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# THE ACTIONS AND ABSORPTION OF SUBARACHNOID BLOOD

## I. EXPERIMENTAL HYDROCEPHALUS
## II. ABSORPTION OF INTACT RED BLOOD CELLS FROM THE SUBARACHNOID SPACE INTO THE BLOOD

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ABSTRACT

(I) Experimental Hydrocephalus: Repeated injections of autogenous blood components into the cisterna magna were given to adult rabbits. Only the whole blood, if injected more than three times, could cause the ventricular dilatation. Hydrocephalus was not produced in animals which received repeated injections of washed red blood cells, plasma or hemolysed red blood cells. However, the breakdown product of red blood cells was able to induce meningeal reaction and fibrosis, and acted as a noxious agent on the CNS. It is concluded that an organization of the clot in the subarachnoid space results in pia-arachnoid adhesion and blockage of the CSF circulation.

(II) Absorption of Intact Red Blood Cells from the Subarachnoid Space into the Blood: Absorption rate of intact red blood cells from the subarachnoid space into the blood was measured after the intracisternal injection of the RBC tagged with radioactive chromium. In normal rabbits, up to 16.6% of the total injected dose was recovered in the systemic blood in 72 hours, but the highest absorption was observed within 24 hours. Paradoxical enhancement of the absorption rate was observed, if the cisternal injections of whole blood had been made prior to the measurement. It is concluded that the absorption mechanism of the RBC is different in some respects from that of CSF. The concept on the valvular structure of the arachnoid villi, which was presented by Welch and Friedman, was taken into account and discussed.

## I. Experimental Hydrocephalus

### INTRODUCTION

There are many etiologies of the subarachnoid bleeding, such as bleeding from the intracranial aneurysm or vascular malformation, a head trauma, brain tumor and inevitably from an operative site. It is surprising, however, that

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little attention has been paied to its noxious action on the central nervous system. The subarachnoid blood may exert an immediate and direct effect on the central nervous system, as well as a late and indirect effect which causes the extraventricular obstructive hydrocephalus. It is generally agreed that this type of hydrocephalus is the result of the blockage of cerebrospinal fluid circulation caused by the meningeal fibrosis. Furthermore, it is generally believed that the agent causing the meningeal fibrosis is the breakdown product of blood cells. But it has not been investigated whether red blood cells alone, free of plasma component, could ever cause a sufficient meningeal fibrosis for development of hydrocephalus. Also it seems necessary to investigate whether the high content of plasma proteins in cerebrospinal fluid can result in a blockage of the fluid circulation causing the hydrocephalus. From the concept widely accepted, it is expected that the introdution of breakdown products of red blood cells (hemolysed red blood cells) will induce, more readily, the meningeal reaction and hydrocephalus. From these points of view, the following experiments were made.

## MATERIALS AND METHODS

Adult rabbits (2.5–3.3 kg) were used. Each blood component was obtained from autogenous blood.

*A. Intracisternal injection of whole blood*

This procedure was carried out on 9 rabbits. 1.5–2.0 ml of whole blood was withdrawn from the ear artery. Blood was not heparinized in some occasions but mostly heparinized in minimum to prevent coagulation during the procedure. This volume of whole blood was injected in the cisterna magna under intravenous anethesia of phenobarbital sodium. Injections were repeated 3–6 times at interals of 7–21 (mostly 7) days. Animals were sacrificed 7–14 days after the last injection except in one which was autopsied after 24 hours because of traumatic tap (No. A 2).

*B. Intracisternal injection of red blood cell suspension*

This procedure was carried out on 9 rabbits. 1.5–2.0 ml of heparinized blood was washed four times with cold saline, and the whole amount of this suspension of red blood cells was injected intracisternally. This procedure was also repeated 3–6 times at intervals of 7–14 days. Animals were sacrificed 7–14 days after the last injection.

*C. Intracisternal injection of plasma*

This procedure was carried out on 5 rabbits. 0.8–1.7 ml of plasma was obtained from heparinized blood and injected intracisternally 3–11 times at intervals of 7–14 days. Animals were sacrificed 10–20 days after the last injection.

*D. Intracisternal injection of hemolysed red blood cell*

This procedure was carried out on 9 rabbits. 1.0–1.5 ml of whole blood was washed four times with cold saline and hemolysed by adding 1.0 ml of distilled water to the red blood cell sediment. After making it isotonic by adding hypertonic (9%) sodium chloride solution, it was centrifuged (3,000–4,000 rpm, for 10 min) and its supernatant, free of gross cellular débris, was injected into the cisterna magna.

## Results

After the brain was perfused through the carotid arteries with 10% formalin which was adjusted to pH 7.0–7.2 with phosphate buffer solution, it was taken out from the cranium and fixed further in this buffered formalin for about 48 hours.

The brain was cut in frontal plane, each about 3 mm thick, and the evidence of the ventricular dilatation was checked on each section. The extent of hydrocephalus was designated as (++) when dilatation was evident in all sections, and as (+) when it was recognized only in some of sections. In cases of no evident dilatation, it was designated as (−).

*A. Intracisternal injection of whole blood (Group A; Table 1).*

Repeated intracisternal injections more than three times of whole blood led to ventricular dilation in 7 out of 9 rabbits. In A 1, A 2 and A 4 on which injections were repeated 5, 6 and 4 times respectively at 7–8 days intervals dilatations were evident in all brain sections. These rabbits were sacrificed within 7 days after the last injection.

A 3, A 5 and A 6 which received 3 injections at 7 days interval showed dilatations only in some of their brain sections. Dilatation was also partial in A 8 which received 5 injections but at longer intervals (7–21 days). On A 3 and others, autopsy was carried out 14 days and 7 days after the last injection, respectively.

