

PASSAGE OF RADIOACTIVE ERYTHROCYTES FROM THE PERITONEAL CAVITY INTO THE BLOOD STREAM DURING EXPERIMENTAL ASCITES*‡

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Peritoneal absorption of intact red blood cells has been recognized for many years (5). It has been shown by differential agglutination (3) and hemolysis (18), and elliptocyte transfusions (10) in human beings, and by tagging with radioiron in dogs (9) that such red cells reach the blood stream intact and apparently in normal condition. The cells probably pass through the stomata in the peritoneum (1, 2), enter lymphatics, and pass through the thoracic lymph nodes without significant phagocytosis (9).

Experimental ascites produced in dogs by constricting the inferior vena cava above the diaphragm has been a useful method of studying ascitic fluid production (11-14). It has been shown that ascitic fluid protein enters the blood plasma from the peritoneal cavity (14). The ascitic fluid produced by these dogs usually contains about 0.1 per cent red cells with no detectable free hemoglobin. It is of interest therefore, to determine whether red cells, as well as protein pass out from the peritoneal cavity of these dogs. Red cells with radioactive iron incorporated into the hemoglobin,—where it remains as long as the cell survives (6, 8),—make a convenient tool for this type of study. The experiments described below indicate that such tagged red cells rapidly cross peritoneal and endothelial barriers and enter the circulating blood in the experimental ascitic dog, though somewhat more slowly than in normal dogs.

Experimental Methods

The animals used were healthy mongrel dogs. Some months before these experiments an aluminum constricting band had been placed about the inferior vena cava above the diaphragm as described in detail elsewhere (12), and the subsequent production of ascites had been proved.

The tagged red cells were prepared by injecting donor dogs intravenously with adequate amounts of radioiron in gelatin (7). After a suitable period for incorporation of the labeled iron into the donors' circulating red cells, blood (50 to 75 cc.) was withdrawn under aseptic conditions, using heparin (liquaemin Roche) as an anticoagulant. This whole blood was

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promptly injected into the peritoneal cavity of the recipient animal with experimental ascites. Before infusion both the donor and recipient were typed,¹ and only compatible blood was injected to guard against possible hemolysis (19).

Two tests were performed on each of three recipient dogs. One of these tests was done when the animal was on a high protein-low salt diet, and therefore producing minimal ascites (13). The high protein-low salt diet was continued during the 3 days of the experiment to ensure minimal ascites formation. The other test was performed when the animal was in active ascites production. Four days before the injection of the tagged cells, 4 gm. of sodium chloride (1 gm. enteric-coated tablets) was given by mouth daily to the recipient dog, in addition to the high protein diet. This amount of sodium chloride was continued during the 3 days of the experiment to stimulate ascitic fluid formation (13).

During the 3 day test period in each series of experiments, blood samples (20 cc.) were drawn at 6, 12, 24, 48, and 72 hours for determination of the radioactivity in the circulating blood. At the end of the 72 hours the peritoneal cavity was emptied as completely as possible of its fluid. The fluid was centrifuged and the amount of the administered radioactivity remaining in the peritoneal cavity was determined on aliquots of the separated red cells. At no time was there detectable hemolysis in the recipients' plasma or ascitic fluid.

The experiments were terminated at 72 hours because it was felt that after this interval significant amounts of radioiron might begin to appear in the recipients' red cells following destruction of aged or injured tagged donated cells with subsequent reincorporation of the released radioiron into newly synthesized hemoglobin (4, 17).

Determinations of the radioactivity of the injected blood and of each of the samples were made by the method described by Yuile *et al.* (20). Blood volumes, using a modified T-1824 dye method (20), were determined prior to or during the course of the experiments. On the basis of these determinations, the per cent of injected red cells which was absorbed into the circulation was estimated for each periodic blood sample. In animals which had previously received radioactive red cells correction was made for baseline activity. Corrections were also made for the radioiron removed in sampling. The dogs were rested 4 to 6 weeks on a high protein-low salt diet between experiments.

