Consequently, the distribution of hexobarbital between plasma and brain tissue in the FD-rats remained unaltered; 15 min after injection of the barbiturate, the quotient liver/plasma was for FD-rats higher than that for controls [1.26 ± 0.13 (n = 7) versus 0.79 ± 0.11 (n = 6), $p < 0.01$].

Undoubtedly, this means an accumulation of hexobarbital in the liver of FD-rats, which, however, is scarcely to be expected to shorten the sleeping time. The liver weight amounts only to 5% of the total body weight (HAGEMANN[4]). Therefore, in FD-rats the liver cannot be expected to take up so much more hexobarbital than in normally fed animals that this would reduce the amount of drug available in the brain.

Also 35 min after injection, when the absolute concentrations were still higher in FD-rats, and had not fallen so much as in normally fed animals, the quotients liver/plasma of the experimental and the control group, respectively, were found identical [1.35 ± 0.27 (n = 5) versus 1.38 ± 0.44 (n = 4)].

Discussion. The results show that in FD-rats hexobarbital concentrations in brain, liver and plasma are higher than those in the normally fed animals, and decrease more slowly. This slowed elimination may be explained by an impaired drug metabolism leading to a reduced breakdown of hexobarbital in the liver. A pharmacological consequence would be the prolonged sleeping time, as already found for pentobarbital by FÖLDI and FÖLDI-BÖRCSÖK[1].

To explain the prolonged sleeping times in FD-rats, it is not necessary to take into account a changed brain susceptibility for hexobarbital. Furthermore, in FD-rats and controls, respectively, the same distribution quotients brain/plasma point to the fact that in both groups hexobarbital is found in the brain tissue in the same portion of the plasma concentration. Obviously the blood-brain barrier for hexobarbital, if it exists at all (OLDENDORF[5]), remains in its previous state.

In flavonoid deficiency, the drug metabolizing systems in the liver microsomes may be disturbed. Such damage would fit into a general disturbance of FD-rats. This view is supported by our own observation that within 2 weeks after the transport by car from Salzgitter to Lübeck (about 300 km) 20 of 35 FD-rats died while the same number of control animals survived.

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An Electron Microscopic Study on the Autonomic Innervation of the Rabbit Parotid Gland[1]

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Summary. As visualized in the electron microscope, the parotid gland of the rabbit has a dual innervation. Both adrenergic and cholinergic nerves are equally distributed in the parenchyma and often run together within the same nerve bundle. Nerve terminals are observed not only subjacent to the basement membrane but interposed between the latter and the acinar cells, where they establish a close membrane to membrane contact with the latter.

The rabbit parotid gland, a purely serous gland[3,4], has been widely employed in experimental physiological and pharmacological studies on secretory responses to various stimuli. Moreover, the rabbit parotid gland has been extensively investigated with respect to cellular synthesis and secretion of salivary gland secretory proteins[5,6]. These functional studies have been supplemented by recent recordings of morphometric data concerning the secretory cells[7,8].

However, there is but little information to be obtained in the literature on the distribution of autonomic nerve elements within this gland. The present paper deals with some electron microscopic findings on the relationship between adrenergic and cholinergic nerves and the acinar cells of the rabbit parotid gland.

Materials and methods. Salivary gland tissue was obtained from 4 adult male rabbits of a mixed strain. The animals were killed by an i.v. injection of sodium pentobarbital (Mebumal®, ACO Drug Ltd., Sweden). The parotid glands were rapidly removed, and small tissue slices were fixed for 90 min in icecold 3% $KMnO_4$ in Krebs-Ringer phosphate buffer (pH 7.0). Following fixation, the specimens were rinsed in Ringer solution and contrasted en bloc for 60 min in 1% uranyl acetate[9]. The specimens were dehydrated in graded ethanol solutions followed by propylene oxide and embedded in Epon 812. Ultrathin sections were cut on an LKB Ultrotome, collected on copper grids, contrasted with lead citrate and examined in a Philips EM 300 electron microscope.

Results and discussion. In the connective tissue between the acini throughout the parotid gland, numerous unmyelinated nerves are observed. The nerves generally appear in bundles, each containing several axons completely or partly enveloped by a Schwann cell. With the present technique, it was possible to distinguish between two types of axons on the basis of the appearance of the neuronal vesicles within axonal varicosities. One group of varicosities contains either small, granular vesicles (500–600 Å in diameter), or slightly larger ones (1,000–1,200 Å). These axons represent adrenergic nerves. The other group contains agranular vesicles (500–600 Å); these are cholinergic nerves. The 2 types of axons are often found within the same nerve bundle, i.e. they are enveloped by the same Schwann cell.

Adrenergic as well as cholinergic varicosities are commonly observed subjacent to the acinar cells, separated from the latter by the basement membrane only. However in addition to these extra-acinar nerve terminals, varicosities are also encountered interposed between the acinar basement membrane and the epithelial cells. These intra-acinar nerve endings are completely free of Schwann cell investment, and contain either granular (adrenergic) (Figure 1) or agranular vesicles (cholinergic) (Figure 2). Within contact areas, the axolemma and the secretory cell plasma membrane run parallell and are separated by a roughly 200 Å wide cleft.

