J. Anat. (1975), **119**, 2, pp. 333–345
With 7 figures
Printed in Great Britain

A study of mitotic activity and the diurnal variation of the epithelial cells in wounded rectal mucous membrane

D. R. E. REEVE

Department of Anatomy, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

(*Accepted* 1 *October* 1974)

INTRODUCTION

In a recent paper the present author reported that peaks of mitotic activity occur in the epithelial cells of wounded rectal mucous membrane, the first peak appearing about 48 hours after the traumatizing event (Reeve, 1974). It was noted that the proliferative cellular response was often less intense in the glands at the immediate wound edge as compared with those a little further away.

As part of a further study it was decided to investigate the first 24 hours after injury in order to examine in more detail the initial response to wounding and to confirm the persistence or otherwise of diurnal variation in the response. This paper reports the results obtained by comparing mitotic activity in groups of animals, some wounded in the morning and some in the evening.

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 5–8 mm from the anal margin by pulling the mucous membrane down through the anus with fine forceps and excising an area of approximately 4 mm^2 with scissors. Animals were sacrificed at 2 hour intervals for 24 hours (two animals at each stage) in both experiments. The ulcer region was removed after dissecting the rectum away from surrounding structures and opening its ventral wall. The tissue was pinned to cork, fixed in 3 % glutaraldehyde, and treated according to a standardized schedule. Sections 6 μm thick were cut serially through the ulcer region and stained with haematoxylin and eosin (H & E). Twenty sections were selected which presented both edges of the ulcer cut in true sagittal section, and mitotic counts were carried out on the first eight glands outwards from the wound margin. The gland *at* the margin was numbered as gland 1, the next as gland 2 and so on. Each gland in sagittal section contained about 120 cells, and the positions of the cells in the glands were numbered, position 1 being at the top of one side of the gland, position 60 at the

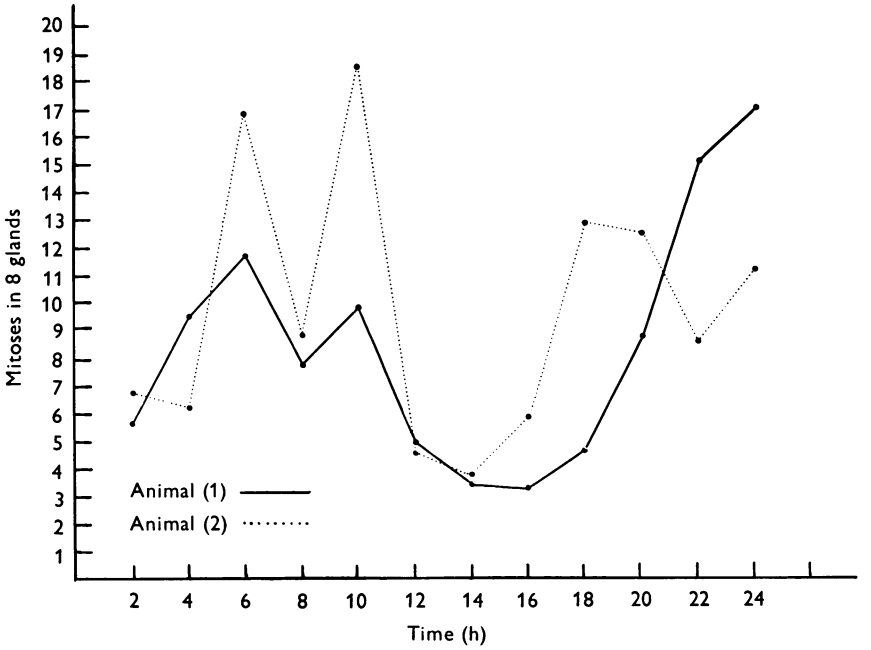

Fig. 1. Results of Experiment 1 (morning series). Note peaks of mitotic activity.

base and position 120 at the top of the other side. The numbers of mitotic nuclei in the epithelial cells of the eight glands were noted and their positions recorded. The total number of cells counted at each ulcer edge was 960 and the total number of mitoses in the group was called the mitotic number and expressed as a percentage. Ten rats, unoperated or sham-operated, were used as controls in each experiment and these gave an average mitotic number of 0·35 %.

RESULTS

Experiment 1

Twenty-four rats were wounded within 20 minutes of each other commencing at 09.00 hours. The animals were subsequently kept in pairs in a quiet, daylit laboratory and disturbed as little as possible. Two animals were killed at 2 hour intervals, mitotic counts being performed on the ulcer edges as described. The results are depicted on a graph (Fig. 1). In both animals an increase in the number of dividing cells above the control rate (0·35 %) was noted within 2 hours. In both animals peaks of activity occurred at 6 and 10 hours after wounding, one animal demonstrating considerably more cellular proliferation than the other. At 12 hours the number of mitoses had fallen to near normal levels in both animals and thereafter showed an increase to the end of the 24 hour period with a mitotic number three to five times the control value (one animal exhibiting a temporary fall in mitoses for 4 hours).

