THE EXTENT OF THE CAPILLARY BED OF THE HEART.

By JOSEPH T. WEARN, M.D.

With the Technical Assistance of LOUISE J. ZSCHIESCHE.

(From the Thorndike Memorial Laboratory of the Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.)

Plates 13 to 16.

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This investigation was begun with the object of determining the relative number of capillaries in normal human hearts as contrasted with the number in abnormal hearts from patients who had shown clinical signs of cardiac failure. In order to approach the problem it was first necessary to establish the size of the capillary bed and the distribution of the capillaries in normal hearts. The present paper, therefore, deals with the capillaries of the normal myocardium as determined by injections through the coronary arteries in man, cats and rabbits, and with the methods used in making these injections.

The coronary vessels have held the attention of numerous investigators for the past 3 centuries. Earlier workers studied their distribution by careful tedious dissections; and during the last few decades the vessels have been injected and outlined with various dyes and masses which have made possible a detailed study of the finer branches. Unfortunately, however, the injection masses used in most instances have failed to penetrate the capillaries and, as a result, the existing knowledge of this important group of vessels is very meagre. The same could be said, moreover, of the capillaries elsewhere in the body until the recent studies of Dale and Laidlaw (1) and Dale and Richards (2) on the effect of histamine upon the circulation, demonstrated the enormous capacity of the capillary bed. Krogh (3) subsequently directed attention to the number and behavior of capillaries in skeletal muscle, and, by his brillant investigations, opened a new pathway to this extensive but obscure branch of the vascular tree. In 1919, Richards (4, 5) made direct observations upon the kidney of a living frog and was able to study the behavior of the capillaries in that organ.

The heart does not permit of direct observation of the circulation in its walls, although Drury and Smith (6) have observed the blood flow in the branch of the coronary artery which supplies the first part of the pulmonary artery in the

turtle. Attempts to inject the capillaries of the heart have met with indifferent and not altogether uniform results. No effort has been made to determine the relative number of capillaries in the heart and very little study has been directed to their relationship to the muscle fibres, their anastomoses with one another or to their connections with the Thebesian circulation. One worker only, A. V. Meigs (7), injected two hearts with Berlin blue and his study led him to the conclusions that the capillaries in the heart were much larger than those elsewhere in the body and were unique in that they actually pierced and entered the cardiac muscle fibres. The sections of heart muscle reproduced in his paper, however, show an incomplete injection of the capillaries, and his evidence is not wholly convincing. Many other workers have failed to fill the capillaries and Nussbaum (8) was led to say that complete injection of the capillaries of the heart was impossible.

Methods.

Several methods for injecting the capillaries have been employed in the course of the present work, some of which have been successful in a few instances only, while one method in particular has been satisfactory in that it has given a uniformly and constantly complete injection of the capillary bed. Both the successful and unsuccessful methods will be described, the former in some detail, inasmuch as many of the experiments which were regarded as unsuccessful and resulted in incomplete injections of capillaries later proved to be of considerable value in a study of the Thebesian vessels.

In selecting materials for the injection it was necessary to choose substances that were soluble or, if in suspension, with particles of such diameter that they would enter the capillaries without difficulty. The other requirements for the injection material were that it remain in the capillaries, show no extravasation and withstand the process of fixation, embedding and staining. Berlin blue and India ink[1] have been employed frequently for injecting blood vessels and in this investigation they proved to be the most satisfactory of the various substances in suspension. Solutions of diazine green and trypan blue were used in some experiments with success, and many other dyes were tried but with disappointing results.

The first observations were made in hearts removed from cats after death and the method of making the injections was that which

[1] Either Weber's or Higgins' India ink was used.

had been commonly employed by most investigators in studying the coronary circulation.

The hearts were kept in the ice box for 48 hours post mortem in order to allow rigor to pass off. In preparation for injection they were perfused with 0.85 per cent salt solution until the perfusate came through practically clear and the heart appeared to be free from blood. This perfusion was carried out at various pressures ranging from 40 to 250 mm. Hg and at a temperature of 45°C. In some of the experiments the temperature was kept constant at 37°C. After washing out the blood with the perfusing fluid, the injection was made at the same temperature and pressure. A 2 per cent suspension of Berlin blue or India ink diluted with an equal part of distilled water was found to be the most satisfactory injection material. Both the saline solution and the injection suspensions were filtered before using in all experiments to be described hereafter. It was soon found, however, that this method, even when most carefully controlled, failed to inject all the capillaries. When the method was applied to human hearts, it proved even less efficient, and in many instances very few of the capillaries were filled. This failure was due in part to the escape of the dye through the Thebesian vessels.[2]

The same procedure was carried out on hearts obtained immediately after death with results slightly more encouraging but far from satisfactory.

In some of these experiments potassium sulfocyanate (3 per cent) was perfused through the coronary arteries to prevent constriction of the vessels, but this addition contributed little or nothing to the success of the method. The results were not dependable, for in some hearts scarcely a dozen capillaries per low power field were injected, while in others there were areas where many of the capillaries were filled.

After many other methods had been tried, partial success was obtained by omitting the washing out of the vessels, and injecting the dye without this preparation. Kerr and Mettier (9) found this procedure satisfactory in their study of the vascularity of the heart valves. The results obtained with this method, though better than the first, were not successful in that all the capillaries of the myocardium were not filled. A possible explanation of the variation in the results of this method was furnished in a series of experiments dealing with the Thebesian vessels. In brief, it was found that India ink, injected into the coronary arteries, escaped for the most part (60 to 90 per cent) through the Thebesian vessels and the capillaries remained unfilled. If, on the other hand, there was scant flow from the Thebesian veins and most of the ink escaped through the coronary sinus

[2] The part played by the Thebesian vessels is included in another paper in this issue.

and veins, the capillaries were apt to be completely or almost completely filled. This method, therefore, in some instances, gave beautiful injections, but it was not dependable and for this reason it was not adopted as the method of choice (see Figs. 1 and 2).

Attention was next directed to living hearts, and the first series of experiments was performed upon cats and rabbits anesthetized with sodium barbital. They were carried out in the following manner.

