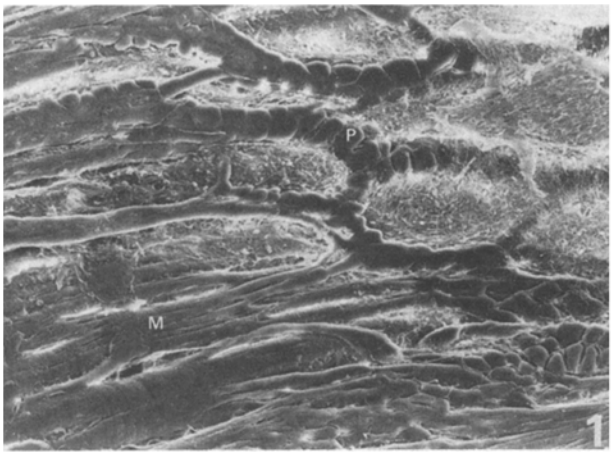

Figure 1. Scanning electron micrograph of the heart treated with NaClO followed by HCl. Purkinje fibers(P) show a delicate network, finally becoming continuous with ordinary myocardial cells(M). × 82.

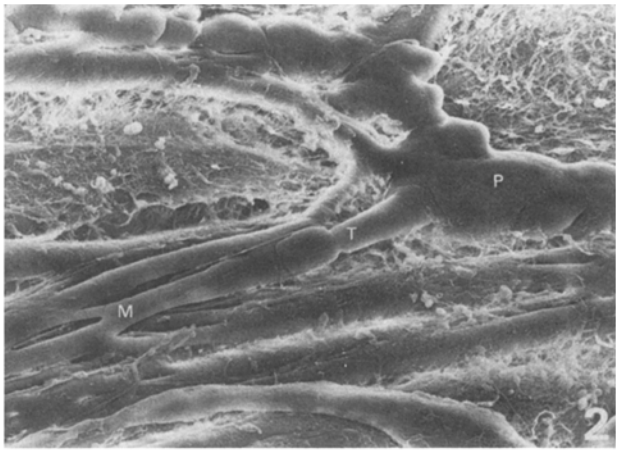

Figure 2. Junctional region between Purkinje fibers(P) and ordinary myocardial cells(M). The former is followed by a transitional cell type(T) which makes contact with the latter. × 232.

and myocardial cells. The stromal surfaces of the transitional cells were relatively smooth, in striking contrast to that of Purkinje fibers, where regularly arranged falt swellings of sarcoplasm were prominent. In addition to such indirect transition, mediated by transitional cells, direct continuity from Purkinje fibers to myocardial cells was occasionally noted. The structural features of this mode of transition will be reported elsewhere.

As is well recognized, the P–M region of the heart is extremely complicated in structure. It has therefore been considered relatively difficult to analyze precisely the functional structures of this region by light and transmission electron microscopy. The combination of scanning electron microscopy (SEM) and chemical digestion used here has made it possible to reveal the precise functional structures of the region. As is apparent from the present particular example, it is certain that a combination of SEM and chemical digestion are a promising mean by which the essential nature of complicated biological structures which are hard to analyze by light and transmission electron microscopy can effectively be elucidated.

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Sequential ultrastructural study of mucosal innervation following parietal cell vagotomy and antrectomy

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Summary. Rats having undergone parietal cell vagotomy (PCV) or PCV with antrectomy were sacrificed and gastric mucosal samples studies by electron microscopy. Degeneration of axons was followed by the appearance of small, neurotubule-rich axons which increased in size and number with increasing postoperative interval. The source of these regenerating fibers is unknown but may have come from the fundus.

Parietal cell vagotomy (PCV) offers many advantages in the treatment of duodenal ulcers. PCV accomplishes a decrease in gastric acid output while avoiding the complications of total vagotomy, i.e. gastric dumping, nausea and diarrhea, which result from the denervation of the antrum, pylorus and upper bowel[1-4]. However, the recurrence rate of ulceration following this procedure increases at a rate of approximately 2% per year. A previous study[5] has indicated that reinnervation of the parietal cell mass can occur at a rapid rate in the rat. The source of these regenerating fibers is unknown. This study was undertaken to ascertain whether the fibers may arise from the intact nerves in the antrum.

