TABLE II. Reaction in Complement Fixation Test Employing Heated Spherule Antigen, Coccidioidin, and an Egg Yolk Antigen and the Sera from Rabbits Immunized Intravenously with Spherule Vaccine.

Antigens	Rabbit	Serum dilutions 1-1	1-2	1-4	1-8	1-16	1-32	1-64	1-128	Controls Antigen	Serum
1	5	—	—	—	—	—	—	—	—	—	—
2		3+	3+	3+	3+	3+	3+	1+	—	—	
3		2+	3+	3+	2+	—	—	—	—	—	
1	1	—	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	2+	—	—	—	—	—	
3		4+	3+	-+	—	—	—	—	—	—	
1	6	4+	2+	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	1+	—	—	—	
3		4+	4+	4+	2+	2+	1+	—	—	—	
1	2*	2+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	2+	—	—	—	
1	4*	1+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	4+	3+	2+	—	—	
1	3	1+	—	—	—	—	—	—	—	—	—
2		4+	4+	4+	4+	3+	3+	—	—	—	
3		4+	4+	4+	4+	2+	—	—	—	—	

4—Complete fixation (no hemolysis); –, no fixation (complete hemolysis).
* Sera from these rabbits were not tested with egg yolk antigen.
1—Coccidioidin. 2—Spherule. 3—Egg yolk.

was found to stimulate low titers to coccidioidin in hyperimmunized animals. 4. The "Spherule antigen" completely absorbed antibodies to the homologous antigen and coccidioidin from the serum of a hyperimmunized rabbit, but failed to absorb antibodies from the serum of a rabbit infected with *Coccidioides immitis*.

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Identification of Ferritin in Blood of Dogs Subjected to Radiation from an Atomic Detonation.* (19441)

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Direct observations on the mesoappendix capillary bed in the roentgen ray irradiated rat demonstrated that a pronounced peripheral vasodepression occurred during the first post-irradiation week. Further investigation showed that this effect was not due to a direct effect of the radiation on the capillaries themselves but was the result of the appearance of a vasodepressor agent in the blood. It was shown that this vasodepressor was

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liberated by the liver and had all of the characteristics of ferritin(1).

The present investigation furnishes presumptive evidence for the presence of ferritin in the blood of dogs subjected to the radiation from an atomic detonation.

Experimental. Male CFW strain rats weighing 125-200 g (average 150 g) were anesthetized with sodium pentobarbital (45 mg/kg intraperitoneally) and prepared for observation of the mesoappendix capillary bed by the method of Chambers and Zweifach(2). Plasma samples were obtained from dogs subjected to a lethal dose of irradiation. The dog plasma or its dilutions with physiological saline were administered intravenously to standardized normal rats in a volume of 0.5 ml. All samples were designated by code to avoid cognition by the assayist and remove personal bias from the observations. Freshly prepared solutions of epinephrine in doses of 0.1 ml were applied topically to the mesoappendix preparation and a selected vessel studied for changes in sensitivity of its precapillary sphincters and in vasomotion. The epinephrine concentrations used for screening sensitivity tests were $1:1 \times 10^{7}$, $1:8 \times 10^{6}$, $1:6 \times 10^{6}$, $1:4 \times 10^{6}$, $1:2 \times 10^{6}$, $1:1 \times 10^{6}$, $1:1 \times 10^{5}$, and $1:1 \times 10^{4}$; while concentrations of $1:3 \times 10^{6}$, $1:1 \times 10^{6}$, $1:9 \times 10^{5}$, $1:6 \times 10^{5}$, $1:3 \times 10^{5}$, $1:1 \times 10^{5}$, $1:9 \times 10^{4}$, $1:6 \times 10^{4}$, $1:3 \times 10^{4}$, and $1:1 \times 10^{4}$ were used in titering vasodepressor activity of the plasma. The time interval between epinephrine applications was 10 minutes and the observation period was 5 minutes. All positive responses were checked to eliminate the possibility of errors in observations, and a number of samples were recoded and rerun to check the general reliability of the observations. Serial dilutions in physiological saline of a number of active samples were evaluated in order to obtain some information on the relative amounts of vasodepressor material present in these active samples. Selected samples of dog plasma whose vasodepressor activity had been previously determined to be high were aerobically incubated with minced rat liver or kidney in a Dubnoff shaking apparatus at 37.5°C and re-evaluated for vasodepressor activity. A highly active sample was dialyzed overnight in the refrigerator against physiological saline and the dialyzate as well as the sample was evaluated for vasodepressor activity. Crystalline dog ferritin was prepared from dog liver by the method of Granick(3). Rabbit anti-dog ferritin serum was prepared by injecting intravenously or subcutaneously well-dialyzed recrystallized dog ferritin into New Zealand rabbits. Precipitin reactions against dog ferritin showed that this antiserum was highly active. Known amounts of dog ferritin or active samples were incubated with various amounts of this antiserum and assayed for vasodepressor activity. Incubation was carried out at 43°C for various time intervals and in all cases for at least one hour before testing.