After complete anesthesia had been obtained, a tracheotomy was done and artificial respiration instituted. The lower part of the sternum was removed with enough of the ribs to expose the entire pericardial sac. Careful hemostasis was practiced and the structures handled as little as possible. Finally, after opening the pericardium in such manner as to bring the left auricle and ventricle into view, the dye was injected through a needle into the left auricular appendage, at a pressure very slightly in excess of that within the auricle. India ink or a dye was injected until the heart showed complete injection grossly, and this after some practise was determined by the appearance of the heart. If ink were used, for instance, the heart became uniformly black only when the capillaries were filled. At this point of maximum filling of the vessels some substance, such as formalin or alcohol, was injected through the same needle in an effort to stop the heart.

The following protocol, which represents the only successful experiment by this procedure, is illustrative.

Experiment Cat 1.—Weight of cat 3 kilos. May 14, 1924. Anesthesia was induced by means of sodium barbital (0.6 gm. per kilo intraperitoneally). Blood pressure was then registered by means of a cannula in the carotid artery. The chest was opened under artificial respiration and the pericardium incised in such a manner that the left auricle and ventricle came fully into view. India ink diluted with an equal part of distilled water and at a temperature of 37°C. was then injected directly into the left ventricle. As the injection began the aorta was clamped at the level of the right carotid artery. The pressure used in injecting the ink was barely sufficient to cause the ink to enter the ventricle against ventricular pressure. When the heart began to turn black, showing a capillary injection, 5 mg. of histamine was injected with the ink. The total amount of ink used was 20 cc. and the injection was made at such a rate that the blood pressure never rose above the original level.

At a certain time during the injection the heart turned very black and at this point a large clamp was placed on the heart in the auriculoventricular groove. The vessels at the base were also clamped, the heart excised and placed in 10 per cent formalin with the clamps in place. This heart ceased to beat almost immediately after the clamp was placed in the auriculoventricular groove. The injection grossly appeared to be perfect.

The experiment reported, however, together with another similar one, Fig. 3, furnishes the only two completely injected hearts obtained thus far in intact animals by injections made directly into the heart with Berlin blue and India ink. The obvious reason why these were successful is that the heart was stopped at the time of the maximum injection of the capillaries. In every other experiment attempts to bring about instantaneous stoppage were unsuccessful[3] and as a result the heart, by continuing to beat, "milked out" the dye from its capillaries. This phenomenon held true when the injected material was a suspension such as India ink or Berlin blue, for in many instances small particles of dye or ink adhered to the capillary walls and outlined them very faintly.

The method which finally gave the most satisfactory and almost uniformly successful injections was found to be the perfusion of the isolated beating heart. The method in brief was carried out in the following manner.

Rabbits or cats were killed instantly by a blow on the head. The heart was excised and a cannula tied into the aorta at a sufficient distance from the aortic valves to prevent blocking of the openings of the coronary arteries. The perfusion was immediately started at a pressure of 45 cm. of water, the temperature being kept at 37°C. The perfusate used was oxygenated Locke-Rosenheim solution (10). The chemicals used in the solution were the purest to be obtained upon the market. The water used was distilled from sodium hydrate, the entire distilling apparatus being made of Pyrex glass. All cannulæ and apparatus employed in the experiment were thoroughly cleaned and finally rinsed with the glass-distilled water before using.

With some practise a heart can be excised and the perfusion started before the heart ceases to beat, but even though it stops beating it is well known that it will begin to beat almost immediately after the perfusion is under way. The method as used will be recognized as a slight modification of the old Langendorff method.

The rate and strength of beat were measured by means of a spring lever tied to the tip of the apex. The coronary flow was collected and measured in a tipping bucket which recorded given amounts upon the smoked drum of the kymograph. When the experiment thus described was properly carried out, the rate of beat and coronary flow soon became constant with a liberal flow through the coronary

[3] Injection of chloroform, digitoxin and very strong currents applied to the heart stopped it in violent systole and this pressed the dye or ink from the capillaries.

vessels. In the event of faulty solution the coronary flow was very scant and the heart soon ceased to beat. When the constant flow level had been reached, a suspension of Berlin blue, 2 per cent, was injected into the stream near the opening of the coronary arteries. If the injection was successful the heart became almost instantly a dark blue color, and at this point it was stopped by the injection of 5 to 10 cc. of glacial acetic acid or formalin (10 per cent) in alcohol (95 per cent) into the perfusion fluid.

Experiment Cat 3.—February 12, 1926. The cat was killed by a sudden blow upon the head, and the heart immediately excised. A cannula was tied into the aorta and perfusion with Locke-Rosenheim solution immediately started. The perfusion pressure was 45 cm. of water and temperature of the perfusing fluid as it reached the heart was 37°C. The Locke-Rosenheim solution was well oxygenated. Coronary flow was registered by means of the tipping bucket, and after the heart rate and flow became constant a suspension of 2 per cent Berlin blue at a temperature of 37°C. was injected into the perfusing fluid near the coronary arteries, and the heart immediately became a deep blue, showing complete capillary injection. This was followed by 10 per cent formalin in 95 per cent alcohol, which caused the heart to stop beating.

Experiment Rabbit 4.—July 23, 1926. The heart used in this experiment was obtained from a rabbit after a procedure similar to that described in Experiment Cat 3. A cannula was placed in the aorta and perfusion was started with oxygenated Locke-Rosenheim solution at a pressure of 45 cm. of water at 37°C. After the flow became constant and the rate had become constant, Berlin blue was injected, a 2 per cent suspension, into the perfusion fluid. This was followed by glacial acetic acid which stopped the heart immediately. The heart became blue with the exception of a small spot which seemed to have blocked vessels. It was immediately placed in 10 per cent formalin in 95 per cent alcohol for fixation (Figs. 4 and 12).

In view of the complete injection of the capillary bed obtained by this method in the hearts of animals, it was applied to human material obtained at necropsy. Hearts obtained within 3 or 4 hours post mortem began to contract regularly when perfused with oxygenated Locke-Rosenheim solution although in some instances the left ventricle remained inactive if rigor had set in.

