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Mitotic activity of epithelial cells in wounded rectal mucous membrane

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INTRODUCTION

In view of the prevalence of human peptic ulceration, repair processes in the mucous membranes of the stomach and small intestine have been the subject of numerous investigations, but until recent years the mucosa of the large intestine has attracted less interest. Early experimental work suggested that lesions of the mucous membrane did not heal with new gland formation (O'Connor, 1954, 1956; Hightower, 1958). More recently, however, it has been found that glands do regenerate (Foley & Wattenberg, 1960; Braucher & Kirsner, 1962, 1966).

Little is known about mitotic activity in surviving colonic epithelial cells after wounding. Florey & Webb (1931) reported an increase in mitotic activity when the mucous membrane was eroded with mustard oil, but McMinn (1958) did not see any obvious indication of such activity in the epithelial margins of an experimental lesion in the cat rectum: however, these workers did not count mitotic figures.

The present work was designed to discover the patterns of mitotic activity in the epithelial cells of the rectal mucous membrane at a wound margin.

MATERIALS AND METHODS

Adult female rats of the Wistar strain were used in all experiments. The animals were bred at the Royal College of Surgeons of England and were all raised on the same standard diet and cared for under identical conditions. The animals used in each experiment were kept in the same laboratory under similar conditions of temperature and lighting. All animals received the same diet with water *ad libitum* and were disturbed as little as possible.

Under ether anaesthesia, small ulcers were made on the dorsal wall of the rectum by pulling the mucous membrane down through the anus with fine forceps and excising an area of approximately 4 mm^2. Animals were killed at intervals between 12 hours and 28 days (2 animals at each stage) over a series of five experiments as undernoted. All animals were killed at 10.00 hours, the time of operation being adjusted accordingly, e.g. for 12 hour specimens the animals were operated on at 22.00 hours. This procedure was adopted to prevent discrepancies in the mitotic counts due to a possible diurnal variation of mitotic activity.

The whole rectum was excised and the ulcer removed and fixed in glutaraldehyde. Serial sections were cut at 6 μm through each ulcer and stained with haematoxylin

and eosin. For epithelial mitotic counts sections were selected from the centre of the lesion where both ulcer margins presented with the glands cut in true sagittal section. Mitotic counts were carried out on the 8 glands bordering each ulcer edge, the total number of mitoses in these 8 glands being referred to as the mitotic number and expressed as a percentage of the total number of nuclei. As each gland on section contained approximately 120 cells, about 960 were included in each count. The gland immediately adjacent to the ulcer margin was designated as gland 1, with gland 2 next to it and so on up to gland 8 which was the eighth gland from the margin.

In a further experiment (Experiment 6 as undernoted) autoradiography was used to see if any correlation existed between the numbers of labelled cells at the wound margin and the mitotic number. The isotope thymidine-6-H_3 was administered intraperitoneally in a dose of 250 μCi for a rat weighing between 150–200 g. Two animals were sacrificed at 10.00 hours daily for 2 weeks, 1 hour after the isotope was administered, and treated using a standard schedule described by Appleton (1972). Counts of labelled cells were made in the same manner as for mitotic counts (the autoradiographs of the 8 and 9 day ulcers were unfortunately spoiled).

RESULTS

Controls

Control animals (20) showed an average mitotic number of 0·30%. Those given thymidine gave an average labelling index of 2·5%.

Experiment 1

In this experiment mitotic activity during the time period from 1 to 14 days was investigated. Mitotic figures increased two- or threefold within the first 24 hours after wounding (Fig. 1), after which counts remained at that level for 6 days (although the 48 hour specimens were spoiled). In the subsequent 6 days the numbers of dividing cells doubled, with a small drop in counts on the twelfth day. In both rats a peak occurred on the fourteenth day which represented a level of cell proliferation approximately six times the normal mitotic number for rat colon. The cell counts showed a marked drop on the fifteenth day. The overall pattern of mitosis exhibited by the two animals sacrificed at each time interval was remarkably similar.

