THE RESISTANCE OF THE VACCINE VIRUS TO FILTRATION.

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Whether the vaccine virus is filterable or not has been the subject of experiment and discussion for many years and since Negri's results published in 1905, from which he deduced that the virus was filterable, numerous experiments dealing with the subject have been made by many workers.

The consensus of opinion appears to be that the vaccine virus is capable of passage through a filter such as a Berkefeld V, and although a few experimenters have failed to obtain positive results it is usual to include the vaccine organism in the list of filter passers. It is so included by Löffler (1913) and Lipschütz (1913), and Paschen (1911) states that the evidence for the filterability of the lymph organism is overwhelming.

The main difficulty throughout with lymph filtering experiments has been that in the absence of artificial culture of the virus, one has had to deal with the ordinary lymph "emulsion," a thick conglomeration of vesicular material, in which the specific organism is embedded. In attempting to use a filter to negotiate this slimy mass the chief difficulty, and a very important one, has been that, after the first few seconds of filtering under the necessary pressure, the bougie has been covered externally with a slimy coat which transforms the character of, say, a Berkefeld V candle to that of a much finer one; almost, perhaps, to the condition of a gelatine filter. The result has been that, while critics of positive filtration results have felt suspicious of the filters

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being intact, critics of negative filtration results have pointed to the fact that a slime-coated bougie is calculated to prevent the passage through it of any kind of micro-organism.

In order to overcome this difficulty the following technique was suggested to me by Professor C. J. Martin. The vesicles of calves, 96 hours after vaccination, were clamped, and portions of their clear fluid contents rapidly transferred, by means of a graduated Pasteur pipette, to a measured quantity of a solution of sodium citrate and distilled water. This expressed fluid consists microscopically of white blood cells and a few staphylococci. The presence of the vaccine virus in an active condition is demonstrated by the vaccination of a suitable animal with the fluid. By using this material for filtration not only was the slimy mass of epithelial cells in various stages of dissolution avoided, but clotting of the expressed lymph was obviated, and a clear fluid was obtained for experiment, which filtered with ease under moderate pressure, passing through the filter almost as readily as water.

0·25 c.c. vesicular exudate was admixed with 2 c.c. of 5 % sodium citrate solution. This small quantity can be dealt with easily for filtration if a sufficiently small filter be used. The smallest pattern Berkefeld V laboratory filter was obtained, and its metal mount and a portion of the adjacent part of the bougie were removed, leaving about 3 cm. of the distal part of the bougie intact. The hollow of the bougie was carefully reamered out so as to make it circular at the orifice and into this prepared hollow surface was fitted one end of a piece of circular glass tubing, which had been covered with thin rubber tubing. A perfect fitting joint was thus obtained, and a small filter prepared capable of introduction into an ordinary test-tube and of dealing adequately with the small quantity of fluid available for the experiment.

Using this technique, the lymphs of nine calves have been experimented with. Six separate samples were taken from each calf, making a total of 54 experiments.

The filtrates were inoculated on guinea-pigs, as also were portions of the unfiltered fluids to act as controls. Subsequently the inoculations were repeated on calves, being stored at 4° C. until opportunity for calf-inoculation arose. In these experiments, in which the filtration of calf vaccine has been reduced in simplicity to the filtration of water, in no single instance has any vesiculation or any visible trace of specific reaction resulted from the inoculation of any of the filtrates, while in every instance, without exception, the controls have set up definite and typical vesiculation.

Criticism may possibly be directed against the use of guinea-pigs as tests for calf vaccine activity. If guinea-pigs are vaccinated on the back or abdomen they are not very satisfactory as test animals. But if only buck pigs are used and these are not already immune and are vaccinated with active virus on the scrotum, typical and usually exceptionally fine vesicles result. The advantages of using these small animals are that one animal can be used exclusively for each experiment—a proceeding not usually possible with calves—and the tests can be carried out in a place isolated from general vaccination work, thus eliminating the chance of contamination with other sources of vaccination. These guinea-pig tests are in themselves so satisfactory that corroboration by calf experiments is scarcely necessary, but as mentioned above, the inoculations were repeated on calves. As was pointed out by more than one speaker at the morning session of the Bacteriological Section of the International Medical Congress in London, on August 11th, 1913, no positive results can be fully accepted when the tests have been carried out where routine work connected with the test virus is in progress; and still less is such a test to be relied on when made on animals, other areas of whose skin are inoculated with a virus of a like nature to the test virus.

To account for the failure on the part of some observers to filter the vaccine virus, Negri has suggested that the virus of vaccine lymph which has been stored will pass through a filter when the virus of recent lymph would not pass. Negri bases this view on the assumption that small filterable forms of the virus occur as the result of two or three weeks' storage. I therefore made a further series of 36 experiments with lymph prepared in the way described above, which had been stored for some weeks. Portions of the expressed lymph of six calves were pipetted into citrate solutions as before, but the lymph dilution in this series was 1 in 13. 36 samples from the six calves were then taken. These were stored at 4° C. for three weeks with the addition of 1 c.c. of pure glycerine to each sample of citrated lymph. At the end of three weeks' storage, the unfiltered control portions produced good vesiculation on guinea-pigs and upon calves, but in no instance was any trace of reaction seen after vaccination with the filtrates.

From the foregoing 90 experiments I am forced to conclude:

(1) That the specific virus of vaccinia, as contained in the clear lymph obtained from vesicles 96 hours after vaccination of the calf, is either of such a size that it is unable to pass through large filter pores such as a Berkefeld V filter possesses, or

(2) It is contained within some body which is of such a size that it is incapable of such passage, or

(3) It loses its virulence by passage through a Berkefeld V filter, which seems improbable.

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