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An X-ray microscopic study of the postnatal development of the vasa vasorum in the human aorta

By JOHN A. CLARKE
Department of Anatomy, The University, Glasgow W.2

INTRODUCTION

Little reference could be found in the literature to the development of the vasa vasorum in the aortic wall of neonates, infants or children. The main paper is by Schlichter (1948), who X-rayed (technical details not given) 10 μ sections of dog, rabbit, chicken and human aorta after the injection of a radio-opaque medium and concluded that while the dog aorta was the most vascular, the human aorta received the richest distribution of vasa vasorum during the neonatal period.

The present work is part of a series of studies on the vasa vasorum of the human arterial wall, using the Coslett Nixon X-ray projection microscope. In a previous communication (Clarke, 1965*a*), X-ray microscopy, as a method for investigating small arteriole and capillary beds, was considered, and the results compared with the routine injection, clearing and histological methods of previous investigators (Pickworth, 1934; Winternitz, Thomas & LeCompte, 1938; O'Neill, 1947; Williams, 1948; Scharrer, 1950; Woerner, 1959).

MATERIAL AND METHODS

Twenty normal neonatal and twenty normal aortae from children in the first 5 years of life were obtained within 12 hr. of death and examined in equally divided groups of 5 at weekly and yearly intervals respectively.

Fifteen normal aortae were obtained as before from children between 5 and 15 years of age and examined in equally divided groups of 5 at 3-yearly intervals.

In no case was there a history or evidence of cardiovascular disease.

In each case the aorta, with the heart attached, was removed in a single block of tissue and the bronchial arteries preserved. The remainder of the collateral branches of the aorta were occluded 1 cm. from their origin.

After irrigation of the specimen with normal saline at 37° C for 2 hr., the aortic vasa were injected with an undiluted suspension of micropaque (particulate diameter 0·5 μ) at manometrically controlled pressures of 100 mm Hg before the fifth year and 120 mm Hg thereafter.

The arterial vasa were demonstrated by injecting through the brachio-cephalic trunk, the coronary sinus being occluded in neonatal and infant specimens to prevent retrograde filling of the venous vasa in the adventitia of the ascending aorta with micropaque, which had tracked through the foramen ovale from the left side of the heart.

The venous vasa on the ascending aorta, arch, descending thoracic and abdominal aorta were injected through the coronary sinus, bronchial, intercostal and lumbar veins respectively.

X-ray projection micrographs of full thickness aortic wall and about 1 mm. thick sections, cut by hand, were taken on Ilford Contrasty Plates, with an exposure time of 5 min. before the fifth year and 7 min. thereafter. The microscope was operated at 10 kV. and 50 μA. with an aluminium target providing the X-radiation from birth until the fifth year, and at 15 kV. and 30 μA. with a copper target from the fifth to fifteenth years.

When comparing the vascular densities of specimens, the arterioles and capillaries were estimated against a radio-opaque grid with 200 grid squares per inch, which was superimposed on the specimen when the micrograph was taken. The arterioles and capillaries in every square were counted as separate vessels. Since the technical advantages of the X-ray microscope allow vessels in whole thickness mounts to remain in focus within limited magnifications, vessel 'counts' were performed at primary magnifications of $\times 20$. For ease of counting, such an X-ray micrograph may be projected on to a viewing screen when the number of vessels in each square of the grid may be recorded. The area of a full thickness mount of aortic wall covered by the grid at each count was 0·5 cm.^{2} and a total of three such fields were examined from each of the ascending, arch, thoracic and abdominal portions of the aorta. The site of these fields was kept constant in different specimens.

The density measurement per square of the grid in each part of the aorta, therefore, came from a total of 1·5 cm.^{2} of aortic wall and was obtained from the total number of vessels being divided by the number of squares.

The intrinsic vascular arrangements were studied histologically in five neonates and ten specimens of aorta from children between the first and fifteenth years of life. Pickworth's (1934) method (sodium nitroprusside benzidene stain) and routine sections stained with Masson and Mallory's trichrome techniques (Culling, 1957) were used to provide controls for the micrographs.

RESULTS

Radiological observations

(*a*) *Ascending aorta*

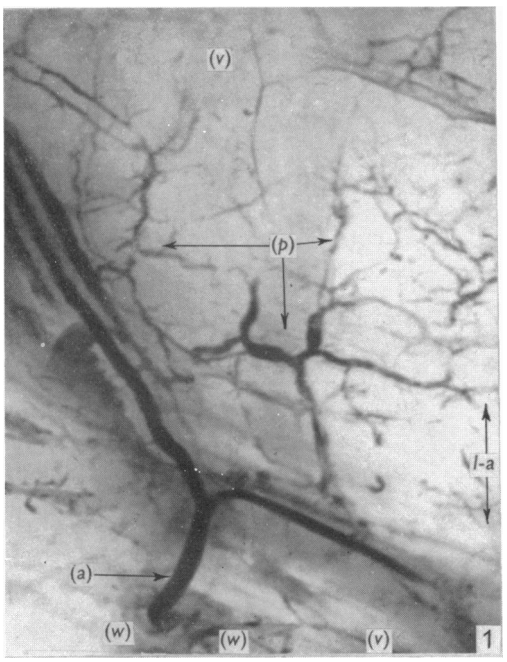
In the neonatal aorta an irregular network of arterioles, 40–60 μ in diameter, originated from the coronary ostia to be distributed to the adventitia (Fig. 1). By the second year longitudinal, sinuous, adventitial arterioles, 60 μ in diameter, had replaced the neonatal picture (Fig. 2). At the distal end of the ascending aorta an anastomosis existed between the adventitial arterioles of the proximal aortic arch and the ascending aorta (Fig. 3) and slight coiling of the adventitial arterioles occurred in the fifth year. Arterial vasa were confined to the adventitia until the fourth year when a secondary network of small arterioles and capillaries, 10–20 μ in diameter, penetrated the outer third of the media (Fig. 4).

