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Regeneration of parietal and visceral peritoneum: an electron microscopical study

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INTRODUCTION

In a previous investigation with the light microscope (Raftery, 1973*a*) it was shown that defects in the peritoneum healed rapidly and that the new mesothelium appeared to arise by metaplasia of subperitoneal fibroblasts. Further it was shown that, following labelling of the peritoneal macrophages with polystyrene spheres, no such spheres were subsequently seen in fibroblasts or the reconstituted mesothelium; this was put forward as strong evidence against the theory of Eskeland (1966) and Eskeland & Kjaerheim (1966) that peritoneal macrophages are transformed into mesothelial cells either directly or via fibroblasts. On the basis of light microscopy it was not possible to discount the theory that mesothelial cells may become detached from adjacent normal peritoneal surfaces and give rise to the new mesothelium (Cameron, Hassan & De, 1957; Johnson & Whitting, 1962; Bridges & Whitting, 1964). A search for detached mesothelial cells in the peritoneal fluid (Raftery, 1973*b*) revealed that a few such cells were present in the peritoneal fluid of rats which had been subjected to abdominal surgery involving excision of areas of peritoneum, but their numbers could not be accurately assessed since they could only be identified with certainty in sections of pellets of peritoneal cells examined by electron microscopy. Also it appeared that some of these detached mesothelial cells were injured or dying. Because the identification of detached mesothelial cells requires electron microscopy, their role in peritoneal regeneration can only be assessed adequately by the same means. The present ultrastructural study was undertaken chiefly for two reasons:

(1) To attempt to confirm the finding, obtained by light microscopy, that the new mesothelium arose by metaplasia of subperitoneal fibroblasts and not by transformation of peritoneal macrophages.

(2) In order to assess the role of detached mesothelial cells in peritoneal regeneration.

MATERIALS AND METHODS

A total of 80 adult male Sprague–Dawley rats weighing 220–250 g was used. The rats were anaesthetized with ether, and the abdominal cavity was opened through a mid-line incision 5 cm long. Wounds approximately 1 mm deep and either $\frac{1}{2}$ cm or 1 cm square were made in the liver capsule by a tangential cut with a scalpel blade. Wounds were also made in the parietal peritoneum on each side of the mid-line; the

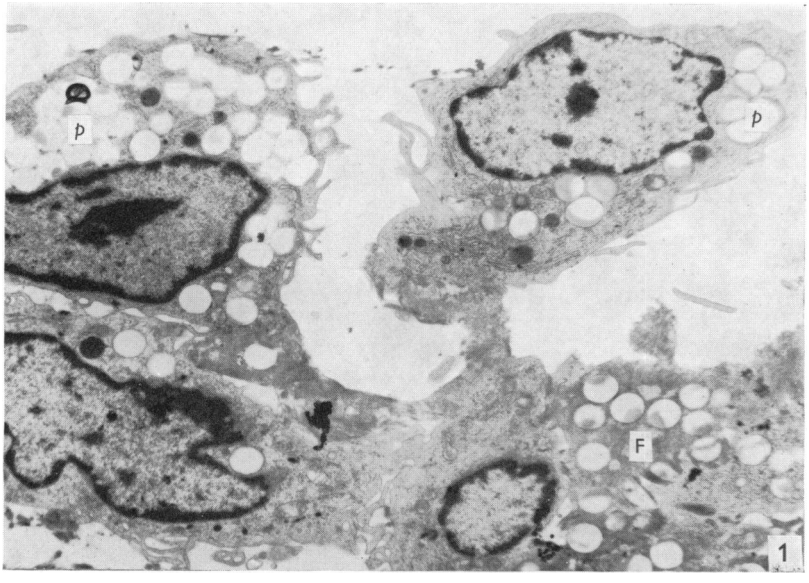
lesion on the left was 2 cm square, and that on the right was 1 cm square. The peritoneum, together with the underlying layer of muscle, was removed within this area. In other animals, the caecum was delivered through the wound, and the peritoneum was stripped from its anti-mesenteric border over areas of about 1 cm by 1 cm or 1 cm by 2 cm. Haemostasis was obtained by applying pressure from a gauze swab. In order to label the peritoneal macrophages, some animals were given an intraperitoneal injection of 2·5 ml of a 0·2% suspension of polystyrene spheres (0·79 μm diameter; Dow – Latex. Serva, Feinbiochemica, Heidelberg) in 0·9% saline at the time of operation. The abdominal incision was closed in two layers, catgut sutures being placed through the musculo-peritoneal layer and silk sutures through the skin. Animals were killed at intervals of 12 hours for the first 3 days, daily until 8 days and then at 10 days, 12 days and 14 days after operation. Material from at least three wounds was examined at each stage. The animals were killed by exsanguination under ether anaesthesia; the peritoneal cavity was opened rapidly and the wound surface was flooded with 2·5% glutaraldehyde in 0·1 M-cacodylate buffer at 4 °C. In some animals, primary fixation was effected by dripping cold (4 °C) 1% osmium tetroxide in 0·1 M-phosphate buffer on to the wound surface. The wounds were excised *in toto*, pinned flat on cork in the case of parietal peritoneum and caecum, and transferred to the appropriate fixative for 2 hours. When fixation was completed, pieces of tissue approximately 3 mm long, 1 mm wide and 1 mm deep were cut from different areas of the wound surface. Tissue fixed in glutaraldehyde was washed overnight in buffered sucrose and then post-fixed for 2 hours in 1% osmium tetroxide in 0·1 M-phosphate buffer. The specimens were subsequently dehydrated in graded solutions of ethanol, passed through epoxy-propane, and embedded in Araldite to allow sectioning perpendicular to the wound surface. Sections, 1 μm thick, were cut on an LKB Ultratome III, stained with methylene blue–Azure II (Richardson, Jarett & Finke, 1960) and examined by light microscopy. Such sections were cut at several levels from specimens from different areas of the wound surface. When appropriate areas were found, thin sections were cut, mounted on uncoated grids and either stained with lead citrate alone (Reynolds, 1963) or double-stained with uranyl acetate (Stempack & Ward, 1964) and lead citrate prior to examination in an AEI type EM 6B electron microscope.

RESULTS

The changes observed on the wound surface and in the base of the wound, in both parietal and visceral peritoneum, were for the most part similar. The findings will therefore be described collectively, and any minor variations will be pointed out at the relevant stage.

12 hours

Numerous cells were seen entangled in fibrin strands. At this stage polymorphonuclear leucocytes were predominant but many macrophages and a few eosinophils and mast cells were also seen. There was relatively little cellular infiltration in the depths of the wound when compared with the surface. Polystyrene spheres were seen in macrophages and polymorphonuclear leucocytes.



