Myocardial indicator content in vitro		in vivo	
Tissue parts		Tissue parts	
Superficial	Central	Superficial	Central
2077	1180	1781	1563
2551	858	1765	1459
3347	1812	1842	1524
2542	913	1714	1474
3108	1615	1745	1345
2895	1371	1923	1560
3090	1529	1783	1480
2430	1000	1701	1517
2782	1189	1879	1575
2301	939	1761	1400
2476	756	1601	1321
2691 ±368	1197 ±327	1772 ±90	1474 ±89

The single data are expressed as dpm/100 mg wet weight of myocardium and summarized as the mean ± S.D. They are based on a level of 10,000 dpm/75 λ of radioactivity in the serum and in the incubation fluid respectively

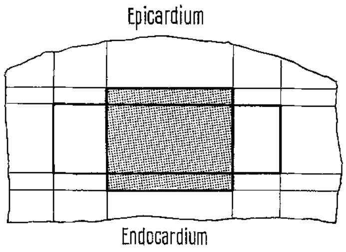

Fig. 1. In previous experiments the myocardial piece was sectioned in a cube-like (:::) central part, while now a rectangular central part (□) was excized. The new slicing pattern corresponds to the morphological characteristics of the ventricular wall, which consists of 3 different layers: endocardium with adjacent myocardial area, pure myocardium, and myocardium with epicardium.

histological phenomena are demonstrated in Figure 2 and are assumed to originate in the standstill of contraction and circulatory functions. There also exist 2 overlapping processes as a function of time, i.e., the tissue deterioration and the indicator penetration into the central parts. Swollen cells as well as material from myolyzed cells occluding the diffusion ways may hinder the indicator in penetrating the tissue. In addition an emigration of red blood cells from the tissue into the surrounding incubation medium can be observed by the naked eye. The same process is also believed to occur for other substances, i.e., hyaluronic acid and other macromolecules, which are known to exclude inulin from a homogenous distribution[3]. Since those particles mainly emerge from peripheral interstitial and vascular spaces, this phenomenon might be attributed to the unequal indicator distribution in vitro.

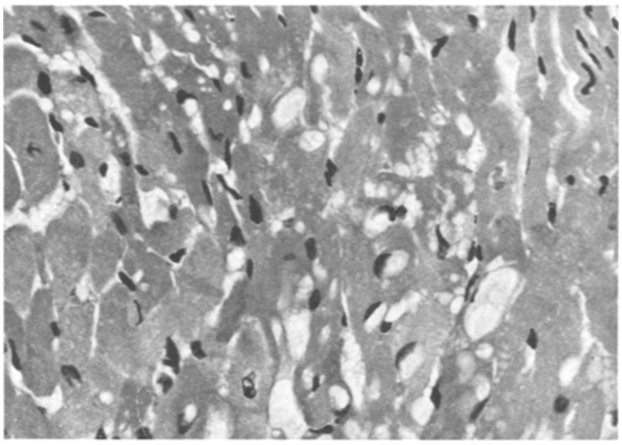

Fig. 2. Swelling, vacuolization and myocytolysis of myocardial cells of the left ventricular wall after 60 min of in vitro-incubation. The heart muscle piece was excized from the heart of a 104-day-old healthy female Syrian golden hamster of the London School of Hygiene-strain (hematoxylin-eosin), × 400.

The findings described above demonstrate that there is no homogenous extracellular space throughout the ventricular wall of the mammalian heart and also that the in vitro approach of extracellular space measurements does not reproduce normal or other in vivo conditions, which are likely to be explored. Therefore in vitro results are highly questionable.

Zusammenfassung. Extrazellulärraumindikatorverteilung ist in vivo und in vitro unterschiedlich, trotz ähnlicher Tendenz. Das Myokard verändert sich während der in-vitro-Inkubation. Deshalb erscheint es unmöglich, in vitro normale beziehungsweise in vivo herrschende Extrazellulärräume zu messen.

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Measurement of Collateral Flow in Experimental Coronary Occlusion

The currently available methods to study coronary collateral circulation after acute or chronic occlusion of coronary arteries, which include backflow measurements, electromagnetic flowmeter methods, clearance studies, cinearteriography and corrosion cast techniques, are imperfet[1,2]. A recently described autoradiographic technique[3] to assess the distribution of blood flow within organs has therefore been used to estimate regional myocardial blood flow after acute ligation of coronary arteries.

The method is based on the application of small radioactivity labelled particles, which do not pass through the systemic capillaries. If a calibrated amount of the particulate material is injected into the systemic circulation, its distribution represents the regional distribution of blood flow, provided the indicator is completely mixed in the blood leaving the left ventricle and completely extracted in one pass through the capillary bed. Its local concentration is an index of local flow[3].

