THE FORMATION OF VILLI FOLLOWING ARTIFICIAL LESIONS OF THE MUCOSA IN THE SMALL INTESTINE OF THE CAT

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INTRODUCTION

The mechanism of growth and the reparative power of tissues were reviewed in 1894 by Bizzozero, who included epithelial coverings among those tissues which could continue to multiply throughout the life of the individual. The fact that the epithelial cells of the alimentary tract could be included in that category was well appreciated, and numerous investigators, both before and after that time, have demonstrated the regeneration of intestinal epithelium following a variety of lesions. In recent decades the stomach and duodenum have been selected for intensive study by many workers, in view of the problems of peptic ulceration in man. In the course of a series of studies on Brunner's glands, Florey & Harding (1935) investigated the healing of artificial defects of the duodenal mucosa of the cat. In these studies it was found that not only did complete epithelialization of the lesion occur but new villi were formed. In this way an almost normal pattern of mucosal architecture was restored with the exception of the muscularis mucosae which did not regenerate. Thus reconstitution of part of an organ occurred as well as regeneration of epithelium. These findings were contrary to views then current on the extent of healing of intestinal lesions, which was commonly believed to be confined to a simple epithelial repair overlying scar tissue. Healed lesions of the small intestine (excluding the duodenum) in man are not commonly examined, because their presence is not suspected or their site is not known. Brown & Sampson (1930), however, have cited evidence to the fact that in certain cases of intestinal tuberculosis, especially where the ulceration has been superficial, a complete epithelial covering has been formed with new, though irregular, gland formation.

The present work was carried out to investigate the mode and extent of villous formation at the site of artificial lesions of the mucosa in the ileum of the cat. At the same time the opportunity was taken to make observations in a number of the experimental animals on the mitotic activity of the regenerating epithelium, using colchicine.

MATERIAL AND METHODS

All experiments were performed on healthy adult cats. An 'artificial ulcer' was created in the small intestine by operation, and the site of this lesion was examined histologically at various post-operative periods. In a number of animals, colchicine was used to arrest mitosis.

Operative technique

The cats were starved for a pre-operative period of 24 hr. They were anaesthetized with pento-barbitone given intraperitoneally, following which the abdominal cavity was opened and a loop of small intestine was brought out through the wound. At

a site 45 cm. proximal to the ileo-caecal junction, an incision 2·5 cm. in length was made along the anti-mesenteric border, and the mucosal surface of the mesenteric border was protruded through the incision by a finger of the assistant. A square, approximately 1 sq.cm. in size, was mapped out on the protruding mucosa surface by four incisions. From this delimited area, the whole thickness of mucosa and submucosa was removed. During this part of the procedure it was found that at the level of the inner circular muscle of the intestinal wall there was a plane of easy cleavage which facilitated the stripping off of the overlying mucosa and submucosa. The intestinal and abdominal wounds were then closed, a continuous Connell suture being used for the intestinal wall. The animals were starved for a post-operative period of 24 hr. before the gradual resumption of normal feeding.

The lesion thus created in the small intestine is referred to throughout this paper as an 'artificial ulcer'.

For the study of mitotic activity in regenerating epithelium, colchicine was used to arrest mitosis in metaphase. The dose was 0·25 mg./kg. of body weight, given intraperitoneally in aqueous solution 5 hr. before death. Using this dosage and time interval it was found that dividing nuclei were in the stage of metaphase and later phases of the mitotic cycle were not seen (Pl. 2, fig. 16).

The animals were killed 1, 2, 3, 4, 7, 10, 14, 42 and 100 days after operation, four animals being used for each time period.

Histological technique

The portion of intestine bearing the ulcer was removed, opened along the original incision and pinned out on cork prior to fixation in 80% alcohol. After embedding in paraffin wax, sections 7 μ thick were cut and stained with either haematoxylin and eosin or with iron haematoxylin and picrofuchsin. Some sections were stained with acid fuchsin and light green. For the demonstration of mucin the Gomori (1946) technique was used following preliminary digestion with ptyalin (saliva).

