

# THE CELL CLUSTERS IN THE DORSAL AORTA OF MAMMALIAN EMBRYOS<sup>1</sup>

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TWO PLATES (11 FIGURES)

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## I. GENERAL STRUCTURAL CHARACTERISTICS OF THE AORTIC CELL CLUSTERS

Figure 1 represents a portion of the ventral wall of the dorsal aorta as seen in a median sagittal section of a 9 mm. pig embryo. Along its endothelial surface may be observed five or six darkly stained cellular masses or clusters ( $ac_1-ac_5$ ). Figures 2, 3 and 4 represent similar clusters as seen in transverse

<sup>1</sup> Some of the present observations were made while engaged in research work at the University of Strassburg and I wish here to express my indebtedness to Professor Weidenreich for the generosity with which the facilities of his laboratory were placed at my disposal. An abstract of the work was also published in the Proceedings of the American Association of Anatomists, Anatomical Record, Vol. 9, p. 77, 1915.

sections of the dorsal aorta. These figures illustrate the general appearance, maximum size, and anatomical relations of these structures. In consequence of their basophilic reaction to Giemsa's stain they stand out in sharp contrast to the red stained erythrocytes of the aortic circulation.

Cytological details in the structure of these clusters are illustrated in figures 6 and 9. The cells usually approximate a spherical form. The nuclei may be round but they more frequently present an indented or kidney shape and generally occupy an eccentric position in the cell body (figs. 6 and 9,  $m_1$ ,  $m_2$ ). Usually, the nucleoplasm takes a lighter stain than that of the cytoplasm. Chromatin granules are rather evenly distributed throughout the nucleus. The cytoplasm takes a basophilic stain of varying intensity. Occasionally the cytoplasm is also vacuolated and contains phagocytized cellular inclusions, the latter being evidently chiefly of an erythrocytic nature. In some instances a reddish tinted or what appears to be a centrosphere is observed in the region of nuclear indentation (fig. 9,  $m_1$ ). Aside from certain structural variations to be subsequently considered, the nuclear and cytoplasmic characteristics of the majority of these cells are comparable to the basophilic and phagocytically active cells or macrophags (mesamoeboids?) present in the circulating blood of the same embryos. That the component cells of these clusters become gradually dissociated and as detached cells contribute to the circulatory elements of the blood is indicated by those relationships in which certain cells are attached by only a slender cytoplasmic pedicle (figs. 6 and 9,  $m_1$ ) and others are apparently entirely free (figs. 6 and 9,  $m_2$ ).

These clusters were studied in pig, mouse and rabbit embryos and at certain developmental stages found in each of these mammals.

## II. THE QUESTION OF THEIR ORIGIN

1. *Statement of problem.* In endeavoring to ascertain the nature and origin of these cell clusters in the aorta certain ques-

tions arise for consideration. Are they merely more or less agglutinated masses of circulatory blood corpuscles incidentally resting against the vascular surface or are they structures arising directly from the vascular wall? Furthermore, can their occurrence be correlated with any special developmental processes of the embryo?

Of preceding investigators who have recorded the observation of similar structures may be noted Maximow ('09, p. 157) for rabbit and cat embryos, Dantschakoff ('07) for the chick embryo and Minot ('12, p. 525) for the human embryo. Maximow and Dantschakoff interpret these cell masses as endothelial derivatives differentiating in situ from the vascular wall. Minot on the other hand did not find the evidence sufficiently convincing to justify the conclusion that they are of endothelial origin. Beyond the immediate question of their origin no data has been advanced as to why and under what conditions these cell clusters occur in the aorta. The present status of the problem and certain hematological questions associated with the subject are consequently such as to justify a further extension of the investigation.

Of the mouse, rabbit, and pig embryos studied in the present work the cell structures in question were found most pronounced and striking in the pig embryo. Consequently the following account is based chiefly on the results derived from the latter mammal, in which it appears that these clusters have not been previously described. The pig material for this purpose consisted of seventeen embryos varying in size from 6 mm. to 25 mm. fixed in Zenker-formalin (Helly's combination), embedded in paraffin or celloidin and the serial sections stained with Giemsa's fluid. Several of the 12 mm. embryos were also stained in toto with borax carmine and the sections counter stained with Lyon's blue.