Experiment Heart 52.—July 15, 1926. This heart was obtained 2 hours post mortem from a boy aged 15 who died of Hodgkin's disease. In the space of another 20 minutes perfusion was begun with oxygenated Locke-Rosenheim solution at 37°C. and 50 cm. of water pressure. In about 5 minutes after the perfusion was begun the auricles began to twitch, this was followed immediately by regular beats, which in turn were followed by ventricular beats. After 10 minutes or so had elapsed the heart was beating normally, ventricular beats following the auricular

beats in regular order. The rate of beat was 62 per minute. The beats gradually increased in strength and finally the left ventricle was spurting the solution through the aorta with good force. When the flow had become steady a 2 per cent suspension of Berlin blue was injected into the perfusing fluid at such speed as to replace the perfusing fluid.

The heart immediately became a deep blue, but the Berlin blue flow was maintained for about 3 minutes, during which time the heart beat gradually weakened but did not cease. Glacial acetic acid was then run into the perfusing fluid and this stopped the heart instantly.

The heart was then sectioned, each section being carefully measured, after which they were placed in aqueous formalin for fixation (Figs. 5, 6 and 7).

Following such injections, the hearts were sectioned, placed in 10 per cent aqueous formalin or 10 per cent formalin in 95 per cent alcohol, for fixation, and, finally, after the standard preparation, were embedded in celloidin and sectioned. If it was desired to count the number of capillaries per sq. mm., careful measurements were made of the blocks of tissue before and after fixation in order that corrections might be made for the shrinkage during fixation.[4]

Sections of the muscle were stained according to the study to be made. When being prepared for counting capillaries per muscle fibre, a van Gieson or lithium carmine stain (11) was employed. If counts of capillaries per sq. mm. were to be made, the muscle was stained with picric acid, or phloxine for contrast to the color of the injection material.

Method of Counting Capillaries.

The number of capillaries in the heart muscle has been determined in two ways. The total number per sq. mm. of muscle has been counted, and the number of capillaries per 1000 muscle fibres has been counted. In counting the capillaries by either method sections were selected in which the fibres and capillaries were cut transversely. The actual counting was then carried out by placing a ruled micrometer field in the eyepiece of the microscope and making the counts of the capillaries and fibres by the same method that one uses in counting erythrocytes in the ordinary chamber.

In counting the muscle fibres that had been cut in cross-sections slight difficulties were encountered because of the branching structure of the fibres. Thus a fibre sectioned at the exact point of branching sometimes appeared so that it

[4] All figures in this paper have been corrected for shrinkage. The method for counting capillaries per sq. mm. is adopted in order to compare the number found in normal hearts with that found in pathological hearts. These studies are now in progress.

might have been interpreted either as one or two fibres. Here the personal equation entered in. No claim is made that the counting of the fibres was absolutely accurate, but rules of counting were adopted and followed throughout and the fact that different people were able to make practically duplicate counts was sufficient to show that this error was not a serious one.

In each heart, capillary counts were made from numerous places in the walls of the ventricles, the auricles, the septum and the papillary muscle. The final figures given in the tables represent the average of many counts, and in most instances, the average of the results of several counters. In almost every instance, a thousand or more capillaries were counted in a given area. The exceptions to this, of course, were in making the counts of the auricle, and of the Purkinje fibres where no single area could be found that contained as many as a thousand capillaries cut in cross-section. The counts were made in areas that appeared to be completely injected, and figures from areas where the injection was obviously incomplete are not included.

The results of counts are shown in Tables I to VII. Table I illustrates variations met with in the counts of capillaries from the same heart by different observers. Tables II to IV show the averages of all counts by all observers of the number of capillaries per 1000 muscle fibres. Tables V to VII show the averages of all counts by all observers of the number of capillaries per sq. mm. of heart muscle. The variations in numbers of capillaries in the normal heart of man, cat and rabbit were very slight—so slight indeed that all these hearts can be said to have approximately one capillary for each muscle fibre.

To say finally that these numbers represent a complete filling of every capillary is not possible, but the finding of the same number in various hearts of man, cats and rabbits is strong evidence in favor of a complete filling of all the capillaries. The even distribution moreover, favored total injection for in many instances when injections were incomplete the distribution of capillaries was uneven and the vacant spaces were easily recognized.

In all parts of the heart, with the exceptions of the auricular walls and the Purkinje system, the capillary supply was practically the same. In the auricles, however, the supply was less abundant and the number of capillaries per muscle fibre varied considerably (*cf.* Figs. 5 and 6). In the thickest part of the auricular wall, where the muscle fibres were large, the count approximated in many instances that of the ventricle, but the auricular muscle fibres were usually distinctly

smaller and of less diameter than those of the ventricle. In such areas the number of capillaries per muscle fibre, therefore, was less than in the ventricle. Moreover, in the very thin portion of the auricular wall the number of capillaries per muscle fibre was distinctly less.

TABLE I.

Human Heart 52.

By whom counted	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle	Purkinje fibres	Muscle immediately adjoining Purkinje fibres
O. A.	1000	1060	1010	1160			
S. W.	828	1196	993	924			
J. T. W.	1091	1133	1040	1079		671	1040
	1108	1100	1018	1083		476	1018
	1046			1058		595	
				1076		607	
						627	
						631	
L. Z.	1004	1080	1090	990	511		
	1050	1120	940	940	478		
	930	1010	960	1009	459		
	930	1040	930	980	484		
	930	1030	960	960	461		
					402		
A. C. E.	1072	1054	1083	1104			
	1067	1069	1074	1101			
	1067	1072	1032	1077			
	1060	1094	1065	1061			
Average of all counts	1013	1081	1015	1040	466	601	1029

In comparing quantitatively the circulation of the Purkinje system to that of ordinary heart muscle, it must be remembered that the actual area of the Purkinje system is small and the number of fibres counted consequently was much less than the number of ordinary fibres counted.

TABLE II.

Human Hearts. Average of All Counts.

Number of Capillaries Per 1000 Muscle Fibres.

