REGENERATION OF PARIETAL AND VISCERAL PERITONEUM IN THE IMMATURE ANIMAL: A LIGHT AND ELECTRON MICROSCOPICAL STUDY

BY A. T. RAFTERY

DEPARTMENT OF ANATOMY, SCHOOL OF MEDICINE, UNIVERSITY OF LEEDS

SUMMARY

1. The healing of visceral and parietal peritoneum has been investigated in the immature animal by both light and electron microscopy.

2. Healing has been found to occur more rapidly in the immature animal when compared with the adult animal. Parietal peritoneum was covered by a new mesothelium in 7 days, while the liver was covered in 5 days.

3. The new mesothelium developed from subperitoneal perivascular connective-tissue cells but it was not possible to determine whether these were primitive mesenchymal cells or fibroblasts.

4. The findings are discussed in relation to the pathogenesis of peritoneal adhesions.

The healing of peritoneum in the immature animal is of interest in view of the fact that intestinal obstruction due to peritoneal adhesions is more common following abdominal surgery in early life (Devens, 1963; Replogle, Johnson, and Gross, 1966), especially in the neonatal period. The greater incidence and severity of adhesions in the infant compared with the adult may reflect a difference in the nature of healing of peritoneum. Ellis, Harrison, and Hugh (1965) have shown that peritoneal regeneration occurred more rapidly in the immature rat than in mature ones, but flattening of the surface layer of cells, as seen in paraffin sections cut perpendicular to the wound surface, was used as a criterion for assessing complete mesothelial regeneration. This has been shown to be an inadequate method of assessment and the need for Hülten preparations in which the surface cells are viewed en face has been stressed (Eskeland, 1966; Raftery, 1973a). The present investigation was undertaken to determine, by both light and electron microscopy, the nature of peritoneal regeneration in the immature animal and to compare the findings with those for the adult animal (Raftery, 1973a, c).

MATERIALS AND METHODS

A total of 92 male Sprague-Dawley rats weighing 50-60 g was used in this investigation. The rats were anaesthetized with ether and the abdominal cavity was opened through a midline incision 3 cm long. The operative technique was clean but not strictly aseptic. Wounds approximately 1 mm deep and either 0.5 x 0.5 cm or 1 x 1 cm were made in the liver capsule by a tangential cut with a scalpel blade. A wound was also made on each side of the midline in the parietal peritoneum, that on the left side being 2 x 2 cm and that on the right side 1 x 1 cm. The peritoneum, together with the underlying layer of muscle, was removed within this area. The abdominal incision was closed in two layers, catgut sutures being placed through the musculoperitoneal layer and silk sutures through the skin. Animals were killed by exsanguination under ether anaesthesia at 12 hours, 24 hours, and at daily intervals up to 8 days after operation. At least 3 animals were examined at each stage.

The liver was removed, rinsed briefly in 5 per cent glucose, immersed in 0.25 per cent silver nitrate for 30 seconds, rinsed again in 5 per cent glucose, and then fixed in 10 per cent formaldehyde for 8-12 hours. The wounds in the parietal peritoneum were removed together with a wide margin of adjacent peritoneum, pinned flat on cork, and treated in the same manner as the liver. Hülten preparations of the wounds were subsequently made as described by Eskeland (1966) and stained with haematoxylin. In other animals the wounds were excised, fixed in formaldehyde, or Bouin's fluid, dehydrated, and embedded in paraffin wax. Serial sections were cut perpendicular to the wound surface at 6 μm. These sections were then fixed with uranyl acetate (Stempack and Ward, 1964) or double stained with uranyl acetate and lead citrate, prior to examination in an AEI type EM6B electron microscope.

In order to label the peritoneal macrophages some animals were given an intraperitoneal injection of 1 ml of a 0.2 per cent suspension of polystyrene spheres (0.79 μm diameter Dow-Latex; Serva, Feinbiochemica, Heidelberg) in 0.9 per cent saline at the time of operation. Specimens from these animals were processed for Hülten preparations in order to check if the inflammatory reaction caused by the polystyrene spheres had any effect on the nature of healing. Some specimens were treated as described above. Some normal animals were given an intraperitoneal injection of 1 ml of a 0.2 per cent suspension of polystyrene spheres in
order to check that normal mesothelial cells and subperitoneal fibroblasts did not ingest polystyrene spheres. Specimens of parietal and visceral peritoneum from these animals were fixed in 2.5 per cent glutaraldehyde, processed as described above, and examined by electron microscopy.

