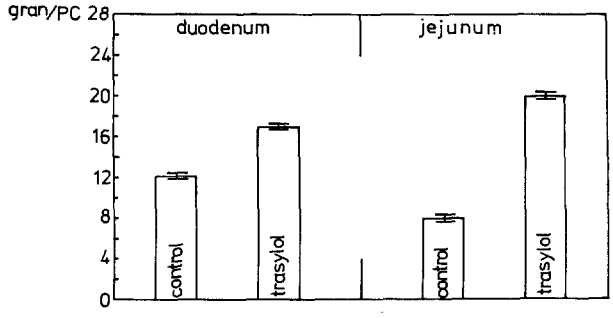

Fig. 3. The mean number (± S.E.) of secretory granules of Paneth cells after Trasylol® administration. The granule count per cell is marked on the ordinate. There is statistically a highly significant increase of the granule count per cell both in the duodenum and jejunum. Abbrevations: gran, granule; PC, Paneth cell.

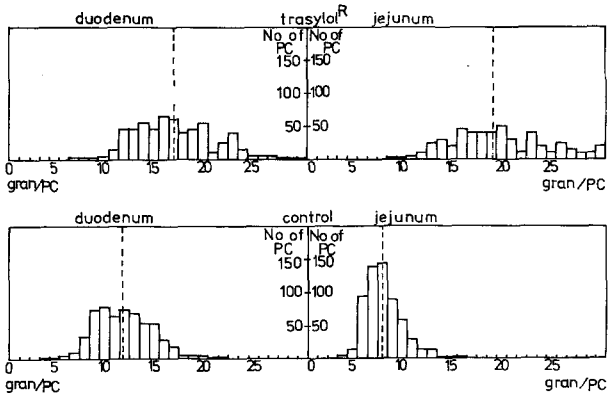

Fig. 4. The distribution of the Paneth cells as a function of the number of coarse cytoplasmic granules after Trasylol® administration. The number of granules per cell is marked on the axis and the number of Paneth cells on the ordinate. The average number of granules per cell is marked with dotted lines. The same material was used for Figures 3 and 4.

for these diverse phenomena have mainly remained obscure. It is shown that Trasylol® is able to inhibit the activity of zymogenic proteases of the acinar cells of the rat pancreas[18]. The present results indicated that Trasylol®, which is an inhibitor of various proteinases and trypsin, has obviously an identical effect on Paneth cells of the intestine. This appeared as an increased number of secretory granules of the cells as well as an increased granule size. Similar phenomenoma have been described to occur in acinar cells of the rat pancreas after soybean ingestion which contains trypsin inhibitors[14]. The origin and the relation of the Paneth cells to other cell types in the gastrointestinal tract is not wholly understood. The present observations further support the view that Paneth cells are morphologically and functionally and even embryologically related to the pancreatic acinar cells, as suggested previously on the basis of numerous morphological studies[13]. The underlying mechanism for the increase of the counts of secretory granules may either result from increased production of material necessary for granule formation or delayed extrusion of the granules from the cytoplasm of the cells into the intestinal lumen. The marked increase of the granule size in the present Trasylol® experiments favours the latter mechanism.

Zusammenfassung. Nachweis, dass Trasylol®, ein Hemmstoff für proteolytische Enzyme, im Duodenum und Jejunum der Maus eine Zunahme der Panethschen Zellen bewirkt und ausserdem eine Vermehrung der Sekretgranula pro Zelle verursacht.

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Stereological Measurements of Atrial Ultrastructures in the Guinea-Pig

Examination of the excitation-contraction coupling process in atrial muscle has suggested that the frequency-dependent mechanical response of the tissue[1,2] is associated with calcium derived from multiple calcium stores[3-5]. These stores have been suggested to be the sarcolemmal membrane, subsarcolemmal cisterns (SSC), sarcoplasmic reticulum (SR) and mitochondria (Mt). Electron microscopic examination of these and other intracellular structures have been performed in several atrial preparations[6,7]. However, in order to develop a better understanding of the contractile behavior of the tissue, a quantitative examination of cell structure is desirable. Previous determinations of this kind have been made on ventricular myocardium[8-11] and skeletal muscle[12,13]. In this paper, we report the results of stereological measurements made on the contraction-related structures of a typical mammalian atrial preparation: the guinea-pig left atrium.

Materials and methods. Left atria from guinea-pigs of either sex, weighing between 400 and 800 g, were isolated in oxygenated Krebs-Henseleit solution at 24 °C. Prior to fixation, mechanical characteristics of the tissue were examined with a standard isometric recording apparatus. Only atria exhibiting normal behavior were prepared for stereological examination.