The rectal gland cell histograms show some interesting points. When the mitotic

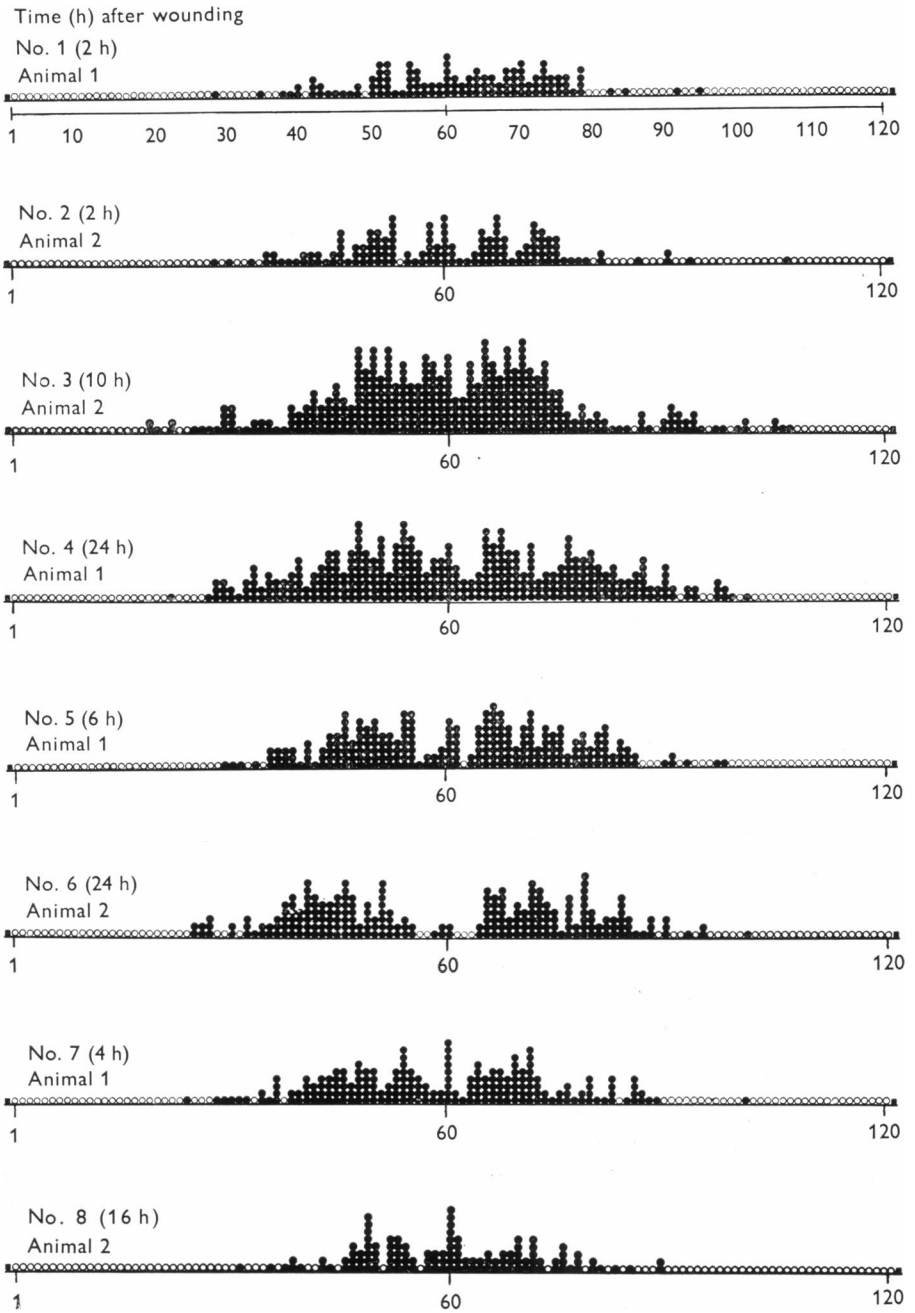

Fig. 2. Rectal gland cell histograms. They represent 'opened out' colonic glands which contain approximately 120 cells when seen cut in sagittal section. Cell 1 is at the surface nearest the ulcer edge and cell 60 is the extreme basal cell of the crypt. The spots represent the total numbers of mitoses found in each cell position in the eight glands at the wound edge in 20 sections for each animal investigated.