There was no evidence of ventricular dilatation in A 7 and A 9 which

TABLE 1. Group A: Intracisternal Injection of Whole Blood

| Rabbit No. | Volume injected | Number of injections | Interval of injections | Days at sacrifice | Hydro-cephalus |
|---|---|---|---|---|---|
| A 1 | 1.4–1.5 ml | 5 | 7 days | 7 days | (++) |
| A 2 | 1.5–2.0 ml | 6 | 7–8 days | 24 hours | (++) |
| A 3 | 1.5 ml | 3 | 7 days | 14 days | (+) |
| A 4 | 1.3–2.0 ml | 4 | 7–8 days | 7 days | (++) |
| A 5 | 1.5 ml | 3 | 7 days | 7 days | (+) |
| A 6 | 1.5 ml | 3 | 7 days | 7 days | (+) |
| A 7 | 1.5 ml | 3 | 7 days | 14 days | (−) |
| A 8 | 1.5 ml | 5 | 7–21 days | 7 days | (+) |
| A 9 | 1.5 ml | 4 | 7–21 days | 14 days | (−) |

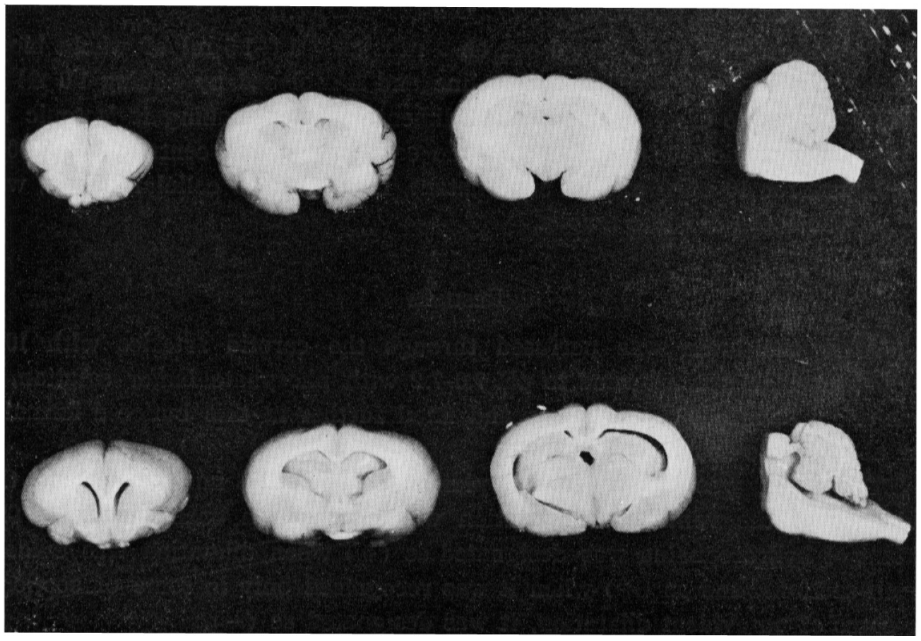

FIG. 1. Sections of the brains of normal (upper) and hydrocephalic (lower) rabbits. On the latter group, slight or moderate enlargement of the cerebral ventricles and aqueduct is noticed as compared with the normal brain sections on which actually no ventricular space can be visulized.

received 3 and 4 injections respectively and were sacrificed after 14 days. In A 9, the injection interval was longer (7–21 days).

Hydrocephalus was of communicating type accompanied with widening of the aqueduct and the fourth ventricle (Fig. 1).

*B. Intracisternal injection of red blood cell suspension* (*Group B; Table 2*).

Up to 6 times of repeated injections of red blood cell suspension which was prepared from 1.5–2.0 ml of whole blood did not induce dilataion of the

TABLE 2. Group B: Intracisternal Injection of Red Blood Cell Suspension

| Rabbit No. | Volume injected as whole blood | Number of injections | Interval of injections | Days at sacrifice | Hydro-cephalus |
|---|---|---|---|---|---|
| B 1 | 1.5–1.7 ml | 3 | 7–9 days | 14 days | (−) |
| B 2 | 1.5–2.0 ml | 6 | 7 days | 7 days | (−) |
| B 3 | 1.5 ml | 5 | 7 days | 7 days | (−) |
| B 4 | 1.5 ml | 5 | 7–14 days | 9 days | (−) |
| B 5 | 2.0 ml | 4 | 7 days | 7 days | (+) |
| B 6 | 2.0 ml | 4 | 7 days | 7 days | (−) |
| B 7 | 2.0 ml | 4 | 7 days | 7 days | (−) |
| B 8 | 2.0 ml | 4 | 7 days | 7 days | (−) |
| B 9 | 2.0 ml | 4 | 7 days | 7 days | (−) |

cerebral ventricles except in one (B 5) that had received 4 injections at 7 days intervals. This dilatation was partial and of minor degree. 7 rabbits including B 5 were autopsied 7 days after, and 2 others (B 1, B 4) 14 and 9 days after, respectively.

The rabbits tolerated injections of the red blood cell suspension better than those of the whole blood.

*C. Intracisternal injection of plasma (Group C; Table 3).*

0.8–1.7 ml of plasma corresponds to the plasma of 1.5 3.0 ml of whole blood. This volume of plasma was injected in the cisterna magna of each of 5 rabbits. Injections were repeated 3–11 times. Intervals between each injection were 7–14 (mostly 7) days. Animals were antopsied 14–20 days after the last injection. In general, longer periods were allowed before sacrificing than in other groups.

As to the hydrocephalus, none of them had even a minimal dilatation of the cerebral ventricles or aqueduct.

Repeated injections of plasma had no notable effect on general conditions of the animals.

TABLE 3. Group C: Intracisternal Injection of Plasma

| Rabbit No. | Volume injected | Number of injections | Interval of injections | Days at sacrifice | Hydro-cephalus |
|---|---|---|---|---|---|
| C 1 | 0.8–1.0 ml | 5 | 7–14 days | 14 days | (−) |
| C 2 | 0.8 ml | 3 | 7 days | 14 days | (−) |
| C 3 | 1.0–1.5 ml | 11 | 7–8 days | 20 days | (−) |
| C 4 | 0.8–1.0 ml | 3 | 7 days | 10 days | (−) |
| C 5 | 1.3–1.7 ml | 7 | 7–8 days | 20 days | (−) |

*D. Intracisternal injection of hemolysed red blood cell (Group D; Table 4)*

It was extremely difficult to repeat this procedure. Following injection, general convulsion occurred almost invariably. D 2, D 4 and D 8 died shortly after the first injection and these were not autopsied. D 1, D 3 and D 7 died from extreme emaciation 13–14 days after the first injection. These were autopsied, but there was no ventricular dilatation.

It was successful to repeat injections (4–5 times) in three rabbits (D 5, D 6 and D 9). Interval of injections was variable (5–12 days) due to unstable conditions of the animals. These were autopsied 7–14 days after the last injection. No evidence of dilatation of the cerebral ventricles or the aqueduct was recognized.

The volume of whole blood, from which hemolysed red blood cell was prepared, was reduced to minimize morbidity and mortality of the animals.

*Microscopic examination with iron staining* (Fig. 2)

Brains fixed in buffered formalin were stained for ferric iron by Perls' method. Brains of Group C (plasma) were not stained for iron.