Examination of the micrographs showed that the venous vasa formed an irregular plexus of veins 50–70 μ in diameter, which was confined to the adventitia until the fourth year, when tributaries drained the outer third of the media.

(*b*) *Aortic arch*

In neonatal specimens an irregular plexus of arterioles, 40–50 μ in diameter, originated from the ostium of the brachio-cephalic trunk immediately after the

parent vessel had left the aortic wall and from terminal branches of the bronchial arteries to be distributed to the adventitia (Figs. 5, 6). By the second year sinuous adventitial arterioles, 60 μ in diameter, had replaced the irregular plexus on the aortic summit. These arterioles became more sinuous in the fifth year and extended on to the sides of the aortic arch, replacing the irregular network which had persisted until this time (Fig. 7). Arterial vasa penetrated the outer third of the media in the fourth year to distribute a plexus of arterioles and capillaries similar to the ascending aorta (Fig. 4).



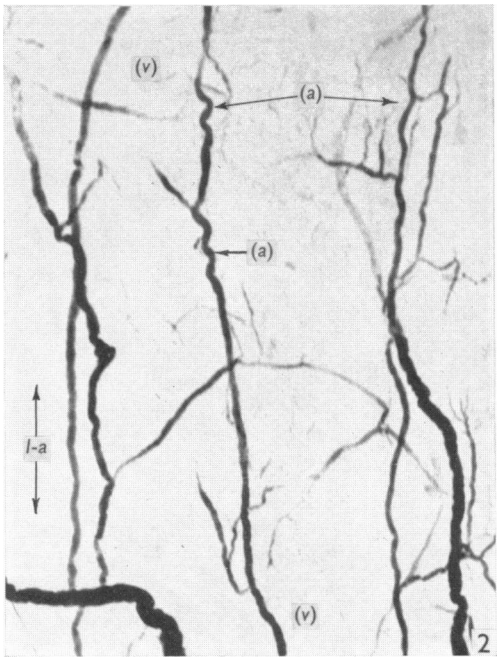


Fig. 1. Micrograph of the ascending aorta, aged 3 weeks. Note the arteriole (*a*) originating from the wall (*w*) of the coronary ostium and the arterial plexus (*p*) in the adventitia (*v*). × 50. All micrographs are of full thickness aorta except figs. 4, 8, 12, 15, and 17. Long axis of aorta (1–*a*).

Fig. 2. Micrograph of the ascending aorta, aged 2 years. Note the longitudinal sinuous arterioles (*a*) in the adventitia (*v*). × 30.

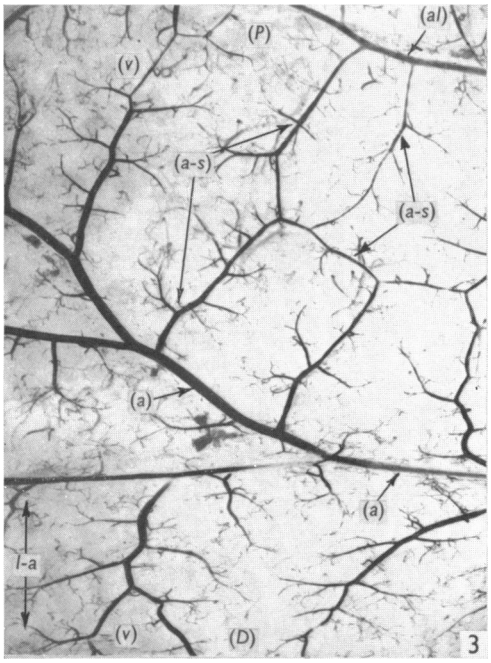
Venous vasa appeared as an irregular plexus of adventitial veins, 50–70 μ in diameter, which received tributaries from the outer third of the media in the fourth year (Fig. 8).

(*c*) *Descending aorta*

In neonatal aortae a spiral network of arterioles, 40–60 μ in diameter, originated from the intercostal, lumbar and mesenteric arteries immediately after the parent vessels had left the aorta to be distributed to the adventitia. Arranged in a spiral fashion almost at right angles to the long axis of the aorta, these arterioles also distributed a few longitudinal branches to the adventitia (Fig. 9).

The spiral network of arterioles was replaced by a dense irregular plexus of arterial vasa, 50–70 μ in diameter, in infant aortae (Fig. 10). Situated predominantly on the lateral sides of the descending aorta, the pattern of these vessels was retained until the fourth year, when longitudinal sinuous arterioles, 60–80 μ in diameter, were formed. As the posterior aspect of the descending thoracic aorta was approached, the arterial vasa became progressively poorer in distribution (Fig. 11).

The proximal adventitial arterioles of the thoracic aorta anastomosed with the adventitial arterioles on the distal aortic arch in a similar arrangement to the anastomosis described at the proximal end of the arch.



