INTRODUCTION

Saint in 1929 documented the experimental studies that had been carried out up to that date on the healing of oesophageal wounds. The reported observations dealt with the relatively long-term results, and little attention was paid to the early changes that occurred during the period immediately following the infliction of the wound. Recently, Malm (1951) made observations following surgical experiments on the oesophagus in dogs, but again no reference was made to the early changes in epithelium or connective tissue. The most recent experimental work on oesophageal healing appears to be that of Picard, Henry, Cotte, & Inglesakis (1956), but their interest lay in the repair of muscular tissue.

The present investigation was designed to study the behaviour of epithelium and connective tissue in the cat's oesophagus, following the removal of small areas of mucous membrane. Routine histological methods were supplemented by a series of histochemical studies on both the normal and regenerating tissues.

MATERIALS AND METHODS

All operations were carried out on healthy adult cats, a total of 31 animals being used.

Operative procedure

Under anaesthesia with intraperitoneal nembutal, the lower end of the oesophagus (i.e. the abdominal part) was approached through an upper abdominal midline incision. The oesophagus was opened by a longitudinal incision in its ventral wall and a piece of mucous membrane about 0.5 cm.² in size was removed. The oesophageal wound was closed by a single continuous catgut suture which did not incorporate the mucosa, and the abdomen closed in layers. Following operation the animals were allowed to survive for periods ranging from 6 hours to 1 year. During the immediate post-operative period, the animals

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were fed with water alone on the first day and milk alone on the second and third days; normal feeding was then resumed.

**Histological and histochemical techniques**

After death by coal-gas poisoning, the oesophagus was opened and the site of the lesion was excised, pinned out on cork, and fixed either in ice-cold 80 per cent. alcohol or in ice-cold Carnoy's fluid. The tissue was embedded in paraffin wax and serial sections were cut at a thickness of 8 μ; every 20th was mounted and stained with haematoxylin and eosin. These sections were then examined and from regions of particular interest serial sections were mounted for histochemical studies. For the demonstration of alkaline phosphatase the Gomori cobalt sulphide technique was employed, the substrate being sodium-β-glycerophosphate, and the incubation periods ranging between 15 minutes and 24 hours. The findings with this technique were confirmed with the azo dye method. The periodic acid-Schiff (PAS) technique was used for the demonstration of polysaccharides, glycogen being identified by comparison of sections treated or untreated with saliva. The presence of ribonucleic acid (RNA) was demonstrated by the methyl green pyronin technique (Kurnick, 1955), and metachromasia by the use of toluidine blue (0·5 per cent. for 30 minutes) or of azure A (0·1 per cent. for 10 minutes).

**RESULTS**

*The normal oesophageal mucosa*

The epithelium in the lower end of the cat's oesophagus is of the stratified squamous variety in which there is no keratinization of the surface cells. Sections stained with haematoxylin and eosin (Plate 1, fig. A) indicate that this epithelium consists of three zones of cells, viz. basal, intermediate, and superficial. The basal zone consists of 2 to 3 rows of small polygonal cells whose nuclei stain densely. The intermediate zone varies considerably in thickness, consisting of many layers of cells where the epithelium protrudes into the lamina propria, and is formed by large polyhedral cells with faintly staining cytoplasm and nuclei. The superficial zone comprises several layers of flattened cells whose nuclei appear pyknotic. This differentiation of the epithelium into three zones can be further demonstrated following histochemical procedures. The reaction for alkaline phosphatase, using the Gomori technique with short incubation periods of 15 to 30 minutes, was confined to the superficial and basal zones, the intermediate zone being invariably negative (Plate 1, fig. B). This appearance was similar with the azo dye method (Plate 1, fig. C), from which the impression was formed that the reaction was confined to the cell-membrane, or intercellular substance, particularly in the basal zone. Staining with toluidine blue again emphasized the division into the three zones by the fact that they stained with different intensities (Plate 1, fig. D). In addition to demonstrating the zoning of the epithelium, toluidine blue (and azure A) revealed the presence of a large number of mast
cells. These were found not only in the lamina propria, where they were usually associated with blood-vessels, but also in the basal zone of the epithelium (Plate 2, fig. G). In no specimen were any mast cells seen in the intermediate or superficial zones. They varied considerably in size and in the extent to which their cytoplasm was filled with metachromatic granules. From the study of the present series it appeared that the number and position of the mast cells in the normal epithelium of the lower part of the oesophagus varied little from one animal to another.

Sections treated by the PAS technique demonstrated that the normal epithelium did not contain any histochemically detectable glycogen. PAS-positive material other than glycogen was concentrated in the superficial zone, and appeared to be intercellular (Plate 1, fig. E).

