Virchows Arch. A Path. Anat. and Histol. 362, 283—289 (1974)

Arterial Cell Kinetics in Experimental Hypertension

O. Hassler

Department of Pathology, University of Umeå, Sweden

Received January 11, 1974

Summary. In experimentally induced hypertension an increased cell renewal was observed in the intimal and medial coats of the aorta. Because the labelled cells seemed to be rather similarly distributed early and late after injection of ^{3}H-thymidine, the increased cell renewal was assumed mainly to be a compensation for increased destruction of cells. One day after the administration of the hypertensive drug Aramine® the cell renewal was normal.

Hypertension is known as one of the most powerful and also most common atherosclerotic factors. How hypertension influences the arterial wall is not fully known. A recent study of cell-turn-over with ^{3}H-thymidine was carried out by Schmitt *et al.* in 1970. They found a remarkably increased cell renewal in the arterial wall in hypertension and their study centered around the problem of the origin of the newly formed cells. They did not investigate the ultimate fate of the newly formed cells. In this respect, at least two possibilities seem to exist, *i.e.*: A) that newly formed muscle cells replace dead muscle cells in the tunica media and B) that newly formed muscle cells go to the thickened intima. Support for the second possibility may be obtained from the fact that, with the aid of electron microscopy, muscle cells can be seen to pass through the windows of the internal elastic lamella (Jurukova and Knieriem, 1970, and many others). Several other works (cf. Hassler, 1970, 1971) also indicate that muscle cells from the tunica media go to the intima in the case of intima lesions and intima thickening.

The object of the present study is: 1) to investigate to where most of the newly formed cells, mainly in the tunica media, go; 2) to study when the increased cell renewal stops after an acute period of hypertension.

Material and Methods

A total of 68 rats, weighing 198–210 g, were used. They belonged to 9 litters and were divided into 4 groups in such a way that the litters and sexes were as evenly distributed as possible.

Group I consisted of 32 animals. The left renal artery was clamped by a silver clip with a distance between the arms of 0.18 mm. A laparotomy was made by a midline incision under anaesthesia induced by intravenous injection of Mebumal® (a short-acting barbiturate produced by ACO, Stockholm, Sweden). After 4 weeks the cardiovascular system of the animals was investigated by means of ^{3}H-thymidine autoradiography as described below.

Group II comprised 12 animals. They were injected intramuscularly with a hypertensive drug, *viz.* Aramine® (=metraradin; Merck Sharp & Dohme, New York, USA) 12,4 and 0 hours before administration of ^{3}H-thymidine. The dose was 0.2 mg/kg body weight.

Group III also comprised 12 animals, receiving intramuscular injection of the same dose of Aramine® as in group II. Now, however, ^{3}H-thymidine was injected 36, 28, and 24 hours after the Aramine® administration.

Group IV (=control group) also consisted of 12 rats. They were maintained in separate cages and were kept on a standard diet similar to that of groups I–III. Nine were killed at the same time as the animals in group I; and 3 at the same time as those in groups II and III, respectively.

Autoradiographic Technique

^{3}H-thymidine (specific activity 5000 mCi/μmol, Radiochemical Centre, Amersham, England) was administered intraperitoneally. The dose was 5 μC per g body weight. The animals were killed with an overdose of ether 40 minutes, 6 hours, 3 days and 7 days (see Table 1) after the administration of ^{3}H-thymidine. The upper part of abdominal aorta, and the hearts, were removed and fixed in 10% formalin. The hearts were weighed, and in group I all material was discarded if the heart weight was less than 0.60 g/100 g body weight. All remaining aortas were embedded in paraffin and sectioned so that ringshaped cross-sections were obtained. The control aortas were embedded together with the aortas of the test animals, so that variations in thickness of the sections and the autoradiographic film should influence the results as little as possible. Dipping autoradiography was then performed as described previously (Hassler, 1966) on 200 cross-sections chosen at random from each animal. A cell was counted as loosely attached to the intima (Table 1) only when less than 30% of its surface bordered on the intima in the section.

Results

Group I. Two animals died in connection with the operations and were excluded. Eighteen rats of this group showed heart weights less than 0.60 g/100 g body weight and were, consequently, excluded. The remaining 12 rats showed heart weights of 0.60–0.96 g/100 g body weight, i.e. a considerable enlargement of the heart, that in all cases was macroscopically found to involve mainly the left ventricle.

