PASSAGE OF INTACT IRON-LABELED ERYTHROCYTES FROM SUBARACHNOID SPACE TO SYSTEMIC CIRCULATION IN DOGS*

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In the course of experiments in dogs in which injection of blood into the spinal subarachnoid space was followed by injection of saline, it was noted that in some instances the subarachnoid space to a very considerable extent was washed free of blood. It was decided to try to determine the disposition of the subarachnoid blood by tagging it with radioactive materials. At first, Cr⁶¹ was used. In 2 animals, 5.2 per cent and 41.1 per cent of the radioactivity was recovered in the blood of the recipients, respectively, within a few hours. In calculating the percentage of radioactivity recovered in the blood of the animal, the blood volume (ml.) was estimated by taking 7.2 per cent of the body weight in grams. However, only 50 per cent of the radioactivity of the Cr⁶¹ blood injected was confined to the red blood cells. In order to obtain blood with high radioactivity confined largely to the red blood cells, a donor dog was prepared by giving 5 daily injections of Fe⁵⁹, 2 microcuries each. Repeated assays showed over 99 per cent of the radioactivity confined to the red blood cells. The donor dog subsequently received additional injections of Fe⁵⁹ (Table 1).

In initial experiments moderately high intraspinal pressures were used, which produced occasional elevations of blood pressure and, at times, transitory respiratory arrest. Blood pressure, spinal subarachnoid pressure and respiration were recorded on a physiograph. Gravity pressures of 130 mm. of mercury and occasional injection pressures of from 300 to 400 mm. of mercury were used.

Animals often tolerated high pressures for short intervals better than more intermediate pressures for longer periods. From 12.4 per cent to 53 per cent of the radioactivity injected intraspinally (Fe⁵⁹ blood) was recovered in the blood of the recipient dogs (Dogs 3–6). By using smaller quantities of blood and more prolonged periods for the injection of saline following the blood (up to 6½ hours), similar percentages of recovery were found using gravity pressures as low as 50 mm. of mercury (Dogs 7–10). Finally, cisternal injections of Fe⁵⁹ blood, 7 to 10 ml., were made after removal of a slightly larger volume of cerebrospinal fluid (Dogs A–E). Up to 27 per cent of the radioactivity was

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### TABLE 1

<table>
<thead>
<tr>
<th>Date</th>
<th>Fe⁵⁹ Intravenous (microcuries)</th>
<th>Blood Withdrawals (ml.)</th>
<th>Hematocrit (per cent)</th>
<th>Net Count per Min. (1 ml.)</th>
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recovered in $5\frac{1}{2}$ hours and up to 51 per cent in 24 hours. No pressures were taken in these dogs, but any elevation of pressure was simply a physiological response to the presence of blood.

When multiple specimens were taken, there was always an increase in radioactivity in succeeding specimens. The radioactivity in the spleen (1 gm.) is usually $\frac{1}{2}$ (or less) of that of the blood (1 ml.). The radioactivity of the plasma of the recipient dog varies between 1 and 5 per cent of that of the blood, but approximates 1 per cent when the experimental error of counting radioactivity is the least.

**METHOD**

Dogs were anesthetized with pentobarbital sodium, 30 mg./kg. intravenously. A lumbar laminectomy was performed just above the sacrum and a polyethylene tube was introduced about 5 cm. upward within the subarachnoid space. A ligature was placed around the dura mater and the contained spinal cord, cauda equina and polyethylene tube. A “T” connector was placed so that the subarachnoid tube was connected to a pressure transducer and to a syringe or a container for gravity injection. Between 10 and 45 ml. of radioactive blood were injected into the subarachnoid space and followed with varying quantities of normal saline, depending upon the flow under the pressure being used. Most of the specimens of blood from the dog that had received the radioactive subarachnoid injection were 4 ml. and some were 6 ml. The larger samples, especially when the count was only slightly higher than background, increased accuracy. Donor blood usually was taken an hour before introduction into the subarachnoid space, and was heparinized when it was taken. All specimens of blood from recipient dogs were heparinized when taken. The record of the dog, donor of Fe$^{59}$ blood, is summarized in Table 1.

