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Cell Renewal in Aortic Necrosis Following Orthostatic Collapse

An Experimental Autoradiographic Study in the Rabbit Using ^{3}H-Thymidine

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Summary. The cell renewal was studied by ^{3}H-thymidine autoradiography in 16 rabbits with aortic necrosis due to orthostatic collapse. As controls, five rabbits without necrosis were investigated in a similar way. At the borders of the necrosis a marked increase in the number of labelled cells resembling smooth muscle cells was recorded. Only a slight increase in the number of labelled endothelial cells was recorded in the neighbourhood of the necrosis.

Changes resembling idiopathic media necrosis of the aorta can be induced in the rabbit by means of orthostatic collapse (Meesen, 1939; de Faria, 1955, 1958, 1962, 1970; d'Aquino and Barone, 1957). The changes consist of areas of band-like necrosis in the middle parts of the tunica media (de Faria, 1955, 1970). In man, similar lesions have also been observed in several cases of circulatory collapse before death (Meesen, 1939; Zink, 1940; de Faria, 1957). One week after the induction of the necrosis in the rabbit, intima thickening develops over the areas of necrosis. In man, a similar intima thickening is often observed in the case of atherosclerosis.

De Faria (1970) made a thorough routine histological study of the regeneration of the tunica media after necrosis. He also studied the origin of the cells in the intimal thickening. He found that the cell regeneration occurred mainly in "fibroblast-like undifferentiated subendothelial cells" and that there was practically no regeneration in the tunica media. These findings are not in accord with the observations I have made in other lesions of the aorta in other animal species (Hassler, 1970, 1971), in which the main part of the regeneration occurred in the tunica media. In order to get some additional light on this difference, a study of the regeneration of the aorta in the case of necrosis following orthostatic collapse was made, using ^{3}H-thymidine autoradiography. This experimental approach does not seem to have been applied previously to this problem.

Material and Methods

Twenty-one male rabbits (Swedish Landrace) belonging to five litters and weighing 1.5–1.7 kg at the start of the experiment were used. Five (one from each litter) were used as controls. In the remaining 16 rabbits orthostatic collapse was induced by the method of de Faria (1958). These animals were restrained in a wooden holder and then put in a vertical position. The collapse appeared in 20–120 minutes and was maintained for about 30 minutes. The signs of collapse were superficial respiration, tachycardia, disappearance of both corneal reflex and muscle tone in the limbs, and cyanosis of the ears and lips. The collapse could be interrupted immediately by putting the animal in the horizontal position. The collapse was induced twice at an interval of one day in the eight animals with the

longest observation times. In the other eight animals (with short observation times) only one single collapse was induced. Two rabbits were killed at each of the following observation times after the first collapse: 1, 12, and 36 hours, 3 days, 1, 2, 4 and 8 weeks. The five controls were kept in similar cages on a similar diet. Two controls were killed at the same time as the two test animals sacrificed 1 hour after collapse. One control was killed with the two test rabbits sacrificed 7 days after collapse. The remaining two controls were killed with the two rabbits sacrificed 56 days after collapse. Forty minutes before death, ^{3}H-thymidine (Radiochemical Centre, Amersham, England) was injected in all animals into an ear vein in a dose of 1 mC/kg body weight. The animals were killed by a blow on the neck, followed by the cutting of the neck vessels. The thoracic and abdominal parts of the aorta were fixed in neutral 10% formalin. Paraffin sections, 4 μ thick, were cut. Autoradiography was performed in the same way as previously (Hassler, 1966). The autoradiographs were post-stained with Harris' haematoxylin. Sections stained by van Gieson's method combined with Gomori's (1950) elastin stain and PAS were also produced from all animals.

In each collapsed animal, the numbers of labelled endothelial cells were determined in 100 cross-sections.

The numbers of labelled media-cell nuclei in the same sections were also determined. In the animals with media necrosis, only sections with such necrosis were studied. The numbers of labelled endothelial and media cells were also determined in the aortas of the control animals. The same numbers of sections chosen at random were studied.