TABLE 4. Group D: Intracisternal Injection of Hemolysed Red Blood Cell

| Rabbit No. | Amount injected as whole blood | Number of injections | Interval of injections | Remarks | Hydro-cephalus |
|---|---|---|---|---|---|
| D 1 | 1.5 ml | 1 | | Convulsion: Died after 14 days | (−) |
| D 2 | 1.0 ml | 1 | | Convulsion: Died after 15 min | |
| D 3 | 1.0 ml | 1 | | Convulsion: Died after 13 days | (−) |
| D 4 | 1.4 ml | 1 | | Convulsion: Died after 10 min | |
| D 5 | 1.0 ml | 5 | 5–10 days | Convulsins: Sacrificed after 14 days | (−) |
| D 6 | 1.0 ml | 5 | 7–12 days | Convulsions: Sacrificed after 7 days | (−) |
| D 7 | 1.2 ml | 1 | | Convulsion: Died after 14 days | (−) |
| D 8 | 1.2 ml | 1 | | Died after 10 min | |
| D 9 | 1.0 ml | 4 | 7–8 days | Convulions: Sacrificed after 7 days | (−) |

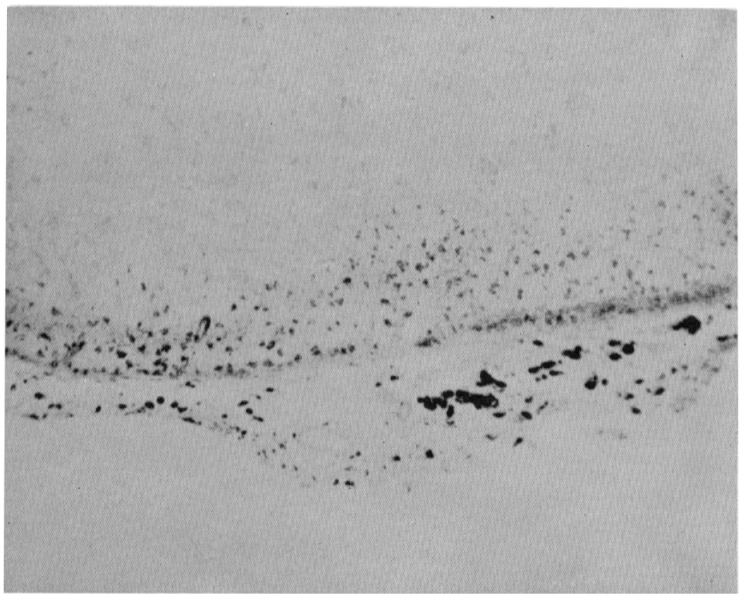

FIG. 2. A section of the basal cortex. Iron staining (Perls' method). Iron-positive in subpial region of the cortex and in thickened pia. (×100)

In Group A and B, Prussian blue reaction was recognized in the leptomeninges over the base of the brain, pons and medulla oblongata, and in the subpial layer of the basal cortex especially of the temporal tips. In the

leptomeninges of the other parts of the brain, namely, the fissura interhemispherica and the upper surface of the midbrain, iron deposits were also recognized, but in minor degree without diffusion into the cerebral tissues. Leptomeninges of the cisterna magna into which the injections were performed directly contained much iron deposit, but this is exceptional. Iron was not demonstrated in the cerebral ventricles, nor in their walls. Leptomeninges were wholly thickened and adherent to the dura mater, which made it difficult to take out the brain together with its leptomeninges especially in Group A. Thickening was remarkable microscopically at the base of the brain.

In Group D which had received injections of hemolysed red blood cells, iron deposit was scarcely noticeable in any part of the brain or leptomeninges, in spite of severe reactions appeared in animals on injection of this solution. Thickening of the leptomeninges was of minor degree.

## DISCUSSION

Hydrocephalus following subarachnoid hemorrhages was experimentally studied. Repeated intracisternal injections of whole blood can result in ventricular dilatation in rabbits. The volume of the blood injected was so determined that it can represent a massive intracranial hemorrhage in rabbits. One ml of liquor can be barely withdrawn from the cisterna magna of rabbit; this gave an estimation how much amount of blood should be introduced at one time.

Blood accumulates mainly in the cisterna magna and basal cisterns. Iron deposits in the basal parts of the brain indicate that red blood cells precipitate in these regions and, in parts, undergo destruction.

Accumulations of blood cause fibrous adhesions and blockage of these parts of the subarachnoid space. Hydrocephalus was of extraventricular obstructive type in every case, accompanied with dilatations of the aqueduct and the fourth ventricle.

Preliminary experiment had shown that one injection of whole blood does not cause any dilatation of the cerebral ventricles. It was necessary to repeat injetions at least three times. One or two injections did not produce arachnoidal adhesion extent enough to result in the obstruction of cerebrospinal fluid circulation. Ventricular dilatation was more evident and extensive, with increase in the number of injections.

The interval of injections seems to be one of the most important factors. The shorter the intervals, the more effective are the injections. The leptomeningeal fibrosis and arachnoidal block may regress when there is no subsequent introduction of blood. In regard to this regression, time of sacrifice after the last injection may be an important factor. The experimental evidence is, however, not enough to conclude the temporary nature of this kind of

hydrocephalus.

The intracisternal injection of the plasma or the washed red blood cell suspension has actually no effect on the development of hydrocephalus. It is true that the red blood cells or even the plasma can induce leptomeningeal fibrosis though in minor degree, but it cannot result in ventricular dilatation; in other words, it is not so extensive and adherent as to cause an arachnoidal block.

Whole blood is most effective to produce hydrocephalus. There is no doubt that this property exists in the clot formation of the whole blood in the subarachnoid space. Presumably the clot acts as a medium into which fibroblasts of the pia-arachnoid proliferate under the irritating influence of blood components such as liberated from the hemolysed red blood cells. This organization of the clot, if permitted to name as such, explains so adherent fibrosis of the leptomeninges that can result in arachnoidal block and ventricular dilatation.

It needs special mentions about intracisternal injection of hemolysed red blood cells. It was unsuccessful to make a hydrocephalic rabbit with red blood cell suspension. Red blood cells *per se* do not induce sufficient leptomeningeal fibrosis that will cause the arachnoidal block. It was presumed that some components released by destruction of red blood cells cause more intense meningeal reaction and fibrosis. Contrary to the expectation, intracisternal injections of hemolysed red blood cells did not produce any dilatation of the ventricles, but almost invariably, they induced tonic convulsion of the animal, so severe that it frequently resulted in death of the animal.

In contrast to the severe effect on the central nervous system, hemolysed red blood cells do not precipitate in the brain tissue or leptomeninge. Iron deposits were not demonstrated in any part of the brain slices. There is no doubt that hemolysed red blood cells intracisernally injected are so rapidly eliminated from the subarachnoid space into the blood stream that it cannot be converted to hemosiderin to be deposited in brain tissues.