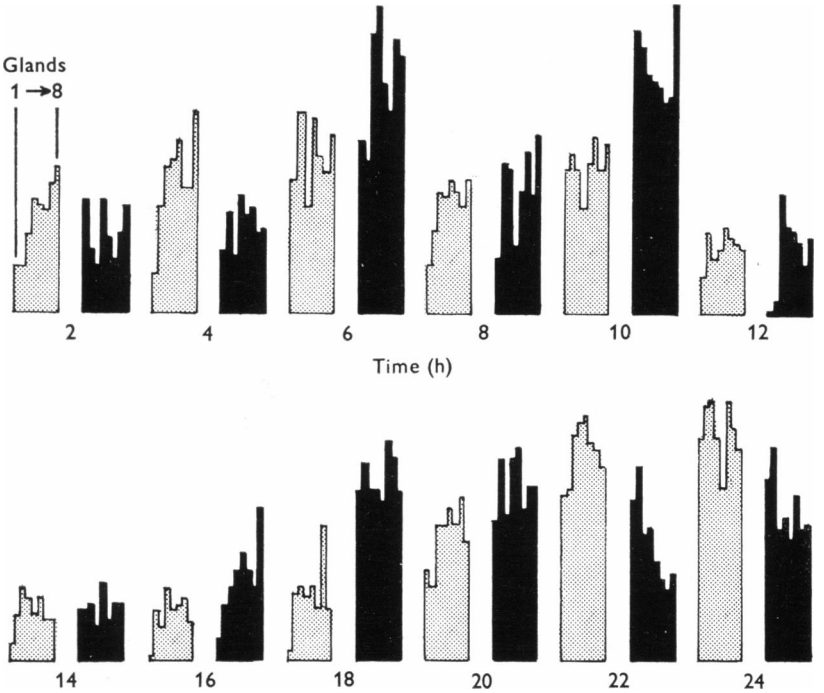

Fig. 3. Eight gland histograms. These show the relative total numbers of mitotic cells in the eight glands at the ulcer edge in 20 sections of each lesion, ▦, Animal 1; ■, animal 2.

activity was low the dividing cells were found near the bases of the glands between cells 40 and 80. A rise in the mitotic number resulted from increased proliferation in these cells (e.g. Fig. 2, nos. 1 and 2). An even higher mitotic number was the result of a combined increase in cell division in these areas and an increased incidence of mitosis higher in the gland (e.g. Fig. 2, nos. 3 and 4). At maximal levels of activity dividing cells began 20 cells from the gland surface. Mitoses were almost never observed, however, in cells 1 to 20 or 100 to 120 (the highest cells of the rectal glands). The highest levels of activity occurred around the gland base, with a decreasing incidence of cell division in the higher aspects of the gland. With such a distribution peaks should have been expected around cell 60 (the very basal cell of the crypt). This did not in fact occur, as the cells on either side of cell 60 often showed less activity than those at slightly higher levels, around cells 50 and 70 (e.g. Fig. 2, nos. 5 and 6). This appears to be particularly true at the lower levels of mitotic activity. On the other hand, cell 60 (the very basal cell) was a common cell to be observed dividing and frequently produced peaks on the cell histogram (e.g. Fig. 2, nos. 7 and 8).

The eight gland histograms (Fig. 3) also provide information about the overall mitotic activity at the wound edge and the peaks which were previously recorded. It was noted that the number of dividing cells counted in gland 1 immediately adjacent to the wound was often less than in the adjacent seven glands. This was also

