PERMEABILITY OF THE BLOOD-BRAIN BARRIER IN APES WITH EXPERIMENTAL POLIOMYELITIS

M. Ya. Maizerlis, L. I. Ravkina and A. V. Tyufanov

From the Institute of Poliomyelitis
(Dir., Active Member of the AMN SSSR M. P. Chumakov) of the AMN SSSR and the Institute of Psychiatry (Dir., Prof. D. D. Fedotov) of the Ministry of Public Health of the RSFSR, Moscow
(Presented by Active Member of the AMN SSSR M. P. Chumakov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9, pp. 53-58, September, 1962.

A number of investigations have been devoted to studying the permeability of the blood-brain barrier (BBB) during inflammatory diseases of the brain. Clinical observations have shown that in acute cerebrospinal meningitis and acute encephalitides the permeability of the blood-brain barrier is markedly elevated [1, 2, 3, 5].

In the chronic stages of epidemic encephalitis the permeability of the blood-brain barrier is either decreased or unchanged [2, 5].

Data on the increase in blood-brain barrier permeability associated with inflammatory diseases of the brain is corroborated by enumerable experimental investigations. A number of authors have noted an increase in the permeability of the blood-brain barrier in allergic [12] and infectious encephalomyelitis in animals (7, 10, 13, 14]. MacCurdy and Evans [13], using monkeys, demonstrated selective staining of foci in the brain with trypan blue, associated with experimental poliomyelitis. In a number of clinical and experimental investigations an increase in the permeability of the blood-brain barrier has been demonstrated following cranio-cerebral trauma and experimental injury of the brain.

However, a search of the available literature failed to disclose experimental or clinical data on the permeability of the blood-brain barrier associated with poliomyelitis and obtained by the use of precise, quantitative methods of investigation.

In this work we utilized the method of artificial radioactive isotopes in an attempt to elucidate how the permeability of the BBB changes in animals inoculated with attenuated poliomyelitis virus of the Sabine strain.

EXPERIMENTAL METHOD

The investigations were carried out on 31 monkeys of the Macacus rhesus breed, both sexes, weighing from 1.5 to 3 kg; 13 animals served as the control, 6 were injected with vaccine intracerebrally (0.5 ml in each optic tubercle) and 12 – intraspinally (0.1 ml into the lumbar portion of the spinal cord, at the level of L_2-L_3). As an indicator of the permeability of the BBB, we used an artificially radioactive isotope of phosphorus (P^{32}), which was injected into the animal intramuscularly with a total activity of 15-30 microcuries, 28 days after the inoculation with vaccine. After an hour the animals were sacrificed by exsanguination, samples of liquor were taken (from the great ventricle), as well as samples of blood and brain tissue from various regions. The radioactivity of the desiccated samples were studied with the aid of a surface counter, BFL-25, on the B-2 apparatus, in a lead housing. The percent relationship of the liquor and brain radioactivity to that of the blood (in units of volume or weight) served as the index of BBB permeability. The obtained results were subjected to statistical analysis.

All the animals were autopsied. The brain was fixed in a 10% solution of formalin, and sections of the brain were stained according to the method of Nissl and with hematoxylin-eosin. We studied the cortex of the motor areas, the optic tubercles, the midbrain, upper and lower divisions of the medulla oblongata, and six cervical segments, four thoracic segments and five lumbar segments of the spinal cord.

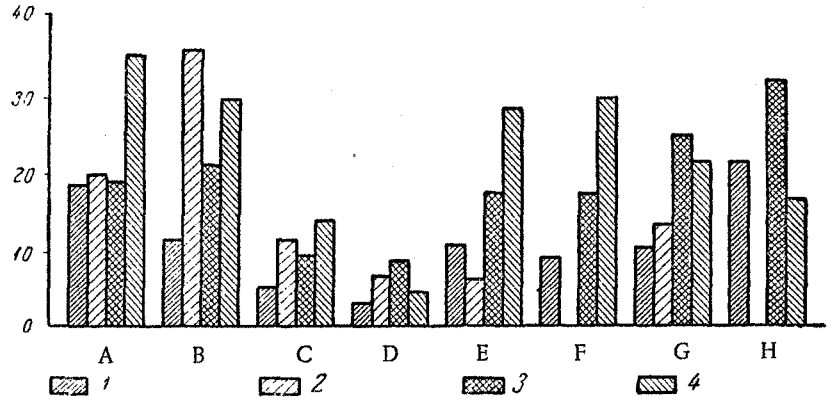
EXPERIMENTAL RESULTS

1. In the control animals, one hour after injection of the P^{32}, its concentration in the liquor was equal to an average of 19.9 ±2.78% in comparison with the blood. The lowest concentration of the isotope was observed in the

subcortical areas (3.34 ± 0.46%) and the white matter (5.5 ± 1.4%); the highest – in the hypothalamic region (21.4 ± 4.88%) (see figure).