When stained by the methyl green pyronin technique for RNA, the normal epithelium gave a strongly positive reaction in the intermediate zone, a faintly positive reaction in the superficial zone, and no reaction in the basal zone (Plate 1, fig. F).

The healing wound

Six hours after operation the site of the lesion (Plate 2, fig. I) could be recognized macroscopically by the absence of mucosa and the presence of blood and fibrin clot. During subsequent days reddish granulation tissue was seen, and by the end of 2 to 3 weeks a white, slightly puckered area offered naked-eye evidence of the region of operation. The sites of lesions after 3 and 6 months and 1 year could only be recognized microscopically after sectioning what was considered to be the area concerned.

Epithelial repair

Within 24 hours epithelial cells had begun to spread or migrate from the wound margin over the floor of the lesion. The migrating cells were several layers thick and no longer displayed the normal zoning. By the end of 2 days this migration had increased and there was considerable mitotic activity in the surrounding epithelium. Any high-power field of epithelium adjacent to the ulcer margin showed several nuclei in different stages of mitosis (Plate 2, fig. H), a state in striking contrast to the normal epithelium where very few mitotic figures were seen.

The cells of the spreading epithelium and those at the margin of the wound were considerably larger than normal, a feature particularly marked when normal basal cells were compared with those that now formed the basal layer of the spreading epithelium. Mast cells were never found among the migrating cells.

From the third to the tenth day the process of epithelial migration accompanied by mitotic activity continued. During this period the epithelium became infiltrated by polymorphonuclear leucocytes and lymphocytes. These cells were
concentrated chiefly in the upper layers and many were found in spaces which made their appearance during this time in these regions (Plate 2, fig. J). The spaces in which white cells were seen varied greatly in sizes and gave a degenerative appearance to the epithelium. They were never seen in the basal layers.

By the tenth day the ulcer floor had become completely epithelialized (Plate 2, fig. K) and the increased mitotic activity had now declined. At this stage the cells were still considerably larger than normal, and in the more superficial regions some inflammatory cells and spaces were still found (Plate 2, fig. L).

Subsequent stages in the epithelial repair consisted of a thinning of epithelium due to a decrease in the size of the cells, and a return to the zoning seen in the normal epithelium, with an absence of infiltration. The normal pattern had been reestablished within 1 month (Plate 2, fig. N), and by this time mast cells were again noted in the basal layer of the epithelium now overlying the original wound area.

The normal distribution of alkaline phosphatase was not seen in regenerating epithelium; the enzyme was completely absent from the migrating cells, even up to the time of complete epithelialization (Plate 3, fig. P). However, when the stratified pattern of the epithelium was becoming restored (during the second week), a reaction for the enzyme occurred in the superficial zone (Plate 3, fig. R). From the third week onwards alkaline phosphatase could be demonstrated in the basal region also; thus the reaction pattern seen in undisturbed epithelium had now returned (Plate 3, fig. T).

Alterations in the distribution of PAS-positive material were also noted in the regenerating epithelium. The hypertrophic cells at the wound margins, and those that were migrating, contained abundant quantities of glycogen, with the notable exception of cells that formed the most basal layer, which never contained this substance (Plate 3, figs. O and Q). The PAS-positive material other than glycogen, which was a prominent feature of the superficial zone of undisturbed epithelium, was no longer present in cells at the margin, nor was it found in those that were spreading over the floor of the wound (Plate 3, fig. S). Following complete epithelialization and the reappearance of stratification, glycogen could no longer be detected histochemically, but non-specific mucopolysaccharide could be demonstrated again in the superficial zone (Plate 3, fig. U).

With the methyl green pyronin stain there did not appear to be any significant increase in pyroninophilia in the regenerating cells.

Connective tissue reactions

The changes that occurred in the floor of the lesion were essentially those of the accumulation and maturation of granulation tissue, together with a considerable degree of infiltration with inflammatory cells. Mitotic activity in connective tissue cells was first noted on the second post-operative day, and was most marked by the fourth or fifth day after which it gradually declined. Metachromasia of the ground substance in the wound area became prominent by the
end of the first week, was still present at the time of complete epithelialization, and thereafter disappeared. Mast cells were absent from the wound site until about the end of 1 month, when a few could be detected in the vicinity of blood-vessels.

At all stages of the healing process there was a complete absence of alkaline phosphatase in the newly forming connective tissue (Plate 3, fig. R). This was so even after prolonged incubation periods of up to 24 hours, when all the tissue elements, including dividing fibroblasts, young blood-vessels, and fibrous extracellular material, still gave a negative reaction.