Bagley[1] introduced whole blood repeatedly into the cisterna magna, the subarachnoid space over the cerebral cortex and the cerebral ventricle of the dogs and pups. Of 14 dogs injected with blood only one which received six injections into the cisterna magna showed a well marked dilatation of the ventricles. Hydrocephalus was more frequently seen in pups. Six of 9 pups showed definite dilatation of the ventricles. Ventricular dilatation was always associated with meningeal thickening.

Into the cisterna magna of dogs, Iwanowski and Olszewski[2] repeatedly introduced heparinized blood, hemolysed red blood cells and iron-dextran complex (Imferon), and found that little untoward effect was caused by introduction of the whole blood or hemolysed red blood cells and that iron-dextran complex was more toxic than blood resulting in marked accumulation of iron

positive granules in nervous tissues. Two dogs injected with iron-dextran complex showed hydrocephalus with meningitis and ependymitis.

Finlayson and Penfield[3] demonstrated in cats the effects of intracisternal injection of fluids from a human subdural hematoma and a cyst of a human cerebellar astrocytoma. The authors found an intense leptomeningeal reaction with polymorphonuclear infiltration and inflammatory exudation, and concluded that the breakdown products of blood caused acute postoperative aseptic leptomeningitis in man.

Jackson[4] injected the whole blood which had been incubated at body temperature for a period from 1 to 12 days, into the subarachnoid space of dogs and found that the most irritative reaction was resulted by the blood incubated for 4-8 days. It was concluded that the toxicity is augmented by liberation of products of hemolysis and that the agent in the blood causing the greatest meningeal irritation was probably bilirubin or pigment alike.

According to Barrows, Hunter and Banker[5], oxyhemoglobin, being liberated by hemolysis, appears in cerebrospinal fluid at subarachnoid hemorrhage, and reaching to its maximum within the first few days and bilirubin appears in two or three days after the hemorrhage.

Behar and Feldman[6] injected talcum powder into the cisterna magna of cats and found an intense pia-arachnoid adhesions in the basal cisternae, but no appreciable hydrocephalus was noted. Bachs and Walker[7] also found it difficult to produce hydrocephalus in cats by obstruction of either the aqueduct or the fourth ventricle. This may indicate a species difference in the development of hydrocephalus.

Schurr, McLaurin and Ingraham[8] produced experimental communicating hydrocephalus in dogs. Five dogs were given 2 or 3 cisternal injections of Pantopaque. In all these animals communicating hydrocephalus developed. Three control animals received only one injection and did not become hydrocephalic. Seven animals received interpeduncular kaolin suspension followed by cisternal Pantopaque. In 6 of them, communicating hydrocephalus developed. In 2 dogs that received cisternal injections of 2 ml of Lipiodol, communicating hydrocephalus developed.

The pathological investigations on the subarachnoid hemorrhages in man are numerous, but little attention has been paid to the hydrocephalus resulting from the subarachnoid hemorrhages.

Noetzel[9] reported two cases of meningitis carcinomatosa and hemorrhagic meningitis in which bleeding resulted in diffusion of the blood pigment into the superficial layers of the central nervous system. According to Rosenthal,[10] the subpial cerebral siderosis results from the diffusion of iron "in the form of micelle" and not from the diffusion of hemoglobin.

Smith[11] discussed the pathological changes of the brain in subarachnoid hemorrhage from the view point of vasospasm and cerebral edema, but there

is no description about the evidence of hydrocephalus in her autopsy materials. Hammes[12] examined 53 necropsied cases of intracranial aneurysm with special reference to the meningeal response to subarachnoid blood. The author did not find any evidence of hydrocephalus in this series of cases. But in this study definite fibrosis developed in ten days or so after the bleeding. Hammes used the term "organization of the clot" and noticed an irregular and patchy distribution of the fibrotic response. He found that the pia-arachnoid adhesion is characteristic of the meningeal fibrosis after subarachnoid hemorrhage.

McGee, Van Patter, Morotta and Olszewski[13] reported two cases of subpial cerebral siderosis, one from ependymoma of the spinal cord with evidence of old hemorrhages and the other from a bleeding of venous malformation in the lateral ventricle. Both of them showed a moderate and symmetrical enlargement of the lateral ventricles. It was postulated that subarachnoid hemorrhages occurred repeatedly or continuously over a long time.

Finlayson and Penfield[3], in their report of cases of "acute postoperative aseptic leptomeningitis", considered that blood or fluid with high protein content is discharged from the operative site into the ventriculosubarachnoid system through the internal fistula and causes meningeal fibrosis with a high incidence of communicating hydrocephalus. Foltz and Ward[14] reported 10 cases of communicating hydrocephalus secondary to subarachnoid bleeding, the etiology of which was operative hemorrhage (2 cases), rupture of an intracranial aneurysm (5 cases) and head trauma (3 cases). The operative procedures were occipital craniotomy and suboccipital craniectomy in which blood escaped into the basal cisterns. Aneurysms were situated at or near the basal cisterns (the internal carotid, anterior cerebral and basilar aneurysms) and two or three episodes of bleeding had been recorded before the hydrocephalus was recognized. Recognition of the hydrocephalus after hemorrhage varied from 2 to 12 weeks. Kibler, Couch and Crompton[15] reviewed 13 cases of hydrocephalus following spontaneous subarachnoid hemorrhage (5 cases of their own and 8 cases from the literature) of which 9 cases were from aneurysms on or near the circle of Willis and 4 cases were secondary to angiomatous malformations. Multiple bleeds were common to both groups of cases. The authors suggested that the mere physical presence of subarachnoid blood might induce acute dilatation of the cerebral ventricles shortly after the bleeding. Shulman, Martin, Popoff and Ransohoff[16] presented 7 cases with aneurysms of the anterior portion of the circle of Willis and 1 case with an arteriovenous anomaly within the lateral ventricle, all of which showed communicating or "extraventricular obstructive" hydrocephalus. In 4 of these 8 cases, there was clear historical evidence of multiple episodes of bleeding.

Hydrocephalus in infancy must be considered separately. Lorber and Bassi[17] reported that in their series of 110 hydrocephalic infants without spina bifida there were 21 cases in which the intracranial hemorrhage in the newborn

period was strongly suggested.

The effect of intrathecally injected steroid on the meningeal reaction which is caused by irritant agents, such as blood, Pantopaque or talcum powder, was studied experimentally or clinically by Smith, Ross[18], Turon, Sêris[19], Feldman and Behar[20)21)]. The possibility of preventing hydrocephalus of this origin with steroid may be worth studying.

