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Arterial Cell Renewal under Hyperlipidemic Conditions

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Summary. The renewal of arterial cells under various hyper- and normo-lipidemic conditions has been studied by ^{3}H-thymidine autoradiography. In hyper-lipidemia the endothelium shows a greater propensity to proliferate than in normo-lipidemia, but in both conditions the smooth muscle cells of the tunica media also proliferate when the intima is injured.

Zusammenfassung. Die arterielle Zellneubildung wurde unter verschiedenen hyper- und normolipämischen Zuständen mit ^{3}H-thymidin-autoradiographie untersucht. Bei Hyperlipämie zeigt das Endothel größere Neigung zur Proliferation als bei Normolipämie. Bei beiden Zuständen jedoch proliferieren auch die glatten Muskelzellen in der Tunica media im Zusammenhang mit Intimaverletzungen.

In a preceding work (Hassler, 1970) the origin of the cells constituting the arterial intima thickening in normo-lipidemic rats was studied by ^{3}H-thymidine autoradiography. It was found that the cells constituting an experimental intima thickening were probably derived mainly from the muscle cells of the tunica media and to a lesser extent from the endothelium. The objection may be made, however, that the intima thickening obtained in the previous work with normo-lipidemic animals resembles more the thickening occurring physiologically at branching points, *i.e.* physiological intima cushions (Hassler, 1962), than atheromatous lesions. The aim of the present work was to study whether the same type of intima proliferation also occurs under hyper-lipidemic conditions.

Material and Methods

Control material was obtained from Group I (see below) and the intact carotid arteries of Groups II–V. Group II was a control group to Group III. In Group II the ligated carotid-artery segment was filled with the dispersion agent of the lipid emulsion of Group III. A local hyper-lipidemic condition was obtained by filling a ligated carotid-artery segment with a lipid suspension for 1 day (Group III), with cholesterin for 4 days (Group IV), or with hyper-lipidemic human serum for 1 day (Group V). In Group VI an alimentary hyperlipidemia was induced. In Group VII a temporary hyper-lipidemia was induced by repeated, heavy, intravenous injections of a lipid suspension for 4 hours.

The study was performed on 70 male white rats (Sprague-Dawley, from Anticimex, Stockholm, Sweden) weighing 100–140 g at the start of the study. The rats belonged to eight litters. The animals were divided into Groups I–V, each comprising 9 rats, Group VI (15 rats), and Group VII (10 rats). When the groups were formed, it was arranged that Groups I and IV and Groups II and III should be as similar as possible with regard to the weights of the animals and their representation among the litters.

Group I. Anaesthesia was produced by intraperitoneal injections of a short-acting barbiturate (Mebumal, ACO, Stockholm, Sweden). On one carotid artery two ligatures were applied: one was situated at the branching point into the internal and external carotid

arteries; the other ligature was situated on the carotid artery, as proximally as possible without injuring the pleura. The carotid-artery segment between the ligatures contained much blood. The wound was closed.

Groups II–V. Anaesthesia was produced as above. One carotid artery was also ligated as in Group I. But the arterial segment situated between the two ligatures was punctured and its contents were evacuated and replaced by Intralipid® 20% (Vitrum, Stockholm, Sweden) in Group III, by the dispersion agent of Intralipid (with no lipids added) in Group II, sterilized cholesterin powder (Merck, Darmstadt, Germany) in Group IV, and hyper-lipidemic human serum in Group V. The hyper-lipidemic serum was obtained from a 54 years old man with hyper-lipidemia (1540 mg/100 ml determined according to Zöllner and Kirsch). (The man had a recent myocardial infarction, but no other relevant data in the case history.) The puncture was made close to the upper ligature and the hole was closed with a third ligature, so that the contents was retained in the artery until death. The other carotid artery was left intact. The wound was closed.

Group VI. Alimentary hypercholesterinemia was induced by keeping the animals for 2 weeks on a diet rich in lipids (Hartroft and Thomas, 1963).

Group VII. Under barbiturate anaesthesia (see above) the rats were given intravenous injections of Intralipid® 20% in a dose of 0.5 mg/kg body-weight every 15 minutes for 4 hours.

