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The Influence of the Autonomic Nervous System on the Ultrastructure of the Parotid Acinar Cells

Experimental Contribution to the Neurohormonal Sialadenosis*

K. Donath, M. Spillner and G. Seifert
Institute of Pathology, University of Hamburg (Director: Prof. Dr. G. Seifert)

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Summary. The influence of the autonomous nervous system on the ultrastructure of the parotidean acini and the terminal neuraxon was tested on 70 Wistar rats under the following experimental conditions: sympathetic stimulation (Aludrin 2 mgm/100 gm of body weight daily), parasympathetic stimulation (Pilocarpine 0.2 mgm) sympathicolysis (Guanaclin 5 mgm), parasympathicolysis (Scopolamine 0.2 mgm) and sympathico-parasympathicolysis (Guanaclin 5 mgm and Scopolamine 0.2 mgm). The following aspects were of primary importance to the comparative analysis: changes in the secretory cycle and cell organelles; a comparison between the acinar structure and changes in the neuraxons; the relationships of the findings to human sialadenosis. In addition, morphometric measurements of the diameters of the acini were carried out in all test groups.

After sympathetic stimulation a rapid secretion and stimulation of the protein resynthesis after secretion results (nuclear swelling, enlargement of the nucleolus, multiplication of the granular endoplasmatic reticulum and the formation of light secretory granula). Secretion also results after parasympathetic stimulation although to a lesser extent than after sympathetic stimulation (light as well as dark secretory granula are present). A sympathicolysis effects a disturbance of the secretory discharge (alteration of secretory granula) and of the protein synthesis (reduction of the protein synthesizing organelles); a parasympathicolysis also disturbs secretory production (concentration of prosecretory granula and formation of granula with a dense mantle and inhomogenous center) and secretory discharge. The greatest acini alterations are found in a combined sympathico-parasympathicolysis and indicate a disturbance of the intracellular cycle. Changes in the preterminal axons occur predominantly in sympathicolysis (loss of granular vesicles, destruction of mitochondria, formation of lysosomal corpuscles, disintegration of the membrane) or combined sympathico-parasympathicolysis. Sympathetic stimulation results in the multiplication of granular vesicles. Morphometric measurements show a significant enlargement of the acini, particularly with sympathetic and parasympathetic stimulation, but also with sympathicolysis. On the basis of the findings one may conclude that the neurohormonally elicited interference in the secretion process results primarily by means of a direct action on the neuroreceptor of the glandular cell. A feedback mechanism between secretory production and secretion exists within the secretory cycle (production, storage, secretion) as does a daily rhythm which is dependent upon food intake. The agreement of experimental and human sialadenosis findings (acini enlargement, alteration of the granula) is further substantiation for the thesis that human sialadenosis is caused by a neurohormonally elicited secretory disturbance.

Zusammenfassung. Der Einfluß des autonomen Nervensystems auf die Ultrastruktur der Parotisacini und der terminalen Nervenaxone wurde an 70 Wistar-Ratten unter folgenden experimentellen Bedingungen untersucht: Sympathicus-Stimulierung (Aludrin 2 mg/100 g K.-Gew. tgl.), Parasympathicus-Stimulierung (Pilocarpin 0,2 mg), Sympathicolyse (Guanaclin 5 mg), Parasympathicolyse (Scopolamin 0,2 mg) und Sympathico-Parasympathicolyse (Guanaclin 5 mg und Scopolamin 0,2 mg). Bei der vergleichenden Analyse standen folgende

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Gesichtspunkte im Vordergrund: Veränderungen des Sekretionscyclus und der Zellorganellen, Vergleich zwischen Acinusstruktur und Veränderungen der Nervenaxone, Beziehungen der Befunde zur menschlichen Sialadenose. Zusätzlich wurden morphometrische Messungen der Acinusdurchmesser in allen Versuchsgruppen durchgeführt.

Nach Sympathicus-Stimulierung kommt es zu einer raschen Sekretabgabe und zu einer Stimulierung der Protein-Resynthese nach Sekretabgabe (Kernschwellung, Nucleolusvergrößerung, Vermehrung des rauhen endoplasmatischen Reticulum und Bildung heller Sekretgranula). Nach Parasympathicus-Stimulierung erfolgt ebenfalls eine Sekretabgabe, allerdings in geringerem Ausmaß als nach Sympathicusreizung (Vorkommen sowohl von hellen als auch dunklen Sekretgranula). Die Proteinsynthese wird angeregt. Eine Sympathicolyse bewirkt eine Störung der Sekretabgabe (Alterationen der Sekretgranula) und der Proteinsynthese (Reduktion des proteinbildenden Organellensystems), eine Parasympathicolyse ebenfalls Störungen der Sekretbildung (Anreicherung von Prosekretgranula und Ausbildung von Granula mit dichtem Mantel und inhomogenem Zentrum) und Sekretabgabe. Bei kombinierter Sympathico- und Parasympathicolyse treten die stärksten Acinusalterationen als Ausdruck einer Störung des intracellulären Sekretionscyclus auf. Veränderungen der präterminalen Axone treten vorwiegend bei Sympathicolyse (Verlust der granulären Vesicel, Zerstörung der Mitochondiren, Ausbildung lysosomaler Körper, Membranzerfall) oder kombinierter Sympathico-Parasympathicolyse auf. Bei Sympathicusreizung kommt es zu Axonvergrößerungen und einer Anreicherung mit granulären Vesiceln.

Die morphometrischen Messungen ergeben eine signifikante Acinusvergrößerung vor allem bei Sympathicus- und Parasympathicusreizung, jedoch auch bei Sympathicolyse.

Aus den Befunden wird der Schluß gezogen, daß der neurohormonal ausgelöste Eingriff in den Sekretionsprozeß vorwiegend durch einen direkten Angriff am Neurorezeptor der Drüsenzelle erfolgt. Bei dem Sekretionscyclus (Produktion, Speicherung, Abgabe) besteht ein Feedback-Mechanismus zwischen Sekretproduktion und Sekretabgeabe, außerdem ein von der Nahrungsaufnahme abhängiger Tagesrhythmus. Die Übereinstimmung der Befunde bei der experimentellen und menschlichen Sialadenose (Acinusvergrößerung, Granulaalterationen) ist ein weiterer Hinweis für die These, daß die menschliche Sialadenose durch eine neurohormonal ausgelöste Sekretionsstörung verursacht ist.

Among the secretory disturbances of the salivary glands (Lit.: Seifert, 1964) a particular form may be differentiated which is distinguished by double sided, non-inflammatory parotid sweeling and is also known as sialadenosis (Lit.: Rauch, 1959; Seifert, 1966, 1967; Buchner and Sreebny, 1972). Etiologically, endocrine, metabolic and neurohormonal factors are discussed. Newer electron microscopical findings on the parotid gland after the influence of various neurohormonal stimuli (Wilborn and Schneyer, 1972; Johnson and Sreebny, 1973; Lillie and Han, 1973; Barka *et al.*, 1972; Donath, 1973; Donath and Seifert, 1973) indicate first and foremost that dysregulations of the autonomous nervous system play the decisive causal role.

A comparative study of rats under standardized conditions was carried out in order to further clarify the question of which structural changes the acinar cells and the autonomous nervous system of the parotid undergo when influenced by various neurohormonal stimulants. For this purpose, pharmaco-chemically induced excitations or paralysations of the sympathetic as well as parasympathetic nervous system were used.

