Repair of Wounds of the Mucosa in the Rectum of the Cat

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WITH THREE PLATES

INTRODUCTION

Investigators of wound healing in the alimentary canal have carried out their experiments mainly in the gastro-duodenal region, in view of the importance of the problem of peptic ulceration in man. Little attention has been paid to repair processes at lower levels of the intestinal tract; histological investigations on this subject in the large intestine appear to have been carried out only by O’Connor (1954, 1956) and by Lumb & Protheroe (1955). Sircus (1956) studied the ulceration that ensued in portions of colon that had been implanted into the stomach wall in dogs, but his interest lay in the mechanism of ulcer production rather than ulcer healing, and Truelove’s (1957) biopsies from patients with ulcerative colitis were used largely to assess a method of treatment and not to investigate repair processes.

The present series of experiments was carried out in order to study the repair of mucosal lesions in the rectum of the cat. Routine histological studies were supplemented by histochemical observations on both the normal and regenerating tissues.

MATERIALS AND METHODS

All experiments were performed on healthy adult cats, a total of 34 animals being used. Under nembutal anaesthesia, a piece of mucous membrane 0.5–1.0 sq. cm. in size was removed per anum from the rectum, at a site on the dorsal wall 2 cm. proximal to the muco-cutaneous junction. The mucosa in this region is very mobile, a factor which allowed it to be picked up with forceps prior to cutting off the required area with scissors. The lesion so created can be conveniently referred to as an ‘artificial ulcer’. The animals were allowed to survive after operation for periods ranging from 24 hours to 6 months.

Following death by coal-gas poisoning, the site of the lesion, together with an

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area of normal surrounding tissue, was removed and fixed in ice-cold 80 per cent. alcohol. Some specimens were fixed in Gendre’s fluid after freeze-substitution. After embedding in paraffin wax, serial sections were cut at a thickness of 8 μ, and every twentieth mounted for routine staining with haematoxylin and eosin. These were examined, and from regions of particular interest serial sections were mounted for further study. The following techniques were used:

1. Iron haematoxylin and picrofuchsin (van Gieson), for the demonstration of connective tissue.
2. The Gomori cobalt sulphide technique for alkaline phosphatase, with the modification of Kabat & Furth (1941). Incubation times varying from a few minutes to 24 hours were used.
3. The periodic acid-Schiff (PAS) technique for the demonstration of polysaccharides (Hotchkiss, 1948). Diastase-labile material, following digestion with saliva, was identified as glycogen.

RESULTS

Histological study of specimens from animals killed 24 hours after operation showed that at the ulcer site the whole thickness of the mucosa, including the muscularis mucosae, had been removed. The floor of the ulcer consisted of the inner layer of the muscularis externa, with some overlying submucosa and a variable amount of blood and fibrin clot. The epithelium and lamina propria at the ulcer margins had fallen towards the ulcer floor, thus covering up the cut ends of the muscularis mucosae, which may have assisted the process by retracting. In some specimens a few epithelial cells were found to be overlying the edge of the ulcer floor.

By the end of 2 days epithelial cells, in continuity with those of the undisturbed mucosa at the wound margins, were found covering the periphery of the ulcer (Plate 1, fig. A). Many of the cells were sufficiently flattened to give the appearance of a squamous epithelium (Plate 1, fig. E). During the next few days the spread or migration of epithelial cells towards the ulcer centre continued, but by the fourth day the very flat type of cell was no longer seen; all were becoming cuboidal or low columnar (Plate 1, fig. B). Among the many serial sections studied from ulcers in the first few days after operation, four sections only were found in which a single mitotic figure was seen among the spreading cells (Plate 1, fig. F). The apparent absence of goblet cells in the migrating epithelium was notable.

In the floor of the wound there was evidence of proliferative activity in connective tissue-cells from the second day onwards. This was not confined to regions near the surface of the wound; mitoses in connective tissue-cells were also found in the septa between bundles of muscle fibres in that part of the muscularis externa that lay below the ulcer area (Plate 1, fig. C). Beneath the spreading epithelium, and in unepithelialized areas in the centre of the ulcer, typical granulation tissue was appearing, as evidenced by the presence of young
fibroblasts with well-marked cytoplasmic processes (Plate 1, fig. B), and buds of capillary blood-vessels.