Autoradiographic Procedure. In Groups I and IV ^{3}H-thymidine (specific activity 2000–3000 mC/mM, Radiochemical Centre, Amersham, England) was administered intraperitoneally 4 days after the operation. In Group II, III, and V the thymidine was administered in the same way 24 hours after the operation. In Group VII the thymidine was given in the same way in immediate connection with the last injection of Intralipid. The dose was always 2 μC/g body-weight. All the animals were killed with an overdose of ethyl ether 40 minutes after the injection of thymidine. From the rats of Groups I–VI blood was taken for lipid determination (Zöllner and Kirsch, 1962). In all the animals both carotid arteries were removed and fixed in formalin. Autoradiographic examination and counting of labeled cells were performed in the same way as in the preceding work (Hassler, 1970) Sections stained with Gomori's elastin stain (Gomori, 1950), combined with van Gieson's stain, were also produced in all cases.

Results

All the animals survived, except one from Group III that died immediately after the operation and was excluded. No unexpected complications could be discovered in the other animals. The animals of Groups I–V all showed normal lipid contents of the blood (values varying between 392 and 455 mg/100 ml). In Group VI, the values varied between 696 and 1822 mg/100 ml. The six animals of this group showing values below 1200 mg/100 ml were excluded.

Group I. The general histological examination of the ligated, blood-filled, carotid artery showed no thrombosis, but a slight, non-specific inflammation of the vessel wall. A slight thickening of the subendothelial intima tissue was seen, so that this layer contained cell nuclei.

The autoradiographic examination showed that no granulocytes or lymphocytes were labeled. The number of labeled endothelial and subendothelial cells was increased (Table). The appearance of the labeled subendothelial cells (Fig. 2A) varied and many of them were difficult to classify. About 40% had the *nuclear characteristics of smooth-muscle cells* (Hassler, 1970), however. There was also an increase in the number of labeled cells in the tunica media (Table). Most labeled cells of this layer (at least 75%) had the nuclear characteristics of smooth-muscle cells, also.

Group II. The general histological examination of the ligated carotid artery filled with the dispersion agent of Intralipid® showed slight non-specific inflammation but no subendothelial thickening. The autoradiographic examination