The following aspects were of primary importance to the ultrastructural comparative analysis:

1. Changes in the secretory cycle and cell organelles under various experimental conditions,

2. a comparison between the acinar structure and changes in the preterminal axons,

3. relationships of the findings to human sialadenosis.

Materials and Methods

The experiments were carried out on 3—6 week old female Wistar rats with an average weight of 120—150 gm. For the duration of the experiment the animals received an Altromin-standard diet and water according to need. A total of 7 experimental groups were formed (see Table 1). In view of the daily rhythm of the secretory cycle (Albegger and Müller, 1973) which is dependent upon eating times (beginning of food intake around 6 p.m., end of food intake around 4 a.m.), the dissection of all animals took place at 8 a.m. The last injection was always given 24 hours before dissection. For this purpose Urethane (0.125/100 gm of body weight) was given intraperitoneally as an anaesthetic. Each experimental group consisted of at least 10 animals. The experimental groups I and II served as control groups. Food was taken away from experiment group II before the beginning of the eating period (around 6 p.m.). The type, dosage and length of the experiment with regard to the individual groups can be seen in Table 1.

For the electron microscopical work the parotid was fixed in a 2% glutaraldehyde-cacodylate buffer mixture (p_H 7.2; 320 m osmols) for two hours and after thorough rinsing for another 2 hours in 1.33% s-collodin buffered solution of osmium-tetroxide. After dehydration in graded series of alcohol the embedding was done in Epon 812.

Table 1. Summary of experimental procedure in the 7 experimental groups

Experimental group	Type of nervous stimulation	Substance	Dosage (mgm/100 gm of body weight)	Length of experiment (maximum)	Notes
I	—	—	—	10 days	Control group
II	—	—	—	10 days	Control group with denutrition at the beginning of the eating period (evening 8 p.m.)
III	sympathetic stimulation	Aludrin i.p.[a]	2 mgm	10 days	Dissection of further animals 6 and 12 days after the last Aludrin injection
IV	parasympathetic stimulation	Pilocarpine s.c.	0.2 mgm	3days	—
V	sympathicolysis	Guanaclin[a]	5 mgm	100 days	For particulars see: Donath (1973) and Donath and Seifert (1973)
VI	parasympathicolysis	Scopolamine s.c.	0.2 mgm	3 days	—
VII	sympathico- and parasympathicolysis	Guanaclin i.p. Scopolamine s.c.	5 mgm 0.2 mgm	10 days	Additional Guanaclin treatment 20 days prior to commencement of experiment

[a] We thank the Boehringer Company, Ingelheim, for their contribution of Aludrin and the Bayer Company, Leverkusen, for their contribution of Guanaclin.

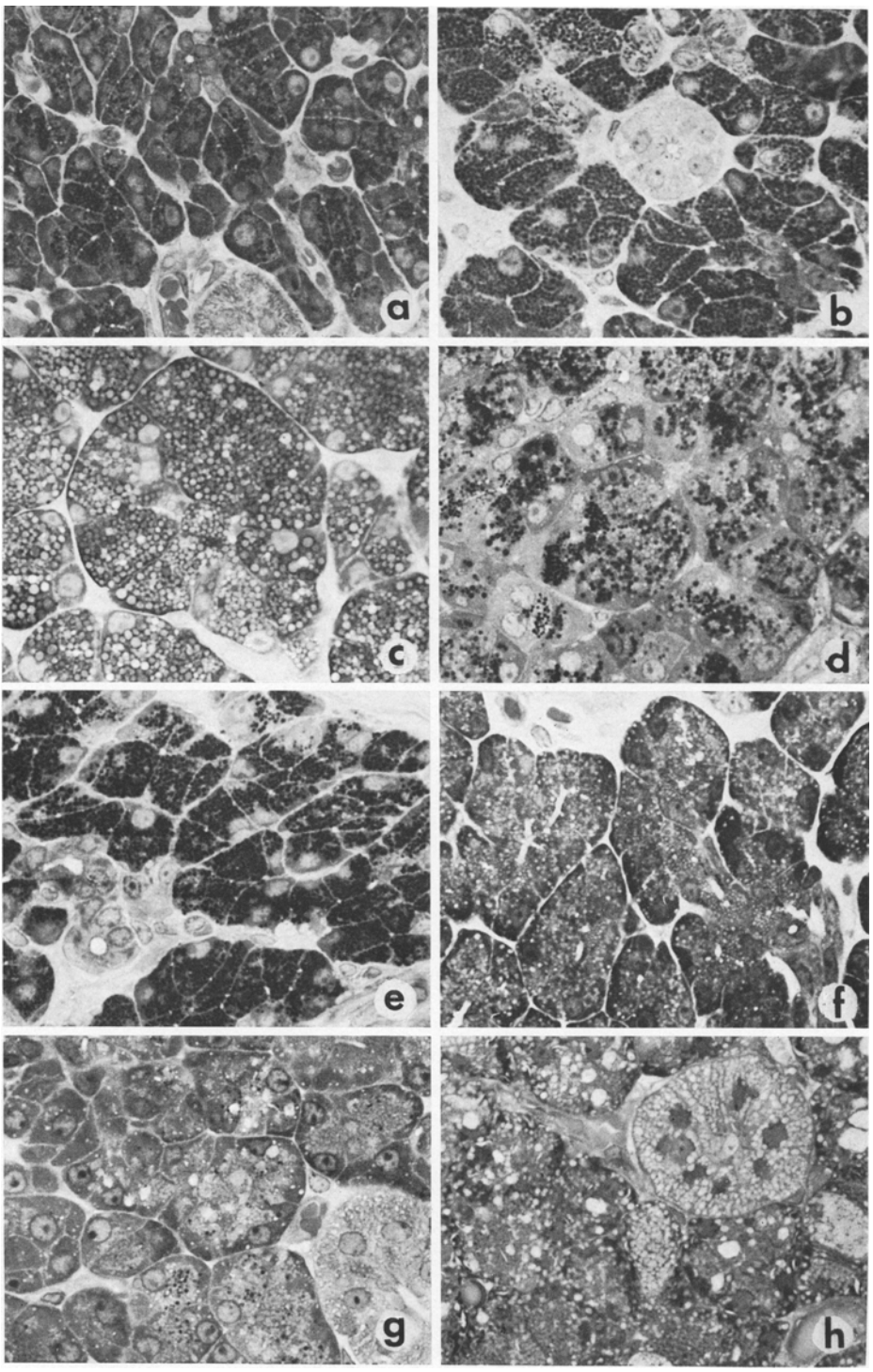

Fig. 1 a—h

Toluidin blue stained semi-thin sections were used for morphometric evaluations, the establishment of pathohistological findings and the selection of ultra-thin sections for further electron microscopical evaluation. The ultra-thin sections were placed on unfilmed microscopic slide nets and contrated with alcoholic uranylacetate and lead-citrate. Analysis was accomplished with a Phillips EM 300 at an accelerating voltage of 60 kV and a Siemens-Elmiskop I at 80 kV.

Morphometry. For each animal in all experimental groups the acini were counted out in various fields ($F = 14.4 \times 10^3\ \mu^2$) and the acinar diameter determined from a total area of $F = 144 \times 10^3\ \mu^2$. The Visopanphotometer of the Reichert Co. was used for measurement. The Gauss' normal distribution and margin of error were determined for all averages. The significance of the differences in average acinar diameters was tested in a t-test after the student distribution.

