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Lipid Metabolism by Rabbit Aortic Intimal and Medial Cells in Tissue Culture*

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Summary. Aortic cells from intimal and medial layers of aortas from both normal and cholesterol-fed rabbits were studied in tissue culture. Three preparations from normal-fed rabbits were used—intimo medial cells prepared either from explants or from collagenase, elastase digests of the inner aortic layer and cells prepared from explants of the medial muscle layer. Two preparations from cholesterol-fed rabbits—collagenase, elastase digests of the inner aortic layer and cells from the medial layer were used. Growth was rapid in secondary cultures prepared from enzyme digests and the cells could be used for isotope uptake and incorporation studies within seven days of primary seeding. Growth from medial explants was slower but secondary cultures were readily prepared and could be used for isotope studies.

All five preparations took up appreciable amounts of (1-^{14}C)-oleic acid and incorporated this precursor primarily into phospholipid (mostly lecithin and phosphatidyl inositol). A smaller proportion of the label was incorporated into triglyceride but only about 3% was incorporated into cholesteryl ester by each of the five cell types studied. A significantly greater proportion of the (1-^{14}C)-oleic acid was incorporated into triglyceride and a lower proportion incorporated into phospholipid by the intimal cells from the cholesterol-fed rabbit than was the case with the intimal cells from the normal-fed rabbit. No other differences in incorporation of the oleic acid into other lipid fractions was demonstrated between the five cell preparations studied.

The uptake and incorporation of ^{32}P-phosphate into phospholipid by the five cell preparations was also demonstrated. Cells grown from intimal digests of the cholesterol-fed rabbit aortas showed significantly lower incorporation into phosphatidyl inositol and higher incorporation into lecithin than the other cell preparations. The synthesis of lipid by normal and cholesterol-fed rabbit aortic cells in relation to the pathogenesis of atherosclerosis is discussed.

Introduction

Numerous investigators have described techniques for growth of arterial wall cells in tissue culture (Rutstein *et al.*, 1958; Branwood, 1964; Kokubu and Pollak, 1961; Robertson, 1961, 1965). Most of these studies have used whole thickness arterial wall as a primary source of cells, although attempts have been made by cloning to separate different cell types from such cultures (Robertson, 1961, 1965). In view of the key role of proliferation of smooth muscle cells in

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atherogenesis, a number of studies have been initiated using specifically arterial smooth muscle cells in culture (Jarmolych *et al.*, 1968; Kao *et al.*, 1968; Ross, 1971). It has been shown that the growth of these smooth muscle cells is accelerated by the addition of hypercholesterolaemic serum to the incubation medium and in particular by the addition of the low density lipoprotein fraction (Florentin *et al.*, 1969; Dzoga *et al.*, 1971).

Most of the studies on tissue culture of arterial wall cells including those on the culture of smooth muscle cells have been concerned primarily with their growth characteristics and morphology. Little information is available regarding lipid metabolism by these cells. In view of the active synthesis of lipid by lipid-containing cells in the atherosclerotic arterial wall (Day *et al.*, 1966; Day and Wahlqvist, 1968; Wahlqvist *et al.*, 1969) a rather more definite approach to the lipid metabolism of various cells in the normal and atherosclerotic arterial wall seems justified. The tissue culture method provides a convenient means of studying such metabolism and in particular in determining changes in lipid metabolism in relation to the transformation of the normal to the lipid-containing cell. In the present work, therefore, different preparations of arterial wall cells from both normal and cholesterol-fed rabbits are investigated in culture and some preliminary observations on the lipid metabolism of these cells reported.

Methods

Tissue Culture Procedure. Rabbits 2 to 6 weeks of age were used for all studies with the exception of those involving cholesterol-fed rabbits. The animals were killed by ether anaesthesia and the thoracic aortas carefully removed under sterile conditions. Following removal of the adventitial connective tissue the aortas were separated by careful stripping into three layers. The inner layer, consisting of endothelium, sub-endothelial connective tissue and some media (termed the intima-media preparation), was reserved for either explant culture or for isolated cell culture following the initial disruption of the layer with collagenase and elastase. The middle layer (media preparation) contained only medial smooth muscle, care being taken in stripping it from the underlying adventitia to exclude tissue where adventitial contamination was a possibility. The adventitia was discarded.

For explant culture the intima-media preparation or the media preparation were cut into 1 to 2 mm squares and plated into Petri plates containing Basal Eagles Medium (B.E.M.) with 10% fetal calf serum added. Plates were incubated at 37°C in a CO_2 incubator. When growth was well-established (9 to 14 days in the case of the intima-media explants, 12 to 21 days in the case of the media explants) the cells were trypsinized (0.25% trypsin in phosphate-buffer solution) and replated into Leighton tubes. When growth was re-established these preparations were used for isotope studies. In later experiments it was found more satisfactory to separate the intima-media cells, prior to culture, by the use of collagenase and elastase. The intima-media layer was cut into small portions and incubated with 5 ml of Media 199 containing 10 mg of collagenase and 2.5 mg of elastase. Disruption of the intima was followed visually with an inverted tissue culture microscope and when almost complete (approximately 1 to 2 hours) the cells were taken up in phosphate buffer solution, separated and washed by centrifugation and finally suspended in B.E.M. containing 10% fetal calf serum and plated into Petri plates. These were incubated at 37° in the CO_2 incubator. When growth was established (4–5 days) the cells were trypsinized as above and replaced into Leighton tubes for subsequent metabolic studies.

Intima-media and media cells obtained from the aortas of cholesterol-fed rabbits were also grown and investigated in culture. The rabbits were fed a diet containing 1 gram of cholesterol and 3 grams of peanut oil daily for periods of 3 to 4 months. At the end of this period they were killed by ether anaesthesia and the thoracic aorta removed and stripped into three layers as set out above. The intima-media layer was disrupted with collagenase and elastase

and the cell suspension plated into Petri plates essentially as described for the normal-fed animals. The media layer was stripped from the adventitia and grown as explants again as described for the normal-fed animals. The adventitia was discarded.

Histological Techniques. Where the morphology of the cells was investigated in fixed preparations cover slips were added to the Leighton tubes and the cells allowed to grow on the cover slips. After fixation in 3% glutaraldehyde the cells were stained with either May-Grunwald-Giemsa or with Sudan III. Radioautographs were prepared using similar preparations by overlaying the cover slips with Kodak AR 10 stripping film. After exposure, the cells were stained through the film with hematoxylin and eosin.