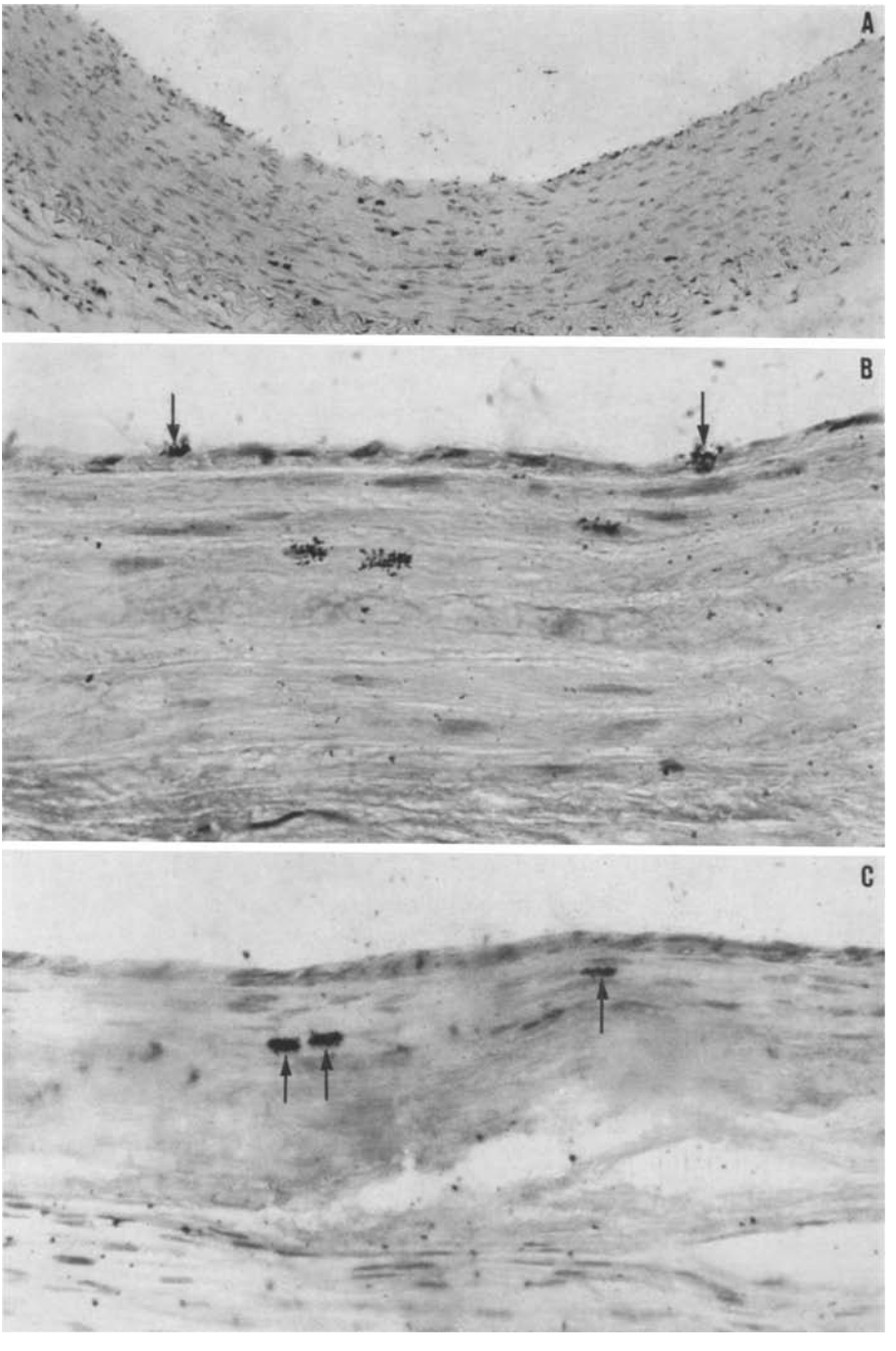
Table 1. Labelled cells per cross-section in the aorta of the animals