Human No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle	Purkinje fibres	Muscle immediately adjoining Purkinje fibres
40	1080	1030	950		550		
		1120	1080		520		
		1110	1110		510		
		1110			570		
		1060					
		1123					
		1126					
		1052					
		1070					
		1160					
		1080					
		1100					
		1000					
52	1000	1060	1010	1160	511	671	1040
	828	1196	993	924	478	476	1018
	1091	1133	1040	1079	459	595	
	1108	1100	1018	1083	484	607	
	1046	1080	1090	1058	461	631	
	1004	1120	940	1076	402	627	
	1050	1010	960	990			
	930	1040	930	940			
	930	1030	960	1009			
	930	1054	1083	980			
	1072	1069	1074	960			
	1067	1072	1032	1104			
	1067	1094	1065	1101			
	1060			1077			
				1061			
57	1058	965	1092	1090	608		
	1170	985	1131	1030	567		
	1214	971	1090	1080	541		
	1177	941	1046	1080	505		
	1183	940	1068	1080	441		
	1165	978	1082	1084	460		
	1070	946	1071	1108			
	1166	970	1038	1040			
	1060		1034	1042			
	1120		1109	1130			

TABLE II—*Concluded.*

Human No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle	Purkinje fibres	Muscle immediately adjoining Purkinje fibres
58	1024	1200	1236	1060	493		
	1104	1080	1180	1080	546		
	1060	1200	1143	1090	508		
	1159	1146	1110	1050	513		
	1135	1081	1095	1080	500		
	1242	1133	1087	1069	482		
	1085	1140	1099	1094			
	1095	1088	1083	1110			
	1233	1072	1139	1080			
	1145	1087	1127	1156			
Average of all counts	1075	1032	1071	1060	503	601	1029

The main branches of the Purkinje system lie immediately beneath the endocardium, and the sections in which the capillaries were quantitated were from the papillary muscles or from the septum. The Best stain for glycogen was first used to identify the conducting system but the hemotoxylin so obscured the blue dye in the capillaries that it was impossible to count them. Consequently the Best stain was modified by Miss Louise J. Zschiesche[5] so that when tissues had

[5] The method was carried out in the following manner:

1. Stain the sections lightly with alum-hematoxylin for 10 seconds or longer, enough to bring out the nuclei.

2. Remove and wash in several changes of water.

3. Stain for 15 to 20 minutes in the following solution as found in Mallory and Wright (Mallory, F. B., and Wright, J. H., Pathological technique, Philadelphia, 8th edition, 1924, 199).

Stock carmine solution freshly filtered	2 cc.
Household ammonia	3 cc.
Methyl alcohol 95 per cent	3 cc.

Filter.

4. Differentiate for 5 to 7 minutes in the following solution, changing the fluid two or three times until it remains uncolored:

Absolute alcohol	80 cc.
Methyl alcohol 95 per cent	40 cc.
Distilled water	100 cc.

been through the process the capillaries remained clearly visible. This stain showed the glycogen as sharply as the Best stain.

The capillary supply to the Purkinje system was found to be much less than that to the heart muscle and the supply decreased rapidly as the endocardium was approached (see Figs. 13 and 8). Thus the Purkinje fibres lying immediately beneath the endocardium had an average of about 600 capillaries per 1000 fibres, while the muscle fibres lying more centrally in a papillary muscle averaged in two counts, 1029 capillaries per 1000 fibres. This is shown beautifully in Fig. 13. In some instances very small clumps of Purkinje fibres, made up of ten or twelve fibres, had a blood supply equal to that of the muscle—one capillary per fibre—but this finding was an unusual one.

When the entire capillary bed was completely injected, the enormous wealth of the heart in capillaries came to light. Practically every heart fibre was in direct contact with one capillary while many were touched by two or more capillaries. The anastomoses between the capillaries were so numerous indeed that these branchings running across the parallel muscle fibres wove a tangled structure of muscle and blood vessels so complex that one fibre was often completely surrounded by capillaries. The longitudinal sections showed this interrelated mesh of fibres and capillaries very strikingly (Figs. 3 and 4).

When the tissue was fixed and embedded in such a way that the shrinkage caused the fibres to stand out separately it was clear that the capillaries lay between and did not actually pierce the muscle fibres, but their intimate entwining about the fibres and through the forks of branching heart fibres frequently gave the appearance of their actually entering the substance of a fibre.

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5. Wash in several changes of 80 per cent alcohol.
6. Wash in one change of 95 per cent alcohol.
7. Stain about 30 seconds in 95 per cent alcohol to which a saturated solution of picric acid has been added (5 cc. picric acid solution; 50 cc. 95 per cent alcohol).

The length of time the sections are left in the picric acid and alcohol depends on the intensity of the color desired for the cytoplasm.

8. Wash in several changes of 95 per cent alcohol.
9. Oil origanum.
10. Balsam.

The long standing controversy over the occurrence of blood vessels in the heart valves has received renewed attention in the past few

TABLE III.

Cat Hearts. Average of All Counts.

Number of Capillaries Per 1000 Muscle Fibres.

Cat No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle	Purkinje fibres
3	1080	1048	1070	1043	701	
	1035	1085	1070	1029	490	
	1006	1068	1008	1024	458	
	1089	1072	1000	1027	463	
	1058	1035	1042	1056	486	
	1085	1102	1057	1030	650	
	1114	1046	1053	1033	558	
	1074	1058	1056	1044	509	
	1094	1050	1066	1046		
	1129		1051	1043		
4	1050	1002	1100	897	370	
	976	1100	1037	970	490	
	1002	833	920	1008	423	
	989	979	942	990	402	
	1023	940	971	1000	455	
	1021	970	910	1061	358	
	1005	979	964	956	485	
	1016	981	1000	957	462	
	978	947	971	1014	454	
	971					
5	1071	1073	1079	1084	608	638
	1021	1087	1115	1110	567	
	1063	1071	1086	1102	492	
	1073	1048	1026	1096	686	
	1063	1051	1064	1018	374	
	1050	1048	1058	1125	405	
	1106	1076	1047	1048	456	
	1058	1069				
	1061					
Average of all counts	1046	1031	1029	1031	491	638

years and several contributions have been made. Numerous methods have been employed to inject the vessels of the valves and the results

TABLE IV.

Rabbit Hearts. Average of All Counts.

Number of Capillaries Per 1000 Muscle Fibres.