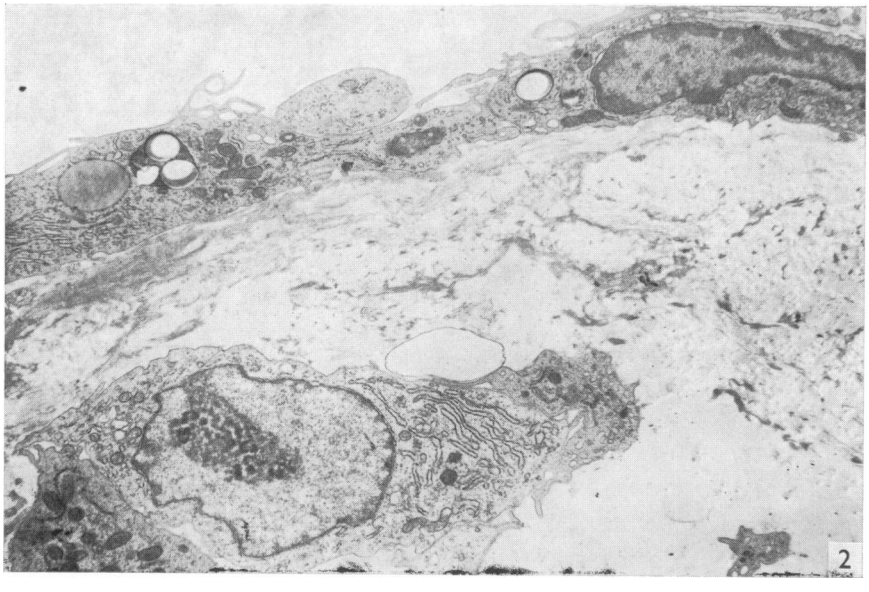


Fig. 1. 1 day. Parietal peritoneum. Macrophages filled with polystyrene spheres (*p*) on the wound surface. Several polystyrene spheres are trapped within the fibrin (*F*). Uranyl acetate (*UA*) and lead citrate (*LC*). ×4500.

Fig. 2. 2 days. Parietal peritoneum. Note the two flattened macrophages, containing polystyrene spheres, on the wound surface. They are in close contact but no junctional complexes occur between adjacent cells. *LC*. ×4500.

24 hours

The number of cells in the superficial parts of the wound was greatly increased and most of these were macrophages. They were tightly packed, and numerous strands of fibrin were seen between the cells. Many polystyrene spheres were seen within the macrophages and also trapped within the fibrin mesh (Fig. 1). The deeper parts of the wound were still relatively acellular. No cells which could be identified as detached mesothelial cells were seen on the wound surface. The appearances were similar at 36 hours.

2 days

At this stage there were marked changes, both on the surface and in the base of the wound. In most areas the wound surface was covered with a single layer of macrophages resting on a fibrin base. Some of the macrophages were round, but others had flattened out on the wound surface and came into close contact with one another (Fig. 2). The macrophages contained polystyrene spheres. No junctional complexes were seen between adjacent macrophages.

Two further types of cell were seen on the wound surface at this stage (Fig. 3).

First type: The cells varied in shape, but were usually elongated and flattened. Their cytoplasm, which was often sparse, was characterized by numerous aggregates of ribosomes (Fig. 4). Otherwise, the cytoplasm contained only a few cisternae of rough-surfaced endoplasmic reticulum, a small Golgi complex, a few mitochondria and the occasional lipid droplet. The nucleus was large and had an even distribution of chromatin, except at its periphery, where the chromatin formed a narrow, dense band on the inner aspect of the nuclear membrane. One or more large, prominent nucleoli were seen. Cells of this type were only rarely seen on the wound surface at this stage: in contrast to the macrophages, they never contained polystyrene spheres.

Cells with similar characteristics occurred in the base of the wound (Fig. 5), mainly in the region of the perivascular connective tissue. Some of them possessed long processes directed towards the wound surface. This type of cell in many respects closely resembled a primitive mesenchymal cell, although descriptions of the latter vary from author to author (Kelley, 1970; Haar & Ackerman, 1971; Dempsey, 1972). It will be referred to as a primitive mesenchymal cell in the subsequent text.

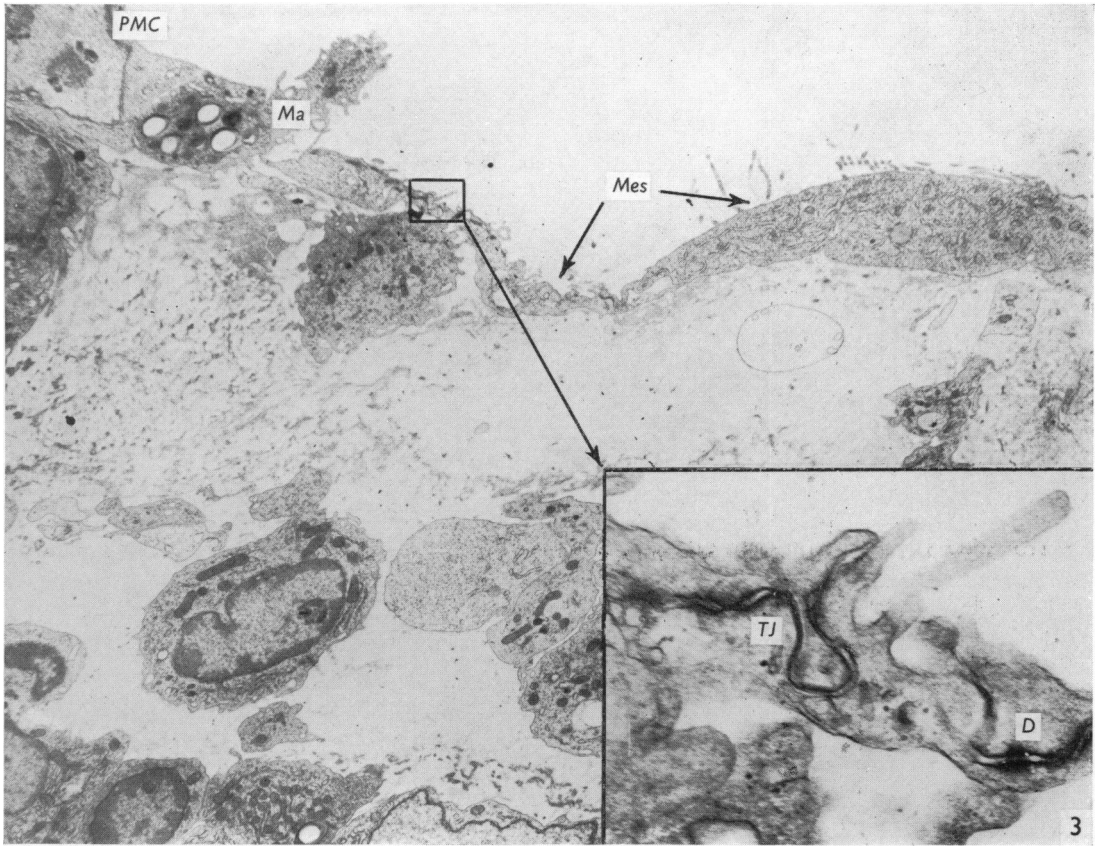
Second type: Cells of the second type were extremely rare, being seen only in two sections from different areas of the same wound. They were identified as islets of mesothelial cells (Fig. 3). They possessed numerous microvilli and pinocytotic vesicles and were joined to adjacent cells by desmosomes and tight junctions (Fig. 3). No basement membrane was seen beneath such cells.