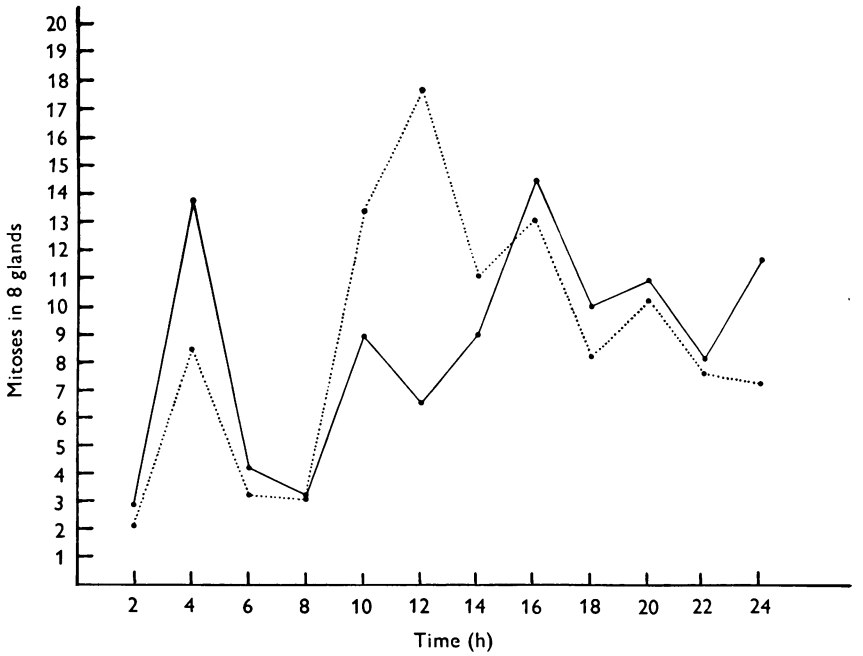

Fig. 4. Results of Experiment 2 (night series). Note peaks of mitotic activity. —, Animal 1; ····, animal 2.

frequently true of gland 2. At lower rates of proliferation activity was highest in the glands farthest from the wound. At higher rates the activity was perhaps more evenly distributed, with less obvious low mitotic levels in glands 1 and 2.

Experiment 2

This was similar to the first experiment except that animals were wounded in the evening instead of the morning. Animals of the same strain, sex, weight and background were selected and given a few days to adapt to quiet conditions. All were operated on within 30 minutes of each other beginning at 21.00 hours and returned at once to their cages and kept there until they were killed. They were caged in pairs, so that removal at 2 hour intervals disturbed the other animals as little as possible. Mitotic counts were carried out at the ulcer edges and the results presented as before (Fig. 4). The graph demonstrates, as in the previous experiment, a number of peaks. In this case the initial increase occurred 4 hours after wounding (control level 0·35 %), which corresponded with the first peak. A second peak, the highest of the experiment, occurred at 12 hours in one animal; a third at 16 hours was noted in both animals; and there was a suggestion of a burst of activity 20 hours after ulceration. The mitotic number in both the animals fell to almost control levels after the first peak, i.e. 6 hours after ulceration. Thereafter, the animals exhibited increasing activity up to 16 hours after wounding, with an overall decrease in the final 8 hour period.