Metabolic Studies. ^{14}C-labelled oleic acid (59.7 mCi/mMol, Radio Chemical Center, Amersham, U.K.) and carrier free ^{32}P-labelled phosphate (Radio Chemical Center, Amersham, U.K.) were used as lipid precursors. ^{14}C-labelled oleic acid was added to the incubation medium combined with the albumin in the serum in a concentration of approximately 1 μCi per ml of incubation medium. ^{32}P-labelled phosphate was added at a concentration of approximately 10 μCi per ml. After incubation (37°C for 20 hours), the radioactive medium was removed, the cells washed 3 to 4 times with 0.9% sodium chloride solution and the labelled lipid in the cells extracted with 2:1 chloroform:methanol. The extracts were washed as described by Folch *et al.* (1957) and the labelled lipid extracts separated into their individual components by thin layer chromatography on Silica Gel G using n-hexane:diethyl ether:acetate acid (100:38:3 v/v/v) as the developing system. A standard mixture of lipids was added to the extracts prior to separation in order to facilitate detection of the lipid spots which were then identified by spraying with 0.2% dichlorfluorescein in ethanol.

Spots were scraped into counting vials and the radioactivity determined directly using the dioxane-water scintillator of Snyder (1964). ^{32}P and ^{14}C-labelled phospholipids were separated into their individual components by thin layer chromatography on Silica Gel (Camag) using the method of Skipski (1964). A mixture of known phospholipids was added as internal standard in order to detect the phospholipid spots and the radio-activity was then counted directly using the dioxane-water scintillator of Snyder (1964).

Results

The morphological characteristics of cells cultured from the intimo-media layer of normal rabbit aortas is shown in Fig. 1. In the earlier studies explants were prepared and the cells allowed to grow out from such explants as shown in Fig. 1 A and B. Growth commenced two to three days from seeding (Fig. 1 A). At this stage, two distinct cell types could be distinguished. The most prominent cell was the fibroblastic type. There were, however, a number of more rounded or polygonal cells present slightly larger than the fibroblastic type cells and probably representing endothelial cells. Growth proceeded quite rapidly and by five days (Fig. 1 B) an extensive network of fibroblastic cells were present. Very few rounded endothelial-type cells persisted at this period. After trypsinization, and replating into Leighton tubes, growth rapidly became re-established. The morphology of the secondary culture cells in fixed and stained preparations is shown in Fig. 1 C and D. The preparation is an essentially homogeneous one consisting of fibroblastic type cells. Few vacuoles are present in their cytoplasm and little lipid is present at this stage as indicated by the absence of Sudan staining.

It was possible to obtain more rapid growth of cells by separating the intimo-media layer into cells or cell clumps prior to primary culture. Using this technique, initial growth occurred within 24 hours of plating. Fig. 2 A shows the outgrowth of cells from a central primary culture clump at 24 hours after seeding. Growth, which was primarily growth of spindle-shaped cells, occurred quite rapidly so that at three days (Fig. 2 B) appreciable coverage of the initial tissue culture vessel

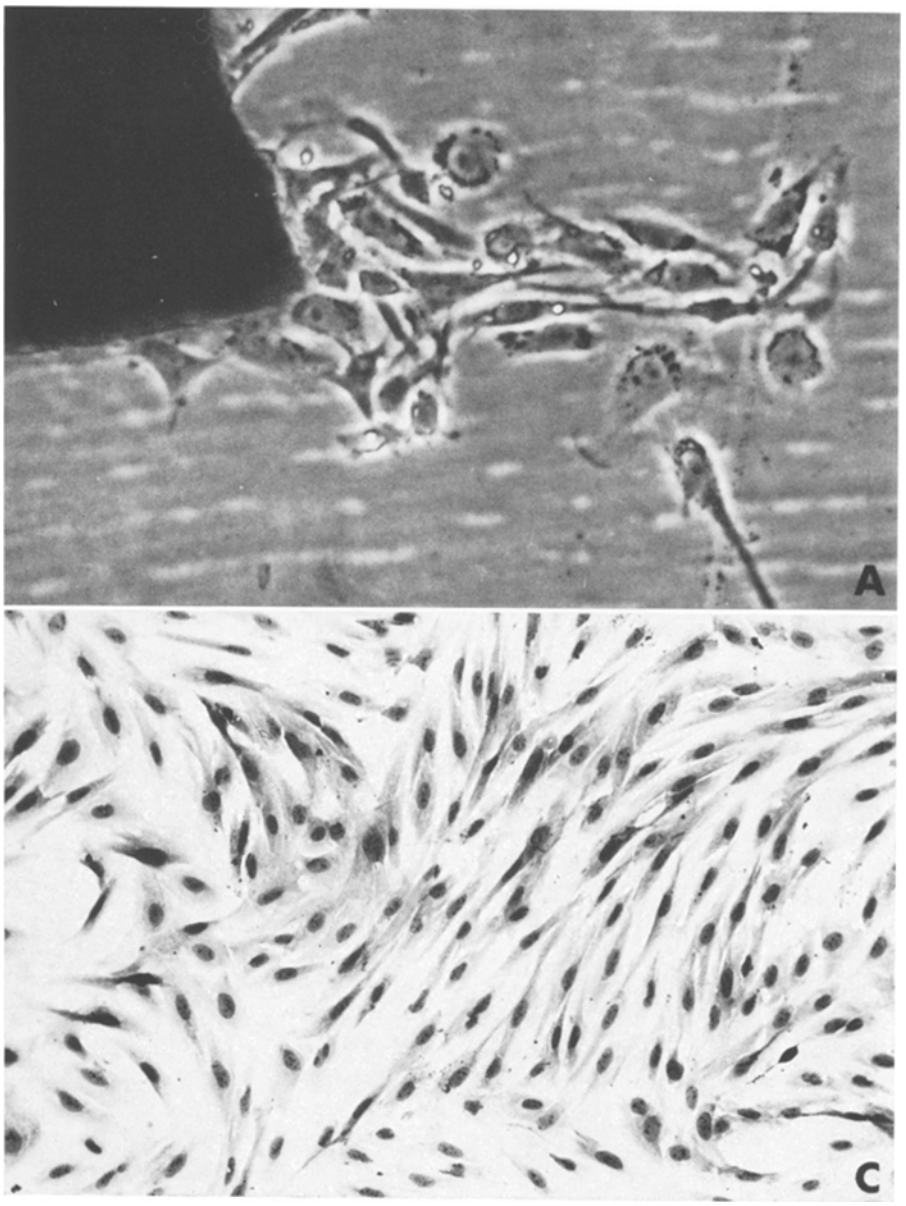

Fig. 1 A—D. Cells cultured from intima-media explants of normal rabbit aortas. A 3 days after seeding, Phase contrast ×280. B 5 days after seeding. Phase contrast ×280. C 6 days after passage. 14 days after primary culture, May-Grunwald-Giemsa ×100. D as for C, ×400

was obtained. Cells could be trypsinized within a week of primary culture and replated into Leighton tubes for metabolic study. Fig. 2C and D show fixed and stained preparations of these cells following trypsinization and secondary culture.

