# PERITONEAL ABSORPTION

## Red Cells Labeled by Radio-Iron Hemoglobin Move Promptly from Peritoneal Cavity into the Circulation

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Radioactive iron can be incorporated in the hemoglobin of red cells by feeding an anemic dog with suitable amounts of radio-iron. These red cells circulating in the anemic dog are labeled or marked by the radio-iron and such red cells taken from the donor dog can be used in a variety of ways—for example to establish red cell circulating volume (4). These red cells containing radio-iron in hemoglobin can be given *intraperitoneally* and the tables below show a rapid and at times almost complete uptake of these red cells into the circulation. One is tempted to suggest that the peritoneum is in reality a fine-meshed sieve through which red cells pass without difficulty. The diaphragmatic portion of the peritoneum is most active but other areas participate in red cell uptake. These red cells pass through the lymph spaces, lymphatic vessels, and lymph glands without injury or phagocytosis in these normal dogs. A variety of questions remain unanswered which are capable of resolution by the use of the radio-iron red cell technique. We hope to resume work on this problem after the national emergency.

Absorption from the peritoneal cavity is not a new subject. An excellent review by Cunningham in 1926 (3) brings the reader up to that date. Much work is reported dealing with absorption of fluids, dyes, particles (india ink), and red cells. Siperstein and Sansby (6) suggested the transfusion of infants and children by intraperitoneal citrated blood. They did experimental work on rabbits to show that red cell hematocrits increased after intraperitoneal red cell injections. Using nucleated red cells (pigeons) they demonstrated the appearance of these red cells in the peripheral circulation in 15 minutes. Allen (1) and Allen and Vogt (2) did experiments on dogs, rabbits, mice, chicks, and frogs. They demonstrate the presence of *stomata* in the diaphragm through which particles and red cells gain entrance to the lymph spaces. This is not a phagocytic response. Movements of the diaphragm stretch these stomata and permit the passage of cells and particles of 2 to 20 $\mu$ diameter.

### *Methods*

The peritoneal cavities of all the dogs used were believed to be normal and dogs had been under observation from birth (Table 1, anemic dogs). The anemic dogs

had been in our anemia colony for years, maintained at a steady anemia level by suitable bleeding as described elsewhere (7). The dogs in Table 2, anemic and hypoproteinemic, had been under observation months or years. This condition is produced by continued bleeding while the dog is on a very low protein diet plus essential diet accessories and ample iron as described elsewhere (5). The normal dogs were healthy mongrels which had been under observation for many months.

Red cells containing radioactive iron in the hemoglobin were obtained from anemic dogs fed radio-iron which was incorporated into the new formed red cells. These donor dogs can be sustained in an active state by periodic feeding of radioactive iron.

The donor blood was received in citrate or heparin and the red cells separated by centrifuge. The packed red cells in amounts noted were diluted with small amounts of normal saline and given into the peritoneal cavity by hypodermic needle.

## EXPERIMENTAL OBSERVATIONS

We became interested in the subject of peritoneal absorption because of experiments with hemoglobin (5) given in large amounts intraperitoneally. These experiments show that the globin of the hemoglobin participates constructively in the protein body metabolism and contributes to the formation not only of hemoglobin but of plasma protein in the doubly depleted dog (anemic and hypoproteinemic).

Red cells *labeled by radio-iron* in the contained hemoglobin seemed to be perfectly suited to answer the question as to whether the *intact red cells* do appear in numbers in the circulation following injection intraperitoneally. One feels confident under such circumstances that the labeled red cells do appear in the circulation and not some other red cells brought out from some hypothetical reserve storage due to the peritoneal stimulus.

Three types of dogs were used—anemic, doubly depleted (anemic and hypoproteinemic), and normal. We expected that the anemic dog might react more promptly and absorb more red cells than the normal dog because of a real need for red cells. Apparently anemia is not a factor in the rapid absorption of red cells from the peritoneum—in fact if we take the 24-hour period for comparison the normal dog runs ahead of the anemic and far ahead of the doubly depleted dogs (Tables 1 and 2).