	Endothelial cells	Subendothelial intima cells	Cells of tunica media
Controls, 1 hour	1.9 ± 0.2	0	6.8 ± 0.6
Control, 1 week	2.1 ± 0.1	0	7.3 ± 0.4
Controls, 8 weeks	2.0 ± 0.2	0	7.0 ± 0.7
1 hour after collapse	2.9 ± 0.2	0	41.8 ± 3.8
12 hours after collapse	3.6 ± 0.3	0	71.2 ± 5.6
36 hours after collapse	4.8 ± 0.4	0	73.4 ± 5.9
3 days after collapse	3.9 ± 0.3	0	69.7 ± 5.5
1 week after collapse	2.6 ± 0.3	16.2 ± 1.1	61.5 ± 5.5
2 weeks after collapse	2.4 ± 0.3	14.1 ± 1.0	41.7 ± 3.7
4 weeks after collapse	2.3 ± 0.3	7.4 ± 0.5	28.1 ± 2.0
8 weeks after collapse	2.4 ± 0.2	4.5 ± 0.4	14.3 ± 1.4

Results

All of the animals survived the collapses and no unexpected complications could be discovered in any of them when killed. Necrosis was observed in all the collapsed animals, except those killed 12 hours or earlier after collapse. The necrosis resembled in all respects those described previously by de Faria (1957). When the cells in the tunica media with necrosis were classified according to Hassler (1970), more than 90% fulfilled the criteria of smooth muscle cells during the first 36 hours after collapse. After that time 75% or more had the criteria.

Fig. 1 A–C. Autoradiograms from rabbit aorta (lumen upwards in all figures). A Sixty minutes after collapse. Clusters of labelled cells occur in the tunica media, but there is yet no visible necrosis. $\times$ 100. B Three days after collapse. Two labelled endothelial cells (arrowed) are seen at the border of the necrosis, which here reaches the endothelium. There are also



three labelled cells classified as smooth muscle cells in the tunica media. × 400. C Seven days after collapse. At the border of the necrosis in the tunica media three labelled cells (arrowed) were found. They were classified as smooth muscle cells. × 400. Haematoxylin-eosin (Fig. 1A–C reduced to 9/10)

A thickening of the intima resembling that described by de Faria (1970) was also recognized 14 days later. Fifty per cent or more of the cells of the subendothelial cell layer fulfilled the criteria of smooth muscle cells.

The autoradiographic examination showed that the number of labelled endothelial cells (cf. Fig. 1 B) was slightly to moderately increased from 12 hours to 14 days and then not clearly increased (cf. Table 1). The labelled cells of the tunica media (Fig. 1 A and C) were markedly increased, especially during the first 14 days. More than 90% fulfilled the criteria of smooth muscle cells during the first 36 hours after collapse. After that time 75 or more fulfilled the criteria.

The number of labelled cells of the intima thickening was highest during the first period of its existence (cf. Table 1). Fifty per cent or more of the cells of this layer fulfilled the criteria of smooth muscle cells.

Discussion

The present study shows that the cell regeneration in the case of aorta necrosis following orthostatic collapse occurs mainly in the tunica media at the borders of the necrosis. The cells that regenerate seem to be smooth muslce cells, because of their morphological characteristics and the fact that they seem to be present in the aorta at the beginning of the necrosis. The newly formed media cells seem to de-differentiate to fibroblast-like cells, because in the healed stage the destroyed area is to a great extent built up of collagenous connective tissue. Only a slight increase in the regeneration of endothelial cells was observed in the neighbourhood of media necrosis.

The present results resemble those of my previous works (Hassler, 1970, 1971), in which the main part of the regeneration occurred in the tunica media around the lesions. The character of the present arterial lesions differed, however. No surgical intervention, which may cause uncontrollable side effects, was used in the present study. The animal species used in the present study was also different from those of my previous studies. These factors therefore do not seem to influence the results. The present results are not in accord with those of de Faria (1970), who found that the cell regeneration occurred mainly in a subendothelial cell layer of the intima. He did not use ^{3}H-thymidine autoradiography, however, and his attention was focussed on the intimal thickening that he observed in the neighbourhood of the lesions. The intima thickening resembled that observed in my previous works. The origin of the cells in that intima thickening was probably the tunica media.

De Faria (1970) observed that, when the necrosis of the media was so great that it bordered on the endothelium, no subendothelial intima thickening occurred. He found this noteworthy. His explanation was that the necrosis had produced a collapse of the subendothelial cells of the intima, which he considered responsible for the intima thickening. My explanation of that phenomenon is that the necrosis had destroyed all the smooth muscle cells so that no cells could regenerate there.

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