTRATION OF POLIOMYELITIS VIRUS IN VACUO

In vacuo concentration of poliomyelitis virus has been attempted by Flexner and Clark (unpublished) and by Amoss and Taylor.[101] But in neither case had the concentration been carried to the extent desired by Dr. Clark in the search for possible organisms.



Apparatus Used for Concentrating Poliomyelitis Virus

A = Inlet; covered with sterile gauze stocking.
B = Reservoir and Safety flask, 500 cc.
C = Stop cocks to regulate flour.
D = Distilling flask, 1000 cc.
E = Thermometer.
F = Large diameter glass tubing
G = Condensing tube.
H = Suction flask, 2000 cc.
I = Glass tubing, packed with cotton.
J = Pressure gauge
K = Electrically heated water bath, with thermostatic control.
L = Safety flask

The apparatus which we have devised and used for our invacuo concentrations is shown in Figure I. In practise this apparatus has proved capable of concentrating 100-200 cc of filtrate to 1cc in one hour with the temperature of the filtrate from 18° - 23° centigrade. For our purposes this speed and temperature of concentration appeared to be practicable, since the virus survives without injury.

The intake tube, A, is covered with a stocking of several layers of gauze. The entire apparatus is autoclaved thoroughly before use to insure sterility. The stocking covering the intake tube is slipped over the flask from which the filtrate is drawn, thus preventing dust contamination of the filtrate while it is being drawn into the apparatus. The transfer of filtrate from one flask to another is accomplished through the negative pressure afforded by the water pump exhauster. Flask B serves as a reservoir of virus and also prevents air from getting directly into distilling flask, D from the intake tube. The water pump used is capable of producing a pressure of 15 mm. of mercury as measured by a spring vacuum gauge, J. At this pressure the filtrate in the distilling flask boils at 18°-22° C. Raising the temperature of the water both increases the intensity of boiling but not the boiling temperature, but in actual practise the water bath temperature was held at about 40° C. Finely drawn, sealed capillaries are placed in the distilling flask to prevent undue bumping. The boiling point is lowered several degrees by running very cold water through the condensing tube and over the collecting flask, the vapor pressure in the apparatus--being thus reduced through rapid condensation.

The filtrate as it is obtained from a 5% suspension of the brain and cord of a poliomyelitic monkey is usually made up in normal physiologic

saline, so that a concentration to 1/40 of its volume would result in a saturated NaCl solution (0.9% x 40 = 36.0%) as it was feared that this NaCl saturation might have an injurious effect upon the virus, the investigation was begun using a distilled water filtrate and a 1/20 normal physiologic saline filtrate.

Distilled Water Filtrate: It was found that the distilled water usspension, because of the absence of an electrolyte, could not be centrifuged clear enough to pass through a Mandler " N " filter. Consequently distilled water was immediately abandoned in favor of a solution containing an electrolyte. A single monkey was inoculated with a six day old distilled water filtrate (secured with difficulty) but did not develop symptoms of poliomyelitis.

1/20 Normal Physiologic Saline Filtrate: The filtrate of a 5% brain and cord suspension made up in 1/20 normal physiologic saline could not be cleared very well even by prolonged centrifugation at high speed, but it was possible, with considerable pressure to obtain a filtrate from the centrifuged supernatant.

The concentrate, in all our concentrations was a brownish, sometimes redish, very viscous, opaque fluid from which a considerable quantity of protein material and NaCl crystals precipitated.

Two series of monkeys were used to determine the potency of the concentrate made from such a filtrate, using the unconcentrated filtrate as a control. In these experiments, and in all following ones, the concentrate, or a portion of it, was rediluted before injection to a volume equivalent to that of the original filtrate, for purposes of a quantitative comparison of the potency of the concentrated virus.

The virus used in all these experiments was one which had passed through a long series of monkeys and was as nearly 100% infective for monkeys as any virus was likely to be. All the monkeys were inoculated intracerebrally. The clinical diagnosis of poliomyelits was in all cases verified by a gross and microscopic pathological examination.

The following is a typical protocal illustrating the preparation of the concentrated virus from brain and cord material.

PROTOCOL OF PREPARATION OF CONCENTRATE

Dec. 13, 1928.

Material from glycerinated brain and cord of monkey #214.

Cerebellum	-	7 gms.
Cord	-	4 gms.
Cerebrum	-	35 gms.
Total		46 gms.

(1) Washed in physiologic saline to remove glycerine.

(2) Ground in sterile mortar with sterile sand and emulsified with 505 cc. 1/20 Physiologic Saline Solution (a 10% suspension was used here; a 5% suspension was more usually used)

(3) Emulsion shaken with glass beads for thirty minutes.

(4) Then centrifuged at 3000 revolutions per minute for one hour.

