gested that the "spreading factor," hyaluronidase causes an increase in capillary fragility by affecting the intercellular cement, other investigators were unable to confirm this observation. Danielli and Stock have stated that in all probability the injury to the capillary wall in toxic conditions is direct and not due to enzymic action.

It is even more unlikely that the "spreading factor" plays a role in the course of radiation injury. This impression is supported by the failure in the work of Rekers and Field(11) on dogs of such potent hyaluronidase inhibitors as dopa and sodium gentizate to influence the course of radiation. These results showed that the effect of vitamin 'P' factors seems also to be directly on the intercellular substance. They obtained no evidence that these factors inhibit heparin, for the heparin content was not altered by vitamin 'P' therapy to any great degree.

Rekers and Field also found that certain flavonoids gave considerable protection against a total body near-lethal dosage of radiation. They concluded that "previous misunderstanding of the nature of vitamin 'P' has arisen from both the failure to recognize that several flavonone analogues possess very similar anti-hemorrhagic activity and that ascorbic acid has the capacity to potentiate activity in other flavonones." They are inclined to conclude that vitamin 'P' factors affect the capillary system directly perhaps participating as a principal in the 'wear and tear' of a part or all of the capillary system, inhibiting degeneration and taking part in its regeneration. The results of our present investigation are in full accordance with their viewpoint and give further support to the original work of Armentano *et al.*(1) as to the role which the factors of vitamin 'P' play in increased capillary permeability. This term should be interpreted, however, as indicative of chemical lesions in the capillary wall, or more exactly as increased capillary fragility.

Conclusion. Administration of leukotaxine, a bacterial polysaccharide or an exposure to ionizing radiation induces increased capillary fragility. Injury to the capillary wall, whether it is caused by ionizing radiation, by leukotaxine, or by a bacterial polysaccharide, can be prevented to a considerable degree by the administration of a vitamin 'P' compound composed of flavonoids naturally present in citrus fruit.

11. Rekers, F. E. and Field, J. B., *J. Clin. Invest.*, 1949, v28, 746.

Received June 9, 1950. P.S.E.B.M., 1950, v75.

Occurrence of Endocarditis with Valvular Deformities in Dogs with Arteriovenous Fistulae.* (18082)

C. W. LILLEHEI,† J. R. R. BOBB,‡ AND M. B. VISSCHER

From the Departments of Surgery and Physiology, University of Minnesota, Minneapolis

The accidental finding of endocarditis in dogs has been reported to be extremely rare (1). Likewise the dog is generally believed to be a relatively resistant animal to the experimental production of endocarditis(2). It is therefore of interest that the application of an increased work load of a particular type to the canine heart was followed by a high incidence of endocardial vegetations without the necessity of intentional injection of any bacteria. The observations herein reported were made incidental to a study of heart failure in the dog induced by an increase in work load. This increase in work load of the heart has been accomplished by large

* This work was supported by a grant from the Life Insurance Medical Research Fund.

† Trainee, National Cancer Institute.

‡ U.S.P.H.S. Post-doctorate Research Fellow.

1. Morehead, R. P. and Little, J. M., *Am. J. Path.*, 1945, v21, 339.

2. Clawson, B. J., Personal Communication, 1949.

arteriovenous fistulae made surgically under aseptic conditions. Where more than one fistula was made in a particular dog, the operations were staged one to 4 weeks apart. Following the construction of arterio-venous fistulae of sufficient size, it was necessary only to allow a minimum time of approximately 4 to 6 weeks to elapse for the development of endocarditis in the animals in which physiological studies as outlined below were carried out.

Method of study. Mongrel dogs previously dewormed and of either sex were used in these studies. All animals were allowed a period of at least 2 months in the animal colony for purposes of acclimatization before they were utilized in these experiments. Animals not in good health for any reason at the end of this period of time were not operated upon. Dogs of two estimated age groups were used. The criteria for estimating age have been described(3). Group 1. Young dogs—2 to 5 yrs. Group 2. Old dogs—10 or more yrs. All dogs not dying during the course of these experiments were sacrificed by intravenous pentobarbital. An autopsy was performed on all animals at the time of death.