Mitotic counts were carried out by counting the resting and dividing nuclei of epithelial cells. The number of nuclei in arrested mitosis was then expressed as a percentage of the total number, at least 2000 nuclei being included in any single count. The counts were carried out using a square mask in one of the oculars of a binocular microscope, the nuclei being counted in contiguous fields from the bases of crypts to the tips of villi. Although mitosis normally occurs only in the epithelium which lines the crypts, the percentages expressed refer to the whole epithelial covering including that of the villi. Counts were undertaken in the epithelium of undisturbed mucosa in regions immediately adjacent to the original ulcer margin, where both crypts and villi were cut approximately throughout their length. Thus it was possible to gauge the mitotic activity of the epithelium at comparable sites during different stages of the healing process. Control counts were carried out on sections cut at 7 μ and stained with haematoxylin and eosin, taken from sites 15 cm. distal to the ulcers. Random fields were chosen and counts undertaken only where the crypts and villi were sectioned approximately throughout their length.

RESULTS

Gross appearance

The site of operation in the intestine was identified externally by unabsorbed catgut, and a varying number of peritoneal adhesions were usually present. In the early post-operative period, the floor of the ulcer resembled reddish granulation tissue, and by the end of the second week a shallow depression with the colouring of the surrounding mucosa was the only evidence of the lesion. There was nothing in the mucosal surface of the 42- and 100-day specimens to suggest that there had been operative interference, and portions of unabsorbed catgut alone indicated the region to be examined histologically, although on palpation a slight thickening of the intestinal wall could be appreciated.

Histological examination

Histological examination of the artificial ulcer 24 hr. after operation (Pl. 1, fig. 1) revealed the presence of blood clot, with round cell infiltration, covering the floor of the ulcer. There was some oedema of the underlying muscle. The periphery of the clot was covered with a single layer of flattened cells with centrally placed nuclei (Pl. 2, fig. 8). When traced towards the margin of the ulcer these cells gradually became taller with more basally placed nuclei and were in continuity with the normal columnar epithelium. After 2 days the cells had extended for a distance of approximately 500μ towards the centre of the ulcer, and the mucosal margin had fallen towards the floor of the ulcer (Pl. 1, fig. 2). An increasingly wide area of ulcer was covered during subsequent days by this single layer of cells which often burrowed beneath the clot. By the fourth day the flattened type of cell was no longer seen; all were cuboidal or low columnar (Pl. 2, fig. 9), and at the periphery of the ulcer occasional globlet cells were seen. Shallow depressions now appeared in this hitherto level covering at the periphery, and examination after 7 and 10 days suggested that these were deepening to form pits, the deepest being those nearest the ulcer margin (Pl. 2, fig. 13). Goblet cells were now more frequent.

At the time when the cellular layer first became pitted (4 days) a number of rounded cyst-like spaces were seen at a deeper level (Pl. 2, fig. 15). These were lined with a single layer of cells similar to those on the surface—usually columnar but in places much flattened. The examination of serial sections revealed that these spaces were continuous with the lumen of the intestine at the bases of the pits, of which they appeared to be the dilated terminations.

While the above changes were taking place, granulation tissue had been accumulating beneath the clot (Pl. 1, fig. 3) and organization was occurring with the formation of a varying amount of fibrous tissue in the base of the ulcer. The organizing tissue showed signs of considerable vascularity, with the larger vessels coursing towards the ulcer surface. The muscularis mucosae could be seen to end abruptly where it had been cut at the original margin of the ulcer.

By the end of the second week almost the whole ulcer had become covered by a single layer of cells (Pl. 1, fig. 4); the surface in the central area remained flat, the cells being of columnar type (Pl. 2, fig. 14), while towards the periphery the

deepening depressions and pits gave rise to a pattern of increasingly long finger-like projections clothed with tall columnar cells and many goblets (Pl. 2, fig. 12).

After 42 days the central area of the ulcer was no longer flat but showed irregularities similar to the projections already well-defined peripherally (Pl. 1, figs. 5, 6).