The rectal gland cell histograms show similar points to those noted in Ex-

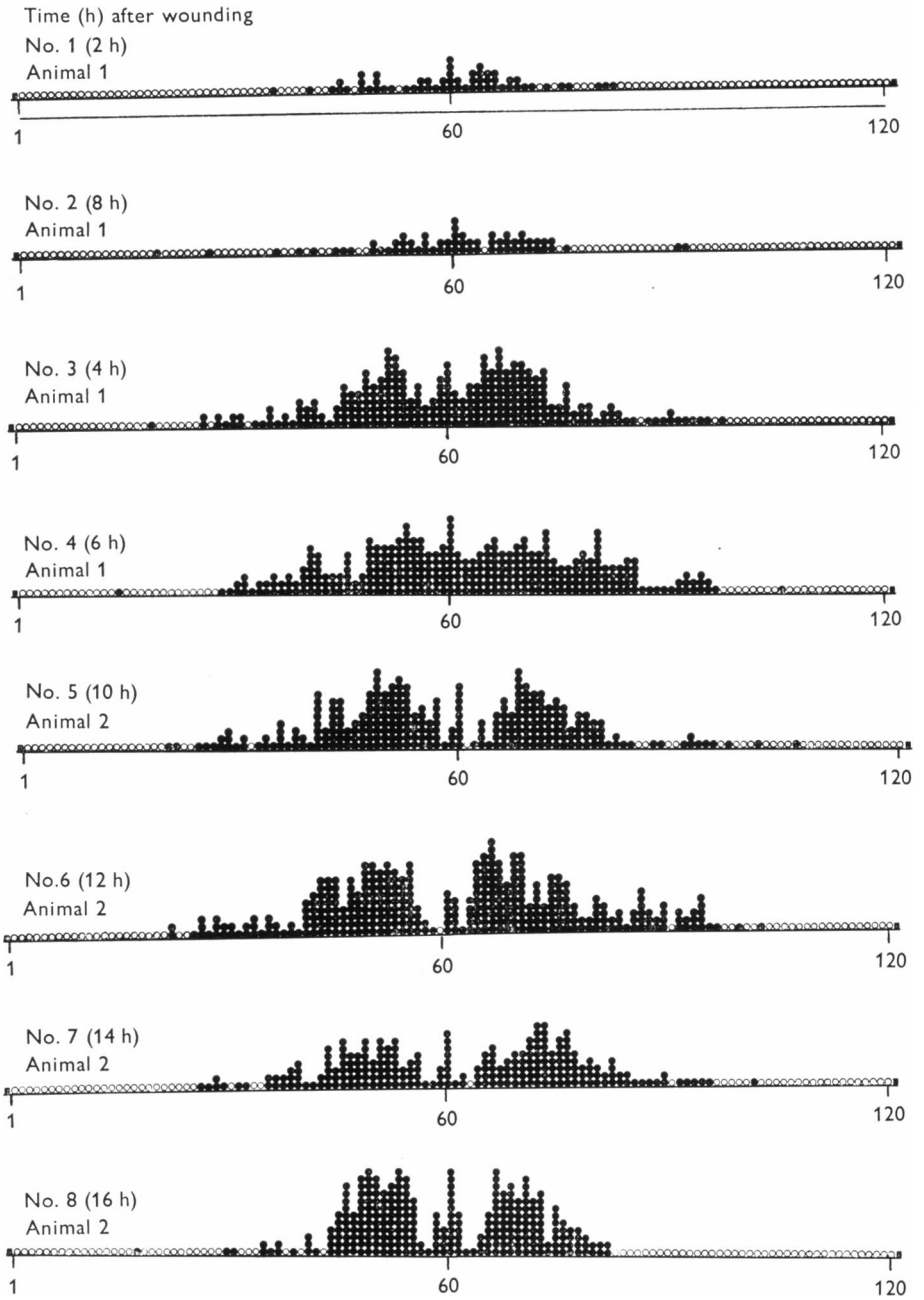

Fig. 5. As for Fig. 2.

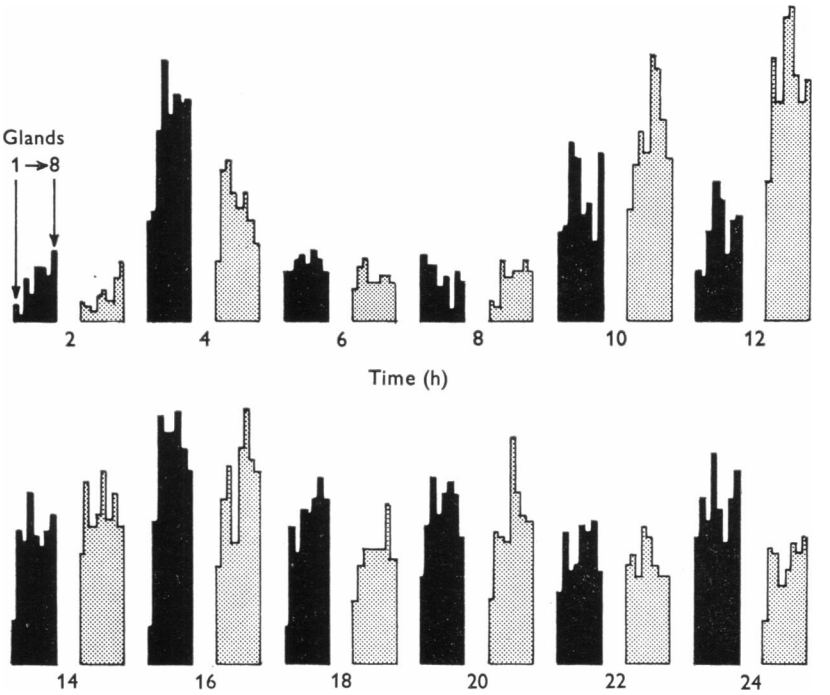

Fig. 6. As for Fig. 3. ▦, Animal 1; ■, animal 2.

periment 1. When the mitotic rate was at normal levels (0·35 % for this experiment) the dividing cells were found in the bases of the colonic glands, between cells 50 and 70 (e.g. Fig. 5, nos. 1 and 2). Greater activity was evidenced by increased division in these cells, whilst with maximal activity dividing cells were also present at a higher level in the colonic glands, between cells 20 and 100 (e.g. Fig. 5, nos. 3 and 4). As before, the cells in the immediate vicinity of cell 60 (i.e. cells 55 to 65) showed less inclination to divide as compared with those slightly higher in the gland (e.g. Fig. 5, nos. 5 and 6). Cell 60 was again a common cell to be observed in mitosis (e.g. Fig. 5, nos. 7 and 8). The other striking similarity with the previous experiment was the fact that only ten mitotic nuclei were found in those cells near the mucosal surface, i.e. cells 1 to 20 and 100 to 120, in all the 240 sections examined.

Gland histograms are equivalent to those of Experiment 1 (Fig. 6). The gland nearest the ulcer edge exhibited the lowest numbers of dividing cells when the mitotic number was near normal. Gland 2 was similar. An increase in the mitotic number was associated with increased activity in glands 1 and 2.