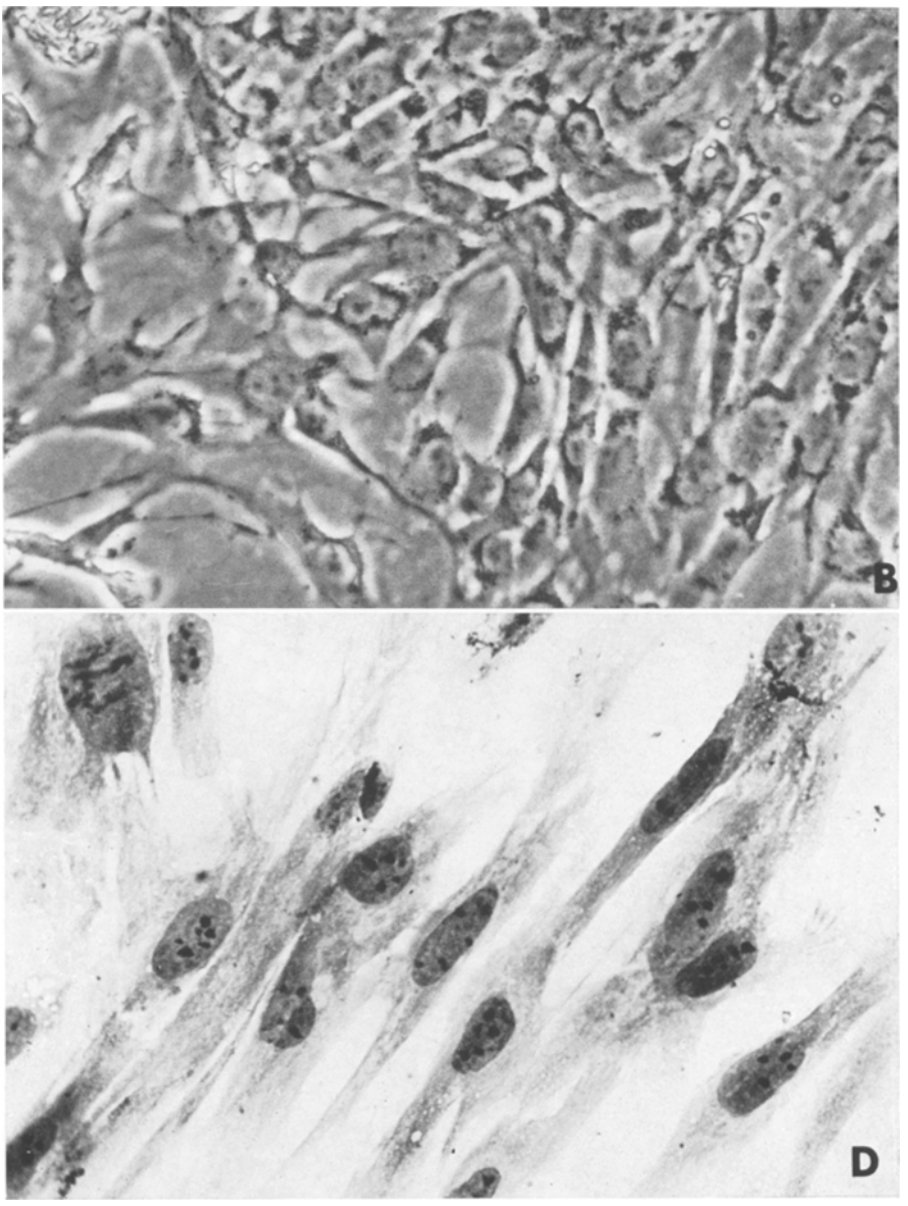

Fig. 1 B and D

Morphologically, the cells are similar to those obtained from explant culture. Little vacuole formation or sudanophilic material is present within them.

Cell growth obtained from the media layer of the normal aortas are shown in Fig. 3. Although it was attempted, it was not possible to obtain growth from media layers disrupted with collagenase and elastase so that the preparation shown