60 hours

The appearances were similar to those at 2 days, except that no detached mesothelial cells were seen on the wound surface.

3 days

Most of the cells on the wound surface were macrophages, but primitive mesenchymal were becoming more common. Patches of fibrin were still present on the



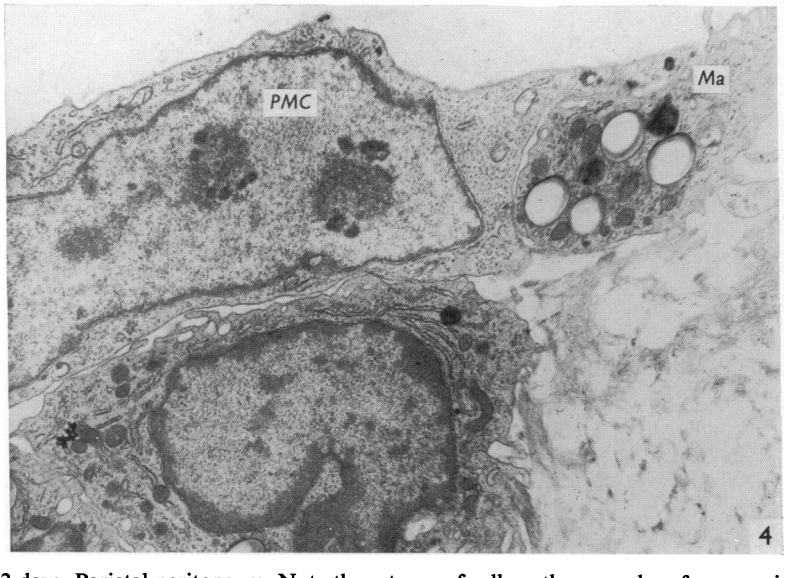


Fig. 3. 2 days. Parietal peritoneum. Note three types of cell on the wound surface; a primitive mesenchymal cell (*PMC*), a macrophage (*Ma*) and a group of mesothelial cells (*Mes*) joined by tight junctions (*TJ*) and desmosomes (*D*) – see inset. Polystyrene spheres are seen only in the macrophage. *LC.* ×4000 (inset ×40000).

Fig. 4. 2 days. Parietal peritoneum. A primitive mesenchymal cell (*PMC*) on the wound surface. Note the prominent nucleoli and the numerous ribosomes in the scanty cytoplasm. Only the macrophage (*Ma*) contains polystyrene spheres. *LC.* ×8000.

wound surface in some areas. Where fibrin was seen, macrophages usually formed the most superficial layer of cells, but where fibrin was absent, primitive mesenchymal cells were usually found on the wound surface (Fig. 6). In some areas, long processes of primitive mesenchymal cells, identified as such since they contained numerous aggregates of ribosomes and few other organelles, were seen extending to the wound surface between macrophages (Fig. 7). Occasional short microvilli arose from their surface.

A rare finding at this stage was a surface cell possessing several short microvilli arising from the peritoneal surface (Fig. 8). Difficulty arose in the identification of this type of cell, for although it possessed microvilli on its free surface, it contained relatively few cytoplasmic organelles, and its nuclear and cytoplasmic characteristics resembled more closely those of a primitive mesenchymal cell than those of a detached mesothelial cell. Few macrophages were seen in the base of the wound at this stage, and the cells in this situation ranged from those containing numerous ribosomes and little else (identified as primitive mesenchymal cells), to cells containing numerous dilated cisternae of rough-surfaced endoplasmic reticulum, several aggregates of ribosomes and a prominent Golgi complex; these resembled proliferating fibroblasts. Sometimes the latter type of cell was separated from the peritoneal cavity only by a single layer of macrophages or the occasional mast cell (Fig. 9).

With the exception of macrophages, the cells on the wound surface at 3 days bore a close resemblance to the cells of the deeper layers of the wound, and were similar to primitive mesenchymal cells. No polystyrene spheres were seen in either primitive mesenchymal cells or proliferating fibroblasts. No detached mesothelial cells were seen on the wound surface.

4 days

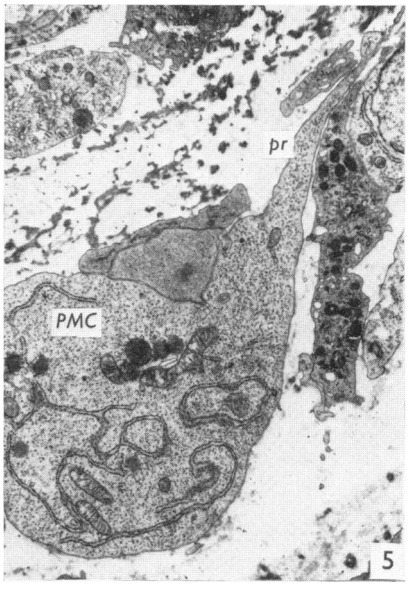
At this stage there was even greater variation on the wound surface. In areas where fibrin remained the superficial cells were clearly macrophages (Fig. 10). The cells immediately underlying the macrophages were often elongated, and varied in appearance. Some closely resembled primitive mesenchymal cells, while others resembled proliferating fibroblasts. Yet others possessed some characteristics common to both types of cell and appeared intermediate in form between the two (Fig. 10). In some areas of the wound surface primitive mesenchymal cells possessing an occasional microvillus and a few pinocytotic vesicles were seen (Fig. 11), while in other areas cells bore a superficial resemblance to the fibroblasts in the base of the wound (Fig. 12).

Fig. 5. 2 days. Parietal peritoneum. Note the large primitive mesenchymal cell (*PMC*) in the base of the wound. A long process (*pr*) leads off in the direction of the wound surface. *LC.* × 3000.

Fig. 6. 3 days. Parietal peritoneum. Part of the cytoplasm of a primitive mesenchymal cell (*PMC*) on the wound surface. Note the free ribosomes and lipid droplets (*L*). With the exception of the polymorph (*PMNL*) the cells below the surface resemble primitive mesenchymal cells. *LC.* × 4000.

Fig. 7. 3 days. Caecum. A long process (*pr*) of a primitive mesenchymal cell passing between two macrophages. Note the occasional microvillus (*mv*) arising from the surface of the process. *UA*+*LC.* × 3000.