Experiments were carried out in 12 mongrel dogs anaesthetized with Nembutal. A wire cuff was placed on the anterior descending coronary artery and a polyethylene catheter was inserted into the left atrium. Following recovery from surgery, the anterior descending coronary artery was occluded. The effect of the ligation was controlled by coronary angiography. After a period of 30 min following the occlusion, 2 mC J^{131} labelled macroaggregated albumin were injected into the left atrium. 4–10 min after the injection, the animal was sacrificed. The heart was rapidly removed and frozen. The frozen organ was slized into 1 mm thick sections employing a heavy sledge microtome. Contact autoradiographs were produced exposing the slices to an Agfa Gevaert Graphic Film O 53. The exposure time was 1–6 days. After exposure the films were separated from the sections and developed in a freshly prepared devel-

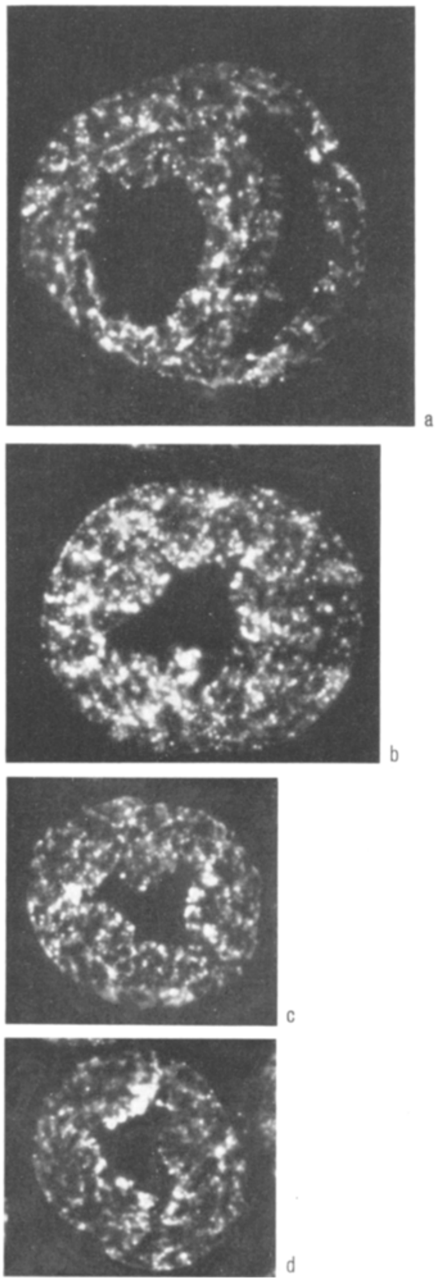

Fig. 1, a–d. Autoradiographs showing the intramyocardial distribution of the labelled indicator under normal conditions.

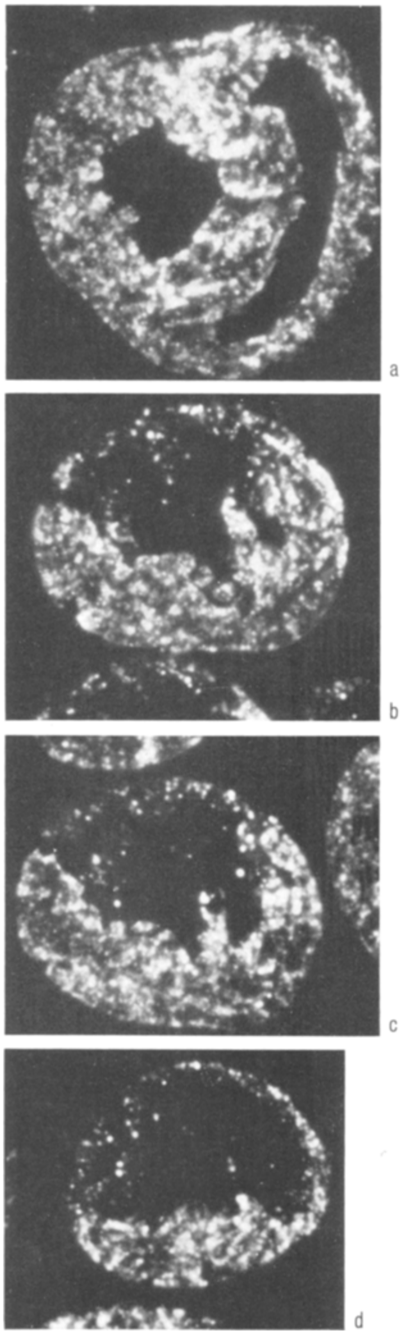

Fig. 2, a–d. Autoradiographs showing the intramyocardial distribution of the labelled material after acute complete occlusion of the anterior descending artery, distal from the septal artery. Large ischemic zone in the anterior wall of the left ventricle; the reduction of indicator uptake is most marked in the subendocardial layers of the left ventricle.

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oper. Photographs were taken of the tissue sections for comparison with the corresponding autoradiographs.

