activity would have produced substantially the same subgroups. It should also be pointed out that if the mean plasma fibrinolytic activity were calculated for all the post-cardiac-infarction subjects it would not differ greatly from the mean obtained from a group of normal subjects. This would agree with the findings of Nestel (1960), Merskey *et al.* (1960), and Goldrick (1961). This calculation, however, obscures the clear differences which exist between the two subgroups.

It would appear that subgroup B consists of subjects whose fibrinolytic activity and mucoprotein levels are essentially normal, while the subjects in subgroup A, with raised mucoproteins and low fibrinolytic activity, have some metabolic disturbance resulting in these and possibly other changes. Goldrick (1961) observed that plasma fibrinolytic activity decreased with increasing body bulk in both healthy and atherosclerotic Australians. Our finding that a decreased height: weight ratio is associated with low fibrinolytic activity tends to confirm this observation. The measurements used in this study to assess body build, however, are insufficient to determine whether the variations are due to differences of somatotype or of nutritional status.

From the finding of previous workers that the serum mucoprotein level is raised in atherosclerosis (Antonini and Salvini, 1957; Schwartz and Gilmore, 1958) it might be inferred that the subjects in subgroup A have more generalized atherosclerosis. Alternatively the subjects in subgroup B may have a different type of coronary-artery disease, since the parameters used in this study do not differentiate them from healthy subjects.

This study of the relationships between serum mucoprotein levels, plasma fibrinolytic activity, and body build in subjects who have sustained a cardiac infarction must be regarded as a preliminary survey. It is clear that further investigation is required to elucidate fully these relationships and to evaluate their importance against the background of coronary-artery disease.

Summary

A group of post-cardiac-infarction subjects could be divided into two subgroups as follows: (A) those in whom the serum mucoprotein level was high and the plasma fibrinolytic activity low, and (B) those in whom these findings fell within the normal range.

The mean height of the subjects in the two subgroups was the same, but the mean weight of subjects in subgroup A was significantly greater than that of those in subgroup B.

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ROLE OF BLOOD-BORNE CELLS IN ORGANIZATION OF MURAL THROMBI

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[WITH SPECIAL PLATE]

No evidence generally regarded as conclusive has yet been produced to show whether or not mononuclear leucocytes are capable, unaided, of organizing mural thrombus into a new intimal layer. Most work apparently demonstrating this occurrence has been open to the criticism that the regenerated vascular endothelium and its supporting elements could conceivably have been an ingrowth from neighbouring tissues. Clear knowledge of the potentials of the mononuclear cell in this respect is fundamental to our understanding, not only of many repair processes throughout the body, but also of events within the organizing thrombus and various phenomena in the ageing artery, notably atherosclerosis.

The study now presented was stimulated by the observation of one of us (A. R. G.) that mononuclear white blood cells in a diffusion chamber appeared capable of transformation into macrophages, fibroblasts, and endothelial cells; the other author (D. J. T.) was impressed by the selective fashion in which mononuclear cells with every appearance of being the precursors of the endothelial cells line the lumen of "dacron" arterial prosthesis from the earliest stage. It was decided to investigate further the potentials of the mononuclear cell in this respect.

Method

It was necessary to evolve a preparation which could be inserted for varying periods into the arterial systems of experimental animals and upon which blood-borne cells would be deposited; at least one portion of the preparation had to be inaccessible to ingrowth by any cell from neighbouring tissue. A woven "terylene" arterial prosthesis (Tibbs and Martin, 1960) appeared to be a good basis for such a preparation. Cellular ingrowth from neighbouring tissues into the lumen of an arterial prosthesis occurs by two processes: (a) infiltration from surrounding tissues through the fabric of the prosthesis; and (b) growth along the lumen from the artery at each end to which the prosthesis is anastomosed. Infiltration through the fabric may be prevented by use of a cell-impervious plastic-film sheath; the centre portion of a prosthesis may be made inaccessible to ingrowth along the lumen if the prosthesis is long enough. Naturally, the length of the prosthesis necessary to ensure an invasion-free centre portion depends on the rate at which ingrowth along the lumen occurs and on the period during which the prosthesis is to be retained in the experimental animal. It was upon these principles that the preparations employed were based.