Sections of ulcers examined 100 days after operation revealed a picture very similar to that of normal small intestine (Pl. 1, fig. 7). Narrow finger-like projections with intervening pits, covered by closely packed columnar cells, many of goblet type, were seen overlying a 'submucosa' composed of collagenous tissue of varying density. The muscularis mucosae was still seen to end abruptly at the original ulcer margin (Pl. 2, fig. 11), and although a few muscle fibres from the inner circular muscular layer of the intestinal wall intermingled with fibrous tissue in the submucosa in the original ulcer area, no muscle fibres were seen to course into the projections. Blood vessels, however, were prominent in the cores of the projections.

The results of mitotic counts are summarized in Tables 1 and 2. Counts at control sites indicated that 3·77 % of the total number of epithelial nuclei were in arrested mitosis 5 hr. after colchicine injection (Table 1). The figures obtained from the epithelium at the margins of ulcers at varying stages during the healing process (Table 2) suggest that mitotic activity was greater 24 hr. after operation than at other times, but the number of animals used for these observations was small and this apparent increase was not statistically significant ($P > 0{\cdot}05$).

DISCUSSION

The results show that, following repair, the site of the artificial ulcer eventually bears a close resemblance to normal intestine, the mucous membrane as a whole (with the exception of the muscularis mucosae) being reconstituted.

The regeneration of the epithelium itself proceeds in a manner which is well known. In epithelial coverings such as the epidermis and the mucous membrane of the intestine there is considerable regenerative activity even under normal conditions (Maximow & Bloom, 1952), in order to replace the frictional loss of cells to which these coverings are constantly subjected. In the case of the intestine, mitoses in the crypts are the source of the cells which make good this loss (Le Gros Clark, 1952). Leblond & Stevens (1948) showed that in the small intestine of the rat this physiological replacement occurred by a migration of cells from the bases of the crypts towards the tips of the villi, from which the cells were eventually shed. The process was continuous and was not significantly influenced by feeding. A similar process has more recently been demonstrated to occur among certain cells of the rat's stomach (Stevens & Leblond, 1953) but at a much slower rate.

The present studies indicate that crypts in the normal mucosa immediately adjacent to the ulcer margins are the source of the cells which become pushed over the floor of the ulcer. There is no evidence to suggest that any cells of the underlying granulation tissue are transformed in order to contribute to this epithelial layer, in a manner that has been described for endometrium (Papanicolaou, 1933). It has been said of epithelia in general that a solution of their continuity leads to 'a local dispersal of cells' which by the 'lessening of their mutual pressure appears to induce their multiplication' (Wright, 1950). To ascertain whether this could be applied to

Table 1. *Percentage of dividing nuclei in intestinal epithelium at control sites, 5 hr. after injection of colchicine*

	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
Cat 36	2220	74	3·33	3·74
	2612	108	4·13	
	2425	81	3·45	
	2510	98	4·06	
Cat 31	2293	85	3·85	3·58
	2223	84	3·95	
	2254	75	3·33	
	2190	70	3·19	
Cat 39	2298	92	4·00	3·99
	2375	95	4·00	
	2377	102	4·29	
	2101	77	3·67	

Mean of average percentage in arrested mitosis: 3·77.

Table 2. *Percentage of dividing nuclei in intestinal epithelium at ulcer sites 5 hr. after injection of colchicine*

	Days after operation	Total no. of nuclei	No. of nuclei in arrested mitosis	Percentage of nuclei in arrested mitosis	Average percentage
		At ulcer margin			
Cat 63	1	2385	103	4·32	4·28
		2313	99	4·28	
		2439	100	4·10	
		2303	102	4·42	
Cat 31	1	2175	115	5·34	5·67
		2176	146	6·71	
		2181	105	4·82	
		2032	118	5·81	
Cat 64	1	2096	110	4·77	4·79
		2132	114	5·35	
		2101	103	4·90	
		2302	96	4·17	
Cat 50	2	2036	64	3·13	4·34
		2086	70	3·35	
		2165	146	6·75	
		2325	96	4·13	
Cat 24	4	2330	86	3·69	4·08
		2382	112	4·70	
		2697	98	3·63	
		2042	88	4·31	
Cat 49	7	2403	75	3·11	3·13
		2119	70	3·30	
		2366	71	2·91	
		2025	65	3·20	
Cat 39	100	2176	84	3·81	3·79
		2267	81	3·57	
		2154	91	4·22	
		2135	76	3·56	
		At centre of healed ulcer			
Cat 36	100	2086	66	3·11	3·49
		2318	85	3·65	
		2132	75	3·52	
		2201	81	3·68	