**SPINAL SUBARACHNOID INJECTIONS**

Dog 1. 5-24-61. 16 kg. male. 25 ml. Cr$^{51}$ blood intraspinally. Net count per min. of 1 ml. Cr$^{51}$ blood was 32,839. Sacrificed in 1 hr. to administration of blood. No saline followed. Spinal pressures to above 300 mm. of Hg. 3.2 per cent of radiation recovered in calculated total blood volume.

Dog 2. 5-25-61. 25.4 kg. male. 45 ml. Cr$^{51}$ blood intraspinally followed by 300 ml. N. saline in total of 2 hrs. Spinal pressures to above 300 mm. of Hg. Sacrificed. Net count per min. of 1 ml. Cr$^{51}$ blood was 32,839. 41.0 per cent of radioactivity recovered in calculated total blood volume.

Dog 3. 6-1-61. 16.5 kg. male. 17 ml. Fe$^{59}$ blood intraspinally. Succumbed in 10.5 min. No saline. Spinal pressures to above 300 mm. of Hg. Net count per min. of 1 ml. Fe$^{59}$ blood was 4,464. 20.0 per cent of radioactivity recovered in calculated total blood volume.

Dog 4. 6-8-61. 18.8 kg. male. 20 ml. Fe$^{59}$ blood intraspinally followed by 308 ml. N. saline. Spinal pressures to above 300 mm. of Hg. Sacrificed in 3.1 hrs. Net count per min. of 1 ml. Fe$^{59}$ blood was 15,176. 33.0 per cent of radioactivity recovered in calculated total blood volume.

Dog 5. 6-15-61. 13.2 kg. female. 15 ml. Fe$^{59}$ blood intraspinally followed by 12.5 ml. N. saline in 1$\frac{1}{2}$ hrs. Succumbed. Spinal pressures to above 300 mm. of Hg. Net count per min. of 1 ml. Fe$^{59}$ blood was 16,182. 21.0 per cent of radioactivity recovered in calculated total blood volume.

Dog 6. 6-22-61. 17.4 kg. male. 22.5 ml. Fe$^{59}$ blood intraspinally. Sacrificed in 3.1 hrs. Spinal pressure of 110 mm. of Hg. and only terminally to 240 mm. of Hg. Net count per min. of 1 ml. Fe$^{59}$ blood was 16,894. Recovery in calculated total blood volume was 4.1 per cent in 1 hr., 6.8 per cent in 2 hrs., 12.4 per cent in 3$\frac{1}{2}$ hrs.

Dog 7. 6-22-61. 30.5 kg. male. 16 ml. Fe$^{59}$ blood intraspinally followed by 75 ml. N. saline. Spinal pressure, 50 mm. of Hg. Sacrificed in 3$\frac{1}{2}$ hrs. Net count per min. of 1 ml. Fe$^{59}$ blood was 19,161. Recovery in calculated total blood volume was 17.6 per cent in 1$\frac{1}{2}$ hrs., 41.6 per cent in 3$\frac{1}{2}$ hrs.

Dog 8. 7-6-61. 13.9 kg. female. 15.5 ml. Fe$^{59}$ blood intraspinally followed by 3.5 ml. N. saline. Spinal pressure, 50 mm. of Hg. Sacrificed in 3$\frac{1}{2}$ hrs. Net count per min. of 1 ml. Fe$^{59}$ blood was 21,073. Recovery in calculated total blood volume was 10.9 per cent in 2 hrs., 16.4 per cent in 3 hrs.