## II. Absorption of Intact Red Blood Cells from the Subarachnoid Space Into the Blood

### INTRODUCTION

Although it was proved experimentally that only the whole blood (blood clot) in the subarachnoid space can produce a sufficient meningeal fibrosis (pia-arachnoid adhesion) resulting in the commuicating hydrocephalus, it is, without doubt, the break-down product released from red blood cells that exerts the most severe effect on the central nervous system, and that causes the hydrocephalus in case it contacts with the meninges continuously or repeatedly. Therefore, it becomes necessary to investigate whether the whole subarachnoid blood must undergo complete hemolysis before it is absorbed from the subarachnoid space, and whether there is any passageway of intact blood cells from the space into the circulating blood.

The whole blood, if introduced repeatedly into the subarachnoid space, could result in hydrocephalus; thus it is quite probable that this procedure caused a blockage of the cerebrospinal fluid circulation. It would be reasonable to expect that the absorption mechanism for red blood cells will also be impeded if a subarachnoid blockage is induced by previous cisternal injections of whole blood.

Evidence for the absorption of intact red blood cells and the variability of its rate in certain conditions seem to provide an important clue in understanding physiology of absorption of the cerebrospinal fluid and particles from the subarachnoid space into the blood.

### MATERIALS AND METHODS

Adult rabbits weighing over 3.0 kg were used. $^{51}$Cr-RBC was prepared from the autogenous blood in every instance.

*Preparation of $^{51}$Cr-RBC*

2.5 ml of heparinized blood was obtained from the ear artery. It was washed once with equal volume of cold saline (0.9%) and gently centrifuged (1,000–1,500 rpm). The top fluid containing most of plasma protein was

discarded. To the sediment (red blood cells) 20 $\mu$C of $Na_2{}^{51}CrO_4$ was added and sturred. In such a way, by removing most of plasma protein, red blood cells were tagged with $^{51}Cr$ efficiently. The mixture was placed in room temperature for 1–2 hours, being sturred occasionally. $^{51}Cr$-RBC suspension was then washed four times with cold saline and made to 2.5 ml with saline.

*Intracisternal injection of $^{51}Cr$-RBC suspension*

Rabbits were anesthetized with intravenous or intraperitoneal administration of phenobarbital sodium, or with ether. Intravenous phenobarbital anesthesia was chosen in most occasions.

The animal was laid on its right side and the neck was flexed to get enough intervertebral space for cisternal puncture. Cisternal puncture was easily succeeded in general and was confirmed by dripping of cerebrospinal fluid from the needle. About 1.0 ml of $^{51}Cr$-RBC suspension was injected slowly into the cisterna magna. The same syringe and needle that were used for a cisternal injection were preserved for preparation of standard diluted solution of $^{51}Cr$-RBC suspension. Nuchal region was compressed with a finger tip for a few minutes after an injection to prevent eventual leakage of $^{51}Cr$-RBC from the needle pore of the meninges.

*Preparation of standard diluted solution of $^{51}Cr$-RBC suspension*

Using the same syringe and needle that had been used for an intracisternal injection, exactly equal volume of $^{51}Cr$-RBC suspension was diluted with water to 500.0 ml and hemolysed. This standard diluted solution ($\times 500$) stands for counting to calculate the dose that was injected intracisternally.

*Sampling of blood and measurement of its radioactivity*

After a cisternal injection, samples of blood were withdrawn from the ear artery every 24 hours for 6 days, 1.4 ml at each time, of which 1.0 ml was taken into a flat based test tube with a whole pipette and its radioactivity was counted. Well-type scintillation counter was used for measurement.

*Measurement of circulating blood volume of rabbits*

The blood volume of each rabbit was measured immediately after the last blood sample was withdrawn. Five minutes after adequate dose of RISA was injected from the ear vein, arterial blood was withdrawn from the ear artery of the opposite side and its radioactivity was counted. Circulating blood volume was calculated as follows:

$$\text{Blood volume (ml)} = \frac{I}{C'-C}$$

$I$: Total injected dose of RISA (cpm)
$C'$: R.A. of blood sample after RISA injection (cpm/ml)
$C$: R.A. of blood sample before RISA injection (cpm/ml)

*Calculation*

Radioactivities recovered in the circulating blood were estimated as per cent of the total injected dose of $^{51}$Cr-RBC. It must take into account the sum of radioactivities contained in blood samples that were withdrawn for measurement (1.4 ml at each day) until the day before a sampling, though it is a relatively small fraction of total radioactivity in circulating blood. Results were calculated as follows:

$$\text{R.A. recovered in circulating blood (\%)} = \frac{\text{R.A. (cpm) of a sample (1.0 ml)} \times \text{Blood volume (ml)} + \text{Sum of R.A. (cpm) withdrawn}}{\text{Total injected dose (cpm)}} \times 100$$

*Measurement on rabbits previously treated with intracisternal injections of whole blood*

Aside from the normal rabbits, the absorption rate of $^{51}$Cr-RBC from the subarachnoid space was measured in rabbits which had received two intracisternal injections of the whole blood before the measurement. This experiment was carried out on 7 rabbits. One and half ml of whole blood was injected into the cisterna magna, tow times at 7 days interval. Measurement was performed 7 days after the second injection. Experimental procedures and calculation were the same as in normal rabbits.

## Results

*Normal rabbits*

Table 5 shows the absorption rate of $^{51}$Cr-RBC from the subarachnoid space of normal rabbits. Ten experiments were performed. Mean values ±S.D. at each day are shown at the bottom. It is noted from large standard deviations (s) that individual variations are great.

