A NOTE ON THE FILTRATION OF THE VIRUS OF HERPETIC ENCEPHALITIS AND OF VACCINIA.

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I.

Although herpetic encephalitis has long been recognized as due to a filtrable virus, the actual filtration of the virus through the ordinary filters has been attended with a certain amount of difficulty. When the brain of a rabbit dying of herpetic encephalitis is emulsified in saline, the emulsion centrifuged and the supernatant fluid passed through a Berkefeld V filter, our own experience has been that the virus sometimes passes through the filter, and sometimes it does not. This doubt about the infectivity of the filtrate and the consequent necessity of working with the virus mixed with an unfiltered, turbid emulsion of brain tissue have been a hindrance to the study of this virus.

Knowing that the virus of herpetic encephalitis in a saline emulsion of brain tissue, especially when diluted, quickly becomes inactive when exposed to the air, the writers thought that if the filtration of the emulsion could be carried out anaerobically, and the filtrate protected from oxidation, more regularly infective filtrates would be obtained. This hope was not realized, and attention was then turned to the menstruum in which the emulsion of brain tissue was made. Bronfenbrenner (1) had pointed out that if particles carrying bacteriophage were deposited on a Berkefeld filter, the bacteriophage could not be washed through the filter with water, but did pass through if broth was used instead of water. Accordingly, the brain of a rabbit dying of herpetic encephalitis was emulsified in broth (5 gm. of brain in 100 cc. of hormone broth) and the emulsion centrifuged. The supernatant fluid was then filtered anaerobically through a Berkefeld V filter. An ordinary aerobic filtration was carried out at the same time. Both

filtrates were infective, the anaerobic filtrate, kept in an atmosphere of hydrogen, remaining infective for at least 8 days at room temperature. Exactly how long an anaerobic filtrate will remain active, if protected from oxidation, has not been ascertained, as attention was then turned to the conditions necessary for regularly obtaining an active aerobic filtrate from the brain of a rabbit dying of herpetic encephalitis.

Numerous experiments have now been carried out, and in our hands an active filtrate has invariably been obtained with the following technique:

1. The Preparation of the Broth Emulsion of Brain Tissue.—The whole brain of a rabbit which has died of herpetic encephalitis is removed within 24 hours of the rabbit's death. A rabbit showing typical symptoms can of course be killed and the brain removed at once, if it is not convenient to wait for the death of the animal. 5 gm. of the brain are then ground up carefully with sand in a mortar. After grinding for some minutes, hormone broth is added, gradually at first, and then in quantity, until 100 cc. of the broth have been added, the grinding being continued the whole time. This emulsion is then centrifuged for 30 minutes at 2000 revolutions per minute, and the supernatant fluid poured off. A few drops of a fresh culture of *B. prodigiosus* are added to the supernatant fluid, which is then ready for filtration.

2. The Preparation of the Filters.—Berkefeld V filters, after washing through successively with a 1 per cent solution of potassium permanganate, a 5 per cent solution of sodium bisulfite and about a liter of distilled water, are tested for their water flow at a constant negative pressure. The majority of these filters will allow 45 to 60 cc. of distilled water to flow through in 5 minutes at a negative pressure of 10 cm. of mercury. Any filters which differ markedly from these figures are not used. After testing, the filters are autoclaved. The filters are further checked during the actual filtration by the culture of *B. prodigiosus* which is added to the supernatant fluid of the centrifuged emulsion before filtering.

Berkefeld V filters were used throughout in these experiments, but the following filters were tried in one experiment—Berkefeld N, Chamberland L_2, Seitz E.K. and Mandler regular. Of these, only the Mandler filter allowed the virus to pass through. In another experiment, the virus passed through the Berkefeld N filter.

3. The Filtration.—This has been carried out at various negative pressures from 20 to 60 cm. of mercury, and the virus has passed through in all cases. Usually about 30 cc. of the centrifuged broth emulsion are filtered.

