

of cholesterol flocculated by our method is proportional to the amount of antibody used. For instance, 10 cc. of a serum diluted 1:10 flocculated 11.4 mg. out of a sol containing 158 mg. % cholesterol, whereas the dilution 1:20 flocculated 5.5 mg., and the dilution 1:40, 2.6 mg. under the same conditions.

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Tests for the Blood-C. N. S. Barrier in Experimental Poliomyelitis.*

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It is well known that the mechanism regulating exchange between the blood and the central nervous system and spinal fluid is disturbed in all types of meningitis, allowing both normal blood constituents and foreign substances to pass into the spinal fluid (reviewed by Katzenellenbogen¹). While a "meningeal stage" has been described in poliomyelitis, the origin of the cells in the subarachnoid spaces appears to be from the perivascular spaces of the medullary substance. This communication will give the results of several tests of the blood central nervous system barrier in experimental poliomyelitis. Such findings have a bearing on specific treatment, since a gross barrier defect would allow free passage of neutralizing substances from blood into the medullary substance and spinal fluid.

Flexner and Amoss² while showing that the virus did not regularly pass from the blood to the nervous system, suggested that a defect in the barrier might be of importance in the natural pathogenesis of this disease in man. The failure to find virus-neutralizing substances in the spinal fluid of man during convalescence,³ although these substances are present in the blood of a large number of human convalescents even during and prior to paralysis,⁴ could be explained

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¹ Katzenellenbogen, S., *The Cerebrospinal Fluid and Its Relation to the Blood*, Baltimore, The Johns Hopkins Press, 1935.

² Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, **25**, 525.

³ Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, **25**, 499.

⁴ Harmon, P. H., Harkins, H. N., Fahey, J. J., and Wasbotten, P. M., *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 585.

by an intact barrier. In testing the barrier in experimental poliomyelitis with several types of antibodies, Shaughnessy, Grubb and Harmon⁵ showed that none of these substances, except diphtheria antitoxin, passed from the blood into either the spinal cord or the spinal fluid. In the clinic, Flatau⁶ found the barrier impermeable to acid fuchsin. The value of this latter observation is not great as the stage of the disease was not stated.

We have utilized 4 materials in tests of barrier permeability: 2 diffusible substances, acid fuchsin^{6, 7} and nitrate⁸ ion and two colloidal dyes, Congo red⁹ and trypan blue.¹⁰ Tests have been done by each method in both the preparalytic and the paralytic stage. The dyes were administered intravenously, acid fuchsin as 5 cc. of 5% solution, Congo red as 10 cc. of 1% solution and trypan blue as 5 to 8 cc. of 1% solution. Sodium nitrate was given subcutaneously as 2 cc. of 5% solution. Cistern punctures were performed respectively after 1½ hours, (fuchsin) 8 to 14 hours (Congo red) 8 to 16 hours (trypan blue) and 4½ to 5 hours (sodium nitrate). Fuchsin color was intensified by acid alcohol,^{6, 7} sodium nitrate was estimated upon a rough quantitative basis by diphenylamine,⁸ and Congo red was brought out as blue by dilute acid, while trypan blue was identified by its color alone. In each instance unused monkeys (*Macaca mulatta*) were tested as normal controls while similar animals to which normal horse serum had been given by way of the cisterna magna to produce an aseptic meningitis served as a second series of controls. After the dyes were given, examination of the blood demonstrated an intense coloration of the blood serum at the time of the cistern puncture. Before the test was again applied in the subsequent paralytic stage, a preliminary cistern puncture was performed to show that no dye had penetrated into the spinal fluid in the interim. If gross or microscopic blood was observed in any fluid, that specimen was discarded. Monkeys of the rhesus variety (*Macaca mulatta*) were used throughout. In several cases the animals were sacrificed and coloration of the gray matter noted.