The following arterial prostheses were put into circuit with the aortas of dogs.

(a) Three 8-mm.-diameter terylene arterial prostheses 7 cm. long without plastic-film sheaths. These were used only for periods up to 90 minutes in the arterial circuit.

(b) Four 8-mm.-diameter terylene arterial prostheses 7 cm. long lined with cell-imperious plastic membrane (Millipore Filter Co.) throughout (Type I). It was appreciated that after 14 days within the experimental animal ingrowth from the ends could conceivably invalidate observations upon the centre portion, and a longer graft was therefore developed.

(c) Three 8-mm.-diameter terylene prostheses 15 cm. long with the centre portion ensheathed in Millipore membrane for 5 cm. (Type II). It became clear that after three weeks within the experimental animal ingrowth occurred from surrounding tissue through the fabric of the unsheathed portion of the "graft," and it might be possible for this to spread via the lumen into the ensheathed portion; total enclosure in nylon film was therefore adopted.

(d) Four 8-mm.-diameter terylene prostheses 17 cm. long with their entire length enclosed in a closely fitting sheath of crimped nylon film of 0.001 in. (0.025 mm.) thickness (Type III) (see Diagram). The nylon sheath was a complete

A Type III preparation consisting of a "terylene" fabric arterial prosthesis 17 cm. long and ensheathed in crimped nylon film. The method of insertion is shown.

barrier to cell ingrowth, and it was carefully inspected for breaks in the welded seam. Our observations indicated that cell ingrowth from the ends could not cross the 8-cm. length from either end to the centre of the graft in the time intervals employed in this study.

All prostheses were inserted by simple division of the dogs' abdominal aorta and end-to-end anastomosis of the graft to the cut ends; since no aorta was removed the graft formed an additional length to the normal arterial tree. In the case of the long grafts it was necessary to coil the grafts within the abdomen, and all but one remained patent (see Diagram).

Cutting sections suitable for histological study proved difficult, since the terylene fabric was easily fragmented by the microtome knife or when floated on water. Eventually a satisfactory technique was evolved; the specimens were embedded in "tissue mat" paraffin, and before each section was cut the surface of the block was coated with celloidin to hold the section together. At a later stage the celloidin was washed away by immersing the section for an hour in equal parts of ether and alcohol. Other steps in the processing of the histological preparation were orthodox and do not require special mention.

Each preparation was studied by longitudinal sections of the anastomoses and by transverse sections close to the midpoint of the prosthesis. Additional sections were taken wherever further study seemed indicated.

Results

An arterial prosthesis which had been merely soaked in whole blood but through which blood had not been allowed to flow showed, as might be expected, a mass of red cells, with occasional white cells, lying on and among the fabric. When, however, flow through the prosthesis had occurred for periods as brief as half an hour a very different picture was seen. The inner aspect of the fabric was coated with true thrombus composed of fibrin and white blood cells laid down selectively from the blood-stream (platelets were not specifically studied in this experiment); red cells were scattered in variable number throughout the thrombus.

The fate of the mural thrombus deposited so quickly upon the foreign material of the graft fabric was studied histologically in two areas.

(a) At the Actual Anastomosis Sites

Here ingrowth from the neighbouring artery may quickly obscure the intrinsic changes within the thrombus. Longitudinal sections revealed that at two days there was no active change in the arterial wall; at eight days the endothelial cells of the artery had regenerated rapidly and invaded the mural thrombus lining the anastomotic site for a distance of 1 mm. The media and adventitia showed early fibroblastic growth, which was beginning to invade the mural thrombus. At two weeks the invasion of endothelium and fibroblasts from the host artery into the thrombus of the prosthesis had progressed to 2 mm., and an occasional slender capillary could be seen sprouting from the vasa vasorum of the artery. The same changes could be seen at slightly more advanced stages at the third and fourth weeks, and it was estimated that endothelial and fibroblastic ingrowth from the living artery progressed at about 1–1.5 mm. per week.