EXPERIMENTAL OBSERVATIONS

The pertinent data of the three experiments are shown in Table A. Dog 47-168 which had had the vena cava constricted 11 months previously weighed 11.1 kilos at the time of the tagged red cell injection in the control test (Experiment 1-A) and 11.2 kilos at the end of the 72 hour period. The blood volume was 1016 cc. The administered tagged red cells, in 50 cc. whole blood, contained 64,600 counts per minute and were given intraperitoneally. The radioactivity, expressed as percentage of the amount administered, as it appeared in the circulation, was 0.0, 8.7, 24.0, 38.2, and 43.4 per cent at 6, 12, 24, 48, and 72 hours, respectively. A paracentesis at 72 hours yielded 480 cc. of ascitic fluid which contained 43.2 per cent of the injected radioactivity. Thus 86.6 per cent of the original amount is accounted for, leaving only 13.4 per cent in other, inaccessible sites.

A second test (Experiment 1-B) with the animal actively forming ascitic fluid was performed after a convenient interval. At the time of the intraperitoneal injection of red cells, the animal weighed 12.2 kilos and 72 hours later,

¹We are indebted to Dr. R. M. Christian for performing the serological tests.

12.6 kilos, indicating an increasing ascitic fluid accumulation. The blood volume was 1019 cc. The administered red cells, in 50 cc. whole blood, contained 53,400 counts per minute. The radioactivity present in the circulating blood was 0.7, 0.7, 3.9, 17.4, and 25.9 per cent after 6, 12, 24, 48, and 72 hours respectively. A paracentesis at 72 hours yielded 1670 cc. of ascitic fluid containing 47.0 per cent of the injected radioactivity. Thus 73.0 per cent of the administered amount is accounted for.

Similar data are available for two other ascitic dogs. Dog 12-02 which had the vena cava constriction performed 2 months previously weighed 15.1 kilos

TABLE A
Passage of Red Cells Labeled with Radioiron from the Peritoneal Cavity of Ascitic Dogs into Circulation

Dog	Experiment	Weight		Activity in circulating blood Per cent of injected dose					Ascitic fluid		Total recovery of radio-active red cells
		0 hr.	72 hrs.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	Volume removed at 72 hrs.	Activity remaining 72 hrs.	
		kg.	kg.							Per cent	Per cent
47-168	1-A*	11.1	11.2	0.0	8.7	24.0	38.2	43.4	480	43.2	86.6
	1-B†	12.2	12.6	0.7	0.7	3.9	17.4	25.9	1670	47.0	73.0
12-02	2-A*	15.1	15.0	1.5	1.0	25.6	62.4	67.0	600	12.5	79.5
	2-B†	17.6	18.2	2.0	10.5	26.4	48.0	51.2	1200	5.3	56.5
47-140	3-A*	8.9	9.0	0.0	12.5	35.8	52.2	56.4	260	8.9	65.3
	3-B†	9.4	10.2	1.2	5.0	20.4	33.2	38.8	1800	35.2	74.0

* A, high protein—low salt diet. Low ascitic fluid production.

† B, high protein diet with 4 gm. of sodium chloride daily. Active ascitic fluid production.

at the start of the control experiment, and 15.0 kilos after 72 hours, indicating low ascitic fluid accumulation during the test (Experiment 2-A). After 6 hours, 1.5 per cent of the injected radioactivity of 80,500 counts per minute was present in the circulating blood, progressing steadily to 67.0 per cent at 72 hours. The 72 hour paracentesis of 600 cc. of ascitic fluid yielded only 12.5 per cent of the radioactivity, thus accounting for 79.5 of the administered amount. Experiment 2-B shows the figures for the same dog in the face of steadily accumulating ascites. After 72 hours, 51.2 per cent of the injected activity of 123,500 counts per minute was present in the circulating blood, and 5.3 per cent remained in the 1200 cc. of ascitic fluid removed at that time. Thus 56.5 per cent of the injected radioactivity is accounted for.