Experiment 2

As the above results demonstrated possible peaks of activity, it was decided to repeat the experiment and extend it to cover a 3 week period (Fig. 2).

The mitotic activity 3 days after ulceration was between two and three times control rates (0·30%), and held this level for the first week. A slight increase occurred in the next 4 days and a sudden peak appeared on the twelfth day, 2 days earlier than in Experiment 1. A rapid drop in counts occurred on the thirteenth day and mitotic numbers were close to normal values by the end of the second week. A subsequent smaller peak was noted on the nineteenth day.

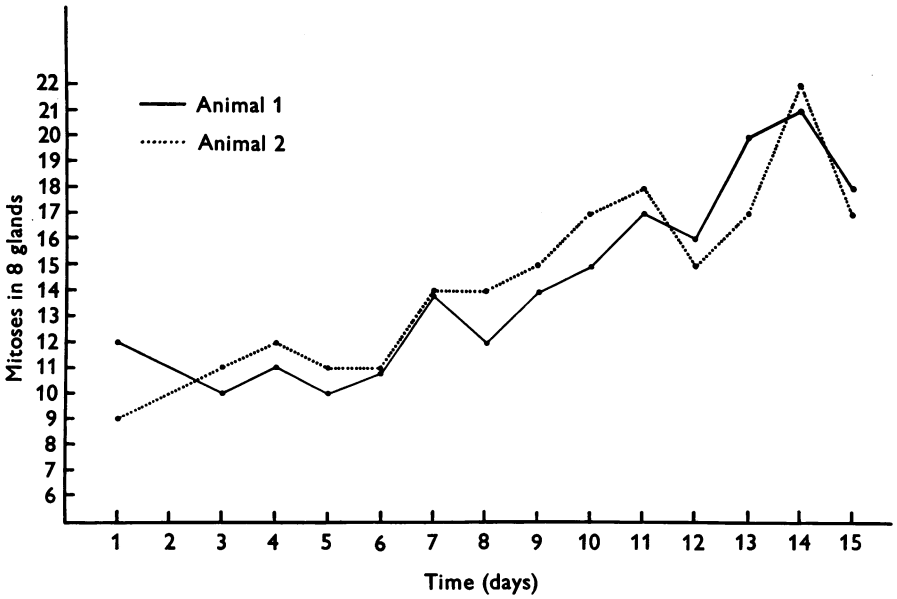

Fig. 1. Results of Experiment 1. Note peak at 14 days.

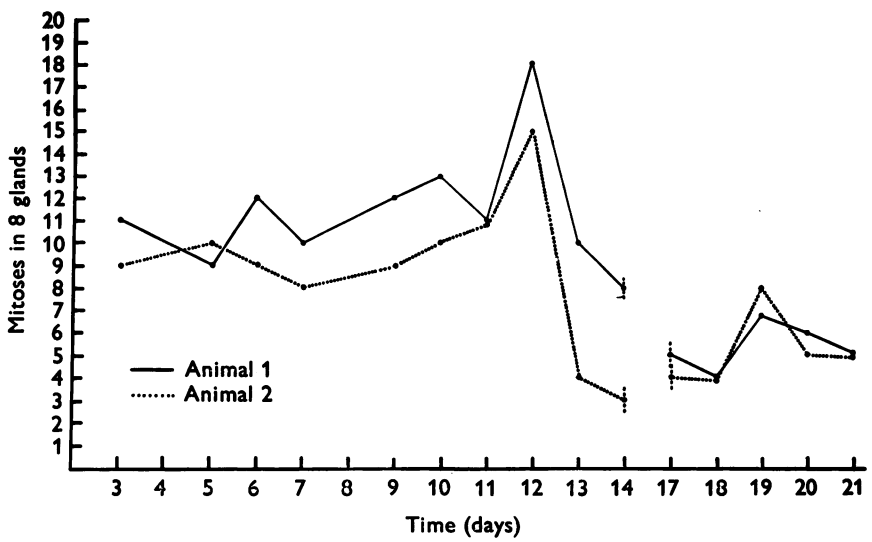

Fig. 2. Results of Experiment 2. Note peak at 12 days and subsequent fall in mitoses. A smaller peak is seen at 19 days.