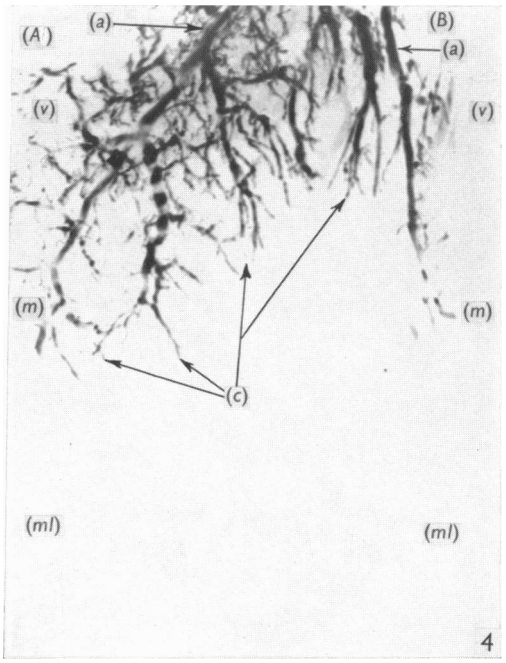


Fig. 3. Micrograph of the distal part of the ascending aorta (*D*) and the proximal part of the aortic arch (*P*), aged 4 years. Note the arterial vasa (*a*) from the ascending aorta anastomosing (*a-s*) with the arterial vasa (*al*) from the aortic arch. Adventitia (*v*). ×40.

Fig. 4. Micrograph of 1 mm thick transverse section of the junction of the ascending aorta (*A*) and the aortic arch (*B*), aged 4 years. Note the arterioles (*a*) in the adventitia (*v*) distributing capillaries (*c*) to the outer third of the media (*m*). No injection medium has entered the inner media (*ml*). ×120.

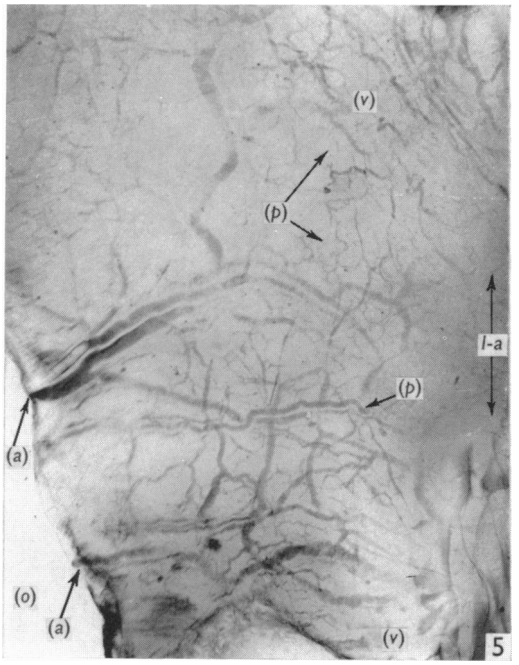
Arterial vasa, which had been confined to the adventitia until the fourth year, penetrated the outer third of the media to form a secondary network of vessels 10–20 μ in diameter (Fig. 12).

The venous vasa formed an irregular network of veins, 60–80 μ in diameter, which received tributaries from the outer third of the media in the fourth year.

Aortae examined between 5 and 15 years of age

No alteration could be demonstrated in the patterns described for the aortic vasa until the tenth year.

Between the tenth and twelfth years the longitudinal sinuous arterioles in the adventitia of the ascending aorta and summit of the aortic arch showed increased sinuosity and coiling, while the arterial vasa on the sides of the aortic arch only revealed increased sinuosity. An anastomosis between the adventitial arterioles of the proximal and distal ends of the aortic arch with the adventitial arterioles of the ascending and descending aorta could no longer be demonstrated (Fig. 13).



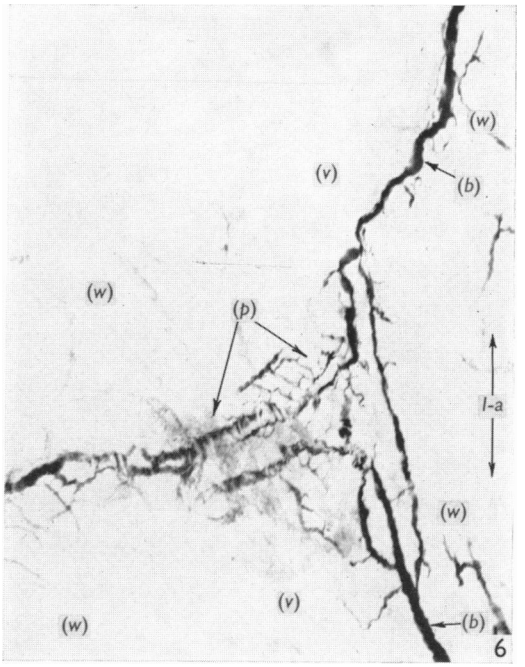


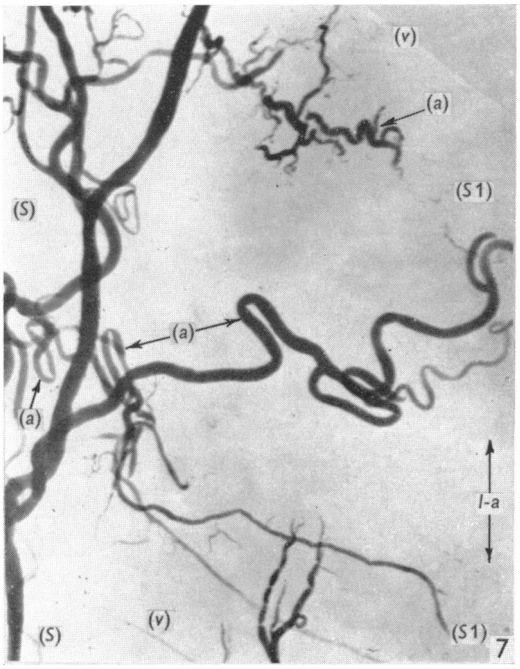
Fig. 5. Micrograph of the summit of the aortic arch, aged 3 weeks. Note the arterioles (*a*) originating from the ostium (*o*) of the brachio-cephalic trunk and the plexus (*p*) of arterial vasa in the adventitia (*v*). × 50.

