well as breeding experiments, may help to answer this question. A more detailed presentation of the histology of the various types of neoplasms will be published subsequently.

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Effect of vagotomy upon the neurohistochemical and ultrastructural integrity of the inbuilt intrinsic nervous apparatus of the choledocho-duodenal junction

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Summary. The neurons of the choledocho-duodenal junction of the cat were shown to be neurohistochemically and morphologically independent of their extrinsic vagal connections. The effect of vagotomies upon the intrinsic nerve nets was also quite negligible.

There is convincing evidence that the integrity of the inbuilt intrinsic nervous apparatus of the choledocho-duodenal junction is of crucial importance for the maintenance of normal biliary dynamics[1-5]. The sympathetic influence upon the choledocho-duodenal junction is mediated through alpha receptors responsible for contraction of the sphincter following sympathetic nerve stimulation, and through beta receptors responsible for relaxation of the sphincter following stimulation by circulating catecholamines[3,4,6,7]. Recently, the presence of contraction-mediating atropine-sensitive cholinoceptors in the sphincter of Oddi of the cat has been confirmed[3]. In addition, it is generally believed that there is, in the regulation of the functioning of the extra-hepatic biliary duct system, a neurohumoral link between the vagal innervation and the effect of cholecystokinin, gastrin and secretin, all these humoral agents being released from the mucosal neuroendocrine cells by vagal nerve stimulation[8]. Finally, it has been shown that the physiological influence of cholecystokinin upon the biliary smooth muscle necessitates functioning of a reflex arch containing at least 1 synapse and procaine-sensitive receptors in relation to the cholecystokinin-releasing cells[5]. This further emphasizes the importance of the intrinsic innervation in the maintenance of normal biliary dynamics. However, until now, surprizingly little is known about the neurohistochemical nature and ultrastructural characteristics of the intrinsic neurons of the choledocho-duodenal junction, and this is true also concerning the neurohistochemical and ultrastructural consequences of vagotomies, although vagal denervation of the upper abdominal viscera has, during the last few years, become a common surgical procedure, and the role of the vagus nerves in the biliary dynamics has received special attention.

The nature of the intrinsic neurons of the choledocho-duodenal junction of the cat was studied: 1. by fluorescence microscopy (formaldehyde-induced and glyoxylic acid-induced fluorescence), 2. by light- and electron microscopical demonstration of the acetylcholinesterase enzyme [a) Gomori's modification of the Koelle-Friedenwald technique, b) the method of Karnovsky and Roots, c) the method of Lewis and Shute], 3. by examining the glutaraldehyde-osmiumtetroxide-fixed ultrastructure, and 4. by studying the effect of unilateral and bilateral subdiaphragmatic abdominal or cervical vagotomies performed 5 days - 3 months before sacrifice. A total of 30 adult cats was used for the present study.

In light microscopy, most of the neurons showed moderate to intense AChE activity (figure 1). At electron microscopy, intra-cellular distribution of the reaction product was