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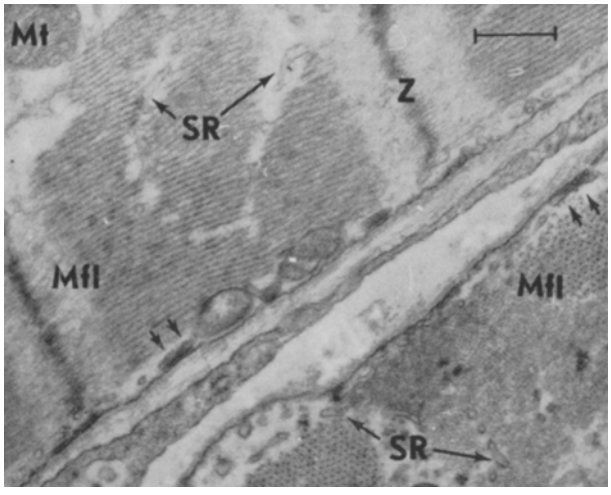

A typical guinea-pig left atrial section employed in morphometric analysis. Mitochondria (Mt), myofilaments (Mf1), Z-line (Z), sarcoplasmic reticulum (SR), subsarcolemmal cisterns (double arrow). Scale, 0.5 μm; ×29400.

After mechanical evaluation, atria were initially fixed for 2 h in ice-cold 3% glutaraldehyde in 0.05 *M* collidine buffer (pH 7.4). Specimens were then washed several times with 0.3 *M* sucrose in 0.05 *M* collidine buffer (pH 7.4) and post-fixed in a 1% osmium tetroxidecollidine buffer solution for 2 h. Atria were dehydrated in a graded series of ethanol washes, followed by propylene oxide, embedded in Epon DDSA and NMA[14] and sectioned on a Reichert ultramicrotome with a diamond knife. Thin unoriented silver sections were stained with a saturated solution of uranyl acetate and Reynolds lead citrate and examined on a RCA model 3H-electron microscope. In order to obtain an unbiased sample, micrographs were recorded from the upper right-hand corner of the squares of the supporting copper grid. For calibration, a carbon replica cross grating (21,600 lines/cm) was photographed. Measurements were made on 19×19 cm prints having a final magnification of 33,600 and on 20×23 cm prints having a final magnification of 18,500.

Point counting and line integration[12,15–17] were performed on the micrographs by covering them with transparent sheets of thin plastic on which a square grid (either 0.65 or 0.3 cm/side) had been photographically imprinted. The grid and print were illuminated by placing them on an X-ray viewing box. The fraction of cell volume occupied by a cellular component i was determined by the relation $V_i/V_{cell} = P_i/P_{cell}$[16], in which P_i is the number of points falling on the component i, and P_{cell} is the total number of points falling on the myocardial cell. The surface to volume ratio of the cellular component i was calculated from the relation $A_i/V_{cell} = (\pi/2)\,(C_i/aP_{cell})$[16], in which A_i is the external membrane area of the component i, C_i is the number of intersections of the external membrane of component i with the vertical lines of the grid, and a is the length of one side of the square on the measuring grid divided by the final magnification of the print. The theoretical considerations involved in stereological measurements of non-oriented but anisotropic tissue and the method of counting necessary to overcome such problems have been discussed by others[12,17].

Results and discussion. The structural organization of the guinea-pig atrial cell (Figure) is similar to other mammalian atrial preparations[6,7], possessing a mean cell diameter of 7.6 μm. The quantitative measurements made of these structures and their relationship to other myocardial cells are summarized in the Table. The contractile proteins occupy 41.4% of the cell volume arranged in irregularly shaped groups of myofibrils. The nuclei (4.1%) are centrally located, being associated with a prominent Golgi complex as well as atrial granules and vesicles. The mitochondria (14.4%) are aligned in clefts between the myofilaments as well as in the juxtanuclear cytoplasm. The sarcoplasmic reticulum (1.7%) is randomly oriented, meandering for short distances across the bands in any direction. The tubules of the sarcoplasmic reticulum make specialized couplings with the sarcolemma, increasing the total SR fractional cell volume to 2.2%. The subsarcolemmal couplings appear as flattened saccules parallel to the sarcolemma and separated by an interspace possessing periodic densities.

Comparison of these fractional cell volumes to those obtained in other myocardial fibres (Table) demonstrates an apparent structure-function relationship. The ventricular cells associated with electrical transmission (i.e., purkinje, bundle branch and pseudotendinous fibre) possess a less developed intracellular structural organiza-

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Comparative stereological measurements of mammalian myocardial structures[a]

Myocardial cell	Volume (μm³/μm³)				
	N	Mt	SR	SSC	Mfl
Guinea-pig l. atrium[b]	0.041 ± 0.012	0.144 ± 0.008	0.017 ± 0.001	0.005 ± 0.000	0.414 ± 0.006
Rat l. ventricle[9]	—	0.34 ± 0.01	0.035 ± 0.002	—	0.481 ± 0.009
Rat l. ventricle[10]	—	0.358 ± 0.006	0.035 ± 0.002	—	0.476 ± 0.007
Calf l. ventricle[11]	0.029 ± 0.008	0.307 ± 0.044	—	—	0.387 ± 0.018
Sheep purkinje[8]	0.009 ± 0.002	0.103 ± 0.006	—	—	0.234 ± 0.009
Calf l. bundle branch[11]	0.024 ± 0.014	0.110 ± 0.016	—	—	0.214 ± 0.030
Calf l. pseudotendinous fibre[11]	0.027 ± 0.006	0.113 ± 0.025	—	—	0.212 ± 0.039