RESULTS

1. Macroscopic.—Adhesions to the wounds were found in 16 animals and in all but one of these the liver was adherent to part of the wound in the parietal peritoneum. The one exception was an adhesion between the omentum and parietal peritoneum.

2. Light Microscopy.—

Control Animals.—Following intraperitoneal injection of polystyrene spheres into normal animals none was seen in mesothelial cells or subperitoneal fibroblasts.

Intraperitoneal injection of polystyrene spheres did not have any discernible effect on the time or nature of peritoneal healing.

a. Parietal Peritoneum.—The cellular changes which occurred on the wound surface, in its base, and

During the first 24 hours the wounds were uneven and haemorrhagic, but by 2 days they were paler, and by 3 days they were usually smooth and glistening. At 5 days it was difficult to distinguish the wound from the normal surrounding parietal peritoneum. The wounds in the liver capsule appeared as greyish-white puckered areas and were easily visible at 8 days.

in the adjacent peritoneum were similar to those reported for the adult animal (Raftery, 1973a) but occurred more rapidly in the immature animal. Thus, by 2 days after operation the initial acute inflammatory response had subsided and two well-defined types of cell were seen on the wound surface (Fig. 1): one was a small round cell with a dark staining,
eccentric, kidney-shaped nucleus which was identified as a cell of the monocyte/macrophage type, and the other was a large cell with an ill-defined boundary, having a large, round, or oval nucleus, containing one or more prominent nucleoli and with pronounced cytoplasmic basophilia. The latter resembled fibroblasts. The above changes were not seen until 3 days in the adult animal. In sections cut perpendicular to the wound surface marked fibroblastic proliferation was seen in the base of the wound at 2 days (Fig. 2), and only occasional macrophages were seen. The number of monocyte-like cells on the wound surface gradually decreased and at 5 days only the fibroblast-like cells remained. These cells came into contact with one another as shown by complete silver lines in Hautchen preparations, and reconstituted the new mesothelium. Healing was completed by 7 days (Fig. 3) compared with 8 days in the adult animal.

Fig. 5.—Parietal peritoneum at 2 days. Macrophages (Ma) containing polystyrene spheres rest on a fibrin base. A process (pr) of a primitive mesenchymal cell extends towards the wound surface. Uranyl acetate (UA) + lead citrate (LC). (x 2000.)

Fig. 6.—Parietal peritoneum at 2 days. Primitive mesenchymal cells (PMC) are seen on the wound surface and in the base of the wound. A process (pr) of a primitive mesenchymal cell and part of a macrophage (Ma) containing a polystyrene sphere are also seen. LC. (x 3000.)

Fig. 7.—Parietal peritoneum at 2 days. Numerous macrophages and primitive mesenchymal cells (PMC) are seen in the base of the wound. Some cells (X) have some characteristics common to both primitive mesenchymal cells and fibroblasts and probably represent intermediate forms between the two. Polystyrene spheres are seen only in macrophages. UA + LC. (x 2000.)

Fig. 8.—Parietal peritoneum at 2 days. A long process of a primitive mesenchymal cell extends along the wound surface. LC. (x 7500.)
Throughout the course of healing polystyrene spheres were seen only in cells of the monocyte/macrophage type (Fig. 4) and never in fibroblasts or mesothelial cells. There was no difference in the time or nature of healing between small and large wounds.

b. Liver.—Healing of wounds in the liver capsule closely resembled the healing of parietal peritoneum for the most part. Again healing of the wound occurred more rapidly than in the adult animal and a complete layer of mesothelium had covered the wound by 5 days compared with 7 days in the adult animal. There was no difference in the time or nature of healing between small and large wounds.

3. Electron Microscopy.—The findings for parietal peritoneum and liver will be described together and where any variation occurred this will be pointed out at the relevant stage.

12 Hours.—Numerous cells were seen entangled within fibrin strands. Polymorphs predominated but macrophages and a few eosinophils and mast cells were also seen. Polystyrene spheres were seen in polymorphs and macrophages. No detached mesothelial cells were seen on the wound surface.

24 Hours.—The majority of cells on the wound surface were macrophages resting on a fibrin base.

No cells which could be identified as detached mesothelial cells were seen on the wound surface.

