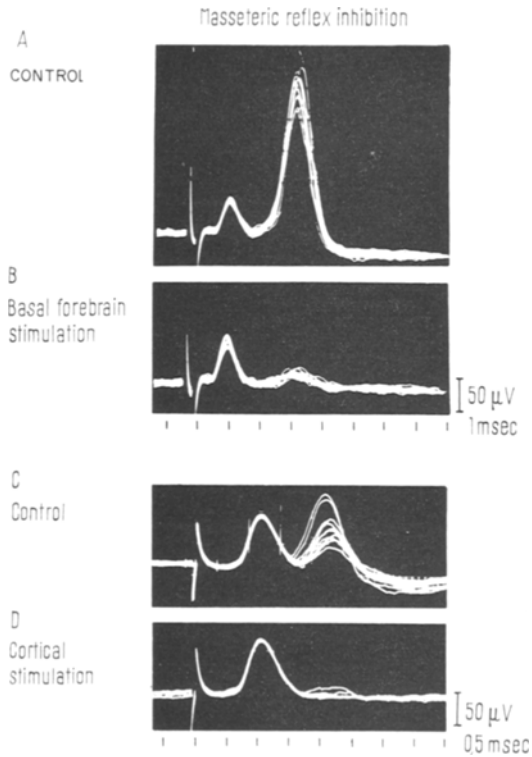

Fig. 2. The control masseteric reflex is illustrated in A and C. Following the artifact, can be observed first the antidromic volley and then the reflex volley as recorded along the masseteric nerve. In B an almost complete inhibition of the reflexive response is observed during the stimulation of the ipsilateral preoptic area, while D shows an even more pronounced effect during stimulation of the ipsilateral orbital gyrus.

were either less effective or ineffective at these sites, and stimulation of different sites resulted in other effects to be described more completely elsewhere.

These findings support an hypothesis developed from our former work which has indicated the existence of a forebrain inhibitory system involving cortical, basal forebrain and limbic structures which descend to reticular areas of the mid- and hindbrain. Stimulation at the rostral end of this system (basal forebrain or orbital gyri) resulted in suppression of motor behavior and the induction of sleep. Inhibition of the masseteric monosynaptic reflex points to the fact that this suppression can be reflected in the most basic of neurophysiological mechanisms as well. This particular finding is especially relevant since relaxation of the antigravity muscles resulting in opening of the mouth in the unsupported lower jaw is usually a behavioral correlate of the initial stages of sleep.

Zusammenfassung. Der monosynaptische Reflex des Nervus massetericus, durch elektrische Reizung des Nucleus mesencephalicus des Trigeminus hervorgerufen und vom Nervus massetericus abgeleitet, konnte durch Reizung bestimmt umschriebener Gebiete des präoptischen basalen Telencephalons oder durch Reizung des Gyrus orbital im Katzen-Cortex effektiv gehemmt werden. Entsprechende Reizung der gleichen Hirngebiete führt zur Synchronisation des Elektrocorticogramms und ruft Schlaf hervor.

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Connective Tissue Degradation and Distensibility Characteristics of the Non-Living Heart

It is well known that the myocardium is infiltrated by a considerable amount of connective tissue. Although collagenous fibers predominate, the myocardium also contains elastic and reticular fibers[1]. These non-muscular components of heart tissue are arranged in such a way as to provide a matrical superstructure upon which the muscular, nervous, and vascular components are arranged. To what extent the connective tissue fibers contribute to the static (much less dynamic) properties of the heart is largely unknown.

Methods. Left ventricular distensibility was compared in 4 groups of rabbit hearts: (1) control, (2) treated with collagenase, (3) treated with elastase, and (4) treated with trypsin.