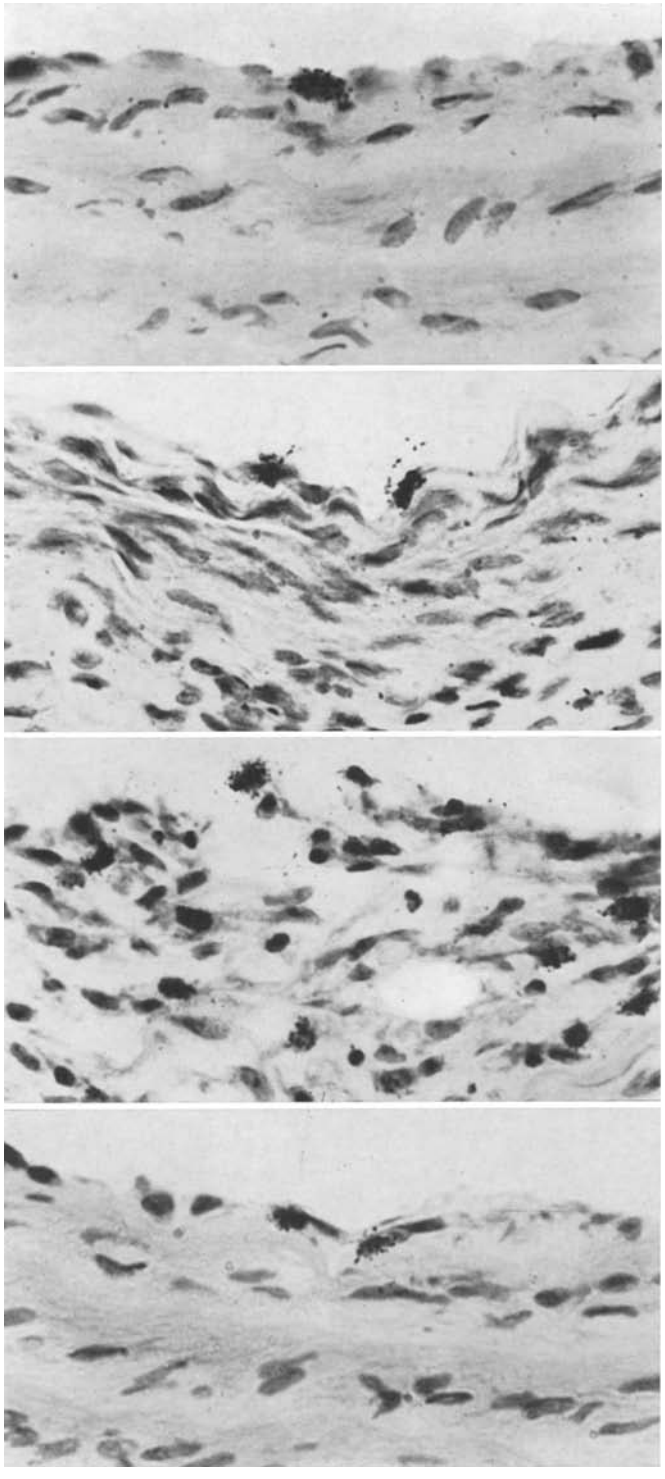

Fig. 1 A–D

Table. *Labeled cells per 100 carotid artery cross-sections. In each case, the mean and the standard deviation are given*

Experimental group No.	Carotid artery	Endothelium	Subendothelial intima	Tunica media
I–V	Normal	19.9 ± 3.1	0	95.8 ± 7.1
I	Ligated, blood-filled, 4 days	51.4 ± 4.9	72.6 ± 5.8	498 ± 28
II	Ligated, filled with the dispersion agent of Intralipid, 1 day	59.2 ± 5.1	0	404 ± 26
III	Ligated, Intralipid-filled, 1 day	287 ± 18	0	384 ± 24
IV	Ligated, cholesterin-filled, 4 days	362 ± 20	169 ± 11	492 ± 26
V	Ligated, filled with hyper-lipemic human serum, 1 day	82.5 ± 6.9	0	401 ± 26
VI	Alimentary hyperlipemia	31.2 ± 4.6	0	140.5 ± 13
VII	Repeated, intravenous inj., Intralipid	28.7 ± 4.1	0	118 ± 9.0

(see Table) demonstrated that there was only a moderately increased number of labeled endothelial cells. The increase in the number of labeled cells in the tunica media was of the same order of size as in Group I. Most labeled cells of this layer (at least 75%) had the nuclear characteristics of smooth muscle cells.

Group III. The general histological picture of the ligated, Intralipid-filled, carotid artery resembled that of the ligated carotid artery of Group II. The autoradiographic examination (see Table) demonstrated that there was a markedly increased number of labeled endothelial cells (Fig. 1 B). The number of labeled cells in the tunica media showed a similar degree of increase to that in Group II. Most labeled cells of this layer (at least 75%) had the nuclear characteristics of smooth-muscle cells.

Group IV. The general histological examination of the ligated, cholesterin-filled, carotid artery showed slight, non-specific inflammation. A slight-to-moderate thickening of the subendothelial intima tissue occurred, so that this layer also contained cell nuclei, which was not the case in the specimens of Groups II and IV. The thickening was generally twice as large in the cholesterin-filled artery, compared with the ligated, blood-filled one in Group I. The cells were difficult to classify, but a great many of them had comparatively voluminous foamy cytoplasm that probably contained lipids (the present material was not sufficient to allow freeze-sectioning and fat-staining, but in a pilot study on a similar material staining for fat was positive). No collagenous fibres could be discovered.

Fig. 1 A–D. ^{3}H-thymidine autoradiograms post-stained with haematoxylin. Representative labeled endothelial cells from: A, a normal carotid artery (Group I), B, a carotid-artery segment filled with Intralipid® (a fat emulsion) for 24 hours, C, a carotid-artery segment filled with cholesterin for 4 days, D, a carotid artery of a rat with alimentary hyperlipidemia. ×380