Fig. 8. 3 days. Parietal peritoneum. Note the surface cell with a few microvilli (*mv*) arising from its superficial surface. It is probably a primitive mesenchymal cell. In contrast to the macrophage (*Ma*) in the depth of the wound, it contains no polystyrene spheres. *UA*+*LC.* × 2500.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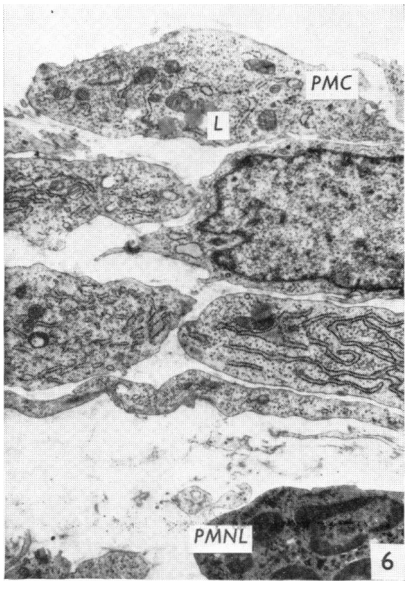
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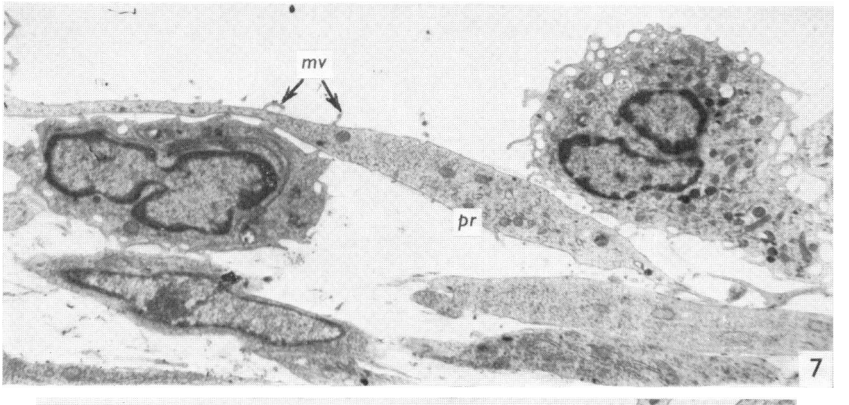
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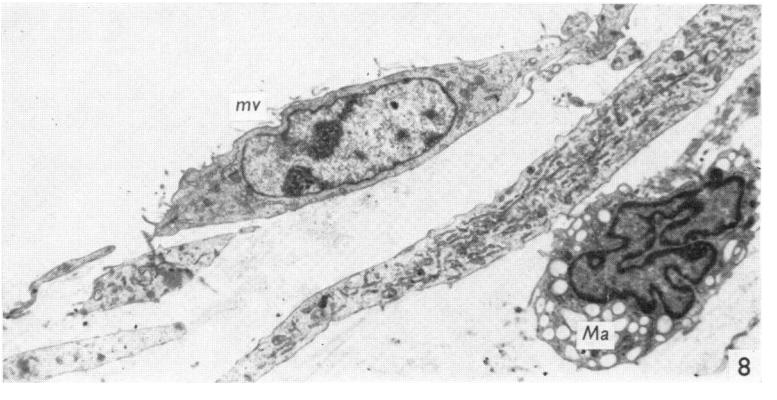
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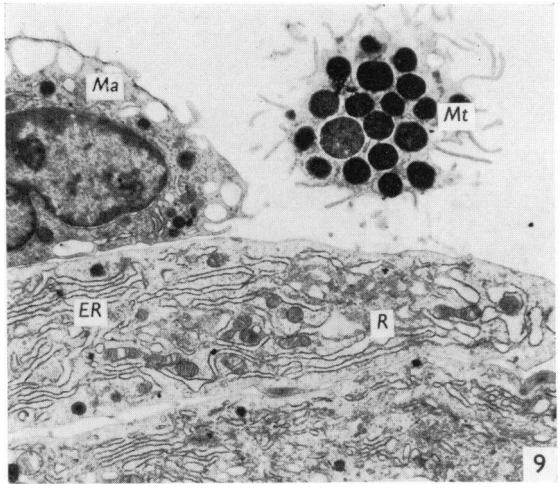


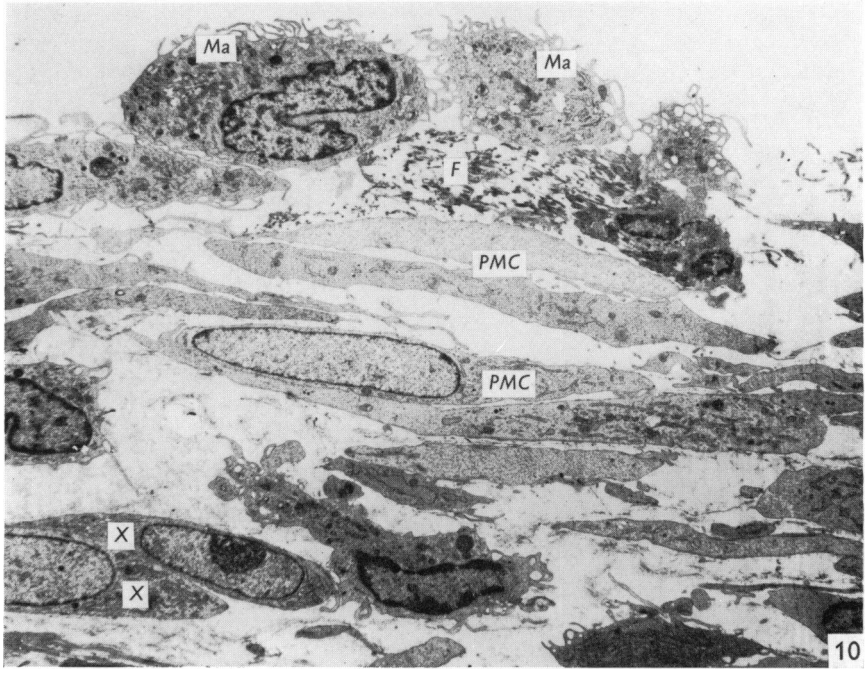
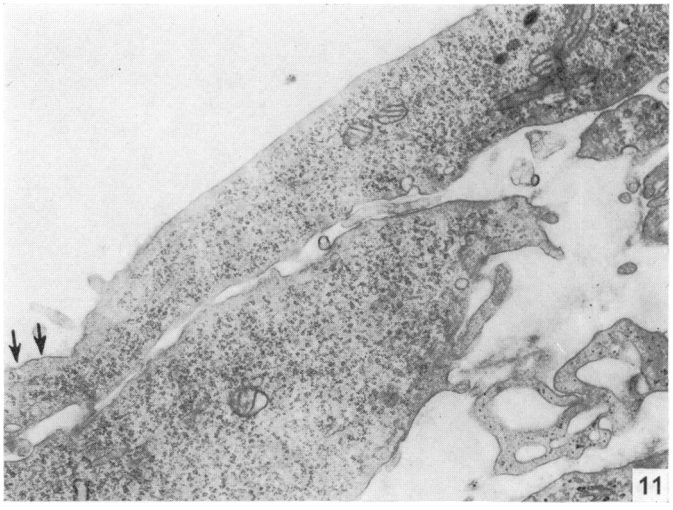


Fig. 9. 3 days. Parietal peritoneum. A macrophage (*Ma*) and a mast cell (*Mt*) rest on a proliferating fibroblast. Note that the latter contains much dilated rough-surfaced endoplasmic reticulum (*ER*) and aggregates of ribosomes (*R*). *LC*. × 5000.