Dog 9. 7-13-61. 18 kg. male. 12 ml. Fe$^{59}$ blood intraspinally followed by 82 ml. of N. saline. Spinal pressure, 50 mm. of Hg. Sacrificed in 6$\frac{1}{2}$ hrs. Net count per min. of 1 ml. Fe$^{59}$ blood was 18,623. Recovery in calculated total blood volume was 13.3 per cent in 1$\frac{1}{2}$ hrs., 16.8 per cent in 3 hrs., 33.0 per cent in 4$\frac{1}{2}$ hrs., 45.8 per cent in 6$\frac{1}{2}$ hrs.

Dog 10. 7-13-61. 13.5 kg. male. 10 ml. Fe$^{59}$ blood intraspinally followed by 4 ml. of N. saline. Spinal pressure, 50 mm. of Hg. Sacrificed in 6$\frac{1}{2}$ hrs. Net count per min. of 1 ml. Fe$^{59}$ blood was 18,623. Recovery in calculated total blood volume was 7.8 per cent in 1$\frac{1}{2}$ hrs., 12.0 per cent in 3 hrs., 14.6 per cent in 4$\frac{1}{2}$ hrs., 15.1 per cent in 6$\frac{1}{2}$ hrs.

**CISTERNAL INJECTIONS**

Dog A. 6-15-61. 16.5 kg. male. 10 ml. cerebrospinal fluid withdrawn from cisterna magna and replaced by equal amount of Fe$^{59}$ blood. Net
count per min. of 1 ml. of Fe$^{59}$ blood was 16,182. Recovery of radioactivity in total blood volume was 2 per cent in 3 hrs., 13.7 per cent in 24 hrs., 21.0 per cent in 6 days.

Dog B. 6.29-61. 15.5 kg. male. 10 ml. cerebrospinal fluid withdrawn from cisterna magna and replaced by equal amount of Fe$^{59}$ blood. Net count per min. of 1 ml. of Fe$^{59}$ blood was 19,161. Recovery of radioactivity in total blood volume was 4.7 per cent in 24 hrs.

Dog C. 16.5 kg. male, Dog D. 11.6 kg. female, and Dog E. 9.8 kg. female, on 7-20-61 received 10.0 ml., 8.0 ml., and 7.0 ml., respectively, of Fe$^{59}$ blood intracisternally after removal of a slightly larger quantity of cerebrospinal fluid. Net count per min. of 1 ml. of Fe$^{59}$ blood was 14,309. Recovery of radioactivity was as follows:

<table>
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<tr>
<th>Dog</th>
<th>Net Radioactivity per Cent</th>
<th>3$\frac{1}{2}$ hrs.</th>
<th>12 hrs.</th>
<th>24 hrs.</th>
<th>32 hrs.</th>
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<tr>
<td>E</td>
<td>4.2%</td>
<td>11.3%</td>
<td>51.6%</td>
<td>64.8%</td>
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</table>

RESULTS

Red blood cells, radioactive as a result of administration of Fe$^{59}$ to a donor dog, when introduced into the subarachnoid space of another dog, have been recovered from the circulating blood in quantities amounting to between 5.2 per cent and 64.8 per cent of the quantity of radioactive blood introduced. The subarachnoid space thus appears to be permeable to red blood cells. High intraspinal pressures appear to accelerate the exodus of red blood cells from the subarachnoid space, but the exodus occurs also with moderate intraspinal pressures and with no pressure from without after an equal volume of radioactive blood has replaced cerebrospinal fluid removed by cisternal puncture. The concentration of radioactivity in the sediment and the freedom of the plasma from significant count indicate the passage of intact red blood cells from subarachnoid space to circulating blood. The fact that the radioactive count of the spleen does not reach that even of the circulating blood in experiments lasting 6$\frac{1}{2}$ hours indicates intactness and lack of important damage to red blood cells.

It is considered likely that a dog receiving Fe$^{59}$ blood in the subarachnoid space will, if the radioactive red blood cells are destroyed all or in part, accumulate the Fe$^{59}$ in his bone marrow and form new red blood cells possessing radioactivity (as in the donor dog). However, this source of ambiguity of interpretation probably is of no significance in the first 8 hours of an experiment and is probably inconsequential until after 24 hours.