Operative procedure. The iliac fistulae were made immediately distal to the trifurcation of the abdominal aorta between the iliac artery and vein. The femoral fistulae were made 2 to 4 cm below the inguinal ligament between the femoral artery and vein. The aorta-vena cava anastomoses were made just proximal to the trifurcation of the abdominal aorta. In all cases the artery-vein anastmoses were made side to side using a single running stitch of fine silk (6-0) on atraumatic needles. The lengths of all fistulae were measured at the conclusion of the anastomosis after release of the blood vessel clamps and again at autopsy. With only one exception (Dog 36)§ all iliac and femoral fistulae were made 23 to 40 mm in length. Since in all of these cases the diameter of the fistula substantially exceeds that of the parent artery these minor differences in the length of the fistula can probably be disregarded in making comparisons. All animals were given prophylactic injections of penicillin in oil 150,000 units daily for one week following each operative procedure.

Diagnosis of endocarditis. The term endocarditis is used in these studies to denote an inflammation of the lining of the heart especially that overlying the valves. The diagnosis of endocarditis in these dogs has been based upon the following criteria:

1. Development of fever. 2. Development of elevated blood sedimentation rate. 3. Development of heart murmurs, systolic and diastolic. 4. Observation of petechial phenomena (in eyes, lungs, kidneys). 5. Recovery of organisms from the blood. 6. Observation of typical gross and microscopic autopsy findings within the heart.

Results. The incidence of endocarditis in all animals with arteriovenous fistulae studied is indicated in Tables I, II and III. Inspection of these tables indicates several factors of probable importance in the development of endocarditis. These factors will be discussed under the following headings: (1) relationship of the arteriovenous fistula load to the incidence of endocarditis, (2) duration of the fistula load, (3) age of the animal, (4) pathology, (5) bacteriology, and (6) adrenal weights.

1. *Relationship of arteriovenous fistula load to incidence of endocarditis.* We observed in these studies that there is a very definite level to which one must increase cardiovascular stress in order to be followed regularly by the development of endocarditis. With degrees of cardiovascular stress less than this level the hearts remained immune to the development of "spontaneous" endocarditis. These relationships between degree of increased fistula load and the occurrence of endocarditis observed in these studies are indicated in Table IV.

There are several measurable factors which influence the degree of cardiovascular stress created by an arteriovenous fistula. (a) The diameter of fistula. (b) The position of fistula. As stated above, all of these animals may be considered to have arteriovenous fis-

3. Lillehei, C. W. and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 129.

§ Iliac fistula = 15 mm.

TABLE I. Dogs with Large* Arteriovenous Fistula Load. Young age group (2 to 5 yr).

	Dog No.	Arteriovenous fistula		Days survival after		Cause of death
		No.	Location	Fist. I	Fist. II	
A. With endocarditis	114	2	Iliac-femoral	42	30	Endocarditis
	250	2	Iliac† aorta-vena cava	98	60	"
	124	2	Iliac-femoral	109	76	Heart failure (induced with NaCl)
	451	2	Iliac-iliac	112	27	Endocarditis
	26	2	Iliac-femoral	120	35	"
	35	2	" "	121	63	"
	24	2	" "	148	137	"
B. Without endocarditis	110	1	Aorta-vena cava	2	—	Acute heart failure
	47	2	Iliac-iliac	31	1	Died postop.
	69	2	Iliac-femoral	48	41	Heart failure
	49	3	" " + aorta-vena cava	49 2 (3rd fistula)	21	" " ‡
	431	2	Iliac-femoral	61	20	Sacrificed§
	71	2	" "	109	81	Heart failure
	130	2	Iliac-iliac	276	33	Sacrificed‡

* Fistula load sufficient for the development of endocarditis.
† Iliac fistula partially closed prior to making aorta-cava fistula.
‡ Dog died 2 days after the 3rd (aorta-vena cava) fistula made, other fistulas partially closed.
§ Sacrificed to obtain weight of adrenal glands.

TABLE II. Dogs with Large* Arteriovenous Fistula Load. Old age group (10 yr old or more).

	Dog No.	Arteriovenous fistula		Days survival after		Cause of death
		No.	Location	Fist. I	Fist. II	
A. With endocarditis	6	1	Iliac	55	—	Endocarditis
	37	2	Femoral-femoral	81	69	"
	36	2	Iliac-femoral	109	87	Sacrificed†
B. Without endocarditis	305	1	Iliac	1	—	Acute cardiac failure
	17	1	"	13	—	Gangrene of leg
	4	1	"	36	—	Progressive heart failure

* Fistula load sufficient for the development of endocarditis.
† Endocarditis cured with aureomycin 2 mo. before sacrificed.