These changes are described only briefly, because they correspond with many previous descriptions (Macpherson and Duthie, 1957; Martinez *et al.*, 1957; Mackenzie and Loewenthal, 1960).

(b) In the Centre Portion of the Grafts

In that portion of the graft believed to be immune from the confusion caused by ingrowth of cells a very different picture from that just described was observed. After two days polymorphonuclear cells were seen to be degenerating and were being absorbed or phagocytosed by mononuclear cells and their numbers sharply decreased. The mononuclear cells, however, survived and showed active phagocytic activity for degenerate cells or particulate matter. They grew larger to measure 20–40 μ in diameter; many showed cytoplasmic vacuoles of different sizes.

At eight days it was possible to trace all these successive structural changes in the mural thrombi in one microscopical section, because in places several layers of thrombus of different ages showed varying degrees of development, the most recent being on the surface exposed to the blood-stream. The deepest and oldest portion—that is, nearest the terylene graft—showed trabeculation and lacunar formation in the skein of the thrombus. Between fibrinous strands and lacunar spaces many large mononuclear cells were found scattered throughout this layer together with binucleate and multinucleate giant cells. The formation of lacunae was consistent with Dible's (1958) description

of this occurrence in thrombus brought about by the absorption of thrombocytes, polymorphs, red blood corpuscles, and the more delicate fibrinous network, to leave only the more dense strands of fibrin.

At 14 days the lacunar spaces had taken on more regular shape, and some had become lined with long, slender fusiform cells, while others were still infiltrated with large mononuclear cells, macrophages, and epithelioid cells. The multinuclear giant cells had increased in size and number and were situated mostly near or on the terylene graft.

After 21 and 28 days an increasing number of the lacunar spaces showed lining by endothelial-like cells and some of the spaces contained fresh red cells which had every appearance of having been in active circulation. At this stage no fibroblasts were observed surrounding or supporting the lacunae. The picture consisted of widely opened sinuses lined by a single layer of fusiform cells reminiscent of a cavernous haemangioma (Special Plate, Fig. 1).

After 28 days Type II grafts were used, so that at this stage no observations were made upon the centre portion of grafts less than 15 cm. in length. The same changes as those just described were noted at five, six, and seven weeks, but the unsheathed portion of the prosthesis showed definite ingrowth of fibroblasts through the graft fabric to give the well-organized neo-intima so often described in studies on plastic arterial grafts (Sauvage and Wesolowski, 1955; Deterling and Bhonslay, 1955; Creech *et al.*, 1957; Bain, 1958).

Further observations were made upon four long grafts enclosed over their entire length with nylon film (Type III) so that they were impervious throughout their length to cells or fluid. In this preparation there is a layer of whole blood trapped between the nylon film and terylene prosthesis—this collects during the first seconds of blood-flow through the graft by simple leakage of blood through the interstices of the fabric into the nylon envelope. This collection was found to behave quite differently from the thrombus laid down selectively on the luminal surface of the prosthesis and which is able to receive direct nourishment from its immediate contact with the blood-stream. Thus the layer between nylon film and prosthesis is different in cell content and structure from the outset; it is relatively inaccessible to the blood-stream, and only slow diffusion of nourishment or infiltration of cells through the graft material is possible; the nylon film forms an impassable barrier to any influences from surrounding tissues. It is not surprising that, except for scattered areas near the prosthesis, the layer between nylon film and terylene prosthesis should in most cases become an amorphous collection. This layer will again be referred to later, but its study is incidental to the experiment, and the changes on the inner surface of the completely ensheathed long terylene grafts must now be considered.

In a seven-weeks preparation a well-formed lining of fibrin was observed with scattered areas of advanced formation of "endothelial"-lined blood sinuses similar to those previously described. In places fusiform cells closely resembling young fibrous-tissue cells were seen.