After a number of months some wound areas remained relatively flat (Plate 2, fig. M) while others developed a convoluted pattern more closely resembling the architecture of normal mucosa and submucosa. In all wounds the muscularis mucosae showed no evidence of regeneration and the cut edges remained to give some indication of the margin of the original lesion (Plate 2, fig. M).

DISCUSSION

The results show that, following repair, the site of a small oesophageal wound eventually bears a close resemblance to the normal mucosa, the muscularis mucosae being the only tissue that shows no evidence of restoration. The absence of regeneration in this component of the alimentary wall has been repeatedly observed both in animals and in man (Ivy, Grossman, & Bachrach, 1952).

The repair process in oesophageal epithelium resembles that in epidermis, the tissue upon which the majority of studies on wound healing have been carried out. In both instances there is a migration of cells from the wound margin, with hypertrophy of the cells concerned, followed by increased mitotic activity in the surrounding epithelium. No convincing evidence of the presence of mitosis in migrating epithelial cells was found in the present work. This contrasts with comparable studies made by the authors on regenerating urinary bladder and gall-bladder epithelia (McMinn & Johnson, 1955, 1957), where cells in division could be found without difficulty among those that were migrating. The infiltration of new oesophageal epithelium by inflammatory cells and the presence of spaces which lend to it a degenerative appearance are phenomena not shared with epidermis, but they may be due to the relatively septic environment of the upper part of the alimentary tract compared with the skin. The fact that the inflammatory invasion was less pronounced in the basal layers of cells may indicate that the nutrition and metabolism of these basal cells is less disturbed than that of more superficial cells which are farther removed from the subepithelial tissue fluid, their probable source of nutriment.

The presence and distribution of glycogen in the regenerating epithelium is also comparable with the findings in hypertrophic epidermis (Bradfield, 1951), including the absence of glycogen from the basal layers of both types of epithelia. It appears that in the oesophagus there is a reciprocal relationship between the
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presence of glycogen and of other polysaccharides. When cells are migrating
the PAS-positive material normally present in the superficial zone of the epithe-
lium disappears, and glycogen is then found in most of the enlarged cells; when
the glycogen disappears, the PAS-positive material returns to the upper layers of
cells. Wislocki, Fawcett, & Dempsey (1951) consider that such PAS-positive
material in squamous epithelia is concentrated in the intercellular spaces and on
cell surfaces, and that its presence varies inversely with the amount of keratin
in any given epithelium. Since the epithelium of the cat's oesophagus is non-
keratinizing, the present finding of PAS-positive material in the superficial zone
is in accord with the observations of these workers. Scothorne & Scothorne
(1953) have reviewed the possible reasons for glycogen accumulation in epi-
dermal cells and concluded that none of the theories so far advanced is entirely
satisfactory.

The finding of alkaline phosphatase in the deep and superficial layers of the
normal epithelium is unexpected. In epithelia, e.g. of the intestine and renal
tubules, its presence has been correlated with absorptive processes, but the
oesophageal lining is not regarded as a tissue through which the passage of
solute normally occurs. No reason can be given for the absence of the enzyme
among the migrating cells; a number of as yet unknown physico-chemical
changes may be occurring in the cell and in the intercellular substance in order
to allow cells the freedom to move, but it is not possible to say whether the
absence of phosphatase is correlated with such changes.

The regularity with which large numbers of mast cells occur in the basal
layer of normal oesophageal epithelium in the cat has already been reported
(Johnson & McMinn, 1957) but Mota, Ferri, & Yoneda (1956), who investi-
gated the distribution of mast cells in the digestive tract of laboratory animals
(including the cat), do not mention them as being a feature of the epithelium.

The reactions in the connective tissue of the floor of the lesion are in general
typical of those of any wound that is healing by granulation. Although mast
cells have been considered to play some part in the formation of new connective
tissue, particularly the metachromatic ground substance (Asboe-Hansen, 1954),
their absence from the wound area does not support this hypothesis. In this
respect the present work is in accord with the results reported by Taylor &
Saunders (1957) who studied the fibrogenesis occurring around implants of
gelatine sponge in rats; mast cells were again notably absent from the develop-
ing and maturing granulation tissue.

The absence of alkaline phosphatase in the newly forming connective tissue
elements is very striking and contrasts with the results of work carried out by
Fell & Danielli (1943) and others, who noted in the healing skin wounds of
rodents a considerable increase in phosphatase activity. However, it is of further
interest that in the study of healing gall-bladder wounds in the cat, McMinn &
Johnson (1957) were again unable to demonstrate the presence of phosphatase.
The fact that a species difference may account for the conflicting results found
in cats and rodents has led to further work on this subject which will be presented elsewhere (Johnson & McMinn, 1958).

