

# CONVULSIONS AND THE PERMEABILITY OF THE BLOOD-FLUID BARRIER

by

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## INTRODUCTION

Since WALTER reported the bromine-method of estimating the permeability of the blood-fluid barrier quantitatively in 1925, many clinical and experimental studies have been made in Japan and shown that the permeability of the blood-fluid barrier increases in cases of meningitis, B-encephalitis, general paresis, pumping of the cerebrospinal fluid, infantile dyspepsy, functional disturbances of the liver and the kidney, fever, convulsions and decreases in schizophrenia. According to TODA<sup>13)</sup>, HIROSE<sup>2)</sup>, KAMEYAMA<sup>6)</sup>, TATSUMI<sup>12)</sup> and SHIMIZUEATA<sup>10)</sup> the factors influencing the permeability of the blood-fluid barrier are as follows: (i) condition of the choroid plexus, the pia mater and their small vessels, (ii) fluctuation in circulation, composition and osmotic pressure of the blood and the cerebrospinal fluid, (iii) alteration in the tonus of the autonomic nervous system, (iv) condition of the reticuloendothelial system, (v) disturbances in the endocrine functions and (vi) age etc.

Recently, demonstrating that in experimental convulsions being caused by the injection of various convulsant drugs into the spinal central canal the drug appeared immediately after the injection in the cerebrospinal fluid, SUHARA et al<sup>14)</sup> concluded that the drug penetrated the spinal substance and appeared in the subarachnoid space.

INOUE<sup>3)</sup>, HIROSE<sup>2)</sup>, MATSUOKA<sup>8,9)</sup> reported that the permeability of the blood-fluid barrier increased in convulsions. I have been studying the vital staining of the central nervous system with methylene blue for some years and recognized that in the animals intravenously perfused with the dye by the drip technique, the grey matter of the central nervous system was stained dark blue macroscopically and many nerve fibers in the white matter were stained blue histologically. In the present study experimental convulsions were induced with cardiazol in such animals and the appearance of the dye in the cerebrospinal fluid was examined.

## MATERIALS AND METHODS

Three hybrid dogs weighing 3.4~6.2 kg were used. After the animals were anesthetized with intravenous injection of isomytal (isoamylethyl-barbituric acid) soda (0.03 g per kg of body weight) laminectomy of the upper thoracic vertebrae was done and the dura mater was exposed. After complete hemostasis a vinyl tube

with inside diameter of 1 mm was inserted 10~20 cm upwards into the subarachnoid space until its tip reached the cerebellomedullar cistern. The vinyl tube was fixed with 2 or 3 knots passing through the muscle and the skin, and its other end was hung into a test-tube to collect the cerebrospinal fluid continuously. On inserting the tube the unnecessary loss of the fluid should be avoided, thus the dura was opened not by a knife cut, but in the following way. Lifting the dura with a pincette, we punctured the dura and the arachnoid with a fine injection needle taking care not to injure the vessels and the spinal cord. After the leakage of the cerebrospinal fluid was recognized, the hole was enlarged with an unhooked pincette and the vinyl tube with an oblique cut end was inserted into the subarachnoid space. The vinyl tube was not fixed on the dura by suturing or ligating, because these manipulations were apt to cause the leakage of the cerebrospinal fluid. The tube remained well fixed even in the convulsive stages by the superficial sutures only. When all were prepared for the collection of the cerebrospinal fluid, 0.5% methylene blue\* solution, made up in 5% glucose solution, was perfused into the saphenous vein of the animals at the rate of 4 to 20 drops per minute.\*\* Thirty minutes after the onset of the perfusion, the skin and the visible mucous membrane became slightly blue colored. In this stage the central nervous system was considered to be stained or to be taking the dye\*\*\*

One hour or more after the beginning of the dye perfusion, cardiazol was intravenously administered and the convulsions were induced. Injections of cardiazol were repeated at intervals of one hour or more, and the cerebrospinal fluid was examined.

Besides, the following control experiment was done. A hybrid dog weighing 8.6 kg was perfused intravenously with methylene blue. The blood of the animal was taken repeatedly at intervals of 15 minutes, and the concentration of methylene blue in blood serum was estimated by colorimetric method.

## RESULTS

Convulsions were caused eight times with intravenous injection of cardiazol, but the appearance of methylene blue in the cerebrospinal fluid could never be recognized. Experimental results in each animal are as follows.

Dog, No. 1, weighing 3.4 kg, male. Anesthetized with 0.12 g of isomytal soda, thoracic laminectomy was done. During the experiment the dye solution was perfused continuously for six hours into the animal's vein at the rate of 1~15 drops per minute. The total amount of perfused dye was 0.8 g. Two hours after the onset of the perfusion 0.06 g of cardiazol was intravenously injected. At first, tonic

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\* Methylenblau B extra (MERCK) was used.