2. *Grounds for regarding these clusters as of greater significance than merely incidental structures.* Upon first impression one may be inclined to discredit any special relationship between these cell masses and the aortic wall. As the result of further investigation it appears clear, however, that the phenomenon

is evidently one of greater significance than that of merely incidental cellular accumulations.

First, the clusters are in many cases at least, evidently rather firmly attached to the aortic wall. This is indicated by the fact that these structures may be found in the aorta even though the vessel is practically empty as may occur for example through the loss of blood during the preparation of the material. Again in some cases in which during the process of fixation almost the entire blood content settles toward one side of the aorta, the clusters instead of being carried along with the rest of the blood, continue attached in what appears to be their original position on the aortic wall. Finally, it may be observed that many of the larger cell masses present a rather elongated shape (fig. 1) with one end of the long axis adherent to the aortic wall and the other end free and directed caudalward (towards the left in the figure); i.e., the relations are such as might be expected in an attached cell mass one end of which was free to be carried down stream by the force of the blood current.

Second, the clusters are of constant occurrence in 6 mm. to 15 mm. embryos. Thus in a count for four 12 mm. embryos there was a total of 45 clusters distributed in the proportion of 11, 9, 13 and 12. On the other hand in embryos beyond about the 15 mm. stage, these cell masses are absent. Such conditions do not appear readily accounted for on the basis of accidental agglutination.

Third, in all the embryos studied the clusters were confined to the ventral half of the aortic tube and with greatest frequency toward the median region of its ventral wall (figs. 2, 3 and 4). In no case was a cluster found in the dorsal aortic wall. Such facts certainly appear indicative of a deeper relationship between these cell structures and the aortic tube.

In connection with this conclusion it is of course to be recognized that an occasional cell from the circulating blood may possibly now and then have been fixed in such a manner by the killing reagents as to be adherent to the vascular surface. On the other hand sections other than these passing through the base of the cluster may also present the deceptive appearance

of an absence of attachment, Finally with the gradual disappearance of the clusters in older embryos an intimate histological relationship with the aortic wall may become less and less evident.

3. *Evidence as to their origin from the vascular endothelium.* The preceding considerations rendered necessary a more detailed investigation of the cytological relations and origin of the aortic clusters. On the basis of the following results the conclusion is drawn that they are endothelial derivations and, as will be subsequently more fully elaborated, arise in relation to certain vascular conditions in the ventral portion of the aorta.

The first notable feature to which attention may be directed is the absence in the majority of cases of a definite continuity of the vascular endothelium in the region of contact between the clusters and the aortic wall. Cell boundaries are not clearly defined and the cells of the endothelium are in evidently syncytial relation (illustrated in figure 9 but not clearly evident in the low power drawings for figures 2 and 3). The cluster in figure 9 cannot be said to be resting upon a continuous sheet of typical flattened endothelium. On the contrary it can in the second place be shown that endothelium at the base of the cluster presents marked cytological modifications. In the vicinity of the cell masses the endothelial cells can be observed usually closer together than normally and the rounded nuclei frequently at one side present a more or less marked indentation or concavity giving it a kidney shaped appearance. (fig. 9). It is also to be noted that such endothelial conditions are most evident in the case of the clusters presenting the more intimate relationship with the vascular wall. Third, what appears to be transitional cytological changes can be traced from the somewhat lighter stained endothelial cells at the base of the cluster to the more sharply outlined and more deeply basophilic cells at the periphery of the mass (figs. 9, 6). It may also be observed in the same figures that many of the more peripheral cells still present a clearly defined cytoplasmic elongation or pedicle attaching them to the more central regions of the cluster. Fourth, that these conditions represent active cellular

differentiation rather than cellular disintegration appears demonstrated by the not infrequent evidence of mitotic activity as well as phagocytic function in the component cells of the clusters (fig. 9).