Results

1. Pathohistology

Group I (Control Group). The nuclei are round, of moderately dense chromatin, contain a conspicuous nucleolus and lie in the basal third of the cell without reaching the limiting membrane of the cell. (Fig. 1a) Numerous secretory granula are in the apical third of the cell. The acinar lumina are clearly recognizable, the intercellular spaces moderately dilated.

Group II (Control Group with Denutrition Prior to Eating Time). The nuclei lie predominantly basal. The entire cytoplasm contains an abundance of secretory granula (Fig. 1b). The acinar lumina are only recognizable in fission form.

Group III (Aludrin). The nuclei lie basally and have large nucleoli. The swollen cytoplasm contains primarily large light secretory vacuoles and only a few regularly configurated secretory granula. The acinar lumina are clearly distended. After several days of treatment the entire acinar cytoplasm is filled with light secretory vacuoles from the basis to the apical cell pole (Fig. 1c).

Group IV (Pilocarpine). The moderately enlarged nuclei contain conspicuous nuclei and are basally located. The apical cytoplasm is filled with numerous, optically partly dark, partly lighter secretory granula (Fig. 1d). The acinar lumina and intercellular spaces are not distended.

Group V (Guanaclin). The nuclei are located at the cell basis. The cytoplasm is filled with dark secretory granula (Fig. 1e). In a longer experiment light secretory granula (30 experiments day) or acinar cellular necrosis (40 experiment days) may be observed.

Fig. 1a—h. Rat parotid (semi-thin sections). a Control animal (group I) without denutrition: dark secretory granula in apical third of cell, ample ergastoplasm in basal third. b Control animal (group II) with denutrition 14 days before dissection: dark secretory granula in entire cytoplasm. c 9 day administration of Aludrin (group III): Greatly enlarged acini with varyingly large light secretory granula in entire cytoplasm. d 3 day Pilocarpine administration (group IV): Numerous light and dark granula in cytoplasm. 25 day administration of Guanaclin (group V): Dense filling of entire cytoplasm with dark secretory granula. f 3 day administration of Scopolamine (group VI): Centrally translucent granula in entire cytoplasm. g and h 10 day administration of Guanaclin-Scopolamine (group VIII): Light and only occasional dark secretory granula. Lacunar cytoplasmic translucence in individual acinar cells. h So-called mucoid transformation of the cytoplasm in individual acinar cells. Toluidine blue: Enlargement $\times$ 560

Group VI (Scopolamine). The nuclei are located basally. The entire cytoplasm is filled with centrally transluscent secretory granula (Fig. 1f). Some lacunary cytoplasmatic translucences may be observed. The acinar lumina are distended.

Group VIII (Guanaclin and Scopolamine). The swollen cytoplasm contains numerable light and occasional dark secretory granula (Fig. 1g). Lacunary cytoplasmic translucences of nucleus size may be observed frequently (Fig. 1h).

2. Morphometry

The findings are summarized in Table 2. In contrast to the control group a significant acinar enlargement was observed particularly after stimulation of the sympathicus and parasympathicus. The denutrition before the beginning of the eating period (group II) also led to an obvious increase of the mean acinar diameter. The results of group VI were somewhat above the norm, whereas in group VII the results—after elimination of the sympathetic and parasympathetic nervous system—were slightly lower (p 0.45) than those of the control group. The range of acinar diameters was particularly great in group II (denutrition) and group V (sympathicolysis).

Table 2. Result of morphometric examinations

Experimental group		Tissue removal after commencement of experiment	Average acinar diameter (μ)	Margin of error (μ)
I	(Control)	Day 10	25.6	± 0.79
II	(Denutrition)	Day 1	32.2	± 0.93
III	(Aludrin)	Day 3	33.0	± 0.53
IV	(Pilocarpine)	Day 3	34.1	± 0.92
V	(Guanaclin)	Day 3	35.0	± 0.54
VI	(Scopolamine)	Day 3	27.8	± 0.3
VII	(Guanaclin and Scopolamine)	Day 10	25.3	± 0.86

3. Ultrastructural Findings

a) Glandular Acini. In the basal cytoplasm of the acini of control group I granular endoplasmatic reticulum in parallel order and adjoining the nucleus (Fig. 2a) is formed. Dark secretory granula may be observed at the apical cell pole, condensing vacuoles (Fig. 2a) in the vicinity of the Golgi areas.

In the *control group with denutrition (II)* numerous electron dense secretory granula are concentrated in the entire cytoplasm (Fig. 2b). In comparison, the Golgi areas are small as are the mitochondria. Occasionally, condensing vacuoles may be found. The acinar lumina are smoothly limited.

After the influence of Aludrin (group III) numerous electron optically light secretory granula (secretory vacuoles) can already be found in the apical cytoplasm on the third experiment day (Fig. 3a). These light secretory granula are considerable larger than the electron dense granula which can still be observed

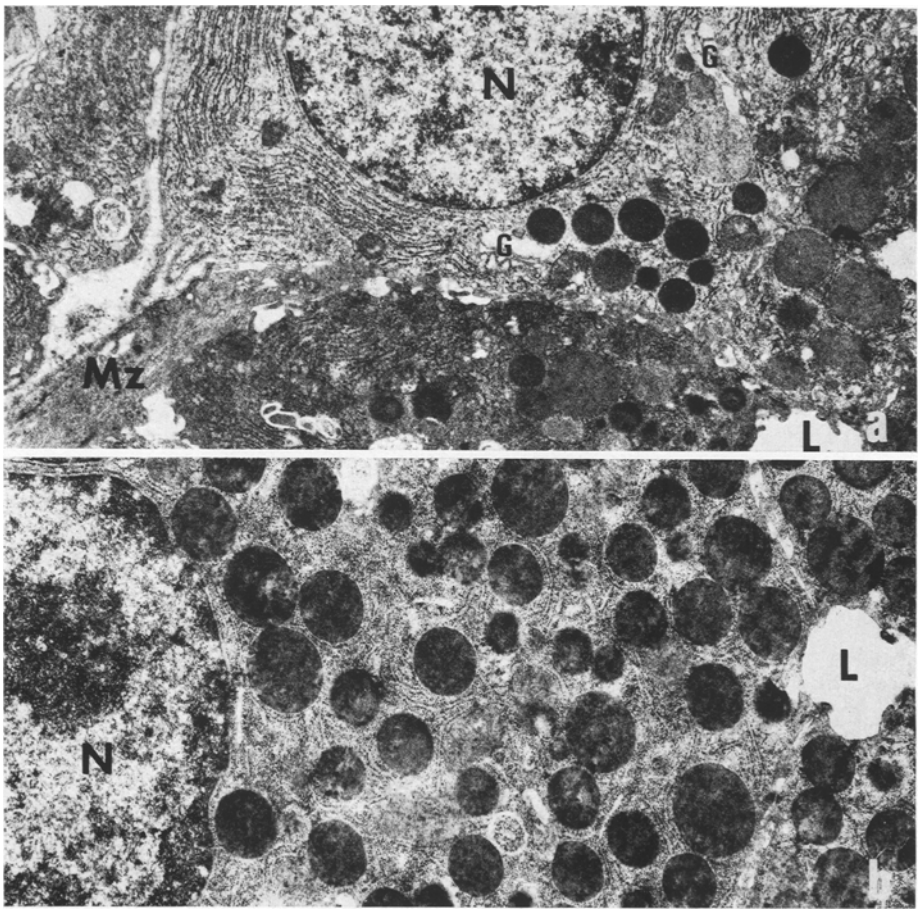

Fig. 2a and b. Rat parotid, acinar sections. a Control animal (group I): Basally and laterally parallel granular endoplasmatic reticulum; apical secretory granula at various stages of maturity. Golgi area (*G*), nucleus (*N*), acinar lumen (*L*), myoepithelial-cell-offshoot (*Mz*). b Control animal (group II): Predominantly dark secretory granula in apical cytoplasm; smoothly limited acinar lumina (*L*). Nucleus (*N*). Enlargement: ×7000; ×9600

in individual cells. The basal granular endoplasmatic reticulum has increased. The numerous mitochondria have a light matrix. The Golgi areas are much larger and in a changed vesicular form.