[a] Values are fractional volumes of structure i as a function of cell volume. [b] Atrial results are given as means ± SEM. In all cases, the standard error has been calculated with $n = 8$, since 8 left atria were employed. For each atrium, observations were made on an average of 7 micrographs.

tion, with the myofibrils and mitochondria comprising only 32% of the cell volume. Similarly, the fractional cell volumes of the myofibrils and mitochondria in rat (82%) and calf (69%) ventricles are greater than in guinea-pig atrium (55%), being associated with their ability to develop greater contractile tension. The fractional cell volumes occupied by the sarcotubular systems in atrial and ventricular muscle are appreciably less than in skeletal muscle (13%)[13]. This implies a decreased storage capacity which is consistent with the rapid effect of altered extracellular calcium concentration on developed tension in the myocardium[18].

Mitochondrial cell volume determined by morphometry exhibits reasonable correlation to volumes calculated by measuring copper content[19]. Guinea-pig left ventricular wall and left atrium possessed copper contents of 0.33 and 0.19 μmoles Cu/g dry weight, respectively. Stereological examination of these cells provided mitochondrial volumes of 0.35 and 0.144 $\mu m^3/\mu m^3$ cell volume (Table), respectively. For amphibian atrial trabeculae, GREEN et al.[20] determined the mitochondrial volume as approximately 15%. The lower mitochondrial volume observed in atrial muscle, as well as the lower myofibrillar content per unit dry weight[21], may reflect a lower total consumption of ATP by the contractile apparatus of the cells.

A_i/V_{cell} for atrial sarcolemmal membrane, determined on randomly oriented sections, was 0.24 ± 0.03 $\mu m^2/\mu m^3$ which was in reasonable agreement with values determined in ventricular cells[9,10], 0.30 ± 0.02 $\mu m^2/\mu m^3$. Our inability to orient the tissue necessitated determination of the sarcoplasmic reticulum surface to volume ratio by the relationship $(A_{sr}/A_{sl})\ (A_{sl}/V_{cell})$, where A_{sr}/A_{sl} is independent of the sectioning angle [12,17]. The ratio determined for the sarcoplasmic reticulum was 0.36 ± 0.04 as compared to 1.2 $\mu m^2/\mu m^3$ for ventricular muscle[9,10]. The lower A_{sr}/V_{cell} determined for atrial muscle, as compared to ventricular muscle, is associated with a smaller sarcoplasmic reticulum fractional cell volume as well as a decreased dependence of atrial muscle on intracellular calcium stores[22].

Zusammenfassung. Mit morphometrischen Methoden wurde beim Meerschweinchen eine quantitative Analyse von Volumen und Oberflächenareal der Ultrastrukturkomponenten der Herzvorhof-Muskulatur durchgeführt, die mit dem Erregungs-Kontraktions-Kopplungsvorgang und der Erschlaffung verknüpft sind.

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Influence of Antihistamine and Compound 48/80 on Healing

The conception of the histamine-forming capacity (HFC) of tissues and the possibility of altering the HFC experimentally, more specifically of elevating it, has provided the possibility of accelerating the rate of wound healing[1–3]. In the tissues from skin wounds of the rat, the HFC has been found conspicuously elevated compared to that of intact skin, whereas the histamine content of wound tissue was low. The HFC of the skin can be experimentally increased: Repeated injections of compound 48/80 or polymyxin depletes the skin of histamine, whereby the histidine decarboxylase activity is concomitantly increased. In rats treated for a 3-day period with daily injections of 48/80 or polymyxin, wound healing has been found to be accelerated; both the tensile strength of the experimental wound and the collagen content in the granulation tissue were significantly increased. This increase persisted throughout the whole period of fibroplasia of the healing wound. The stimulation of healing was tentatively accounted for by an earlier formation of fibroblasts and thereby an earlier onset of collagen formation[4].

The pharmacologically liberated histamine, which reaches the skin via the blood-stream, i.e. extracellular histamine, does not enhance healing, since, on injecting long-acting histamine, the tensile strength of the wound was the same as the control wound in the untreated rat, as shown by KAHLSON et al.[5].

Material and methods. In order to strengthen further the evidence of the ineffectiveness of extracellular histamine in enhancing healing, rats were treated with the antihistamine shortly before receiving the histamine liberating drug. The tensile strength of 5-day-old healing skin incisions was measured with a tensiometer; the technique and procedures involved have been described in detail[4,6]. A control wound was first tested on each animal. The antihistamine was then injected followed by 48/80 15–20 min later. The antihistamine mepyramine (neoantergan®) was given i.p. in a dose of 25 mg/kg and 48/80 i.p. 1 mg/kg. This treatment was given 3 days before the experimental wound was inflicted.

Results. The dose of antihistamine was effective against extracellular histamine, because 48/80 alone produced signs of histamine shock; within 15–20 min the rats were lying still in their cages with stridor, cyanosis, signs of itching and sometimes oedema of nose and paws. The mortality in histamine shock in our previous studies was 20%[4]. When the rats had antihistamine prior to 48/80, no signs of shock were seen; the rats moved about in the normal way, looked unaffected and no deaths occurred.

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