Fig. 6. Micrograph of the side of the aortic arch, aged 3 weeks. Note the arteriolar plexus (*p*) in the adventitia (*v*) originating from terminal branches (*b*) of the bronchial arteries. Wall of aortic arch (*w*). × 50.

In the adventitia of the descending thoracic and abdominal aorta, a well-defined longitudinal plexus of sinuous and coiled arterioles was observed, which showed a segmental anastomosis on the lateral aspects of the aorta (Fig. 14). In addition, the arterial vasa from the mesenteric arteries appeared as sinuous arterioles, 80–100 μ in diameter, on the anterior aspect of the abdominal aorta.

Arterial vasa began to penetrate the middle third of the media in all parts of the aorta from the tenth year, and by the thirteenth year a well-defined capillary plexus could be seen at the junction of the middle and inner thirds of the media (Fig. 15).

Well-marked venous patterns began to emerge in the aortic adventitia in the thirteenth year. Longitudinal venous channels, 100–140 μ in diameter, could be demonstrated in the adventitia of the ascending aorta, while a circumferential pattern of adventitial veins emerged in the adventitia of the descending thoracic and abdominal aorta (Fig. 16). The veins in the adventitia of the aortic arch retained their plexiform appearance during this period.



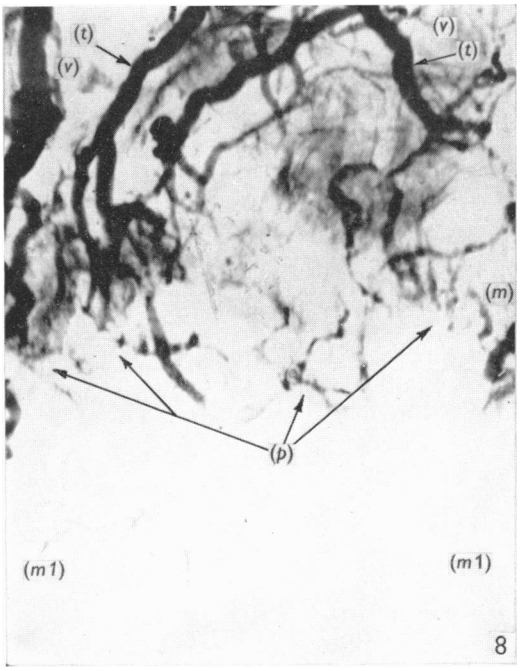


Fig. 7. Micrograph of the summit (*S*) and side (*S*1) of the aortic arch, aged 5 years. Note the sinuosity of the arterioles (*a*) in the adventitia (*v*). × 50.

Fig. 8. Micrograph of 1 mm. thick longitudinal section of the aortic arch, aged 4 years. Note the origin of the venous plexus (*p*) in the outer third of the media (*m*) draining into tributaries (*t*) of the adventitial veins. Adventitia (*v*). No injection medium has entered the inner media (*m*1). × 110.

Venous tributaries of the adventitial veins drained an ever-increasing portion of the media as the arterial vasa penetrated the aortic wall from the tenth year. By the thirteenth year vessels 10–20 μ in diameter could be demonstrated in the inner third of the media when the venous vasa were injected (Fig. 17).

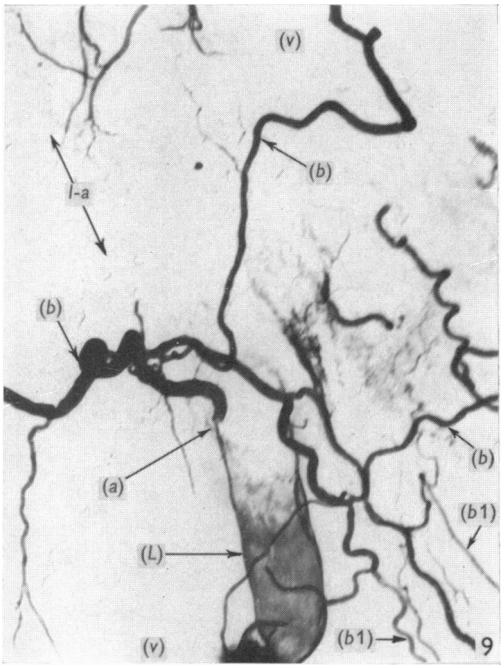
No injection medium entered the intima of any specimen examined.

Histological observations

In general the vasa vasorum conformed to previous descriptions in neonatal, infant and childrens' specimens. After the tenth year, however, no vessels could be demonstrated in the inner third of the media and only occasionally at the junction of the middle and inner third (Fig. 18).

Vascular densities in the aorta

When the arteriole and capillary distribution was examined on the grid, it was found that, while the density of arterioles and capillaries was greater during the neonatal period than at the end of the first year of life in the ascending aorta and arch, the reverse was true in the descending thoracic and abdominal aorta. The results of arteriole and capillary 'counts' recorded in different parts of the aorta between birth and 15 years of age are shown in Fig. 19.