\*\* It is difficult to maintain the constant rate of perfusion. As a rule the number of drops decreases gradually.

\*\*\* Since EHRLICH<sup>1)</sup> for the first time stained the nervous system with methylene blue vitally, the fact is well known that methylene blue is reduced to colorless leucomethylene blue by the nerve cells in good conditions.

convulsions were induced for 10 seconds and then clonic convulsions for 8 minutes. Three and a half hours later, momentary convulsions and succeeding clonic convulsions of 5 minutes duration were induced following the second injection of 0.1 g cardiazol. The cerebrospinal fluid had been collected during the dye perfusion, but it remained colorless throughout.

Dog, No. 2, weighing 5.5 kg, male. Under the anesthesia with 0.25 g isomytal soda, laminectomy of the lower cervical and upper thoracic vertebrae was done. With the injection of 0.5 g cardiazol one hour after the onset of the dye perfusion, tonic convulsions for 30 seconds followed by clonic convulsions for 2 minutes were induced. With the second injection of 0.3 g cardiazol two hours later, clonic convulsions were induced for 1½ minutes without remarkable tonic phase. The animal died four hours after the onset of the dye perfusion. Total amount of the perfused dye was 1.37 g. The cerebrospinal fluid remained colorless during the experiment.

Dog, No. 3, weighing 6.2 kg, female. Anesthetized with intravenous injection of 0.3 g isomytal soda. Upper thoracic laminectomy. One hour after the onset of the dye perfusion 0.3 g of cardiazol was injected. Tonic or clonic convulsions appeared for 5 minutes. One hour later, 0.5 g of cardiazol was injected for the second time without being followed by convulsions. By the third injection of 0.4 g cardiazol two hours later tonic convulsions of 1 minute duration succeeded by clonic convulsions for 3 minutes were induced. One hour later, 0.3 g of cardiazol was injected for the fourth time. Then came momentary tonic convulsions followed by clonic convulsions lasting for 1 minute. The animal died five and a half hours after the onset of the dye perfusion. Total amount of the perfused dye was 1.25 g. The cerebrospinal fluid of 14.5 cc was collected during the entire experiment. But the coloration could never be recognized.

Fig. 1 indicates the time relation between the onset of the dye perfusion and injections of cardiazol in these three animals.