The protocol for all experiments was as follows: (1) As much fluid as possible was withdrawn from the left ventricle and pressure at this 'zero' volume was recorded. (2) Continuous pressure records were obtained while infusing physiological saline into the left ventricle at a rate of 4.12 ml/min until the pressure reached 40–50 mm Hg. (3) The pump was then reversed and withdrawal was continued at the same rate as infusion. (The hydrostatic level of the saline bath was maintained constant throughout both infusion and withdrawal.) This constituted the first pressure-volume (P-V) curve and it was always obtained within 10 min after isolation of the hearts. (4) After 60 min time lapse (when stiffening was maximal) the second P-V curve was obtained. (5) Immediately after the second P-V curve was obtained, the hearts were stretched one time with the same volume of fluid that was used to obtain the first P-V curve and then the third P-V curve was obtained. (6) The hearts were then incubated for 90 min at 36.5 °C in a solution which contained either collagenase, elastase, trypsin, or buffered physiological saline (pH 7.1, phosphate buffer). The enzymes were obtained from the Nutritional Biochemical Co., Cleveland, Ohio, USA; their concentrations were: collagenase 1.5 mg/ml (pH 7.1, phosphate buffer), elastase 0.4 mg/ml (pH 8.8, carbonate buffer), and trypsin 2 mg/ml (pH 7.95, phosphate buffer). The left ventricular chamber and the coronary circulation were perfused with the solution. The hearts were then immersed in the digestion mixture.

[1] R. H. Licata, in *Cardiology* (Ed. A. A. Luisada, McGraw-Hill, New York, N.Y., USA) vol. 1, p. 61 (1959).

Following incubation, the hearts were washed in saline, resuspended in the experimental apparatus and P-V curve (number 4) recorded.

At the termination of the experiments, samples of myocardium were taken for histological observation and, in some experiments, for estimation of collagen. Sections were stained with Mallory's aniline blue (collagen), Gomori's aldehyde fuchsin (elastin), and hematoxylin-eosin. Collagen was extracted by the method of FITCH, HARKNESS, and HARKNESS[2] and estimated by the hydroxyproline method of NEUMAN and LOGAN[3] as modified by MARTIN and AXELROD[4].

Results. Changes in P-V curves under control conditions are depicted in Figure A (this figure is representative of those obtained from 6 experiments). As expected, distensibility was greatly decreased 60 min following isolation (curve 2, Figure A). Immediately following passive stretch (curve 3, Figure A), distensibility was approximately half that of control. 90 min after the passive stretch, the organ's P-V curve had shifted farther to the left (Figure A, curve 4). The fact that curves 2 and 3 are dissimilar indicates some physicochemical change; that this change was partially reversible is indicated by curve 4.

FigureB shows P-V curves that are representative of 10 hearts incubated in collagenase. Curves 1, 2, and 3 are similar in shape to the same curves presented in Figure A. Following incubation for 90 min in collagenase, the P-V curve was displaced to the right (curve 4, Figure B). This stands in sharp contrast to the curves similarly obtained after incubation in saline (Figure A), elastase (Figure C) or trypsin (Figure D).

Figure C shows a P-V plot of a typical result obtained when 6 hearts were incubated for 90 min in a solution containing elastase. This figure does not differ substantially from the control. Since some degree of tissue maceration was noted in those hearts subjected to collagenase and elastase, another group of 6 hearts was incubated in a solution which contained trypsin. This was an attempt to produce approximately the same degree of tissue maceration as seen in the other hearts. Thus the trypsin group served as an additional control. Representative P-V curves are shown in Figure D. Following 90 min incubation in a solution which contained trypsin (Figure D, curve 4) the distensibility was not changed, essentially, from that obtained immediately following passive stretch.

When a statistical comparison of volumes required to obtain pressures of 0, 10, 20, 30, and 40 mm Hg was made, the only group that differed from the control was the group treated with collagenase. Only in the pressure range from 0–20 mm Hg was the intraventricular volume significantly greater ($p < 0.01$) in the collagenase treated group.

Discussion. What contribution do collagen and elastin make towards the distensibility characteristics of the mammalian ventricle? It seems clear that collagenase treatment of the rabbit heart results in a significant increase in distensibility of the left ventricle and that this increased distensibility is more evident in the low pressure range. On the other hand, treatment with elastase produced no significant change in the distensibility characteristics of the isolated ventricle. These findings stand in sharp contrast to those of ROACH and BURTON[5] for arteries. This in itself does not necessarily indicate any qualitative difference in the properties of the collagens in the 2 loci but can reflect differences in the geometrical arrangement of the fibers in arteries and the heart.