Table 1 illustrates a variety of responses in different dogs. There are individual variations between dogs but also variations in the same dog on different occasions separated by intervals of weeks or months. We have no adequate explanation for many of these observed differences and further experiments are needed. The last experiment in Table 1 (dog 34-148) shows no significant absorption over a 5-day period but there was hemoglobinuria during the first 24 hours. This probably means the escape of much of the labeled hemoglobin by way of the urine. Wright (8) studied the factors which may be present in some dogs and lead to red cell hemolysis of donor red cells. No other experiments in Table 1 showed any significant hemolysis but there was

TABLE 1

*Red Cells Containing Radio-Iron Hemoglobin Given Intraperitoneally Appear in Circulation All Dogs Anemic for Long Terms*

| Dog No. | Red cells containing radio Fe injected | Percentage of intraperitoneally injected red cells found in circulation | | | | | | | Red cell hematocrit | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 hr. | 6 hrs. | 24 hrs. | 2nd day | 3rd day | 4th and 5th days | 6th and 7th days | 1 hr. | 24 hrs. | 5th day |
| | *cc.* | | | | | | | | *per cent* | *per cent* | *per cent* |
| 32-5 | 15 | 3 | 48 | 83 | 84 | — | 80 | 62 | 20.9 | 20.0 | 21.8 |
| 32-5 | 10 | — | 35 | 81 | 68 | 77 | 57 | 64 | 21.8 | 21.1 | 20.0 |
| 32-5 | 15 | — | 62 | — | — | — | — | — | 21.8 | — | — |
| 34-145 | 15* | 5 | 9 | 13 | 28 | — | 70 | 88 | 17.7 | 20.9 | 21.1 |
| 34-145 | 15 | — | 0 | 17 | — | — | — | — | 23.2 | 21.7 | — |
| 37-89 | 15 | 2 | 5 | 8 | 13 | — | 59 | 79 | 21.9 | 23.6 | 20.8 |
| 37-21 | 15 | 2 | — | 4 | 6 | — | 23 | 46 | 21.5 | 20.3 | 19.1 |
| 34-148 | 35 | 2 | 2 | 2 | 3 | 3 | 4 | — | 20.8 | 21.1 | 23.6 |
| Average..... | | | 26 | 34 | (Exclusive of Dog 34-148) | | | | | | |

* Preceded by normal saline solution 200 cc. intraperitoneal 30 minutes before red cell injection.

TABLE 2

*Red Cells Containing Radio-Iron Hemoglobin Given Intraperitoneally Appear in Circulation Dogs Anemic and Hypoproteinemic**

| Dog No. | Red cells containing radio Fe injected | Percentage of intraperitoneally injected red cells found in circulation | | | | | | | Red cell hematocrit | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 hr. | 6 hrs. | 24 hrs. | 2nd day | 3rd day | 4th and 5th days | 6th and 7th days | 1 hr. | 24 hrs. | 5th day |
| | *cc.* | | | | | | | | *per cent* | *per cent* | *per cent* |
| 40-33 | 15 | 3 | 6 | 17 | 29 | 35 | 33 | 53 | 23.4 | 24.2 | 23.2 |
| 41-53 | 15 | 3 | 3 | 5 | 6 | — | 17 | — | 26.0 | 23.7 | 30.7 |
| 40-34 | 35 | 1 | 2 | 5 | 11 | 11 | 29 | — | 28.6 | 29.8 | 28.8 |
| Normal control dogs | | | | | | | | | | | |
| 42-1034 | 21 | — | 55 | 115 | 113 | 88 | 95 | — | 41.6 | — | — |
| 42-1034 | 25 | — | 18 | 18 | — | — | — | — | 47.0 | — | — |
| 42-351 | 22 | — | 27 | 52 | — | — | — | — | 47.5 | — | — |
| 42-450 | 22 | — | 7 | 16 | 20 | 25 | 31 | — | 47.0 | — | — |
| 42-450 | 25 | — | — | 29 | — | — | — | — | 50.8 | — | — |
| Average for normal dogs.. | | | 27 | 43 | | | | | | | |

* Plasma protein levels dog 40-33 = 4.3 per cent, dog 41-53 = 4.3 per cent, dog 40-34 = 4.8 per cent.

a faint trace of hemoglobin in the plasma (dog 37-89) 6 and 24 hours after the peritoneal injection. Administration of normal saline before the red cell peritoneal injecton in a single experiment (dog 34-145) does not seem to modify the usual pattern of absorption.