Fig. 3 shows the mean values plotted on a semilogarithmic paper; per cents of the total injected dose are on the ordinate and days elapsed after

TABLE 5. Absorption Rate of $^{51}$Cr-RBC from Subarachnoid Space in Normal Rabbits (Per Cent of Total Injected Dose)

| Days No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 11.0 | 12.3 | 11.6 | 12.3 | 12.3 | 12.0 |
| 2 | 11.4 | 19.1 | 20.0 | 20.0 | 19.0 | 19.2 |
| 3 | 8.7 | 12.6 | 12.8 | 13.3 | 12.5 | 13.0 |
| 4 | 24.1 | 33.4 | 33.5 | 31.6 | 29.1 | 28 9 |
| 5 | 10.3 | 14.3 | 14.4 | 14.6 | 14.9 | 15.2 |
| 6 | 5.6 | 6.8 | 6.6 | 8.9 | 9.2 | 8.6 |
| 7 | 21.8 | 29.0 | 31.7 | 29.7 | 27.7 | 27.3 |
| 8 | 5.9 | 7.4 | 7.0 | 5.6 | 7.0 | 7.0 |
| 9 | 5.7 | 11.1 | 11.1 | 9.4 | 8.8 | 9.7 |
| 10 | 13.7 | 16.5 | 17.5 | 16.7 | 16.0 | 15.4 |
| Mean±s | 11.8±6.1 | 16.3±8.3 | 16.6±8.9 | 16.2±8.2 | 15.7±7.2 | 15.6±7.1 |

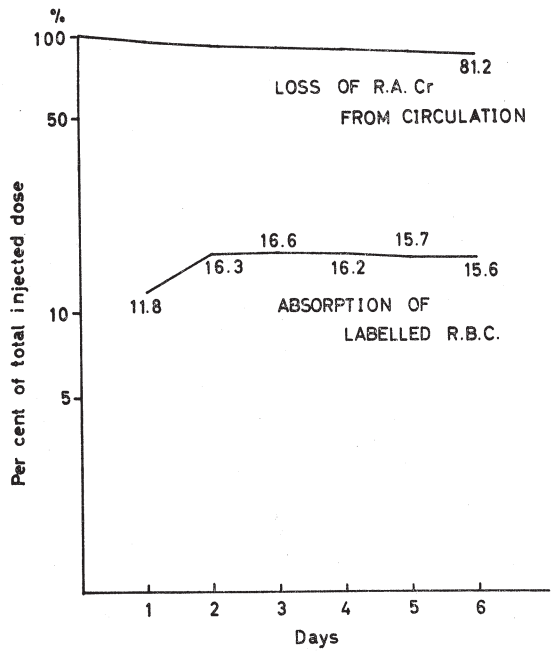


FIG. 3. Absorption curve of $Cr^{51}$-RBC from the subarachnoid space into the blood (lower curve) and disappearance curve of radioactive chromium from the blood (upper curve)=survival curve of $Cr^{51}$-RBC. *Normal rabbits.*
Ordinate; per cent of total injected dose (logarithm).
Abscissa; days after injection.
See the text.

cisternal injection on the abscissa. It is known from this curve that radioactivity in the circulating blood reaches its maximum (16.6%) on the 3rd day after the cisternal injection, and it declines insidiously (15.6% on 6th day).

It is, however, absolutely necessary to compare this curve with the curve of extinction of radioactivity after $^{51}$Cr-RBC is injected intravenously. The latter was obtained from 5 experiments, in which 1.0 ml of $^{51}$Cr-RBC suspension was injected into the ear vein and radioactivities recovered in circulating blood were counted every 24 hours for 6 days.

Assuming it as 100% the radioactivity of blood sample which was obtained 5 minutes after intravenous injection of $^{51}$Cr-RBC, radioactivities of subsequent blood samples were counted and calculated. Mean values on each day were as follows:

| Days | 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| % | 100.0 | 92.6 | 91.5 | 88.0 | 88.1 | 84.1 | 81.2 |

These values are plotted in the top part of Fig. 3.

The curve of $^{51}Cr$ RBC absorption from the subarachnoid space declines a little more slowly than that of $^{51}Cr$-RBC loss from the circulating blood. Probably it indicates that absorption of $^{51}Cr$-RBC from subarachnoid space still continues after the 3rd day of cisternal injection, even though it is very scanty.

*Rabbits previously treated with intracisternal injections of whole blood*

Table 6 shows the absorption rate of $^{51}Cr$-RBC from the subarachnoid space

TABLE 6. Absorption Rate of $^{51}Cr$-RBC from Subarachnoid Space of Rabbits Previously Treated with Intracisternal Injections of Whole Blood

| Days No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 26.0 | 37.6 | 37.8 | 35.4 | 34.9 | 31.6 |
| 2 | 12.7 | 25.1 | 23.9 | 22.3 | 23.8 | 20.2 |
| 3 | 16.4 | 24.4 | 18.4 | 19.1 | 17.3 | 23.9 |
| 4 | 25.8 | 32.5 | 32.6 | 28.8 | 30.1 | 29.5 |
| 5 | 29.9 | 31.5 | 32.4 | 33.3 | 30.1 | 29.1 |
| 6 | 20.0 | 23.9 | 25.0 | 23.2 | 23.0 | 22.5 |
| 7 | 12.7 | 20.5 | 20.5 | 18.8 | 16.1 | 15.0 |
| Mean±s | 20.5±6.4 | 27.9±5.6 | 27.2±6.6 | 25.8±6.2 | 25.0+6.5 | 24.5±5.5 |

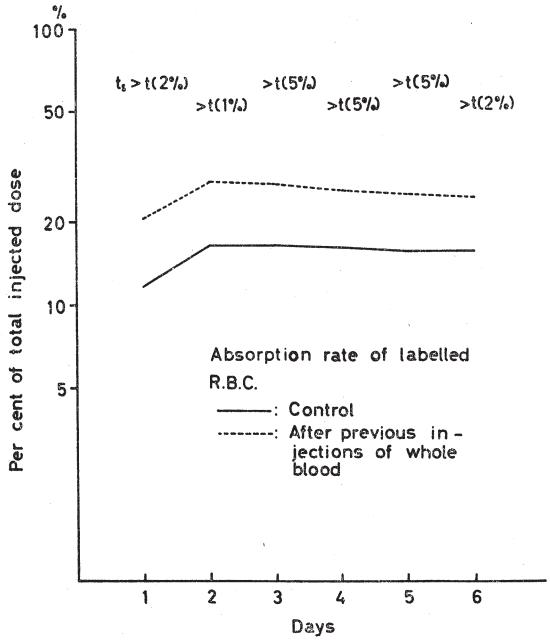


FIG. 4. Absorption curve of $Cr^{51}$-RBC from the subarachnoid space into the blood; upper curve: *rabbits previously injected with whole blood,* lower cnrve: ncrmal rabbits (the same as in Fig. 3). Differences between the two curves are statistically significant ($P<0.01$-$0.05$), as figured above the curves ($t$-distribution).

of rabbits which had been treated previously with intracisternal injections of the whole blood.

As are seen on the smaller standard deviations, the individual differences are not so great as in normal rabbits. Mean values are uniformly greater than in normal rabbits. This elevation of the absorption rate is statistically significant ($P<0.01$–$0.05$).

Mean values were plotted on a semilogarithmic paper in comparison with the curve of normal rabbits (Fig. 4). Recovery of radioactivity in the circulating blood reaches its maximum (27.9%) on the 2nd day of cisternal injection. Declining of the curve is also very gradual as in normal rabbits. Two curves run almost in parallel on semilogarithmic paper, that is, there is little difference between them as to course of absorption of $^{51}$Cr-RBC. Differences between the mean values of the normal and treated groups on each day were statistically estimated and figured on the top of Fig. 4.

