TABLE I.
Dog P-1. Second adrenal removed Jan. 17, 1931. Assay of Feb. 1 extract lot.

Date 1931	Weight kg.	NPN blood mg.%	Dose ext. cc. per kg. per day	Clinical condition.
Feb. 4	6.4	46	0.25	Lively. Eats well.
15	6.3	36		
20	5.9	60	0.16	Lively. Eats poorly on 2/18 and 2/19.
24	5.5	92	0.12	Symptoms of severe insufficiency.
25	5.3	44	2.0	Recovering. Food fairly well taken.
26	5.8	38	2.0	Lively. Eating well.

A dog unit is defined as the minimum daily kilogram dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days; the two criteria of normal physiological condition being maintenance of body weight and blood level of non-protein-nitrogen (or urea). According to this definition the hormone content of different batches of our stock cortical extract varies from 4 to 10 dog units per cc.

Recently Kutz[2] has suggested the use of the adrenalectomized rat as a test animal since adrenalectomy is uniformly fatal in young rats of his colony. Obviously the use of a small animal form would have very definite advantages in standardization work, but the findings of various workers on the percentage mortality in the rat following adrenalectomy are so confusing that for the time being we prefer to use the dog. All workers are agreed that the occurrence of accessory tissue in the dog is exceedingly rare.

5925

Experiments on the Filtrability of the Rosenow Streptococcus of Anterior Poliomyelitis.

RUTH D. RICHARDSON AND R. R. MELLON.

From the Institute of Pathology, Western Pennsylvania Hospital, Pittsburgh.

This paper deals with the successful filtration through Berkefeld candles of 2 strains of the Rosenow anterior poliomyelitis streptococcus. These strains were designated 1630.30 and 1694.50. 0.5 cc. of a 20-hour brain infusion broth culture of 1630.30 was inoculated intracerebrally into each of 3 rabbits. Two of these animals died in less than 24 hours, and the third in 36 hours. Autopsies showed

[2] Kutz, R. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 91.

typical cerebral meningitis. From cultures of the brain, meninges, and cerebral exudate, the original streptococcus was recovered in each case. Heart blood cultures were negative.

The brains of these 3 animals were emulsified in broth and 0.5 cc. reinoculated intracerebrally into fresh rabbits. Death again resulted in approximately 18 hours, with recovery of the streptococcus. Animals inoculated with 0.5 cc. of the Berkefeld N filtrate of these emulsions suffered no ill effects. By intensive cultivation of these 3 brain emulsion filtrates using the Hauduroy transplant method on blood agar plates, we were able to recover from 2 of them an organism resembling both microscopically and culturally the 1630.30 streptococcus. In all cases it appeared on the 4th to 6th plate in the series.

Identically this same procedure was carried out using the 1694?50 strain Three rabbits were inoculated intracerebrally. All showed meningitis symptoms but only one died (48 hours). The streptococcus was recovered from brain cultures of this animal, but not from the heart blood. An organism believed to be 1694?50 was also recovered from the brain filtrate in the same way as for the 1630.30 strain.

Then each of the 4 strains was treated in the following manner: 20 cc. of a 24-hour brain heart infusion broth culture was injected intraperitoneally into a small half-grown rabbit and 50 cc. into a large rabbit. Eighteen hours later 50 cc. of broth was injected by the same route and after thorough manipulation of the abdomen the animals were killed and the peritoneal exudate removed aseptically and made up to a volume of 35 cc. with sterile broth. These lysates were then filtered through Berkefeld N candles and the filtrates cultivated on blood agar plates by the Hauduroy transplant method. On the 3rd to 6th plate, in every case, a streptococcus was recovered. This growth appeared at first as a very minute colony, not affecting blood, and closely resembling a pleomorphic diphtheroid upon microscopic examination. Further cultivation in each case gradually developed a streptococcus giving a faint greening action on blood and resembling the Rosenow strains in every way. Agglutination tests have not been carried out between these 2 strains. We do not consider it necessary that the streptococcus recovered from the filtrates be exhaustively tested for identity with the original Rosenow streptococcus. Very commonly filtrates do not yield immediately the identical organism and the crucial point here is not this, but whether the filtrable or virus form, so-called, is identical with the virus of polio.