Sample autoradiographs show the distribution of the labelled material under normal conditions (Figure 1, a–d) and after acute complete ligation of the anterior descending coronary artery (Figure 2, a–d). In this, as in most cases, flow beyond the occlusion does not fall to zero. Within the area of reduced flow the indicator is distributed inhomogeneously. Reduction of flow is most marked in the inner layer of the left ventricular wall. This agrees with the findings of MOIR and DE BRA[4] and seems to indicate a primary nonhomogeneity of collateral supply.

Zusammenfassung. Es wird ein Verfahren zur autoradiographischen Darstellung der intramyocardialen Durchblutungsverteilung nach experimenteller Coronarocclusion angegeben. Im Versorgungsbereich akut verschlossener Coronararterien wird eine inhomogene Reduktion der Durchblutung beobachtet, wobei die Innenschichten regelmässig stärker als die subepicardialen Myocardanteile betroffen sind.

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A Protective Mechanism for the Tongue: Suppression of Genioglossal Activity Induced by Stimulation of Trigeminal Proprioceptive Afferents

Biting on the tongue during mastication is a relatively rare incident in the healthy adult. The tongue, particularly its anterior portion, appears to be well protected by a very reliable mechanism. One could hypothesize that the masticatory activity itself plays a major role in the protection of the tongue by sending to the brain stem impulses which regulate the activity of certain portions of the tongue. It was the purpose of this study to verify this assumption. The genioglossus muscle offered itself as an ideal test organ since its activation leads to protrusion of the tongue beyond the occlusal zone of the teeth. Evidence will be presented here showing that the protrusive action of the tongue is inhibited by proprioceptive afferent activities from masticatory muscles. Portions of this work were previously presented in the form of an abstract[1].

Methods. 18 adult cats were used for this study. Surgery (tracheotomy; nerve dissections) was carried out under general anesthesia produced by short-acting sodium methohexital. Following all surgical procedures, a light anesthetic state was maintained by i.p. application of chloralose. Gallamine triethiodide was injected i.v. as a muscle relaxant. The method of proper ventilation of immobilized preparations was described earlier[2]. The body temperature was kept constant at 36–37 °C with radiant heat. Peripheral nerves were carefully dissected and suspended in a small pool of mineral oil. For recording and stimulating purposes, bipolar silver hook electrodes were applied to the proximal portion of the severed nerves. The following nerves were prepared: hypoglossal branches to the genioglossus muscle, lingual nerve, glossopharyngeal nerve branches to tongue mucosa, masseteric nerve, and the nerve to the anterior belly of the digastricus muscle. Reflexes to the genioglossus were elicited by electrical test stimuli (single pulse: 0.1 msec) to the ipsilateral lingual or glossopharyngeal nerve at the rate of 0.5/sec. Reflex depression was induced by applying conditioning stimulation (burst of 3 pulses; 500/sec; 0.3 msec; 3–5 V) to either the masseteric or the anterior digastric nerve. The conditioning interval is defined as the time elapsed between the beginning of the conditioning stimulus and the beginning of the test stimulus. The reflex potentials were amplified with a Tektronix RM 122 preamplifier and displayed on a Tektronix RM 565 oscilloscope. Averages of the polysynaptic reflex discharges were computed with the aid of a 'Computer of Average Transients' (CAT model 400A) and written out by an X–Y plotter. The area underlying the averaged reflex pattern served as an index of reflex activity. Areal measurements were carried out with the aid of a compensating polar planimeter.

Results. The effects of electrical stimulation of trigeminal proprioceptive afferents on reflexes to the protruder of the tongue (genioglossus) are illustrated in Figure 1. The computer-determined 'mean control' reflexes are shown in the upper part of the figure. If conditioning stimulation to the ipsilateral anterior digastric nerve (A, C) or the masseteric nerve (B) preceded the test stimulus by the optimal conditioning-test interval (15 msec; compare Figure 2), the reflex activity was considerably depressed ('conditioned responses'; lower part of Figure 1). Similar but slightly weaker effects were obtained when conditioning stimulation was applied to the contralateral masseteric nerve or anterior digastric nerve. Conditioning stimulation of the masseteric nerve also induced depression of reflexes elicited by glossopharyngeal input.

The relationship between the conditioning-test interval and the degree of reflex depression is demonstrated in Figure 2. The reflexes were elicited by test stimuli to the lingual nerve. Conditioning stimulation was applied to the ipsilateral anterior digastric nerve. The time course of reflex inhibitions shows an initial depressive effect at a conditioning interval of 5 msec. Maximal depression was observed at 12–15 msec. The total duration of the inhibitory phase ranged from 400–500 msec. Time courses with similar characteristics were obtained when conditioning stimulation was applied to the masseteric nerve, or when the genioglossal reflex was elicited by glossopharyngeal input.

Discussion. The preceding experimental observations have shown that electrical stimulation of the masseteric or anterior digastric nerve induces depression of reflexes to the genioglossus muscle. It is our contention that

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