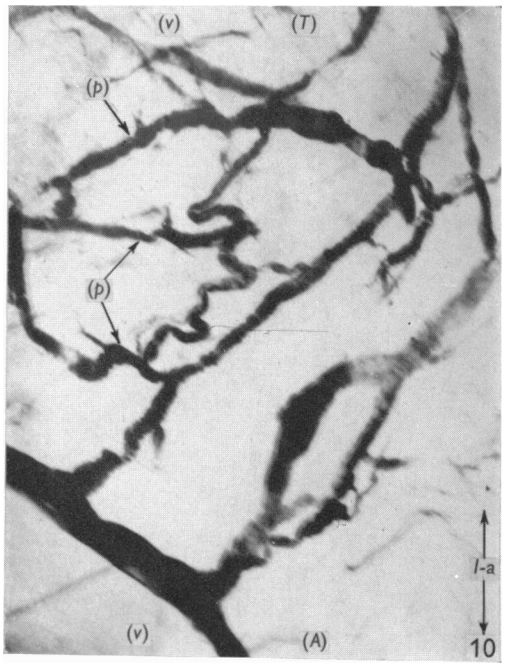


Fig. 9. Micrograph of the abdominal aorta, aged 4 weeks. Note the arteriole (*a*) originating from the lumbar artery (*L*) and branches (*b*) being distributed almost at right angles to the long axis of the aorta. A few longitudinal branches (*b*1) can be seen. Adventitia (*v*). × 50.

Fig. 10. Micrograph of the junction of the thoracic aorta (*T*) and abdominal aorta (*A*), aged 1 year. Note the irregular plexus (*p*) of arterial vasa in the adventitia (*v*). × 25.

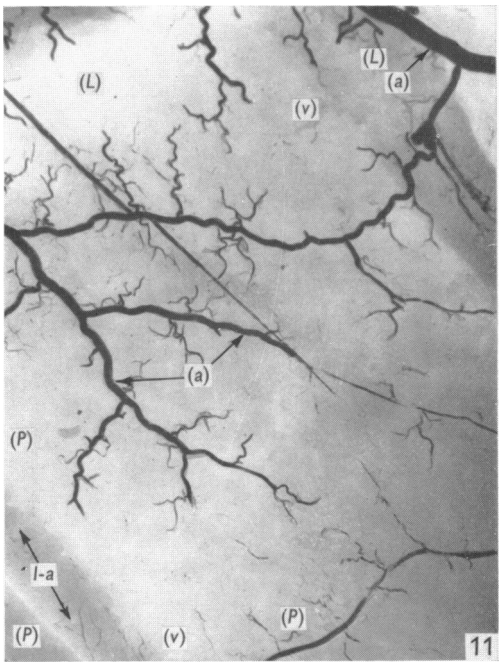
DISCUSSION

The technical difficulties encountered in demonstrating the patterns of the vasa vasorum constitute a limiting factor in studying the vessels in the wall of the aorta.

In contrast to injection methods, techniques which require the staining of red cells depend upon the uniform filling of the capillaries with red cells at the time of death, and failure to demonstrate vasa vasorum by Pickworth's (1934) method may be due to lack of blood within the mural vessels. Demonstration of vasa by routine staining techniques or by examining alkaline phosphatase in endothelial cells does

not allow the pattern of the vasa vasorum to be appreciated, in contrast to the routine X-ray micrograph.

Staining methods are suited to the investigation of adaptive changes in the circulation, demonstrating the actual state of the circulation at that time. Injection methods, on the other hand, intend to present the vascular pattern at its maximum capacity, and are particularly suited for anatomical investigations. For this reason along with the technical advantages of X-ray microscopy, which allows an examination of small arterioles and capillaries in unfixed, full thickness specimens, the vasa vasorum of the aorta were investigated by this method.



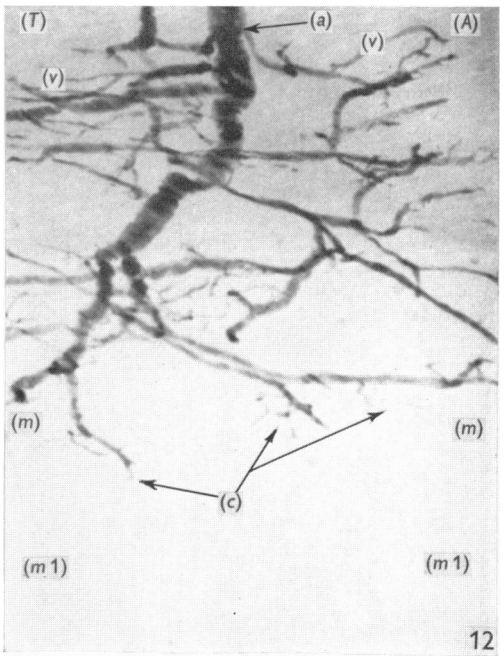


Fig. 11. Micrograph of the lateral aspect (*L*) and posterior aspect (*P*) of the thoracic aorta, aged 4 years. Note the longitudinal plexus of arterioles (*a*) on the lateral part of the aorta and the diminished number of arterial vasa on the posterior part. Adventitia (*v*). ×50.