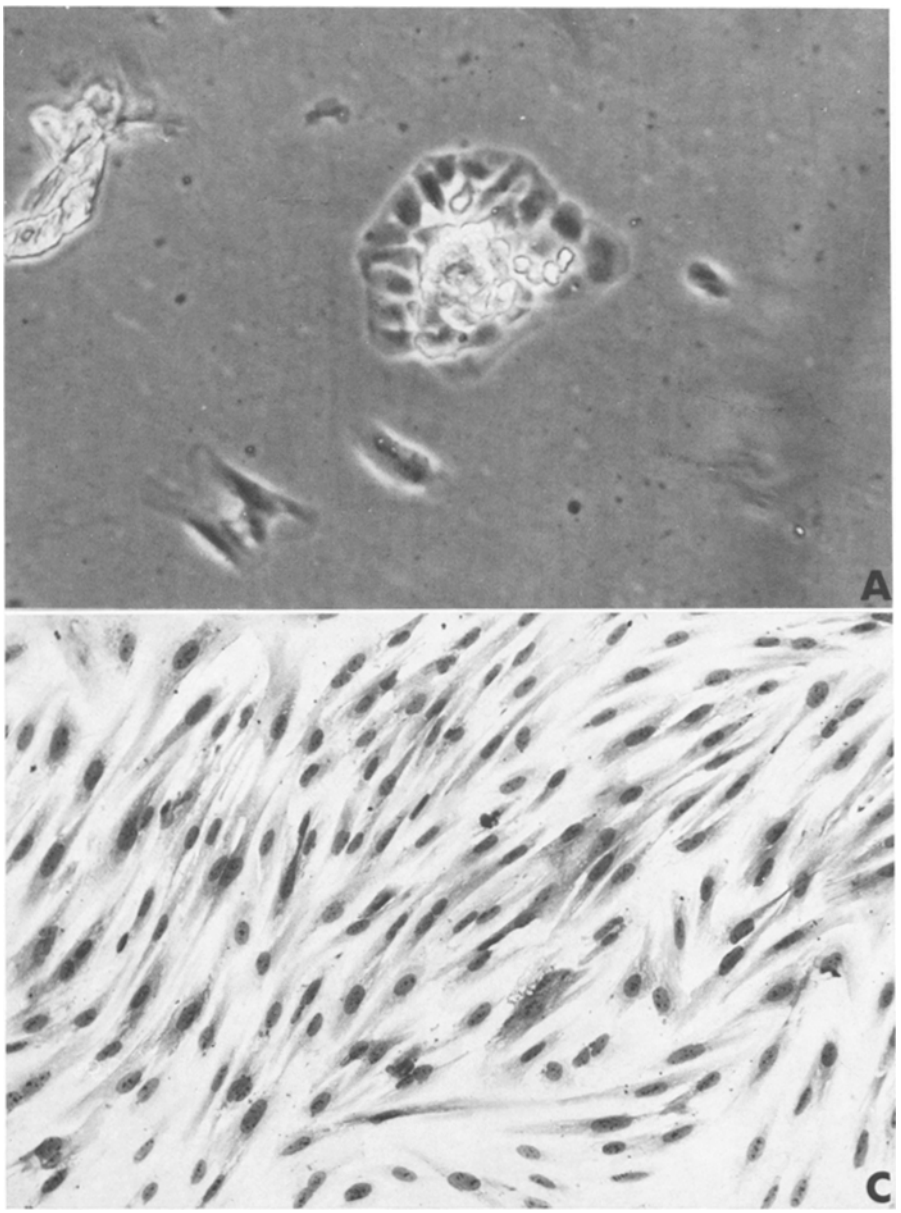

Fig. 2A—D. Cells cultured from intima-media of normal rabbit aortas following disruption with collagenase, elastase. A 1 day after seeding. Phase contrast ×280. B 3 days after seeding. Phase contrast ×280. C 7 days after passage, 11 days after primary culture, May-Grunwald-Giemsa ×100. D As for C, ×400

in Fig. 3 and the cells in tissue culture used for subsequent metabolic study were obtained by primary culture from explants. New cell growth started to appear at about the fifth day (Fig. 3A and B). The cell population was essentially homo-

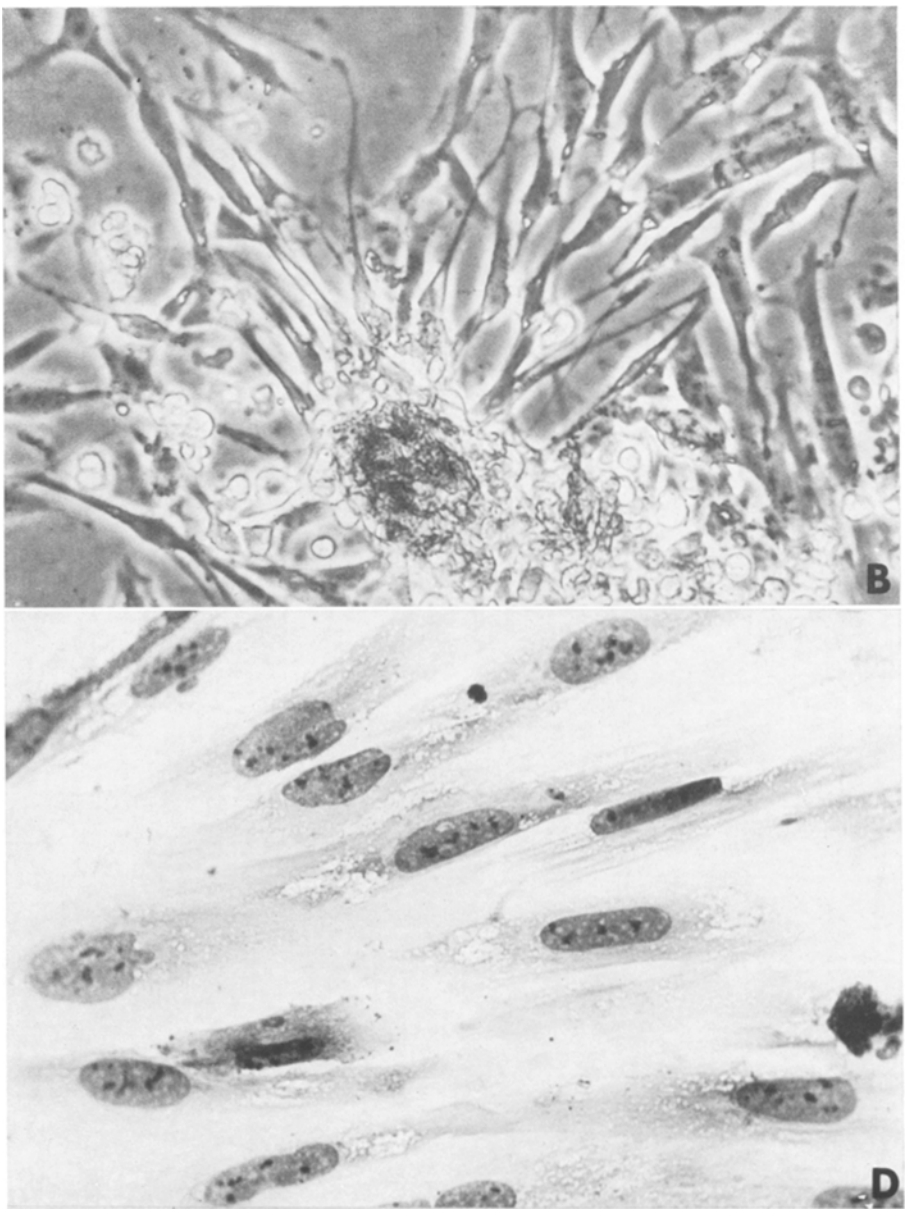

Fig. 2 B and D

geneous at the light microscope level and there was no evidence of the round or polygonal endothelial-type cells at any stage during this preparation. Not only was growth slower to appear but the cells proliferated less rapidly than was the case with cells obtained from the intimo-media layer. Trypsinization was carried out at about 21 days from primary culture and resulted in a secondary culture