Table 1. Mean number of labelled cells in various parts of the arterial walls of 200 cross sections at different times after injection of ^{3}H-thymidine

	40 minutes	6 hours	3 days	7 days
Group I. Renal hypertension				
Tunica media	44.9	138.5	91.6	79.8
Subendothelial intima	9.8	19.7	15.3	13.1
Endothelium	17.2	46.1	36.9	33.7
Loosely attached	0.6	4.4	1.9	1.3
Group II. Aramine® administration				
Tunica media	40.4	123.5	81.9	70.7
Subendothelial intima	1.2	1.5	1.8	0.9
Endothelium	15.2	39.6	22.1	21.9
Loosely attached	0	3.9	2.2	0.7
Group III. One day after Aramine® administration				
Tunica media	19.6	67.3	46.9	40.0
Subendothelial intima	0.9	0.3	0.7	0.7
Endothelium	9.4	19.7	17.1	16.1
Loosely attached	0.7	1.2	0.9	0.9
Group IV. Control				
Tunica media	20.1	59.3	40.8	37.9
Subendothelial intima	0.9	0.7	0.3	0.7
Endothelium	8.9	22.3	17.5	15.8
Loosely attached	0.3	1.9	1.0	0.7

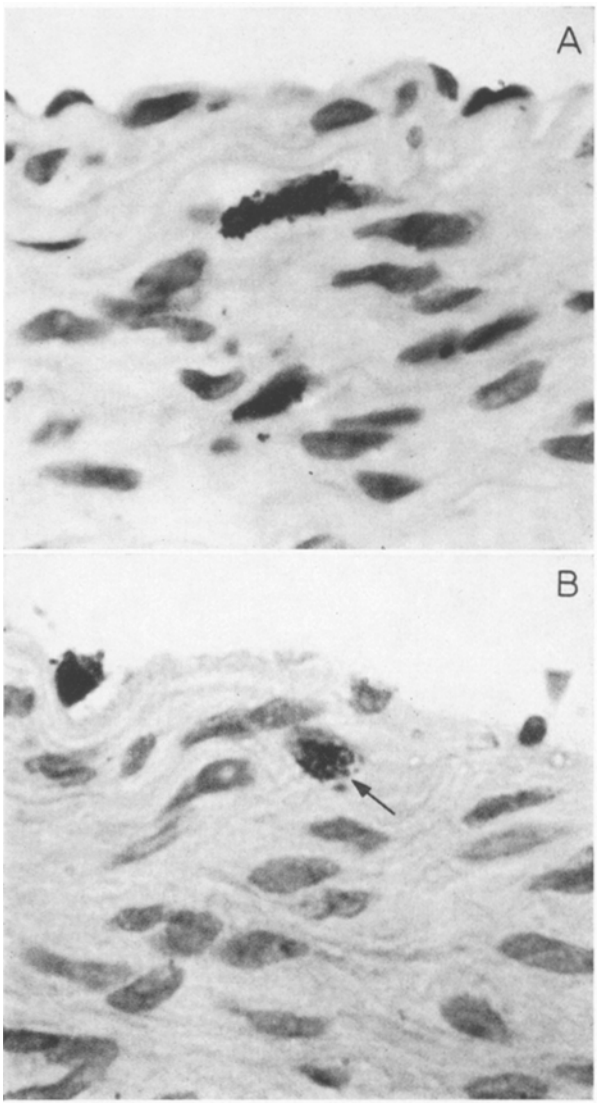

Fig. 1 A—E. Autoradiograms from rat aorta (lumen upwards in all figures) demonstrating labelled: A) smooth muscle cell; B) subendothelial cell; C) endothelial cell; D and E loosely attached intima cells; F) entirely loose blood cells in the lumen. Magnification in all cases ×800. Haematoxylin

The autoradiographic findings of the 12 rats are given in Table 1. After 40 minutes there was an increased number of labelled cells in the intima and tunica media when compared to those in control group IV. The labelling of the cells was strong, i.e. more than 90% of the labelled cells were covered with 6 or more silver grains. Only a few labelled cells in the intima were loosely attached and protruded into the lumen (cf. Fig. 1).

