Table 2. Differential inhibition of mitochondrial respiratory control by N-1-substituted, 3-, 4-substituted pyridinium halides

Compound	Glutamate respiration I <sub>50</sub> *	Succinate respiration I <sub>50</sub>
N-1-Hexadecylnicotinamide bromide	36 μM	31 µM
N-1-Dodecylnicotinamide chloride	31 µM	36 μM
N-1-Dodecylisonicotinamide bromide	17 μ <b>M</b>	8 μΜ
N-1-Dodecylpyridinium bromide	2 μΜ	19 μ <b>M</b>
N-1-Octadecylpyridinium bromide	5 μΜ	21 μM

<sup>\*</sup>I<sub>50</sub> refers to the concentration of inhibitor that diminished respiratory control by 50%.

Table 3. Selective inhibition of succinate oxidation in mitochondria by N-1-dodecylisonicotinamide

Inhibitor (µM)	Respiratory velocity*		RCR
	State 3	State 4	
0	$115.0 \pm 4.0$	22.1 ± .4	5.21 ± .16
5	$59.5 \pm 2.6$	$17.2 \pm .4$	$3.46 \pm .14$
10	$29.4 \pm 2.6$	$13.9 \pm .5$	$2.10 \pm .12$
15	$16.5 \pm 1.5$	$11.9 \pm .6$	$1.38 \pm .07$
20	$11.8 \pm 1.0$	$10.0 \pm .7$	$1.17 \pm .07$
25	$8.8 \pm 0.7$	$9.3 \pm .6$	$0.95 \pm .04$

<sup>\*</sup>Nanogram atoms oxygen per min per mg mitochondrial protein ± SEM. RCR is the ratio of respiratory velocity in presence of phosphate acceptor (ADP) to that after exhaustion of ADP.

pounds was a selective inhibition of the respiration stimulated by phosphate acceptor. Table 3 shows the dose related behavior of N-1-dodecylisonicotinamide bromide on succinate respiration. Phosphorylating oxidation (state 3) was markedly depressed whereas respiration in the resting state (state 4) was relatively unaffected. Similar characteristics were observed with each of the substituted pyridinium halides and with either succinate or glutamate as substrate. Since these compounds did not stimulate resting state respiration, they do not have uncoupling activity and consequently they are classified as inhibitors of phosphorylating oxidation.

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## The Purkinje fiber-myocardial cell region in the goat heart as studied by combined scanning electron microscopy and chemical digestion<sup>1</sup>

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Summary. The 3-dimensional architecture of the junctional region between Purkinje fibers and ordinary myocardial cells has been closely studied by combined scanning electron microscopy and chemical digestion in the goat heart. It was revealed that the Purkinje fibers forming the terminal arborization of the atrioventricular bundle are followed by transitional cells which are in contact with ordinary myocardial cells.

It is well known that Purkinje fibers are electrophysiologically linked to ordinary myocardial cells of the ventricle in the heart. The transitions between the 2 types of cardiac cells have not, however, yet been perfectly elucidated on a morphological basis. In the light-microscopic studies on the Purkinje fiber -myocardial cell junction of the heart made so far, a number of authors have observed a direct continuity from Purkinje fibers to ordinary myocardial cells<sup>3-6</sup>, whereas other authors could not confirm its existence<sup>7</sup>. Further, another group of authors recognized direct continuity only in man out of the 3 animal species examined<sup>8</sup>. Kugler et al., 9 using the light microscope, detected an indirect transition between Purkinje fibers and ordinary myocardial cells mediated by transitional cells, in addition to direct continuity. Later, Palomo et al. 10 examined using an electron microscope the cardiac cells which could be identified as transitional cells on the basis of their electrophysiological features. To the best of our knowledge, however, there has not been any electron-microscopic evidence which shows unequivocally direct or indirect transition between the Purkinje fibers and ordinary myocardial cells.

In the present study, a combination of scanning electron microscopy and a chemical digestion procedure<sup>11</sup> have enabled us to study the 3-dimensional architecture of the junctional region (P–M region). As a result of the study, examples of indirect transition were found, and the steric structure of the transitional cells could be described.

Materials and methods. Hearts of adult goats were excised under nembutal anesthesia and were immersed in Karnovsky's fixative for 3 h or longer. The tissue specimens containing endocardium were dissected out from the free wall of the ventricle. The endocardial endothelium and connective tissue elements were subjected to digestion with NaClO followed by HCl<sup>11</sup>. The specimens were then throughly washed in physiological saline, postosmicated, dehydrated in graded ethanol series, dried by the critical point method and the examined in a JSM-25 scanning electron microscope.

Results and discussion. As a result of chemical digestion, the endocardial endothelium and the connectve tissue elements of the subendocardium were effectively removed and Purkinje fibers, myocardial cells and transitional cells were clearly visualized 3-dimensionally by examination with a scanning electron microscope. The Purkinje fibers were broader and shorter than ordinary myocardial cells. The former was found to form a subendocardial network, whereas the latter was arranged in parallel rows (fig. 1). Transitional cells frequently occurred in the P-M region, either singly or in rows of two or more cells (figs. 1 and 2). A transitional cell or rows of these cells were attached at one end to the terminal arborization of Purkinje fibers and to be in contact with ordinary myocardial cells at the other. Transitional cells were somewhat cylindrical in shape and of a thickness intermediate between Purkinje fibers

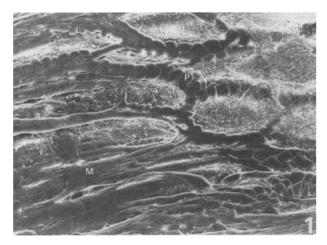


Figure 1. Scanning electron micrograph of the heart treated with NaClO followed by HCl. Purkinje fibers(P) show a delicate network, finally becoming continuous with ordinary myocardial cells(M). × 82.

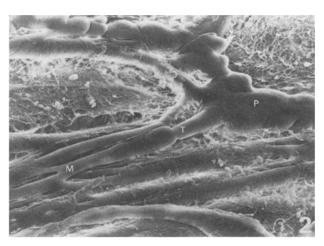


Figure 2. Junctional region between Purkinje fibers(P) and ordinary myocardial cells(M). The former is followed by a transitional cell type(T) which makes contact with the latter.  $\times$  232.

tively 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

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 rela-

- 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<sup>1-4</sup>. However, the recurrence rate of ulceration following this procedure increases at a rate of approximately 2% per year. A previous study<sup>5</sup> 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