4. Testing of the Filtrate.—About 1 cc. of the filtrate is removed sterilely from the filter flask, transferred to a tube of blood broth and incubated for several days to test for the presence of *B. prodigiosus.* If present, growth often does not show before 48 hours, and occasionally even longer than that. We have found quite a number of Berkefeld V filters which allow this organism to pass through. 0.5

cc. of the filtrate is injected intracerebrally into a normal rabbit. If the filtrate contains the virus, the temperature rises on the 3rd or 4th day, and the animal dies from the 4th to the 7th day after injection, after showing the usual symptoms of encephalitis. No systematic experiments have been carried out to ascertain how long and under what conditions the filtrate remains active.

II.

The virus of vaccinia has been filtered by several workers, but it is generally agreed that filtration of this virus is attended with great difficulty. If the same technique which has been described for the filtration of the virus of herpetic encephalitis is applied to the fresh scrapings from the lesions of a vaccinated calf, the virus of vaccinia can be demonstrated in the filtrate by the usual method of scarifying the shaven skin of a normal rabbit. It is clear that there must be a great adsorption of virus by the filter, because the undiluted filtrate gives rise only to discrete papules instead of the confluent eruption caused by the unfiltered emulsion.

From both a practical and theoretical point of view, it is of interest to note that the filtrate is still active after 6 weeks in the ice chest, whether it is in an open tube or sealed with a layer of vaseline or in an open tube diluted with an equal amount of pure glycerol. During this period, no diminution in the activity of the filtrate could be detected.

It has been found that if the scrapings are kept on ice for 2 or 3 days before emulsifying in broth and filtering, the virus can no longer be demonstrated in the filtrate, although the unfiltered, centrifuged emulsion is still very active. Further, if the fresh scrapings are emulsified in 50 per cent glycerol broth (in a proportion of one part of scrapings to four parts of 50 per cent glycerol broth) and this emulsion kept on ice for some days before adding fifteen additional parts of broth (*i.e.* a final 5 per cent emulsion of the scrapings), the filtrate is again negative.

While these experiments were in progress, Mills, Shibley and Dochez (2) in America, and Garrod (3) in England have published papers on a method of obtaining filter-passing organisms from the nasopharynx, using broth instead of saline or phosphate buffer solution to obtain the nasal washings for filtration. The filtrates of the saline washings were almost uniformly negative, while organisms were regularly found in the filtrates of the broth washings.

DISCUSSION.

This note is published in the hope that it will help in the study of filtrable viruses in general. The difficulty in filtering certain of the filtrable viruses has been sometimes ascribed to the intracellular location of the virus, *e.g.* vaccinia, the filter removing the cells and therefore the infecting agent. It is clear from these experiments that the virus of vaccinia, even if it is ordinarily intracellular, can easily be separated from the cells without destroying its infectivity. Further, if careful centrifugation experiments were done on the filtrate, as Gordon (4) has done on the emulsion, it might be possible to throw some more light on the probable size of the infecting agent. Again, the question of whether or not there are complement-fixing and precipitating antibodies in neutralizing serum might be reinvestigated, with an antigen which is free of organisms and cells. From the practical point of view, the possibility is opened up of preparing vaccine for human vaccination quickly and easily, removing the contaminating organisms completely by filtration, instead of incompletely removing them by the long drawn out process of partial sterilization.

We have not been able so far to throw very much light on the size of the herpes virus by these filtration experiments. The fact that the virus invariably passed through a Berkefeld V filter and in the first experiment was stopped by a Berkefeld N filter was encouraging, but in a second experiment the virus passed through the N filter. Perhaps a second filtration of the clear Berkefeld V filtrate through graded filters might give a truer indication of the size of the virus.

SUMMARY.

The virus of herpetic encephalitis and the virus of vaccinia can be demonstrated in the filtrate, if a broth emulsion of fresh tissue containing the virus, is passed through a Berkefeld V filter.

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