(5) The supernatant was filtered through a Mansler "N" filter (Beckefeld filters were found to give a clearer filtrate) The filtrate amounted to 250 cc.

(6) 225 cc. of the filtrate was concentrated in vacuo to 5 cc. or

to 1/45 of original volume.

Time	Temperature in Bath	Temperature in Flask	Negative Pressure
4:00 P.M.	25°	18°	28
4:05	40°	18°	28
4:45	42°	17°	28 +
5:00	Finished with 5 cc.		

TABLE I

EXPERIMENT USING 1/20 NORMAL PHYSIOLOGIC SALINE FILTRATE

Monkey No.	Source of Inoculum	Form of Inoculum	Degree of Concentration	Amount Injected	Date of Inoculation	Date of First Symptoms	Incubation Period	Subsequent Course
							Days	
First Series:								
216	214	Concentrate	30-1	3cc.Equiv*	Dec. 14, 1928	Jan.4,1929	21	Recovered
217	214	"	30-1	1cc.Equiv*	Dec. 14, 1928	Jan.6,1929	23	Died Jan.7
218	214	Filtrate		2cc	Dec. 14	Died of colitis Dec. 22,1918		
Second Series:								
219	214 & 215	Filtrate		2cc	Dec. 27, 1928	Continued	a normal	course
221	214 & 215	Concentrate	50-1	2cc Equiv.*	Dec. 27, 1928	Continued	a normal	course
Physiologic Saline Filtrate Control:								
220	214 & 215	Filtrate		2cc	Dec. 27, 1928	Jan.7,1929	10	Killed on Jan. 12.

* The concentrate was in all instances rediluted to the original filtrate volume and 1cc, 2cc, or 3cc, as the case might be, of this filtrate equivalent was injected intracerebrally.

In the first series the animals receiving the concentrate developed poliomyelits only after extremely protracted incubation periods. Although #216 had a partial paralysis, i.e. a tonic paralysis, of the hind limbs he subsequently recovered completely, and by March all residual paralysis had disappeared.

In #217, following a protracted incubation period, the disease appeared in a remarkable fulminating manner, the animal dying one day after the first symptoms of poliomyelitis had developed.

Since #218, the filtrate control animal, died of colits, it was impossible to draw any conclusions concerning the relative strength of the filtrate and concentrate.

The second series proved conclusively that a 1/20 physiologic saline filtrate is much weaker in virus than a normal physiologic saline filtrate. The animals receiving the 1/20 physiologic filtrate, or a concentrate thereof, did not develop poliomyelitis, whereas the animal receiving the physiologic saline filtrate developed typical poliomyelitis. The filtrate of the 1/20 physiologic suspension is not highly infective because of incomplete precipitation by centrifugation of filter clogging material. That it is not due to the absence of a certain minimal salt concentration is shown by the fact that we later produced poliomyelitis by distilled water preparations.

NORMAL PHYSIOLOGIC SALINE FILTRATE

Two series of monkeys were used to determine the potency of the virus concentrate made from a normal physiologic saline filtrate of a brain and cord suspension. As usual the concentrate was rediluted in sterile distilled water before injection to a volume equivalent to that of the original

TABLE II

EXPERIMENT USING NORMAL PHYSIOLOGIC SALINE FILTRATE:

Monkey No.	Source of Inoculum	Form of Inoculum	Degree of Concentration	Amount Injected	Date of Inoculation	Date of First Symptoms	Incubation Period	Subsequent Course
First Series:								
220	214 & 215	Filtrate		2cc	Dec.27,1928	Jan.7,1929	10 days	Killed Jan. 12
222	214 & 215	Concentrate	30-1	2cc Equiv.	Dec.27,1928	Died Dec.31	of unknown cause	
Second Series:								
223	217	Concentrate	30-1	2cc Equiv.	Jan.9,1929	Jan. 17	8 days	Died Jan. 19
224	217	Filtrate		2cc	same	Jan. 13	4 days	Died Jan. 16
225	217	Concentrate	30-1	1cc.Equiv.	same	Jan. 14	5 days	Died Jan. 19

filtrate, for purposes of quantitative comparison and because the saturated saline content of the undiluted concentrate might very possibly be fatal when injected directly into the brain.

In the first series #222, receiving the concentrate, developed symptoms not unlike those of poliomyelitis. But because the incubation period had been extremely short, three days, we were led to believe that the case was not poliomyelitis. In view of the fact that we have since observed a case with a four day incubation period following inoculation with the concentrated virus, it is quite possible that #222 died of poliomyelitis. Unfortunately the histologic sections were accidentally destroyed.

The second series gave very definite results indicating that the saturated saline content of the concentrate did not affect the virus after twenty-four hours.

It now seemed advisable to determine how long the virus remained active in the saturated salt solution, and to make a rough quantitative comparison between the amount of virus in the concentrate and the amount of virus in the original suspension before filtration.