Experiment 3

It was decided to investigate the first week more closely with particular reference to the first 2 days which had been previously missed (due to spoilt specimens) and to the period between days 5 and 7 where a small peak appeared likely.

Within 24 hours the mitotic number had increased threefold over control values

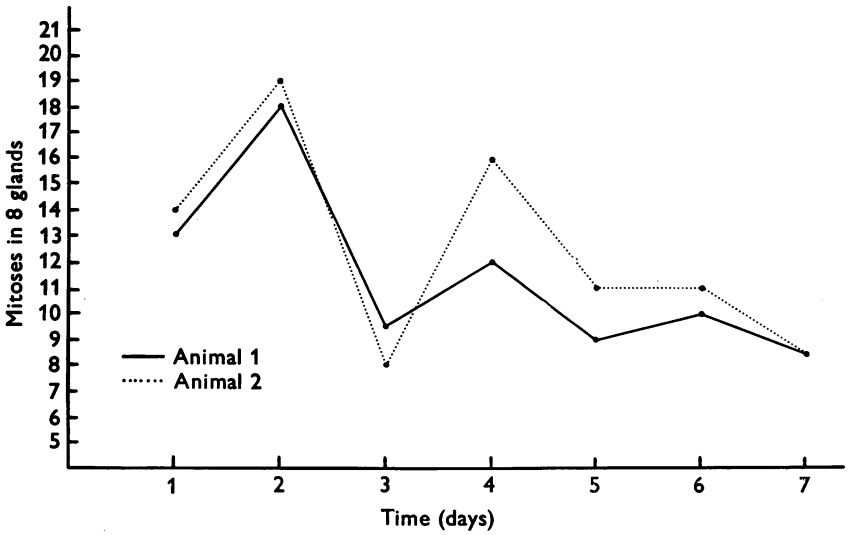

Fig. 3. Results of Experiment 3. Note peaks at 2 and 4 days.

and a peak occurred at day 2 which represented a mitotic number five times the normal control (Fig. 3). The level fell rapidly the next day, but rose to a smaller peak of activity on the fourth day. The next 3 days showed the levels falling to pre-peak values and remaining constant.

Complete healing was obtained in the above experiment in 4 weeks and the second half of this 4 week period was now examined in more detail.

Experiment 4

The experiment was commenced on the fourteenth day, at which time mitotic levels (Fig. 4) corresponded to those expected from the prior experiments. A third large peak appeared on the eighteenth or nineteenth day, falling by the twentieth day. A fourth smaller peak was recorded around the twenty fourth day, which also dropped to previous levels a day later. The two groups of animals corresponded reasonably well.

Experiment 5

In this experiment animals were sacrificed at 12 hourly intervals to investigate mitotic activity during the first 3 days. The initial two- or threefold increase in the mitotic number, previously recorded in Experiment 3, occurred at 12 hours (Fig. 5) and a high peak was found at 36 hours in one animal and 48 hours in the other, representing a five- to sevenfold increase respectively in the mitotic number over control levels (control 0·30%). At 60 hours the rate fell to pre-peak levels and remained so to the third day.

