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Nitrate and Bromide Tests for Blood-CNS Barrier Permeability in Experimental Poliomyelitis.*

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(Introduced by G. M. Dack.)

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Changes in the permeability of the blood-CNS barrier are known to occur in a number of pathologic conditions involving the central nervous system.¹ Of the many tests devised for measuring such changes in permeability, that of Mestrezat and Gaujoux,² based on the passage of nitrate ions through the barrier into the spinal fluid, is one of the simplest. This test, which is roughly quantitative, proved in our hands to be so crude that the results in any single instance were difficult to interpret; however, by employing a large series of monkeys we³ have been able to demonstrate that an increased barrier permeability to sodium nitrate occurs in experimental poliomyelitis.

Because the crudeness and unreliability of the nitrate test far outweighed any advantages accruing from the simplicity of the analytical procedure, we⁴ later replaced this method with the Walter bromide test,⁵ adapted by us to meet the needs of experimental work on monkeys. This test was found to be much more accurate than the nitrate method; in fact, it was usually possible for the analyst, who had no previous knowledge of the clinical status of the animals tested, to report whether or not the animals were infected, something which could only occasionally be done with the nitrate test. For this reason we thought it desirable to compare the two tests, barrier permeability being tested on the same animal by both methods simultaneously.

The comparative tests were done on 54 *rhesus* monkeys (*Macaca mulatta*), 33 of which were in the acute stages of poliomyelitis. The control series consisted of 10 normal, healthy monkeys and 11 animals which had been injected intracerebrally with 1.0 cc of normal

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

² Quoted by Loberg, K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1926, **106**, 164.

³ Lennette, E. H., and Reames, H. R., *J. Immunol.*, 1938, **34**, 215.

⁴ Lennette, E. H., and Campbell, D. H., *Science*, 1937, **86**, 160.

⁵ Walter, F. K., *Z. f. d. ges. Neurol. u. Psychiat.*, 1925, **95**, 522.

monkey spinal fluid, 1.0% normal monkey cord suspension or 2.0% starch to determine the effect of trauma on the permeability of the barrier.

Sodium bromide was administered subcutaneously twice daily for 2 consecutive days, 45 mg per kg of body weight being given. On the 3rd day 35 mg per kg of sodium nitrate was injected intravenously. One hour later spinal fluid was withdrawn by cisternal tap, blood being obtained at the same time by cardiac puncture.† The nitrate content of a portion of the spinal fluid was determined by a method described elsewhere;⁶ the remaining spinal fluid and the blood serum were analyzed for bromide by a modification⁷ of the original Walter technic.

In Table I are summarized the results; the upper figures represent the mean of the determinations made, the lower figures the extremes of the range over which the determinations varied. The table shows that poliomyelitic monkeys tended to have a larger amount of nitrate in the spinal fluid than did the control animals; the extent of overlapping, however, was so great that unequivocal results were obtained only when barrier permeability was so marked that relatively large amounts of nitrate appeared in the spinal fluid. Essentially

TABLE I.
Comparison of the Nitrate and Bromide Tests for Measuring Blood-CNS Barrier Permeability in Experimental Poliomyelitis.

History of monkeys	No. of monkeys	Conc.* NaNO ₃ in spinal fluid	Conc.† of NaBr in		Bromide P.Q.
			Spinal fluid	Serum	
Normals	10	27.0	24.2	38.4	1.58
		20-30	20.2-30.8	28.9-43.0	1.34-1.88
Trauma controls	11	29.1	30.0	36.6	1.22
		20-40	22.2-33.2	29.3-41.7	1.15-1.33
Preparalytic	17	31.8	33.8	33.8	1.00
		20-50	25.6-46.6	27.1-47.1	.91-1.02
Paralytic	16	40.0	35.2	35.1	.99
		30-70	26.9-41.9	28.6-42.3	.94-1.02

* Expressed in mg per liter.

† Expressed as mg %.

† Both procedures were done under deep ether anesthesia.

⁶ Lennette, E. H., and Reames, H. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 769.

⁷ Lennette, E. H., and Campbell, D. H., *Am. J. Dis. Child.*, 1938, **56**, 756.

the same was found to hold true with bromide when only the spinal fluid concentration was considered; when the degree of permeability, however, was expressed as a ratio (serum bromide/spinal fluid bromide), usually referred to as the permeability quotient or P.Q., the difference in barrier permeability between the various groups at once became apparent. It is probable that similar differences might be shown with the nitrate test were it more precise and were the method applicable to serum analyses.

It appears, then, that even exact determinations of the spinal fluid concentration of any substance used to test barrier function may give little information as to the degree of altered permeability present, except when permeability is so greatly increased that unduly large amounts of the test agent are permitted to pass the barrier. A more precise evaluation of barrier function can be achieved by determining the concentration of the test agent in both blood and cerebrospinal fluid, and expressing the degree of permeability as a quotient (P.Q.).

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Change in Amino Acid Oxidation Following Dissociation of *Staphylococcus aureus*.

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Using the Methylene Blue technic, Bach¹ has reported that in the presence of *Staphylococcus aureus*, the amino acids with the exception of cysteine and glutamic acid are not active as hydrogen donors. The amino acids in the present study are classed by him as "doubtful or inactive." Soru^{2, 3} compared the ability of the dissociated and undissociated *B. coli* to oxidize glucose. He found that the fermentation of glucose, various other substrates, and other sugars is slower with the R form than with the S form. Alanine and serine were so slowly oxidized that no difference in reduction time could be noted. Under similar conditions, using Bacille d'aertrycke, glycine was not attacked, and other substances such as

¹ Bach, D., *Compt. rendu de Soc. Biol.*, 1935, **120**, 608.

² Soru, E., *Compt. rendu de Soc. Biol.*, 1936, **121**, 1647.

³ Soru, E., *Compt. rendu de Soc. Biol.*, 1935, **120**, 232.