Materials and methods. I. Surgical Procedure. 36 fasted male Wistar rats (150–250 g) were anesthetized with ether and a 2 cm midline abdominal incision made. The stomach was delivered into the wound and the esophago-gastric junction and

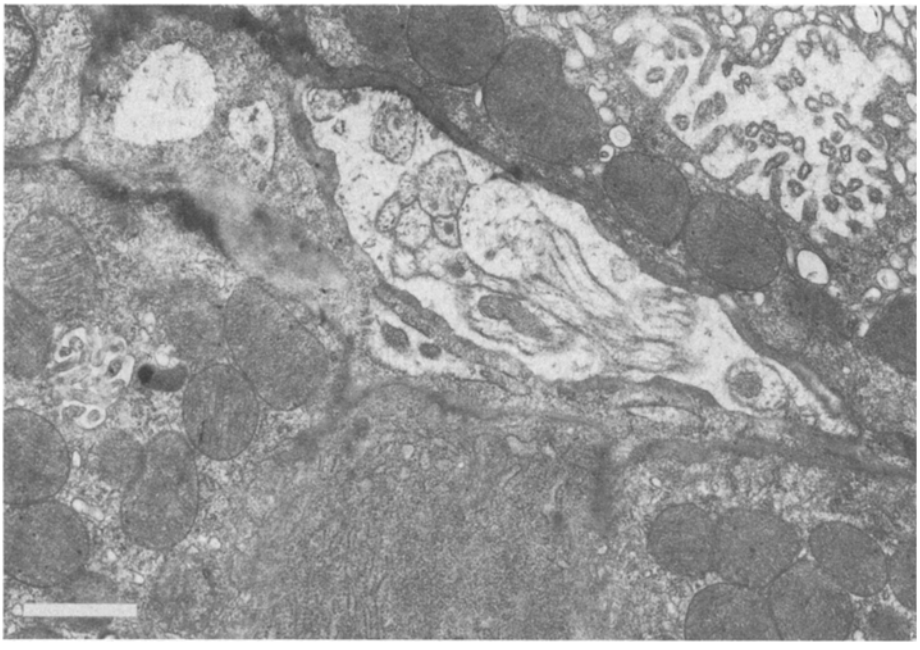

Figure 1. 6 weeks postop. Axons can be classified as either small with densely packed neurotubules, or large with relatively few neurotubules. The smaller axons appear to be regenerating fibers. Bar represents 1 μm.

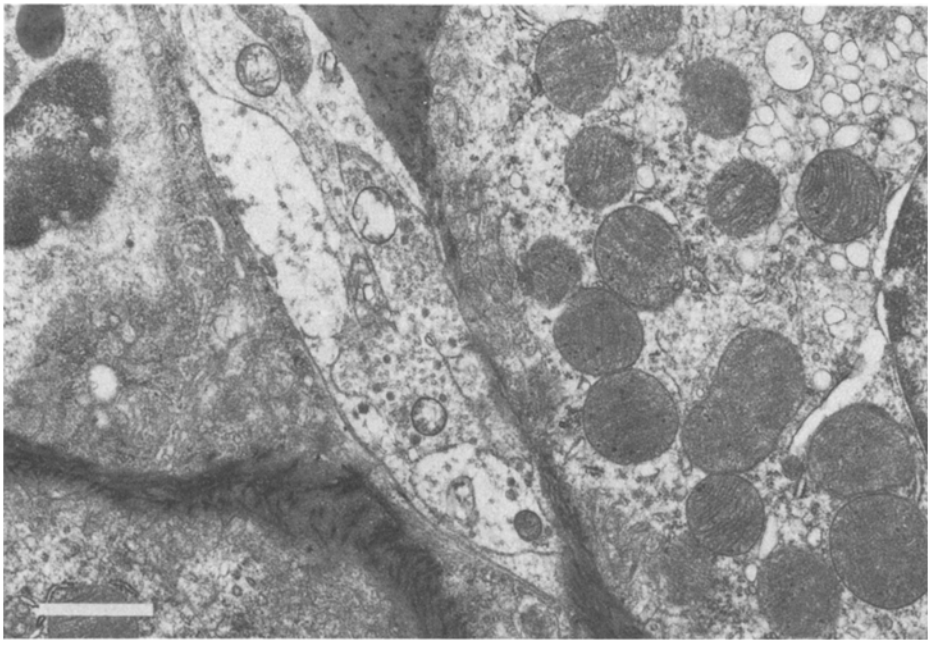

Figure 2. 15 weeks postop. Area of synaptic contact with the basal region of parietal cell. Vesicles appear to be of the cholinergic transmitter variety. Bar represents 1 μm.

anterior neurovascular bundle exposed using a 16 x magnification. The anterior nerves to the fundus and body of the stomach were divided, leaving the gastric blood vessels and vagus nerve to the antrum and pylorus intact. The stomach was reflected to expose the posterior neurovascular bundle which was then similarly divided. An antrectomy was then performed in 18 of these rats and the fundus anastomosed to the duodenal bulb by Billroth I gastrectomy. The stomach was returned to its normal position and all incisions closed. Ten rats underwent a sham operation with similar mobilization of the stomach and exposure time and served as controls.

II. Electron microscopy: Groups of 3 PCV and 3 PCV + antrectomy rats were sacrificed by cervical dislocation at 3 days, and at 3 week intervals up to 15 weeks following surgery. A 1 cm square portion of the ventral wall of the fundic stomach was removed at necropsy. It was immediately immersed in cold 2.5% glutaraldehyde in caceodylate buffer. After a short period of fixation, each sample was cut into small cubes and allowed to fix overnight at 4 °C. Sham operated rats were treated in a similar fashion. The tissue cubes were then postfixed in buffered 2% osmium tetroxide, dehydrated and embedded in Spurr epoxy resin. 0.5 μm thick sections were

made from each block, stained with toluidine blue and used for selection of areas for ultrastructural observation. Thin sections were stained with uranyl acetate and lead citrate prior to examination in a JEOL JEM 100C transmission electron microscope.

