docarditis and the adrenal hypertrophy are interrelated. Further studies of this relationship are in progress.

The occurrence, in one dog, of a typical glomerulonephritis associated with endocarditis is perhaps of significance in the light of the frequent clinical association of these two entities.

Summary. Observations have been reported in which after the creation of large arteriovenous fistulae in dogs endocarditis occurred without intentional introduction of bacteria. This result occurred in about 8 out of 10 dogs in which sufficiently large shunts existed for more than 4 weeks. Adrenal gland enlargement occurred following creation of a large fistula. Other concomitant findings have been reported and discussed.

Received June 13, 1950. P.S.E.B.M., 1950, v75.

Plasma and Blood Volumes of Mouse Organs, As Determined with Radioactive Iodoproteins.* (18083)

NATHAN KALISS†‡ AND DAVID PRESSMAN.† (Introduced by Frank Maltaner.)

From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me., and Sloan-Kettering Institute for Cancer Research, New York City.

In connection with the investigations of the localization of radioactive antibodies in various tissues as carried out in this laboratory (1-4), it was necessary to know the plasma and blood volumes of several organs of the mouse. These volumes were determined by injecting mice with radioiodinated proteins and assaying the organs for their radioactivity content. The results are reported here(4a). Similar use of radioiodinated proteins has been made by Fine and Seligman(5) and Gibson *et al.*(6-8) in studies on plasma volumes in traumatic shock in dogs.

We used 3 different preparations of radioiodinated protein. These were the globulin fraction of rabbit antiovalbumin serum, and 2 preparations of bovine serum albumin which were iodinated with iodine containing radioactive I^{131},§ according to a method described previously(1). The bovine serum albumin was the crystallized product obtained from the Armour Co., Chicago. The globulin fraction of the antiovalbumin serum was the same preparation described previously(4). The mice used were 6 to 7 week old males, weighing 17 to 25 g, from the inbred Akm strain. The hematocrit was determined on the heparinized blood of 3 males weighing 19 to 20 g. These animals had received an intravenous injection of 0.1 ml of a heparin solution 3 minutes before drawing the blood. Two samples, one each from the caudal and jugular

* This research was jointly supported by the Office of Naval Research contract No. N6-ori-99 T.O.1, and the Atomic Energy Commission and the James Foundation of New York, Inc.

† Senior Fellow in Cancer Research, American Cancer Society Fellowship recommended by the Committee on Growth of the National Research Council.

‡ Visiting Fellow, Sloan-Kettering Institute, Jan.-July, 1948.

1. Pressman, D., *Cancer,* 1949, v2, 697.

2. Pressman, D., *J. Immunol.,* 1949, v63, 375.

3. Pressman, D., Hill, R. F., Foote, F. W., *Science,* 1949, v109, 65.

4. Pressman, D., and Keighley, G., *J. Immunol.,* 1948, v59, 141.

4a. See also Wish, L., Furth, J., and Storey, R. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 644.

5. Fine, J., and Seligman, A. M., *J. Clin. Invest.,* 1944, v23, 720.

6. Gibson, J. G., II, Peacock, W. C., Seligman, A. M., Sack, T., *J. Clin. Invest.,* 1946, v25, 838.

7. Gibson, J. G., II, Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J., and Evans, R. D., *J. Clin. Invest.,* 1946, v25, 848.

8. Gibson, J. G., II, Seligman, A. M., Peacock, W. C., Fine, J., Aub, J. C., and Evans, R. D., *J. Clin. Invest.,* 1947, v26, 126.

§ The radioactive iodine was obtained from the U. S. Atomic Energy Commission, Oak Ridge Operations, Isotopes Division, Oak Ridge, Tenn.