Experiment 6

Autoradiography in a further series of animals confirmed the peak and trough pattern (Fig. 6), the labelled cells appearing mainly in the lower one third of the

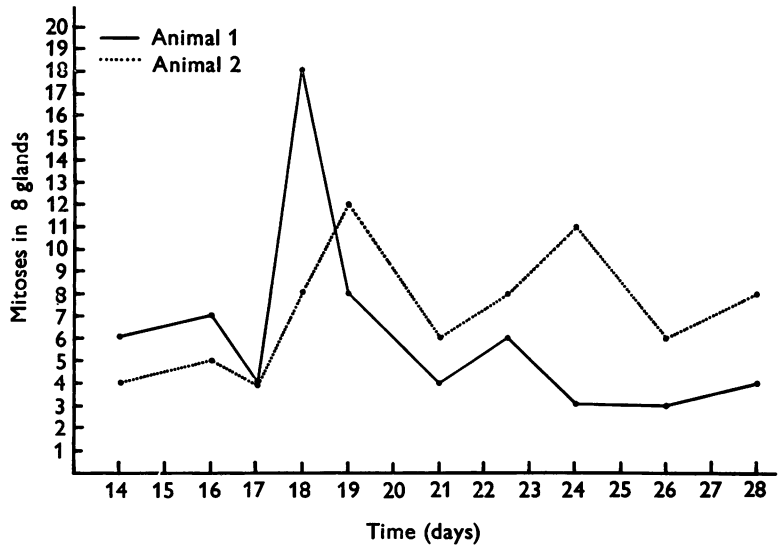

Fig. 4. Results of Experiment 4. Note peaks at 18–19 days and 23–24 days.

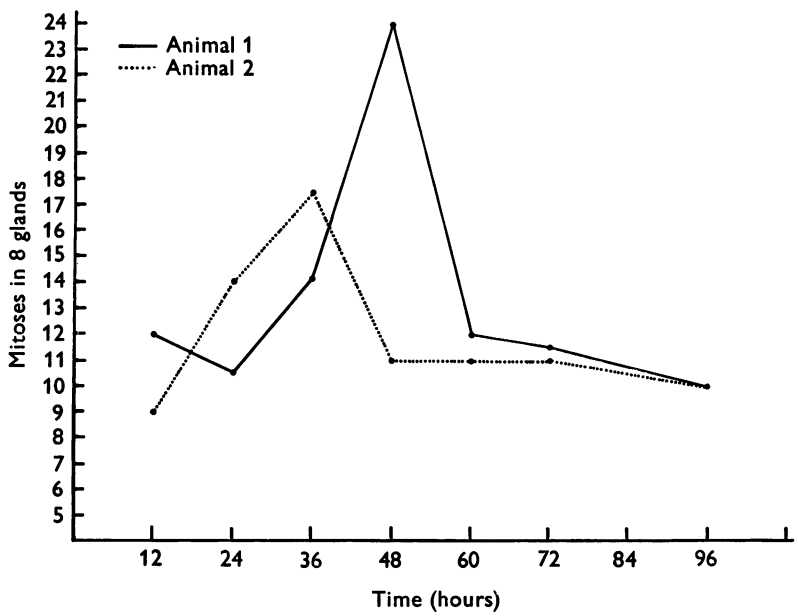

Fig. 5. Results of Experiment 5. Note peaks at 36–48 hours.

glands. At peaks of activity there were increased numbers of labelled cells in the upper regions of the glands, although the top 10–15 cells of the glands and the surface epithelium were not labelled.

A composite graph of the first five experiments illustrates the overall findings (Fig. 7). In summary, there is, after wounding, an obvious increase in mitotic activity

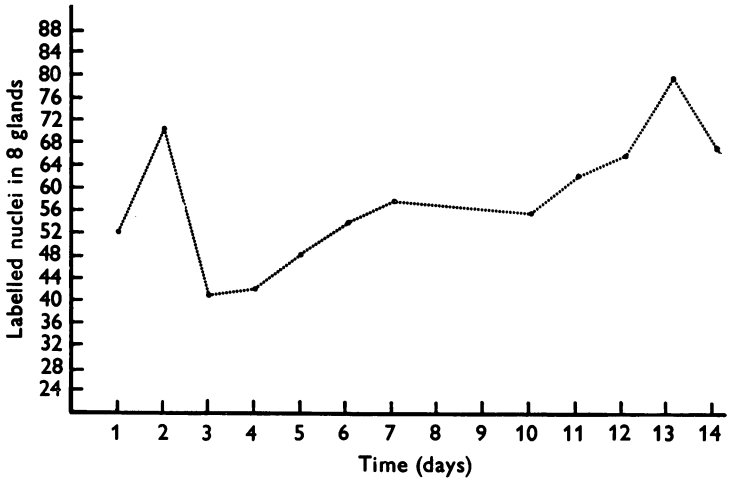

Fig. 6. Counts of labelled cells (thymidine-6-H_3) showing peaks at 2 and 13 days.