There was considerable variation in the subsequent course of the healing process from one cat to another, particularly in regard to the amount of granulation tissue formed in the ulcer floor. Where the amount of such tissue was relatively small, ulcers examined at the end of one week after operation revealed that the epithelium had migrated for about 1,000 μ towards the centre of the lesion, and that in some places near the original ulcer margin it dipped down into the underlying granulation tissue, forming shallow epithelialized depressions (Plate 2, fig. 1). All the epithelial cells were now tall columnar in form (Plate 1, fig. G), and although some goblet cells were seen they were not numerous. Between the second and fourth weeks such lesions had become completely epithelialized, and showed over the entire wound area numerous shallow depressions (Plate 1, fig. D) whose epithelium contained occasional mitotic figures (Plate 1, fig. H) and some goblet cells (Plate 2, fig. J). These goblets were not as numerous as in normal crypt epithelium. The granulation tissue had become organized and mitotic figures in connective tissue-cells were almost absent.

In wounds that showed an exuberance of granulation tissue, shallow epithelialized depressions were not seen, and deep crypts, with many goblet cells, abutted against the mound of granulation tissue, the surface of which was not covered by epithelium (Plate 3, fig. O).

At the end of 3 and 6 months the whole ulcer area was covered with a mucosa that closely resembled the normal, but the muscularis mucosae was absent from the region of the wound and its cut edges remained to give some indication of the original margin of the lesion. Some specimens exhibited a rather shallow mucosa in which the crypts were a little less deep and less closely packed than normal (Plate 2, fig. K). Others showed strands of fibrous tissue that passed obliquely from the submucosa into the mucosa between groups of crypts (Plate 3, fig. P).

**Histochemical reactions**

The epithelium in the undisturbed mucosa gave a strong reaction for alkaline phosphatase at the striated borders of the cells (Plate 2, fig. L). The reaction could be seen with incubation periods of only a few minutes, and was present even in the deepest parts of the crypts, although it was less intense here than at more superficial levels. Specimens examined within the first few days after operation showed that the migrating epithelial cells gave a completely negative reaction for phosphatase (Plate 2, fig. L). At the end of the first week the reaction in the new epithelium overlying the ulcer floor was still negative, even in the cells that lined the shallow depressions near the ulcer margins (Plate 2, fig. M). However, by the end of the third week a reaction of normal intensity
was seen in all epithelial cells (Plate 2, fig. N), and this degree of phosphatase activity was present at all subsequent stages of healing.

In the developing and maturing granulation tissue of the ulcer floor, there was a complete absence of phosphatase at all stages of the healing process examined, including those early stages at which proliferative activity of connective tissue-cells was observed (Plate 2, fig. M). Even after prolonged incubation periods of 24 hours this new tissue was still negative.

With the PAS technique, the epithelium in the normal rectum showed the presence of PAS-positive material in the position of the striated border (Plate 3, fig. Q). As with alkaline phosphatase, the reaction was also present in cells in the deepest parts of the crypts, though of diminished intensity. The material was not diastase-labile following digestion with saliva and hence was not glycogen, nor could the presence of glycogen be demonstrated in any other part of normal epithelial cells. The spreading epithelium that was seen in ulcers a few days old showed little or no PAS-positive material at the free borders of most of the cells (Plate 3, fig. R); the absence of PAS-positive material was not as complete as the absence of phosphatase. After 7 days a strong reaction had returned to the position of the striated border (Plate 3, fig. S), and as in the normal rectum the material resisted digestion with saliva. At the same time, a number of small granules, intranuclear in position and diastase-labile, were revealed in the tall columnar cells of this spreading epithelium (Plate 3, figs. T, U). Some of the cells lining the shallow depressions near the ulcer margin contained these glycogen granules (Plate 3, fig. V). It was not possible to demonstrate glycogen at any later stages, but the PAS-positive border was seen in all epithelial cells throughout the remainder of the healing process. Although a number of workers, e.g. Culling (1957) and Hale (1957), have recommended fixation in Gendre's fluid for the preservation of glycogen, the use of ice-cold 80 per cent. alcohol in the present circumstances appeared to be equally efficacious.