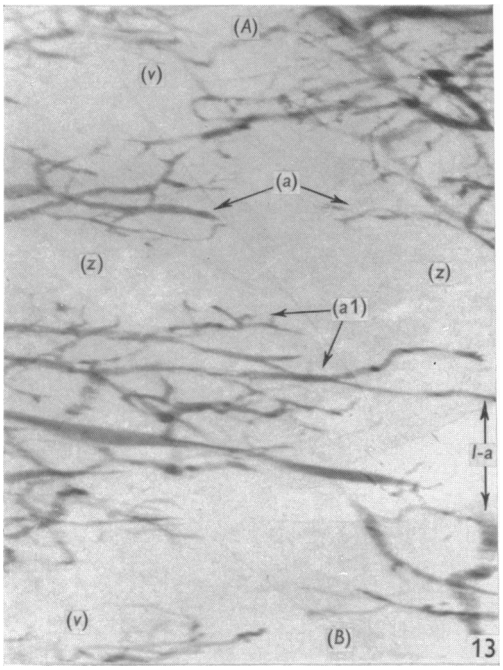
Fig. 12. Micrograph of 1 mm. thick transverse section of the junction of the thoracic aorta (*T*) and abdominal aorta (*A*), aged 4 years. Note the arterioles (*a*) in the adventitia (*v*) distributing capillaries (*c*) to the outer third of the media (*m*). No injection medium has entered the inner media (*m*l). ×110.

In this study, the criterion for accepting an area of the young aortic wall as devoid of vasa vasorum was the repeated failure to introduce the injection medium into the arterial wall under conditions which filled capillary beds elsewhere in the same speecimen, and subsequently proved satisfactory in these areas in older specimens. When assessing the depth the vasa vasorum penetrated, specimens showing 'stub end' arterioles were discarded. Only when a reasonable extent of the capillary bed had been revealed was any interpretation made, and although 'stub ends' of

capillaries were observed it was felt that this was inevitable with any injection method sooner or later no matter how 'complete' it was.

The present study has shown that there is an extensive vascular supply to the aortic wall, the arrangement and distribution of which alters considerably in early postnatal life.

The first year of life was characterized by an irregular adventitial network of arterial vasa on the ascending aorta and arch, with a well-defined spiral pattern on the descending aorta. This picture was similar to the appearance of the aortic vasa described in the later months of pregnancy (Clarke, 1965*b*).



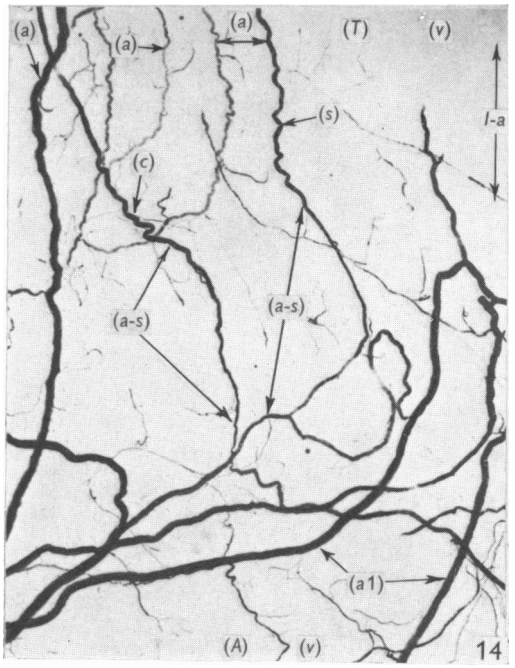


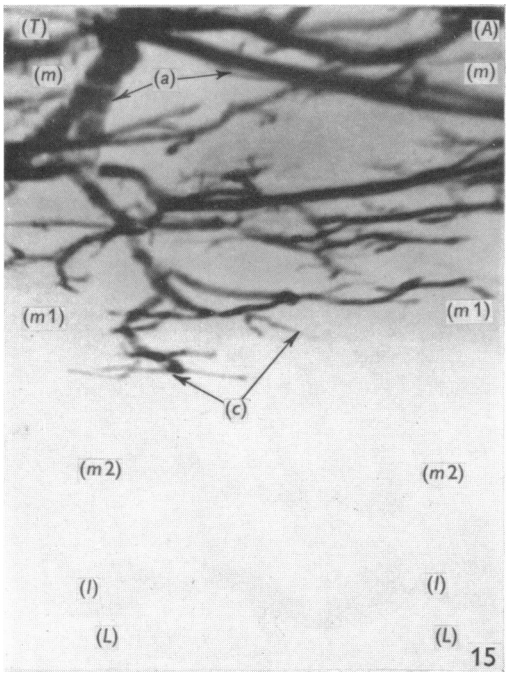
Fig. 13. Micrograph of the junction of the ascending aorta (*A*) and proximal aortic arch (*B*), aged 12 years. Note the arterioles (*a*:*a*1) of the ascending aorta and arch in the adventitia (*v*) and the zone (*z*) where no injection medium has entered. ×75.

Fig. 14. Micrograph of the junction of the thoracic aorta (*T*) and abdominal aorta (*A*), aged 12 years. Note the anastomoses (*a-s*) on the lateral aspect of the aorta between the segmental arterioles (*a*:*a*1) of the thoracic and abdominal aorta. Note coiling (*c*) and sinuosity (*s*) of the arterioles. Adventitia (*v*). ×20.

Between the second and fifth years sinuous or coiled arterioles replaced the irregular plexiform arrangement seen in the adventitia of the ascending aorta and arch, while an irregular network replaced the spirally arranged arterioles in the adventitia of the descending aorta, before a longitudinal sinuous or coiled pattern emerged. The functional significance of these alterations in the pattern of the adventitial arterioles is obscure. The common picture seen in the adult aorta has been shown to be that of coiled or sinuous arterioles generally orientated in the long axis of the aorta (Clarke, 1965*c*, *d*). It is suggested, therefore, that as the blood

pressure increases during early childhood and approximates to adult measurements, the stretch effect of systole on the aortic wall will become greater. The development of sinuous or coiled arterioles from irregular arteriolar plexuses may simply be a defence mechanism against vasal stretch as the pressure increases.