Fifth, areas of the aortic wall are found in which the endothelial cells present structural characteristics comparable to those already described in the endothelium adjacent to the larger cell masses (fig. 10a). Such areas are not, however, in any direct juxtaposition to the aortic clusters nor are they to be explained as deceptive appearances due to oblique or tangential sections of the endothelial surface. It may be observed that the endothelial cells are closer together, project above the general level of the vascular surface, and not infrequently take a more basophilic stain. The nuclei may be either rounded in form or approximate a kidney-shaped contour. No evidence was observed of mitotic cavity indicative of merely an incidental increase of endothelial cells in such regions through ordinary endothelial growth and cell multiplication. It is also noteworthy that such conditions were not found in the dorsal aortic wall as is illustrated in a comparison of figures 10a and 10b. Indeed the cytological structure and vascular relations of these cells appear identical with that of cells occurring in the aortic clusters. The fact that the aortic cell clusters present a great difference in size varying from the large masses in figures, 2 6 and 9 to these smaller accumulations of only a few cells and that they are also no longer present beyond certain stages of embryonic development, suggests that such areas as shown in figure 10a may represent end stages in the gradual dissociation and final disappearance of the aortic clusters, in which, however, there still remain indications of the endothelial reaction which has given rise to these structures. Sixth, of the two fixed tissue elements of the aortic wall which could possibly take part in the cell activities in question it is evidently primarily the endothelium rather than the mesenchyma which participates in the formation of these cell masses. The demarcation between the mesenchyma and the aortic clusters is fairly well defined, nor was there obtained any conclusive evidence of a

possible migration of free cells from the mesenchyma into the clusters.

Finally, it is important to note that not infrequently the aortic clusters are in direct relationship or continuity with cell masses situated within certain atrophying arterial branches of the aorta. These intra-arterial masses have evidently arisen *in situ* from the endothelium of the artery in question and as described elsewhere (pp. 409-411) probably constitute the primary source of origin of the aortic clusters.<sup>2</sup>

### III. CONCERNING THE CORRELATION OF THE CLUSTERS WITH CERTAIN AORTIC DEVELOPMENTAL PROCESSES

1. *Degeneration and caudal wandering of aortic rami.* In the development of the mammalian aorta there occur two important vascular changes involving a shifting or caudal wander-

<sup>2</sup> An additional observation which may be conveniently recorded here relates to a type of structure illustrated in figure 5. This group of cells is attached to the ventral aortic wall and projects into the lumen of the vessel but differs from the typical aortic cluster through its enclosure by a more or less definitely marked peripheral membrane (*en*). In close relation to the membrane are a number of cells some of which present the flattened endothelial form while others are more rounded in shape. In other respects the component cells appear similar to those of the clusters. Such structures are apparently of rare occurrence for in the present material they were found in only one embryo, a 12 mm. specimen (W. U. coll. No. 3), in which there were two of these bodies, both ventrally located. (This embryo had been stained with borax-carmin and the present statements are made without having data derived from Giemsa stained material.) It is of interest to observe that this same embryo was also deficient in the usual number of aortic clusters, for only three of the latter were found instead of the 9-13 clusters in each of four other embryos of the same size.

The aorta of the same embryo also contained three elongated cellular strands evidently of endothelial nature. Two of these strands (about 60 micra in length) were attached to the left umbilical artery near its origin from the aorta. The third strand about 300 micra in length and varying from one to several cells in thickness, was connected by only a slender cytoplasmic strand to the aortic wall. Two such structures were also found in a second 12 mm. embryo (W. U. coll. No. 5), one in the aorta and the other in the region of origin of the umbilical arteries. Nothing conclusive was ascertained as to the significance of these strands, but the suggestion merits further investigation as to whether they may possibly be associated with the fusion of the two original dorsal aortae. With reference to our present purpose, however, it is of interest to note that many of their component cells present rounded form, kidney shaped nuclei, and phagocytic activities comparable to that of the component cells of the aortic clusters.

ing of certain arterial branches of the aorta and an extensive degeneration of others.

Directing attention first to the degenerative changes it will be recalled that the early embryonic aorta has three sets of eighteen to twenty or more paired branches—dorsal, lateral, and ventral. Of these rami, nearly all the dorsal vessels persist throughout embryonic development, whereas practically all the remaining vessels, with certain exceptions, subsequently atrophy and disappear. Thus in the case of the human embryo it has been shown that the primitive lateral branches of the aorta which form an extensive system in the twenty-three somite embryo, have in the 16 mm. to 19 mm. stage embryos become largely atrophied. The single median arterial stems which have replaced the extensive series of paired ventral arteries of younger stages (Tandler, '03) and in a 5 mm. embryo extend as a "complete series of unpaired or median ventral segmentals from the seventh cervical to the second lumbar segment inclusive," in a 7 mm. embryo "have been reduced to three main trunks" the coeliac, superior, and inferior mesenteric arteries. (Keibel-Mall, '12, pp. 603, 611, 643, 653).