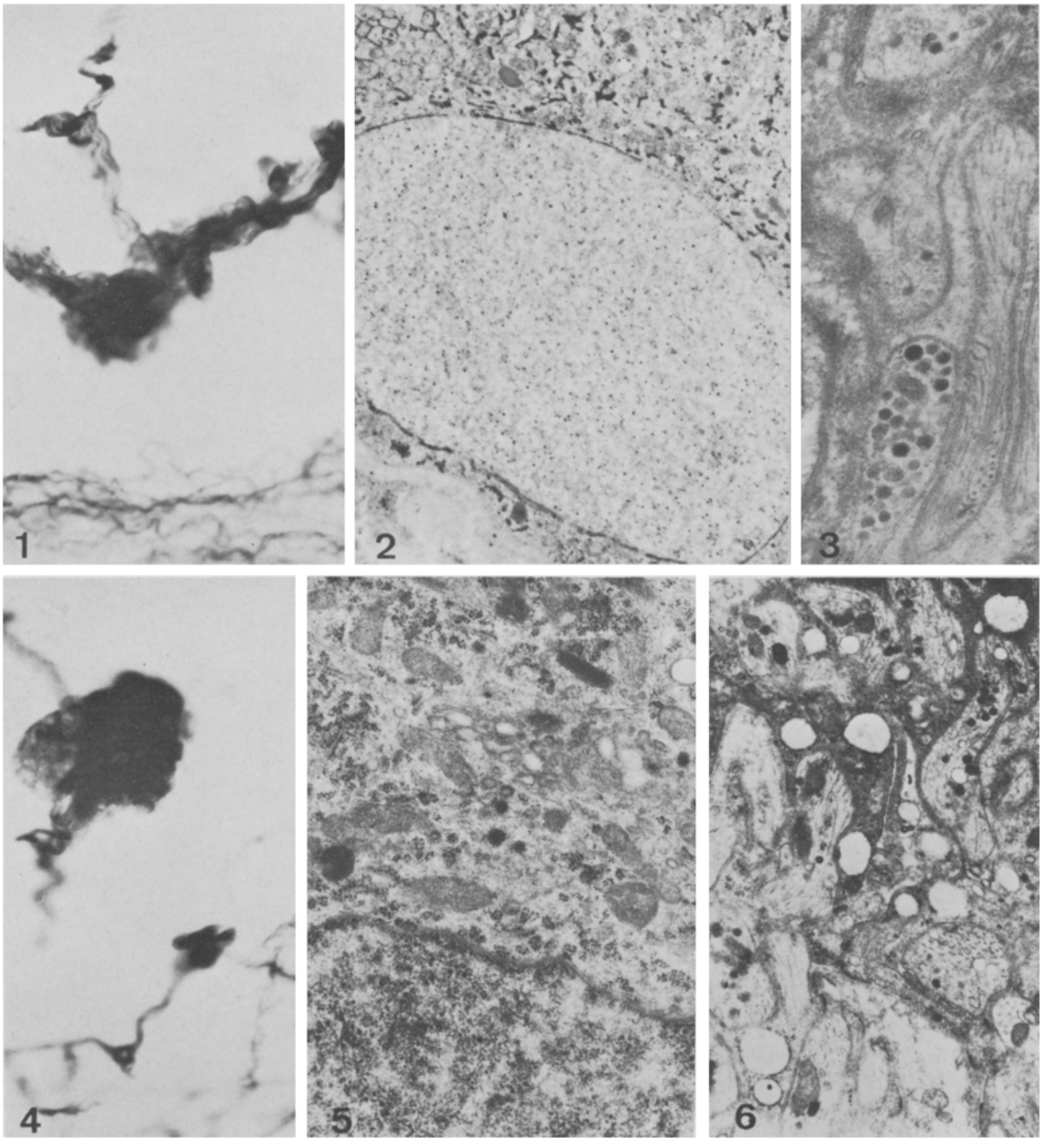

Fig. 1. Ganglion (of the submucous plexus of Meissner) containing acetylcholinesterase-positive nerve cells. Gomori's method. × 100. Fig. 2. Intracytoplasmatic distribution of acetylcholinesterase activity in a nerve cell (of a ganglion of the submucous plexus of Meissner). × 10,000. Fig. 3. Nerve terminal crowded with extra-large (purinergic?) vesicles. × 14,760. Fig. 4. Acetylcholinesterase-positive ganglia (in the submucous plexus of Meissner) 3 months after bilateral truncal vagotomy. Gomori's modification. × 100. Fig. 5. Normal fine structure of a nerve cell (belonging to the submucous plexus of Meissner) 3 months after bilateral truncal vagotomy. × 14,760. Fig. 6. Mosaic-like destruction in a large nerve fascicle coursing within the sphincter of Oddi, 3 months after bilateral truncal vagotomy. × 12,054.

demonstrated (figure 2). Because fluorescing neurons were not seen, the conclusion is reached that these neurons obviously are cholinergic. However, the possibility remains that some of the intrinsic neurons might be non-cholinergic and non-adrenergic. In fact, there is considerable evidence that the mammalian gastro-intestinal smooth muscle is supplied also by non-adrenergic intrinsic inhibitory neurons, and the studies of Persson[7] indicate that also the sphincter of Oddi of the cat has a non-adrenergic inhibitory innervation. The non-adrenergic inhibitory innervation has been attributed to 'purinergic' neurons, the terminals of which are characterized by membrane-bound electron-opaque spherical bodies, usually with no discernible 'halo', ranging from 1200 Å to 2000 Å in diameter[9]. Such terminals, possibly originating from intramural neurons, were occasionally seen in the present study, but they were not of common occurrence (figure 3). So, it may be possible that some of the intramural neurons are purinergic, although it is not possible, at present, to identify the purinergic nerve cell bodies morphologically or neurohistochemically. The

ultrastructure of the neurons was typical of that generally described for enteric neurons. After vagotomies, the ganglia exhibited a normal AChE activity (figure 4), and the ultrastructure of the neurons remained completely intact (figure 5). So, the neurons of the duodenal submucosal ganglia in the region of the choledocho-duodenal junction are neurohistochemically and morphologically independent of their extrinsic vagal connections, which would obviously suggest a functional independence as well.

The effect of vagotomies upon the intrinsic nerve nets was quite negligible, too: at electron microscopy, only little mosaic-like destruction was observed (figure 6); at light microscopy, corresponding changes were observed in the form of fragmentation and minor changes in the intensity of the cholinesterase activity of the intramural nerve plexuses; the number and fluorescence intensity of the adrenergic axons remained unchanged. The destructive changes following vagal denervation, although always quite limited in extent, were clearly individually variable, which may possibly give some explanation for why the extra-gastric consequences of vagotomies seem to be quite unpredictable, both in clinical and in experimental surgery.