Results. Observations on the capillary effects of thirteen control plasma samples obtained from 8 dogs prior to irradiation showed only one plasma sample had vasodepressor activity. However, after irradiation a pronounced vasodepressor activity of 5 to 6 days' duration was observed starting irregularly on the first, second or third post-irradiation in 6 of the dogs' plasma samples. The other 2 dogs' plasma samples showed the presence of a vasodepressor material but did not have the same overall pattern of appearance. The loss of vasodepressor activity occurred on the sixth, seventh or eighth post-irradiation day when a normal epinephrine sensitivity and vasomotion response was obtained in the standardized test animal. These results are summarized in Table I.

The irregularity of appearance of the vaso-

TABLE I. Sequence of Appearance of Ferritin in Dog Plasma After Atomic Irradiation.

Dog No.	Control 1	Control 2	Post-irradiation day 1	2	3	4	5	6	7	8
51	—	—	—	I	I	I	A	I	A	A
B-81	I	I	I	I	I	A	I	I	—	—
18	—	I	A	A	A	A	A	I	I	—
19	I	I	A	A	A*	A	A	A	I	I
A-82	I	I	A	A	I	I	—	A	—	—
B-83	I	I	I	A	A	A	A	A	I	—
A-90	I	I	I	I	A	A	A	A	A	—
A-73	I	A	A	A	A	I	A	A*	A	I

I—Inactive, no ferritin present.
A—Active, ferritin present.
* Results equivocal.

TABLE II. Titration of Ferritin (VDM) in Plasma of Irradiated Dogs.

Post irradiation day*	Dog 19		Dog B-83		Dog A-90	
	No. vasodepressor† doses/ml plasma	Dog Ferritin N equivalents, γ/ml	No. vasodepressor doses/ml plasma	Dog Ferritin N equivalents, γ/ml	No. vasodepressor doses/ml plasma	Dog Ferritin N equivalents, γ/ml
1	>15<50	>.015<.50	>50<500	>.05<.50	>300	.30
2	>15	.015	>10<50	>.01<.05	>50<300	>.05<.30
3	>15	.015	<10	.01	>15	.01
4	0	0	~2	.002	>15	.015
5	0	0	~2	.002	>15	.015

* Time is marked from the first day of appearance of vasodepressor activity.
† One vasodepressor dose = 0.001 γ dog ferritin N/ml.

depressor material (Table I) prompted an evaluation of the amount liberated and the duration of its presence in the plasma. Table II gives the titers on the plasma of three dogs. These evaluations are based on the fact that 0.001 γ N/ml is the smallest amount of ferritin which can be consistently detected by the mesoappendix text(1,4). The results presented in Table II indicate that initially a large amount of VDM is released after irradiation and the amount is reduced as the organism begins to recover from the initial damage produced by the irradiation.

