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Filtration Experiments With Bacteriophage Employing a Physiological Filter-Pia, Dura and Arachnoid.

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Much has been written concerning filtration experiments with various types of artificial filters such as the Berkefeld, Chamberlain and collodion membranes. Recently a new type of filter has come upon the market, the Seitz-Werke filter, and for certain types of work is apparently quite efficient. The efficiency of all these types of artificial filters, as far as the mere removal of bacteria is concerned, is beyond question. It is only when investigators attempt to draw fine conclusions from filtration experiments employing such filters that their value is questioned. A substance, for example, which is filterable through a Berkefeld Type N or W filter in the hands of one investigator may not be filterable when presumably the same type of filter in every respect is employed by another. Such results are very confusing and often discouraging. This has been particularly true in the field of filterable viruses and indeed, there are many viruses so classified at the present time upon the sole basis that they have, in the hands of some workers, been able to pass through one of these artificial filters. In the first place it is well known that filters of this type, even when new and unused, though prepared by standard methods, still vary one from the other. No two are precisely the same. Again the amount of pressure employed by different workers against these filters is never the same and further in many instances the filter is not controlled. The time element of filtration is known to be important and there are always the variations in individual technique which add to the general unreliability of results. These criticisms are well known to all laboratory workers and as a result we have come to regard filtrability through such filters in the same sense as diffusibility, as only a relative concept.

Upon the basis of such methods, then, we have come to classify what we regard as the etiological agents of a host of diseases affecting man, animals, insects and plants. The term "filterable" has become general in use and based purely upon the methods employed, is becoming more and more burdensome. The great temptation at present is to consider all those diseases in which the causative agent

is unknown as disease due to a "filterable virus." Taken together with our general lack of definite information regarding the nature of these agents, we appear to have adopted a term, perhaps because of its convenience largely, which is exceedingly indefinite.

In the present communication experiments are described concerning the filtration of bacteriophage from the blood stream to the spinal fluid and from the spinal fluid to the blood stream in rabbits. Conceiving of the pia, dura and arachnoid as a physiological filter system one should be able here to think of the filter as representing uniform conditions as near as is possible.

It is well known that physiological saline solution is absorbed rapidly when injected into the sub-arachnoid space even in large quantities. In several diseases therapeutic serum is administered by this route with beneficial results. The substances introduced into the sub-arachnoid space have been limited largely to these two as untoward results have followed when certain dyes and drugs have been administered this way.¹ It is also well known that the amount of therapeutic immune serum, drugs (such as salvarsan) and certain dyestuffs, reaching the spinal fluid when injected intravenously, is comparatively small, if any at all. According to the physiologists the cerebrospinal fluid may be considered a dialysate from the blood or an ultra-filtrate. Its specific gravity ranges from 1.007 to 1.008 while that of blood varies from 1.041 to 1.075. When freshly drawn the pH of cerebrospinal fluid is 7.4 which is approximately that of fresh blood. Both blood and spinal fluid become more alkaline on standing. According to some authors the cerebrospinal fluid is quite similar to blood in many other respects. It contains from 17 to 26 mg. of non-protein nitrogen, 7 to 13.5 mg. of urea nitrogen, .07 to 0.1 per cent of dextrose, and .02 per cent of protein, all based on 100 cc. The trace of protein is globulin in nature, it contains no albumin or fibrinogen but there are traces of other organic substances to be found in it. Hewett⁴ in a recent paper concludes that in normal cerebrospinal fluid there is about .02 per cent albumin and about .003 per cent globulin, of which by far the greater part is pseudoglobulin. While it appears fundamentally that most of the constituents of the cerebrospinal fluid come from the blood stream which carries nutrition to the tissues of the body, it is evident that some of the blood elements are unable to pass through the filtration apparatus, notably the protein aggregates. At least they do not appear there in any great quantities and some of them not at all (albumin and fibrinogen). The explanation for this phenomenon might explain why drugs, certain dyes and immune substances fail to pass since it

is possible they may become compounded with blood proteins when injected intravenously. Flateau³ has recently been able to pass fuchsin from the blood stream of rabbits to the spinal fluid by first injecting various substances, such as atropine or glucose solution intravenously or intraspinaly. Whether the actual permeability of the membranes is altered in this way is still questionable, however. Le Fèvre⁵ has shown that certain dyes, such as methylene blue, trypan blue, neutral red and others exert a favorable influence upon the fixation by the central nervous system of certain neurotropic viruses such as the virus of herpes and of rabies, presumably by acting on the vascular endothelium of the meninges.