At the same time that the animal inoculations were being performed a Berkefeld N filtration was made of a 24-hour broth (brain-heart infusion) culture of each of the strains as received from Rosenow. Prolonged plate to plate cultivation of these filtrates gave no growth. Each of the filtrates was then divided into 2 parts and sealed in ampoules. One part was incubated at room temperature and the other at 37°C. Five months later a slight sediment was noted in the filtrate of the 1630.30 strain which had been kept at 37°C. A slight opalescence was also noted in the 1694?50 filtrate kept at the same temperature. Plates made from these ampoules at this time gave minute colonies—the 1630.30 filtrate grew on the first plate, while the 1694?50 required from 2 to 3 serial transplants. These colonies likewise proved to be indifferent pleomorphic diphtheroids, but upon 3 days' growth in whole blood regained their streptococcus characteristics, and we believe them to be identical with the original strains filtered.

The filtrate samples kept at laboratory temperature have failed to give any growth with even the most careful and prolonged cultivation. As a result of these experiments we feel justified in concluding that these 2 strains of streptococci recovered by Rosenow from cases of anterior poliomyelitis in human beings are, at certain phases of their growth, filtrable through Berkefeld N candles.

Through the courtesy of Dr. C. W. Jungeblut of the Department of Bacteriology of the College of Physicians and Surgeons the filtrates from these 2 cultures were injected intracerebrally into monkeys with entirely negative results after a month's incubation. This would seem rather definite evidence that these filtrable forms are not identical with the virus of poliomyelitis. In fact, they probably have no cyclic relation to it, a view made conceivable by the discovery of filtrable forms of bacteria.

Nevertheless, the presence of these organisms in the disease, with which they react specifically in serologic and immunologic ways, still remains to be explained. One of us (Mellon) wishes to postulate a new point of view, which is a compromise between the 2 extreme views now held, viz, that the streptococcus is a pure secondary invader having no relation at all with the disease, and on the other hand the view that the streptococcus is but the visible stage of the causative virus.

My suggestion is that as a result of the altered conditions brought about in the host by the virus there is dissociated *in vivo* a form of streptococcus which reacts specifically with the host. I am led to make this suggestion partly as the result of a similar situation that

has recently been shown to prevail in typhus fever and in small-pox. Fisher of this laboratory, in a study still unpublished, has shown that in hemorrhagic small-pox there is a hemolytic streptococcus in the blood with characters as specific for the disease as is the *Streptococcus scarlatinae* for scarlet fever.

Still more striking in this connection is the situation with Proteus X-19 in its relation to typhus fever. Silber[1] has shown that *B. proteus vulgaris,* which does not react serologically with the serum of typhus cases, can be made to do so as the result of its *in vivo dissociation* in collodion sacs in the abdominal cavities of typhus infected guinea pigs.

As knowledge of the biologic nature of the filtrable phases of bacteria increases it becomes clearer that their designation as viruses —for example, the tuberculosis virus—is apt to be misleading. On the other hand, the validity of these filtrable phases of bacteria is not in the slightest doubt, regardless of the specious explanations that have been raised regarding leaky filters, etc.

5926

Mass Cultures of Streptococcus Hemolyticus in Broth.

J. HOWARD MUELLER AND KATHARINE S. KLISE.

From the Department of Bacteriology and Immunology, Harvard University Medical School.

One of us[1] found that a small amount of glucose appeared to be essential in media to be used for the cultivation of hemolytic streptococci and pneumococci. It seems likely that by a splitting of this substance to lactic acid, these organisms are able to derive energy for their growth. The amount of growth was roughly proportional to the percent of sugar, up to the point where sufficient acid was produced to check the growth of the organisms. This use of a phosphate buffered broth to delay the culture in reaching a toxic pH, thus allowing the utilization of more glucose and the development of heavier growth, is now very common. However, even a heavily buffered medium will yield not more than 2 or 3 times the volume of bacteria that can be obtained from the ordinary unbuffered infusion broth.

---

[1] Silber, L., *Z. f. Hyg. and Infekt.*, 1928, **108**, 146.

[1] Mueller, J. H., *J. Bact.*, 1922, **8**, 309.