Fig. 10. 4 days. Parietal peritoneum. Note the macrophages (*Ma*) and fibrin (*F*) on the wound surface. Many of the cells in the deeper part of the wound resemble primitive mesenchymal cells (*PMC*). Some cells (*X*) have characteristics common to primitive mesenchymal cells and proliferating fibroblasts and probably represent intermediate forms between the two. *UA*+*LC*. × 2500.



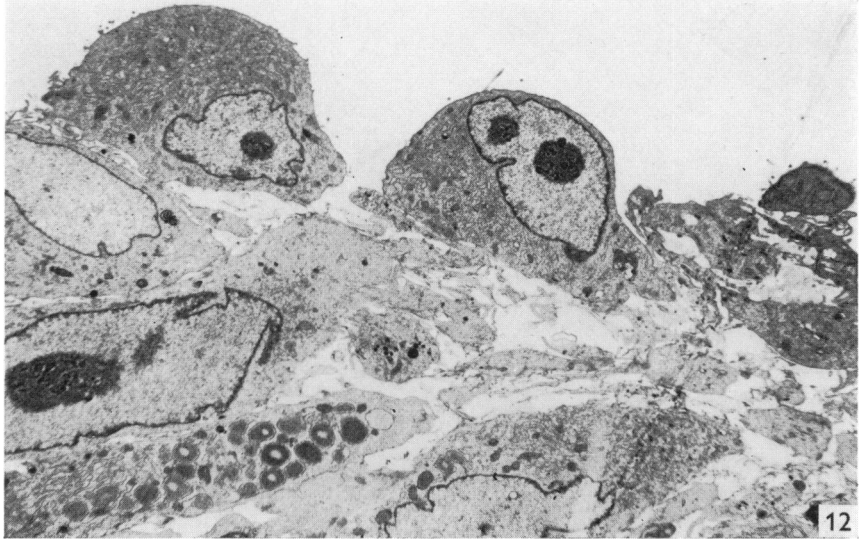
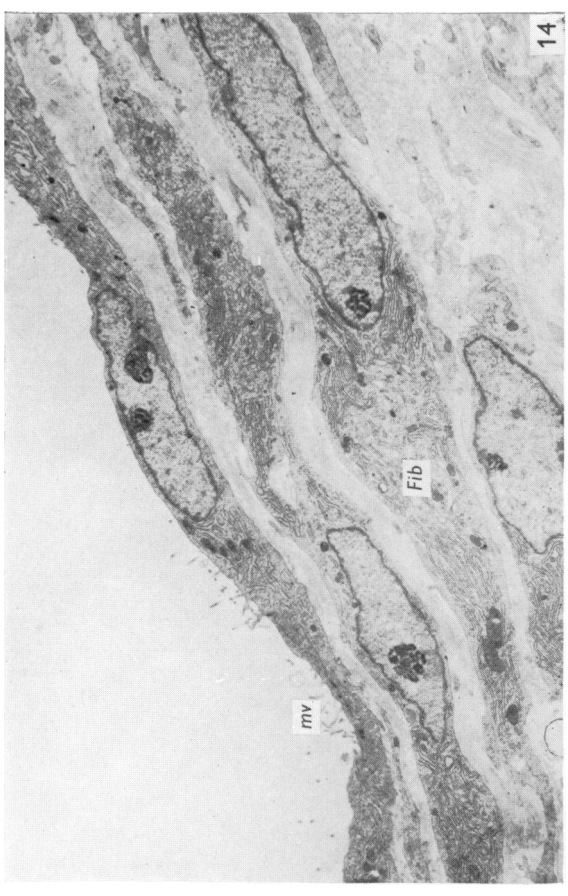


Fig. 11. 4 days. Parietal peritoneum. A primitive mesenchymal cell on the wound surface and one immediately below. Note that they contain mainly ribosomes. A few pinocytotic vesicles (arrows) are seen in relation to the superficial surface. *LC.* × 12500.

Fig. 12. 4 days. Parietal peritoneum. Two cells which have some characteristics common to both primitive mesenchymal cell and proliferating fibroblasts are seen on the wound surface. Note that they resemble the cells in the deeper parts of the wound in nuclear and cytoplasmic characteristics. *UA+LC.* × 2500.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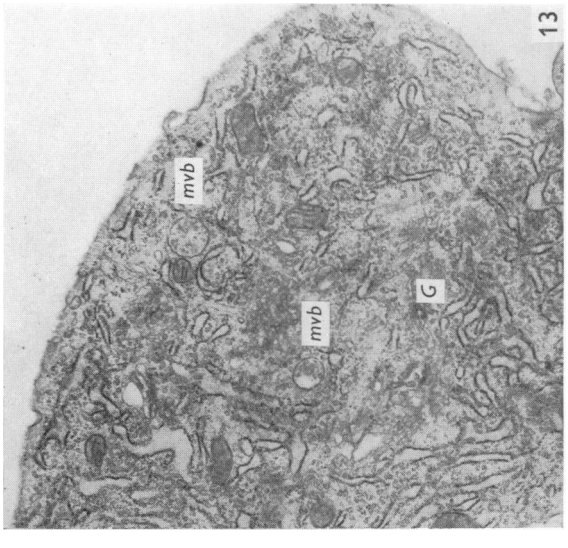
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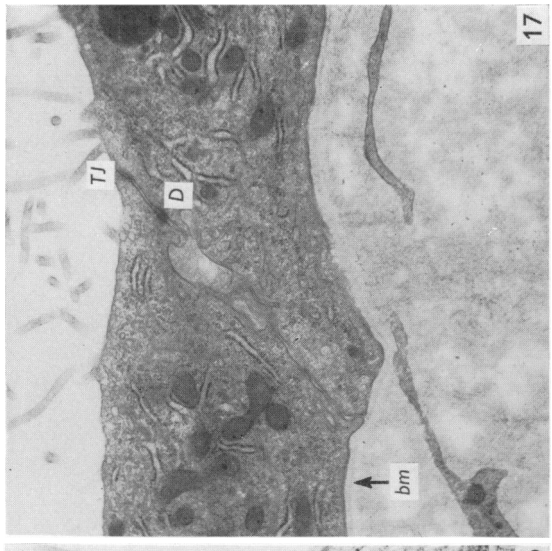
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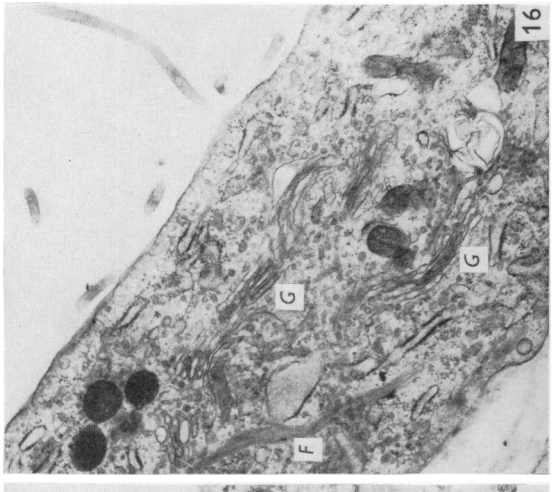
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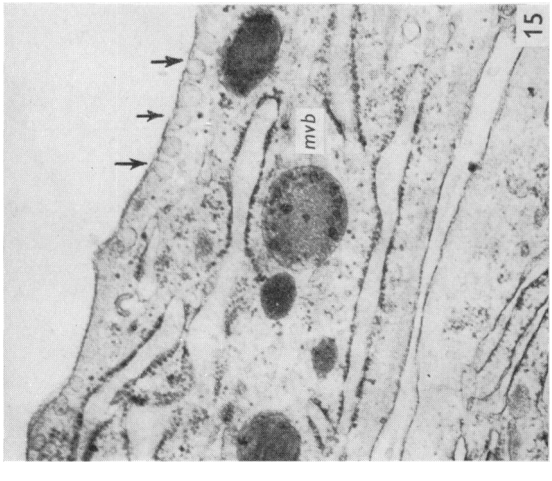
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However, high power examination of this latter group showed that they possessed numerous dilated cisternae of rough-surfaced endoplasmic reticulum, numerous aggregates of ribosomes, a prominent Golgi complex, a few multivesicular bodies (Fig. 13) and occasional pinocytotic vesicles, but only the rare microvillus and no basement membrane. These cells had many characteristics in common with both primitive mesenchymal cells and fibroblasts or mesothelial cells, and probably represent an intermediate form of cell. No junctional complexes were seen between the above cells at this stage, although cells resembling primitive mesenchymal cells or proliferating fibroblasts on the wound surface came into close contact with one another.