After the *influence of Pilocarpine (group IV)* the granular endoplasmatic reticulum is particularly conspicous (Fig. 3b). The cysternae of the granular endoplasmatic reticulum are distended and contain granular material. Condensing vesicles and occasional electron dense secretory granula may be found in the remaining cytoplasm. The nucleus contains a well defined nucleolus with ring shaped translucent areas.

After *administration of Guanaclin (group V*: Lit.: Donath, 1973) an alteration in the secretory granula (spotty translucence, lumpy disintegration) is ob-

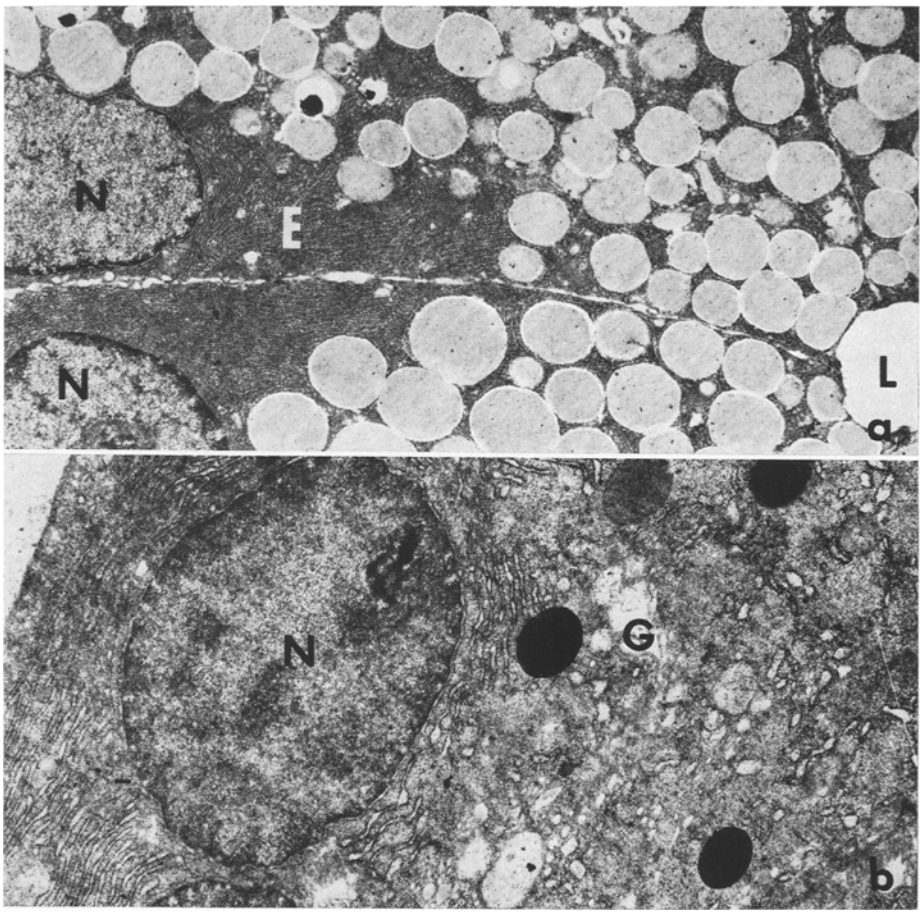

Fig. 3a and b. Rat parotid, acinar sections. a 4 day Aludrin treatment (group III): Varyingly large light secretory granula; nucleus (*N*), acinar lumina (*L*), ergastoplasm (*E*). b 3 day administration of Pilocarpine (group IV): Mature secretory granula and condensing vacuoles in apical part of cell. Distended cysternae of the granular endoplasmatic reticulum. Golgi field amply filled with vesicles (*G*); nucleus (*N*). Enlargement $\times 5800$; $\times 9000$

served. The Golgi areas have changed to a vesicular form. The granular endoplasmatic reticulum is limited to the cell basis and contains a moderate amount of granular material. After 20 experiment days (Fig. 4a) non-circular, inhomogeneous electron dense secretory granula and only occasional condensing vacuoles may be found in the numerous acinar cells.

Scopolamine (group VI) results in a concentration of prosecretory and altered secretory granula in the cytoplasm. Homogeneous electron dense secretory granula are lacking. Instead one finds (Fig. 4b) secretory granula which can be recognized by an electron dense mantle and an inconsistently dense inhomogeneous center. The granular endoplasmatic reticulum contains extended cysternae with fine granular material. The mitochondria have a light matrix. The Golgi areas are variably large.

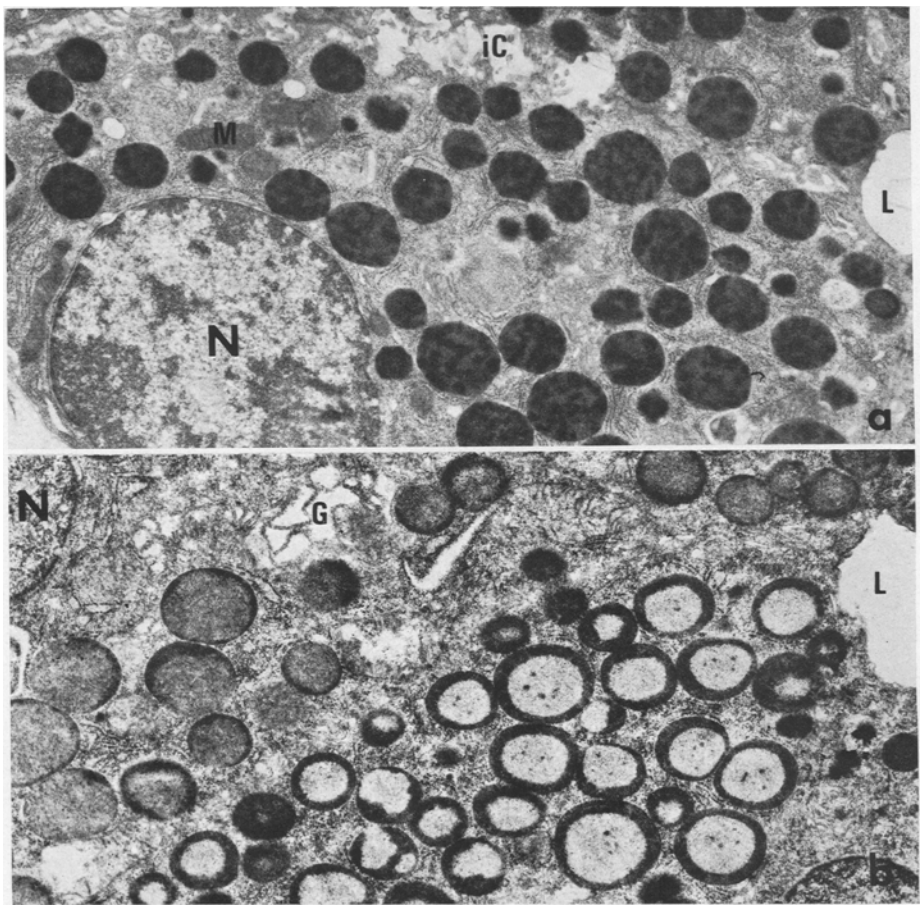

Fig. 4a and b. Rat parotid, acinar sections. 25 day Guanaclin treatment (group V): Dark, inhomogenous secretory granula in entire cytoplasm. Small, dark mitochondria (*M*); nucleus (*N*), intercellular canaliculus (*iC*); acinar lumen (*L*). b 3 day Scopolamine administration (group VI): Numerous secretory granula with dark mantle and inhomogeneous center. Golgi field (*G*), nucleus (*N*), acinar lumen (*L*). Enlargement $\times 6400$; $\times 9500$