2 Days.—At this stage there were marked changes, both on the wound surface and in its base. In some areas the wound surface was covered by a layer of macrophages resting on a fibrin base (Fig. 5), while...
in others, primitive mesenchymal cells were seen (Fig. 6). Even in areas where macrophages pre-
dominated on the wound surface, numerous primitive
mesenchymal cells were seen in the base of the wound
(Fig. 7). Many of the latter possessed long processes
which extended towards the wound surface (Fig. 5).
These processes contained chiefly aggregates of
ribosomes and few other organelles. In areas where
primitive mesenchymal cells were present on the
wound surface long processes often extended from
them along the wound surface (Fig. 8). There was a
close similarity in nuclear and cytoplasmic character-
istics between primitive mesenchymal cells on the
wound surface (Fig. 8) and perivascular connective-
tissue cells in the base of the wound (Fig. 9). No
detached mesothelial cells could be identified on the
wound surface at this stage. Polystyrene spheres
were seen only in macrophages and never in primitive
mesenchymal cells.

3 Days (Fig. 10).—At this stage very few macro-
phages remained on the wound surface. The majority
of cells seen on the wound surface resembled the
proliferating fibroblasts or primitive mesenchymal
cells in the base of the wound in nuclear and cyto-
plasmic characteristics except that adjacent cells
were often joined by tight junctions. In many cases
it was not possible to draw a sharp distinction
between primitive mesenchymal cells and fibroblasts
and many cells appeared intermediate in form
between the two. Small amounts of collagen were
now seen in the base of the wound. Macrophages
were the only cells to contain polystyrene spheres.

4 Days.—The appearances were similar to those at
3 days.

5 Days.—At this stage there were differences
between the wounds in the parietal peritoneum and
the liver. The cells on the surface of the wounds in
the parietal peritoneum were of uniform appearance
and, apart from microvilli and tight junctions,
closely resembled proliferating fibroblasts in the base
of the wound in both nuclear and cytoplasmic
characteristics (Fig. 11). However, tight junctions
were not always seen between cells on the wound
surface. In the case of the liver there was a con-
tinuous layer of mesothelial cells on the wound
surface and either tight junctions or desmosomes
were seen between adjacent cells. A basement
membrane was seen beneath some mesothelial cells
covering the liver at this stage but frequent breaks
were observed in it. No basement membrane was
observed beneath mesothelial cells covering the
wound in the parietal peritoneum.

6 Days.—The appearances at 6 days did not differ
from those at 5 days in the case of both the liver and
the parietal peritoneum.

7 Days (Fig. 12).—A continuous layer of mesothelial
cells was seen on the surface of both the liver and the
parietal peritoneum. A basement membrane was
seen beneath the mesothelial cells in most areas but
gaps in it were still visible. Dense bundles of collagen
were seen in the base of the wound together with
fibroblasts and macrophages. No polystyrene spheres
were seen in mesothelial cells although some were
still present in macrophages.

8 Days.—The appearances were similar to those at
7 days except that a continuous basement membrane
was now seen beneath the mesothelial cells.

**DISCUSSION**

This study of peritoneal wound healing in the immature animal has in part confirmed the findings
of Ellis and others (1965) that peritoneal healing is
more rapid in the immature animal. The earlier
authors noted a flattened layer of cells on the wound
surface at 3 days as well as numerous elongated
fibroblasts in the base of the wound. The present
study has shown that, while cells on the surface of
the wound in the parietal peritoneum were mainly
flattened by 3 days, complete mesothelial regeneration
did not occur until 7 days, as shown by Häutchen
preparations, compared with 8 days in the adult
animal. In the case of the visceral peritoneum covering
the liver healing was complete by 5 days,
compared with 7 days in the adult animal (Raftery,
1973a).

Previous investigations have led to the conclusions that the new mesothelium arises by three possible
mechanisms:—

1. Transformation of peritoneal macrophages.
2. Metaplasia of subperitoneal connective-tissue
cells.
3. Seeding of mesothelial cells from the adjacent
normal peritoneum.

It has been stated (Raftery, 1973a, c) that there is
no evidence to support the theory that seeding of
mesothelial cells from adjacent surfaces makes
a significant contribution to peritoneal healing,
although a few mesothelial cells are present in the
peritoneal fluid of operated animals (Raftery, 1973b).
Also no evidence was found to support the theory
that peritoneal macrophages are transformed into
mesothelial cells either directly or via fibroblasts. It
was concluded that the new mesothelium arose from
subperitoneal perivascular connective-tissue cells but it
was not possible to determine whether these were
primitive mesenchymal cells or fibroblasts (Raftery,
1973c). Undifferentiated primitive mesenchymal
cells are considered to be present in the perivascular
connective tissue and to have the potentiality of
becoming transformed into other types of cells
(Maximow, 1927).

