The Journal of Immunology
RESEARCH ARTICLE | MARCH 01 1938

Further Studies on the Permeability to Sodium Nitrate of the Blood-CNS Barrier in Experimental Poliomyelitis[1] FREE

Edwin H. Lennette; ... et. al

J Immunol (1938) 34 (3): 215–220.
https://doi.org/10.4049/jimmunol.34.3.215

FURTHER STUDIES ON THE PERMEABILITY TO SODIUM NITRATE OF THE BLOOD-CNS BARRIER IN EXPERIMENTAL POLIOMYELITIS[1]

EDWIN H. LENNETTE AND HAROLD R. REAMES

From the Department of Bacteriology and Parasitology, The University of Chicago, Chicago, Illinois

Received for publication October 2, 1937

Disturbances in the permeability of the blood-CNS barrier are known to occur in a number of pathologic affections of the central nervous system (1), and hence there exists the possibility that similar changes may occur in poliomyelitis. Studies on the blood-CNS barrier in this disease have been few, although Flexner and Amoss (2) long ago suggested that defects of the barrier may be of some importance in the pathogenesis of poliomyelitis. Equally important is the possibility that alterations in permeability of the barrier may constitute an approach to specific or non-specific therapy.

The literature concerning permeability of the barrier in poliomyelitis is contradictory and confusing, due in part to the various reagents used in testing function of the barrier and in part to the small number of cases, human or monkey, studied. Flatau (3) found the barrier impermeable to acid fuchsin in human cases. Parr and Goodale (4) were unable to demonstrate isohemagglutinins in the spinal fluid of human cases. The value of these two sets of observations is marred, however, by failure of the authors to state the stage of the disease in which these studies were made. The results of Shaughnessy, Grubb and Harmon (5) indicated that intravenously injected antibodies could rarely be demonstrated in the spinal fluid of monkeys in either the preparalytic or paralytic stages of the disease. Len-

[1] This work was supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

nette and Campbell (6) noted that a crystalloid, sodium bromide, passed into the spinal fluid more readily in poliomyelitic than in normal monkeys.

In a previous report (7) we presented data which led us to believe that a slight increase in permeability of the blood-CNS barrier may occur in experimental poliomyelitis, although the results were difficult to interpret because the number of animals studied (twenty-eight) was small and chance variation in distribution could not be ruled out. We have since extended our observations to include 111 monkeys, the data on which we wish to present in this report.

MATERIAL AND METHODS

Animals. Rhesus monkeys (*Macaca mulatta*) were used throughout this study. The test group was composed of animals in the preparalytic and paralytic stages of poliomyelitis following intracerebral or intranasal inoculation of virus. Those monkeys referred to as normal were healthy, unused animals and constituted one group of the control series; the other control groups comprised monkeys receiving intraspinous injections of horse serum (to produce an aseptic meningitis) or intracerebral inoculations of normal monkey cord, spinal fluid or starch (to study the effect of cerebral trauma on permeability). Poliomyelitis convalescent monkeys were included for completeness; these animals had passed through an attack of the disease 3 or more months before the test.

Preparation of animals. Each animal received intravenously 35 mgm. of sodium nitrate per kilogram of body weight. To study the effect of aseptic meningitis on the passage of nitrate into the spinal fluid, the test was done 24 hours after intrathecal injection of sterile normal horse serum.[2] In attempting to evaluate the rôle of cerebral trauma on barrier permeability, the test was done from 5 to 8 days after cerebral injection of normal monkey cord suspension, spinal fluid or starch in order to make the time interval comparable to that elapsing between

[2] We are indebted to Parke, Davis and Company for a generous supply of horse serum.

inoculation and appearance of symptoms in the poliomyelitic group.

Analysis of spinal fluid for NO_3^-. Spinal fluid obtained by cisternal puncture one hour after intravenous injection of sodium nitrate was immediately analyzed for its content of nitrate as follows. Standard solutions of sodium nitrate containing from 10 to 100 mgm. per 1,000 cc. were prepared from a 1.0 per cent stock solution. Two cubic centimeters of Merck's reagent sulfuric acid (NO_3 content 0.0002 per cent) were pipetted into the bottom of clean serological tubes and overlaid with 0.2 cc. of 1.0 per cent diphenylamine in 75 per cent sulfuric acid. Finally, 0.2 cc. of a standard solution or of centrifugated spinal fluid was added, the two top layers carefully mixed and the tubes allowed to stand until a blue ring appeared; the contents were then shaken to obtain a uniform distribution of color and the tubes containing spinal fluid were compared with the standards.

RESULTS

The results are summarized in table 1, which shows the distribution of the animals according to the concentration of nitrate of the spinal fluid. As with all tests which are only roughly quantitative, there is considerable overlapping between the values obtained for any one group and those of any other; were a more delicate and quantitative analytical method used, more apparent differences might be brought out between poliomyelitic and control animals.