*Injection of $^{51}$Cr-RBC in the epidural tissue*

It is of critical importance to prove that $^{51}$Cr-RBC is absorbed from the subarachnoid space but not from the epidural tissue into which a leakage of any amount of $^{51}$Cr-RBC might take place.

1.0 ml of $^{51}$Cr-RBC suspension was injected into the epidural tissue, and radioactivity in the ciculating blood was measuered (Table 7, a). Radioactivities in all blood samples were very scanty (0.0–3.1%). It is inconceivable that

TABLE 7

a. Recovery of radioactivity in circulating blood after *epidural* injection of $^{51}$Cr-RBC suspension (per cents of total injected dose)

| Days No. | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 0.0 | 0.6 | 0.6 | 0.8 | 1.4 | 0.8 |
| 2 | 1.1 | 0.5 | 3.1 | 2.2 | 1.0 | — |
| 3 | 0.6 | 0.9 | — | — | — | — |

b. Recovery of radioactivity in circulating blood after intracisternal injection of *hemolysed* $^{51}$Cr-RBC

| Days No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| 1 | 2.7 | 1.1 | 1.2 | 0.2 |
| 2 | 4.1 | 3.4 | 1.3 | — |
| 3 | 3.4 | 2.0 | — | — |

c. Residual radioactivity after intravenous injection of *hemolysed* $^{51}$Cr-RBC (R.A. of blood at 5 min after injection=100.0%)

| Time No. | 5 min | 15 min | 35 min | 75 min | 24 hours | 48 hours |
|---|---|---|---|---|---|---|
| 1 | 100.0 | 79.2 | 59.0 | 42.0 | 4.2 | — |
| 2 | 100.0 | — | — | — | 6.5 | 5.9 |

$^{51}$Cr-RBC leaks into epidural tissue and is absorbed so rapidly in intact state thus maintaining its high level of the radioactivity in circulating blood. Recovery of radioactivity is insignificant after an epidural injection of $^{51}$Cr-RBC.

*Intracisternal injection of hemolysed $^{51}$Cr-RBC.*

There may be some doubts whether $^{51}$Cr-RBC can be absorbed from the subarachnoid space in intact state. It must be investigated whether $^{51}$Cr-RBC is completely hemolysed and then absorbed from the subarachnoid space.

The $^{51}$Cr-RBC was hemolysed with distilled water and injected intracisternally after being made isotonic with sodium chloride. Recovery of radioactivity in the circulating blood was significant for 24 hours (2.7–4.1%), but it decreased rapidly to insignificant level in the following days (0.2–1.3%) (Table 7, b). This indicates that the hemolysed $^{51}$Cr-RBC or liberated radioactive chrome which was absorbed from subarachnoid space does not remain for hours in circulating blood. It is also inconceivable that the hemolysed $^{51}$Cr-RBC is absorbed from the subarachnoid space and maintain such a high level of radioactivity as in cases of intact $^{51}$Cr-RBC injection. Some portion of the $^{51}$Cr-RBC are absorbed from the subarachnoid space in intact state.

*Intravenous injection of hemolysed $^{51}$Cr-RBC*

To confirm more completely the fact that the hemolysed $^{51}$Cr-RBC, if it is absorbed from the subarachnoid space, remains only for a short period of time in circulating blood, the $^{51}$Cr-RBC hemolysed in distilled water was injected into the ear vein and radioactivity was measured (Table 7, c).

Assuming that the radioactivity of the blood at 5 minutes after the injection to be 100%, 93-96% of the total injected dose disappeared from circulating blood within 24 hours.

## DISCUSSION

The absorption mechanism of water, ions, proteins and other molecules from the subarachnoid space has been the object of many works. Few studies, on the other hand, have been devoted to the absorption of blood cells or other particles introduced into the subarachnoid space.

Sprong[22] found, in clinical observation of cases with the traumatic subarachnoid hemorrhage and on animal experiments with dogs, that blood cells disappeared within 5 or 6 days from the spinal fluid. In 5 days, numerous mononuclear phagocytes were seen engorged with the red blood cell débris and their abundance was sufficient to indicate strongly that their activity may play a major part in cerebrospinal fluid clearance.

Simmonds[23] studied the rate and pathways of removal of red blood cells and protein from the cerebrospinal fluid of rabbits and cats after intracisternal introdution of dyed blood (stained with T 1824). Analysis of the cerebrospinal

fluid suggested that erythrocytes began to leave the subarachnoid space 1 to 3 hours after injection and that most of them disappeared within 48 hours. He found that phagocytosis of erythrocytes was not striking for at least 24 hours after introduction of the blood, and concluded that phagocytosis could not account for the large proportion of cells which disappeared during that period. Plasma protein was removed more readily than the red blood cells. Consequently, he postulated that the greater proportion of erythrocytes must pass intact through the meninges into the venous system. Furthermoer, Simmonds[24] developed his study with the use of red blood cells labelled with $P^{32}$. Rabbits were injected with $P^{32}$-RBC into the cisterna magna and afterwards radioactivity in the circulating blood was counted for a period of several hours. In his study the labelled red blood cells were steadily absorbed into the blood stream at a rate of about 1% of the injected dose per hour for the fist 10 hours. Ligation of the cervical lymph ducts did not measurably affect the rate of absorption of labelled red blood cells. The author hypothesized that the arachnoid villi constitute the non-lymphatic pathway for absorption of labelled red blood cells. $P^{32}$ was an unsatisfactory label for red blood cells, since the cells exchange $P^{32}$ fairly rapidly. Namely loss of radioactivity from labelled red blood cells was 40% after 8 hours and 45% after 16 hours. Therefore, the analysis of the data beomes complicated and the usefulness of measuring the radioactivity is limited for only a short period of time.

Dupont, Van Wart and Kraintz[25] injected intracisternally on dogs radioalbumin, $Cr^{51}$-RBC and radiocolloidal gold, and investigated the rates of clearance from the cerebrospinal fluid. Radioalbumin was detectable in the plasma after 1 to 2 hours reaching a plateau in 16 to 20 hours. $Cr^{51}$-RBC and radiocolloidal gold did not appear in the circulating blood in significant level. It was concluded that red blood cells and the colloidal gold particles were trapped by the mesothelial lining cells of the subarachnoid space, while albumin was cleared rapidly by a very efficient mechanism.