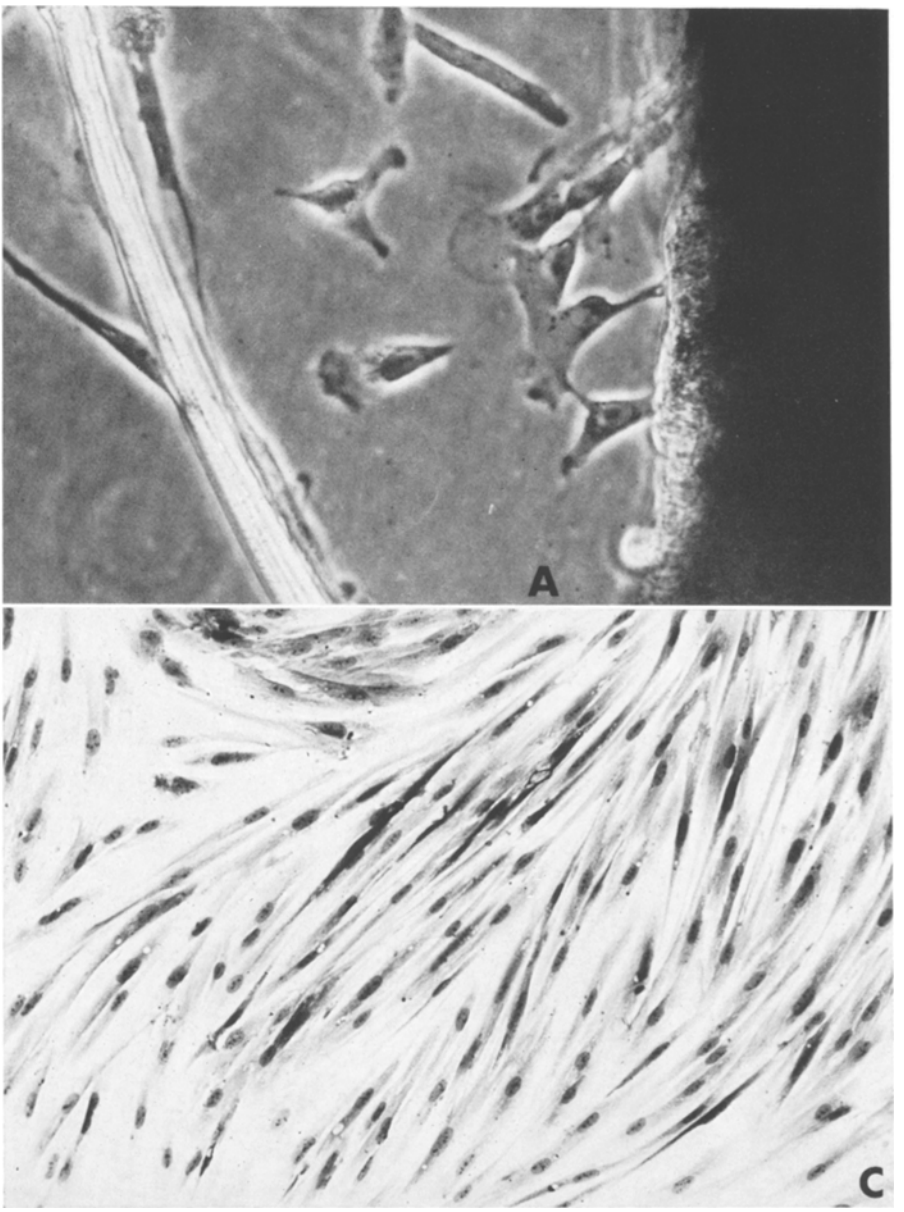

Fig. 3A—D. Cells cultured from medial explants of normal-fed rabbit aortas. A and B 2 preparations, 5 days after primary seeding, phase contrast × 280. C 6 days after passage, 18 days after primary culture, May-Grunwald-Giemsa × 100. D As for C, × 400

in Leighton tubes as shown in Fig. 3C and D. Cells obtained were similar morphologically to those reported by others using similar methods (Jarmolych *et al.*, 1968). While there is some vacuole formation (Fig. 3D) this is not a prominent

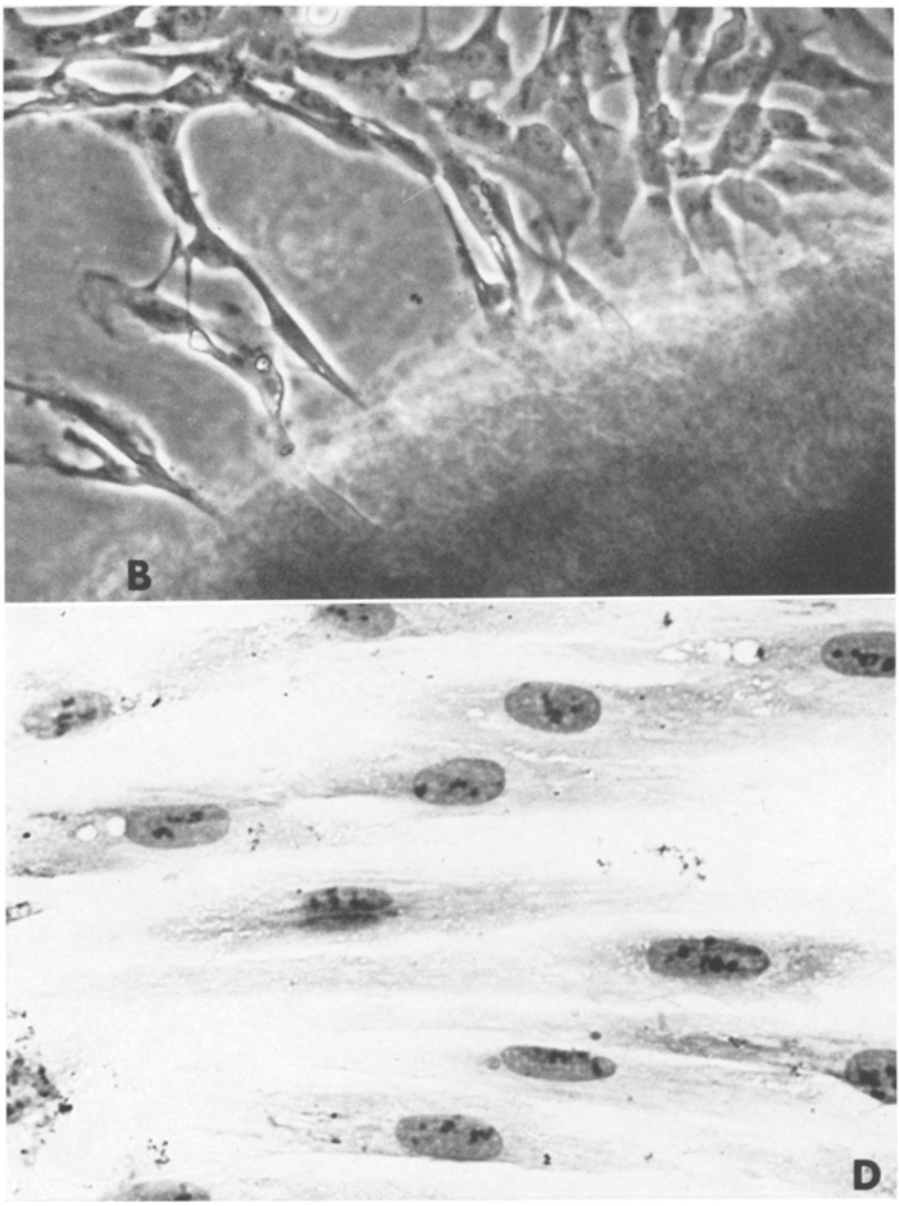

Fig. 3 B and D

feature nor is the presence of sudanophilic lipid prominent in the cell at any stage. The proportion of explants yielding growth was much lower than was the case for the intima-media preparation.