intestinal epithelium, observations on the regenerative capacity of the epithelium were made by mitotic counts. These were carried out on animals receiving colchicine in preference to those to which it was not administered, as it was throught more desirable to compare mitotic activity in different sites over a period of time—in this case 5 hr.—than at the time of death. Although at first sight the figures (Tables 1 and 2) suggest that mitotic activity is greater 24 hr. after the production of the gap in the epithelial covering than at other times, this is not confirmed by statistical analysis. It would appear, therefore, that the presence of a defect in the intestinal epithelium does not serve as an added stimulus to the cell multiplication which is already in progress.

The observations with colchicine confirm that in the cat, as in other animals, epithelial mitosis is confined to cells in the crypts; only rarely is a dividing nucleus seen near the base of a villus, and none is found over the more distal areas of a villus or in the advancing epithelial layer. It is to be noted that multiplication does not occur among those cells which are in the fore-front of the advance to re-establish continuity. During the first 2 days the cells over the periphery of the ulcer bed have a flattened appearance, but at later stages this type of cell is no longer seen and they gradually assume a columnar form (Pl. 2, figs. 8–10). It is of interest to draw a parallel between the mechanism of repair taking place in the epithelium of the intestine and in that of the cornea. In these two tissues epithelial regeneration is comparable only in so far as the cells which cover the defect in the first instance do not show mitotic activity; the intestinal lesion is covered by newly formed cells from adjacent crypts, but in the case of stratified corneal epithelium the wound is made good by the migration into the gap of 'old' cells from the more superficial layers (Mann, 1932; Arey & Covode, 1943). With the formation of new crypts (see below) mitotic activity is brought nearer the centre of the ulcer, and arrested mitosis in their epithelial lining was first observed on the seventh post-operative day. It follows that up to this time the epithelial covering of the ulcer has been derived from 'old' crypts, and that subsequently the newly forming and newly formed crypts are responsible for the addition of cells to this covering, with the 'old' type then reverting to the physiological replacement of cells covering villi.

The precursors of new crypts are seen as early as the fourth post-operative day, as gradually deepening depressions of the epithelial covering into the underlying granulation tissue. The presence of cyst-like spaces under the epithelium near the ulcer margin at this time (Pl. 2, fig. 15) was an unexpected finding. It was first thought that these might be similar to the cysts seen by Giani (1907) in regenerating bladder epithelium, but the study of serial sections revealed their continuity with the lumen of the gut. In later stages they assume the slit-like character of the normal crypt. The flattening of their epithelial lining that is sometimes observed may be attributed to transient blocking of the narrow neck of the space, which may have caused a temporary increase in pressure.

It is by the deepening of primitive crypts that a villous configuration is first achieved, rather than by the upward growth of projections into the lumen of the gut (Pl. 2, fig. 13). While the epithelial downgrowths are developing, the underlying granulation tissue is becoming organized. Such organizing tissue as remains between the deepening crypts is destined to become the stroma of villi. In contrast to epi-

thelial cells, which are derived from parent cells as a result of considerable mitotic activity, only very rarely is a cell in division seen in the differentiating stroma; here movement of cells rather than mitosis is the keynote. By the end of 2 weeks villi approaching the normal pattern are readily identified (Pl. 2, fig. 12). Subsequent elongation and the development of a characteristic vascular pattern in the core give rise to the typical finger-like appearance of the fully formed villus. The site of the lesion after 100 days demonstrates the fact that a restoration of mucosal architecture closely resembling the normal has occurred. Crypts and villi have been reconstituted, and although the crypts are less deep and the villi less closely packed than is usual, individual villi appear similar to those in the surrounding mucous membrane, except that they are not seen to contain prolongations of musculature from the muscularis mucosae. The cut edge of the muscularis mucosae can be identified at the original margin of the ulcer at all stages of the healing process examined (Pl. 2, fig. 11). The absence of regeneration in this component of the intestinal wall has been repeatedly observed both in animals and in man.