TABLE III. Dogs with Small* Arteriovenous Fistula Load. Control series (young age group, 2-5 yr).

Dog No.	Arteriovenous fistula		Days survival after		Result	Cause of death
	No.	Location	Fist. I	Fist. II		
34	1	Femoral	30	—	No endocarditis	Sacrificed
70	1	Iliac	115	—	Living	No evidence of endocarditis
62	1	"	124	—	"	"
100	1	"	125	—	"	"
56	1	"	126	—	"	"
59	1	"	202	—	"	"
135	1	"	237		No endocarditis	Sacrificed
79	1	"	289		"	"
591	2	Femoral-femoral	355	350	"	"

* Dogs with arteriovenous fistula load *insufficient* for the occurrence of endocarditis.

tulae of the same approximate length. The position of the arteriovenous fistula appears to be of importance. The larger the vessels in the lower extremity in which the fistulae were produced the greater was the incidence of endocarditis. The fistulae in the larger vessels obviously have the larger blood-shunting sections.

2. *Duration of the fistula load.* The duration of the arteriovenous fistula is of some

TABLE IV. Relationship of Arteriovenous Fistula Load and Endocarditis.

Dog age (yr)	Sufficient for endocarditis	Not sufficient for endocarditis
Young (2-5)	One iliac + " femoral, *or* 2 iliac, *or* aorta-cava A.V.F.	One iliac, *or* 2 femoral A.V.F.
Old (10 or more)	One iliac, *or* 2 femoral A.V.F.	1 femoral A.V.F.

importance. Following the introduction of an arteriovenous fistula load sufficient[‡] to be followed by endocarditis the earliest that we have been able to diagnose endocarditis is approximately one month. Moreover, in the group of 10 animals (Tables I and II) with the larger arteriovenous fistula loads, which were sufficient in size to result in the spontaneous development of endocarditis, all had died within 148 days. In the ten other animals (Tables I and II) with sufficient fistula loads but which did not develop endocarditis, the duration of life was significantly shorter, although there are several exceptions. It was necessary also to sacrifice several of these dogs with large arteriovenous fistula loads at a time when they did not have endocarditis in order to obtain information as to the relationship of adrenal hypertrophy to valvulitis. It is possible that they also would have developed endocarditis had they been allowed to survive longer. On the other hand in the nine dogs with an insufficient load (Table III), none has developed endocarditis although we have observed them as long as 355 days under exactly similar conditions. Thus, it is apparent that the size, position, and duration of the fistulae are all important determining factors for the total arteriovenous fistula load on the organism.

3. *Age of the animal.* In these experiments the age of the animal was important only in regard to the position of the fistulae. In old dogs bilateral femoral fistulae or a single iliac fistula resulted in endocarditis, whereas larger vessels needed to be involved in young dogs in order to be followed by endocarditis. However, by adjusting the size, position, and number of arteriovenous fistulae it has been possible to observe endocarditis in an equally high incidence in all age groups.

4. *Pathology.*¶ Grossly, the valvular lesions varied in appearance from the soft friable vegetation morphologically similar to those seen in bacterial endocarditis in man to the firm smooth vegetations typical of rheumatic endocarditis (Fig. 1 and 2). Damage to the point of actual rupture of the valve cusp has occurred frequently. Likewise, microscopically the pathological changes in the heart valves show striking similarities to bacterial and to rheumatic endocarditis as seen in man. Often one leaflet may show only bacterial vegetations while another within the same heart shows only the rheumatic type. And occasionally these two types of lesions were seen within different parts of the same leaflet. Vegetations were also found sometimes on the mural endocardium. All valves have been involved except the pulmonary, although not all valves were involved in every animal. Of the ten dogs which developed endocarditis during this study, at the time of death three had vegetations at the site of their arteriovenous fistulae while in the remainder the fistulae were clean. Hemorrhage into valves sometimes occurred. These pathological findings in the heart are summarized in Table V.

One animal in this series with endocarditis (Dog No. 114) has been found to have a typical microscopic picture of acute proliferative glomerulonephritis** in the kidneys.