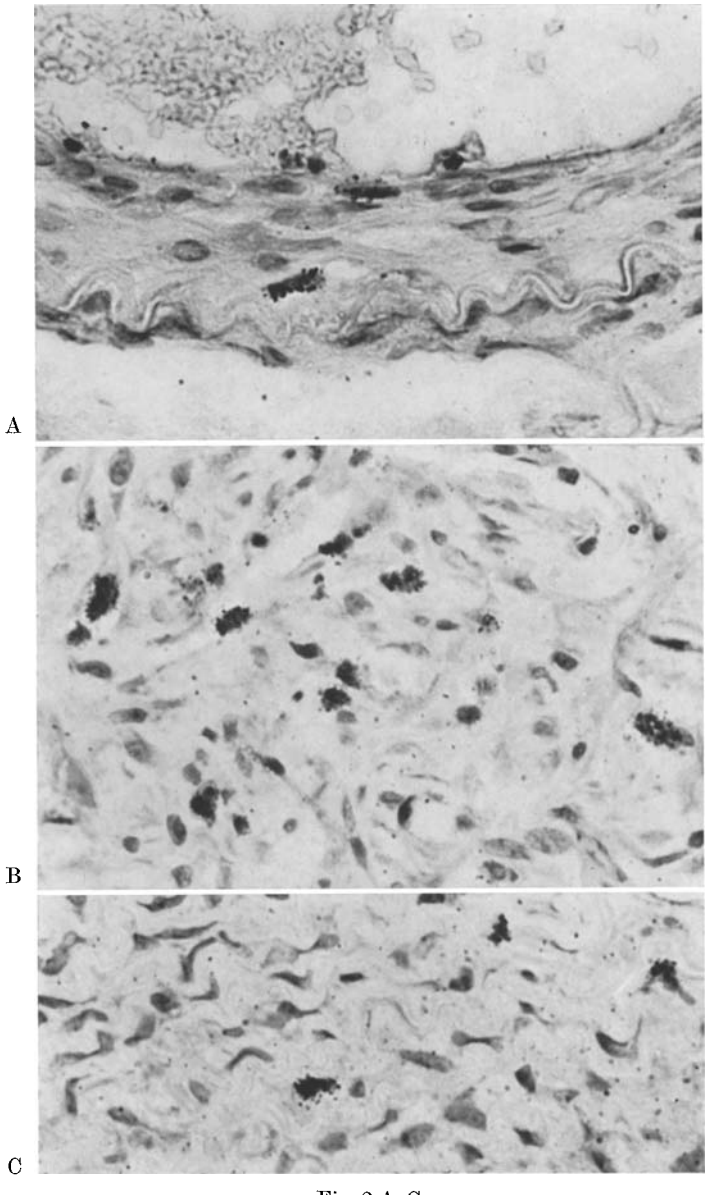

Fig. 2 A–C

Fig. 2 A–E. ^{3}H-thymidine autoradiograms post-stained with haematoxylin. A and B. Representative labeled subendothelial intima cells from: A, a carotid-artery segment ligated and filled with blood for 4 days, B, a carotid-artery segment ligated and filled with cholesterin 4 days (there were somewhat more cells resembling smooth-muscle cells in A than in B). C–E. Representative labeled cells in the tunica media of: C, a normal carotid artery, D, a carotid-artery segment filled with human hyper-lipemic serum for 1 day, E, a carotid artery of a rat which received repeated intravenous injections of Intralipid®. ×380