In the fourth year arterial vasa, which were demonstrable in the adventitia alone until this time, could be seen in the outer third of the media. A lapse of 6 years occurred before this picture changed, when arterioles and capillaries were displayed in the middle third of the media. On account of the relatively small size of the



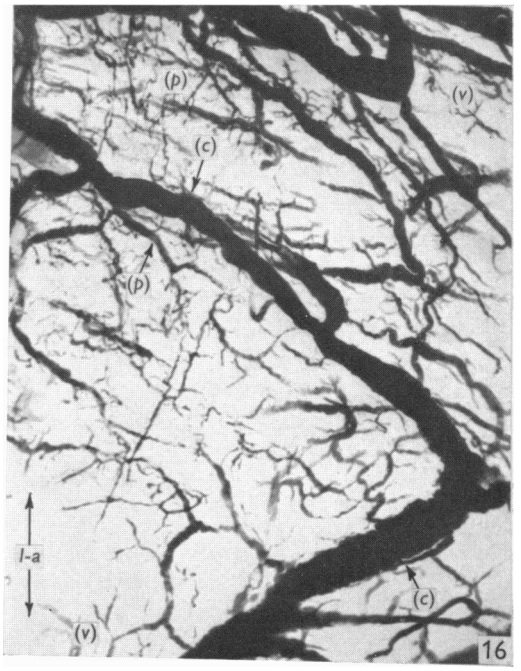


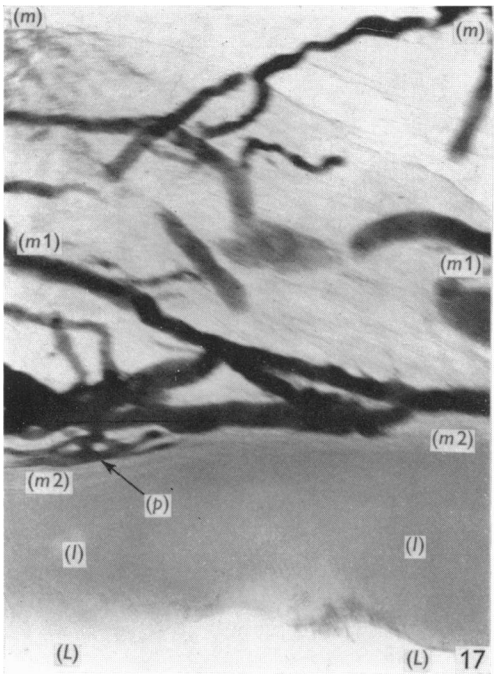
Fig. 15. Micrograph of 1 mm. thick transverse section of the ascending aorta, aged 13 years. Note the arterioles (*a*) penetrating the outer third of the media (*m*) to divide and supply a capillary bed (*c*) to the junction of the middle and inner third of the media (m1: m2). No injection medium has entered the inner third of the media or intima (*I*). Lumen (*L*). ×110.

Fig. 16. Micrograph of the abdominal aorta, aged 13 years. Note the irregular plexus of veins (*p*) draining into circumferential veins (*c*) in the adventitia (*v*). ×45.

sample studied (specimens of these age groups, unaffected by cardio-vascular disorders, being difficult to obtain) these observations could be interpreted as a failure of injection technique. On the other hand, since the number and thickness of the elastic membranes gradually increase in the aorta after birth (Bloom & Fawcett, 1962) the ingrowth of vessels may be a nutritional necessity accompanying the increased girth of the aorta.

While a good arterial supply could be demonstrated at the proximal and distal ends of the aortic arch in specimens until the tenth year, anastomoses between the

adventitial arterioles of the aortic arch and ascending and descending aorta were not demonstrable. It was also difficult to show a good arterial supply to the posterior aspects of the thoracic aorta, arterial vasa becoming progressively fewer as this area was approached. While a failure to demonstrate vessels in these areas may be due to lack of penetration by the injection medium, the micrographs showed that capillaries had been injected. The association of these findings with the occurrence of aortic aneurysm at the proximal and distal ends of the arch and the difficulty experienced with suture lines in proximal aortic grafting is, therefore, an interesting speculation.



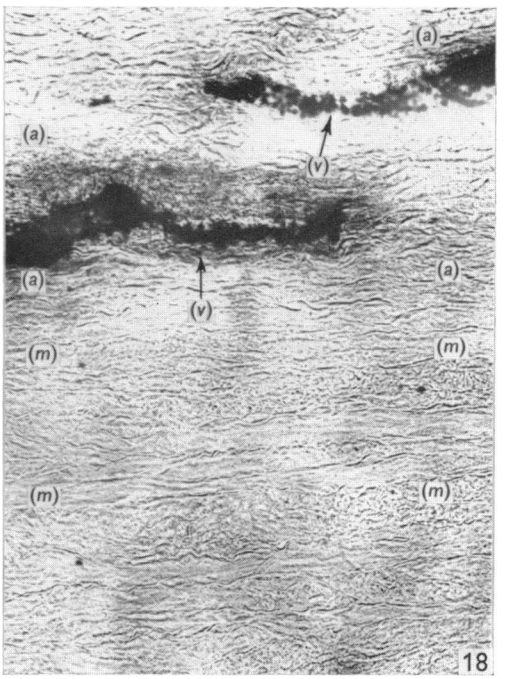


Fig. 17. Micrograph of 1 mm. thick longitudinal section of the aortic arch, aged 13 years. Note the origin of the venous plexus (*p*) in the inner third of the media (*m*2) draining into tributaries of the adventitial veins. Middle and outer third of media (*m*1:*m*). Intima (*I*): lume (*L*). ×200.