Although by the second day the mucosal margin has fallen towards the floor of the ulcer (Pl. 1, fig. 2) there is no other evidence to indicate that the defect is being made good by a sliding of the mucosa, with or without the submucosa, towards the ulcer centre. The new submucosa contains a greater proportion of collagenous fibres than in regions not subjected to operative interference. As in the case of the cat's duodenum (Florey & Harding, 1935) the lesion has healed not only by epithelialization but by a reconstitution of mucous membrane comprising both crypts and villi. In both duodenum and ileum the new villi are less closely packed than in normal mucosa; in the duodenum Brunner's glands also regenerated but the muscularis mucosae, as in the ileum, showed no evidence of repair.

These experiments indicate that it is possible for a very complete repair of the intestinal mucous membrane to take place. In these experimental wounds there is a minimum of inflammatory reaction unaccompanied by the infective elements which so often complicate the healing of disease processes. The endarteritis of pathological lesions of the alimentary tract mitigates against an optimum response, and it seems probable that the relatively high vascularity of the experimental lesion contributes in no small measure to the degree of repair that can ultimately be achieved.

SUMMARY

1. The healing of mucosal lesions in the small intestine of the cat has been studied following the operative removal of areas of mucosa and the underlying submucosa, in order to examine the mode and extent of villous formation at the site of such a lesion.

2. Epithelialization occurs by cell division in the crypts; in the early stages in the crypts of the normal mucosa at the ulcer margins and later in the new crypts formed in the floor of the ulcer. Mitosis does not occur in the advancing epithelial layer. Histological observations using colchicine suggested that mitotic activity in the epithelium was greater 24 hr. after the production of a gap in the epithelial covering than at other times, but this apparent increase was not found to be statistically significant.

3. The precursors of new crypts and villi are first seen on the fourth post-operative day. Downgrowths of the epithelial surface into the underlying granulation tissue gradually deepen to form the crypts; the organizing tissue that remains between the developing crypts becomes the stroma of new villi.

4. Mitosis is first seen in newly forming crypts on the seventh post-operative day.

5. The muscularis mucosae does not regenerate and the submucosa contains a greater proportion of collagenous fibres than normal.

6. After 100 days the reconstituted villi are less closely packed and the crypts less deep than is usual, but the pattern of mucosal architecture bears a close resemblance to the normal.

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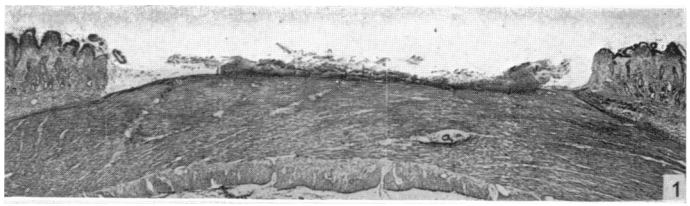
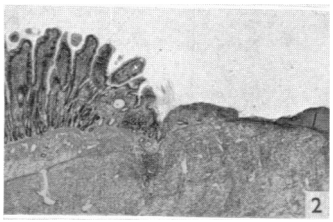
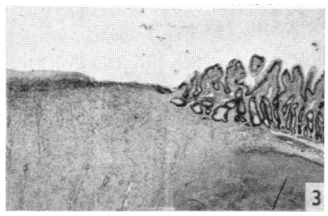
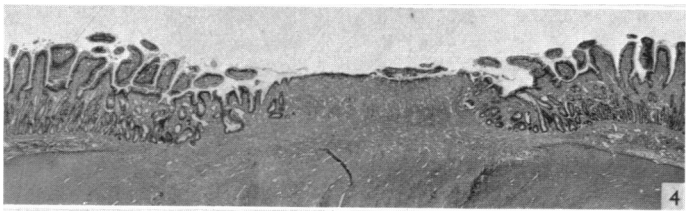
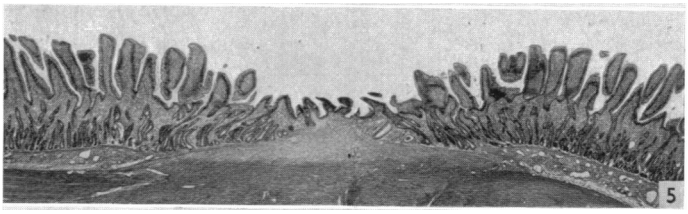
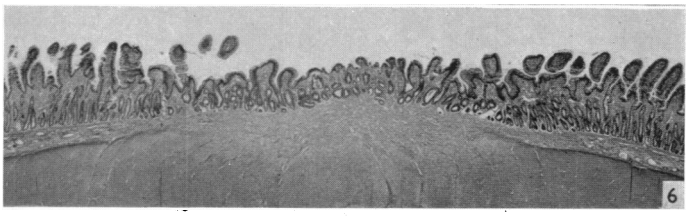