VIABILITY OF CONCENTRATED POLIOMYELITIS VIRUS IN SATURATED NaCl SOLUTION

The concentrate of #217 which had been shown to be highly infective (Table II) was used to determine the viability of the concentrated virus in the saturated NaCl solution which resulted by concentrating the normal physiologic saline filtrate to one forty fifth of its volume. This concentrate was a rather light brown viscous fluid from which there settled a precipitate, the lower part of which was a dark grey and the upper portion a dark brown.

The virus was stored in the ice box and inoculated intracerebrally after the usual dilution at the end of one month and at the end of two months.

TABLE III

EXPERIMENT DETERMING THE VIABILITY OF CONCENTRATED POLIOMYELITIS VIRUS IN A SATURATED SALT SOLUTION

Small quantities of the concentrate of #217 prepared Jan. 8, 1929 was rediluted before inoculation to normal physiologic and 2 cc of this material inoculated intracerebrally.

Monkey No.	Date of Inoculation	Date of First Symptoms	Incubation Period	Subsequent Course
226	Feb. 16, 1929	Feb. 25	8-9 days	Killed on Feb. 27
233	March 8, 1929	March 14,1929	6 days	Killed March 29.
243				

The concentrated virus is apparently not affected by a saturated salt solution in the concentrated media after a period of two months. Further tests with the same concentrate will be made at the end of four months. It is quite possible that this remarkable resistance may be due to the protecting effect afforded by the large amount of coagulated protein in the media. Olitsky and Boez (Jr. Exp. Med, 1927, 45,815) showed that this factor was responsible for the apparent resistance of the virus of foot and mouth disease to coagulating chemicals.

VIABILITY OF ROSENOW'S POLIOMYELITIC STREPTOCOCCI IN SATURATED SALINE

On March 8, 3 cc. of a turbid broth culture of Rosenow's poliomyelitic streptococci nos. 1,2, and 3, were mixed into 750 cc of normal physiologic saline and concentrated to 10 cc. in four hours at a flask temperature of 23° C. Cultures of this concentrate were made on blood agar plates at intervals with the following result:

9/III/29 - Positive

10/III/29 - Positive

19/III/29 - Positive

29/III/29 - Positive though less in number

17/IV /29 - Positive though less in number

23/IV /29 - Negative

Rosenow's streptococcus was thus found to be viable for a month and a half in a saturated salt solution containing 30% beef infusion broth.

In order to determine the viability of the streptococcus under conditions more nearly like those which pertain in the case of the concentrated virus, 1½ cc. of a turbid broth culture of Rosenow's streptococcus was added to the 350 cc. of sterile filtrate of a 5% rabbit brain and cord suspension and concentrated to 10 cc. in four hours at a flask temperature of 22° C. Cultures were made on blood agar plates of this concentrate at intervals with the following results:

13/III/29 - Concentration of material

14/III/29 - Positive culture

19/III/29 - Positive culture

29/III/29 - Positive culture

17/IV /29 - Positive culture

The streptococcus in a concentrated brain suspension filtrate remained viable for not longer than a month. Too much importance should not be attached to this result either for or against the dissimilarity of the streptococcus and the virus, unless it should develop that the virus can remain viable indefinitely in a saturated salt solution, a matter upon which it is not yet possible to make a statement.

THE POTENCY OF A CONCENTRATE AS COMPARED TO THE 5% SUSPENSION

If the concentrate is to be used for bacteriological or other purposes requiring a maximum of virus in a minimum volume, it is obviously important to know whether or not it contains more virus than the 5% brain suspension. Certainly the concentrate contains less foreign material, which is a distinct advantage in itself.

To determine whether the concentrate contained more virus per unit volume the following experiment was conducted:

Monkey No.	Source of Virus	Form of Inoculum	Dilution	Amount Injected	Incubation Period	Subsequent Course
232	#225	5% Suspension	1-30	1cc	No effect	
232 a	#225	Dialysate of Concentrate	1-30 on basis of Concentrate volume	2cc	12 days	Died 4 days later
242	#225	"	"	0.5cc	12 days	Died 3 days later

From this single experiment it appears that the 5% suspension contains less virus per unit volume than does the concentrate. The probable explanation for this fact is that the virus is considerably water soluble in itself or because of its attachment to soluble proteins, being therefore removed from the suspension in a large measure. This point concerning the water solubility of the virus aside from possible protein attachments is under investigation at the present time.

THE USE OF THE CONCENTRATION METHOD IN DETERMINING THE INFECTIVITY OF THE FECES AFTER FEEDING MONKEYS POLIOMYELITIS

Virus: Three monkeys, 219,221, and 230 were given large weekly doses (20-25cc) of a 5% Poliomyelitic brain suspension by stomach tube. Although they received 405 cc of the virus apiece none of the three monkeys developed poliomyelitis.