Fig. 18. Photomicrograph of 50 μ thick longitudinal section of the thoracic aorta, aged 3 years. Note the vasa vasorum (*v*) in the adventitia (*a*). Media (*m*) shows no mural vessels. Sodium nitroprusside and benzidene. ×100.

By the fifteenth year vessels, 10–20 μ in diameter, could be demonstrated in the inner third of the media when the venous vasa were injected. In contrast, injection of the arterial vasa only demonstrated a capillary plexus at the junction of the middle and inner third of the media. The deeper penetration of the injection mass when the venous route was used was a constant finding in all parts of the aorta. It was concluded, therefore, that the capillary-venule bed of the aortic wall lay in the inner third of the media.

The appearance of well-defined venous patterns in the adventitia was delayed until the thirteenth year. Longitudinally arranged adventitial veins and circumferential veins have been shown to be characteristic of the pattern found in the adventitia of the adult ascending and descending aorta respectively (Clarke, 1965*c*, *d*). The venous arrangement in the adventitia of the aortic arch remained plexiform. It is suggested that this pattern might offset the occlusive effect of systole, which is maximal in this region.

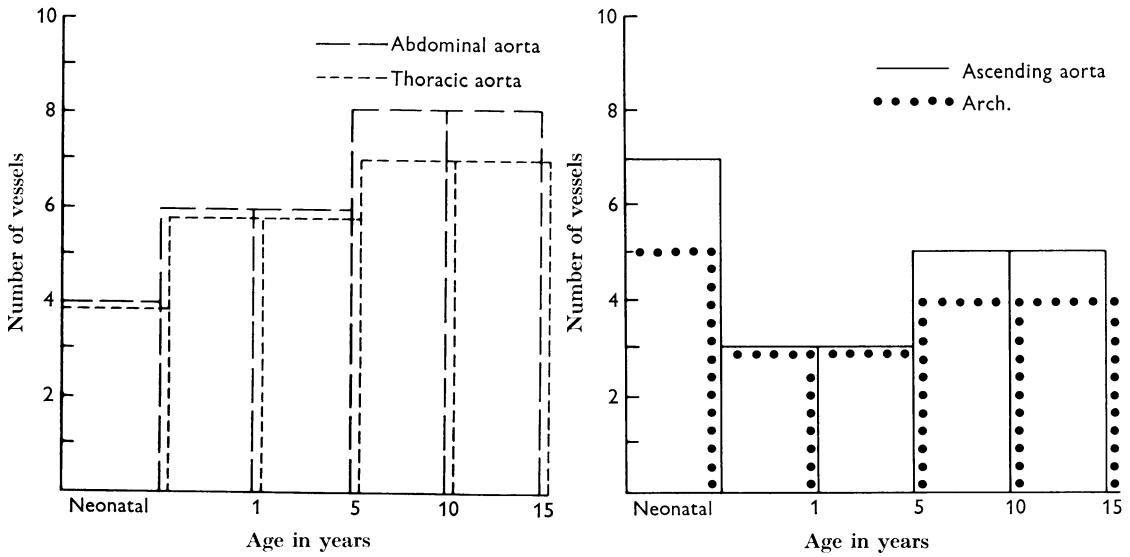


Fig. 19. Histograms to show the density of arterioles and capillaries in different parts of the aorta between birth and fifteen years of age. Symbols for histograms: ascending aorta, ——; aortic arch, • • • • •; thoracic aorta, - - - - -; abdominal aorta, — — —.

Schlichter (1948) was of the opinion that the mural vessels were densest in their distribution during the first 4 weeks of life, but did not comment upon which part of the aorta this conclusion was based on. As indicated by the X-ray microscope the neonatal distribution of arterioles and capillaries was denser in the adventitia of the ascending aorta and arch than at the end of the first year of life, while these observations were reversed in the descending thoracic and abdominal aorta. After the first year of life the vascular density of arterioles and capillaries remained constant until the fifth year when there was a gradual increase in the number of vessels in all parts of the aorta, the abdominal aorta showing the greatest vascularity by the fifteenth year. Since the sample studied was small no formal statistics could be performed, and these observations can only be regarded as a guide to the range of variation which appears to occur.

It would appear that the morphological pattern and distribution of the vasa vasorum approximate to the adult picture by the thirteenth year, and it was concluded that maturity of the vasal architecture had been achieved by the limit of the age group studied.

SUMMARY

1. The arterial and venous vasa of the human aorta were examined between birth and 15 years of age.

2. The arterial vasa originated from the coronary ostia; ostium of the brachiocephalic trunk; bronchial; intercostal; lumbar and mesentric arteries, to be distributed to the adventitia.

3. In the fourth year the outer third of the media received arterial vasa, and the middle third from the tenth year.

4. Venous vasa, demonstrable in the inner third of the media by the thirteenth year, drained through adventitial venous plexuses, into the cardiac veins, bronchial, intercostal, and lumbar veins.

5. The capillary-venule bed lay in the inner third of the media by the thirteenth year.

6. Various patterns were found among the arterial and venous vasa in the adventitia as the aorta developed, adult appearances being demonstrated from the thirteenth year.

7. No injection medium entered the intima.

8. Arteriole and capillary 'counts' showed that the ascending aorta and arch had a greater vascular density in the neonatal period than at the end of the first year; that the reverse was true for the descending thoracic and abdominal aorta, and that the abdominal aorta possessed the greatest vascularity of any part of the aorta by the fifteenth year.

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