Table 2 shows that the anemic and hypoproteinemic dogs do not absorb more red cells than the normal controls. The experiments are too few to be wholly convincing but the evidence suggests that these doubly depleted dogs use red cells in other ways than by gross peritoneal absorption. Experiments unpublished and published (5) indicated that these dogs use hemoglobin very effectively to supplement the depleted blood proteins. Therefore we may suspect that the doubly depleted dogs of Table 2 break down the red cells and use the hemoglobin rather than take up the whole red cells from the peritoneum as is done by the normal dog. No evidence of hemolysis was observed in plasma or urine.

Normal control dogs (Table 2) show a considerable peritoneal absorption of labeled red cells in 6 and 24 hours. The hematocrits indicate clearly a normal red cell concentration—about double the hematocrit of the anemic and doubly depleted dogs.

Quite apart from the gross and histological evidence, rapid appearance of the labeled red cells in the circulation could hardly be explained on the hypothesis that the red cells were broken down in the peritoneum, absorbed and rapidly reconstituted in the marrow utilizing the radio-iron or labeled hemoglobin set free in the peritoneum.

### *Gross and Histological Picture of Peritoneal Red Cell Absorption*

It is very easy to demonstrate the lymphatic absorption of red cells from the normal dog's peritoneum. Three experiments were done, each dog receiving 15 cc. of packed red cells suspended in about 20 cc. of normal saline. Dogs were killed under ether anesthesia by exsanguination 6 hours after the peritoneal injection of red cells. Tissues were taken for fixation from the diaphragm, gall bladder wall, omentum, without handling, to facilitate histological study.

The findings can be described as for one dog since there was complete uniformity in all three experiments. The peritoneal surfaces were normal except for a film of red cells. Other serous surfaces normal. The viscera were all normal. The lymphatics of the anterior mediastinum stood out as beaded vessels filled with blood (lymph and red cells). The lymph glands about the hilum of the lungs and in the upper mediastinum were swollen and cherry red or mottled red and gray. The main thoracic duct showed no red color in mid-thorax. The mesenteric lymph glands showed no red color in gross. In one dog 150 cc. of cottonseed oil was given by mouth 2 hours after red cell intraperitoneal injection. This dog presented conspicuous milky abdominal lymphatics but otherwise resembled the other two dogs.

Histological sections show beautiful injection of the mediastinal lymphatics with lymph and red blood cells. The related lymph glands show distention of the marginal and central sinusoids with red cells which appear normal. There is no phagocytosis of the red cells and no pigment other than a little coal pigment.

The lymph spaces in the diaphragm are distended with lymph and normal red cells. We did not attempt to demonstrate the stomata described by Allen and Vogt (2). Lymphatics in the wall of the gall bladder and in the omentum sometimes contained lymph but again contained lymph and red cells. The large lymph gland in the ileocecal region contained much lymph but always some normal red cells indicating participation in red cell absorption of lymphatics other than those of the diaphragm.

It came as a surprise to us that normal red cells can pass so readily through the normal lymph gland which is rated as a fairly efficient filter. One who is familiar with the lymph glands adjacent to an area of acute inflammation, anticipates active phagocytosis of red cells within the lymph gland. Perhaps red cells do go readily through such inflamed glands and only the damaged red cells are picked up and digested by the lymph gland phagocytes. Or the inflamed lymph gland may phagocytose normal red cells due to the large surplus of phagocytes responding to the stimulus of damaged tissue.

## SUMMARY

The absorption of red cells from the normal peritoneum of the dog can be demonstrated by means of red cells labeled with radio-iron incorporated in the hemoglobin of these red cells. Absorption in normal dogs runs from 20 to 100 per cent of the amount given within 24 hours.

Dogs rendered anemic by bleeding absorb red cells a little less rapidly—ranging from 5 to 80 per cent of the injected red cells. Doubly depleted dogs (anemic and hypoproteinemic) absorb even less in the three experiments recorded.

This peritoneal absorption varies widely in different dogs and even in the same dog at different times. We do not know the factors responsible for these variations but there is no question about active peritoneal absorption.

The intact red cells pass readily from the peritoneal cavity into lymph spaces in diaphragm and other areas of the peritoneum. The red cells move along the lymphatics and through the lymph glands with little or no phagocytosis and eventually into the large veins through the thoracic ducts.

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