A summary of these data is given in Table I. In no instance was

⁵ Shaughnessy, H. J., Grubb, T. C., and Harmon, P. H., Proc. Thirty-eighth An. Meeting Soc. Am. Bact., Indianapolis, Dec. 28-30, 1936, *J. Bact.*, 1937, Preprint, **32**, 47.

⁶ Flatau, E., *Rév. Neurol.*, 1926, **1**, 5.

⁷ Nador-Nikitis, I., *Zentralbl. f. Haut. u. Geschlechtskr.*, 1926, **20**, 478.

⁸ Mestrezat, W., *Le liquide céphalo-rachidien normal et pathologique*, A. Maloine, Paris, 1912.

⁹ Stern, L., Kassil, G. N., and Lockschina, E. S., *Compt. rend. Soc. de biol.*, 1928, **99**, 448.

¹⁰ Doinikow, B., *Folia Neurobiol.*, 1913, **7**, 731.

TABLE I.
Summary of Tests of the Blood-CNS Barrier in Experimental Poliomyelitis.

Substance Used as Test of Barrier	Poliomyelitic Monkeys				Normal Controls		Controls with Sterile Horse Serum Meningitis	
	Infected with		Total No.	No. with Test Substance in Spinal Fluid	Total No.	No. with Test Substance in Spinal Fluid	Total No.	No. with Test Substance in Spinal Fluid
	Virus from Man	PMV Strain						
Acid Fuchsin	5	1	6	0	6	0	2	2
Congo Red	3	6	9	0	5	0	7	6
Trypan Blue	1	5	6	0	4	0	6	0
Sodium Nitrate	6	2	8	3*	4	0*	3	2*

*Signifies concentration of sodium nitrate greater than 10 mg. per L. Three poliomyelitic monkeys showed a concentration of sodium nitrate in the spinal fluid of 20 mg. per L., while 2 of 3 animals having sterile horse serum meningitis had a concentration of 30 and 40 mg. per L., respectively.

dye found in the cerebro-spinal fluid in either the preparalytic or paralytic stage of acute poliomyelitis or in normal animals, whereas passage was noted in several instances in animals having sterile meningitis. Congo red, acid fuchsin and nitrate ion passed more readily than trypan blue. Indeed, passage into the cerebrospinal fluid by nitrate ion was demonstrated late in the paralytic stage. Examination of spinal cords at the intervals stated previously revealed coloration of the gray matter in preparalytic and paralytic poliomyelitis. This coloration was most intense with Congo red and acid fuchsin. No coloration was observed in normal monkeys.

Discussion and Conclusions. These tests demonstrate that the poliomyelitic monkey does not differ profoundly from the normal animal in regard to permeability of the blood-CNS barrier to colloids introduced into the blood. Most important of all, the increased permeability of the vessels of the meninges and choroid plexus seen in meningitis is not observed in the former disease. The coloration of the gray matter demonstrates an increased permeability of the small blood vessels of this region in poliomyelitis as contrasted to the normal animal. Indeed, Faber,¹¹ who recently has visualized by vital coloration, with trypan blue, the early lesions of experimental poliomyelitis induced by nasal inoculation of virus has been able to trace progressive vascularity through higher to lower levels of the central nervous system. This author concludes that increased vascularity precedes cellular infiltration in the reaction of the nervous system to this virus.

This data from experimental poliomyelitis suggests the application of these and other tests, that have already been standardized, for use in man to aid in clinical differentiation of encephalitis and poliomyelitis on the one hand from tuberculous meningitis and lymphocytic choriomeningitis on the other.

¹¹ Faber, E. K., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 10.

ERRATA

Article in the March, 1937, number, by C. R. Schmidt and A. C. Ivy, page 91, table 2, should read

$$\frac{\text{Common Duct Diameter}}{\text{Body Weight}}$$

Effect of Freezing on Transmitting Agent of Neoplasms, by C. Breedis, W. A. Barnes and J. Furth, the abscissa, page 223, should read seconds, not minutes.

Article 9192, page 259, should read, "Studies in the Physiology of Blood," etc.