An eight-weeks preparation showed a thin fibrin lining containing a sparse population of degenerate white blood cells; very occasional blood sinus formation was seen, but this was minimal and not to be compared in quality or extent with the previous preparation. This

preparation was unlike the other preparations in that the layer between nylon film and prosthesis contained a great number of macrophages, binucleate and multinucleate giant cells. The whole appearance suggested a toxic reaction, possibly bacterial infection; however, after appropriate staining no bacteria could be seen, but since culture for bacteria was not performed when the graft was removed from the experimental animal no definite conclusion can be reached on whether infection was or was not responsible.

A preparation of 19 weeks was found to have been occluded. At what stage this had occurred could not be ascertained, but it cannot have been very recent. The whole preparation was filled with amorphous material, and no cellular structure was recognizable. Presumably when flow ceased the one available source of nutrition, the blood-stream, was removed and all living cells perished. This comes as a useful hint of the role of the blood-stream in this particular preparation and the effectiveness of the nylon-film barrier.

At 21 weeks the "intimal" layer showed a well-organized layer composed of fusiform "endothelial" cells supported by a thick layer indistinguishable from fibrous tissue (Special Plate, Fig. 2). Staining by silver (Special Plate, Fig. 3) and van Gieson stain showed reticulin and collagen to be present in easily recognizable amounts. The blood sinuses seen in earlier preparations were less evident and appeared to have been largely replaced by fibrous tissue to give a well-organized new intima. This preparation showed certain other striking changes, superimposed on those just described. The inner lining contained, in the layer next to the prosthesis, a massive deposition of calcium forming a complete ring within the graft. Von Kossa's staining, moreover, showed crystals of calcium salt scattered throughout the new "intima." Staining with Sudan IV showed fat to be deposited in fine droplets both free and within macrophages throughout the "intimal" layer and in the layer between nylon film and prosthesis; the significance of these changes, so reminiscent of atherosclerosis, will be considered further presently.

Thus, of these four totally ensheathed long graft preparations, one showed well-developed blood sinuses and endothelial-like cell development with evidence of early fibrous-tissue formation, and another showed apparent endothelium and extensive fibrous-tissue formation. The latter preparation also showed heavy deposition of calcium and fat. One preparation appeared abnormal in its response, the appearance suggesting local toxic reaction, and one preparation was occluded and unhelpful.

Discussion

Though it could be argued that in preparations of Types I and II ingrowth was possible from the neighbouring artery or through the fabric of the prosthesis and then along the ensheathed portion, we believe that in fact the changes found were not in any part due to this. Our reasons are as follows.

(a) In all preparations longitudinal sections from the anastomosis were used to locate the limit of ingrowth from the artery. Study of the centre portion was made well clear of this.

(b) Studies of the rate of ingrowth again made it clear that ingrowth could not have reached the centre portion in the time during which the graft was in the experimental animal.

(c) The changes appeared to be multifocal and gradations of change from mononuclear cell to "endothelial" cell and fibrous elements could be recognized. The local change gave every appearance of being an independent progressive process.

(d) The changes occurred through the depth of the fibrin lining and not merely as a surface layer.

(e) The appearances where ingrowth was known to occur were distinctive, and quite different from the characteristic changes in the centre portion.

The preparations employed in our experiments, especially Type III, appear to provide a medium in which cells deposited from the blood-stream may be nourished by the blood-stream and yet be immune from interference by ingrowth of extrinsic tissue. The complete accessibility of the blood-stream to the cells under study seems to be a very important advantage, and failure of the blood-stream causes death of all cells in the centre part.

This study appears to demonstrate that a thrombus receiving adequate nutrition from the blood-stream can undergo spontaneous intrinsic changes to form from its mononuclear cells an organized tissue with "endothelial" covering and formation of capillary-like vessels. In the oldest preparation fibrous tissue had made its appearance, and it is hard to explain how this could have arisen from other than the blood-borne cells, probably mononuclear cells. Thus the mononuclear white blood cell can apparently develop into phagocyte, "endothelial" cell, and fibroblast as occasion demands, and it takes on these functions in a well-regulated sequence in the organizing thrombus. This idea is not novel and has been put forward by many authorities (Maximow, 1924, 1927; Higgins and Palmer, 1929; Braunsteiner *et al.*, 1948; Moschowitz, 1950; Dible, 1950, 1953; Downey, 1955; Rebuck and Crowley, 1955; Allgöwer, 1956).