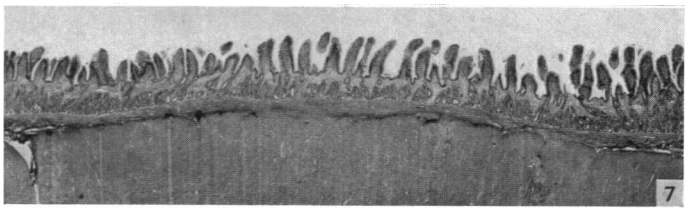




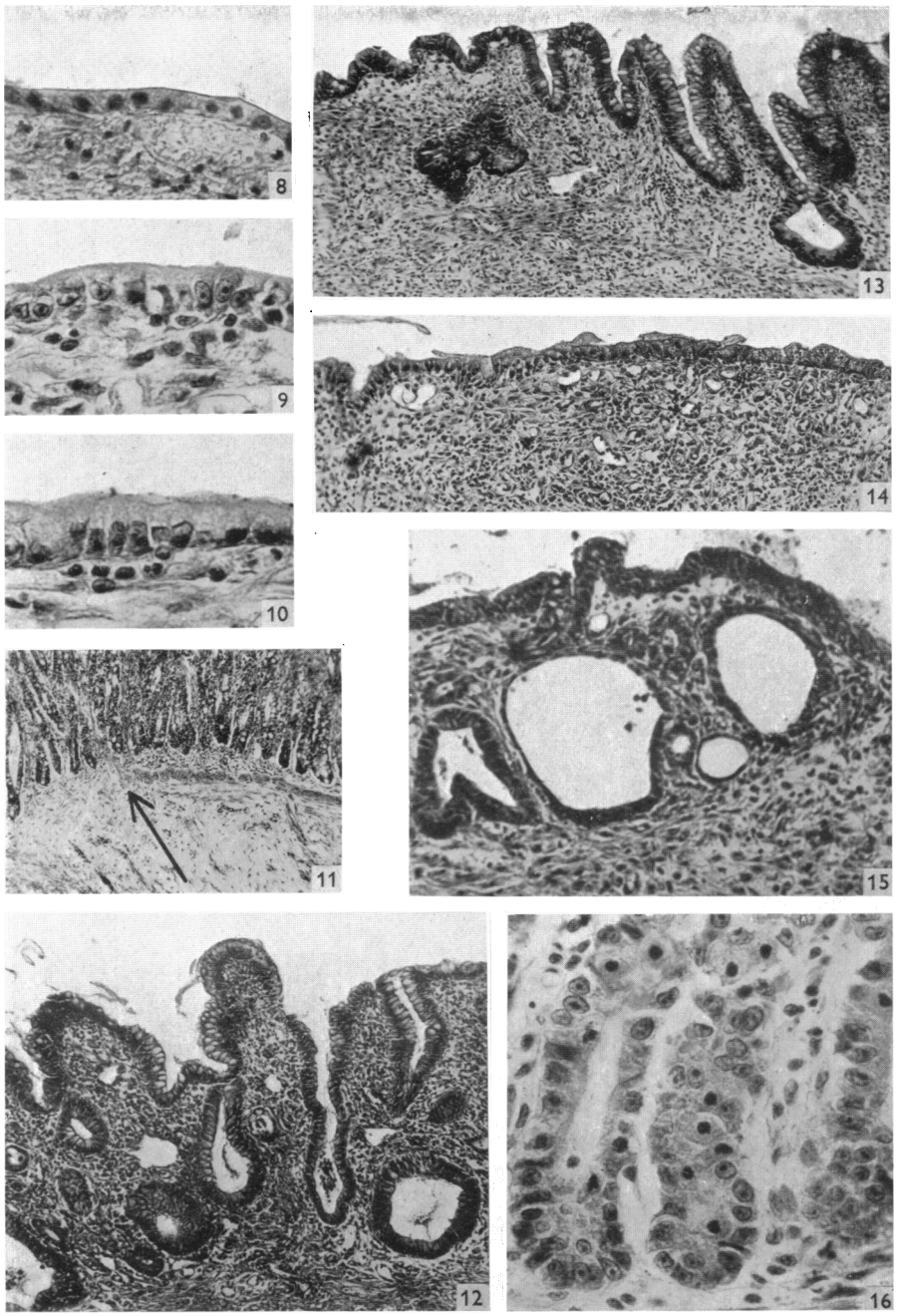








McMINN AND MITCHELL—FORMATION OF VILLI FOLLOWING ARTIFICIAL LESIONS OF THE MUCOSA



McMINN AND MITCHELL—FORMATION OF VILLI FOLLOWING ARTIFICIAL LESIONS OF THE MUCOSA

EXPLANATION OF PLATES

PLATE 1

All sections were stained with haematoxylin and eosin. Magnification, × 11.

Fig. 1. Section through the centre of an artificial ulcer 24 hr. after operation, showing that mucosa and submucosa have been removed, leaving the inner circular layer of muscle and overlying blood clot to form the floor of the ulcer. A thin layer of epithelium is seen over the periphery of the floor on the right.

Fig. 2. Ulcer margin after 2 days. The edge of the mucosa has fallen towards the floor of the ulcer.

Fig. 3. Ulcer margin after 7 days. Granulation tissue has been accumulating in the floor of the ulcer, and new crypts and villi are forming at the periphery.

Fig. 4. Section through the centre of an ulcer after 14 days. The epithelial covering is almost complete and remains flat in the central area. Towards the periphery new crypts and villi are seen.

Fig. 5. Section through the centre of an ulcer after 42 days. The whole surface shows irregular projections into the lumen of the gut, with some underlying fibrous tissue.

Fig. 6. After 42 days, from the same ulcer as fig. 5 but nearer the periphery. The formation of crypts and villi is more clearly defined.