Polystyrene spheres were seen in macrophages only and never in primitive mesenchymal cells or fibroblasts. Occasional multinucleate cells were seen on the wound surface at this stage. In both nuclear and cytoplasmic characteristics they resembled fibroblasts.

5 days

In some areas healing appeared complete in that a single layer of mesothelial cells was seen on the wound surface (Fig. 14). With the exception of microvilli and junctional complexes, these resembled the underlying fibroblasts in both nuclear and several cytoplasmic characteristics. The mesothelial cells possessed several microvilli, numerous pinocytotic vesicles, several dilated cisternae of rough-surfaced endoplasmic reticulum, the occasional multivesicular body (Fig. 15), a prominent Golgi apparatus, and bundles of filamentous material (Fig. 16). Desmosomes and tight junctions were seen between adjacent cells. No basement membrane was seen beneath the mesothelial cells of parietal peritoneum or caecum at this stage, although one was often present beneath those covering the liver (Fig. 17).

In other areas healing was far less advanced, and primitive mesenchymal cells were seen both on the surface and in the base of the wound, although many of the cells in the base of the wound resembled fibroblasts (Fig. 18). The occasional microvillus arose from the free surface of the primitive mesenchymal cells.

Macrophages were occasionally seen in other areas of the wound surface. Polystyrene spheres were seen only in macrophages and never in primitive mesenchymal cells, fibroblasts or mesothelial cells.

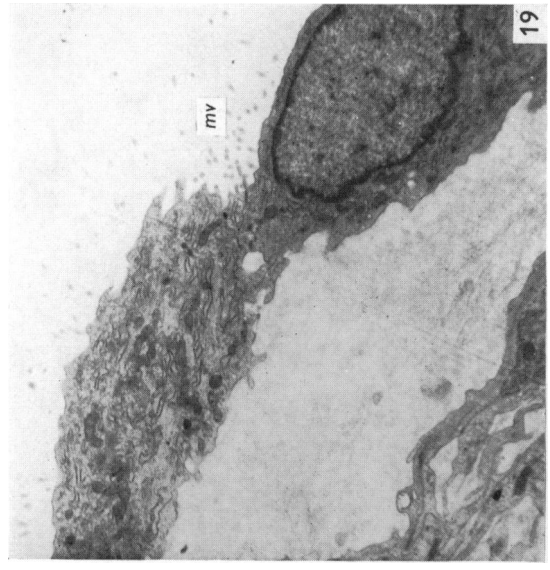
Fig. 13. 4 days. Parietal peritoneum. High power view of a cell similar to those shown in Fig. 12. Note the numerous dilated cisternae of rough-surfaced endoplasmic reticulum, the prominent Golgi complex (*G*), two multivesicular bodies (*mvb*) and numerous aggregates of ribosomes. This type of cell appears intermediate in form between primitive mesenchymal cell and proliferating fibroblast. *LC.* × 14000.

Fig. 14. 5 days. Parietal peritoneum. A single layer of mesothelial cells on the wound surface. Note the microvilli (*mv*) arising from the free surface. Note also the similarity between mesothelial cells and underlying fibroblasts (*Fib*). *LC.* × 3000.

Fig. 15. 5 days. Parietal peritoneum. Note the numerous pinocytotic vesicles (arrows), dilated cisternae of rough-surfaced endoplasmic reticulum, and multivesicular body (*mvb*). *LC.* × 40000.

Fig. 16. 5 days. Parietal peritoneum. Note the prominent Golgi complex (*G*) and bundle of filamentous material (*F*). Note also the absence of a basement membrane at this stage. *LC.* × 25000.