Whether the "endothelial" cells seen in the present study were true endothelium or merely cells with certain histological resemblances to endothelium cannot be decided without further investigation, including electron microscopy. The true endothelial cell has a number of characteristic structural and physico-chemical properties (Buck, 1958), and presumably these must be identified before it can be decided whether a cell has not only the appearance but the function of an endothelial cell. Our experiments suggest a means for settling the long-standing dispute on the possibility of monocytes undergoing transformation into endothelial cells. The Type III preparation would seem capable of providing material derived from the monocyte in a form suitable for detailed study.

The 21-weeks preparation provided an interesting study of the way in which all the features of atherosclerosis can arise purely from elements deposited by the blood-stream. This preparation, when removed from the experimental animal, showed to the unaided eye small scattered whitish nodules throughout the intima, particularly in the centre portion, exactly similar in appearance to the fatty streaks seen in the human aorta. The histological appearances in the organizing thrombus lining the prosthesis closely resembled the atherosclerotic plaque seen in the human artery, and the calcium and fat deposits provided other characteristics of atherosclerosis. Since all the features were observed at a site remote from any tissues other than those deposited from the blood-stream, they provide evidence that the cellular and acellular components of

atherosclerosis need not be dependent upon any process inherent in the wall of an artery (Duguid, 1948). These findings are also in keeping with the experimental observation of Wesolowski *et al.* (1961) that calcification in arterial prostheses is the direct result of a poor porosity of the walls.

Summary

In plastic arterial prostheses there is, from an early stage, a selective laying-down of cells from the blood-stream in the mural thrombi lining the plastic material. The present study has followed the behaviour of these cells in arterial prostheses inserted into canine aortae. These grafts were of sufficient length to prevent ingrowth from the ends reaching the centre portion during the period of the experiment, and were ensheathed in nylon film to prevent ingrowth from surrounding tissues. It was found that, though other cells quickly degenerated, mononuclear cells persisted and took on the characteristics of phagocytes which cleared lacunae within the thrombus; mononuclear cells also underwent progressive transformation into endothelial-like cells, so that vascular spaces and primitive capillaries were formed.

In at least one of the later preparations fibrous tissue supporting the new intima appeared to have originated from blood-borne cells. Gross deposits of calcium and fat were observed in one preparation at a site remote from any tissues other than those deposited from the blood-stream.

This study may help in the understanding of the potentialities of mononuclear blood cells, and incidentally provides evidence that the development of atherosclerosis need not be dependent on any process inherent in the wall of an artery.

We wish to express our appreciation of the encouragement given to us by Professor J. B. Duguid. The work embodied in this paper was principally carried out in the Departments of Pathology and of Surgery of the Medical School, Newcastle upon Tyne; it was completed in the corresponding departments of the Royal Free Hospital, London, and the Radcliffe Infirmary, Oxford. We are indebted to Miss M. McLarty for the drawing.

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G. WESTBURY *ET AL.*: TREATMENT OF RECURRENT CANCER OF HEAD AND NECK

FIG. 1.—Before treatment.

FIG. 2.—After treatment.

FIGS. 1 and 2.—Case 4. Extensive necrosis following intra-arterial infusion of methotrexate.

FIG. 3.—Before treatment.

FIG. 4.—After treatment.

FIGS. 3 and 4.—Case 23. Recurrent carcinoma of palate treated with methotrexate.

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FIG. 1.—Sinuses lined by endothelial-like cells at 28 days. (×66.)

FIG. 2.—Appearance of fully organized mural thrombus at 21 weeks. (H. and E. ×66.)

FIG. 3.—Reticulin network shown by silver stain in 21-week preparation. (×66.)