Second, it is to be observed that the three remaining arterial trunks to which the ventral segmental aortic arteries have been reduced, undergo a remarkable shifting or caudal wandering as first described by Mall in 1891 and subsequently confirmed by Tandler ('03) and Broman ('08). In the human embryo for example "the coeliac artery thus wanders from the seventh cervical to the twelfth thoracic segment, a displacement of some eleven segments, and the superior mesenteric artery almost equally as far (ten segments, second thoracic to third lumbar); whereas the inferior mesenteric artery wanders through but three segments (twelfth thoracic to third lumbar)" (Evans '12, p. 647).

Referring again to the human embryo it is of especial interest with reference to our present purpose to note that all of these vessels "usually attain the adult levels by the time the embryo is 17 mm. long." Furthermore this caudal wandering of the intestinal arteries is not by a displacement of the aorta on the



vertebral column, but is an actual shifting of these ventral branches when compared with the dorsal branches of the same trunk (Evans, '12, p. 648).

Although the details have not, so far as I am aware, been as carefully ascertained as in the case of the human embryo, essentially the same conditions evidently maintain for the pig embryo as in other mammals. The ventral and lateral aortic rami undergo a similar degeneration. The shifting of the ventral or intestinal vessels is indicated by the fact that the coeliac artery which in a 6.5 mm. pig embryo is at the level of the eighth cervical segment, in a 12 mm. embryo is at the level of the fifth thoracic segment. Again, the superior mesenteric artery in the 6.5 mm. embryo is at the level of the third thoracic segment, but has descended to the level of the eighth thoracic segment in the 12 mm. embryo. Both the atrophy of vessels and caudal wandering appear practically complete at about the 15 mm. stage.

2. *Correlation of the aortic clusters with these vascular changes.*

In a comparative analysis of the preceding data for the vascular changes and cell clusters in the aorta certain striking relationships become evident. First, both phenomena occur within the same period of embryonic development—between the stages of about 5 mm. to 15 mm. in the pig embryo. Again, both the formation of the clusters and the degenerative changes and caudal wandering of the arteries, as already indicated, are confined to the same region of the aorta, namely, its ventral wall. Third, in a linear direction within this ventral region the cell clusters are furthermore fairly evenly distributed between the coeliac and umbilical arteries as shown in the following data for four 12 mm. embryos:

SPECIMEN	(W. U. COLL. NO.)	NUMBER OF CLUSTERS BETWEEN:		TOTAL NUMBER OF CLUSTERS
		The coeliac and superior mesenteric arteries	The superior mesenteric and umbilical arteries	
1	(4)	5	6	11
2	(1)	3	6	9
3	(2)	7	6	13
4	(5)	5	7	12

Fourth, there are certain important cytological conditions to be considered in the degenerating arteries themselves. Many of these vessels are found compactly filled with basophilic staining cells (cf. figures 1 da, 7 and 11). Such conditions are found near the aortic origin of the artery and may extend for short distances into the ramus, occasionally continuing to a point where the degenerating vessel is lost in the mesenchyma. Erythrocytes are strikingly deficient in such regions and may indeed be entirely lacking throughout the vessel, a condition evidently indicative of the reduction if not complete cessation of the circulation through these retrograding arteries. The component cells of these intra-arterial cell masses may take a somewhat lighter stain but they otherwise appear cytologically identical with those of the aortic clusters. They are phagocytically active (fig. 11, in) and undergo cell multiplication (fig. 7, d). In regions of the artery not thus occluded the endothelial cells are frequently rounded up or swollen and project into the lumen of the vessel. The transitional stages to be found between the still intact endothelial cells and the intra-arterial masses seem to leave no doubt but that the latter have arisen in situ from the lining endothelium of the retrograding vessel. Finally, there remains the crucial fact of an intimate relationship between these intra-arterial masses and the aortic clusters. This is illustrated in figure 6 in which the intra-arterial cell mass (*iam*) when followed toward the aorta is found to terminate in an aortic cluster situated within the lumen of the aorta. It may be observed that the component elements merge into each other with no evident line of demarcation between them. Indeed the cytological conditions and morphological relations are such as to justify regarding the phenomenon as essentially comparable to a partial evisceration of the contents of the degenerating artery into the lumen of the aorta. Such a relation of aortic clusters and intra-arterial masses is of frequent occurrence. It is also noteworthy that in many cases where such a relationship is apparently lacking the aortic cluster is, however, situated in a well marked depression or concavity in the aortic wall, and that some of these depressions are in relation