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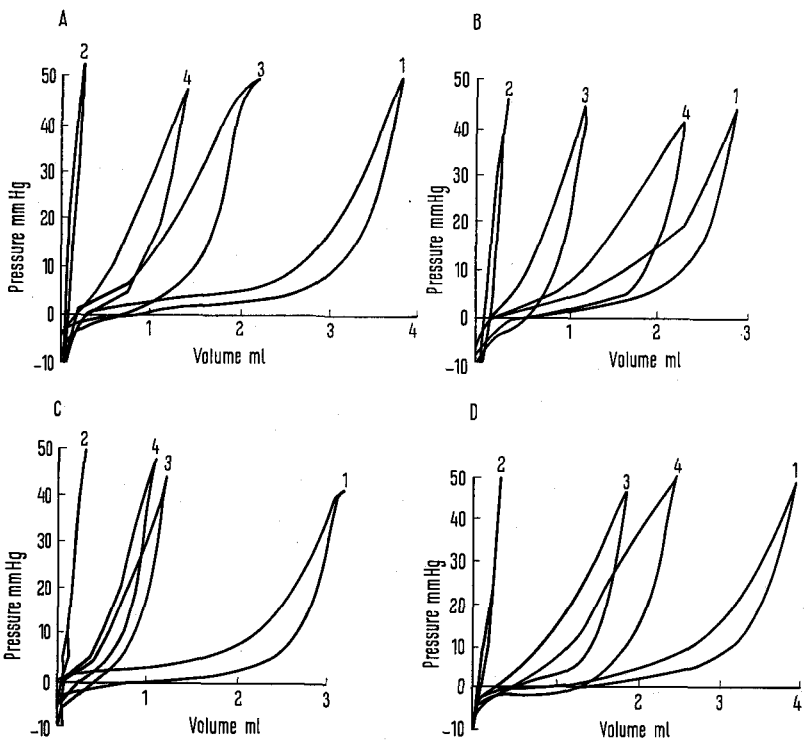
[3] R. E. NEUMAN and M. A. LOGAN, J. biol. Chem. *184*, 299 (1950).
[4] C. J. MARTIN and A. E. AXELROD, Proc. Soc. exp. Biol. Med. *83*, 461 (1953).
[5] M. R. ROACH and A. C. BURTON, Can. J. Biochem. Physiol. *35*, 681 (1957).



A, Saline control (time after isolation): curve 1, 10 min; curve 2, 60 min; curve 3, 62 min; curve 4, 150 min. (Heart weight 6.9 g.) – B, Collagenase (time after isolation): curve 1, 8 min; curve 2, 61 min; curve 3, 64 min; curve 4, 163 min. (Heart weight 7.3 g.) – C, Elastase (time after isolation): curve 1, 8 min; curve 2, 50 min; curve 3, 54 min; curve 4, 161 min. (Heart weight 5.6 g.) – D Trypsin (time after isolation): curve 1, 9 min; curve 2, 60 min; curve 3, 62 min; curve 4, 150 min. (Heart weight 7.7 g.) – Temperature = 37.5 °C. (See text for protocol.)

It might be properly argued that the increased distensibility produced by collagenase action was the result of destruction of the sarcolemma by the enzyme, but there was no microscopical evidence of such destruction. More important, however, is the fact that trypsin activity, which indeed produced tissue maceration, failed to produce any significant change in distensibility. Thus it would appear that the effect seen was due to degradation of extracellular collagen and not solely to destruction of the sarcolemma. The collagen content of hearts treated with collagenase was 0.077 ± 0.011 (SE_m) g soluble collagen/100 g wet tissue while the collagen content of the control group was 0.118 ± 0.017 g soluble collagen/100 mg wet tissue. The difference between the 2 means was significant at the 0.05 level (Student's t test).

No chemical evaluation of the efficacy of elastase or trypsin activity was undertaken other than the histological sections mentioned previously. There was some histological evidence of beading of elastin and of tissue maceration in those hearts subjected to elastase and trypsin respectively. Concentration levels of the enzymes used were in excess of those known to produce significant enzymatic effects[5-8].

Zusammenfassung. Isolierte Hasenherzen wurden im Inkubator in 3 Gruppen von Enzymgemengen behandelt, ihre Ausdehnungscharakteristika durch Druckvolumen-Messungen bestimmt. Von den verwendeten Enzymen Collagenase, Elastase und Trypsin, erzeugte nur Collagenase einen Wechsel in der Ausdehnung.