To show the comparative activity in the eight glands examined at the ulcer edge more clearly, results were tabulated for Experiments 1 and 2 in the following manner. The glands were 'valued' in an ascending order, depending on the proliferative cellular activity in the individual gland for all the 24 animals investigated in each experiment. For example, the gland with the lowest number of mitoses in a total of 20 sections per animal was awarded an arbitrary value of 1, whilst the gland with the

Table 1. *Experiment* 1: *two animals sacrificed at* 2 *hour intervals for* 24 *hours*

Time after wounding (h)	Mitotic number	Gland number 1	2	3	4	5	6	7	8
2 (animal 1)	0·68	1	1	3	6	5	4	7	8
2 (animal 2)	0·57	7	3	1	7	4	2	5	6
4	0·95	1	2	5	6	7	3	3	8
4	0·63	2	6	1	8	5	7	3	4
6	1·18	3	7	7	1	6	4	2	5
6	1·68	2	1	7	8	4	3	6	5
8	0·73	1	2	5	4	7	5	3	7
8	0·89	1	6	5	2	4	7	3	8
10	1·08	2	6	2	1	5	8	2	8
10	1·86	7	6	5	4	3	1	2	3
12	0·49	1	6	2	3	7	5	4	3
12	0·46	1	2	8	7	6	4	3	5
14	0·34	1	4	8	6	5	6	2	2
14	0·33	3	3	5	1	8	2	5	5
16	0·33	1	4	2	8	4	6	7	3
16	0·59	1	2	3	5	7	5	3	8
18	0·47	1	5	6	4	6	2	8	2
18	1·29	2	6	2	2	1	8	7	2
20	0·86	2	1	4	4	7	4	8	3
20	1·25	1	6	2	6	8	3	4	4
22	1·50	1	2	6	7	8	5	4	3
22	1·86	7	8	5	6	4	2	1	3
24	1·70	3	6	7	3	1	8	5	2
24	1·12	7	8	2	5	6	1	2	4
		59	103	103	114	128	105	99	111

Mean = 103

Standard deviation = ±21

highest number of mitotic cells was awarded a value of 8. Totals were made for each gland and are compared (Tables 1, 2). The activity in gland 1 in both experiments was considerably (80% and 150%) below average levels, which was considered significant. On the other hand, gland 2 exhibited no statistically significant decrease in activity as compared with the other six glands.

When averages were taken for the two experiments and the results plotted on a graph (Fig. 7), the following points were noted:

(1) The initial rise in mitotic activity occurred earlier in the group ulcerated in the morning (morning series), namely at 2 hours as compared with 4 hours in the group ulcerated at night (night series). The initial rise in the night series could, of course, have occurred considerably before the ulcer was sampled 4 hours after wounding, in which case the difference may not have been significant. It is clear, nevertheless, that the initial mitotic response is rapid.

(2) In both experiments there were increases in mitotic activity after wounding and these occurred in peaks; the first within 4–6 hours of ulceration and the second

Table 2. *Experiment* 2: *two animals sacrificed at* 2 *hour intervals for* 24 *hours*

Time after wounding (h)	Mitotic number	Gland number							
		1	2	3	4	5	6	7	8
2 (animal 1)	0·29	2	1	4	3	6	6	4	8
2 (animal 2)	0·22	3	2	1	5	6	3	7	8
4	1·38	1	2	3	8	4	7	5	6
4	0·84	1	7	8	5	4	5	3	2
6	0·42	1	1	5	7	4	8	5	1
6	0·33	1	7	8	2	2	5	5	2
8	0·33	8	6	6	2	4	1	5	3
8	0·33	2	1	7	3	4	4	7	4
10	0·89	2	3	8	6	4	5	1	7
10	1·35	1	2	5	3	8	7	6	4
12	0·66	2	1	4	8	7	3	5	6
12	1·77	1	6	2	7	8	4	3	5
14	0·90	1	5	3	8	4	2	5	7
14	1·11	1	7	2	5	8	4	6	2
16	1·46	1	2	7	5	5	8	4	3
16	1·32	1	3	6	2	7	8	4	5
18	1·00	1	3	2	5	4	6	8	6
18	0·82	1	2	4	4	4	4	8	3
20	1·10	1	3	8	4	5	7	5	2
20	1·03	1	4	3	2	8	7	6	5
22	0·82	1	5	2	4	7	5	8	2
22	0·76	4	6	1	8	7	4	1	1
24	1·17	2	6	3	5	4	1	6	8
24	0·73	1	5	4	1	3	6	7	8
		43	90	106	116	127	120	124	108

Average = 104

Standard deviation = ±21

6 hours later. The peak times in the two animals corresponded reasonably well, although mitotic levels were often at variance.