In Experiments 3-A and 3-B (Table A) the results of experiments with dog 47-140 are listed. The vena cava of this animal had been constricted 3 months

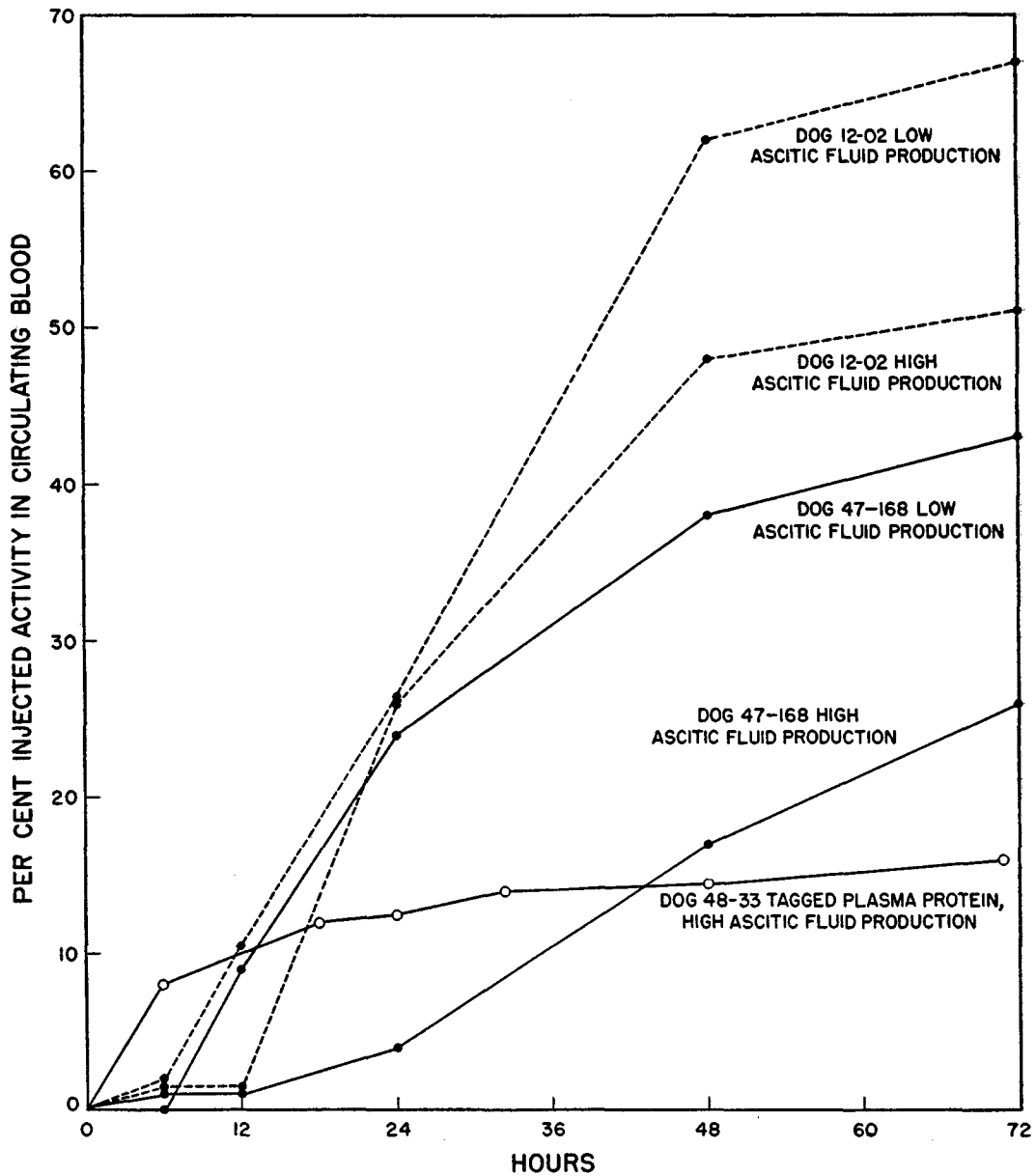


FIG. 1. Appearance of tagged red cells and plasma proteins in the circulating blood following intraperitoneal injection in dogs with experimental ascites.