L. J. O'Brien and C. M. Moore

Medical College of Georgia, Augusta, Georgia and Civil Aeromedical Research Institute, Oklahoma City (Oklahoma, USA), July 15, 1966.

[6] I. Mandl, S. Keller, and B. Cohen, Proc. Soc. exp. Biol. Med. *109*, 923 (1962).
[7] H. Tint, Arch. Biochem. Biophys. *92*, 154 (1961).
[8] P. Fry, M. L. R. Harkness, R. D. Harkness, and M. Nightingale, J. Physiol. *164*, 77 (1962).

Experimentally Produced Ependymal Inclusions in the Brain of *Xenopus laevis*

In the course of an investigation on the influence of telencephalic injuries on hypothalamic neurosecretion in *Xenopus laevis*[1], it was noted that portions of the lateral ventricles, partially or completely isolated from the remainder of the ventricle system, develop strongly chrome hematoxylin (Gomori) positive inclusions in the ependymocytes. These Gomori positive inclusions (GPI) are present in the perikarya and prolongations of the ependymocytes, being frequently found even in the most distal parts of the prolongations which form meningeal end-feet at the brain surface (Figures 1–3).

In this study similar lesions were performed as those previously made in the telencephalon of adult specimens of *X. laevis*[1]. As the result of the operation, the olfactory lobes were isolated from the rest of the brain. The cut caudal end then healed; a small isolated ventricle remaining in the posterior part. Sometimes only a trace of a ventricle was seen; but in every case where ependymocytes were present, GPI developed. In the olfactory lobes, GPI were also seen in glial cells (Figure 6). The caudal extremities of the hemispheres, when isolated, were also frequently the site of GPI. Most frequently, GPI were situated basally with respect to the cell nucleus (Figures 2 and 4). However, at sites where they were particularly abundant, GPI could be seen also in the apical portions of the ependymocyte perikarya. In such cases, the lumen of the isolated ventricle showed the presence of intensely Gomori positive amorphous precipitates (Figure 1). GPI were never found in the ependyma of a normal brain.

GPI are oval, spherical, or bean-like shaped granules, 0.5–2.0 μ in size, situated in the cytoplasm. They frequently form aggregates which coalesce into large Gomori positive masses (Figures 2 and 6). In preparations stained with Gabe's aldehyde fuchsin, GPI are also strongly positive. They are strongly performic acid-Alcian blue positive but negative or only weakly positive in the PAS procedure. In unstained preparations GPI are colourless. They do not contain acid mucopolysaccharides and are distinct from lipofuscin, as shown by the negative result of the staining with 0.1% Alcian blue in 3% acetic acid and the negative outcome of the Hueck and Lillie methods. Thus, GPI are probably of protein character with a cystine content of over 4%.

As shown by the results presented above, the GPI in the ependymocytes of the isolated ventricles of the *X. laevis* brain are, at least with all the methods so far employed, histochemically similar to the Gomori positive inclusions normally found in the periventricular glial cells of the hypothalamus and some other brain areas in

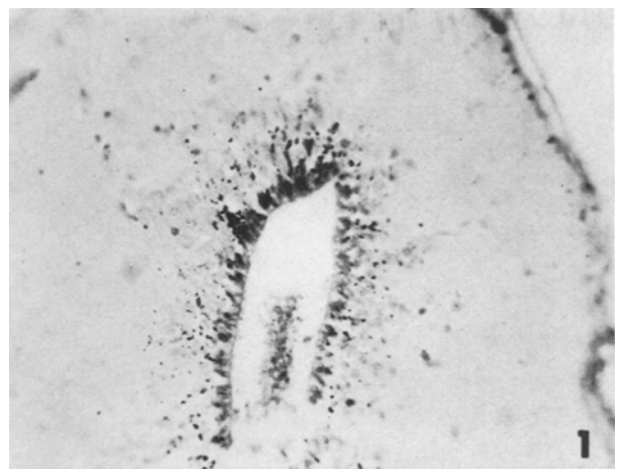


Fig. 1. Isolated ventricle with ependymal Gomori positive inclusions (GPI) in a hemisphere remnant. In the lumen Gomori positive precipitate can be seen. Chrome hematoxylin-phloxin (CHP). × 110.

[1] Z. Srebro, Folia biol. Kraków *13*, 397 (1965).