In addition to the cells obtained from the normal rabbit aorta, studies were carried out with cells obtained from the intimo-media and the media layers

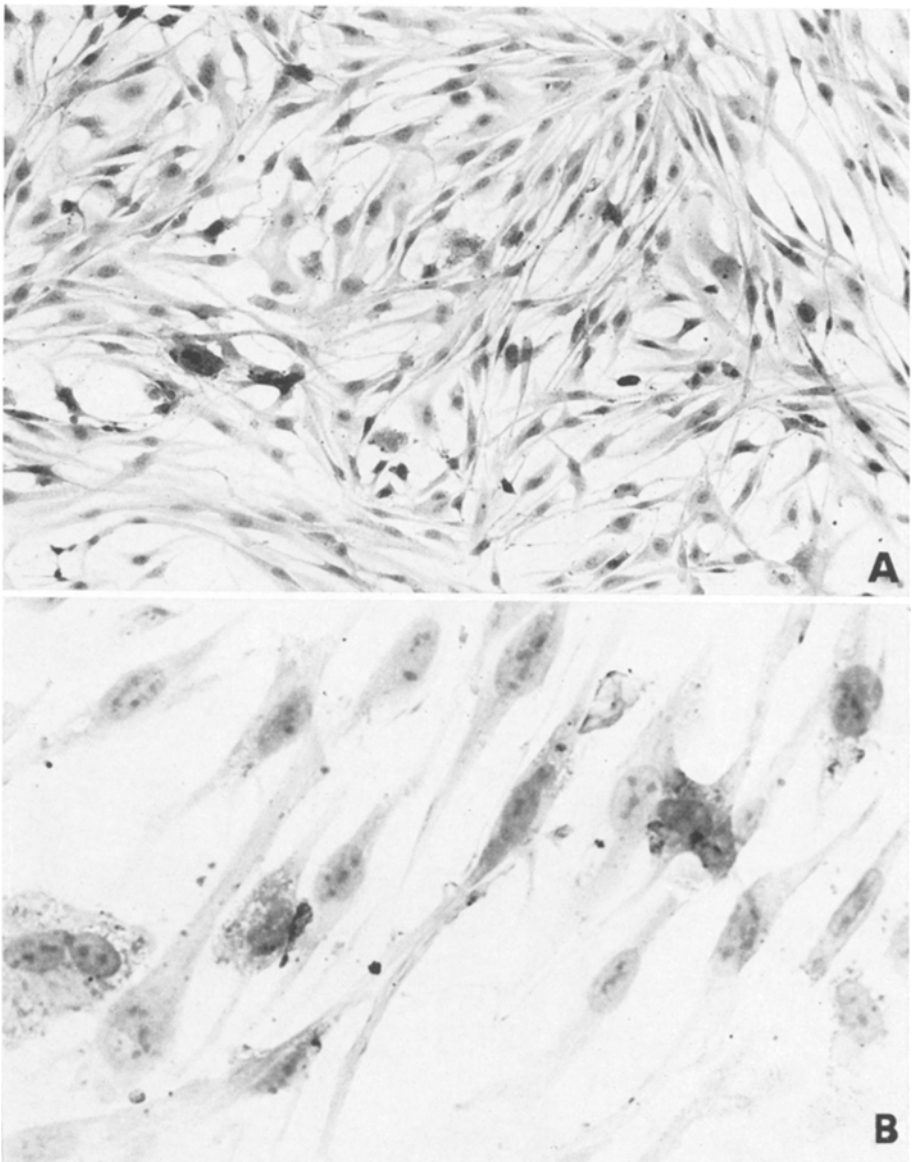

Fig. 4A and B. Cells cultured from intima-media of cholesterol-fed rabbit aortas following disruption with collagenase, elastase. A 3 days after passage, 10 days after primary culture, Sudan-haematoxylin $\times 100$. B As for A, $\times 400$

of rabbits fed cholesterol for three to four months. In these studies the aortas from the cholesterol-fed rabbits were stripped and the intima digested with collagenase and elastase as for the normal rabbit aorta. Explant culture was not used for the intima media layer. The pattern of growth was essentially the same

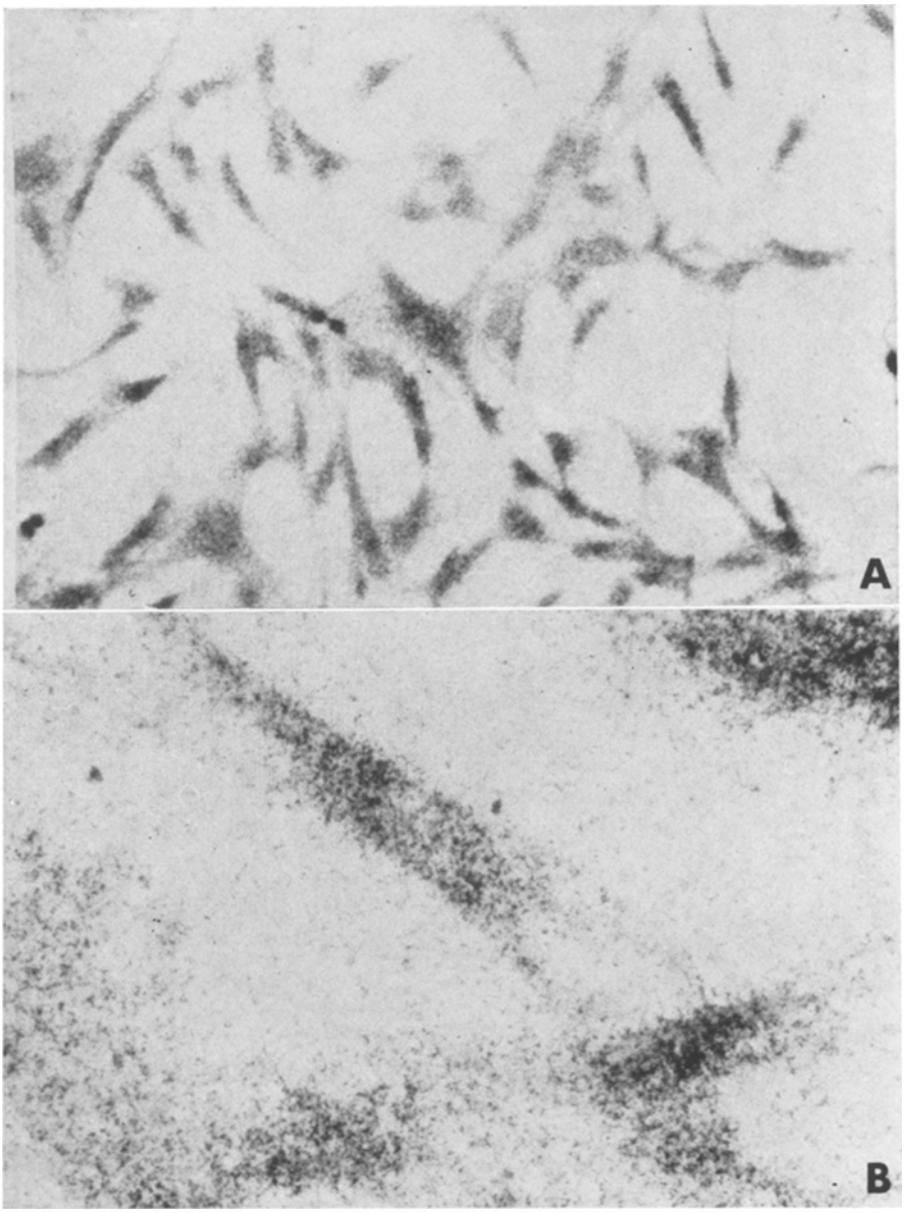

Fig. 5A and B. Autoradiographs of intima-media cells in culture following incubation with (1-^{14}C)-oleic acid. A Cells 5 days after passage from explant culture; Haematoxylin and Eosin $\times$ 100. B 6 days after passage from enzyme disruption primary culture; Haematoxylin and Eosin $\times$ 400