DISCUSSION

The results indicate that an effective restoration of mucous membrane occurs at the site of a mucosal lesion in the rectum, but the muscularis mucosae shows no evidence of restoration. The absence of regeneration in this component of the alimentary wall was noted during wound healing in the cat's oesophagus (McMinn & Johnson, 1958) and small intestine (McMinn & Mitchell, 1954), and has been repeatedly observed both in other animals and in man (Ivy, Grossman, & Bachrach, 1952). The migration of epithelium from normal mucosa at the ulcer margins and the accumulation of granulation tissue in the floor of the ulcer are phenomena that are typical of wound healing, but there are a number of features of interest that call for some discussion.
The problem of mucosal restoration

Concerning the problem of whether the mucosa is made good by the formation of new crypts, or by a rearrangement of old crypts as a result of contraction exerted by the fibrous tissue that develops in the floor of the lesion, it is pertinent to compare the results of the present work with the observations made by O'Connor (1956) on mucosal repair in the large intestine of the mouse. He concluded that the gap in the mucosa was filled by fibrous tissue that contracted and so approximated the cut edges of the mucosa. In the rectum of the mouse, the outgrowth of epithelium from the wound margins never exceeded 600 µ, and he found no evidence of new gland formation.

From the present work on the cat it could be suggested that the conditions seen in Plate 2, figs. O and P are comparable with the findings in the mouse. On the other hand, those in Plate 1, fig. D and Plate 2, figs. I–K might be cited as evidence of new crypt formation. It could still be argued that the shallow depressions seen for example in Plate 2, fig. J are merely old crypts that have been flattened and pulled towards the ulcer centre by the newly forming fibrous tissue. However, the relative absence of goblet cells, abundant in normal crypts, does not support this contention. Furthermore, the histochemical findings offer evidence that new crypt formation does occur, at least in some specimens, for the following reasons. It has been seen that during the first week 'new' epithelium, i.e. epithelium that grows out from the ulcer margins over the periphery of the floor, gives a negative reaction for alkaline phosphatase, in contrast to the epithelium of undisturbed mucosa that is always strongly positive. By the end of 7 days new epithelium still gives a negative reaction for phosphatase, but may now contain glycogen granules that are never present in undisturbed mucosa. Thus the absence of phosphatase and the presence of glycogen appear to be criteria of 'new' epithelial cells. Such criteria are present in cells lining shallow depressions near the wound margins, and since 'old' crypt epithelium always gives a positive reaction for phosphatase and never contains glycogen, the present histochemical findings support the concept of new crypt formation. It is not possible to assess the extent to which fibrous tissue contraction may be assisting the closure of the defect. The fact that after the end of 2–3 weeks the histochemical reactions of the epithelium had returned to normal renders these tests of no value in determining whether new crypt formation occurred at later stages.

It should be noted that in those specimens in which there are no shallow depressions near the ulcer margins, the granulation tissue is profuse and has reached the level of the tops of the normal crypts, or has even exceeded it, as in Plate 3, fig. O. Where such depressions are present and regarded as the precursors of new crypts, the granulation tissue is much less exuberant (Plate 2, fig. J). It seems possible that an end result, such as is illustrated in Plate 3, fig. P, where there are fibrous tissue strands between the crypts, may occur when
granulation tissue is abundant and subsequently contracts; the shallower type of mucosa illustrated in Plate 2, fig. K may be a later development of the pattern of healing seen in Plate 2, fig. J, which with the histochemical evidence suggests new crypt formation. While either of these two postulated courses of events may occur, the factors that determine which of them takes place in any particular wound are unknown.