to the atrophied remnant of a small artery which soon terminates blindly in the adjacent mesenchyma. (Such depressions are inadequately shown in *ac2* and *ac4* of figure 1, but can be readily demonstrated in serial sections.) Not infrequently in instances where such depressions are lacking there may still be observed a clearly evident irregularity, sometimes of a more or less whorled character, in the arrangement of the mesenchymal cells at the base of the cluster as compared with the adjacent regions of the aortic wall (figs. 2, 3, *s*). Occasionally the clusters occur in pairs (fig. 4) as if they had arisen in connection with the simultaneous atrophy of two paired aortic rami. In apparent corroboration of these results certain conditions are occasionally found at the aortic entrance to an as yet relatively intact arterial ramus in which the cytological structure, form and relations of the component elements of the vascular surface suggest an early stage in the endothelial activities involved in the production of the intra-arterial and aortic cell masses (fig. 8).

On the basis of the preceding data the conclusion is drawn that the formation of the cell clusters in the aorta are not only intimately associated with, but are also evidently correctly interpreted as a direct result of the developmental processes involved in the atrophy of the ventral and lateral aortic rami and the establishment of the permanent intestinal arteries of the adult organism.<sup>3</sup>

<sup>3</sup> Concerning the remarkable caudal wandering of the visceral rami of the aorta a number of hypotheses have been advanced to account for the phenomenon (Broman, '08, Tandler, ('93), Evans, '12) but the exact manner in which the process takes place has apparently as yet not been established. In connection with the present study it may be observed that the depressions in the aortic wall and the relations of the atrophied arterial stems and the aortic clusters are such as to suggest that arterial remnants of the former aortic rami are ultimately incorporated into the aorta itself. Such additions and consequent inequalities of growth in the ventral region of the aortic wall as contrasted with its dorsal portion may in a final solution of the problem be found to contribute materially to the caudal shifting of the coeliac and mesenteric arteries. Evans ('12, p. 649) does indeed express the opinion that a primary factor in these vascular changes is an unequal growth of the dorsal and ventral walls of the aorta but has not, so far as I am aware, elaborated the specific nature of the process.

#### IV. DISCUSSION CONCERNING ENDOTHELIAL TISSUE AS CONTRIBUTING TO THE CELLULAR ELEMENTS OF THE BLOOD

On the basis of the present results it appears evident that the cell clusters in the embryonic aorta furnish an instance of the vascular endothelium contributing cellular elements to the circulating blood. Since this is not entire agreement with one of the postulates of the angioblast theory, namely that all the blood cells of the organism are direct descendants from the early embryonic blood islands, it becomes of interest to note the conditions under which this endothelial activity is taking place. The close association of the aortic clusters with degenerating vessels directs attention to the occurrence in these retrogressive vessels of stimulative factors to which the endothelium reacts in the manner under consideration. As stated by Thoma ('93) and elaborated by Mall ('06) in the case of the embryonic liver, a vessel in which there is a reduction of the circulation below normal tends to shrink and disappear. The occurrence of such a retardation of circulation in the atrophy of the aortic rami is indicated by the marked absence of red blood corpuscles (p. 410). With the circulation practically at a standstill it is not improbable that diminished oxidation and inhibition of gaseous interchange are in part at least productive of mildly abnormal chemical and toxic conditions conducive to the phagocytic activities, endothelial proliferation and consequent formation of intra-arterial cell masses and aortic clusters.