SUMMARY

1. Histological and histochemical techniques have demonstrated that the epithelium in the lower end of the oesophagus of the cat consists of three well-defined zones, namely superficial, intermediate, and basal.

2. Wound healing in the lower end of the oesophagus has been studied following the removal of small areas of mucosa.

3. Epithelial repair occurred by migration of cells, accompanied by increased mitotic activity in the surrounding undisturbed epithelium.

4. The alkaline phosphatase and PAS-positive material (other than glycogen) that were present in normal epithelium were not seen in migrating cells, which contained considerable quantities of glycogen.

5. The newly forming connective tissue elements in the floor of the wound did not contain alkaline phosphatase at any stage of the healing process.

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REFERENCES


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EXPLANATION OF PLATES

PLATE 1

**Fig. A.** Normal oesophageal epithelium. Note the small basal cells, the larger cells of the intermediate zone, and the layers of flattened cells that form the superficial zone. There is no keratinization. Haematoxylin and eosin. × 170.

**Fig. B.** Normal epithelium, showing the presence of alkaline phosphatase in the basal zone, and to a lesser extent in the superficial layers. Gomori technique, incubation time 30 minutes. × 170.

**Fig. C.** Normal epithelium, showing a similar distribution of alkaline phosphatase to that in fig. B. Azo dye method. × 170.

**Fig. D.** Normal epithelium, showing the three zones of cells. Toluidine blue. × 170.

**Fig. E.** Normal epithelium, showing PAS-positive material in the superficial zone. PAS after saliva digestion. × 170.

**Fig. F.** Normal epithelium. Note that pyroninophilia is most marked in the intermediate zone and absent from the basal layer. Methyl green pyronin. × 500.

PLATE 2

**Fig. G.** Normal epithelium, showing mast cells lying in the basal layer. The granules are strongly metachromatic. Azure A. × 540.

**Fig. H.** Normal epithelium near the wound margin after 2 days, showing a number of mitotic figures. Haematoxylin and eosin. × 500.

**Fig. I.** Section through the centre of a wound 6 hours after operation. Note that the whole thickness of the mucosa including the muscularis mucosae has been removed. Haematoxylin and eosin. × 25.

**Fig. J.** Epithelial cells migrating towards the right over the floor of the wound, after 3 days. Note the size of the basal cells compared with fig. A, the cellular infiltration, and the spaces in the upper layers. Haematoxylin and eosin. × 170.

**Fig. K.** Completely epithelialized wound site after 10 days, showing the thickened epithelium overlying maturing granulation tissue. Haematoxylin and eosin. × 30.

**Fig. L.** Hypertrophic epithelium overlying a wound site after 10 days at the same magnification as figs. A and J. Note the absence of zoning. Haematoxylin and eosin. × 170.

**Fig. M.** Section through a wound site 6 months after operation. Note the cut edges of the muscularis mucosae and its absence from the flattened wound area. Haematoxylin and eosin. × 25.

**Fig. N.** Epithelium from a wound area 1 month after operation, showing the return of the normal zoning. Compare with figs. A and L at the same magnification. Haematoxylin and eosin. × 170.

PLATE 3

**Fig. O.** Epithelium from a wound margin after 3 days, showing abundant quantities of glyco- gen, but note its absence from the basal layer. PAS. × 750.

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FIG. P. Epithelium from an epithelialized wound after 10 days. Compare with fig. B and note the absence of alkaline phosphatase from the epithelium. Gomori technique, incubation time 30 minutes, lightly counterstained with haematoxylin. ×120.

FIG. Q. An adjacent section to that illustrated in fig. O, showing that the material identified as glycogen in fig. O has now been removed. PAS after saliva digestion. ×750.

FIG. R. Part of an epithelialized wound area after 15 days. Compare with figs. B and P and note that the superficial zone of epithelium gives a reaction for alkaline phosphatase, and that the subepithelial granulation tissue gives a completely negative reaction. Gomori technique, incubation time 30 minutes, lightly counterstained with haematoxylin. ×120.

FIG. S. Section of a wound margin after 10 days. Normal epithelium, above, gives a PAS-positive reaction in the superficial zone. The large, migrating epithelial cells, below, contain glycogen. PAS. ×120.

FIG. T. Epithelium from a healed site after 6 months, showing a normal distribution of alkaline phosphatase. Gomori technique, incubation time 30 minutes. ×170.

FIG. U. Epithelium from a healed site after 6 months, showing a normal distribution of PAS-positive material other than glycogen. Compare with fig. E. PAS after saliva digestion. ×170.

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