At higher levels of the alimentary tract it would appear that new gland formation is the rule during the repair of mucosal lesions. In ileal wounds in the cat McMinn & Mitchell (1954) noted the new formation of crypts and villi without any excessive fibrosis, while Florey & Harding (1935) found that new crypt and villous formation took place in healing duodenal lesions. In stomach wounds of the cat and other animals, a number of investigators have described the formation of new glands (Longmire, Beal, Lipmann, & Bishop, 1952; Williams, 1953; Myhre, 1956). Since O'Connor (1956) found no evidence of new crypt formation at any stage of healing in the rectum of the mouse, it would be interesting to know the nature of the healing process in the stomach and small intestine of this animal.

It should be noted that Lumb & Protheroe (1955), who studied repair following surgical trauma in the human rectum, considered that outgrowing epithelium was unlikely to form new crypts. The pattern of mucosal healing in the upper part of the human alimentary tract (e.g. stomach and duodenum) has led to the belief that new gland formation does occur (Ivy, Grossman, & Bachrach, 1952), findings that are similar to those in the experimental animal at comparable sites.

**Histochemistry of the epithelium**

The accumulation of glycogen granules, in epithelium wherein glycogen is not normally detectable histochemically, may be comparable with the increase that occurs in other regenerating epithelia, e.g. in the skin and oesophagus (Bradfield, 1951; McMinn & Johnson, 1958), although the amount seen in the rectal cells is small compared with that found in the other epithelia mentioned. The epithelium of the crypts of both small and large intestines normally displays a considerable amount of proliferative activity, but the newly formed cells that result from such mitoses never contain glycogen, so that mere youth of cells would not appear to be a factor concerned in glycogen accumulation. It seems more likely that a nutritional disturbance occurs due to an abnormal environment.

**Mitoses in migrating epithelium**

No mitotic counts were carried out in the present study, but there did not appear to be any grossly recognizable increase in mitotic activity in epithelium at the wound margins, whereas most other epithelia do show such an increase when regenerating. However, on the basis of studies with colchicine, it has been shown (McMinn & Mitchell, 1954; McMinn, 1954) that in the ileum there is no significant increase in mitotic activity at the margin of a healing wound, and that
the presence of a gap in continuity does not serve as a stimulus to increased cell-
division in the epithelium of that organ. It may be that the rectum is similar in
this respect. The presence of mitotic figures in the migrating epithelium in the
rectum is a phenomenon that has not previously been described in epithelium
of the small or large intestines, but the finding during the present investigation
of four mitoses in layers of spreading cells indicates that such cells are capable of
dividing. Although the absence of proliferation in spreading cells has been held
to be one of the characteristic features of wound healing in epithelia (Arey, 1936),
the present authors have found many mitotic figures in the migrating cells of
regenerating urinary bladder and gall-bladder epithelia (Johnson & McMinn,
1955; McMinn & Johnson, 1955, 1956, 1957). In the light of this evidence, some
revision of existing concepts becomes necessary.

Alkaline phosphatase of granulation tissue

A number of workers (e.g. Fell & Danielli, 1943) have provided evidence sug-
gestig that alkaline phosphatase is concerned with the elaboration of fibrillar
protein. The complete absence of phosphatase in the maturing granulation tissue
of rectal wounds in the cat does not support this hypothesis, but is in keeping
with the findings of the present authors during wound healing in other hollow
viscera in this animal. The matter is fully discussed elsewhere (Johnson &
McMinn, 1958).

SUMMARY

1. Wound healing in the rectum of the cat has been studied following the
creation of artificial ulcers by removing small areas of mucosa.
2. During the first few days of the repair process epithelial cells migrated
from the wound margins over the floor of the ulcer, in which granulation tissue
began to accumulate.
3. Mitotic figures were occasionally found in migrating epithelium.
4. The striated borders of normal epithelial cells gave a strong reaction for
alkaline phosphatase and were PAS-positive. These reactions were not present
during migration in the early stages of repair. Glycogen granules, not normally
detectable histochemically in this epithelium, were found in migrating cells at
the end of the first week.
5. The pattern of healing at later stages showed considerable variation,
according to the amount of granulation tissue formed. Where the amount of such
tissue was relatively small, increasing epithelialization occurred, with evidence
of new crypt formation; where granulation tissue was exuberant, the margins of
the original lesion may have been drawn together by the contraction of maturing
connective tissue.
6. Alkaline phosphatase was absent from the developing and maturing con-
nective tissue.
ACKNOWLEDGEMENTS

The authors wish to thank Professor Francis Davies for his helpful criticism during the preparation of this paper. They also thank Mr. J. H. Kugler for the preparation of photomicrographs, and Mr. J. H. Morrill and Miss C. J. Crockford for technical assistance.