2. Clinical signs of the illness were not observed in any of the investigated apes of the experimental group subjected to intracerebral inoculation with attenuated poliomyelitis virus. The coefficient of P^{32} permeability in the liquor of these animals was equal to from 6.3 to 26.5% with an average of 20.2 ± 3.26%, i.e., did not differ from the normal.

The concentration of P^{32} in the majority of the brain areas was increased, especially in the cerebral cortex (37%), the white matter of the hemispheres (11.3%), and the subcortical portions (6.3%) (see figure).



Penetration of P^{32} into the cerebrospinal fluid of apes. 1 – control experiments; 2 – intracerebral inoculation with the vaccine; 3 – intraspinal inoculation without clinical signs of poliomyelitis; 4 – intraspinal inoculation with clinical signs of the illness. A – liquor; B – cortex; C – white matter; D – subcortical portions; E – pons; F – medulla oblongata; G – cerebellum; H – hypothalamus.

On pathohistological investigation of the brain and spinal cord, traumatic foci were observed in the optic tubercles of all the animals. Morphological signs of poliomyelitis were not encountered in any of the cases. Signs of focal meningitis were found in the brain of only one of the six monkeys, and these animals showed the highest coefficient of P^{32} permeability in the liquor.

Thus, after intracerebral inoculation the P^{32} permeability coefficient in the liquor corresponded to the normal, while the accumulation of P^{32} in the brain tissue was elevated.

3. After intraspinal inoculation, clinical signs of poliomyelitis were noted in 7 of the 12 monkeys (pareses, paralyses, predominantly of the lower extremities). In these animals the P^{32} permeability coefficient in the liquor was markedly elevated to an average of 45.6% (30.5-62%). In the stricken animals, the concentration of P^{32} was sharply increased in the majority of the divisions of the brain, particularly in the white matter (14%) and the brain stem (28-29%).

Histological investigation showed that with the highest permeability coefficient (62%) were observed more severe and disseminated pathomorphological changes, characteristic of poliomyelitis – neuronophagia, focal fliosis, perivascular infiltrates, and meningitis at the levels of the spinal cord. In two cases, with relatively low coefficients of permeability (30-31%), changes in the central nervous system were only observed in the lumbar portion of the spinal cord, plus isolated perivascular infiltrates in the brain (see table).

In 2 of the animals that recovered the permeability coefficients did not differ from the norm (17.3 and 21%). However, even in these cases neuronophagia, gliosis and perivascular lymphoid infiltrates were observed in the lumbar division, at the level of $L_2 - L_3$, and in one the apes – focal meningitis.

In 5 of the animals without clinical signs of illness, the P^{32} permeability coefficients did not differ from the norm (19.2%) Penetration of the isotope into divisions of the brain, however, was markedly elevated in them as compared with the healthy animals. On the other hand, in many regions of the brain (cortex, white matter, pons, medulla oblongata) the concentration of P^{32} was lower than in the animals stricken with poliomyelitis. In the subcortical portions and the hypothalamic area this relationship was not observed. On histological investigation, a traumatic focus was present in the lumbar division at the level $L_2 - L_4$ in all the monkeys (connective tissue scar), arising as a result of the vaccine injection. In one case, limited poliomyelitis was noted in the lumbar region, near the traumatic focus.

Clinico-Anatomical Relationships in Monkeys Inoculated Intraspinally with the Sabin Strain, and the Coefficient of P^{32} Permeability

Clinical signs	Permeability coefficient (in %)	Morphological changes																			
		brain				spinal cord															
		cortex	mid-brain	optic tubercles	brain stem	C_2	C_3	C_4	C_5	C_6	C_7	T_4	T_6	T_8	T_{10}	L_1	L_2	L_3	L_4	L_5	S
Paralysis of the foot	62	M							+	+M		+	M	++	+			++	++ +M	+	M
Paresis of extremities	43	M	+	+	+	+			+	+	+					++	++ +	++ +M	+		
Paresis of the lower extremities	51				+													+M	++ M	++ +	+
Paresis of the lower extremities with recovery	30				+												+	+	++ +		
Paresis of the lower extremities with recovery	31																		++		
Healthy	17.3																++ +	++ +			
Healthy	19.4																	++ +	+		
Healthy	21																	+			
"	19																	+			
"	33																				
"	29																				
"	5.8																				

Symbols: M—meningitis, +, ++, +++—mild, intermediate and severe signs of damage characteristic for poliomyelitis.

Investigation of P^{32} penetration into the liquor and its distribution in the brain of the healthy animals showed that the permeability coefficient of the isotope in the apes was very high, being equal to an average of approximately 20% in relation to the activity of the blood. Accumulation of P^{32} in all the divisions of the brain investigated was relatively low in comparison with other species of animals—rats, rabbits [1, 4, 11]—which attests to high resistance of the BBB in monkeys.