as for the normal-fed rabbit aortas. Growth appeared within 24 hours and the cells were trypsinized within 7 days and secondary cultures prepared in Leighton tubes. There were, however, several cell types present. The morphology of the cells in

Table 1. Uptake of (1-^{14}C)-oleic acid and its incorporation into lipid by arterial cells in tissue culture[a]

	% Uptake[b]	Percentage distribution of (1-^{14}C)-oleic acid				
		Phospholipid	Diglyceride	Fatty acid	Triglyceride	Cholesteryl ester
Normal fed						
Intimal cells – explants (6)[c]	5.1 ± 1.78	77.8 ± 1.95	2.3 ± 0.20	3.0 ± 0.85	13.4 ± 1.68	3.5 ± 0.40
Intimal cells – enzyme disruption (5)	10.0 ± 3.75	72.4 ± 3.02	2.8 ± 0.21	1.6 ± 0.33	19.7 ± 3.75	3.9 ± 0.62
Medial cells – explants (5)	11.4 ± 4.06	69.0 ± 4.13	1.9 ± 0.39	1.8 ± 0.66	23.6 ± 4.63	3.6 ± 0.55
Cholesterol fed						
Intimal cells – enzyme disruption (3)	22.0 ± 4.10	52.1 ± 5.43	1.9 ± 0.22	0.8 ± 0.12	42.4 ± 5.41	2.9 ± 0.19
Medial cells (1)	9.9	75.1	3.4	1.0	17.7	2.8

[a] Mean with the standard error of the mean is given.
[b] Uptake is expressed as % cpm present in cells in relation to total amount present in the incubation medium.
[c] Number of experiments given in parenthesis.

secondary culture in fixed and stained preparations is shown in Fig. 4A and B. Several grossly lipid loaded cells were present but these were outnumbered at this stage by larger numbers of fibroblastic-type cells. Many of the latter contained vacuoles which Sudan staining revealed to be filled with lipid.

Media layer preparations from cholesterol-fed rabbit aortas were also prepared and grown for metabolic study. The morphology of these cells was similar to those of the normal rabbit shown in Fig. 3 above. Little lipid was present in the cytoplasm and passage of cells in secondary culture could be carried out and these cells used for metabolic studies.

The uptake of ^{14}C-labelled oleic acid by preparations of normal intima-media and media cells in culture is indicated by the autoradiographs presented in Fig. 5A and B. The ^{14}C-labelled oleic acid is taken up avidly by the cells and incorporated very generally throughout the cytoplasm in both types of cells shown.

The uptake and metabolism of ^{14}C-labelled oleic acid and its incorporation into combined lipid by the various types of arterial wall cells in culture is shown in Table 1. The percentages of the material present in the incubation medium taken up and incorporated into lipid by the cells are not strictly comparable from one cell type to the other since rigid control of cell numbers was not attempted. Nevertheless, it is apparent that very considerable uptake of the oleic acid present in the incubation medium had occurred and that much of this had been incorporated into combined lipid by the cells during the incubation period. The distribution of the oleic acid and its percentage incorporation into phospholipid, triglyceride and cholesteryl ester by the different types of arterial

Table 2. Uptake of ^{14}C-oleic acid and its incorporation into individual phospholipids by arterial cells in tissue culture. Percentage distribution of (1-^{14}C)-oleic acid[a]

	Lyso-lecithin	Sphingo-myelin	Lecithin	Phos-phatidyl inositol	Phosphatidyl ethanolamine
Normal fed					
Intimal cells – explants (6)[b]	0.3 ± 0.06	0.6 ± 0.14	79.6 ± 0.82	5.3 ± 0.30	14.3 ± 0.57
Intimal cells – enzyme disruption (5)	0.03 ± 0.04	0.9 ± 0.16	78.9 ± 1.32	5.3 ± 0.42	14.6 ± 1.25
Medial cells (5)	0.2 ± 0.04	0.6 ± 0.10	81.3 ± 0.88	5.8 ± 0.52	12.1 ± 1.05
Cholesterol fed					
Intimal cells (3)	0.3 ± 0.07	0.7 ± 0.13	81.8 ± 0.74	5.0 ± 0.91	12.3 ± 1.24
Medial cells (1)	0.3	0.8	77.0	8.5	13.4

[a] Mean figures together with the standard error of the mean is given.
[b] Figures in parenthesis are the number of experiments.

wall cells in tissue culture is also shown in Table 1. In all of the cells most of the oleic acid taken up was incorporated into phospholipid (approximately 70%). A smaller proportion of triglyceride was labelled and in all cell types only about 3% of the oleic acid taken up was incorporated into cholesteryl ester. It will be noted that the distribution of oleic acid in the different lipid fractions is very much the same in the intima-media cells obtained from either explant culture or from enzyme disruption, and in turn is similar to that obtained for the media cells from the normal-fed rabbits. There are, however, distinct differences in the incorporation of the ^{14}C-labelled oleic acid into lipid by the cholesterol-fed rabbit intimal cells when compared with the equivalent normal cells. Incorporation into phospholipid is relatively less and into triglyceride relatively more than the corresponding intima-media cells from the normal animals.

The uptake of the ^{14}C-labelled oleic acid and its incorporation into the individual phospholipids by the five different types of arterial wall cells in culture is shown in Table 2. Most of the oleic acid is incorporated into lecithin (approximately 80%) with lesser amounts of phosphatidyl inositol and phosphatidyl ethanolamine. Almost no lysolecithin or sphingomyelin is labelled under these circumstances.

Incorporation of ^{32}P-labelled phoshate into phospholipid and its distribution into individual phospholipids following its incubation with the arterial wall cells is shown in Table 3. Again, very appreciable uptake of ^{32}P-labelled phosphate from the incubation medium has occurred during the 20 hour incubation period. In all cell types lecithin is the primary phospholipid labelled. There are differences, however, between the cholesterol-fed rabbit intimamedial cells on the one hand, and the other cell types on the other with respect to the incorporation into phosphatidyl inositol. In the cholesterol-fed rabbit intimal cells a significantly lower proportion of label is diverted to phosphatidyl inositol and higher proportion diverted to lecithin as compared to the other cell types.