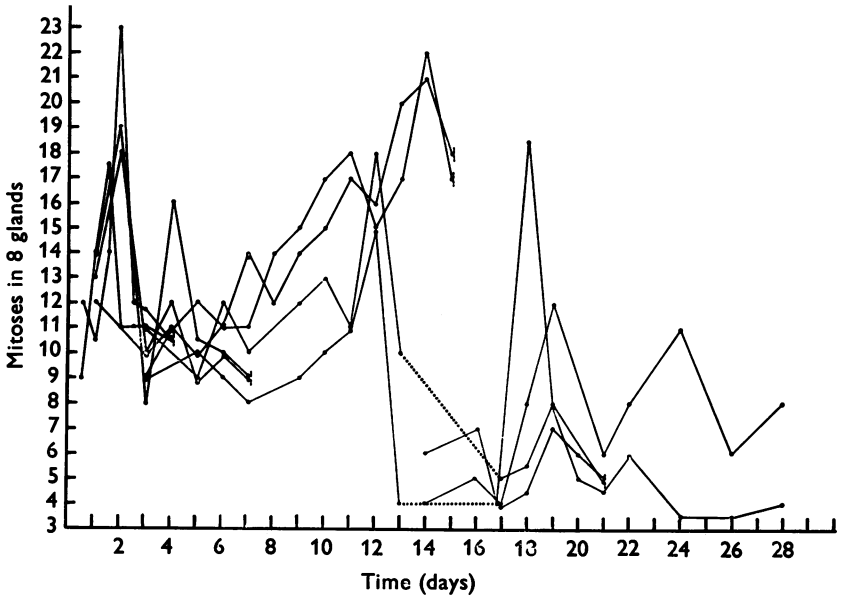

Fig. 7. A composite graph of all experimental results.
Note peaks at 2, 12–14, 18–19, and 24 days.

which does not take the form of a simple rise and fall but occurs in a series of peaks. The peaks occur at reasonably constant times after wounding, viz. 2, 11–12, 18–19 and 22–24 days. There is also an overall rise in the level of mitotic activity up to day 14, after which the level falls to almost normal values. Dividing cells are normally found in the basal region of a colonic gland. On wounding, the increased mitotic activity results from increased cell division in this part of the gland and also in the middle third of the gland. Only rarely was a dividing cell noted in the upper part of

the gland even with the highest peaks of activity. The mitotic activity in gland 1 and to a lesser extent gland 2 (those glands nearest the wound edge), was often lower than in glands 3–8. This was not backed by formal counts as it was decided to investigate this more fully in a future investigation. The highest overall peak obtained was on day 2; thereafter the levels dropped with each subsequent rise until complete healing had been established after some 28 days, when the mitotic number remained stable at its normal control value. Autoradiography, as noted in Experiment 6, confirmed the peak and trough pattern.

DISCUSSION

It is well known that most lining epithelia and parenchymal cells show an increase in mitotic activity when regenerating. Sulkin (1949) noted an increased mitotic rate which was maximal between 3 and 10 days in the tubular epithelium of the normal kidney after a unilateral nephrectomy in the rat. Franck (1960) confirmed this rise in cell activity and found the increase 48 hours after operation. Weinbren (1959) found increased mitotic activity in the parenchymal cells of the liver 24 hours after partial hepatectomy. There are many reports of increased cell division at the margins of skin wounds with the initial increase occurring between 24 and 72 hours after wounding (e.g. Gelfant, 1959; Giacometti, 1967).