The feces of all three monkeys were on one occassion collected for forty-eight hours after they had received 20 cc of the virus apiece. A 5% suspension was made of the feces (75 grams), and the suspension was shaken, centrifuged and filtered through Mandler filters. Five hundred cubic centimeters of the filtrate was concentrated to 10 cc. This concentrate was dialyzed free of NaCl, the volume of the dialysate being 30 cc.

Monkey number 239 was inoculated intracerebrally with $1\frac{1}{4}$ cc of the dialyzed concentrate. Following an incubation period of six days, the monkey developed symptoms of poliomyelitis with paralysis and prostration, dying two days after the onset of symptoms.

DISCUSSION

The filtrate of a suspension of poliomyelitic brain and cord in physiologic saline can be concentrated in vacuo to a small fraction of its volume with out apparently injuring the virus of poliomyelitis in spite of the fact that the resulting concentrate is a saturated NaCl solution. The use of distilled water or 1/20 physiologic saline, instead of physiologic saline, in preparing the brain and cord suspension, is not feasible since such suspensions will not centrifuge clear enough to give a supernatant which can be passed through a filter readily. The absence of sufficient electrolyte in the suspension is probably responsible. The result is that the filtrate made from the cloudy supernatant contains very little virus. The use of a physiologic saline suspension obviates the difficulty of centrifugation, a fairly clear supernatant being uniformly obtained, which when filtered contains a considerable quantity of the virus of poliomyelitis.

That the concentrate has an exceedingly high content of the virus is shown by the fact that a unit volume of the concentrate is more infective than a unit volume of the original 5% brain and cord suspension. This fact suggests two things. First that very little virus is lost by filtration through the Berkfelt and Mandler filters which were used. And secondly that the virus must in a very considerable degree be soluble in physiologic saline of itself or in conjunction with the soluble proteins.

The concentrated virus remains viable for a surprisingly long time in saturated salt solution at the end of two months it was as highly in-

fective as it had been originally. Tests at the end of four months have just been made, the results of which are not yet clear, but either the virus has been so attenuated as to produce mild symptoms after an incubation period of two days, or then it is destroyed completely. This point has yet to be determined.

The comparison between the viability of the concentrated virus and Rosenow's poliomyelitic streptococcus is interesting. A heavy suspension of Rosenow's streptococcus in a brain filtrate, which was subsequently concentrated to give a saturated NaCl solution, remained viable for one month, whereas the streptococcus in a plain saturated NaCl solution remained viable for a month and eight days. Too much importance should not be attached to the greater viability of the virus as compared with the streptococcus in a saturated salt solution. It indicates a smaller size for the virus rather than a necessary difference in character, since by virtue of smaller size the virus is conceivably protected to a greater extent by the proteins present in the solution.

The method of invacuo concentration was successfully used in demonstrating the passage of the virus through the alimentary tract of the monkey. Whether or not all of the virus remained unaffected could be determined only with a careful series of titrations involving the use of a considerable number of monkeys. Certainly a very great amount passes through undestroyed; since 30 cc of the prepared material, representing 20 cc of virus fed to the monkeys, was highly infective. This is quite remarkable since not all of the feces could be procured, and since only about 1/3 the total filtrate was used in preparing the concentrate.

A concentrate of this kind, representing a great deal of virus in a small volume, is admirably adapted for the further study of the proper-

ties of the virus. One line of investigation which has been under way is the fractionation of the virus along with the various protein elements of the concentrate. Significant results have been obtained which, however, must await confirmation. Another line of investigation has been conducted upon the use of the use of the concentrate in producing active immunity in monkeys. The series is not yet adequate for a report.

Thus for microscopic studies and cultivation of the concentrated virus have yielded only negative results. Several new approaches toward the possible cultivation are being made.

SUMMARY

(1) The virus of poliomyelitis can be concentrated without injury by the evaporation in vacuo of a filtrate of a 5% suspension of brain and cord in physiologic saline. The use of a distilled water or 1/20 physiologic saline suspension in making up a concentrated filtrate has not proven feasible.

(2) A given unit of the concentrate contains more virus than a given unit of the original 5% brain and cord suspension; how much more has not been ascertained.

(3) The concentrated virus retains a high degree of infectivity for at least two months, in spite of the fact that the concentrate is a saturated NaCl solution.

(4) At the end of four months the virus is either peculiarly attenuated, or more or less partially destroyed, it is not yet decided which.

(5) Rosenow's poliomelitic streptococci remain viable about one month in a similar NaCl saturated brain and cord concentrate.

(6) The virus of poliomyelitis has been recovered after it has been fed to monkeys and passed through their alimentary tract.

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