The ultrastructural study of peritoneal regeneration
in the immature animal did not provide any evidence
to support the theory that macrophages were trans-
formed into fibroblasts or mesothelial cells. If the
latter were true it is likely that at some stage polystyrene
spheres would be seen in fibroblasts and
mesothelial cells. At a time when macrophages were
filled with polystyrene spheres none was seen in
fibroblasts or mesothelial cells. It is concluded that
in the immature animal, as in the adult animal, meso-
thehial cells do not arise by transformation of peri-
toneal macrophages. No detached mesothelial cells
were seen on the wound surface in the early stages
of healing and it is therefore concluded that the new
mesothelium does not arise by seeding of mesothelial
cells from the adjacent peritoneum. The results of
the light microscopical study suggested that the new
mesothelium arose by metaplasia of subperitoneal
fibroblasts. However, correlation of the results
obtained by light and electron microscopy indicated
that many of the cells which were designated as
fibroblasts on the basis of light microscopy repre-
sented a spectrum of cells ranging from primitive
mesenchymal cells to mature fibroblasts. The results
suggest that the new mesothelium arises in the immature animal in the same manner as it does in the adult animal, namely from the subperitoneal perivascular connective-tissue cells, but it has not been possible to determine whether these are primitive mesenchymal cells or fibroblasts. Proliferation of primitive mesenchymal cells and the appearance of fibroblasts and collagen occur more rapidly in the immature animal. It is suggested that because proliferation of primitive mesenchymal cells and the appearance of fibroblasts occur more rapidly mesothelial regeneration is correspondingly more rapid.

The healing of the visceral peritoneum covering the liver is faster than the healing of the peritoneum of the abdominal wall. This finding is similar to that in the adult animal. The most likely explanation for this finding is that the liver provides a fairly firm substrate for healing while the peritoneum of the anterior abdominal wall is subject to distension.

Adhesions occurred more commonly in the immature animal than in the adult animal. Thus, while in adult animals adhesions were seen in only 6 of 271 wounds and these adhesions between the liver and the midline laparotomy wound (Raftery, 1973a), in the immature ones adhesions occurred in 16 out of 92 animals and involved adherence between the liver wound and part of the wound in the parietal peritoneum. It has been reported (Howes and Harvey, 1932) that fibroplasia occurs earlier in the immature animal, and in the present study a more rapid proliferation of fibroblasts and appearance of collagen have been observed compared with the adult animal. This may have a bearing on the greater incidence of adhesions in the immature animal. The wounds made in the immature animal were the same size as those made in the adult animal (Raftery, 1973a) and it is possible that the relatively large size of the wound in the immature animals resulted in ischaemia in the base of the wound and adhesions arose as the result of ischaemia, as suggested by Ellis (1962).

How can the concept of mesothelial regeneration from subperitoneal perivascular connective-tissue cells be related to the pathogenesis of peritoneal adhesions? Ellis (1971) stated in his review of the causes of peritoneal adhesions that it has been accepted in the past that absorption of a fibrinous exudate depended on the presence of an intact mesothelium. If this remained intact, it was argued, fibrin disappeared. If it was destroyed persistent fibrous adhesions would develop. It has been shown (Robbins, Brunschwig, and Foote, 1949; Trimpi and Bacon, 1952; Williams, 1955; Ellis, 1962), however, that large peritoneal defects would heal without adhesion formation in the majority of cases, but when an attempt was made to appose the edges of the wounds by sutures, adhesions were formed to the wound (Thomas and Rhoads, 1950; Ellis, 1962; Hubbard, Khan, Carag, Albites, and Hricko, 1967). On the basis of his studies Ellis (1962) concluded that it was not the peritoneal defect itself that was a stimulus to adhesion formation but the presence of ischaemic tissue which probably resulted from pulling the edges of the wound under tension with sutures. Hartwell (1955) wrote:—

‘the characteristics of serosal cells being what they are, it is only logical to believe that they prevent adhesions by combining their fibrinolytic power with their epithelial-like function of extending themselves as a solid sheet of cells to cover any raw surface. This gives them the ability to come between any two apposed musculo-fibrous surfaces which are stuck together only by fibrin, which has not been organised by the growth of granulation tissue or laying down of collagen. Adhesions are composed of collagen fibres. If fibroplasia occurs between two surfaces before motion or before serosal cells grow in to separate them a permanent adhesion will be formed. If serosal covering of one or both of the apposed denuded surfaces be rapid and permanent no adhesions will form.’