(3) The mitotic number fell close to the control values 12 hours after wounding in the morning series, the equivalent interval being 6 hours in the night series.

(4) It was noted that the highest levels of activity in the morning series occurred between 6 and 10 hours after ulceration, i.e. between 15.00 and 19.00 hours. The equivalent period in the night series, however, produced the lowest levels of activity between 15.00 and 19.00 hours.

(5) The lowest levels of activity in the morning series were noted between 12 and 16 hours after wounding, i.e. between 21.00 and 1.00 hours. This interval showed maximal levels of activity in the night series between 09.00 and 13.00 hours.

(6) After 01.00 hours the morning series showed a gradual rise in mitotic activity, whereas the night series after 13.00 hours showed a gradual fall in mitotic levels.

These results indicate that the mitotic activity in wounded rat rectal epithelium exhibits a diurnal variation with maximal activity during the day and minimal activity

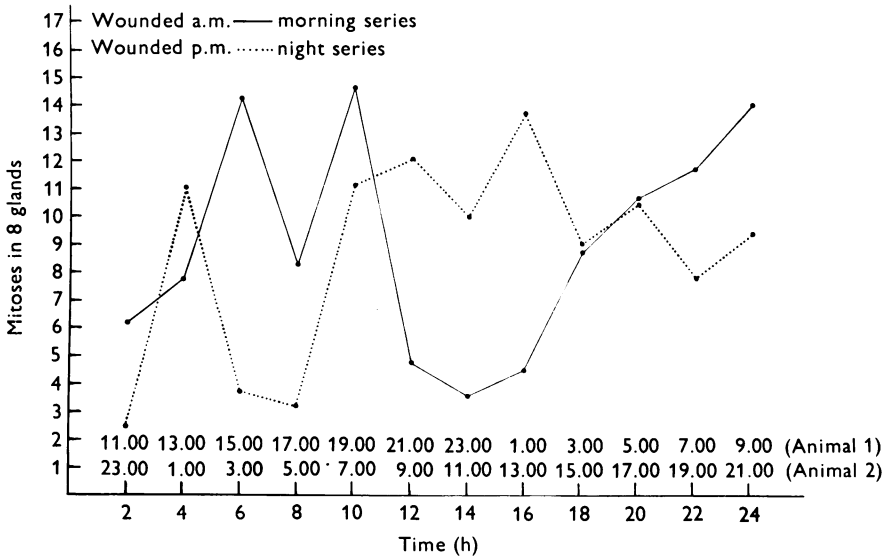

Fig. 7. The average results of Experiment 1 (morning series) and Experiment 2 (night series). —, Animal 1; ····, animal 2.

during the night. The initial response to wounding, on the other hand, may not have been influenced by the time of day or night.

DISCUSSION

It has been known for a long time that many tissues exhibit a diurnal variation in mitotic rates. Blumenfeld (1943), using rabbits, was among the first to show that such variation was present in regenerating epidermal epithelium, with a low point during the day-time and a high point during resting periods at night. Gololobova (1960), in contrast, found no diurnal variation in regenerating skin epithelium in rats, although it was noticed in the adjacent normal epithelium. Williams (1961) noted diurnal variations in the tubules of the remaining kidney of nephrectomized rats, with a maximum between 14.00 and 16.00 hours and a minimum at 06.00–08.00 hours.

Phillips & Leong (1967), also working on the rat kidney, noted a high point at midnight and a low point at noon. On the other hand Jaffe (1954) found, in regenerating rat liver, that high rates of mitosis occurred in the mornings and low rates at night, and this was confirmed by Weinbren & Tarsh (1964).

On this evidence it seems likely that the diurnal rhythm is strain-specific. It is possible that this variation is responsible for the peaks of mitotic activity which occur after wounding, as noted in rectal epithelium in this work and in the other tissues mentioned above.

In Experiment 1 the animals ulcerated at 09.00 hours demonstrated high activity between 15.00 and 19.00 hours and again at 09.00 hours the following morning. The animals ulcerated at 09.00 hours in Experiment 2 showed an initial peak and maximal

activity between 11.00 and 13.00 hours. The time interval between peaks was approximately 12 hours, and in both groups activity during the night was low whilst during the day it was high. In previous experiments in which peaks of mitotic activity were recorded 2, 11–12, 18–19 and 22–24 days after wounding rectal mucous membrane (Reeve, 1974), animals were killed at 10.00 hours when cell division was approaching a maximum. Therefore it is tempting to speculate that those bursts may have been a reflexion of the individual animal's diurnal mitotic rhythm. On the other hand, peaks were not obtained every day, as might be expected if this theory is correct. After the initial peaks neurons were recorded at roughly weekly intervals: no explanation can be given for this periodicity. Sampling would need to be done at, say, 4 hour intervals if the true picture is to be revealed.

