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# Penetration of Antiserum into the Central Nervous System of Monkeys Infected with Poliomyelitis.*

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In the treatment of poliomyelitis, both convalescent and normal serum have been used, despite the fact that the virucidal property of such serum seldom exceeds a titer of 1:30. Since the virus in poliomyelitis is present and spreads within the CNS, antiserum to be effective must be able to penetrate readily into this area.

It is generally recognized that a physiological barrier prevents the entrance of blood proteins into the CNS tissues. Lennette and Campbell[1] have recently shown that the permeability of the blood brain barrier to the bromide ion was increased in experimental poliomyelitis in monkeys. They suggest the possible significance of such increased permeability on serum treatment of the disease. It seemed desirable to examine directly the permeability of the blood brain barrier to serum. Fox[2] has employed hemolysin to investigate the pulmonary permeability in normal animals, and in animals with sterile inflammation of the lungs.

With this work in view, monkeys previously inoculated intracranially with 0.5 to 1.0 cc of a 10% saline suspension of cord infected with poliomyelitis virus, Aycock strain, were injected after paralysis had been noted, with 10 cc of rabbit hemolysin serum against sheep red blood cells, having a titer of 1:40,000. After a definite time interval had elapsed (Table I), the animals were sacrificed by bleeding from the carotid artery, and in some instances, the residual blood was removed by perfusion with physiological saline solution. Spinal puncture was then performed, and specimens of spinal fluid withdrawn. Subsequently, various portions of the brain and spinal cord were removed, and approximately 0.5 g samples mixed with 3 cc of physiological saline solution and ground with glass beads in a shaking machine. The resulting suspensions were centrifuged and the supernatant fluid saved.

Serial dilutions were made of each of the tissue extracts, and also of the monkey's serum, spinal fluid, and the last sample of per-

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1 Lennette, E. H., and Campbell, D. H., *Am. J. Dis. Child.*, 1938, **56**, 756.

2 Fox, J. P., *J. Immunol.*, 1936, **31**, 7.

TABLE I.
Penetration of Hemolysin into Tissues of Monkey with Poliomyelitis.

| Monkey | Stage of disease when hemolysin was given | Hrs after injection before sacrificing | Titer of hemolysin in blood | Titer of hemolysin expressed as percent of that in corresponding blood sample* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Perfusion fluid | Spinal fluid | Lumbar cord | Cervical cord | Medulla | Site of inoculation of lesion | Lobe opposite inoculation site |
| 1 | Arm paresis, leg paresis | 9 | 1:2560 | 12 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 |
| 2 | Arms and right facial paralysis; paresis both legs | 11.5 | 1:5120 | 12 | 0.2 | 1.7 | 0.8 | 3.4 | 1.7 | 1.7 |
| 3 | Generalized weakness | 24 (2 inj.) | 1:2560 | † | 0.7 | 6.0 | 3.0 | 3.0 | 6.0 | 6.0 |
| 4 | Quadriplegia | 19 | 1:1280 | † | 13.8 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| 5 | Paresis both legs | 38 (2 inj.) | 1:2560 | 0.4 | 1.5 | 3.9 | 3.9 | 3.9 | 3.9 | 3.9 |

*If hemolysin were detected in no dilution of the tissue extract, the highest dilution found to be anticomplementary in the control test was assumed to contain hemolysin.

†Not done.

fusion fluid collected. These were tested for the presence of hemolysin in the usual manner, by the addition of normal guinea pig complement, 1% suspension of sheep red blood cells, followed by incubation in a waterbath at 37°C for one hour. As a control on the anticomplementary action of the tissue extracts, tests were carried out with similar dilutions to which a small amount of hemolysin (0.001 cc) had been added.

It was found that the titer of hemolysin in the blood was consistently high (Table I). Only a small percentage of this amount could be detected in the spinal fluid, although in the animal in which the longest time interval elapsed before sacrificing, there was a titer of 10% of that of the corresponding blood specimen. Considering only the lowest dilution shown not to be anticomplementary, it was found that less than 6% of the circulating hemolysin was present in any part of the CNS examined. In those instances where the tissue extracts gave a high titer of hemolysin the animals had not been perfused, and the values indicated probable contamination with blood.

Olitsky and Harford[3] found that when the virus of Eastern equine encephalomyelitis was injected intranasally or intracranially in guinea pigs, antiserum given intraperitoneally was without effect. However, if the virus were not localized in the CNS, but introduced intraperitoneally, similar amounts of serum protected against the disease.

On the basis of the aforementioned findings an attempt was made to account for the ineffectiveness of serum therapy in poliomyelitis. If we assume that 100 cc of normal or convalescent serum is injected intravenously into a 20 kilogram child with a blood volume of approximately 2000 cc, the concentration of antiserum in the blood becomes one-twentieth of the amount injected. Since the concentration in the spinal cord is less than 6% of that in the blood, it should be 6/100 of 1/20; or a dilution of 1 :333 of the original antiserum. If the titer of the latter is 1:30, the amount in the spinal cord is only one-tenth of an adequate concentration.

Although it might be questioned whether it is the serum volume alone rather than the total volume of blood which dilutes the antiserum, we have found that calculations based on total blood volume agree with the resulting titer in the blood serum when hemolysin was injected in the monkey.

Since the potency of antiserum available for administration in poliomyelitis is low and since the amount administered to patients (seldom over 100 cc) is relatively 10 times less than that used in these experiments, it appears on a theoretical basis that antiserum

[3] Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 761.

administered intravenously would be without virucidal effect in the CNS.

These studies are being continued to include the effect of antiserum administered intrathecally.

*Summary.* Studies on the penetration of antiserum into the tissues of monkeys infected with poliomyelitis have shown that only a very small percent of circulating antibody is found in the spinal cord, thus suggesting little possible value in the use of antiserum in preventing the spread of poliomyelitis if such occurs other than by the blood stream.

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## Effects of Insulin Treatment on the Cerebrospinal Fluids of Schizophrenics.

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Changes of the non-electrolyte/electrolyte ratio (INE/IE) in cerebrospinal fluids were observed in cases of spontaneous convulsions[1] and in schizophrenics in which convulsions had been induced by metrazol treatment.[2] A marked parallelism was found between the values of INE/IE and the intensity of the convulsions. Evidence has been given that the increases of the ratio are due to an increase of the non-electrolytes (cleavage products of proteins and lipoids) rather than to a decrease of the electrolytes. The increase in cleavage products after convulsions was explained as due, at least partly, to changes of the cellular and vascular permeability.

In order to test some of these conclusions it seemed of interest to extend these investigations to the cerebrospinal fluids of schizophrenics treated with insulin. Although insulin has in such cases therapeutic effects similar to the ones of metrazol, the occurrence of convulsions is not paramount.[3] Moreover, observations have been reported indicating differences in the effects of the two methods of

1 Spiegel-Adolf, M., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 92.

2 Spiegel-Adolf, M., and Freed, H., 94th Meet. Am. Psych. Assn., San Francisco, June, 1938. Will appear in *Confinia Neurol.*, 1939, **2**.

3 Cobb, S., *Arch. Int. Med.*, 1937, **60**, 1098.