In support of this conclusion attention may be called to the emphasis being more recently attached to such abnormal intra-vascular conditions as stimulative to endothelial activity. Thus Mallory ('00 and '14, pp. 165-166, 183) advances grounds for the conclusion that certain dilute and weak toxines stimulate endothelial proliferation and phagocytes and maintains that in this manner arise the macrophags encountered in many diseases. Batchelor ('14) and Scott ('14) record marked proliferative endothelial changes and phagocytic activity in hepatic vessels occluded by artificially produced emboli and wounds.

Finally the experimental results of a number of investigators of whom may be mentioned Tschaschin ('13, p. 370) and Mac Curdy and Evans ('12, p. 1695) may be adequately summarized in the recent statement by Evans ('15, p. 254) "that occurrences which place the endothelium of the most various vessels under conditions, such, for instance, as a direct injury of the endothelium, cessation of the adjacent current, in short in all cases of thrombosis or embolism, lead to the proliferation of endothelium . . . ." "Probably no area of the body can be excluded in this respect."<sup>4</sup>

In conclusion, therefore, it may be stated that while the original assumption of the angioblast theory—that vascular endothelium does not give rise to cellular elements of the blood—may under normal conditions be true for the general systemic vascular system, it appears that in both embryo and adult mammals, endothelial tissue ordinarily passive may under certain abnormal conditions, however, assume proliferative activities contributing to the free cellular elements of the circulating blood.

#### V. RÉSUMÉ

1. During the development of mouse, rabbit, and pig embryos certain well defined cell masses or clusters are found in the aorta of these mammals.

2. The majority of the component cells of these clusters are in their cytological characteristics comparable to the basophilic and phagocytically active cells or macrophags (mesamoeboids?) in the embryonic circulation.

3. Their constancy of occurrence, firm attachment and restriction to the ventral wall of the aorta indicate that these cell clusters are not merely chance cellular accumulations but structures having a significant relationship to the vascular conditions in the aortic artery.

<sup>4</sup> The participation of the mesothelium in the origin of macrophags in the embryonic coelom (Emmel, '15) is not improbably also a reaction to stimulative conditions arising in part at least through degeneration and disintegration of erythrocytic and other foreign elements escaping into these cavities.

4. Certain structural modifications and frequent discontinuity in the endothelium at the base of these masses, the cytological characteristics transitional between these endothelial cells and the component cells of clusters, the evidence of mitotic activity, variation in size, and relationship to certain degenerating aortic rami, support the conclusion that the aortic clusters have arisen from the vascular endothelium.

5. An intimate association and fundamental causal relationship can be demonstrated between the formation of the aortic clusters and the developmental processes involving the atrophy of certain aortic rami and the establishment of the permanent intestinal arteries of the adult mammal. The endothelium in degenerating stems of the aortic rami is stimulated, (evidently through certain toxic conditions arising in the retrogressive vessels), to phagocytic and proliferative activities giving rise to infra-arterial cell masses constituting a primary source of origin of the aortic clusters.

6. On the basis of the cumulative evidence of various recent investigators it appears evident that the original assumption of the angioblast theory that the endothelium of the general systemic vascular system does not contribute to the cellular elements of the blood, while possibly true under normal conditions, requires the qualification that under certain abnormal conditions endothelial tissue ordinarily passive may in both embryo and adult assume such proliferative activities.<sup>5</sup>

<sup>5</sup> While the present paper was in press an article appeared in the *Anatomical Record*, Vol. 10, p. 417, by Jordan on the "Evidence of Hemogenic Capacity of Endothelium." It is of especial interest to note that Jordan records the observation of cellular structures in the aorta of mongoose and turtle embryos apparently similar to the clusters occurring in the pig, mouse and rabbit embryos. Here again the clusters are confined to the ventral region of the aorta. In the mongoose and turtle, just as in the pig, "the clusters show a progressive increase in size corresponding with the age of the embryos, between 5 and 10 mm., indicating an intrinsic growth" p. 419. It is emphasized that "similar clusters are found nowhere else either in the yolk sac or the embryonic vessels or sinusoids" (p. 418) and in agreement with the results of the present paper cogent reasons are advanced for regarding these clusters as being not merely chance cellular accumulations, but as structures arising from the vascular endothelium.