Rabbit No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle
1	1140	1143	1118	1073	675
	1114	1131	1140	1084	655
	1070	1150	1030	1030	528
	1080	1200	1082	1030	521
	1030	1122	1100	1040	461
	1100	1040	1150	1030	542
	1140	1219	1102	1235	604
	1033	1117	1117	1096	574
	1121	1084	1122	1103	649
	1080	1091	1108	1104	623
	1081	1107		1108	
	1029				
	1126				
2	1128	1103	1160	1107	634
	1020	1100	1099	1057	730
	1038	1100	1047	1087	666
	1086	1129	1038	1080	747
	1070	1060	1121	1017	581
	1094	1050	1118	1170	463
	1107	1070	1093	1093	761
	1070	1000	1070	1032	568
	1025	1010	1086	1047	747
	1097	990	1090	1000	
		1128			
3	1027	1130	1049	1127	528
	1021	1084	1024	1220	604
	1066	1192	1048	1023	408
	1024	1081	1020	1044	429
	1021	1066	1017	1072	407
	1106	1020	1064	1008	403
	1021	1061	1046	1046	
	1106	1054	1075	1020	
	1021	1049	1068	1065	
	1069	1064	1120	1008	
	1035				
	1036				

TABLE IV—*Concluded.*

Rabbit No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle
4	1130	1093	1082	1150	666
	1098	1061	1077	1031	436
	1010	1009	1092	1028	385
	1070	1051	1038	1043	394
	1080	1050	1056	1063	463
	1041	1014	1070	1049	413
	1019	1029	1092	1024	511
	1028	1019	1060	1009	
		1030	1031	1016	
			1100		
5	1223	1039	1224	1135	597
	1100	1057	1037	1104	414
	1095	1064	1044	1050	506
	1122	1041	1044	1091	590
	1087	1030	1081	1090	511
	1047	1043	1053	1070	477
	1038	1020	1068	1008	
	1022	1035	1038	1022	
	1060	1019	1059	1046	
	1048	1054	1105	1041	
Average of all counts	1071	1072	1075	1044	520

TABLE V.

Human Hearts. Average of All Counts.

Capillaries Per Sq. Mm.

Human No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Purkinje fibres	Muscle immediately adjoining Purkinje fibres
52	5852	5330	4480	4600	2360	4920
	5674	5824	4427	4960	1800	5400
	5720	6023		5600	1524	
	5808	5416		4640	2176	
	5674	5590		5464	2840	
	5632	5893		5240	2880	
	5776			5600		
				5360		
				5600		
				4920		
				5400		
Average of all counts	5734	5679	4453	5216	2263	5160

TABLE VI.

Cat Hearts. Average of All Sq. Mm. Counts.

Cat No.	Left ventricle	Right ventricle	Septum	Papillary muscle	Auricle	Purkinje fibres	Muscle immediately adjoining Purkinje fibres
5	3400	4668	4676	4800	3204	1802	4340
	5040	4015	3181	3240	3131		
	3040	4316	3040	3080			
	4408	4216	4480	4436			
	4424	3857	4340	4340			
	4224	3997					
Average of all counts	4089	4178	3943	3979	3167	1802	4340

TABLE VII.

Rabbit Hearts. Average of All Sq. Mm. Counts.

Rabbit No.	Left ventricle	Right ventricle	Septum	Papillary muscle
3	6336	5676	4160	5413
	6292	5806	4096	5600
	6248	5763	4000	6393
	5896	5720	4000	5413
	5764	6196	4000	7000
	5896	6673	4064	5693
	5852	6933	4064	5413
4	5720	6504	7100	5600
	5490	6461	6480	6461
	5320	6030	6800	5240
	5120	5772	6100	6480
	5160	6076	6300	5480
	5400	5987	6600	5680
	4960	5815	7150	5400
				5680
				5960
Average of all counts	5674	6101	5351	5806

obtained have been very variable. No one has found the vessels constantly present in all valves and this is about the only point upon which all workers seem to agree.

Bayne-Jones (12), Kugel and Gross (13) and Kerr and Mettier (9) have helped to bring order from the mass of contradictory evidence, by demonstrating clearly the presence of vessels in many valves. The difference in the methods of injection used in the past probably accounts for the conflicting claims made. Kugel and Gross have used a method which injects only the finer arterioles as they were not interested in the capillaries. These workers have found the vessels to be inconstant but present in many valves. Kerr and Mettier, on the other hand, have made beautiful injections of the capillaries in the heart valves of pigs.

Before any final verdict can be rendered upon the subject a sufficiently large number of hearts must be studied in order to establish the average occurrence. By injecting the beating heart it has been possible to demonstrate vessels in the auriculoventricular valves in many instances, and in the semilunar valves in a few instances, and the distribution of the vessels showed the injection to be complete (Fig. 9). Instead of ending blindly in the valve the capillaries could be seen to go down onto the leaflet of the valve, make an unbroken loop and return to anastomose with the vessels at the base of the valve. Similarly the vessels in the papillary muscles ran up to the chordæ tendineæ, looped over and returned toward the base of the muscle. In rare instances the vessels ran through the chordæ tendineæ to anastomose with the capillaries on the free portion of the valve (Fig. 10).

It was also true, on the other hand, that in other perfectly injected hearts in which the muscle capillaries and vessels at the bases of the valves were completely filled, the valves showed no vascularity. These valves when cleared showed beautifully injected intact capillary loops at their bases, but none of the capillaries ran down upon the valve proper.

In view of the fact that the first part of the aorta receives a blood supply from a branch of the coronary artery, it was not surprising to find its wall well filled with capillaries (Fig. 11). The parallel course of the arteries and veins in the aortic wall is very striking and characteristic of the vasa vasorum in the wall of this vessel. This rich blood supply in the general region where aortitis is most common may be of important clinical significance.

SUMMARY.

By means of injections made into the coronary arteries of beating hearts it has been possible to determine the number of capillaries in the normal heart muscle. This study has shown a very rich blood supply with an average of approximately one capillary for each muscle fibre in the ventricular walls and papillary muscles, and a less abundant supply in the auricular muscle and Purkinje system. The number of capillaries per sq. mm. of ventricular wall or papillary muscle is about twice that found by Krogh in skeletal muscle. Capillaries were not found constantly in the valves of hearts in which there was apparently a complete injection of the capillary bed. The method described for injecting the capillaries of the heart also provides a means of studying the blood supply to the muscle, valves and aortic wall in pathological hearts.