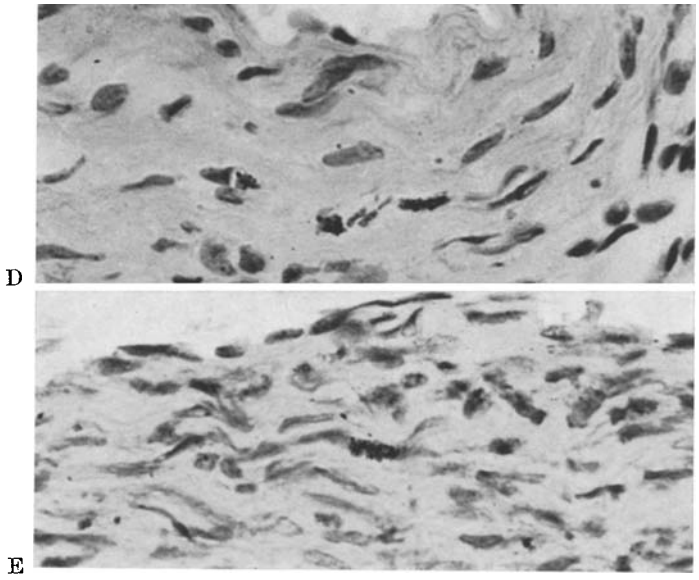

Fig. 2D and E

The autoradiographic examination showed that the number of labeled endothelial cells (Fig. 1C) was markedly increased in the ligated, cholesterin-filled artery (Table). As is apparent from the Table, there were more labeled endothelial and subendothelial cells in the ligated, cholesterin-filled artery than in the ligated, blood-filled artery of Group I. The appearance of the labeled subendothelial cells (Fig. 2B) varied somewhat, but many had a large, foamy cytoplasm and their nuclei resembled endothelial cells. Only about 10% had the nuclear characteristics of smooth-muscle cells. The number of labeled cells of the tunica media showed a similar increase to that of Group I (Table).

Group V. The general histological examination of the ligated carotid artery showed signs of a moderate non-specific inflammation. There was no subendothelial thickening of the intima. As is apparent from the Table, the autoradiographic examination showed only slight increases in the number of labeled cells in the endothelium and the tunica media. The appearance of the labeled cells (cf. Fig. 2D) resembled that in Groups II and III.

Group VI. The six animals, which showed not so high values of the lipid contents of the blood, were excluded (see above). The general histological examination of the other ones showed solitary foam cells in the intima, but no other changes. The autoradiographic examination (Table) showed moderate increases in the number of labeled endothelial and media cells. The appearance of the labeled cells (cf. Fig. 1D) resembled that in Groups II, III and V.

Group VII. The general histological examination showed no special changes in these arteries. The autoradiographic examination (Table) gave similar results to that in Group V. The appearance of the labeled cells (cf. Fig. 2E) resembled that in Groups II, III and V.

Discussion

The advantages and limitations of the autoradiographic methods used have already been discussed in the preceding work (Hassler, 1970). The connection between hyper-lipidemia and atherosclerosis is at present well documented, but the pathomechanisms behind this connection are to a great extent obscure.

The present results seem to indicate that the endothelium plays a greater role in the intima proliferation under hyper-lipidemic than under normo-lipidemic conditions. Thus, the numbers of labeled endothelial cells were markedly greater in the ligated arterial segments containing Intralipid, cholesterin or hyper-lipidemic serum than in the segments containing normal blood. On the other hand, the media cells showed an equal degree of proliferation, irrespective of whether the lumen contained Intralipid, cholesterin, hyper-lipidemic human serum, or normal blood.

The present work resembles somewhat that of Florentin *et al.* (1969) who performed in vitro incubation in a ^{3}H-thymidine solution of en-face (Häutchen) preparations of the endothelium of swine aortas. Animals fed on a normal diet were then compared with animals fed on a cholesterol diet for 3 days. The latter had significantly higher ^{3}H-thymidine indices than the former. The authors concluded that some constituent of the blood of the cholesterol-fed swine (cholesterol per se or other) may act directly on the endothelial cells to produce the observed effect. My study supports this conclusion and seems to indicate that not only cholesterin but also other lipids possess this property.

In several previous works (Strong *et al.*, 1968) during recent years it has been shown that the smooth-muscle cells of the tunica media take up lipid droplets in the case of hyperlipidemia. It has also been suggested that these cells are transformed into the foam cells of the intima (Constantinides, 1970). Electron microscopy has revealed that one cell type in atherosclerotic lesions is the smooth muscle cell (Geer *et al.*, 1961; Balis *et al.*, 1964). The present results seem to be best explained by the hypothesis that both endothelial cells and smooth-muscle cells have the potential to be transformed into foam cells. Subendothelial intima cells do not occur in normal carotid arteries in the rat.

In Groups V–VII fundamentally similar but less significant changes were obtained, probably because the lipid concentration in the vascular lumen was much lower and in Group VII of shorter duration. Probably the conditions for Groups VI and VII resemble to a greater extent those for human beings developing atherosclerosis. But this part of the study could unfortunately not be extended for several reasons: (1) the high cost of the isotope; (2) several practical difficulties and complications in giving the animals Intralipid-infusions over an extended period; (3) the large time-consuming countings, because the differences from the normal values are not so great and no control material could be obtained from the test animals, as in Groups I–V.

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