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## PLATE I

### EXPLANATION OF FIGURES

Figures 1, 6 to 11, inclusive, are from 9 mm. pig embryos fixed in Zenker-formalin and stained with Giemsa. Figures 2 to 5, inclusive, are from 12 mm. embryos fixed in Zenker-acetic and stained with borax carmine and Lyon's blue. All figures are from sagittal sections, except figures 2 to 5 which are from transverse sections of the aorta. The drawings, reduced one-fifth in reproduction, were originally made with the magnifications obtained in the following combinations of Zeiss apochromatic lenses and compensating oculars:

Figs. 1 to 4, oc. 8, No. 3, obj.

Fig. 5, oc. 4, 2 mm. obj.

Figs. 6 to 11, oc. 6, 2 mm. obj.

Recognition is due the artist, C. D. Jarrett, for faithful reproduction of cytological details.

#### ABBREVIATIONS

<i>ac</i> , aortic cluster	<i>la</i> , lateral aortic ramus
<i>d</i> , mitosis	<i>m</i> , the more highly differentiated basophilic cells (or macrophags) in the aortic clusters
<i>da</i> , degenerating aortic ramus	<i>r</i> , erythrocyte
<i>dw</i> , dorsal aortic wall	<i>s</i> , mesenchyma
<i>e</i> , endothelium	<i>va</i> , ventral aortic ramus
<i>iam</i> , intra-arterial cell mass	<i>vw</i> , ventral wall of the aorta
<i>in</i> , phagocytic inclusion	

The direction of circulation and the long axis of the aorta is indicated by an arrow.

A portion of the ventral region of the aorta as seen in longitudinal section, showing the general appearance and morphological relations of the aortic clusters (*ac*<sub>1</sub>-*ac*<sub>6</sub>). A caudalward projection of the free end of the clusters is illustrated in *ac*<sub>4</sub>. Cluster *ac*<sub>6</sub> is situated at the entrance to a root of the superior mesenteric artery. *da* is a section of a degenerating aortic ramus packed with basophilic cells similar to those of the clusters.

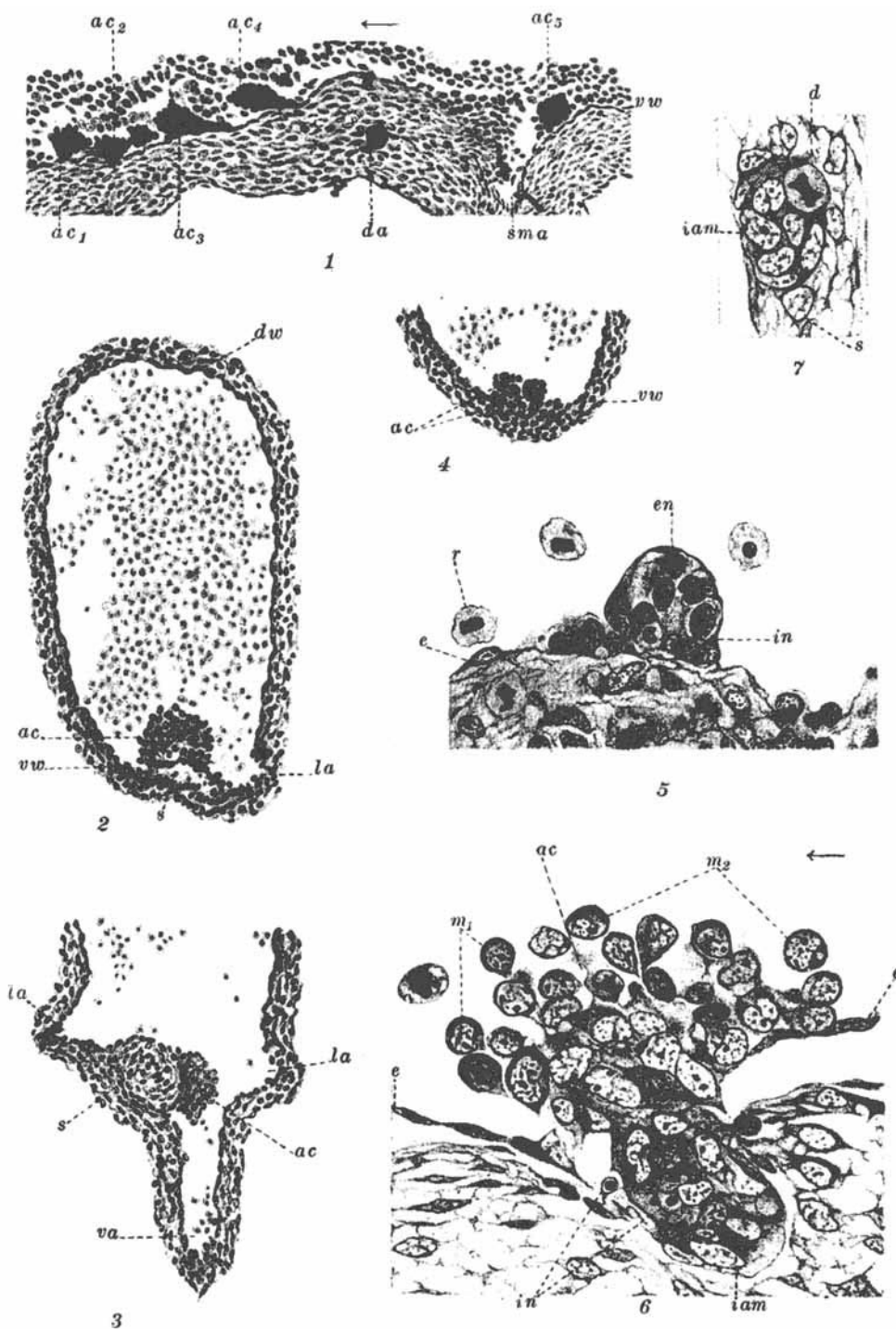
2 and 3 Show the form and structural relations of the aortic clusters as seen in transverse sections of the aorta. Adjacent to the base of these clusters may also be observed a variation in the general arrangement of the mesenchymal cells (*s*).

