

## Use of the Isolated Heart of a Freshwater Mussel (*Anodonta cygnea* L.) for Biological Estimation of 5-hydroxytryptamine<sup>1</sup>

It has been shown that 5-hydroxytryptamine (serotonin)<sup>2</sup> has a strong stimulatory influence on the heart of many mollusc species (ERSPAMER and GHIRETTI<sup>3</sup>, WELSH<sup>4</sup>). The isolated heart of certain marine forms (*Venus mercenaria*, *Buccinum*) has been made use of for bioassay of this substance (TWAROG and PAGE<sup>5</sup>, WELSH<sup>6</sup>).

In order to find out whether the heart of the common freshwater mussel, *Anodonta cygnea*, could be of value as a test organ for identification and estimation of 5-hydroxytryptamine and similar substances, the reaction of the *Anodonta* heart to 5-hydroxytryptamine and some other substances has been studied. The experiments were performed on isolated ventricles containing a short piece of intestine. They were suspended in a bath (volume 10 ml) which was aerated. In accordance with PILGRIM<sup>7</sup>, a mixture of distilled water and sea water (salinity 30‰) in the proportions 96:4 was used as the environmental fluid<sup>8</sup>. The *Anodonta* ventricle preparations, either immediately or after an initial standstill of varying duration, began to beat with a slow even rhythm. Generally the isolated ventricles would continue beating for more than 48 h.

It was observed that 5-hydroxytryptamine, even in rather low concentrations, causes a marked increase in amplitude and frequency of isolated *Anodonta* ventricle. The threshold concentration seemed to be about  $10^{-9}$  gm/ml. The inotropic response took place immediately after addition of the drug and could always be repeated with 5–8 min intervals and change of suspension fluid. Graded responses were obtained with concentrations up to about  $10^{-6}$  gm/ml.

Positive inotropic effects were also seen after dihydroergotamine (conc.  $10^{-8}$ ), cocaine (conc.  $10^{-4}$ ), vasopressin and oxytocin, but the amplitude increase in these cases was slower and less reversible than after 5-hydroxytryptamine.

Adrenaline and noradrenaline in high concentrations ( $10^{-4}$  gm/ml) have a slightly negative inotropic effect, but weaker doses seemed to be without any effect. After tyramine, dopamine, substance P, histamine or acetylcholine in varying concentrations, no distinct reactions were seen. However, TEN CATE and REESINCK<sup>9</sup> have observed an inhibitory effect from acetylcholine in high concentration.

Because the *Anodonta* ventricle is highly sensitive to 5-hydroxytryptamine, but relatively insensitive to a lot of other physiologically active substances, this preparation ought to be well suited for the biological estimation of 5-hydroxytryptamine.

A more detailed account of the present studies will be published later.

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### Zusammenfassung

Die rhythmischen Kontraktionen des isolierten Herzventrikels von *Anodonta* werden von 5-Hydroxy-Tryptamin niedriger Konzentration beschleunigt und verstärkt. Adrenalin, Noradrenalin, Histamin, Acetylcholin, Tyramin usw. wesentlich höherer Konzentrationen haben keine oder nur geringe Wirkungen. Das *Anodonta*-Herz-Präparat scheint deshalb für den biologischen Nachweis von 5-Hydroxy-Tryptamin (Serotonin, Enteramin) geeignet.

## Propagation of a Virus of the Encephalomyocarditis Group in Roller Tube Cultures of Mouse Testicular Tissue

The encephalomyocarditis group of viruses is composed of immunologically related viruses of Columbia-SK, MM, Mengo encephalomyelitis, and encephalomyocarditis. These viruses have been shown, from an analysis of their known physical and biological properties, to be different strains of a single virus<sup>1</sup>. The recent work of VERLINDE *et al.*<sup>2</sup>, suggests the possibility that a significant relationship between this group of viruses and poliomyelitis may occur in nature. This report deals with the propagation of MM virus in roller tube cultures of mouse testicular tissue.

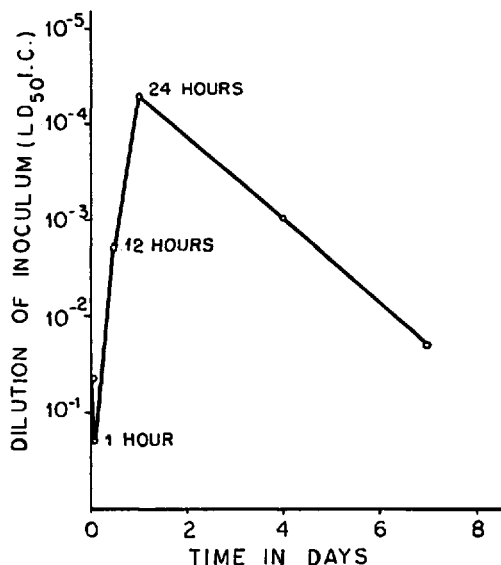


Fig. 1.—Growth pattern of MM virus in roller tube cultures of mouse testicular tissue in lactalbumin hydrolysate medium. Inoculum LD<sub>50</sub> of 0.03 ml of  $10^{-1.6}$  I.C. Mouse titrations of virus in fluids harvested from infected tissue cultures at 1 hour, 12 hours, 24 hours, 4 days, and 7 days.

<sup>1</sup> J. WARREN, J. E. SMADEL, and S. B. RUSS, *J. Immunol.* **62**, 387 (1949). — G. W. A. DICK, *J. Immunol.* **62**, 375 (1949).

<sup>2</sup> J. D. VERLINDE and J. H. MOLRON, *Antonie Van Leeuwenhoek* **20**, 129 (1954).

<sup>1</sup> Aided by a grant from the Kungl. Fysiogr. Sällsk., Lund.

<sup>2</sup> Serotonin creatinine sulfate was provided by Abbott Laboratories, Chicago.

<sup>3</sup> V. ERSPAMER and F. GHIRETTI, *J. Physiol.* **115**, 470 (1951).

<sup>4</sup> J. H. WELSH, *Arch. exper. Path. Pharmacol.* **219**, 23 (1953); *Nature* **173**, 955 (1954).

<sup>5</sup> B. M. TWAROG and I. H. PAGE, *Amer. J. Physiol.* **175**, 157 (1953).

<sup>6</sup> J. H. WELSH, *Nature* **173**, 955 (1954).

<sup>7</sup> R. L. C. PILGRIM, *J. Exper. Biol.* **30**, 297 (1953).

<sup>8</sup> According to PILGRIM the mixture ought to be buffered by M/400-NaHCO<sub>3</sub>.

<sup>9</sup> J. TEN CATE and M. J. REESINCK, *Physiol. Comp. Oecolog.* **3**, 337 (1954).