At the time that this statement was written there was no evidence to support the claim that serosal cells had fibrinolytic power, but recently Myhre-Jensen, Larsen, and Astrup (1969) have demonstrated fibrinolytic activity in peritoneal mesothelium. Also, the statement regarding the epithelial-like function of serosal cells in extending themselves as a solid sheet to cover any raw surface is erroneous (Ellis and others, 1965; Eskeland, 1966; Raftery, 1973a). Ellis (1962) attributed adhesion formation to ischaemia but does not appear to have considered closely the effect of ischaemia on the mesothelial cells. They would probably undergo necrosis, but firm evidence on this point is lacking. A possible relationship exists between the theory of Ellis and the findings of the present investigation showing that mesothelial cells arise from perivascular cells, be they mesenchymal cells or fibroblasts. Ischaemia could result from either inadequate proliferation of vessels in the base of the wound or, if adequate proliferation of vessels did occur, an inadequate blood-flow in these vessels. In either case it is conceivable that the perivascular connective-tissue cells may not proliferate owing to ischaemia, and mesothelial cells would not appear, or appear more slowly, in the area of ischaemia. The absence of definitive mesothelial cells, with associated fibrinolytic activity, could lead to adhesion formation due to fibroplasia before definitive mesothelial cells grow between the two apposed surfaces. While this is largely conjectural it would suggest that an investigation of the fibrinolytic activity of regenerating mesothelium is required.

The present study has shown that regeneration of peritoneum occurs more rapidly in the immature animal. It has shown also that the new mesothelium arises from subperitoneal connective-tissue cells, but it has not been possible to determine whether these are primitive mesenchymal cells or fibroblasts.

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REFERENCES


PINWORM INFESTATION OF THE APPENDIX

BY P. B. BOULOS* AND A. G. A. COWIE

UNIVERSITY COLLEGE HOSPITAL, LONDON

SUMMARY

Eight cases of pinworm infestation of the appendix presenting with symptoms simulating acute appendicitis are reported from a study of 293 appendicectomy operations performed at University College Hospital, London, in the past 2 years.

In each case a diagnosis of appendicitis was made on clinical grounds but in 6 patients the appendix was normal histologically.

Symptoms mimicking appendicitis but caused by *Enterobius vermicularis* infestation merit recognition as a disease process and treatment to eradicate the worm must not be overlooked.

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The vermiform appendix is an anatomical blind loop of the gastro-intestinal tract within which it is possible for pathogenic organisms to become established, later to manifest their presence in disease. Among the pathogens which may behave in this way are schistosome ova (Turner, 1909; Abdel Biguet, and Capron, 1958; Richmond and Guthrie, *Histolytica* of the gastro-intestinal tract within which it is normal histologically. The vermiform appendix is an anatomical blind loop on clinical grounds but in 6 patients the appendix was possible for pathogenic organisms to become established, later to manifest their presence in disease. Among the pathogens which may behave in this way are schistosome ova (Turner, 1909; Abdel Biguet, and Capron, 1958; Richmond and Guthrie, *Histolytica* of the gastro-intestinal tract within which it is normal histologically.

Eggs transferred from an infected patient on the fingers or on towels and linen are ingested and hatch in the gastro-intestinal tract. Mature pinworms inhabit the caecum and appendix and the male worms perish in the bowel. Gravid females, however, migrate to the anus and appear on the perianal skin. Where they die liberating their eggs. This process, especially at night, causes intolerable itching and inevitably in time contamination of fingers and fomites. Usually infestation of one member of a family will result in the infestation of the remainder.

Appendicitis caused by obstruction of the appendix by a bolus of worms is recognized, but symptoms simulating appendicitis in the absence of obstruction or secondary infection have not often been reported.

MATERIALS AND METHODS

Surgical specimens removed at operation for appendicitis performed either as an emergency procedure or electively after a mass had settled were studied. Appendices removed in the course of other surgical procedures were excluded. The specimens were fixed by immersion in formol-saline and set in paraffin wax. Microtome sections were cut and stained with haematoxylin and eosin. In the 2 years of this study 293 appendicectomies were performed.

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RESULTS

Two hundred and seventeen specimens showed the histological changes of acute bacterial inflammation, 68 specimens were normal, and 8 specimens contained pinworms. Six of the 8 specimens containing pinworms showed no evidence of inflammation despite the presence of the worms and the preoperative clinical picture of appendicitis. Even if the worms lie within the mucosa there may be no active inflammation. (Fig. 1).

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DISCUSSION

Symptoms which closely mimic those of appendicitis may be found in association with pinworm infestation (Gross, 1953). Nausea and vomiting, abdominal pain, and tenderness in the right iliac fossa may cause the clinician to decide that appendicectomy is the proper course of action and in a proportion of such patients the appendix will contain pinworms.

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* Present address: Department of Surgery, Faculty of Medicine, Khartoum, Sudan.