We wish to express our appreciation to Misses Sylvia Warren and Olivia Ames, and to Dr. A. C. Ernstene for their assistance in making many of the capillary counts shown in the tables.

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EXPLANATION OF PLATES.

PLATE 13.

FIG. 1. 500 diameters. Human Heart 57. Ventricle. Good injections of capillaries made 48 hours post mortem. The injection was made through the veins without previous washing out of the vessels.

FIG. 2. 500 diameters. Human Heart 57. Ventricle. An incompletely injected area from a point near that shown in Fig. 1.

FIG. 3. 700 diameters. Cat 2. Ventricle. Longitudinal section of capillaries showing numerous anastomoses.

FIG. 4. 500 diameters. Rabbit 4. Papillary muscle. Cross-section of capillaries showing a field similar to that in Fig. 12.

FIG. 5. 500 diameters. Human Heart 52. Shows irregular and less abundant circulation of the auricle. Compare with Fig. 6.

PLATE 14.

FIG. 6. 500 diameters. Human Heart 52. Longitudinal section of ventricle showing several anastomoses of capillaries.

FIG. 7. 500 diameters. Human Heart 52. Ventricle showing cross-section of muscle fibres and capillaries.

FIG. 8. Approximately 150 diameters. Human Heart 52. Papillary muscle showing Purkinje fibres at the upper edge of the section. Note the poorer supply of capillaries in the Purkinje tissue when contrasted with the adjoining muscle.

PLATE 15.

FIG. 9. Human Heart 37. Cleared mitral valve, aortic cusp, showing clumps of capillaries in the valve. Note that the capillaries do not end blindly, but anastomose by loops with others nearby.

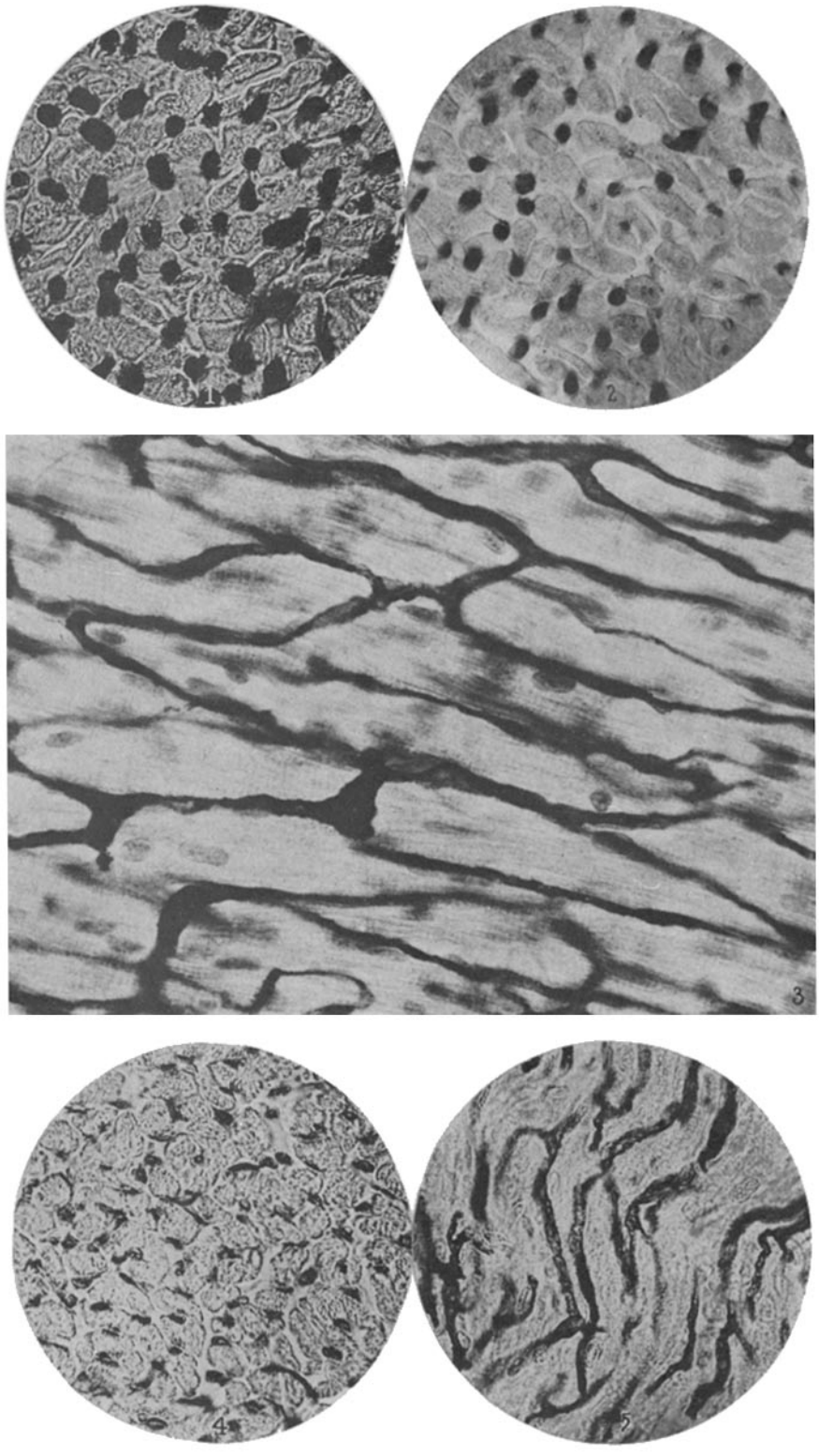
FIG. 10. Human Heart 37. Mitral valve showing anastomoses of vessels in chorda tendinea at the lower part of the plate with vessels on the free portion of the valve.

FIG. 11. Human Heart 52. Cleared section of aortic wall 1 cm. from the aortic valves.