The greatest acinar alterations may be observed in experiment group VII after the *influence of Guanaclin-Scopolamine*. The secretory granula have differing shapes and are of varying electron optical density. Fine granular areas are included as well. Numerous acinar cells (Fig. 5a) contain optically light lacunae which obviously incorporate the content of condensing vacuoles or secretory granula. One can also find cells with numerous lacunae which show a certain confluence (Fig. 5b). Other acinar cells resemble mucigenous glandular cells with diffuse distribution of prosecretion in the apical cytoplasm. The granular endoplasmatic reticulum contains distended cysternae with granular material (Fig. 5c).

b) Preterminal Axons. The normal structure of the autonomous nervous system in the parotid gland of the rat has already been presented in an earlier effort

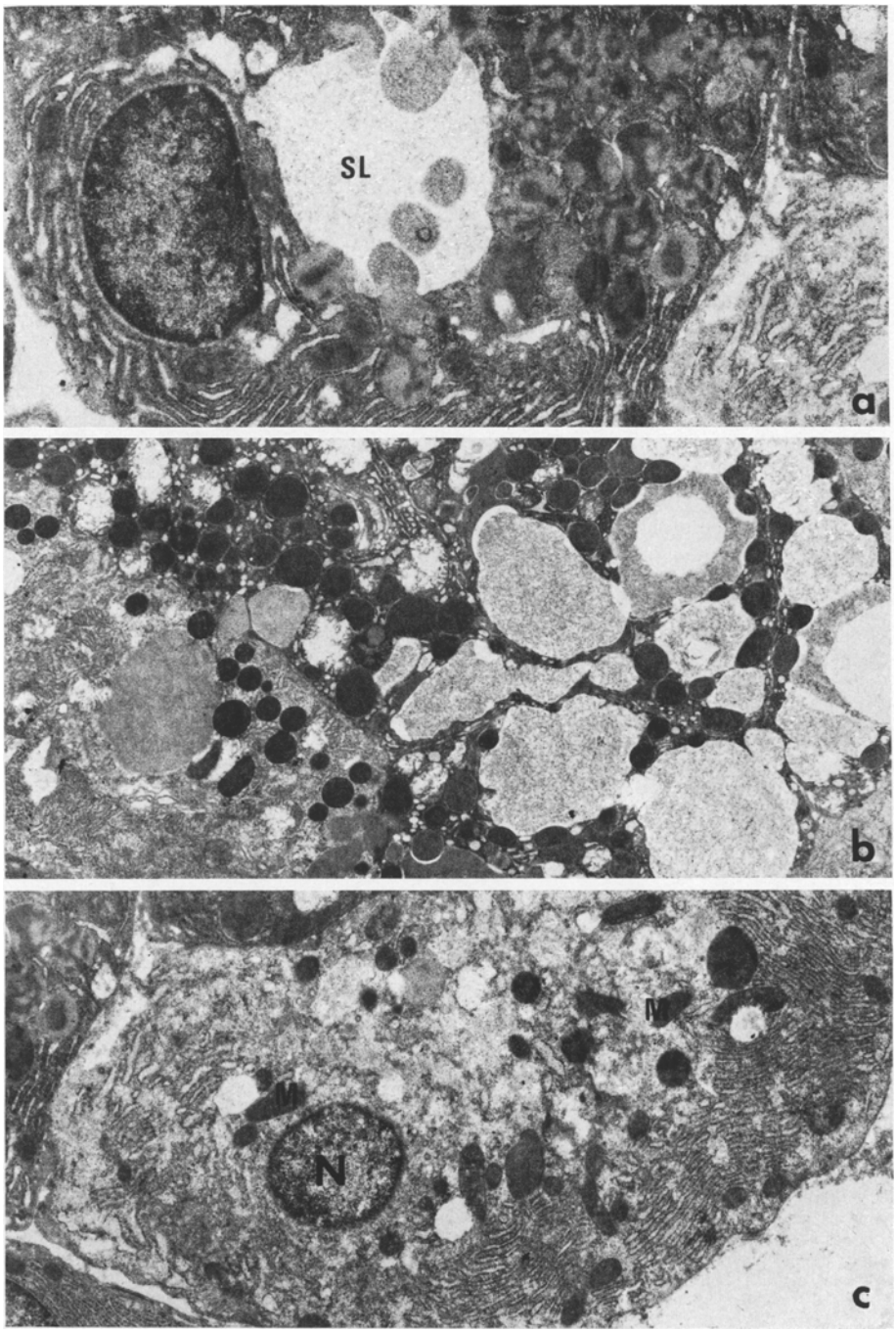

Fig. 5a—c. Rat parotid following 10 day Guanaclin-Scopolamine treatment (group VII): acinar sections. a Inhomogenous electron dense secretory granula in apical cytoplasm. Supranuclear lacuna (*SL*) with fine flaky content in which the altered secretory granula and condensed vesicles are included. b Numerous lacunae with varyingly dense content (prosecretion) and tendency toward confluence. c Diffuse spreading of prosecreta in apical cytoplasm; irregular course of granular endoplasmatic reticulum with distended cysternae. Nucleus (*N*); mitochondriae (*M*). Enlargement $\times 8400$; $\times 4800$; $\times 6800$