It is known that the rate of adrenaline secretion varies from hour to hour in both men and rats (von Euler, 1956), the rate being lowest during periods of rest and sleep and highest during periods of exercise and stress. It has been suggested that this effect of stress and exercise may provide the basis of the diurnal cycle of mitotic activity which is seen in tissues such as the epidermis (Bullough & Laurence, 1961). In effect, Bullough (1964) demonstrated that adrenaline acted in conjunction with a cellular protein (which he called a chalone) to effect a mitotic depression which was maximal during the stress and activity of the day.

The experiments in this work seem to indicate that it is most unlikely that an adrenaline–chalone complex acts on rectal mucosa, for with this theory most activity would be expected at night when adrenaline secretion is (presumably) low. It would also be expected that the diurnal variation would be abolished by wounding if the regulation of mitotic control in rectal mucosa is effected by the chalone complex, as Bullough (1965) has shown that it is in the skin.

In the work done on liver (e.g. Jaffe, 1954) the diurnal variation persisted after wounding and there were high points during the day and low ones at night. It is possible, therefore, that the alimentary tract epithelium and its derivatives, such as liver parenchymal cells, with a common endodermal origin and presumably arising from the same stem cell line, exhibit a common diurnal variation controlled by similar factors. Work is required on the levels and fluctuations of hormones, other than adrenaline, such as the adrenal steroid hormones and sex hormones, which have effects on mitotic activity, before these complex problems can begin to be answered.

It is well known that the increase in mitotic rate in epithelial cells after wounding is limited to a zone 2–3 mm out from the wound edge. Studies on mouse ear skin (e.g. Bullough & Laurence, 1960; Gelfant, 1959) indicated that the highest incidence of metaphases was observed at the *immediate* wound edge. On the other hand Hell & Cruickshank (1963), working on the skin of guinea-pig ear, did not find many mitoses in the first hundred or so cells bordering the cut. This view conforms with the present work, where activity in the first two glands which immediately bordered the wound was frequently found to be less than in those glands more distally placed. This difference was less obvious during peak mitotic activity (Figs. 3, 6).

The possible origins of cells which are responsible for eventual healing of the defect are several. The cells could arise from surface epithelium, from an adjacent damaged gland whose cells revert to a more primitive form, or from the newly dividing

cells associated with the bursts of mitotic activity. Melnyk, Braucher & Kirsner (1966) thought surface cells replaced an epithelial defect, although they did not see mitoses in adjacent surface epithelium producing new cells to compensate for those lost in migration. The cell histograms (Figs. 2, 5) indicate that the progenitor cells are situated in the depths of the mucosal gland, a fact noted by Lipkin, Bell & Sherlock (1963) in the normal colon and rectum of man. Lipkin and his associates envisaged these cells migrating from the gland bases to the surface. The cells at or near the surface rarely divide and are presumably irreversibly mature. With increased demand, however, cells in the mid-regions of the gland appear capable of division. Hence, it would appear that gland cells replace the surface epithelial cells lost to the migrating epithelial sheet that bridges the defect.

SUMMARY

Excision ulcers of the rectal mucous membrane were made in two groups of rats. One group was wounded at 09.00 hours and the second group at 21.00 hours. Mitotic counts were carried out in the glandular epithelium at the ulcer edges at 2 hour intervals over a period of 24 hours. Mitotic activity increased in 2–4 hours and thereafter showed a peak-and-trough pattern. The wounded rectal epithelial cells exhibited a diurnal variation with a peak of activity during the day and a low period of activity at night. It would seem unlikely that the adrenaline–chalone complex acts on the rectal epithelium, as this would entail maximal mitotic activity during periods of rest, when the circulating levels of adrenaline in the rat are at their lowest. The experiments clearly showed that the diurnal variation was not abolished by wounding. The increased mitotic activity occurred in the epithelial cells in the lower and mid thirds of the colonic glands; dividing cells were rarely seen in the top twenty cells or so of the glands, or in the surface epithelium. Mitotic activity was often lower in the first one or two glands at the immediate wound edge, which is difficult to explain by present theories of mitotic control.

I wish to thank Professor R. M. H. McMinn for his support and interest in this work. Thanks are also due to Mr S. Geschmeissner and Mr T. Meyers for technical assistance.

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