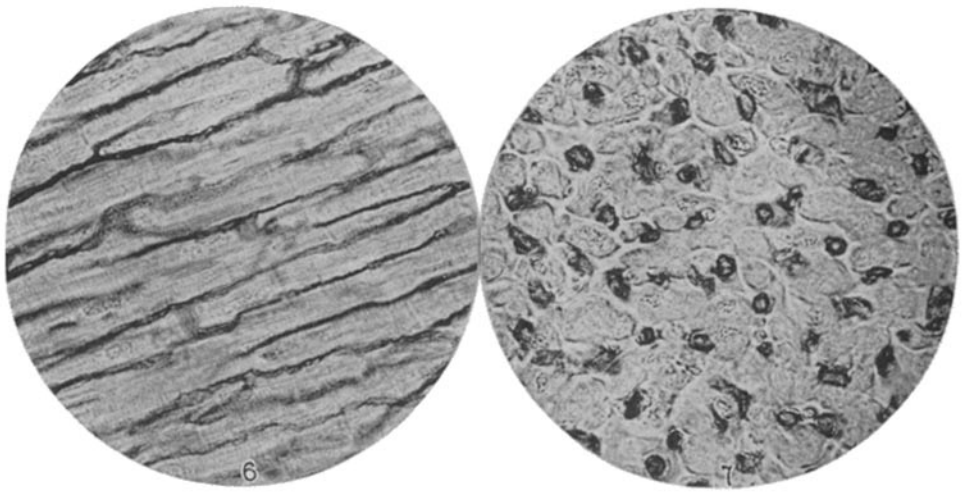
PLATE 16.

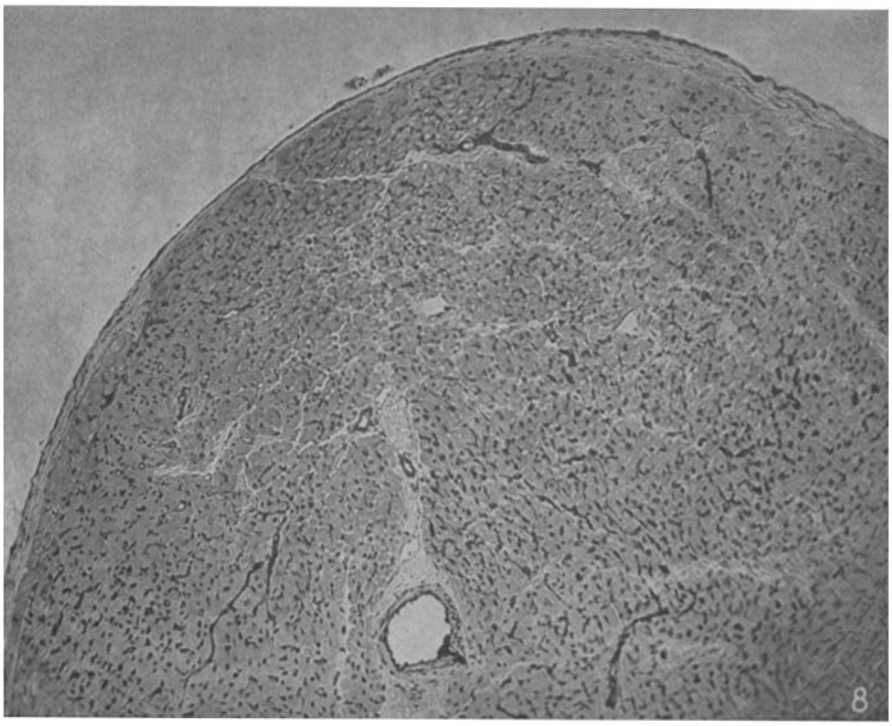
FIG. 12. Ventricle of Rabbit 4. Lithium carmine stain, showing red nuclei and red muscle fibres. The capillaries are filled with Berlin blue. This stain was employed to differentiate capillaries and cell nuclei, in the sections in which the capillaries were counted. This prevented confusion of nuclei and capillaries, and differentiated more clearly the single muscle fibre.

FIG. 13. Human Heart 52. Papillary muscle. Zschiesche modification of Best's stain. Capillaries are blue, Purkinje fibres are red and the ordinary heart muscle fibres are yellow. This plate shows much more clearly than Fig. 8 the difference in blood supply to the heart muscle and the Purkinje system.



(Wearn: Capillary bed of heart.)