5. *Bacteriology.* In all but the earliest animals studied repeated blood cultures have been made.†† These findings are summarized in Table V. In all dogs with endocarditis whose blood was cultured, organisms have

‡ Refer to Table IV for definition of terms "sufficient" and "insufficient" as used in context in reference to arteriovenous fistula load.

¶ A morphological study of these materials is nearly complete and will be published in cooperation with Professor B. J. Clawson, Department of Pathology, University of Minnesota.

** Verified by Professor E. T. Bell, Department of Pathology, University of Minnesota.

†† With the cooperation of Professor Wesley W. Spink, Department of Medicine, University of Minnesota.

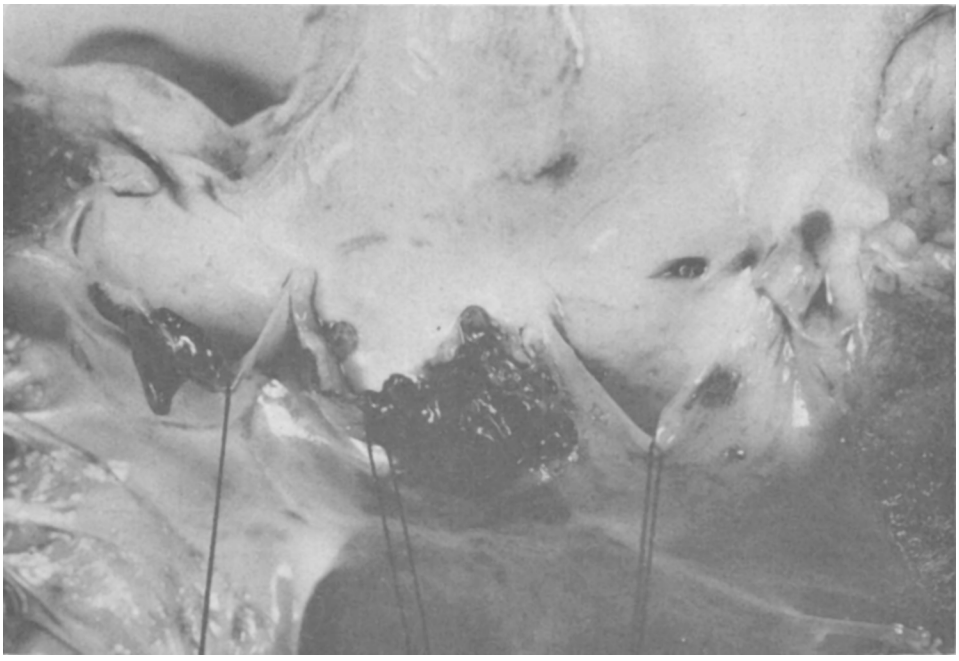

FIG. 1.
Aortic valve, Dog #35. (Right iliac and left femoral arteriovenous fistulas). Note: Soft friable vegetations typical of bacterial endocarditis.