Another investigation on the absorption rate of red blood cells using $Cr^{51}$-RBC was performed on dogs by Adams and Prawirohardjo[26]. At the end of 48 hours after the cisternal injection, 24.2% apeared in the circulating blood, 1.5% remaining in the cerebrospinal fluid and 73.2% in the brain, meninges and spinal cord; 98.9% of the total iniected dose could be accounted for. The highest rate of absorption into the circulating blood was noted within the first 24 hours. Examination of the cervical lymph nodes and lymph obtained from the cervical lymph duct failed to reveal any radioactivity; thus eliminating the lymphatic system as a route of absorption. The authors also injected hemolysed $Cr^{51}$-RBC into the cisterna magna of 2 dogs and noted the maximum counts to be less than 0.01% of the total injected dose in the circulating blood in 24 hours. All of the radioactivity was present in the plasma. Ebaugh[27] found that the intravenously injected hemoglobin tagged with $Cr^{51}$ was not

incorporated into the circulating red blood cells for at least one month and also that $Cr^{51}$ released from the hemolysed $Cr^{51}$-RBC could not be incorporated into the recipient's red blood cells.

Bradford and Johnson[28)29)] introduced the homologous $Fe^{59}$-RBC into the cisterna magna of dogs with the injection pressure of 130 to 400 mm of mercury and measured radioactivity in the circulating blood. The absorption rate of the $Fe^{59}$-RBC was accelerated with high subarachnoid pressure and from 12.4 to 53.0% of the injected dose was recovered in the circulating blood within three and a half hours or less. When volume for volume replacement of the cisternal fluid by the $Fe^{59}$-RBC was made, up to 20% within five and a half hours and up to 51% in 24 hours were recovered in the circulating blood. Negative results were obtained, when the radioactive blood was injected into the muscles. Homologous red blood cells have a disadvantage as compared with the animal's own cells. Simmonds[24)] noted that homologous cells were absorbed less than the animal's own cells.

It seems to be very difficult to find directly the absorption pathway of red blood cells or other particles through the meninges into the blood stream[24)30)]. There are a few hypotheses based on experimental observation.

Bradford and Sharkey[31)] found that, if erythrocytes or carbon particles of India ink were injected within the subarachnoid space prior to an injection of normal saline, the flow of saline out of the subarachnoid space was blocked, and hence the flow of saline into the subarachnoid space was also impeded. The colloidal gold had the same effect if injected into the subarachnoid space before an introduction of saline. Methylene-blue and toluidine-blue solutions which were injected into the subarachnoid space stained the orbital tissues, nasal drippings or both of them. The radioactive colloidal gold injected intrathecally under high pressure were recovered in nasal drippings. The authors emphasized the perineural spaces along the optic and olfactory nerves as a pathway out of the subarachnoid space in dogs.

Welch and Friedman[32)] presented a newer concept of the anatomy of the arachnoid villi. Contrary to the concepts presented by Schaltenbrand[33)] and Turner[34)] who described the essential structure of the arachnoid villi as a blind diverticulum of the arachnoid into a venous ohannel, it was shown, based on the experiment in African green monkeys, that an arachnoid villus is a labyrinth of coapted tubes which connect, from place to place, with each other and which open to the subarachnoid and subdural spaces on the one hand and to the venous channels of the dura on the other. The structure is so delicate that should the pressure surrounding it exceed that within, the villus will be collapsed, the tubes effaced and a passageway will no longer exist. Thus it forms the structural basis for a valved canalicular system allowing the passage of whole cerebrospinal fluid and various particles such as erythrocytes, colloidal gold (0.2–0.3 $\mu$), yeast (3–6 $\mu$) and polystyrene latex spheres (1.17–1.8 $\mu$, 6.4–

12.8 $\mu$)[30], into the blood. The flow of the cerebrospinal fluid and particles through a dural disc which has villi related to the lacunae laterales of the superior saggital sinus, was shown essentially unidirectional from the subarachnoid space into the sinus.

Although fascinating, it is difficult to interprete, by the concept of valved mechanism of the villi, the fact that the absorption of red blood cells is accelerated after the previous injections of blood, while that of cerebrospinal fluid is impeded resulting in hydrocephalus. It seems necessary to take into account a biological mechanism such as diapedesis that can be activated in some pathological conditions.

Thomas and Kerr[35] demonstrated that the radioactive colloidal gold injected into the cisterna magna of cats appears in the blood stream within 5 minutes. Electron microscopic studies showed that the radioactive colloidal gold penetrates through the capillary endothelium of arachnoid villi and does not through the cerebral cortical capillaries. Moreover, the cytoplasmic organelles containing a large amount of colloidal gold are present only in the vessels of the villi and never in cortical capillaries.

This electron microscopic study, however, cannot explain the passage of large particle such as erythrocytes, though it provides a specialized absorptive function for the arachnoid villi in passage of cerbrospinal fluid, proteins and very small particles such as colloidal gold.

It seems most likely that the absorption mechanism of the cerebrospinal fluid and very small particles such as colloidal gold on the one hand, and that of large particles such as erythrocytes on the other hand, are different from each other, but the latter also participates in the former mechanism.

## SUMMARY

1) A genesis of hydrocephalus from subarachnoid hemorrhages was experimentally studied in adult rabbits.

2) Whole blood, red blood cells, plasma and hemolysed red blood cells were repeatedly introduced into the cisterna magna, and the evidence of hydrocephalus was investigated.

3) Hydrocephalus was noted only in rabbits which received whole blood more than three times, and not in other groups.

4) It is concluded that an organization of the clot in the subarachnoid space causes the pia-arachnoid adhesion and blockage of the cerebrospinal fluid circulation which results in hydrocephalus.

5) However, it is the breakdown products of red blood cells that cause the meningeal reaction which results in meningeal fibrosis and the severe convulsion of the animals.

6) Iron pigments were present in the leptomeninges especially over the

base of the brain and in subpial layers of the basal cortex. They were absent in the brains of rabbits which were injected with hemolysed red blood cells, suggesting rapid elimination of the breakdown products of blood cells from the subarachnoid space.

7) Absorption of intact $Cr^{51}$-RBC from the subarachnoid space into the blood was investigated in adult rabbits after the cisternal injection of $Cr^{51}$-RBC suspension.

8) Up to 16.6% of the total injected dose was absorbed in 3 days but the highest absorption was noted within 24 hours.

9) Absorption rate of the $Cr^{51}$-RBC was elevated significantly in rabbits which previously had had two intracisternal injections of the whole blood.

10) This paradoxical enhancement of RBC absorption suggests that the absorption mechanism of large particles is different from that of CSF and very fine particles, in some respects.

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