Table 3. Incorporation of ^{32}P-phosphate into phospholipids by arterial wall cells in tissue culture[a]

	% Uptake[b]	Percentage distribution of ^{32}P-phospholipid					
		Lyso-lecithin	Sphingo-myelin	Lecithin	Phosphatidyl inositol	Phosphatidyl ethanolamine	Ratio: Phosphatidyl inositol / Phosphatidyl ethanolamine
Normal fed							
Intimal cells – explants (2)[c]	0.23 ± 0.00	0.4 ± 0.05	4.2 ± 0.20	66.5 ± 2.05	17.1 ± 2.70	12.0 ± 4.55	1.8 ± 0.90
Intimal cells – enzyme disruption (3)	0.41 ± 0.30	0.5 ± 0.03	3.4 ± 0.39	63.4 ± 0.50	19.1 ± 0.93	13.6 ± 1.07	1.4 ± 0.17
Meidal cells – explants (4)	0.33 ± 0.00	0.45 ± 0.06	5.2 ± 0.79	63.8 ± 1.01	13.7 ± 1.70	16.9 ± 1.17	0.9 ± 0.15
Cholesterol fed							
Intimal cells – enzyme disruption (2)	0.92 ± 0.10	0.4 ± 0.0	4.4 ± 0.20	72.1 ± 2.55	7.4 ± 2.85	16.1 ± 0.35	0.45 ± 0.20
Medial cells – explants (1)	0.47	0.7	6.2	64.4	11.7	17.0	0.70

[a] Mean with the standard error of the mean is given.
[b] Uptake is expressed as % cpm in cell phospholipid relative to total cpm P^{32}-phosphate present in the incubation medium.
[c] Number of experiments given in parenthesis.

Discussion

The morphology of the cells grown from the intimo-medial region of normal rabbit aortas was similar to those described by other workers for cells from whole thickness rabbit aorta (Kokubu and Pollak, 1961; Pollak and Adachi, 1968). As described in the present paper, the latter workers describe endothelial and fibroblastic-type cells, the former appearing early after explant but becoming overgrown by spindle-shaped cells later in the culture period. The cells obtained from the medial layer were similar in morphology both to the intimo-medial preparations from the inner layer and also to the cells described by other workers using essentially the same techniques for smooth muscle cell culture as that used here (Jarmolych *et al.*, 1968; Kao *et al.*, 1968; Ross, 1971). Although no ultrastructural studies were carried out in the present work, the identity of cells obtained in this way as smooth muscle cells has been adequately documented by other workers (Jarmolych *et al.*, 1968; Ross, 1971).

In the present work, the cells were passaged after varying periods in order to remove the original explants or other primary tissue and thus to be able to study the metabolism of the cultured cells. For the intimo-medial cells, the disruption of the primary tissue by collagenase and elastase proved to be a very satisfactory procedure for subsequent culture. It was possible, by this means, to prepare cells for metabolic studies within one week from primary culture. Comparison of the uptake and incorporation of (1-^{14}C)-oleic acid or (^{32}P)-phosphate showed no differences between the cells obtained by this method and those obtained by more conventional explant culture. The reason for the failure to grow medial cells following enzyme disruption is not clear. It may be necessary to have these cells in apposition in order to obtain growth.

The morphology of the cells obtained from the intimo-medial region of the cholesterol-fed rabbit aortas were again similar to those described by Kokubu and Pollak, 1961. In the present study, no proliferation of the grossly lipid-laden foam cells present in the cholesterol-fed rabbit intimal digest was apparent and the spindle-shaped cells soon overgrew the apparently non-proliferating foam cells. Many of the spindle-shaped cells, however, contained large numbers of lipid droplets. The failure of the foam cells to proliferate is consistent with the previous report of Tume *et al.* (1969) where foam cells isolated from cholesterol-fed rabbit intima were maintained under a number of tissue culture conditions but without any evidence of proliferation. It contrasts to the recent report of Yamamoto *et al.* (1971) who claim that such disrupted preparations of cholesterol-fed rabbit aorta can be diluted and proliferation of foam cells demonstrated. It is possible that the proliferating cells demonstrated by Yamamoto *et al.* (1971) were similar to the spindle-type cells grown in the present work where lipid droplet formation was a marked feature. No metabolic studies were reported on these cells by Yamamoto *et al.* (1971) and in the present work the metabolism of the cells which grew from the cholesterol-fed intima did not resemble at all that for isolated foam cells previously reported (Day and Tume, 1969). Foam cells on the one hand incorporate very large amounts of oleic acid into cholesteryl ester whereas the intimo-medial cells studied in the present work incorporated only about 3% of the oleic acid taken up into the cholesteryl ester fraction.

Little information is available regarding the lipid metabolism of aortic cells in culture although some preliminary studies have been reported (Robertson, 1965; Murata, 1970; Avigan, 1972). In the present paper it has been shown that both (1-^{14}C)-oleic acid and (^{32}P)-phosphate are taken up by the aortic cells (both intimo-medial and medial) and predominantly incorporated into the phospholipid fraction, primarily lecithin. The radioautographic observations reported demonstrate fairly general incorporation over the cell and are consistent with the conclusion that the incorporation is primarily into cell membrane phospholipid.

Two differences were observed, however, between the intimo-medial cells cultured from the cholesterol-fed rabbit aorta and those cultured from the normal rabbit aorta. The cholesterol-fed cells paralleled their morphological sudanophilia with an increased incorporation of (1-^{14}C)-oleic acid into the triglyceride fraction. It seems likely that the droplets present were, therefore, triglyceride droplets and that the increased incorporation into triglyceride reflected a changed metabolic pattern in these cultured cells. The other difference between the cholesterol-fed and the normal-fed rabbit aortic cells was the decreased relative incorporation of P^{32} phosphate into phosphatidyl inositol in the cholesterol-fed aortic cell. It has already been noted (Newman *et al.* 1966) that the transformation of the normal to the atherosclerotic arterial wall is associated with reduced relative incorporation of P^{32} phosphate into phosphatidyl inositol. Although the major phospholipid synthesized in the present study by the cholesterol-fed cells was lecithin, presumably an indication of cell membrane turnover, there was a marked reduction in the proportion of P^{32} phosphate giong to phosphatidyl inositol in these cells compared to that in cells grown from the normal rabbit aorta. The significance of the synthesis of phosphatidyl inositol in the arterial cell in relation to the pathogenesis of atherosclerosis is not clear. However, it would seem that in the present work another instance has been described whereby changes in phosphatidyl inositol synthesis are associated with the development of atherogenesis. In this case, a change which persisted throughout cultured cell generations.

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