veins of each mouse, were taken in Van Allen hematocrit tubes. These were centrifuged for 30 minutes with a force of 1000 times gravity. The mean hematocrit was 44.6 ± 0.5. For determination of the plasma and blood volumes, the animals were injected intravenously with the radio-protein containing about 0.5 μc of radioiodine after they had been kept in an incubator at 37°C for a short while (at least 15 minutes) to produce peripheral vaso-dilatation which facilitated injection. The radio-protein was allowed to circulate for at least 15 minutes (and as high as one hour) in order to obtain complete mixing and to permit the animal to equilibrate with the ambient room temperature. In the meantime the total radioactivity in the mouse was determined, by measuring the gamma ray activity, by placing the live mouse in a tube surrounded by a multiple section gamma ray counter according to the method described previously(2). The animal was chloroformed, the tail cut off and a measured volume of blood was obtained from the stump. The tail was immediately ligated and the total volume of blood drawn was kept less than 100 mm^3 to minimize alteration in the blood volume of the animal. The specific activity of the measured sample, as determined by beta ray count as described below, was used as the base for calculating plasma volumes in the organs. In the first six animals assayed, the volume of the blood sample was determined with a 0.25 ml tuberculin syringe. For the other 3 animals, a 20 mm^3 Sahli pipette was used. The animal was then killed with chloroform and opened ventrally. The blood vessels of each organ to be assayed were ligated with nylon thread as close to the organ as possible to prevent loss of blood, and the organs were then excised. Ligating was not feasible for the small intestine, long bones of the hind legs and their associated muscles, and the brain. These organs were, therefore, excised last on the assumption that sufficient stasis would set in to prevent any significant loss of blood.

After excision, the organs were weighed and then the beta ray count determined according to the method described previously (2). The organs were homogenized and the homogenate made alkaline with sodium hydroxide. The homogenate was poured into metal caps, evaporated to dryness under infra-red lamps, and each cap then assayed for its radioactivity count with a thin window G.M. tube. Where the total wet weight of an organ was over 500 mg (which would result in a sample thicker than about 10 mg per cm^2 for a single cap), the homogenate was poured into several caps, and the total of the counts for the caps was determined. The plasma volumes of the organs were determined as the ratio of the radioactivity per unit weight of the organ to the radioactivity per unit volume of plasma. The radioactivity per unit volume of plasma was calculated from the hematocrit and the radioactivity count of the blood sample from the tail. The plasma volume of the whole mouse was determined from the dilution of the injected radioactivity. The injected radioactivity was established from the gamma ray count for the whole mouse, as described above. The blood volume for the various organs and the whole mouse were calculated from the plasma volumes and the hematocrit, obtained as described above. No correction was made for the probable difference in the hematocrits for the various organs.

The data are presented in Tables I through IV. In Table I are listed the mean wet weights of the various organs and the average percentages of total body weight which each organ comprised (with the standard

TABLE I. Wet Weight of Mouse Organs.

	Mean wt in mg	% of total body wt	
		Mean & S.E.	Range
Whole animal*	22,200		
Brain†	380	1.74 ± .08	1.47-2.25
Kidneys*	375	1.70 ± .07	1.42-2.04
Liver*	1,670	7.6 ± .2	6.8 -8.2
Long bones‡	265	1.18 ± .10	.46-1.53
Lungs§	156	.71 ± .05	.59-1.02
Spleen†	85	.37 ± .05	.12- .56
Sub-maxillary glands§	125	.56 ± .06	.23- .74
Testes*	110	.49 ± .04	.33- .82

* Avg values for 9 Akm male mice of 17.4 to 25.2 g.
† 8 mice.
‡ Femur, tibia, and fibula of both hind legs.
§ 7 mice.

TABLE II. Injection Protocol for Radioiodoprotein.

Animal No.	1	2	3	4	5	6	7	8	9
Animal wt (g)	17.4	25.2	19.6	21.8	23.7	21.3	23.0	24.5	23.3
Type of protein inj.	Anti-ovalbumin serum	Preparation 1		Radioiodinated bovine serum albumin Preparation 2					
Amt of protein inj. (mg)		2.3	3.8	0.4	0.7	0.8	1.3	2.6	5.0
Amt of I^{131} inj. (μc)	.063	.071	.066	.048	.049	.055	.048	.114	.050
Vol. of fluid inj. (mm^3)	500	150	250	20	35	40	65	130	250
Time between inj. of protein and death (min.)	15	60	15	15	15	15	20	20	15

TABLE III. Mean Plasma and Blood Vol. of Mouse Organs in ml per 100 g of Wet Tissue.