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Histochemical characterization of the red fibres in pigeon pectoralis muscle

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Summary. Red fibres of the pigeon pectoralis muscle showed high ATPase reaction at pH 9.4. Veronal-acetate pretreatment completely inhibited the ATPase reaction in these red fibres but not in type I fibres of the gastrocnemius. The former are type II red muscle fibres and hence are unlike type I red, the so-called slow-twitch muscle fibres.

The 3 major categories of vertebrate skeletal muscle fibres are type I red, type II red and type II white. The histoenzymatic characterization of muscle fibres in pigeon pectoralis major muscle shows only 2 types of fibres, viz., narrow-red and broad-white[1,2]. In the literature, however, the red fibres of pigeon pectoralis muscle are also referred to as type I or slow and white fibres as type II or fast[3–7]. The purpose of this study was to evaluate veronal-acetate preincubated ATPase reaction in red fibres of the pigeon pectoralis major muscle and compare with the characteristic type I red (presumably slow-twitch) fibres of the gastrocnemius muscle. The present results have shown that the red fibres of pectoralis muscle are type II red.

Materials and methods. Normal pectoralis major and gastrocnemius muscle from 6 adult pigeons *(Columba livia)* were used. The birds were anaesthetized with ether and small blocks of the muscle were excised, dipped in talcum powder[8] and quenched in liquid nitrogen. 10 micra frozen sections were cut on a cryostat (−22 °C), placed on clean dry slides without any adhesive and dried for at least 15 min at room temperature (20 °C). The tissue sections were immersed in cold (4 °C) acetone for 30 min, again dried for 2–3 min and then processed as mentioned below.

The tissue sections were fixed for 20 min in 2% paraformaldehyde and 0.2% sodium azide (pH 7.4)[10]. Some sections were preincubated in 0.2 M veronal-acetate buffer at pH 4.3 for 10 min at 20–21 °C[9]. Fixed sections were washed for 20 min in several changes of cold distilled water. The preincubated sections were washed for a few minutes in distilled water. All sections were incubated for ATPase activity at pH 9.4[10].

Results and discussion. The regular ATPase reaction (i.e. nonpreincubated) showed that both white (broad) and red (narrow) muscle fibres of pectoralis major stained heavily (figure 1). Preincubation in veronal-acetate completely abolished the ATPase reaction in all fibres of this muscle (figure 2). The regular ATPase reaction in the gastrocnemius muscle exhibited high ATPase activity in type II but very low activity in type I fibres (figure 3). Preincubation strongly enhanced the ATPase reaction in all type I fibres

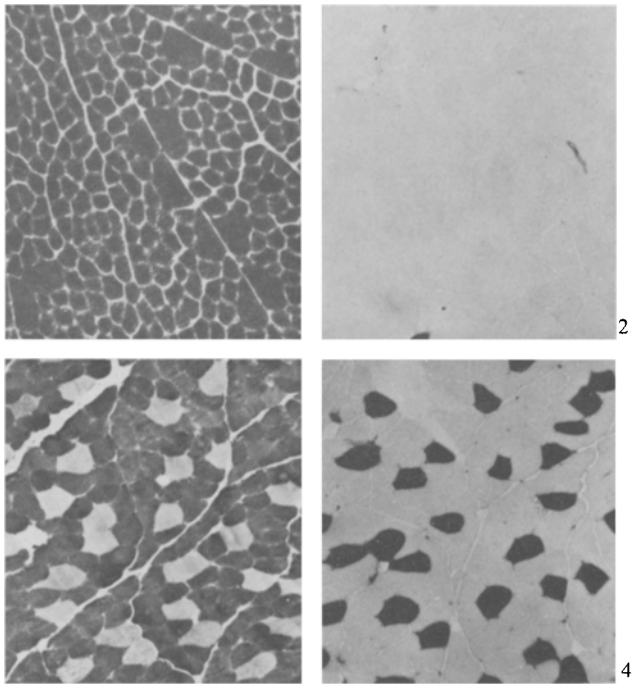

Fig. 1. Transverse section of the pigeon pectoralis major muscle incubated for ATPase reaction at pH 9.4. Both white (broad) and red (narrow) fibres display high reaction. × 250.

Fig. 2. Transverse section. Pigeon pectoralis major muscle incubated for ATPase reaction at pH 9.4 following preincubation. Note the very low reaction in both white and red fibres. × 250.

Fig. 3. Transverse section of the pigeon gastrocnemius muscle incubated for ATPase reaction. Note type I fibres are poorly stained. × 250.

Fig. 4. Transverse section. Pigeon gastrocnemius muscle showing ATPase reaction following preincubation. Note the intensely stained type I but labile type II fibres. × 250.