Intraspinal Injection of Bacteriophage.

3 cc. Coli "D" bacteriophage in broth pH 7.6 were injected intraspinaly into a rabbit. Two hours and 24 hours later the blood, feces, urine and spinal fluid were tested for the presence of bacteriophage. The bacteriophage was active in a 1:10,000,000 dilution.² The first concentrated serum cerebro-spinal fluid and urine were tested for bacteriophage by the test tube method and were found to be negative. This does not prove conclusively that the bacteriophage is absent, since we have shown elsewhere that due to the particulate nature of the bacteriophage in great dilutions that the test tube method is unreliable as a test for its presence or absence.² Other fractions of the serum, cerebro-spinal fluid, urine and feces were added to broth and incubated with the susceptible Coli "D" culture and repeated sub-passages were then tested.

TABLE I.

Material Tested	1/23	1/24	1/25	1/26	1/27	1/28	1/29
Conc. serum	—	*	*	*	*	*	*
Passage serum	*	—	±	+	+	+	+
Passage feces	*	—	—	—	—	—	—
Conc. urine	—	—	•	*	*	•	*
Passage urine	*	—	—	—	—	—	—
Conc. cerebro-spinal fluid	—	*	*	*	*	*	*
Cerebro-spinal passage fluid	*	—	±	+	+	+	+
Control bacteriophage	+	+	+	+	+	+	+

* Not determined.

It will be noted in Table I that the serum from blood removed two hours after intraspinal injection of bacteriophage became questionably positive after the second passage with the sensitive strain while it was definitely positive after the third passage. The

same was true for the cerebro-spinal fluid. The feces or urine never became positive, even after five passages with the sensitive strain. The control was, of course, positive throughout the experiment. This experiment serves to demonstrate that a substance such as bacteriophage which can be detected in high dilutions is capable of passing from the cerebro-spinal fluid to the blood stream. This we might expect since the normal direction of flow is in this direction.

In a second experiment the blood serum, cerebro-spinal fluid, urine and feces of a normal rabbit were first tested for bacteriophage against the sensitive B. Coli "D". They were found to be negative. The animal was then given 5 cc. intravenously of B. Coli "D" bacteriophage active in a dilution of 1:10,000,000. The bacteriophage could not be demonstrated in the spinal fluid, feces or urine after seven passages with the sensitive strain though it was present in the blood serum in the third passage. The control bacteriophage remained positive during each passage.

These experiments indicate that a particular substance such as the bacteriophage is filterable from the cerebro-spinal fluid to the blood stream in rabbits but not from the blood stream to the cerebrospinal fluid. Our failure to detect the bacteriophage in the urine and feces is not conclusive evidence that it is not excreted through both of these channels, since the factor of dilution in both instances is quite different from that of the blood and cerebrospinal fluid.

¹ McKinley, E. B., and Holden, M., *J. Am. Med. Assn.*, in press.

² McKinley, E. B., and Holden, M., *J. Infect. Dis.*, 1911, xxxix, 451-456.

³ Flateau, E., *Revue Neurologique*, 1911, xxxiii, 281-360.

⁴ Hewett, L. F., *Brit. J. Exp. Path.*, viii, 1, 84-92.

⁵ Le Fèvre, M. de A., *Comp. rend. de la Soc. de Biol.*, t, xevi, 206-207.

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Liver Injury in Acute Alcoholic Poisoning.

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Although in most text books of Pharmacology and Medicine the statement is made that alcohol has a directly injurious effect on the liver, recently much doubt has been expressed as to an etiological relationship between alcohol and liver disease. An opportunity of