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

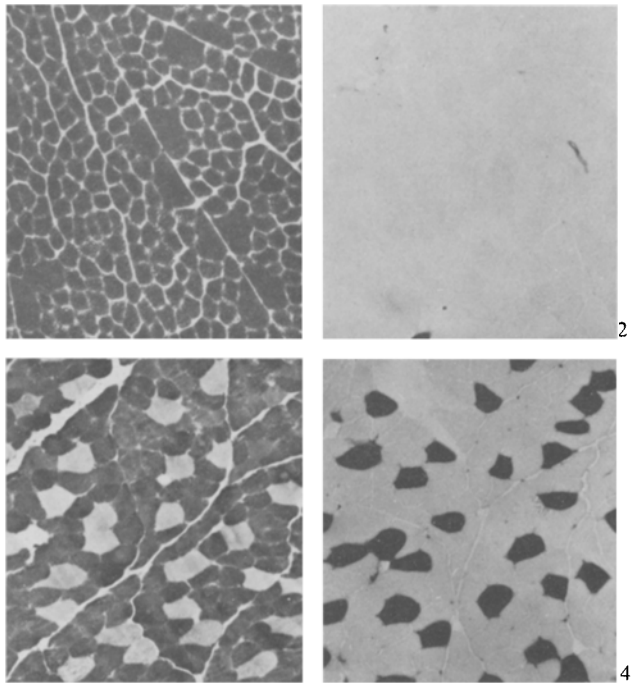

Fig. 1. Transverse section of the pigeon pectoralis major muscle incubated for ATPase reaction at pH 9.4. Both white (broad) and red (narrow) fibres display high reaction. × 250.

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.

and inhibited that in all type II fibres of the gastrocnemius muscle (figure 4).

This study has shown clearly that reciprocity of the ATPase reaction as displayed by type I fibres of the gastrocnemius muscle was absent in red fibres of the pectoralis major. The present results have substantiated that unlike the typical type I fibres, red fibres of pigeon pectoralis display high ATPase reaction, as do the type II red and type II white muscle fibres. Since the speed of contraction of a given muscle is directly proportional to its myosin ATPase content[10-13], and the myofibrillar ATPase of slow muscle is alkali-labile[15-17], it is generally assumed that individual muscle fibres with low ATPase reaction, histochemically, are slowtwitch; conversely muscle fibres showing high ATPase reaction are fast-twitch[14].

The avian[18] or mammalian[10,14,19,21] type I muscle fibres of mixed muscles or slow-twitch muscles[19] exhibit the characteristically activated ATPase reaction following acidic preincubation. The red fibres of pigeon pectoralis do not share this important property. By virtue of the presence of high ATPase and its acid lability, greater number of mitochondria, high SDH[2], and focal en plaque pattern of nerve endings[22], the red fibres of pigeon pectoralis are possibly type II red, i.e. not slow-twitch but fatigue resistant. There may be a spectrum[20] of fastness in the different sub-populations[10,18,21] of this particular variety of muscle fibres. Thus labelling red fibres of the pectoralis muscle as type I or slow is not justified[3-7].

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Effect of prostaglandins on skin tumorigenesis

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Summary. Concomitant administration of prostaglandins E_2 (PGE_2) and $F_2\alpha$($PGF_2\alpha$) with a carcinogen, 3-methylcholanthrene (MCA) to mice for 2 months markedly enhanced the occurrence of squamous cell carcinomas. Only epidermal cell hyperplasia occurred in mice treated with MCA alone by that time. Radioactivity measurements and electron microscopic autoradiography revealed that prostaglandins stimulate DNA, RNA and protein synthesis in neoplastic cells. These findings indicate that PGE_2 and $PGF_2\alpha$ can act as cocarcinogens on skin tumorigenesis.

Recently increased amounts of prostaglandins, especially PGE_2 were detected in human cancers as well as in cultured mouse fibrosarcoma cells[1]. Also an elevated prostaglandin synthetase activity was found in microsomal fractions of transformed cells from methylcholanthrene-treated mice[2]. However, the role of prostaglandins in carcinogenesis is still undetermined. The present report deals with the effect of prostaglandins E_2 (PGE_2) and $F_2\alpha$ ($PGF_2\alpha$) on the chemically induced skin tumors by 3-methylcholanthrene (MCA) in mice.

Material and methods. The experiments were carried out on male albino Swiss mice weighing 20–25 g and which were divided in 6 groups, 20 mice of each as follows: I) Mice which received only the diluent and served as controls; II) Mice topically treated with 0.4% acetone solution of MCA, each mouse received 0.2 ml by pippeting in a marked region of his shaved dorsal skin, 3× weekly for 2 months. III) Mice treated with MCA as above and concomitantly injected i.m. with 10 µg of PGE_2 3× weekly for 2 months. IV) Mice concomitantly treated with MCA and injected i.m. with $PGF_2\alpha$, a dose of 10 µg, 3× weekly for 2 months. Groups V and VI were treated only with PGE_2 and $PGF_2\alpha$ respectively as above. At the end of 2 months and 2 h prior to the sacrifice, 5 mice from each group received i.m. 10 µCi/g b.wt of ^{3}H-thymidine for the study of DNA synthesis; another 5 mice from each group were injected i.m. with 10 µCi/g b. wt of ^{3}H-uridine for the study of RNA synthesis; other 5 mice from each group received 10 µCi/g b.wt of ^{3}H-leucine for the study of protein synthesis and another 5 mice from each experimental group received i.m. 10 µCi/g b.wt of ^{3}H-proline for the study of collagen synthesis. We selected 2 h for the isotope studies, because we found in previous experiments that prostaglandins (PG) exert their maximum effects on cell metabolism in that time[3]. Radioactivity measurements were performed with Nuclear Liquid Scintillation Counting System, efficiency 40% using (^{3}H) as internal standard; the skin specimens were trimmed, the s.c. fat and dermis were removed and the epidermis was homogenized with a Potter-Elvehjem tissue grinder and transferred in vials with scintillation fluid. Results were expressed as cpm and per g of homogenates (mean±SE). For light microscopy and autoradiography, the specimens were fixed in Bouin's fluid, dehydrated and embedded in paraplast; the 5-µm thin sections were stained with hematoxylin and eosin and examined under light microscope. For EM autoradiography, small specimens from tumors were diced, fixed in 2.5% phosphate glutaraldehyde, then postfixed in 1% phosphate buffered 0_s0_4, dehydrated and embedded in a mixture of eponaraldite. Thin sections were covered with Ilford Nuclear Emulsion L_4 using a wire loop procedure[4] for 6–8 weeks, then developed in Microdol-X, fixed, washed and stained with uranyl acetate and lead citrate.

Observations and discussion. Multiple and large tumors, sometimes necrotic and hemorrhagic in the center, occurred after 2 months in almost 90% in the MCA and $PGF_2\alpha$ or