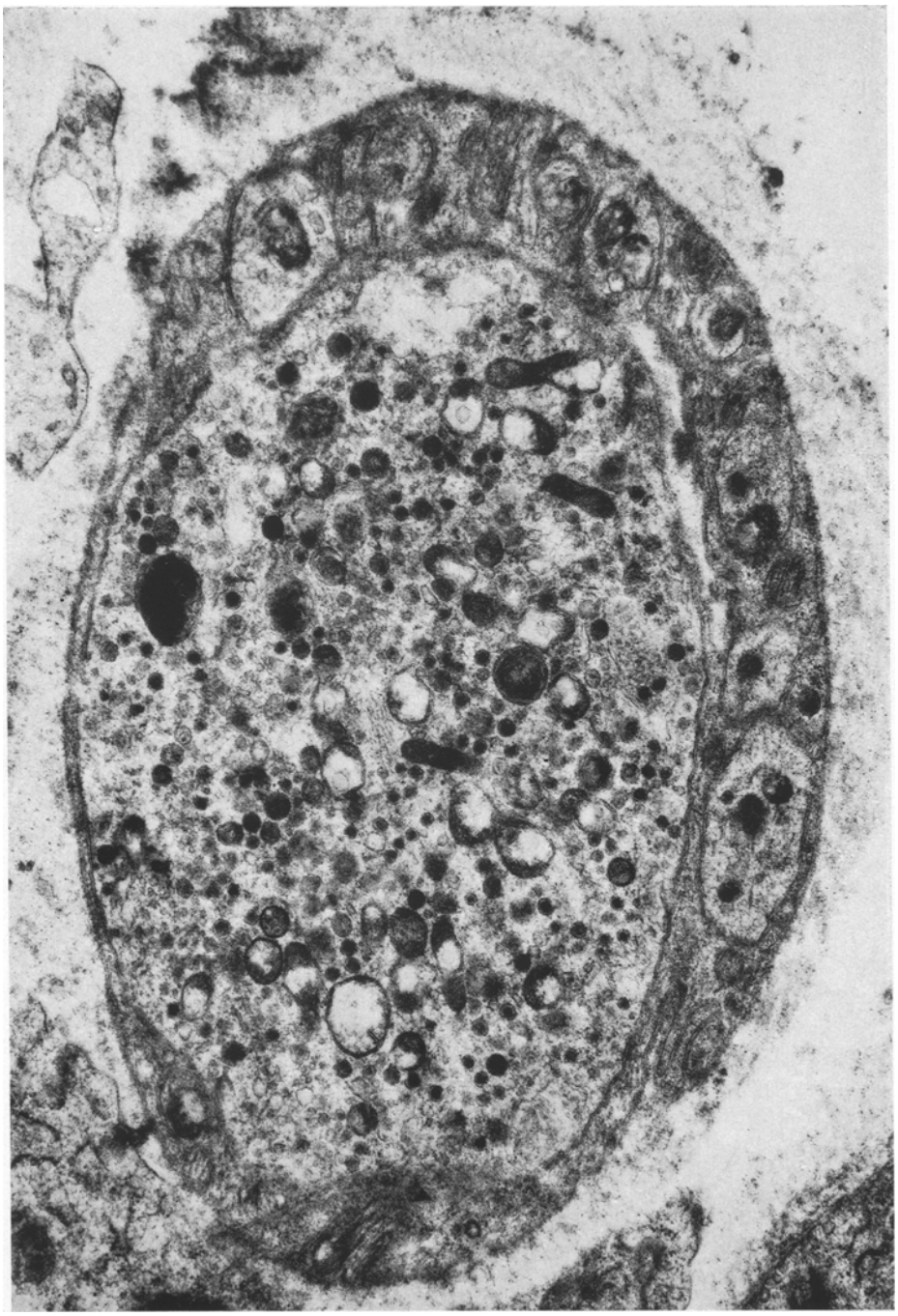

Fig. 6. Small bundles of rat parotid nerve fibres 2 hours after administration of Aludrin (group III): Giant axon with numerous, varyingly large granular vesicles and osmiophilic bodies. Numerous axons in the cytoplasm of the Schwann's cells rest hood—like on the swollen sympathetic axon. Enlargement × 20100

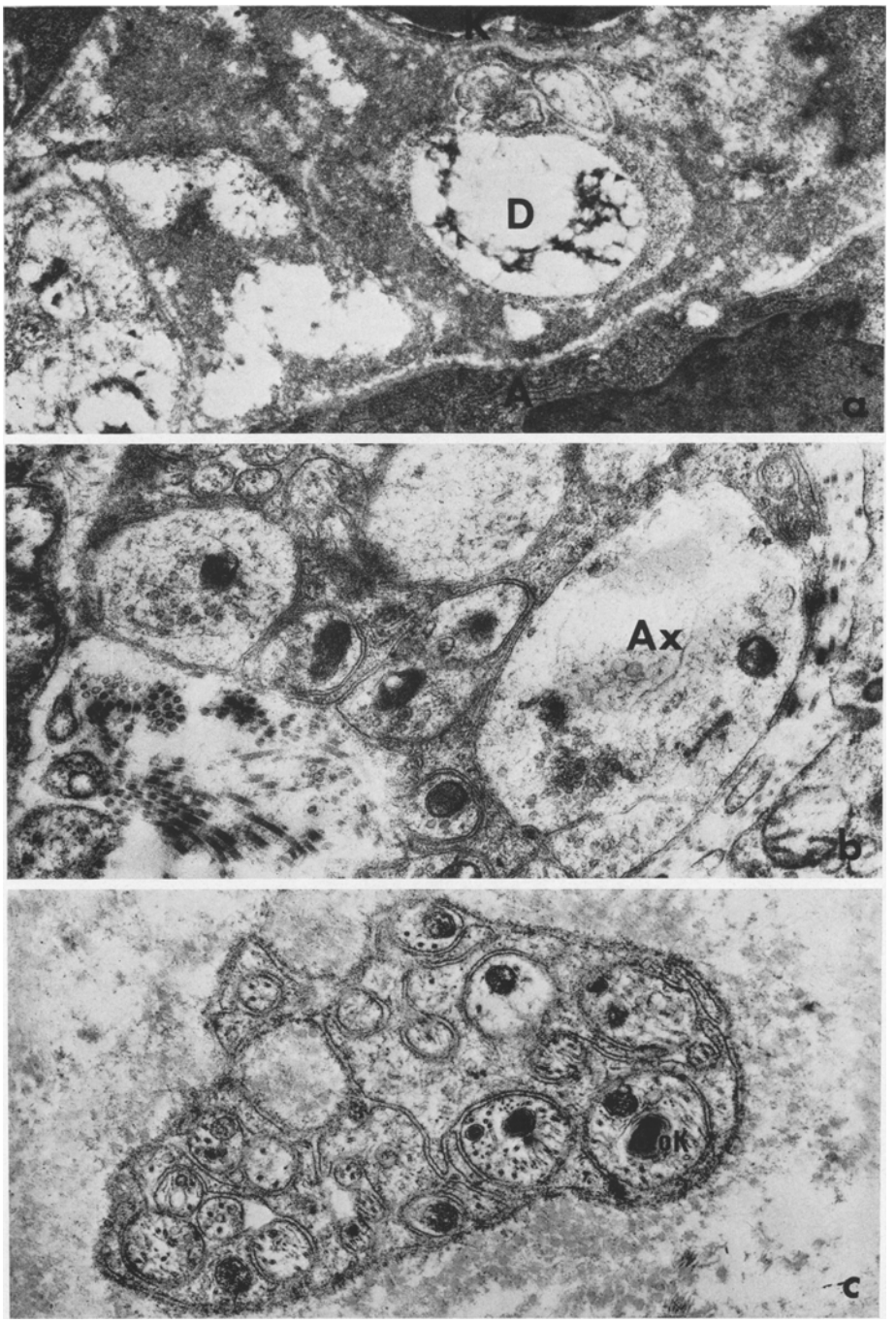

Fig. 7a—c. Autonomic nerves of the rat parotid. a 4 day Aludrin treatment (group III): a Axon degeneration (*D*) with axolemma disintegration. Capillary (*K*); acinar cell (*A*). b 3 day Pilocarpine administration (group IV): Small bundles of nerve fibers with hydropically swollen axons (*Ax*) and aggregated agranular vesicles. c 3 day administration of Scopolamine (group VI): Single axons within the bundle of nerve fibers contain spiral-formed osmiophilic bodies (*oK*). Enlargement $\times 15400$; $\times 24000$; $\times 19100$