Neither in these experiments nor in others in which fluids have been injected under pressure into the subarachnoid space has there been any evidence that rupture or break through the subarachnoid space has occurred after which the fluid injected passes more readily from the subarachnoid space. Dogs vary considerably in the time required for saline or blood to exit from the subarachnoid space. However, blood invariably impedes the exodus of saline which follows it. If it is possible to inject a considerable amount of saline (25 to 50 ml.) after blood, the acceptance of saline by the subarachnoid space increases. The marked rise in cerebrospinal fluid and blood pressures resulting from the addition of 0.5 ml. of blood in Dog 4 is shown in Fig. 1.

The radioactivity not recovered in the circulating blood is largely in the subarachnoid space in those animals whose premature death occurred on attempt to inject blood or saline following blood too forcefully.

Fig. 1. Dog 4. Blood pressure (BP) and cerebrospinal fluid pressure (CSF P) are recorded in mm. of mercury. Respiration is recorded on line 3 and time in minutes on line 4. Fe$^{59}$ blood, 14.5 ml., has been administered by lumbar subarachnoid catheter. An additional 0.5 ml. is injected forcibly where line 2 becomes elevated, and the tube is clamped. The resulting rise in blood pressure results in further rise in cerebrospinal fluid pressure. Respiration is arrested temporarily.
Bloody nasal drainage occurred in Dogs 2, 3 and 4. The hematocrit was 2 per cent on the nasal drippings from Dog 4. The presence of radioactivity equivalent to 4 per cent of that of the injected blood (ml. for ml.) was demonstrated in the nasal drippings of Dog 4. The dogs subjected to less drastic elevations of pressure within the subarachnoid space showed no nasal drainage. Wolff and Davies demonstrated both nasal dripping and retro-orbital edema in dogs subjected to subarachnoid injection of saline under considerable pressure.

Blood in variable amounts was present inside the muscle cone about the sleeves (mencinges) covering the optic nerves. Counts as high as 35 per cent of the count of 1 ml. of injected radioactive blood were found in approximately 1 gm. of tissue from about the optic nerve. Considerable radioactivity was present in all specimens of orbital tissue removed (Dogs 4–10) except on the left side in Dogs 9 and 10 when the sleeve of the optic nerve was clamped at the time that radioactive blood was introduced within the subarachnoid space.

REVIEW OF LITERATURE

Study of the circulation of the cerebrospinal fluid was made by Quincke (1872) by introducing emulsion of cinnabar into the subarachnoid space of animals and by Key and Retzius (1875) by introducing Richardson's blue into the subarachnoid space of dead animals. Weed made more refined studies using isotonic solutions of potassium ferrocyanide and iron ammonium citrate in equal amounts. This technique permitted flow of a true solution within the subarachnoid space, but allowed precipitation by dilute hydrochloric acid at the end of the experiment producing Prussian blue granules which remained unchanged during various histologic techniques. Weed also used finely divided carbon particles suspended in fluid (India ink). The introduced materials accumulated about nerve roots in these experiments and, in addition, Weed noted migration of material to the arachnoidal granulations, whose importance he emphasized.

Faber traced Thorotrast and other substances from the subarachnoid space of rabbits to the nasal mucous membrane and nasal cavity. Cervical lymph nodes were also filled with Thorotrast. Field and Brierley, after repeated injection of fine carbon particles (0.5 to 1.5 μ) in the living rabbit under care to avoid increased pressure, were able to demonstrate carbon particles in the retro-orbital fat, in the nasal mucosa, about emerging cranial and spinal nerves, and in lymph nodes of the neck and trunk. Woolam and Millen injected 1.0 ml. of India ink daily into the subarachnoid space of newborn rats, and sacrificed the animals at intervals of up to 3 weeks. They emphasized the presence of colloidal carbon in the paravertebral lymph nodes, the nerve-root cuffs, and the perivascular spaces of the spinal cord. Svane-Knudsen introduced 0.3 ml. of iron solution into the cisterna magna of guinea pigs, precipitating the iron salts at the end of the experiment. In addition to flow to the perivascular spaces of the brain, the Pacchionian granulations, the nasal mucosa and the orbit, he noted flow through the aqueduct of the vestibule and flow about N. VIII.

