Use of the Isolated Heart of a Freshwater Mussel (*Anodonta cygnea* L.) for Biological Estimation of 5-hydroxytryptamine [1]

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

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[7], a mixture of distilled water and sea water (salinity 30‰) in the proportions 96:4 was used as the environmental fluid[8]. 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^{-5}), 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[9] 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.

[1] Aided by a grant from the Kungl. Fysiogr. Sällsk., Lund.

[2] Serotonin creatinine sulfate was provided by Abbott Laboratories, Chicago.

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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[1]. The recent work of VERLINDE *et al.*[2], 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.

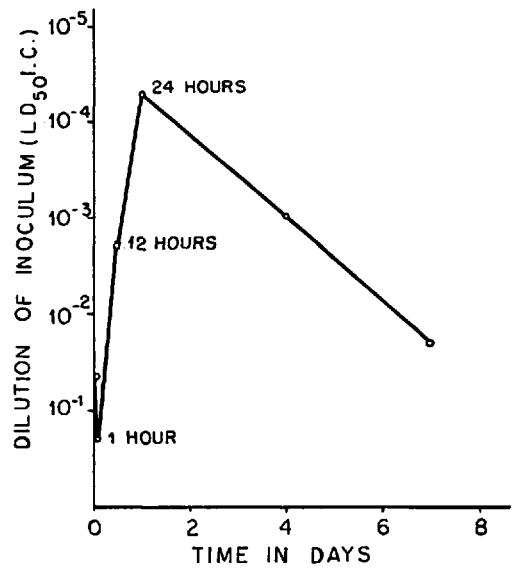

Fig. 1.–Growth pattern of MM virus in roller tube cultures of mouse testicular tissue in lactalbumin hydrolysate medium. Inoculum LD_{50} of 0·03 ml of $10^{-1.6}$, I.C. Mouse titrations of virus in fluids harvested from infected tissue cultures at 1 hour, 12 hours, 24 hours, 4 days, and 7 days.

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The strain of virus used was obtained from Dr. WERNER HENLE of the Children's Hospital, Philadelphia, and has had 15 mouse intracerebral passages in this laboratory. Roller tube cultures were prepared with minced testes from 5–12-week old Swiss white mice freshly killed with ether, by following essentially the methods of CARREL[1] and GEY[2]. Two types of culture media were employed: (a) 0·5% lactalbumin hydrolysate and (b) a medium consisting of 10% chick embryo juice (1:1 minced chick embryo and buffered HANKS' solution), 10% inactivated horse serum, 20% bovine serum ultrafiltrate, and 60% buffered HANKS' solution. To each were added 100 units penicillin and 100 μg streptomycin per milliliter medium. The cultures were incubated on roller machine (15 revolutions per hour) at 37°C, and confirmed to show good growth of fibroblasts before inoculation with virus.

An initial set of 3 culture tubes, designated T-1, was inoculated with 0·1 ml of 10^{-5} dilution of 10% brain emulsion from mice typically infected with MM virus by an intracerebral route. After 4 days fluids from the tubes were pooled and 0·1 ml of 10^{-1} of this fluid was inoculated into each tube of a second set of the cultures. Successive transmissions in this manner were carried out through the 10th tissue passage (T-10). Amount of virus in each inoculum was determined by intracerebral inoculation of 0·03 ml of ten-fold serial dilutions of the virus-containing fluid into 3-week old Swiss white mice. The 50% lethal doses were calculated by the method of REED and MUENCH[3]. At the 11th tissue culture passage, the growth pattern of the virus was investigated (Fig.1). Samples of fluid in the virus-infected tissue cultures were taken out at 1 h, 12 h, 24 h, 4 days, and 7 days respectively after virus inoculation, and titrated into Swiss white mice for viral activities. All Infected cultures were checked daily for characteristic cytonecrosis[4] by a microscope with 3×, 10×, and 43×, objectives, and a 10× eye piece.

As control, similar examinations were made with uninfected tissue cultures. All fluids harvested were confirmed to contain no bacteria by culturing on tryptone-soybean-yeast extract agar slants for 7 days at 37°C.

In order to ensure that MM virus was actually transferred but not another virus' neutralization tests with immune rabbit serum were performed.

In this study a calculated final dilution of $4{\cdot}89 \times 10^{-26}$ of the original infected mouse brain was obtained at T-11. Virus containing fluid from the eleventh serial tissue passage was capable of initiating infection in Swiss white mice to give characteristic symptoms of paralysis and death and an LD_{50} of $10^{-4{\cdot}6}$. The maximum degeneration of infected fibroblasts was at 5 days after virus infection. All mice inoculated with fluids from uninfected cultures (control cultures) survived.

The findings in this study compare favorably with the results obtained by SMITH and EVANS[4] in their study on the propagation of MM virus in monkey testicular tissue in roller tube cultures, but seem better than the results obtained by CHAMBERS *et al.*[5], who used flask cultures with mouse testicular tissue. Because of the easiness of getting the material as well as the capacity to support viral propagation as evidenced above, the roller tube culture method of mouse testicular tissue is very useful for the study of MM virus. These findings emphasize moreover that this neurotropic virus propagates readily in extraneural tissue.

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Zusammenfassung

11 Passagen von MM-Virus der EMC-Gruppe in Mäusetestikel-Gewebekultur werden beschrieben. Maximale Virustiter von etwa 10^{-4} wurden erreicht; maximale Degeneration der infizierten Fibroblastenkulturen wurde nach 5 Tagen Inkubation beobachtet.

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The Prevention of Experimentally Induced Reticulo-Sarcoma by Hypothyroidism

The injection of trypan blue into rats promotes a high incidence of reticulo-sarcoma involving the liver[1], lymph nodes[2], kidneys and other organs[3]. In a large group of animals some 15 to 18% failed to respond to trypan blue. The reason for this refractoriness has been the subject of our enquiry.

Hypothyroidism[4] as well as inanition[5] can retard the emergence of hepatocellular carcinoma in rats fed butter yellow and can also prevent the development of pituitary tumours in rats in response to chronic oestrogen stimulation[6]. We have also demonstrated that severe malnutrition can almost completely suppress the reticulosis induced by trypan blue, an observation which offers a possible explanation for the decreased incidence of human reticulosis in France reported by CAZAL[7] during World War II. In view of the fact that inanition depresses basal metabolism, the possibility thus presented itself that hypothyroidism would also prevent the emergence of the reticulo-sarcoma induced by trypan blue.

Experimental. 121 adult rats weighing between 250 and 300 g were injected subcutaneously at fortnightly intervals with 1 cm³ of 1% of an aqueous solution of trypan blue (Grubler). Twenty of these rats were fed

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