It is interesting to note in this present study of the large intestine that the increased mitotic rate did not merely take the form of a gradual rise over a 2 week period followed by a fall to normal as the epithelial defect was covered, but occurred in a series of peaks. The peaks were reasonably constant in time and were found at 2, 11–12, 18–19, and 22–24 days with perhaps smaller, less definite rises in between.

Peaks of mitotic activity have been noted in other tissues. Williams (1961) and Phillips & Leong (1967) found them in the kidney of the rat after unilateral nephrectomy. Hübner (1967), also using the rat kidney, noted peaks at weekly intervals at 2, 9, 16, 23 and 20 days after unilateral nephrectomy. The much-studied epidermis has also revealed peaks of mitotic activity after wounding. Giacometti (1967), working on the skin of monkeys, found that cell division at the wound edge was maximal at 32–48 hours and 64 hours. Similar findings were made by Hell & Cruickshank (1963) in the skin of guinea-pigs and by Sullivan & Epstein (1963) and Epstein & Sullivan (1964) studying the epidermal mitotic activity in wounded human skin.

The peak and trough pattern suggests that one effect of injury is to induce a temporary synchrony in the cell cycle so that cell division occurs 'en masse'. Patterns of activity in different animals and experiments in this work did not correspond exactly, possibly because of differences between individuals in their initial responses to injury resulting in their being out of phase with each other in their proliferative cellular activity.

It has long been known that the post-traumatic increase in mitotic activity is confined to the wound edge. Hunt (1958), working on gastric ulcers produced by cauterization, found such increase in mitosis which was confined to a zone 2–3 mm wide around the wound margin. Hell & Cruickshank (1963) and Bullough & Laurence (1960), using epidermis, found increased rates of mitosis only within 1 mm of the wound edge. The rectal mucous membrane in the present study exhibited a

similar localization of mitotic activity, but it must be noted that frequently the proximal 2–3 colonic glands in the *immediate* vicinity of the wound showed markedly less activity than the distal 5–6 glands. This is difficult to explain, because if a so-called 'wound hormone' produced locally by damaged cells is responsible for the rise in mitotic rate then one would expect maximum activity in those glands in the immediate vicinity of the wound.

In opposition to the concept of wound hormones, Bullough & Laurence (1960) and Bullough (1964) support the idea that epithelial cells are ever ready to divide but are held in check by some local tissue-specific inhibitor. When the hypothetical inhibitor is removed or neutralized, subsequent to wounding for example, the mitotic rate automatically rises until the inhibitor is restored. Bullough suggests that the inhibitor is a protein which acts in combination with adrenalin, a combination he called a chalone. Such a substance, provided it was limited in its diffusibility, would explain the localization of the mitotic effects after wounding, but not why the glands *nearest* the wound are apparently less capable of increased mitotic activity, nor why peaks of activity occur.

Stone (1962) suggests that the substance responsible for regulating mitosis may be a substrate, perhaps a lipid or a nucleoside, whose depletion might be a stimulus for cell division after wounding. This theory could also account for the facts in the short term, but does not explain the long term findings.

Perhaps the control of mitosis depends on both local factors and general factors such as the circulatory levels of adrenalin and the adrenal steroid hormones, both of which are known to have a profound effect on mitotic activity. Much more work is required on the levels of these circulating hormones in health and disease before their role can be assessed.

SUMMARY

Ulcers were made by excising small portions of the rectal mucous membrane of rats. The mitotic response to the injury in the epithelium of the glands adjacent to the wound margin was studied over a period of 4 weeks. A rise in the mitotic number lasting 2 weeks began within 12 hours of the injury. There were peaks of mitotic activity at 2, 11–12, 18–19, and 22–24 days. After 2 weeks the numbers of dividing cells rapidly fell to almost normal values, apart from the two later peaks.

As a rule mitotic cells are confined to the basal parts of the colonic glands, but as the mitotic number approaches a peak, mitoses are found in the middle parts of the glands as well.

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