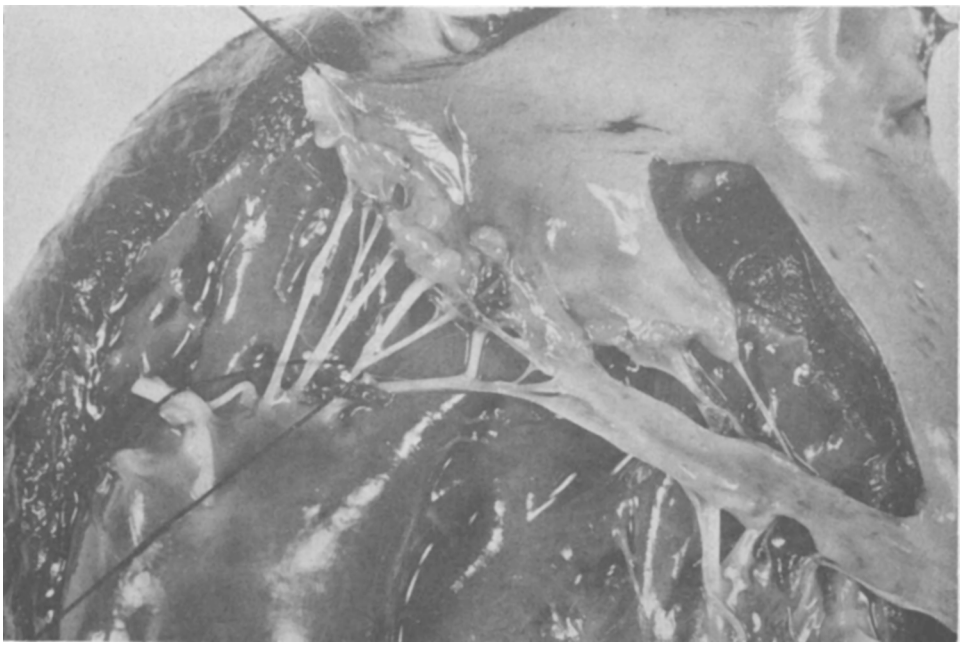

FIG. 2.
Mitral valve, Dog #6. (Old dog with single arteriovenous fistula). Note: Firm smooth vegetations resembling rheumatic endocarditis, and thickened chorda tendinae.

TABLE V. Valve Involvement and Infecting Organism in Endocarditis.

Dog No.	Age	A.V.F. No.	A.V.F. Location	Aortic	Mitral	Tricusp.	Organism
451	Yg.	2	I,I	0	+	0	Not done
114	"	2	I,F	+	+	+	*Strep. viridans*
26	"	2	I,F	+	0	0	*Aerob. aerogenes*
250	"	1	A-Vc	0	+	0	Not done
35	"	2	I,F	+	+	0	*Aerob. aerogenes*
124	"	2	I,F	0	0	+	Not done
24	"	2	I,F	0	+	+	Hemolytic strep.
36*	Old	2	I,F	0	+	0	*Aerob. aerogenes*
6	"	1	I	+	+	+	Not done
37	"	2	F,F	0	+	0	*Aerob. aerogenes*
	Total			4	8	4	

* Blood cultures became negative and clinical condition indicated abatement of the active infection after aureomycin therapy.
I = Iliac A.V.F.
F = Femoral A.V.F.
A-Ve = Aorta-vena cava A.V.F.

been present in the blood. It has been of interest to us, however, that there appears to be nothing specific about the type of organism necessary to cause endocarditis nor does there appear to be any relationship between the type of infecting organism and the type of lesion within the heart, *i.e.* rheumatic-like or bacterial-like. However, it may be significant that the animal mentioned above which developed glomerulonephritis was the only animal in the series with a *Streptococcus viridans* septicemia. Upon histologic examination bacteria were found in the valve lesions.

6. *Adrenal weights.* It was noted early in the course of these studies that the animals which developed endocarditis had large adrenal glands. In all animals studied the adrenal glands have been weighed to the nearest milligram on an analytical balance soon after death. Results of these observations are denoted in Fig. 3. From these data it is apparent that endocarditis was associated with heavier than normal adrenal gland weights and also that this increase in adrenal gland weight preceded the occurrence of endocarditis. Further, those animals with arteriovenous fistulae insufficient to cause endocarditis appear to have adrenal glands of normal weight.

Discussion. From the data presented it is apparent that under the conditions of this experiment dogs "spontaneously" developed a high incidence of severe endocarditis which progressed usually to death. This sequence of events differs materially from the type of experimental endocarditis which has been reported in the past(4-8). In the usual types of experimental endocarditis the infections have been produced by injection of large numbers of often supposedly specific organisms with or without some kind of mechanical damage to the heart valve. This type of endocarditis has usually been characterized by a lower incidence of animals infected, relatively less severe lesions within the heart, and a marked tendency for the endocarditis to heal.

The dogs in this study were subjected to various physiological measurements. In addition to the stress of the arteriovenous fistulae some of the animals were subjected to extra sodium chloride and/or work on the treadmill. However, not all of the endocarditis dogs were subject to either of the above added stresses. All but 2 of the dogs developing endocarditis had physiological studies performed upon them involving venapuncture. Of the animals with fistulas of smaller vessels, showing no endocarditis, all but one had comparable physiological studies. In all probability organisms were introduced adventitiously by these maneuvers which were performed with clean but not aseptic technics. However, the fact that the animals with the lesser cardiovascular stresses imposed showed no endocarditis, seems to indicate that the primary factor in the susceptibility to endocardial infection is the cardiovascular stress.

4. Nedzel, A. J., *Arch. Int. Med.*, 1938, v62, 247.
5. Clawson, B. J., *Arch. Path.*, 1945, v40, 153.
6. Rosenow, E. C., *J. Infect. Dis.*, 1910, v7, 41.
7. Rosenow, E. C., *J. Infect. Dis.*, 1912, v11, 210.
8. Murphy, G. E. and Swift, H. F., *J. Exp. Med.*, 1949, v89, 687.