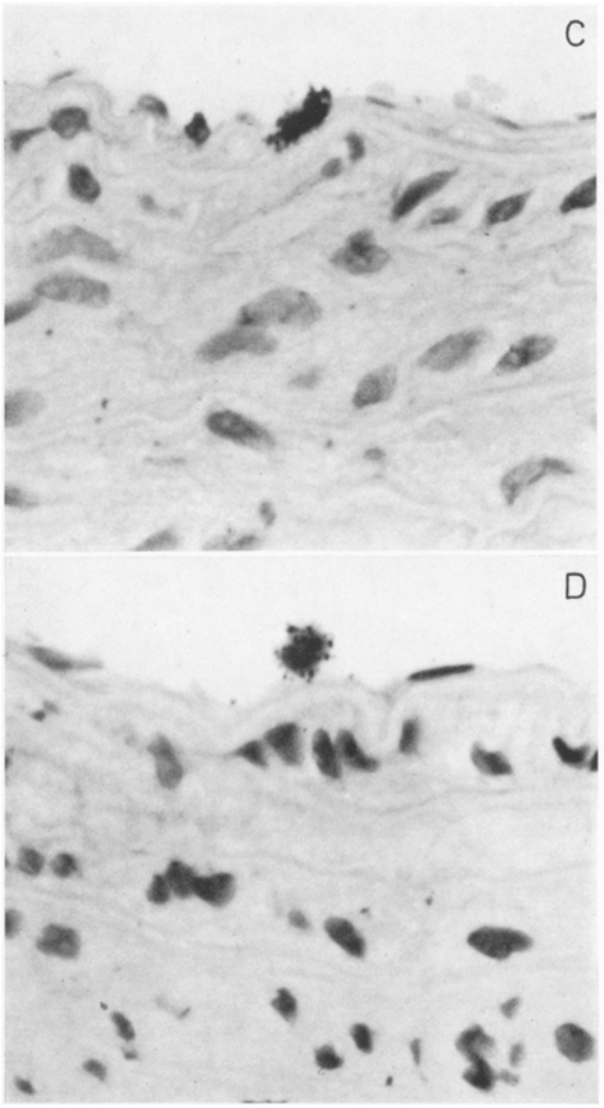

Fig. 1C and D

After 6 hours the results resembled those after 40 minutes but somewhat more labelled cells, loosely attached and protruding into the lumen, were observed.

After 3 and 7 days, respectively, there was also an increased number of labelled cells in the intimal and medial coats compared to control group III. Only a few were loosely attached. The labelled cells showed a rather weak labelling, i.e. roughly 40% had 3–6 silver grains and the remaining 60% had more than 6 grains.

Group II. No animal of this group had a heart weight exceeding 0.60 g/100 g body weight. The results of the autoradiographic study resembled those of group I.