Fig. 17. 5 days. Liver. Note the basement membrane (*bm*) beneath mesothelial cells covering the liver wound. Note also the tight junction (*TJ*) and desmosome (*D*). *LC.* × 14000.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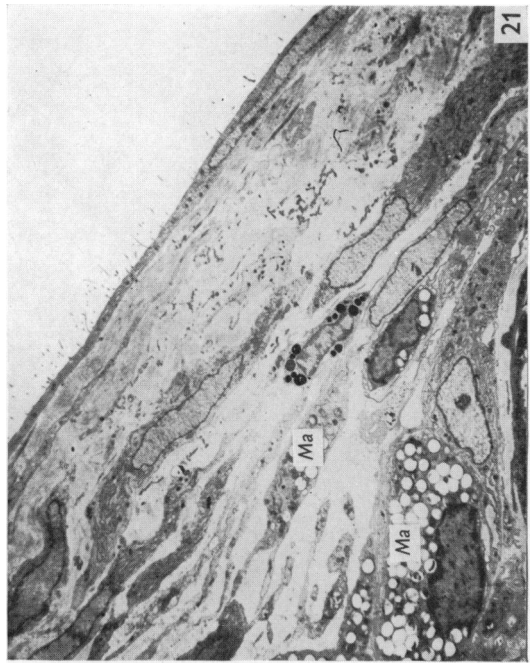
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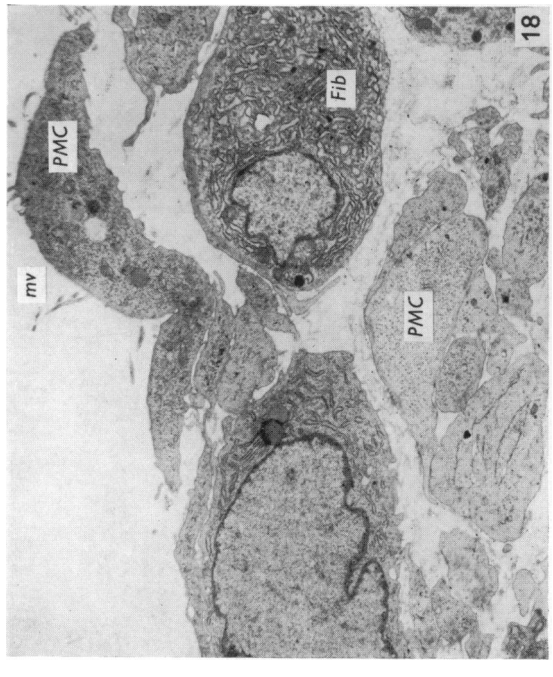
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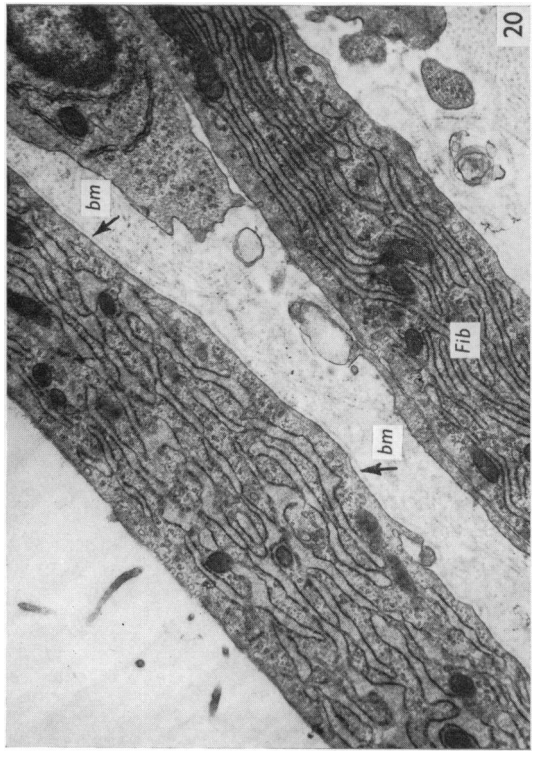
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6 days

Macrophages were only rarely seen on the wound surface. Most of the surface cells possessed the characteristics of mesothelial cells (Fig. 19), but no basement membrane was seen except in relation to areas of the liver.

7 days

The appearances resembled those at 6 days except that a discontinuous basement membrane was now seen beneath mesothelial cells lining the parietal peritoneum and covering the caecum.

8 days

A continuous layer of mesothelial cells was present on the wound surface. In nuclear and several cytoplasmic characteristics they resembled the underlying fibroblasts, except that the mesothelial cells possessed microvilli and junctional complexes. No continuous basement membrane was seen at this stage beneath the mesothelial cells of the parietal peritoneum and caecum.

10 days

A single layer of mesothelial cells resting on a continuous basement membrane was seen at this stage (Figs. 20, 21). Fibroblasts in the base of the wound were arranged with their long axes parallel to the wound surface, and bundles of collagen were present between the fibroblasts. A few macrophages containing polystyrene spheres remained in the base of the wound, but no spheres were seen in the mesothelial cells or fibroblasts (Fig. 21). A notable feature was the close similarity in nuclear and several cytoplasmic characteristics between mesothelial cells and subperitoneal fibroblasts (Fig. 20). Tight junctions were seen between mesothelial cells but desmosomes were rare at this stage.

12 days

The appearances were similar to those at 10 days.

Fig. 18. 5 days. Parietal peritoneum. Compare with Fig. 14. Healing is much less advanced. Note the primitive mesenchymal cells (*PMC*) both on the wound surface and in its base. Note also the fibroblast (*Fib*) in the base of the wound. *LC.* ×4000.

Fig. 19. 6 days. Parietal peritoneum. A single layer of mesothelial cells with numerous microvilli (*mv*). No basement membrane is present at this stage. *LC.* ×4000.

Fig. 20. 10 days. Parietal peritoneum. A single layer of mesothelial cells resting on a continuous basement membrane (*bm*). Note the similarity in cytoplasmic characteristics between mesothelial cell and subperitoneal fibroblast (*Fib*), especially the numerous cisternae of rough-surfaced endoplasmic reticulum. *LC.* ×10500.

Fig. 21. 10 days. Parietal peritoneum. A single layer of mesothelial cells on the wound surface. A few macrophages (*Ma*) containing polystyrene spheres remain in the base of the wound but none is seen in the mesothelial cells or fibroblasts. *LC.* ×2000.

14 days

The cytoplasm of the mesothelial cells was extremely thin in some areas. The cells rested on a continuous basement membrane. Some macrophages in the base of the wound contained polystyrene spheres but spheres were never seen in mesothelial cells or fibroblasts.

DISCUSSION

This study of peritoneal regeneration has revealed that on only two occasions were detached mesothelial cells seen on the wound surface in the early stages of healing; in both cases the sections examined were from wounds in the parietal peritoneum of the same animal at 2 days post-operatively. If mesothelial cells of the peritoneal fluid made any major contribution to peritoneal wound healing it seems likely that they would have been observed more frequently on the wound surface in the early stages of healing. It is concluded that the contribution made by detached mesothelial cells to peritoneal wound healing is negligible. This study therefore has not substantiated the claim of Cameron *et al.* (1957), Johnson & Whitting (1962) and Bridges & Whitting (1964) that mesothelial cells become detached from the intact peritoneum adjacent to the wound and implant on the wound surface as free grafts which proliferate and join together to form a new mesothelial layer. In a previous study (Raftery, 1973*b*) evidence was put forward to show that some of the detached mesothelial cells present in the peritoneal fluid of operated animals were injured or dying. Taking this evidence in conjunction with the findings of the present study it appears that mesothelial cells are detached at the time of operation, enter the peritoneal fluid, and probably undergo necrosis subsequently.

It has not been possible to confirm the theory of Eskeland (1966) and Eskeland & Kjaerheim (1966) that peritoneal macrophages become transformed into mesothelial cells either directly or via fibroblasts. The findings of Eskeland & Kjaerheim (1966) are not disputed on the point that macrophages, present on the wound surface in the early stages of healing, flatten out and come into close contact with one another; nor is it disputed that, in the later stages of healing, there are no reliable criteria for distinguishing many of the cells in the most superficial layer from those in the deeper parts of the wound: they all resemble proliferating fibroblasts. However, if macrophages gradually became transformed into fibroblasts and mesothelial cells it is likely that:

(1) Intermediate forms between the different types of cell would be seen at some stage.