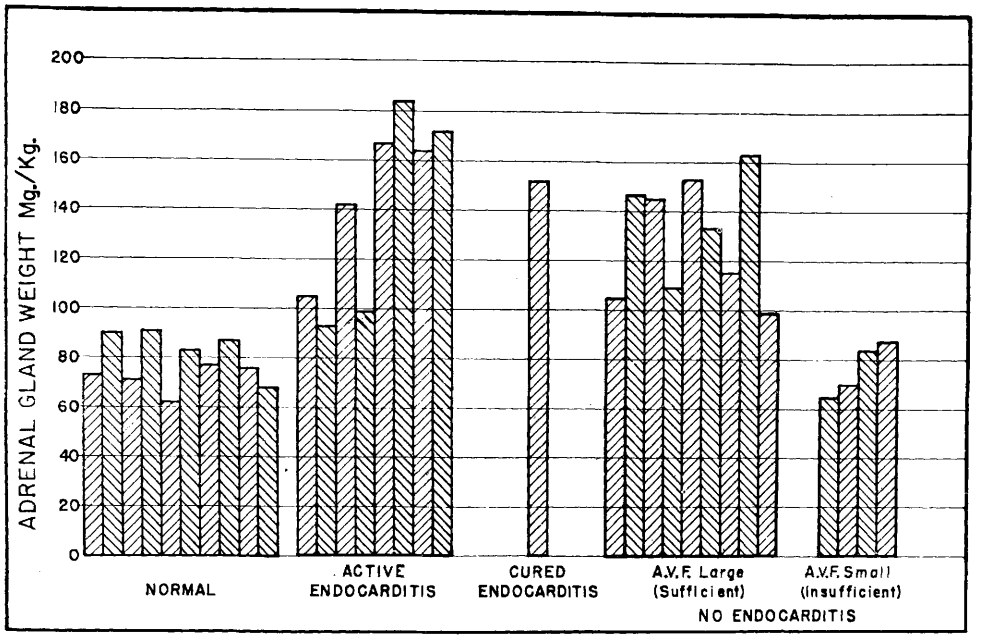

FIG. 3.
Relationship of Adrenal Gland Weight and Endocarditis.
The dogs dying or sacrificed with active endocarditis had heavier than normal adrenal glands. Also animals with large arteriovenous fistulas sufficient in size* to be followed by endocarditis had adrenal enlargement when sacrificed prior to the development of endocarditis. This adrenal enlargement was not seen in animals with smaller* arteriovenous fistulas in whom there was no increased susceptibility to endocarditis.

Mechanical trauma to the valves associated with the increased cardiac output may be a conditioning factor.

An arteriovenous fistula produces a lowering in the total peripheral resistance, the compensation for which is an increase in cardiac output(9). In our studies the cardiac outputs were elevated as much as six-fold, as measured by the direct Fick procedure. The cardiac silhouette as measured by planimetry from 6″ plates was increased. The plasma volume and total extracellular fluid space determinations also showed great elevations, the former up to 100% and the latter by as much as 30%. These observations confirm earlier studies. A large fistula also produces a corresponding rise in pulmonary artery pressure. The average value of the mean integrated pulmonary artery pressure measured with a Statham strain gauge was 16.2 ± 1.1 mm Hg in the normal state as compared with 31.3 ± 1.4 mm Hg in the same animals after creation of the fistulae in dogs developing endocarditis. The mean carotid artery pressure as measured by mercury manometer was not significantly altered by a fistula. Detailed reports on these observations will be made later.

The present study does not indicate what changes as outlined above may be determining in the results observed. However, the increased mechanical work of the heart is obviously a central factor. The question is by what intimate mechanisms this change might influence the ultimate production of endocarditis. One observed fact, namely the increased adrenal weight at death in dogs developing endocarditis, is of interest in this regard. The dogs sacrificed early after bilateral fistula production and without endocarditis showed comparable large glands, indicating that the enlargement precedes the development of valvulitis. It would not serve a useful purpose to speculate at this time about the mechanism by which the stress, the en-

* See text for definition of size.

9. Holman, E., Arteriovenous Aneurysm. The Macmillan Co., New York, 1937.

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.