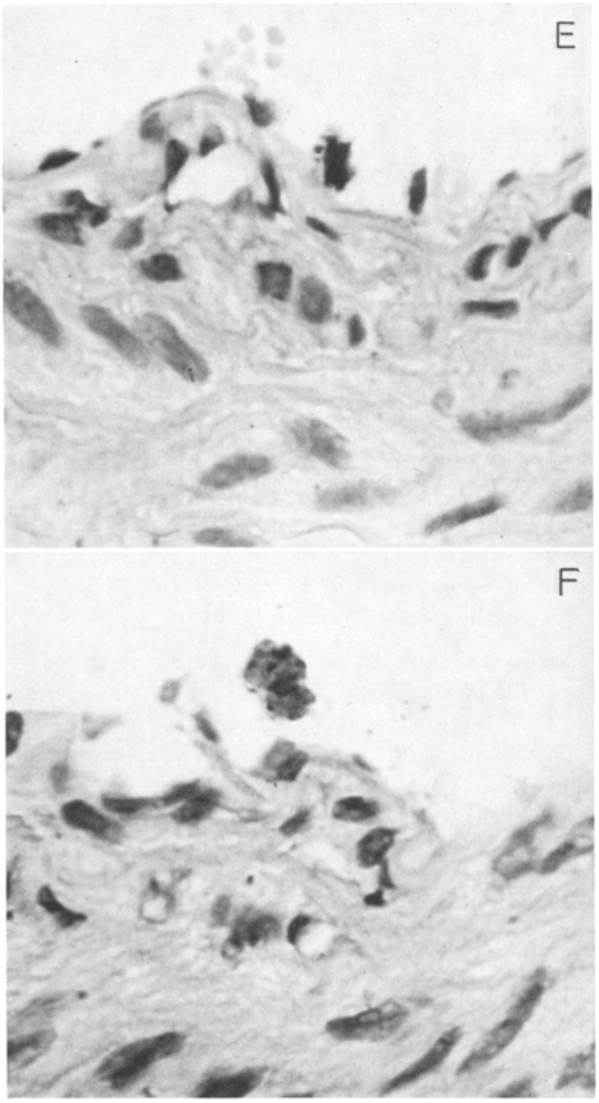

Fig. 1E and F

The subendothelial cell layer was more weakly developed than in group I and resembled that of groups III–IV.

Group III. No animal in this group had a heart weight excending 0.60 g/100 g body weight. The results of the autoradiographic study resembled those of group IV.

Group IV. No animal of this group died spontaneously. No animal had a heart weight exceeding 0.60 g/100 g body weight. The mean heart weight was 0.43 g/100 g body weight. The results of the autoradiographic study is given in the Table 1.

Discussion

In the present study a less marked increase in cell renewal in hypertension was encountered than in the preceding study (Schmitt *et al.*, 1970). The reason for this discrepancy may be differences in the autoradiographic technique and the way of inducing hypertension. There is, however, a similarity in the proportions between intimal and media cells. When the ^{3}H-thymidine was injected 1 day after administration of Aramine no increased cell renewal could be observed any longer. The blood pressure was not recorded in the Aramine rats, but Aramine is known as a reliable hypertensive agent in the rat (Stone *et al.*, 1966) and had a "hypertensive" effect on the cell renewal in group II.

The results of the present study seem to indicate that the main part of the increase in cell renewal is to provide the tunica media with new cells. This layer shows hyperplasia in hypertension and for this purpose some, but apparently a rather small, increase in the number of cells is necessary. A probably much greater part of the newly formed cells may substitute muscle cells that have degenerated and died.

On the basis of the findings in previous works (cf. Hassler, 1973) it is probable that some newly formed cells go to the thickened intima, but firm support for this hypothesis was not obtained in the present study.

Loosely attached labelled cells were not mentioned in the work of Schmitt *et al.* (1970). These cells were much more commonly observed in the present study than in my previous autoradiographic studies on arteries (cf. Hassler, 1970, 1971, 1973). The cells may be newly formed endothelial cells. It seems possible that they may loosen and be carried away by the bloodstream. They may also be cells from the blood that are entering the arterial wall. Schmitt *et al.* (1970), however, focussed their study on the problem of wheter haematogenous cells enter the arterial wall in hypertension, and they considered they had demonstrated that haematogenous cells do not enter the wall.

The reason why the adventitia was not included in this study is that its external border is difficult to delineate, when cells are to be counted.

This study was supported by the Swedish Medical Research Council Project No. B74-12X-561-10C.

Refererences

Hassler, O.: The origin of the cells constituting arterial intima thickening. An experimental autoradiographic study with the use of H^3-thymidine. Lab. Invest. **22**, 286–293 (1970)

Hassler, O.: Arterial cell renewal under hyperlipidemic conditions. Virchows Arch. Abt.A **352**, 26–33 (1971)

Hassler, O.: Cell renewal in aortic necrosis following orthostatic collapse. An experimental autoradiographic study in the rabbit using ^{3}H-thymidine. Virchows Arch. Abt. A **358**, 295–299 (1973)

Jurukova, Z., Knieriem, H.-J.: Elektronenmikroskopische Untersuchungen über die Organisation arterieller Thromben. Virchows Arch. Abt. A **349**, 368–381 (1970)

Schmitt, G., Knoche, H., Junge-Hülsing, G., Koch, R., Hauss, W. H.: Über die Reduplikation von Aortenwandzellen bei arterieller Hypertonie. Z. Kreisl.-Forsch. **59**, 481–487 (1970)

Stone, C. A., Stavorski, J. M., Ludden, C. T., Wenger, H. C., Torchiana, M. L.: Some direct and indirect sympathomimetic actions of metaraminol. Arch. int. Pharmacodyn. **161**, 49–60 (1966)

Professor Ove Hassler
Institute of Pathology
University of Umeå
S-901 87 Umeå 6, Sweden