Results. At 3 days, axon bundles in both the lamina propria and submucosa of all experimental rats were composed of a mixture of normal and swollen degenerative axons. These swollen axons were electron translucent, contained few neurotubules and small scattered strands of axoplasm. Phospholipid membrane debris was common, usually in the form of myelin figures. A mixture of normal and degenerating axons within the same Schwann cell sheath was common. Fibroblasts were frequently seen near degenerating axon bundles.

By 3 to 6 weeks, degenerating axons were found in decreasing numbers. Healthy axons could be divided into 2 types. These were either small and densely packed with neurotubules or large with sparse neurotubules (fig. 1). Areas of apparent synapse were seen between adjacent nerve fibers on rare occasions. Collagen fibers were abundant around the nerve bundles.

At each 3-week interval postoperatively, from the ninth to the fifteenth, an increasing number of normal appearing axon bundles could be seen in the mucosa and submucosa. Small fibers with numerous neurotubules and large fibers with relatively few neurotubules were seen in the same Schwann cell sheaths. Synaptic contact was noted between adjacent fibers within bundles and also between individual nerve fibers and parietal cells. The synaptic vesicles were electron translucent and similar in morphology to cholinergic neurotransmitter vesicles (fig. 2).

The nerve fibers in the mucosa of the sham operated rats were normal in appearance. While some variation in diameter was common, the degree of this variability was less than that seen in the experimental groups. No degenerating axons were seen in any sham rats.

Discussion. The changes noted in the axons of the gastric mucosa at 3 days were typical of nerve degeneration following injury. Degeneration could be found up to 6 weeks after surgery, decreasing with time. As degeneration proceeded, small axons with densely packed neurotubules appeared in both the PCV and PCV + antrectomy experimental rats. The numbers of these fibers showed a definite increase up to the 9–12 week intervals. These were interpreted as regenerating axons. At 12 to 15 weeks the diameter of the smaller axons was greater than in earlier weeks. Schwann cells of these vagotomized rats contained a larger number of axons than those of the controls and the size and shape of the fibers was more heterogeneous. Increased fibroblast activity and collagen deposition was noted in the early period of healing[5]. The rapid appearance of the small axons (2–3 weeks) raises some question as to their source. Division of the vagal nerve fibers is accompanied by retraction of the cut end of the nerves. While regeneration along the vasculature is a possibility, it seems unlikely that the regenerating axons could advance that rapidly in an organized way from outside the walls of the stomach. The findings in the PCV rats and the PCV + antrectomy rats were identical which would indicate that these fibers do not arise from the nerves located in the antrum. It would appear that recruitment of axons from some other nearby source such as the fundus may be involved in this rapid regeneration.

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Light dependent accumulation of macrophages at the photoreceptor-pigment epithelial interface in the retina of albino mice

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Summary. In the subretinal space of albino mice, macrophages appear from the time of eye opening and increase in number for 6 months; thereafter they decline with age. Dark rearing retards the accumulation of these cells, and exposure to constant light results in a rapid increase. Observations suggest that macrophages appear as a response to visual cell decay in albino mice and supplement the phagocytic activity of the pigment epithelium.

In the developing retina of mice, macrophages appear during vascular growth and remain located within the vascularized part of the inner retina, up to the level of the outer plexiform layer[2,3]. As a result of visual cell death due to hereditary degeneration[2,4,5], similar cells appear in the outer retina including the photoreceptor-pigment epithelial interface. In the albino rat Ling[6,7] has observed that macrophages are present in the inner retina but are noticeably absent from the receptor layer and has commented[7] that the pigment epithelial cells normally carry out the phagocytic activity. However, in the course of pathogenesis in dystrophic[8] and light damaged[9,10] retinas in the rat macrophages have been observed in the outer retina. Braekevelt[11] has shown that phagocytic cells occur at the photoreceptor-pigment epithelial interface of teleostean fishes under normal conditions. Recently, we observed similar cells in the retina of albino mice. Light and electron microscopic observations and enzyme histochemical studies were undertaken and a survey was made for the presence of such cells in mice and rats from different strains. In addition, groups of mice were reared in darkness or exposed to constant light and their eyes were examined for the presence of similar cells. In this report, we describe the macrophage nature of the cells and their changes with age and light conditions.

Material and methods. Eyes from albino and pigmented mice and rats from many inbred strains were examined for the presence of phagocytic cells. For other studies albino mice of the Balb/cLiA strain were used. For electron microscopy eyes were fixed in aldehyde mixture and post-fixed in 1% osmium tetraoxide. Small pieces were dissected out and embedded in epoxy resin as described earlier[12]. For the localization of peroxidase