With respect to duct cells, the situation appears to be quite different, as no autonomic nerve endings are ob-

served inside the basement membrane of the epithelium. Autonomic nerve fibres are also associated with glandular vessels. In this case, they are only observed in the adventitia, within an area close to the muscular media. The varicosities of these vascular nerves are of both adrenergic and cholinergic type.

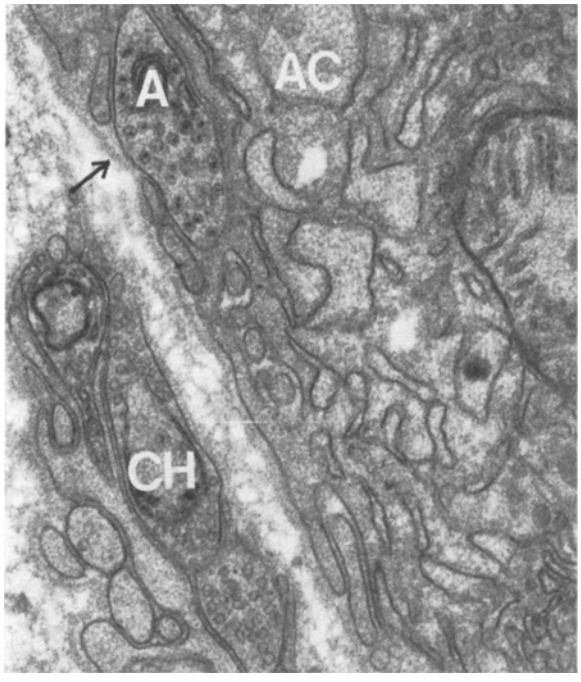

Fig. 1. Rabbit parotid gland. An adrenergic nerve ending (A) with granular vesicles is interposed between an acinar cell (AC) and basement membrane (arrow). In periacinar connective tissue a nerve bundle is seen, the varicosities of which contain agranular vesicles (CH). × 31,850.

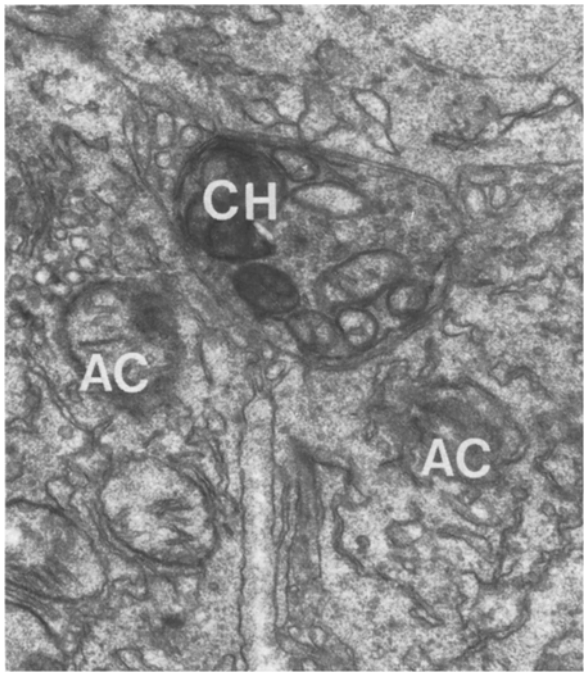

Fig. 2. Rabbit parotid gland. A cholinergic nerve ending (CH) containing agranular vesicles enveloped by cytoplasm of adjacent acinar cells (AC). × 24,850.

In the peripheral autonomic nervous system, it is well established that noradrenaline and acetylcholine are the transmitter substances of the sympathetic and parasympathetic divisions respectively. Employing light microscopic enzyme histochemical techniques as well as fluorescence microscopy, adrenergic and cholinergic nerves have been demonstrated separately in the rabbit parotid gland[10, 11]. Both catecholamine fluorescence and cholinesterase activity appear in nerve fibres throughout the gland including those encircling the acini. Furthermore, nerve fibres of both types are present around the media of muscular blood vessels.

Employing the permanganate fixation-staining technique, it is possible to identify sympathetic as well as parasympathetic nerve endings at the ultrastructural level[9]. From the findings reported here, it is evident, with respect to acinar cells, that both adrenergic and cholinergic nerves establish a close membrane to membrane contact in the rabbit parotid gland. Whereas intra-acinar nerve endings have also been reported in salivary glands of some other species[12, 13], the autonomic nerves do not seem to penetrate the acinar basement membrane in others[14, 15]. In these latter cases, the transmitter substances must necessarily diffuse over a longer distance to reach the effector cells. The classification of the glands as serous, seromucous or mucous does not seem to have any bearing on this matter. It should be stressed that intra-acinar nerve terminals have also been reported in exocrine glands of other location, e.g. the lacrimal gland[16], the exocrine pancreas[17] and bronchial glands[18].

The present findings establish that both divisions of the autonomic nervous system are equally distributed in the rabbit parotid gland. In no distinct portion of the gland is only one or the other type of axon present.

The morphological findings are in accordance with results from previous physiological studies, viz. both adrenergic and cholinergic agents markedly stimulate fluid secretion from the rabbit parotid gland[19]. Furthermore, there is a good correlation between the ultrastructural evidence presented and the light and fluorescence microscopic findings mentioned above[10, 11].

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