	Plasma volume		Mean blood vol.*	% distribution of total plasma
	Mean and S.E.	Range		
Whole animal†	6.7 ± .4	4.6- 8.3	12	100
Brain‡	1.6 ± .1	1.1- 1.8	3	.4
Kidney†	19.1 ± 1.5	13.0-24.2	34	4.8
Liver†	20.2 ± 1.8	12.7-29.4	36	23
Long bones†	6.4 ± .7	3.5- 9.9	11	1.2
Lung§	27.4 ± 2.5	17.9-40	49	2.6
Small intestine†	5.0 ± .6	1.4- 7.5	9	—
Spleen‡	9.2 ± 1.3	5.8-15.8	17	.5
Striated muscle†	1.6 ± .1	.9- 2.1	3	—
Sub-maxillary gland§	5.9 ± .9	3.7-11.1	11	.5
Testes†	3.4 ± .6	1.9- 8.0	6	.2

* Based upon a hematocrit value of 44.6 ± 0.5 for heparinized blood from the caudal and jugular veins of 3 mice. † Avg for 9 mice. ‡ 8 mice. § 7 mice.

error and range). The averages are for 9 mice weighing from 17.4 to 25.2 g. These organ weights are comparable to the weights in mature mice which have been reported previously by Kopec and Latyszewski(9,10).

Table II gives the injection protocols for each animal. It shows the radioprotein injected, the amounts injected, and the time lapse between the injection of protein and death of the animal.

Table III gives the mean total plasma volume and the mean plasma volumes of the various organs in ml per 100 g of wet weight along with the range of values obtained and the standard error. The mean blood volumes are also given as calculated from the plasma volumes and the hematocrit of the blood from the jugular and caudal veins. The last column gives the percentage distribution of the total plasma among the various organs. In general, there was no significant variation in any of the values determined for the animals of different body weight or when different proteins or protein preparations were used, nor did it appear to matter whether an animal were analyzed either at one hour, or at 15 to 20 minutes, after injection. It was felt that a 15 minute interval was sufficient to insure thorough mixing of the iodinated protein and the blood. Longer circulation times were generally avoided, since Fine and Seligman(5) have shown that in dogs, iodinated bovine serum albumin was removed from the circulation to the extent of 10-25% within the first hour after injection. The effects of such a removal would

9. Kopec, S., and Latyszewski, M., *Memoires de l'Inst. Nat. Polonais d'Economie Rurale a Pulawy,* 1929, v10, 509.

10. Kopec, S., and Latyszewski, M., *Memoires de l'Inst. Nat. Polonais d'Economie Rurale a Pulawy,* 1931, v12, 462.

TABLE IV. Total Blood Volume in the Mouse, as Reported in the Literature(4a).

Authority	Methods used for determining blood vol.	No. and type of mice	Avg blood vol. in ml/ 100 g body wt
Dreyer and Ray(12)	Mincing and extraction of hemoglobulin	19	5.8
Oakley and Warrack(13)	Perfusion and exsanguination	46 ♂ & 54 ♀	6.3
Taylor(14)	Exsanguination and mincing	40 strain dba 16 strain C57	5.23 ± .31 4.9 ± .17
Furth and Sobel(11)	a. Exsanguination and perfusion	a. 12	a. 5.2
	b. Dye (T-1824)	b. 9	b. 9.0
	c. Simultaneous dye (T-1824) and exsanguination methods	c. "Several"	c. 5.7 by exsanguination, 10 and 11.7 by dye
Kaliss and Pressman, 1949	Circulating radioiodoprotein	9 strain Akm	12.1 ± .8

be reflected in an apparent increase in the total plasma volume.

Previous reports of total blood volumes in the mouse are summarized in Table IV. These values vary from 4.9 to 11.7 ml per 100 g of tissue. The single important cause of the wide differences in the values obtained is apparently due to the methods used. This is clearly shown by the data of Furth and Sobel(11) who obtained values by the dye (T-1824) dilution technic which are in agreement with the figures reported here, but are nearly double those obtained by them with perfusion and exsanguination methods. It is most certainly true that the dye technic gives a much closer approximation to a true value than does exsanguination, and that the values for the former technic and those obtained with circulating radio-proteins are in agreement. In a comparison of plasma volumes in dogs, determined by the use of the dye T-1824 and radioiodoalbumin circulating in the plasma, Gibson *et al.*(6) found the values to correspond within ±10%, showing good agreement between the two methods.

There are several possible reasons for the variations shown from animal to animal in the plasma and blood volume values that were obtained here, such as the "normal" variations that must exist, particularly for the blood vascular system as a whole, which is so sensitively affected by the physiological and nervous inter-relationships of the body; the handling of the animals; the anaesthetics used; the method of killing the animals prior to dissection and the surrounding air temperature. These as well as other factors may influence both the total blood volume, and the blood volumes of the individual organs. The dissection technics can lead to variations in estimates due to possible loss by hemorrhage, particularly from the large blood vessels. This is especially true for the lungs, where it was difficult to tie off all large vessels at a point close to the lungs. This type of error would operate particularly for the brain, small intestine, and striated muscles, where no attempt was made to tie off the blood vessels prior to dissection. A source of discrepancy in estimating the whole blood volume of an organ lies in the differences of hematocrits between the larger blood vessels and the capillaries. This has been well brought out by the studies of Gibson *et al.*(6) on the distribution of plasma and red cells in the large and minute vessels of the dog.