Distribution of the P^{32} in the brain of the monkeys basically corresponded to the selective localization of the isotope seen in the brain of rabbits, rats, dogs, and even the human [1, 4, 9, 11 and others].

Comparison of the data obtained in the control animals and those inoculated with the attenuated strain of poliomyelitis virus showed that after intracerebral and intraspinal inoculation without clinical signs of illness, as well as in animals that recover, the coefficient of P^{32} permeability does not differ from the norm, despite posttraumatic scarification at the site of the vaccine injection. Along with this, an increase is noted in the P^{32} penetration of divisions of the brain in these animals, which can be explained by the presence of traumatic foci in the region of the optic tubercles.

It should be taken into consideration that, despite the normalization of P^{32} penetration into the liquor, the accumulation of isotope in the brain can be elevated for a prolonged period of time after trauma. Apparently, under certain conditions, changes in the penetration and accumulation of the indicator in the brain tissue do not have to correspond to its concentration in the liquor [4].

In the monkeys with clinical signs of poliomyelitis, a sharp rise was noted (by almost two times) in the coefficient of P^{32} permeability. The highest elevation in P^{32} penetration into the liquor was characteristic for animals with signs of focal meningitis. In the monkeys with clinical signs of illness, along with an increased penetration of P^{32} into the liquor, we observed a greater accumulation of the isotope in almost all the brain divisions investigated.

Thus, the data obtained serve as evidence of a significant increase in the permeability of the blood-brain barrier with development of clinical signs of the illness, when monkeys are inoculated with an attenuated strain of the poliomyelitis virus. This corroborates the point of view of a number of authors [8, 15], that specifically the paralytic form of poliomyelitis can serve as a criterion of significant residual neurotropism in live vaccine against poliomyelitis.

On the basis of our observations, it may be postulated that disruption of the permeability of the blood-brain barrier plays a definite role in the development of pathological symptoms associated with experimental poliomyelitis. The practical significance of these experiments lies in the possibility of using these methods of investigating the permeability of the blood-brain barrier as a diagnostic index for this disease. The data obtained also are of interest in regard to the control of live anti-poliomyelitis vaccine. Thus, the absence of an increase in the blood-brain barrier permeability of monkeys with aparalytic poliomyelitis testifies to the decreased manifestation of the pathological changes in the central nervous system in association with this form of the disease.

SUMMARY

The authors studied the entry of P^{32} into cerebrospinal fluid and parts of brain in control monkeys, after intracerebral and intraspinal injection of attenuated virus of poliomyelitis Sabin. It has been shown that in monkeys with clinical symptoms of poliomyelitis, penetration of P^{32} into cerebrospinal fluid and brain largely increased. There was the same concentration of P^{32} in the cerebrospinal fluid in monkeys without clinical symptoms of the disease as in control animals, but the penetration of P^{32} into the brain increased. There was a definite correlation between the degree of disturbance of the blood-brain barrier permeability and pathomorphological alterations in the central nervous system.

LITERATURE CITED

1. A. M. Vein. The Blood-Brain Barrier in Certain Diseases of the Central Nervous System. Diss. kand. Moscow, 1957.
2. N. D. Kuznetsov. Med. zh. Uzbekistana, 1958, No. 10, p. 10.
3. A. E. Kul'kov. Klin. med., 1931, vol. 9, No. 15, p. 593.
4. M. Ya. Maizelis. Med. radiol., 1960, No. 5, p. 52.
5. N. I. Popov. Kazansk. med. zh., 1937, No. 11, p. 1319.
6. E. A. Fedorova. Works of the Medical Faculty of the Byelorussian University [in Russian]. Minsk, 1928, No. 22, p. 93.
7. G. Ya. Khvoles and I. I. Labutin. Byull. éksper. biol., 1936, vol. 2, No. 6, p. 431.

8. M. P. Chumakov et al. Theses from the Reports of the 4th Scientific Session of the Institute for the Study of Poliomyelitis and the International Symposium on Live Anti-Poliomyelitis Vaccine [in Russian]. B.M., 1960, No. 77.
9. L. Ya. Shargorodskii and N. M. Madzhidov. Zh. nevropatol., 1958, No. 5, p. 567.
10. V. I. Yakushev. Byull. éksper. biol., 1946, vol. 21, No. 5, p. 29.
11. L. Bakay. The Blood-Brain Barrier with special regard to the use of radioactive isotopes. Springfield, 1956.
12. C. F. Barlow. J. Neuropath., exp. Neurol., 1956, vol. 15, p. 196.
13. J. T. MacCurdy and H. M. Evans. Berl. klin. Wschr., 1912, Bd. 49, S. 1695.
14. W. Mestrezat. Le liquide céphalo-rachidien, normal et pathologique. Paris, 1912.
15. D. B. Sabin. J.A.M.A., 1956, vol. 162, p. 1589.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