Inasmuch as similar radiation studies with roentgen ray irradiated rats indicated that the vasodepressor material appearing in the blood after irradiation was presumably ferritin(1), we applied several of the tests for ferritin proposed by Mazur *et al.*(5). Active plasma samples from dogs on the first, second and third post-irradiation days (dogs 19, B-83 and A-90 respectively) were incubated aerobically for 3 hours at 37.5°C with minced kidney and with minced liver. These samples were inactivated by liver but not by kidney. When 3 ml of active dog plasma containing at least 40 vasodepressor doses per ml was dialyzed overnight in the refrigerator against 13 ml of physiological saline, there was no decrease in the vasodepressor activity of the sample, nor any appearance of activity in the dialysate. Injection of crystalline dog ferritin or vasodepressive dog plasma into previously standardized normal rats gave a vasodepression which could be entirely prevented by pre-injection or entirely counteracted by post-injection of 1 ml of rabbit antidog ferritin serum. These highly successful *in vivo* inactivations were in contrast to numerous less successful attempts at *in vitro* inactivation in which various dilutions of the antiserum were incubated with samples of active plasma. Normal rabbit serum did not inactivate either the ferritin or the active plasma.

Discussion. The following text table gives the criteria established by Mazur, *et al.*(5) for the identification of ferritin and shows the similarity between ferritin and the vasodepressor material found in the blood plasma of dogs subjected to radiation from an atomic detonation.

Comparison of Radiation VDM and Ferritin

Criteria	*Ferritin and Dog Plasma VDM*
Effect on capillary bed	Decreased epinephrine sensitivity
Effect on vasomotion	Greatly decreased
Dialysis	Does not dialyze
Aerobic incubation	
Liver	Inactivates
Kidney	Does not inactivate
Effect of antiserum	Inactivates

The completely parallel findings taken together with the fact that no other compound has been shown to produce such profound peripheral vascular effects at such a low dose (6) provides presumptive evidence for the identity of the VDM in the blood of dogs subjected to radiation from an atomic detonation as ferritin.

Summary. Studies on blood plasma sam-

ples obtained from eight dogs subjected to a lethal dose of radiation from an atomic detonation demonstrated that a material was present which, when administered intravenously, decreased the rate of vasomotion and decreased the epinephrine sensitivity of the mesoappendix capillary bed of the normal rat. After its appearance in the blood, the concentration of this vasodepressor material decreased as a function of time. This VDM has been shown to fit the criteria established for ferritin.

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Portal Venipuncture. A Percutaneous, Trans-Hepatic Approach. (19442)

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Many clinicians and physiologists have wanted a simple method of access to the portal venous system. If direct access were possible, not only could studies be made on portal venous blood, but also the portal venous system could be demonstrated by injecting a contrast medium and making roentgenograms. Hitherto, samples of blood from the portal vein have been obtained during laparotomy and, in a few instances, polythene tubing has been tied into the portal vein and brought out through the wound for sampling at a later time. In a few patients with hepatic vascular obstruction, small catheters have been passed into dilated abdominal veins and portal blood thus obtained. Portal venograms have been obtained by similar methods. These methods have evident limitations and preclude a more general investigation of the portal blood circulation in man. It occurred to one of us (H.L.S.) that access to the portal vein through the intact abdominal wall should be possible, and the senior author (H.R.B.) conceived the idea of an approach through the liver. He subsequently developed the percutaneous, trans-hepatic technic of portal venipuncture described below with the assistance of the two junior authors (L.P.W. and K.H.K.).

The portal vein is 7 to 8 cm in length, extending from the level of L2 to the porta hepatis at the junction of L1 and T12(1). The portal vein divides to the right and left just before it enters the liver(2), the right branch being shorter and thicker than the left. The division of the portal vein usually takes place at the level of T12, in the transverse plane at approximately 15 degrees caudal to the right in the frontal plane(3). In a transverse cross-section(2) which passes through the twelfth thoracic vertebra the larger radicles of the portal vein within the liver substance lie to the right of the midline so that lines to midpoint of the anterior surface make angles of 10 to 45 degrees with the medial plane. At this level the liver abuts directly upon the anterior abdominal wall so that no other organ is interposed. On the basis of these anatomical considerations, a needle was successfully inserted into the hepatic portal vein of a cadaver. This was followed by studies on living patients in some of which contrast media was injected and venograms