previously. While in the control or non-accumulating state, 56.4 per cent of injected activity of 87,000 counts per minute was absorbed into the circulation in 72 hours, with 8.9 per cent recovered by the final paracentesis which yielded

260 cc.; in the stage of active ascitic fluid formation, only 38.8 per cent of the injected activity of 80,900 counts per minute was absorbed in a similar 72 hour period, and 35.2 per cent was recovered by the terminal paracentesis which removed 1800 cc.

The radioactive red cells were present in the circulation in detectable amounts after only 6 hours in several instances. The level rose rapidly for 2 days and then more slowly the 3rd day.

The effect of active accumulation of ascitic fluid was constant in these experiments. The animals uniformly absorbed a smaller number of tagged red cells when ascitic fluid was being actively produced.

Fig. 1 presents the data obtained in Experiments 1 and 2 in graphic form. A previously published (14) curve of appearance of protein, tagged with C^{14} and absorbed into the circulation from the peritoneal cavity, adjusted for total plasma volume has been superimposed for comparison.

DISCUSSION

It is clear from the data that injected red cells are removed rapidly, but not completely, in 3 days from the peritoneal cavity of dogs with experimental ascites. The amount (26 to 67 per cent) appearing in the circulation in this 72 hour period compares favorably with that of normal dogs (25 to 100 per cent) (9), though there is a tendency for lower values and slower uptake. However, the amount of red cells remaining in the peritoneal cavity (5 to 47 per cent) is much higher than in normal dogs, in which the cells have usually disappeared completely from the peritoneal cavity by this time (16). It will be noted that dog 47-168, which had the constricting band about the vena cava for the longest period (11 months), showed the highest percentage retention.

It is also of interest to note the rather uniform difference, in terms of percentage, in amounts of radioactivity absorbed when the animals were in a state of low ascitic fluid production compared with the active ascitic fluid formation. Thus in three experiments 17.5, 15.8, and 17.6 per cent more was found in the blood stream after 72 hours when the animal was not actively producing ascitic fluid.

Also of note is the over-all failure to recover 13 to 43 per cent of the injected radioactivity in either blood or ascitic fluid. Presumably this discrepancy is due in part to retention of red cells in the lymphatics, lymph nodes, and general reticulo-endothelial system draining the peritoneum.

The production of ascitic fluid apparently does not seriously interfere with the peritoneal stomata so convincingly demonstrated by Allen (1) and Allen and Vogt (2). However, it is obvious that the removal of red cells is somewhat slower in the ascitic dog, even when not actively producing ascitic fluid, than in the normal dog. This may be due in part to the greater dilution of the cells by the peritoneal fluid, particularly when the animal is producing large amounts of ascitic fluid. The effect of continued ascites on the peritoneum, as noted in

some of the experimental ascitic dogs already sacrificed (12, 13), is a thickening and formation of many adhesions particularly in the area of the diaphragm where the absorption of particulate matter is most rapid. It may be that this long continued ascitic accumulation with its attendant chronic inflammatory manifestations may impair the normal absorptive mechanism sufficiently to account for the observed discrepancy between ascitic dogs and normal dogs.

A comparison of the red cell absorption and protein absorption curves reveals an early rapid rise in the amount of protein appearing in the circulation, which reaches a plateau in 12 to 24 hours, while the red cell curve rises more slowly at first and approaches a plateau after 48 hours. The difference in these curves for protein and red cell uptake from the peritoneum may well be related to the rapid removal of the plasma protein from the circulation after parental administration (15), as well as subsequent replacement in the peritoneal cavity by untagged protein (ascitic fluid circulation) (14). Thus, though the tagged protein is transferred more rapidly into the circulation, it does not build up as high a percentage concentration, because of diffusion into extravascular spaces and tissue cells. The red cells are removed more slowly from the peritoneum and except for obsolescence remain in the circulation.