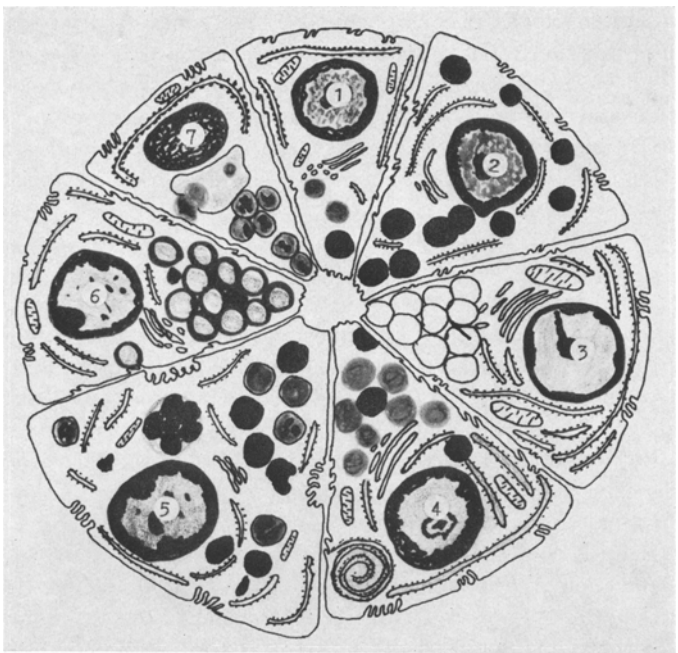

Fig. 8. Synopsis of acinar changes in groups II—VII in comparison to control group I. For particulars see text. *1* Control, *2* withdraw of nutrition, *3* Aludrin, *4* Pilocarpine, *5* Guanaclin, *6* Scopolamine, *7* Guanaclin + Scopolamine

(Donath and Seifert, 1973). For this reason, only findings which deviate from the norm in experiment group III—VII will be dealt with here.

After two hours *Aludrin (group III)* effects a dilation of the preterminal axons filled with granular vesicles of varying sizes and osmiophilic bodies. The mitochondria are swollen. The axons lying in the cytoplasm of the Schwann's cells rest hood-like on the enlarged axon (Fig. 6). After 2 experiment days aggregated and spiral corpuscles can be observed in the dilated axons, on the 4th experiment day degeneration of the axon with axolemma disintegration. The remains of the axons lie as filamentous material in larger vacuoles (Fig. 7a). 12 days after Aludrin treatment osmiophilic corpuscles may be found in the cytoplasm of the Schwann's cells. At this point the Schwann's cells contain axons with agranular vesicles almost exclusively.

Pilocarpine (group IV) does not effect axon degeneration. The axons with granular vesicles are not altered (Fig. 7b). The mitochondrial show no changes. The agranular vesicles are located in the axon periphery. An increased number of osmiophilic corpuscles are formed in the area of individual preterminal axons.

Administration of Guanaclin (group V) results in a loss of granular vesicles, a destruction of the mitochondria and the development of lysosome-like corpuscles in the altered axons and in the cytoplasm of the Schwann's cells. These findings have already been treated in a previous work (Donath and Seifert, 1973).

Scopolamine (group VI) results in alterations distinguished by a swelling of the mitochondria and a concentration of agranular vesicles in the varicosities

of the preterminal axons. The preterminal axons contain spiral formed osmiophilic corpuscles besides neurotubuli and mitochondria (Fig. 7c).

After the influence of Guanaclin-Scopolamine (group VII) considerable axon distintegration and numerous osmiophilic corpuscles may be observed. The destruction of the membranes is clearly recognizable even after potassium-permanganate fixation. The preterminal axons contain only agranular vesicles.

Discussion

The *autonomous regulation of the salivary secretion* has been confirmed in numerous physiological and biochemical investigations (Lit.: Burgen and Emmelin, 1961; Schneyer and Schneyer, 1967). The doubled adrenergic and cholinergic innervation of the salivatory glands could also be supported by ultrastructural and autoradiographic studies in the past few years (Bogart, 1970, 1971; Hand, 1970, 1972; Bogart and DeLemos, 1973). Particularly in the case of the rat parotid, norepinephrine or acetyl cholinesterase can be observed in the vesicle area of the preterminal axons. A particularity of the parotid as opposed to the submandibular gland is that the axons are arranged in direct contact with the basal membrane and acinar cell membrane (Bogart and DeLemos, 1973; Hand, 1970, 1972; Donath and Seifert, 1973). In the transfer of the autonomous nervous stimulants to the glandular cells of the parotid the α- and β-receptors play a decisive role. Biochemical investigations show that an adrenergic stimulation of the rat parotid results in amylase and potassium output and that blockage of the β-receptors hinders the liberation of amylase. Blockage of α-receptors hinders the potassium output (Batzri *et al.*, 1971).

Corresponding to the actual autonomous stimulation one can find a *varying composition of the salivary secretion* (Mangos *et al.*, 1973). Because of the species dependent properties of salivary secretion the relationships in the rat parotid should only be mentioned here. In the primary acinar secretion acquired by micropuncture an adrenergic stimulation effects a greater protein and potassium content than cholinergic stimulation. On the other hand the chlorine and bicarbonate content is substantially less than after the influence of acetylcholine. Taking the maximal effective dosage into consideration, the greatest rate of salivation occured after parasympathetic stimulation.

The morphological *secretory cycle* is distinguished by the triad: "secretory production, secretory accumulation and secretion". By means of combined autoradiographic, ultrastructural and biochemical analyses the process of secretion has been largely explained (Castle *et al.*, 1972; Lillie and Han, 1973). The protein synthesis may be compared to production on a conveyor belt system as in the pancreas. The difference, however, is that in comparison to the pancreas the protein rate is lower and the storage as well as the discharge of secretion longer. The main activity of the marked leucine occurs in the first 6 minutes after incorporation in the granular endoplasmatic reticulum. After that, activity occurs until the 36th minute in the Golgi complex, until the 116th minute in immature secretory granula and, finally, until the 356th minute in mature secretory granula.

The secretory cycle is influenced by the *daily rhythm of food intake* (Albegger and Müller, 1973). With rats in particular, the eating period begins in the evening

(around 8 p.m.) and ends in the morning (around 4 a.m.). Each time structure corresponds to an ultrastructural representation of the glandular cells with a different relationship between the amount of secretion (amount of secretory granula) and ergastoplasm (formation of the granular endoplasmatic reticulum). At the beginning of the eating period a secretory storage phase with massing of secretory granula and a minimum of granular endoplasmatic reticulum may be observed. By contrast, the granular endoplasmatic reticulum achieves maximal development at the end of the eating period whereas secretory granula are mostly lacking. The protein synthesis is greatest at the end of the eating period. This can be recognized from the large Golgi complexes and nuclear swellings with conspicuous nucleoli.

A feedback mechanism may be observed between *secretory production and secretion*. In other words, secretory synthesis and secretory output are in reciprocal relationship on a cellular level. This conclusion may be drawn from experimental conditions chosen by us as well as from numerous other experimental findings. In view of the questions posed initially, the changes in the secretory cycle and the cell organelles under various influences will be analyzed first (Fig. 8). The physiological secretion from the acinar cell does not take place in the case of *denutrition* (group II). The acinar cell remains in a state of secretory storage; this can be recognized by the concentration of electron optically dense secretory granula in the cytoplasm (Fig. 1b and 2b) and by the missing activity of the nucleus and cell organelles involved with protein synthesis (granular endoplasmatic reticulum, Golgi complex, mitochondria). Longer hunger periods of 2—3 days resulted in an intracellular autodigestion of formed secretory granula by the lysosomes (Hand, 1972). Here, an increase of lipid droplets in the basal cytoplasm and lysosomal aggregation with the inclusion of decomposed secretory particles may be observed.