(2) Following labelling of peritoneal macrophages with polystyrene spheres, some spheres would be seen in fibroblasts and mesothelial cells at some stage.

No cells intermediate in type between macrophage and fibroblast or mesothelial cell were observed in the present study. However, it is possible that if transformation of macrophage to fibroblast did occur, it could have been a rapid process which was not observed in static pictures of specimens obtained at intervals of 12 hours. This seems improbable, since if this occurred, the numerous sections examined would surely have revealed at some stage a macrophage in a process of transformation. Also at no time were polystyrene spheres seen in fibroblasts or mesothelial cells.

Further, there was no evidence to suggest that macrophages could have discharged their polystyrene spheres prior to becoming transformed into fibroblasts or mesothelial cells. It is concluded that mesothelial cells do not arise by transformation of peritoneal macrophages.

The present study has demonstrated that another type of cell, closely resembling a primitive mesenchymal cell, was present both on the surface of the wound and in its base in the early stages of healing. No mention was made of this type of cell by Eskeland & Kjaerheim (1966). At the 2 day stage only a few such cells were recognized on the wound surface, and those in the base of the wound were found in relation to blood vessels in the perivascular connective tissue. Long processes of these cells often extended towards the surface of the wound. As healing progressed these cells became more numerous both on the wound surface and in its base, but it became more difficult to distinguish between primitive mesenchymal cells and proliferating fibroblasts. Indeed, in both situations, many cells were seen which appeared intermediate in form between primitive mesenchymal cells on the one hand and proliferating fibroblasts or mesothelial cells on the other. By 5 days the majority of cells on the wound surface resembled the fibroblasts in the underlying wound, except that some surface cells possessed microvilli, tight junctions and desmosomes.

The problem arises as to whether it is justified, on the basis of this evidence, to conclude that the new mesothelium is derived from the subperitoneal fibroblast, which has many nuclear and cytoplasmic characteristics in common with the mesothelial cell. Or does the mesothelial cell arise directly from an undifferentiated primitive mesenchymal cell in the perivascular tissue adjacent to the wound? Such undifferentiated mesenchymal cells are considered to be present in the perivascular connective tissue of the adult animal, and to have the potentiality of becoming transformed into other types of cell (Maximow, 1927). Much of the available evidence at the present time favours the view that fibroblasts are derived from the cells in the perivascular connective tissue (MacDonald, 1959; Dunphy, 1963; Grillo, 1963; Hadfield, 1963; Ross & Odland, 1968; Ross, Everett & Tyler, 1970). What is not clear is whether these perivascular cells are differentiated but resting fibroblasts, or whether they are undifferentiated multipotential mesenchymal cells and the process represents an example of 'modulation' of resident mesenchymal cells as proposed by Weiss (1950). Static observations on the evolution of cells present difficulties in morphological identification. It is, however, tempting to suggest that the primitive mesenchymal cells identified on the wound surface in the early stages of healing differentiate into mesothelial cells, particularly since there is a striking similarity between the observations on mesothelial repair presented in this study and those made by Haar & Ackerman (1971) on the development of mesothelium from mesenchymal cells. Their ultrastructural studies on the mouse embryo showed that mesothelium was directly derived from mesenchymal cells which elongated, became flattened, acquired a basement membrane, and were united to similar adjacent cells by typical desmosomes. They possessed oval nuclei with an even distribution of chromatin, and a prominent nucleolus which gradually decreased in size. A few lipid droplets were present in the young mesothelial cells and the cytoplasm showed a progressive increase in the amount of rough-surfaced endoplasmic reticulum. Pinocytotic vesicles were seen in relation to both surfaces of the cell. However, it must

be concluded that the precise origin of the new mesothelium has not been conclusively demonstrated in the present study, mainly because of difficulty in distinguishing between primitive mesenchymal cells and proliferating fibroblasts in the later stages of healing. It is possible that the former give rise to the latter, but unequivocal proof of this is lacking. The following suggestions are put forward as possible explanations for the origin of the new mesothelium:

(1) It arises *directly* from primitive mesenchymal cells present in the perivascular connective tissue.

(2) It arises *indirectly* from primitive mesenchymal cells via fibroblasts.

(3) It arises from the subperitoneal fibroblasts, which in turn arise from differentiated, but resting, fibroblasts in the perivascular connective tissue.

The results of a previous study of the problem with the light microscope (Raftery, 1973*a*) suggested that the new mesothelium arose by metaplasia of subperitoneal fibroblasts. Correlation of the results obtained by light and electron microscopy indicated that many of the cells, which were designated fibroblasts on the basis of light microscopy, represented a spectrum of cells ranging from primitive mesenchymal cells to mature fibroblasts. In specimens stained with either haematoxylin or methylene blue–Azure II and examined by light microscopy, similar nuclear characteristics, namely a thin rim of peripheral chromatin, one or more prominent nucleoli, and pronounced cytoplasmic basophilia, were common to both primitive mesenchymal cell and fibroblast.

The visceral peritoneum appears to differ little in its healing properties from the parietal peritoneum. Light microscopy (Raftery, 1973*a*) indicated that the liver acquired a new mesothelial covering one day earlier than either caecum or parietal peritoneum. In the present ultrastructural study a discontinuous basement membrane was seen beneath many mesothelial cells covering the liver at 5 days. A discontinuous basement membrane was never seen beneath the mesothelial cells of the parietal peritoneum or caecum until 7 days. The only explanation that can be put forward to explain the earlier appearance of a basement membrane in the case of the liver is that the latter provides a firmer substrate for development of a new mesothelium than either the parietes or the caecum, both of which are more subject to distension.

The present study lends no support to the theories that the new mesothelium arises from transformation of peritoneal macrophages, or that it arises by seeding of mesothelial cells from adjacent peritoneal surfaces. It has shown that the new mesothelium arises from the subperitoneal connective tissue cells, and has thus confirmed the light microscopical studies of Williams (1955), Ellis, Harrison & Hugh (1965) and Hubbard *et al.* (1967). It has not been possible to determine whether the new mesothelium arises from primitive mesenchymal cells or fibroblasts.

SUMMARY

The healing of wounds in parietal and visceral peritoneum has been studied by electron microscopy. During the early stages of healing, macrophages were the predominant type of cell on the wound surface, but cells which resembled primitive mesenchymal cells were occasionally seen. Gradually primitive mesenchymal cells and cells resembling proliferating fibroblasts became more numerous on the wound

surface, while the number of macrophages decreased. By 8 days after operation a continuous layer of mesothelial cells was present on the wound surface. In nuclear and cytoplasmic characteristics they resembled the underlying fibroblasts, except that the mesothelial cells possessed microvilli and junctional complexes. No evidence was obtained to support the theories that the new mesothelium arises from transformation of peritoneal macrophages, or that it arises by seeding of mesothelial cells from adjacent peritoneal surfaces. The new mesothelium arises from subperitoneal connective tissue cells, but it has not been possible to determine conclusively whether these are primitive mesenchymal cells or fibroblasts.

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