Fig. 7. Section through the centre of an ulcer after 100 days. Crypts and villi have been reconstituted. The crypts are less deep than normal and the villi less closely packed. The submucosa is more fibrous than normal.

PLATE 2

All sections were stained with haematoxylin and eosin, except fig. 11 where the stain was acid fuchsin and light green.

Fig. 8. 1 day. Flattened cells with centrally placed nuclei are overlying the periphery of the ulcer. × 380.

Fig. 9. 4 days. The cells covering the periphery of the ulcer are now cuboidal or columnar. × 380.

Fig. 10. 7 days. In the advancing epithelial layer, the cells are becoming more columnar (towards the left) as the ulcer margin is approached. Compare with figs. 8 and 9. × 380.

Fig. 11. 100 days. The section, from the region of the original ulcer margin, shows the abrupt termination of the muscularis mucosae (indicated by the arrow). × 40.

Fig. 12. 14 days. Near the ulcer margin deepening crypts are seen with the accompanying formation of villi. Compare with fig. 13 which shows earlier stages of this process. × 75.

Fig. 13. 7 days. The depressions in the epithelial covering have deepened to form pits as the ulcer margin is approached (towards the right). × 70.

Fig. 14. 14 days. The even columnar epithelial covering near the centre of the ulcer is shown, with early crypt formation beginning on the left, nearer the ulcer margin. From the same section as fig. 4. × 95.

Fig. 15. 4 days. Cyst-like spaces are seen near the ulcer margin under the epithelial covering. × 150.

Fig. 16. Normal mucosa showing typical colchicine mitoses in crypts. × 380.