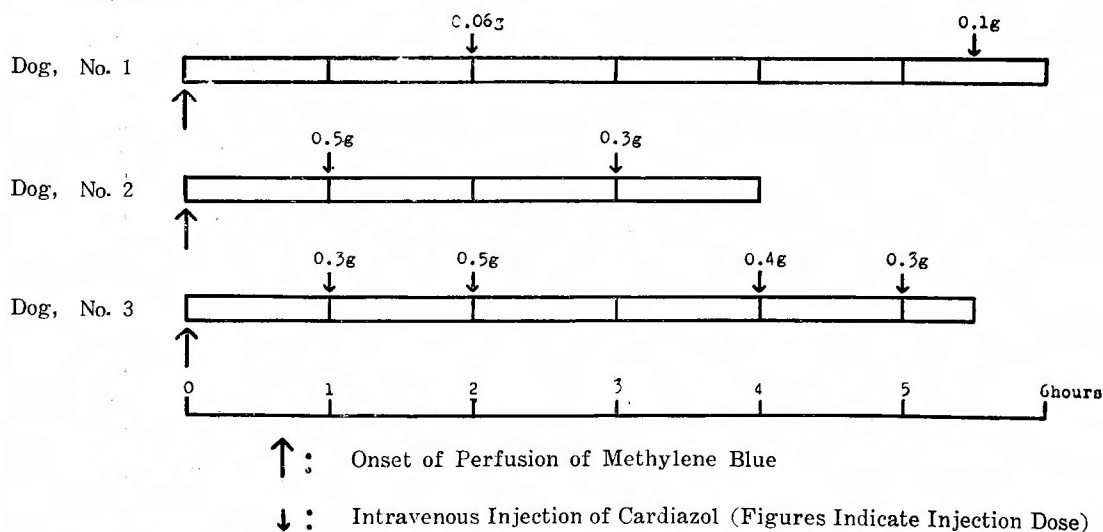
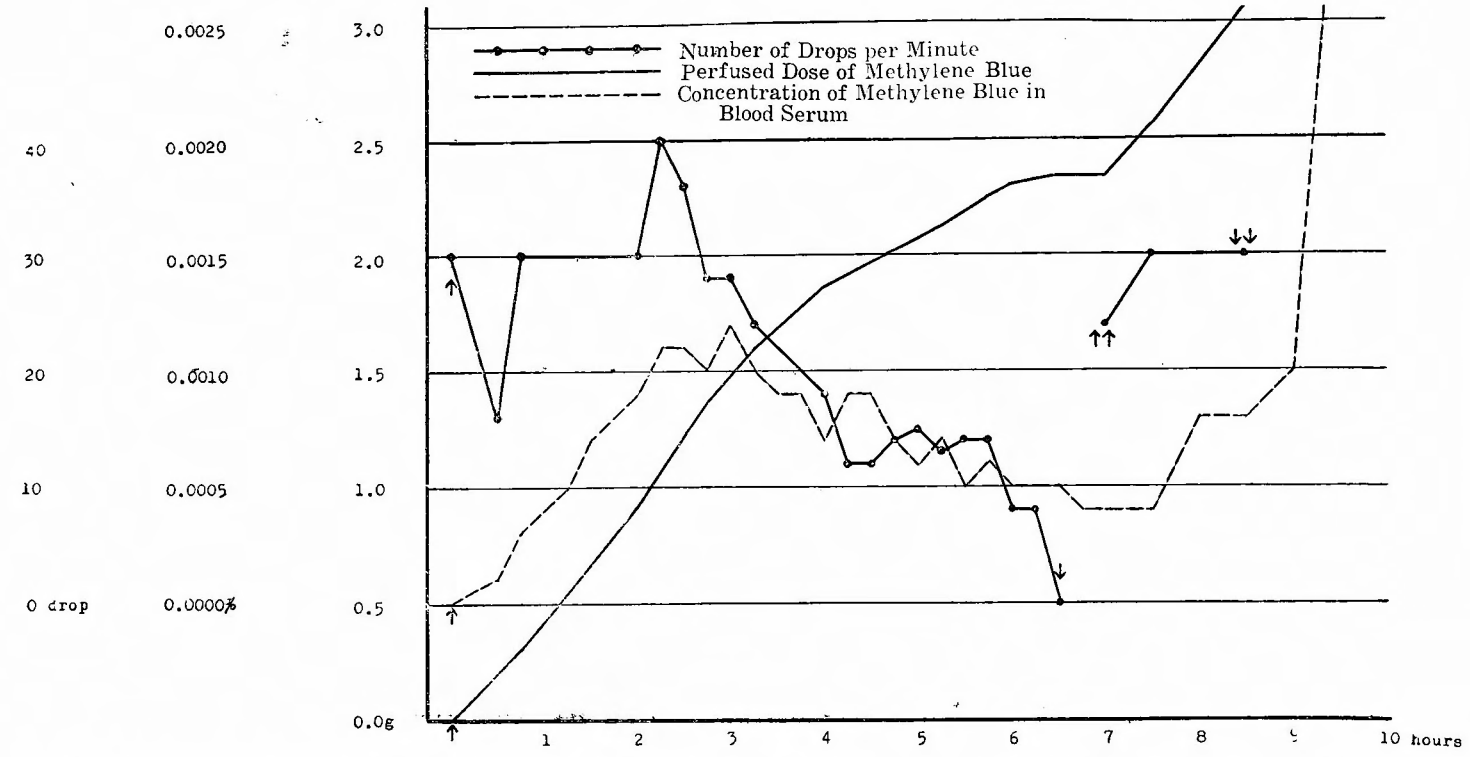


Fig. 1 Time Relation between Onset and Duration of Perfusion of Methylene Blue and Intravenous Injections of Cardiazol



Number of Drops per Minute    Concentration of Methylene Blue in Blood Serum    Perfused Dose of Methylene Blue  
 ↑ : Onset of Perfusion of Methylene Blue    ↓ : Temporary Stoppage of Perfusion of Methylene Blue  
 ↑↑ : Reopening of Perfusion of Methylene Blue    ↓↓ : Stoppage of Perfusion of Methylene Blue

Fig. 2 Relation among Perfused Dose, Number of Drops per Minute and Concentration of Methylene Blue in Blood Serum

Fig. 2 indicates the relation among the total dose, the number of drops per minute and the concentration of methylene blue in blood serum during the dye perfusion without experimental convulsions.

Dog, No. 4, weighing 8.6 kg, male. Blood was taken for the first time fifteen minutes before the perfusion and then repeatedly at intervals of 15~30 minutes from the contralateral saphenous vein. The amount of the blood taken was 5 cc at a time. The concentration of methylene blue in serum was examined by colorimetric method. The total amount of the perfused dye was 3.07 g, and the duration of the perfusion was eight hours. The concentration of the dye in serum became gradually higher after the onset of the perfusion. The concentration of the dye in serum was nearly constant so far as the renal and the reticuloendothelial functions were normal. It rapidly increased just before the animal's death. The concentration of the dye in the evacuated urine eight hours after the onset of the perfusion, the concentration in ascites and in the bladder urine after the animal's death were also examined. The concentration in ascites was nearly equal to that in serum. But the concentration in urine was nearly in accord with that of the perfused solution, and was much larger than that in serum. The fact was, of course, due to the concentration ability of the kidney.