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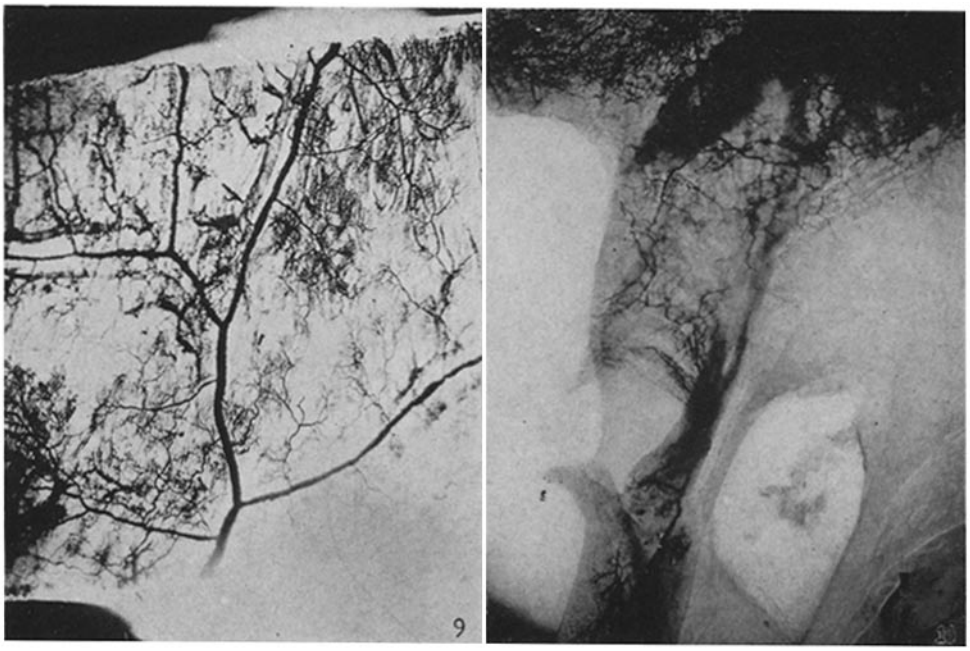
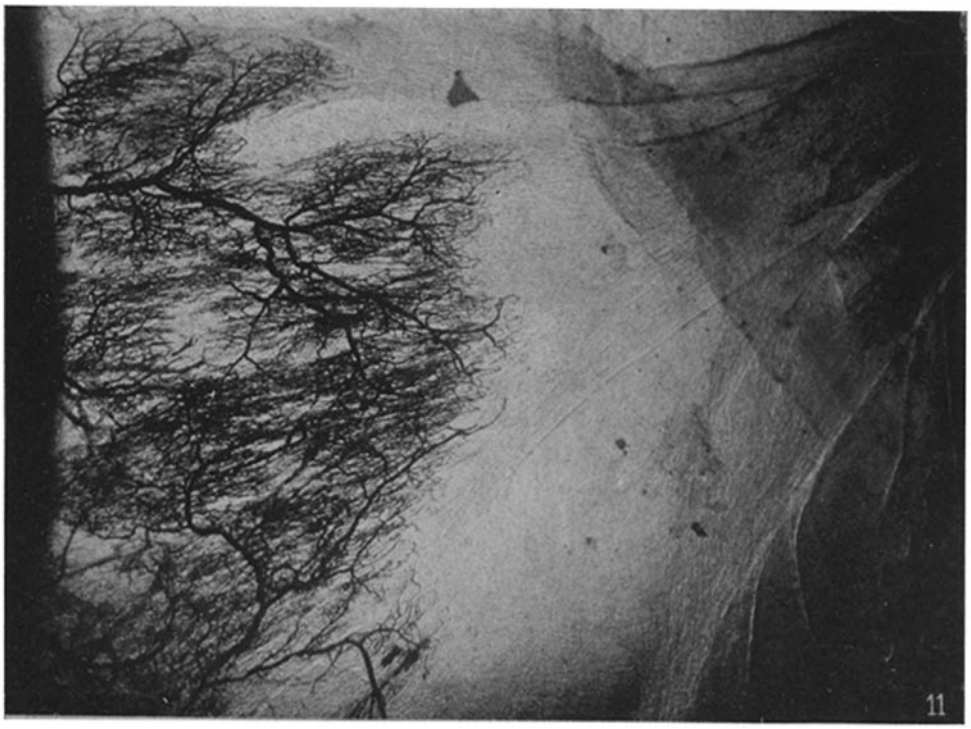
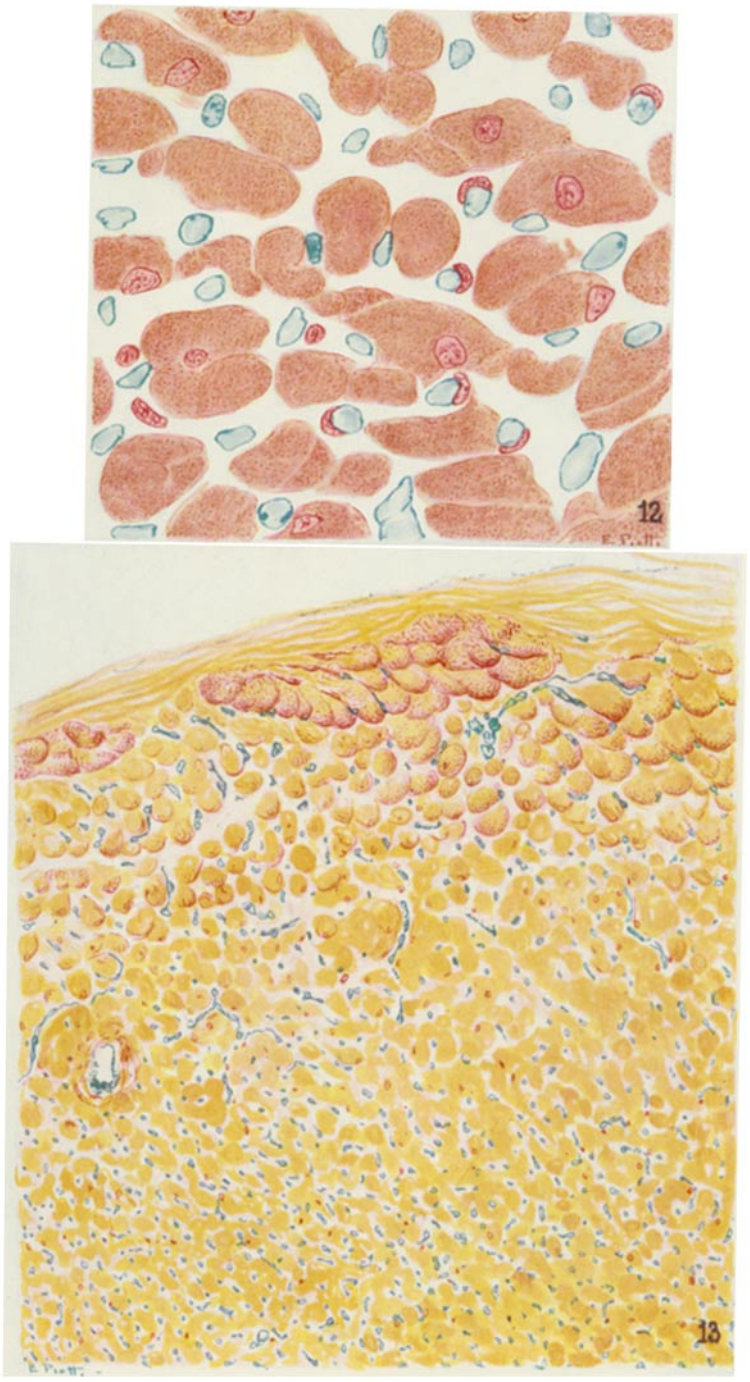




(Wearn: Capillary bed of heart.)



(Wearn: Capillary bed of heart.)