11. Furth, J., and Sobel, H. I., *J. Nat. Cancer Inst.*, 1946, v7, 103.

12. Dreyer, G., and Ray, W., *Roy. Soc. London Philos. Trans.*, 1910, v201B, 133.

13. Oakley, C. L., and Warrack, G. H. *J. Path. Bact.*, 1940, 50, 372.

14. Taylor, A. Univ. of Texas Publication No. 4507 *Cancer Studies*, 1945, p95.

They found hematocrit ratios of 0.85 for the blood of the body, and 0.7 for the minute blood vessels as compared with arterial hematocrits.

Furthermore, the hematocrits differ from organ to organ. Gibson *et al.*(6) found average hematocrit values in the small blood vessels of the dog ranging from 15% for the kidneys to 82% for the spleen. If similar differences hold true for the mouse, as they most certainly must, then estimates of whole blood volume values for a specific organ obtained with the help of hematocrits for venous blood (or arterial blood) must suffer from attendant errors.

Summary. The plasma and blood volumes of mice and of various mouse organs were determined by injecting mice with a protein iodinated with iodine containing tracer quantities of radioactive iodine, and determining the amount of radioactivity present in the various organs. The average plasma and blood volumes of the mice were found to be 6.7 ml and 12.7 ml per 100 g of body weight, respectively. The average plasma volume in ml per 100 g of wet tissue for the brain was 1.6; kidney, 19.1; liver, 20.2; lung, 23.9; small intestine, 5.0; spleen, 9.2; submaxillary gland, 5.9; testes, 3.4.

Received June 14, 1950. P.S.E.B.M., 1950, v75.

Sex Differences in Weight-stimulating Effect of B_{12} in Rats on Diets of Varying Composition.* (18084)

ERNESTINE B. MCCOLLUM AND BACON F. CHOW

From the Department of Biochemistry, School of Hygiene and Public Health, Johns Hopkins University

In this laboratory as elsewhere(1) it has been observed that young rats depleted of B_{12}, and receiving either vitamin-free casein or soybean protein as the protein moiety of an adequate diet, show superior weight gains when given daily intramuscular injections of B_{12}. This has been noted in the case of both proteins fed at high and low levels. The greater gains were not accompanied by greater nitrogen retention(2).

In an effort to determine whether the source of the calories plays a role in the weight gains achieved with and without B_{12}, as indicated by the investigations on the growing mouse by Bosshardt, Paul, and Barnes(3), diets were fed that were high, low or moderate in fat and/or carbohydrate content. The protein level was so adjusted that the percentage of calories from the protein was approximately the same in each ration.

Experimental procedure. The composition of the rations used is given in Table I. Twenty young rats of both sexes, 26 to 30 days old,

TABLE I. Composition of Rations Used.

	A %	B %	C %	D %
Casein (1)	40.	53.5	34.	34.
Salts #4 (2)	4.	4.	4.	4.
Primex (3)	12.	41.2	—	—
Cottonseed oil (4)	1.	1.	1.	1.
Maize dextrin (5)	42.7	—	60.7	10.
Dextrimaltose (6)	—	—	—	50.7
l-cystine	0.3	0.3	0.3	0.3

(1) Vit.-test casein, General Biochemicals.
(2) Hegsted-Mills, Elvehjem and Hart, *J.B.C.*, 1941, v138, 459.
(3) Hydrogenated cottonseed oil, Swift & Co.
(4) Wesson oil.
(5) Made from cornstarch, Corn Products Sales Co.
(6) Mead's Dextri-Maltose #2, Mead Johnson & Co.

* This work was partially supported by grants-in-aid from E. R. Squibb and Sons, Hoffmann-La-Roche, Inc., and Sharp and Dohme and Co.

1. Hartman, A. M., Dryden, L. P., and Cary, C. A., U.S.D.A. BDIM-Inf-76, July, 1949.

2. Chow, B. F. and Barrows, *L. Fed. Proc., (Am. Inst. Nutr.)*, 1950, v9, 354.

3. Bosshardt, D. K., Paul, W. J., and Barnes, R. H., *J. Nutr.*, 1950, v40, 595.