4 Illustrates the double aortic clusters occasionally found.

5 One of two spherical masses of cells found in a 12 mm. embryo. The mass appears in some respects comparable to the aortic clusters, but is surrounded by a more or less definite endothelial membrane (*en*). Situated in the ventral wall of the aorta.

6 Section of an aortic cell cluster situated at the entrance to a ventral aortic ramus and in intimate relationship with the intra-arterial cell mass (*iam*) filling the latter vessel. Also illustrates the more definite differentiation of the peripheral cells (*m*<sub>1</sub>) of the clusters, some of which appear entirely detached from the main mass (*m*<sub>2</sub>).

7 Section of an intra-arterial cell mass in a degenerating ventral aortic ramus. Note absence of a definite lining endothelium and the evidence of active cell multiplication (*e*).



## PLATE 2

### EXPLANATION OF FIGURES

Region of the vascular endothelium at the entrance to an aortic ramus (*ar*). Is of interest as showing cytological changes in the endothelial cells (*e*<sub>1</sub>, *e*<sub>2</sub>, *e*<sub>3</sub>) suggestive of an initial stage in the formation of a cell cluster. The drawing includes only the caudal side of the orifice of the smaller vessel, the entrance to which is indicated by the arrow at the right.

9 Illustrates the cytological structure of an aortic cluster, the discontinuity of the aortic endothelium at its base, evidence of mitotic cell multiplication (*d*), the gradation in structural characteristics from the basal (*e*<sub>1</sub>) to the more peripheral cells of the cluster (*e*<sub>2</sub>, *m*<sub>1</sub>) and the apparent detachment of some of the latter as free cells (*m*<sub>2</sub>). The anatomical relations appear brought out to advantage in this particular case, through the artificial separation of the endothelial surface (*e*) from the underlying mesenchyma (*s*) during the histological preparation of the material.

10a and 10b Are respectively from directly opposite regions of the ventral and dorsal walls of the aorta and illustrate striking differences in endothelial structure. Note the flattened form and much wider separation of the endothelial cells in 10b as contrasted with 10a and, in the latter case, the kidney shaped nuclei and rounded cells (*e*<sub>1</sub>, *e*<sub>2</sub>) raised above the general level of the vascular surface.

11 Transverse section of a ventral arterial branch of the aorta filled with basophilic cells. Some of the cells are phagocytically active (*in*). A definite lining endothelium is no longer evident. (cf. fig. 7 and fig. 1, *da.*)