Blue coloration of the central nervous system was confirmed by autopsy in each experiment.

## DISCUSSION

In the above experiments, the appearance of methylene blue in the cerebrospinal fluid could not be demonstrated in the animals with or without convulsions, even in the stage when a considerable quantity of the dye was contained not only in the blood but also in the brain substance. In this connection some comments will be made in the following.

The first problem is in what dose the brain and the spinal cord may contain methylene blue as a result of the perfusion for 4~6 hours. A small piece of transverse section of a lumbar segment of the control dog No. 4 was excised and immersed in several changes of alcohol, until no more dye was dissolved out from the tissue piece. Determining the dye content in the alcohol by colorimetric method, it was proved that a lumbar segment contained the dye of about 1/900 of its weight. As seen from the fact that the grey matter was stained more deeply than the white matter, the dye may be contained unhomogeneously in the central nervous system. But we may roughly assume that the dye content in the whole central nervous system is about 1/900 of its weight.

The second problem is the minimal recognizable concentration of methylene blue, when diluted with distilled water. 0.1% solution of the dye colored deep blue, 0.001% light blue, 0.00005% slight blue, 0.000025% faint blue. But, 1 cc of 0.000025% solution could hardly be distinguished from that of the distilled water. 0.000005% solution was quite colorless. Accordingly, it is sure that methylene blue in the cerebrospinal fluid in our experiment should be, if present, less than 0.00005%. As

can be seen in Dog No. 4, the concentration of the dye in blood serum was about 0.0005~0.0012% during the perfusion. Thus it is evident that the concentration of the dye in the cerebrospinal fluid is less than 1/10~1/24 of that in blood serum. According to ISHIBASHI<sup>1)</sup>, the normal P. Q. (permeability quotient) is 2.90~3.30 by WALTER'S method, i. e. the amount of bromine in the cerebrospinal fluid is about 1/3 of that in blood. Considering that the amount of methylene blue in the cerebrospinal fluid in our experiment is less than 1/10~1/24 of that in blood, it may be assumed that the dye has hardly appeared in the cerebrospinal fluid.

As the third problem, it may be necessary to consider how the reduction power of glucose, which is contained in blood, in the cerebrospinal fluid and in the perfused solution, may have some influence upon the dye. For the quantitative determination of glucose in the cerebrospinal fluid, there is the methylene blue method of KASAHARA et al<sup>2)</sup>. This method is based upon the principle that glucose in the cerebrospinal fluid, which is weakly alkaline, reduces the dye to colorless leucomethylene blue. But, leucomethylene blue is soon oxidized to blue colored methylene blue again by oxygen in the air. In the present experiment the cerebrospinal fluid was examined after being kept in room temperature for a while. In KASAHARA-HATTORI'S method, the cerebrospinal fluid is boiled after addition of a small quantity of methylene blue and of caustic potash, and then the fading of blue color of the fluid is observed. In our experiment it is unnecessary to take into consideration the reduction power of glucose in the perfused solution, in blood or in the cerebrospinal fluid, because such procedures as boiling or addition of caustic potash were not followed by us.

### CONCLUSION

The experiment was done in three dogs. The animal was perfused with methylene blue solution from the saphenous vein for 4~6 hours, and the cerebrospinal fluid of the animal was collected through a vinyl tube inserted into the subarachnoid space. Cardiazol convulsions were induced repeatedly one hour or more after the onset of the perfusion. In three dogs the appearance of methylene blue in the cerebrospinal fluid could never be demonstrated.

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\* Written in Japanese.

## 和文抄録

# 痙攣と血髄関透過性

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大学院学生 安藤協三

1) 犬を用いて、0.5%メチレン青5%葡萄糖液を長時間にわたって静脈内に点滴注入し、之と平行して蜘蛛膜下腔内にビニール管を挿入して髄液の持続的採集を行った。

2) 点滴注入開始後1時間以上を経過して、カルチアゾールの静注により痙攣を誘発せしめたが、メチレン青の髄液内移行は終始認められなかつた。

3) 此の際脳脊髄実質は明かに相当量のメチレン青

を含有しているのであるから、メチレン青に関する限りは、脳脊髄実質間を通つて速やかに髄液腔内に達する通路があるとする栖原等<sup>1)</sup>の見解と一致をみなかつた。

4) 対照実験としてメチレン青の血清内濃度も測定し、実験成績について若干の考察を加え、血髄関の機能を再確認した。