Aside from the fact that nearly one-half the poliomyelitic monkeys had a spinal fluid nitrate falling within the same range of concentration as that of the controls with an aseptic meningitis, the table reveals no noteworthy differences between any of the other groups. Application of the Chi Square Test to the data in table 1, however, brings out a number of significant points. Comparing the preparalytic and paralytic series of monkeys, P (probability) was found equal to approximately 0.19 i.e., there are 19 chances in 100 that a difference in the nitrate-values of the spinal fluid in these two groups as great or greater than that observed might arise by chance alone. Apply-

ing this same test to the controls (excluding the animals with an aseptic meningitis) P was found equal to 0.80. Since the probabilities in both cases are large, we may consider the poliomyelitic animals as constituting a single group and the controls (again excluding those with an aseptic meningitis) as constituting another, homogeneous group. Applying X^2 to these two large groups, P was found equal to 0.005; since the probability of a difference as great as this occurring by chance alone is small, the difference may be considered significant.

TABLE 1

Results of nitrate-test for permeability of blood-CNS barrier in experimental poliomyelitis

HISTORY OF MONKEYS	CONCENTRATION OF NO_3 IN CEREBROSPINAL FLUID (MGM. PER 1000 CC.)								NUMBER OF MONKEYS IN GROUP
	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	
Acute poliomyelitis:									
Preparaltyic		6	14	7	1	1			29
Paralyzed	1	4	9	3	5	3	1	1	27
Convalescents	4	1	1						6
Controls:									
Normals	2	4	17	2					25
Cerebral trauma*	1	3	9	1					14
Aseptic meningitis†				3	4	1	1	1	10
Total animals	8	18	50	16	10	5	2	2	111

* Injected intracerebrally with 1.0 cc. of either 1.0 per cent normal monkey cord suspension, 2.0 per cent starch suspension or normal monkey spinal fluid

† Injected intrathecally with 2.0 cc. sterile normal horse serum 24 hours before test.

DISCUSSION

The data presented suggest that there is a significant difference in barrier-permeability between poliomyelitic and normal monkeys, and that the trauma of intracerebral inoculation does not increase permeability of the barrier to the same extent as does the damage resulting from infection with the virus. Considering the results of the nitrate-test in any one animal, it is impossible to judge its clinical status so far as poliomyelitis is concerned unless the permeability is so great that large amounts of

nitrate pass into the spinal fluid. This is due, we believe, in major part to the crudeness of the test, which does not permit of exact determinations of the amount of nitrate present to be made, and also to the fact that the analytical procedure is prone to give rather erratic results. We are convinced that a method involving simultaneous determination of the nitrate-concentrations of blood and spinal fluid, with the findings expressed as a ratio or quotient (concentration in serum/concentration in spinal fluid) would give much more clear-cut results. Lennette and Campbell (8) found that the bromide-content of the spinal fluid of poliomyelitic animals differed in no constant manner from that of control monkeys, but when permeability was expressed as a quotient the difference in permeability between the two groups was at once apparent. Such a procedure takes into account natural variations in permeability in the animals, and permits of finer measurement of the degree of permeability. Even so, the permeability quotients occasionally diverge widely from a mean around which most of the animals in the group tend to fall.

In our opinion the nitrate-test is of little clinical value in poliomyelitis, since the results are not constant and do not serve, in any given instance, to delineate a poliomyelitic animal from a normal one. Taken as a whole, however, the data suggest that poliomyelitic monkeys *as a group* differ from normal monkeys in having an increased barrier-permeability to sodium nitrate.

SUMMARY AND CONCLUSIONS

1. The permeability of the blood-CNS barrier to sodium nitrate was studied in 111 *rhesus* monkeys.

2. Statistically evaluated, the results suggested that an increased barrier-permeability to nitrate occurs in monkeys in the acute stages of poliomyelitis.

3. The spinal fluid of nearly one-half (22 of 56) of the poliomyelitic monkeys showed a nitrate-concentration as great as that of the control group in which an aseptic meningitis had been produced by intraspinal injection of horse serum.

4. The trauma incident to intracerebral inoculation had little

effect in increasing the permeability of the barrier under the conditions in which the test was applied.

5. The test of nitrate-permeability has a limited clinical value, since it is too crude to permit fine determinations of nitrate in the spinal fluid.

REFERENCES

(1) KATZENELBOGEN, S.: The cerebrospinal fluid and its relation to the blood. Baltimore, The Johns Hopkins Press, 1935.
(2) FLEXNER, S., AND AMOSS, H. L.: Jour. Exper. Med., 1917, **25**, 525.
(3) FLATAU, E.: Rev. Neurol., 1926, **1**, 5.
(4) PARR, L. W., AND GOODALE, R. H.: Proc. Soc. Exp. Biol. and Med., 1934, **32**, 315.
(5) SHAUGHNESSY, H. J., GRUBB, T. C., AND HARMON, P. H.: Proc. Thirty-Eighth Ann. Meeting Soc. Am. Bact., Indianapolis, Dec. 28–30, 1936, J. Bact., 1937, Preprint, **32**, 47.
(6) LENNETTE, E. H., AND CAMPBELL, D. H.: Science, 1937, **86**, 160.
(7) LENNETTE, E. H., AND REAMES, H. R.: Proc. Soc. Exp. Biol. and Med., 1937, **36**, 769.
(8) LENNETTE, E. H., AND CAMPBELL, D. H.: In preparation.