REFERENCES

EXPLANATION OF PLATES

PLATE 1

Fig. A. Margin of an ulcer after 2 days. Epithelial cells are migrating over the ulcer floor. Note the cut edge of the muscularis mucosae. H. & E. ×65.

Fig. B. Epithelial cells overlying typical young fibroblasts of an ulcer floor, after 4 days. H. & E. ×640.

Fig. C. Two connective tissue cells in metaphase, lying deep to a bundle of muscle fibres of the muscularis externa below the floor of a wound, after 2 days. H. & E. ×65.

Fig. D. Ulcer site after 4 weeks, showing that the whole area is covered by shallow epithelialized depressions. H. & E. ×35.

Fig. E. Flattened epithelial cells migrating over the floor of an ulcer after 2 days. H. & E. ×640.

Fig. F. Migrating epithelium after 3 days, showing a nucleus in prophase. PAS. ×1340.

Fig. G. Migrating epithelial cells after 7 days. Compare with figs. B and E at the same magnification. H. & E. ×640.

Fig. H. A mitotic figure in the epithelium lining a shallow depression in an ulcer site after 2 weeks. PAS. ×640.

PLATE 2

Fig. I. Margin of an ulcer after 7 days, showing epithelial cells that have spread from the margin (on the left) dipping down into granulation tissue. Note that goblet cells are scanty in the new epithelium. H. & E. ×70.

Fig. J. An adjacent section to that illustrated in Plate 1, fig. D, at higher magnification. On the right is the mucosa of the original wound margin; on the left is the spreading epithelium lining shallow depressions and showing some goblet cells. H. & E. ×100.

Fig. K. An ulcer site after 6 months. The position of the cut edges of the muscularis mucosae is indicated. The area of the ulcer is occupied by crypts that are less deep and less closely packed than in the undisturbed mucosa. H. & E. ×15.

Fig. L. Above, normal rectal epithelium, showing a strong reaction for alkaline phosphatase at the striated border of the cells. Below, migrating epithelium after 3 days, giving no phosphatase reaction. Gomori technique, incubation time 30 minutes. ×640.

Fig. M. Epithelialized depression from an ulcer after 7 days, giving no reaction for phosphatase. Note that the granulation tissue is also negative. Gomori technique, incubation time 3 hours. ×255.

Fig. N. Epithelium from the centre of an ulcer after 3 weeks, giving a phosphatase reaction of normal intensity. The subepithelial tissue remains negative. Gomori technique, incubation time 30 minutes. ×640.

PLATE 3

Fig. O. Centre of an ulcer site after 4 weeks, showing exuberant granulation tissue that is not covered by epithelium. H. & E. ×50.

Fig. P. Ulcer site after 3 months. A strand or septum of fibrous tissue is seen passing from the submucosa between groups of crypts towards the surface. H. & E. ×35.

Fig. Q. Normal rectal epithelium, showing a PAS-positive border. PAS. ×640.

Fig. R. Migrating epithelium from an ulcer after 3 days, showing the absence of a PAS-positive border. PAS. ×640.

Fig. S. Epithelium from an ulcer after 7 days. A PAS-positive border has now returned. PAS. ×255.

Fig. T. Migrating epithelium from an ulcer after 7 days, showing infranuclear granules of glycogen. PAS. ×640.

Fig. U. An adjacent section to that illustrated in fig. T, showing that the material identified as glycogen is no longer present. PAS after saliva digestion. ×640.

Fig. V. Epithelial cells, with some perinuclear glycogen granules, lining a depression in an ulcer after 7 days. PAS. ×640.

(Manuscript received 27: ii: 58)