Courtice and Simmonds showed rapid removal of plasma protein from the subarachnoid space in rabbits by labeling the molecules of plasma protein with the blue dye T1824. Twenty per cent of the injected protein was in the blood stream 5 hours after injection. Simmonds established the removal of erythrocytes from the subarachnoid space in rabbits and cats by studying the subarachnoid space for evidences of residual blood at different times after the injection of autogenous heparinized blood into the cisterna magna. He also demonstrated large numbers of erythrocytes in lymphatic ducts and lymph nodes. In rabbits, red blood cells appeared in the nasal submucosa and in the retro-orbital tissues within 1 to 3 hours of cisternal injection. Simmonds concluded that erythrocytes probably entered the blood stream directly by way of arachnoid villi because he could not account entirely for their disappearance from the subarachnoid space by other routes. In 1933 Simmonds labeled
autologous erythrocytes with P\textsuperscript{32} as phosphate and injected them cisternally into rabbits. He calculated a recovery of 7 per cent of the cells from the circulation in 5 hours and 13 per cent in 16 hours. As Simmonds\textsuperscript{7} acknowledged, P\textsuperscript{32} is not an ideal label. Five per cent of the phosphorus is lost from the erythrocytes each hour, but of the radioactivity remaining in the blood stream of the recipient rabbit, from 85 to 90 per cent remains in the erythrocytes from 5 to 16 hours after injection. It was demonstrated that a negligible amount of the free label entered unlabeled circulating cells. Simmonds\textsuperscript{7} considered P\textsuperscript{32} a more satisfactory label than Fe\textsuperscript{59} because with P\textsuperscript{32} he could label the recipient’s own red blood cells while with Fe\textsuperscript{59} a donor’s cells would have to be used. He found that at 5 hours autologous and homologous cells reached the blood stream of the recipient in equal numbers, but at 16 hours autologous cells exceeded slightly homologous cells. In his experiments Simmonds\textsuperscript{7} found that ligation of the cervical lymphatics did not alter the rate of appearance in the systemic circulation of labeled erythrocytes.

**DISCUSSION**

The experiments presented verify the work of Simmonds\textsuperscript{6,7} who, interestingly, made a strong experimental case for the entrance of erythrocytes directly into the blood stream from the subarachnoid space before he confirmed this occurrence by labeling erythrocytes with P\textsuperscript{32}. In our experiments the Fe\textsuperscript{59} label proved very satisfactory for dogs. Incompatibility of blood in dogs is rare, so the use of donor cells should not cause complications frequently. However, some of the variability in percentage recovery of erythrocytes shown in our experiments could have been caused by partial incompatibility of blood. Fe\textsuperscript{59} is quite superior to P\textsuperscript{32} as a label in that it is bonded firmly within the hemoglobin with less than 1 per cent of radioactivity in the plasma. It is freed only upon destruction of the labeled erythrocytes, and then is rapidly removed from the circulation. Fe\textsuperscript{59} has the handicap of its re-use in the recipient animal so that titers of radioactivity in erythrocytes days after administration cannot be interpreted as indicating the presence of the original labeled erythrocytes.

**SUMMARY**

Evidence for the passage of intact, radioactive red blood cells from the subarachnoid space to the systemic circulation is presented. Observations are made of drainage of radioactive red cells from the nostrils and extravasation of radioactive red cells into the orbit.

**REFERENCES**