SUMMARY

Intraperitoneal injection of red cells tagged with radioiron into dogs with experimental ascites demonstrated that such cells were rapidly transferred into the circulating blood.

When the experimental animals were not actively producing ascitic fluid, 43.4, 67.0, and 56.4 per cent respectively, of the administered radioactive red cells passed to the blood in 72 hours. In the same three dogs during active ascitic fluid formation, 25.9, 51.2, and 38.8 per cent of the administered radioactivity was removed in a similar period.

The amount of radioactivity in the blood stream, consequent on the passage of red cells from the peritoneal cavity into the circulation, becomes nearly constant in 48 hours, whereas for radioactive plasma proteins the plateau is attained in 24 hours (Fig. 1).

In normal dogs (16), the passage of red cells from the peritoneal cavity was complete in 72 hours, while in ascitic dogs, 5 to 47 per cent of the injected tagged red cells remained behind in the peritoneum after the same period.

BIBLIOGRAPHY

1. Allen, L., *Anat. Rec.*, 1936, **67**, 89.
2. Allen, L., and Vogt, E., *Am. J. Physiol.*, 1937, **119**, 776.
3. Clausen, J., *Acta paediat.*, 1939, **27**, 24.
4. Cruz, W. O., Hahn, P. F., and Bale, W. F., *Am. J. Physiol.*, 1942, **135**, 595.
5. Cunningham, R. S., *Physiol. Rev.*, 1926, **6**, 242.
6. Finch, C. A., Wolff, J. A., Rath, C. E., and Fluharty, R. G., *J. Lab. and Clin. Med.*, 1949, **34**, 1480.

7. Hahn, P. F., *J. Biol. Chem.*, 1946, **163**, 435.
8. Hahn, P. F., Bale, W. F., Ross, J. F., Hettig, R. A., and Whipple, G. H., *Science*, 1940, **92**, 131.
9. Hahn, P. F., Miller, L. L., Robscheit-Robbins, F. S., Bale, W. F., and Whipple, G. H., *J. Exp. Med.*, 1944, **80**, 77.
10. Hedensted, S., *Acta chir. scand.*, 1947, **95**, suppl. 128, 5.
11. McKee, F. W., Hyatt, R. E., Wilt, W. G., Tishkoff, G. H., and Whipple, G. H., *J. Exp. Med.*, 1949, **90**, 447.
12. McKee, F. W., Schilling, J. A., Tishkoff, G. H., and Hyatt, R. E., *Surg., Gynec. and Obst.*, 1949, **89**, 529.
13. McKee, F. W., Schloerb, P. R., Schilling, J. A., Tishkoff, G. H., and Whipple, G. H., *J. Exp. Med.*, 1948, **87**, 457.
14. McKee, F. W., Wilt, W. G., Hyatt, R. E., and Whipple, G. H., *J. Exp. Med.*, 1950, **91**, 115.
15. Miller, L. L., Bale, W. F., Yuile, C. L., Masters, R. E., Tishkoff, G. H., and Whipple, G. H., *J. Exp. Med.*, 1949, **90**, 297.
16. Snead, C. R., *Arch. Surg.*, 1948, **56**, 249.
17. Stewart, W. B., Stewart, J. M., Izzo, M. J., and Young, L. E., *J. Exp. Med.*, 1950, **91**, 147.
18. Winzeler, H., *Schweiz. med. Woch.*, 1946, **76**, 325.
19. Young, L. E., Ervin, D. M., and Yuile, C. L., *Blood*, 1949, **4**, 1218.
20. Yuile, C. L., Bly, C. G., Stewart, W. B., Izzo, A. J., Wells, J. C., and Whipple, G. H., *J. Exp. Med.*, 1949, **90**, 273.