Sympathetic stimulation (group III) influences protein synthesis as well as secretion. The morphological changes in the glandular acini are dependent on the time of adrenergic stimulation, dosage and the extent of the application. The Aludrin (Isoproterenol) used as a sympathomimetic by us has been the object of numerous experimental investigations in the past few years. It is generally agreed that Aludrin possesses two active components. During secretory storage almost the entire amylase is freed from the glandular acini within two hours (Amsterdam *et al.*, 1969 Simson,1969; Lillie and Han, 1973; Johnson and Sreebny, 1973). After secretion the resynthesis of amylase is stimulated (Enwonwu, 1972). If Aludrin is administered at the height of the synthetic phase, no further increase in the rate of synthesis can be achieved (Johnson and Sreebny, 1973). The feedback mechanism between protein synthesis and secretion remains, irregardless of Aludrin influence. The latency phase of a temporary Aludrin ineffectiveners may be accounted for with the explanation that after secretion, ATP (as the source of energy) as well as the cell organelles and membrane systems must be reorganized (Lillie and Han, 1973). The morphological findings on glandular acini are well correlated to the biochemical results. The light secretory granula (Fig. 1c and 3a) are the morphological equivalent of a suden secretion; the nuclear changes (sweeling, large nucleoli) and the increase of granular endoplasmatic reticulum suggest protein synthesis.

Parasympathetic stimulation with Pilocarpine (group IV) also results in secretion from the parotid. In this case, however, the loss of amylase content amounts to only 50% (Shear *et al.*, 1973) and is less than the loss effected by Aludrin. The presence of partially translucent secretory granula but predominance of numerous, optically dark secretory granula (Fig. 1d) substantiate this finding. The simultaneous indication of a distinct protein synthesis may be deduced from the strongly developed granular endoplasmatic reticulum with numerable cysternae and the nucleus with conspicuous nucleolus (Fig. 3b).

Sympathicolysis by means of Guanaclin (group V) results in an intracellular secretional disturbance which primarily affects secretion. Initially, dark secretory granula are concentrated in the acinar cells (Fig. 1e). In due course alterations of the secretory granula with spot-formed translucences and lumpy disintegration occur. The protein synthesizing organelles (granular endoplasmatic reticulum) are reduced. Disturbances in the granula production have also been observed on the secretory granula following a sympathectomy (Wilborn and Schneyer, 1972).

The development of a heterogeneous granula population with contrasting light and dark secretory granula indicates that the sympathicus plays an important role in the synchronisation of the secretory process in the glandular cell. Morphologically similar alterations of the granula—as in secretory disturbances—can, however, also develop if the synthetic phase is already damaged. Here one can assume changes in the acini because of a shortage of amino acids, blockage of the DNA or RNA metabolism or intoxications (Lit.: Donath *et al.*, 1971).

After *parasympathicolysis* (group VII) one may find suggestions of a disturbance in the protein synthesis as well as in secretion. A concentration of prosecretory granula results as does the development of a type of granula recognized by an electron optically dense mantle and an inhomogeneous center (Figs. 1f and 4d).

In a *combined sympathico- and parasympathicolysis* (group VII) the acinar alteration are most strongly emphasized. The secretory granula have an inconsistent size, form and density. In addition, confluent lacunae develop in the cytoplasm expressing a major disturbance in the intracellular secretory process (Figs. 1g, 1h, 5b, 5c).

Further information concerning the mechanism of the *neurotransmitters and neurolytica* may be deduced from a *comparison* between the *acinar structure* and *changes in the preterminal axons.*

Administration of Aludrin results in a substantial enlargement of the axon (Fig. 6). In due course, a partial axon degeneration may also be observed. As confirmed in other experiments (Lillie and Han, 1973; Barka *et al.*, 1972) the Aludrin effect is based less upon a concentration in the axons than upon a direct action on the β-receptors of the acinar cells. The effect of Aludrin may be stopped by blockage of the β-receptors but not by sympathectomy. Depending on the dosage one can reduce or completely prevent the effect of Aludrin on salivary secretion by administering 6-hydroxydopamine. Small doses result in injury to the superior cervical ganglion without influencing the effect of Aludrin. Large doses eliminate the effect of Aludrin. The explanation for this phenomenon is that large doses also destroy the β-receptors of the acinar cell. After these observations one must assuar that the acinar alterations following Aludrin

administration are best explained by the direct effect upon the acinar cell through the β-receptors and that the transformations of the axons only play a participatory role. In contrast to Aludrin Pilocarpine effects no axon damage.

Axon changes are most pronounced under the influence of *Guanaclin* (Donath and Seifert, 1973). They manifest themselves in an absence of granular vesicles, destruction of the mitochondria and the development of lysosomal bodies in the altered axons and Schwann's cells. Analogous findings after the influence of 5,6 dihydroxy-tryptamine have also been described (Baumgarten *et al.*, 1972). In a combined administration of *Guanaclin-Scopolamine* membrane destructions and axon disintegrations are also clearly visible whereas *Scopolamine* alone results in only slight alterations.

A summary view of the findings made on the glandular acini and preterminal axons permits no direct relationship between glandular structure and axon construction. One may thus draw the conclusion that the neurohormonally elicited interference in the secretory process results primarily from a direct interference at the neuroreceptor of the glandular cell. A further mechanism by way of the preterminal axons is clearly possible although this principle appears to be less important in the parotid than in the submandibular and other glands where no direct relationships exist between the limiting membrane of the glandular cell and the pretermianl axons. Substantiation for the hypothesis that the neuroreceptors of the acinar cell can be directly reached by neurohormonal influences over the blood stream is found in our own experimental observations on the chemically sympathectomised rat parotid. According to these investigations, the administration of α- and β-adrenergic drugs permits the extrusion of granula despite elimination of the sympathetic nervous system.

A final *comparison of the experimental findings with parotid changes in human sialadenosis* yields a number of accordant morphological findings. Human sialadenosis is marked by a swelling of the glandular acini and alteration of the cytoplasm as well as of the secretory granula (Lit.: Seifert, 1966). In the meantime, microscopic findings have been supplement with ultrastructural observations (Donath and Seifert, 1974). Likewise, analysis of parotid saliva in the case of sialadenosis has shown that the amount and composition of salivary secretion changed. The degree of secretory disturbance and the morphological state of the acinar structure is in relationship to the length and extent of the illness.

Morphometric measurements of the glandular acini made under experimental conditions chosen by us show clearly that in contrast to the control group, a significant acinar enlargement occurs primarily after sympathetic and parasympathetic stimulation although after denutrition as well. Acini swelling increases with the duration of the experiment. This applies to long term Aludrin studies as well as to the influence of Guanaclin. From this one may assume that all interferences in the normal secretory process lead to an intracellular secretory disturbance in the course of which secretory production, secretory storage and secretion are individually or even complexly changed. In the case of severe and chronic secretory disturbances one may observe partial cytoplasmatic degradation or complete single cell cytoclasis. Observations made on experimental sialadenosis are further substantiation for the thesis that human sialadenoses are caused by a neurohormonally elicited secretory disturbance. Similar to experi-

mental sialadenosis one may assume that in the course of the illness the main damage results by means of the neuroreceptors of the acinar cell and that changes in the preterminal axons only play a potentiating role.

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Dr. K. Donath
Prof. Dr. G. Seifert
Pathologisches Institut der Universität
D-2000 Hamburg 20
Martinistr. 52 UKE
Federal Republic of Germany