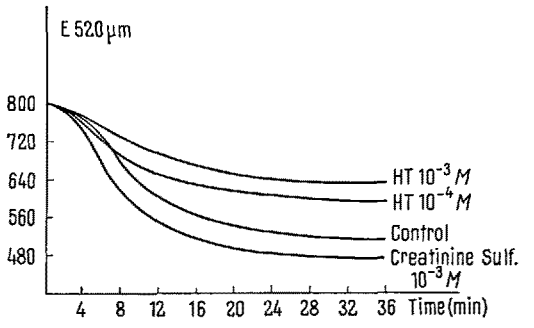

Inhibition by Serotonin on Mitochondrial Spontaneous Swelling as Estimated by the Optical Method, and Swelling Effect of Creatinine-Sulfate.

Percentage Decrease (D%) of Optical Density on Spontaneous Swelling of Rat Liver Mitochondria in 35 min

	No. of experiments	D%
Control	15	29,4 ± 6,4
Mitochondria with serotonin 10^{-3} M	10	20,8 ± 4,5
Mitochondria with serotonin 10^{-4} M	7	22,3 ± 5,1
Mitochondria with creatinine-sulfate 10^{-3} M	3	32,7 ± 5

amount of HT. In addition they observed that reserpine has an effect on the serotonin of these mitochondria which is parallel to that demonstrated on blood platelets[8]: it therefore seemed interesting to see whether this neurohumoral amine influenced mitochondria morphology.

Mitochondria were prepared from liver of albino rat, Wistar strain (150–200 g) body weight) in 0.25 *M* sucrose, containing 0.02 *M* Tris-chloride, pH 7.4 at 4°C, by the method of Schneider[9]. 1 ml of the final concentrated suspension of mitochondria was equivalent to 0.25 g of fresh liver. Changes in extinction at 520 mµ read in a Beckmann model DU spectrophotometer, were taken as measure of swelling as described by Cleland[1]. Each test tube contained 3 ml of 0.25 *M* sucrose, buffered with 0.02 *M* Tris-chloride, pH 7.4: HT was added to the sample examined. Then 0.3 ml of the mitochondria suspension were quickly added, the tube was shaken to mix its contents and the optical density was determined. Usually the first reading was taken within 20–30 sec after mixing and then at given times up to 35 min. The zero time or initial optical density was obtained by extrapolation. The whole experiment was made within 75 min from the killing of the animal.

The 5-hydroxytryptamine-creatinine sulfate (it is the only salt available) used was supplied by Sigma and Roche Laboratories. The results are given in the Table: a significant difference was observed in the % decrease of optical density in samples containing HT; the Figure gives the effect of serotonin on spontaneous mitochondrial swelling at two different concentrations. We also verified the effect of creatinine-sulfate on mitochondria: it was found that it has a swelling effect and is therefore an antagonist to HT.

Consequently it appears that serotonin has a protective action on mitochondria swelling *in vitro*. Further studies are being carried out in order to see if there is a correlation between ATPase activity and protective action of serotonin. This is supported by the fact that the concentration of HT in blood platelets depends on the ATP present[10] (according to Born[11], serotonin would actually be bound to ATP as a complex) and that inhibitors of HT binding by mitochondria such as reserpine, dibenamine, and phenylether, inhibit oxidative phosphorylation[9].

Anna Casu

Istituto di Patologia Generale dell'Università di Genova (Italy), June 1, 1960.

Riassunto

È stato studiato l'effetto della serotonina sul rigonfiamento dei mitocondri *in vitro:* si è trovato che la serotonina protegge i mitocondri dal rigonfiamento spontaneo che si ha quando vengono sospesi in soluzione isotonica di saccarosio.

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The Kinetic of Induction of Plaque Formation in Cell Monolayers by Ribonucleic Acid from Poliovirus

We have published previously[1] a test system for quantitative studies on the infectivity of isolated ribonucleic acid (RNA) from poliovirus. The method was developed to provide a tool for physico-chemical analysis of biological active RNA, in a manner comparable to the analysis of desoxyribonucleic acid (DNA) carried out with the aid of studies on transforming DNA. Other investigators have already applied this method for further characterization of infectious RNA[2,3]. It was of interest to learn more about the basic mechanism of the RNA test system. We present here results on the kinetic of plaque induction in cell monolayers by RNA.

The methods used for preparation and assay of RNA are essentially the same as those described previously[1]. Poliovirus type I strain Mahoney is used for isolation of RNA. The virus suspensions are obtained partially purified from the Cutter Laboratories. Highly purified poliovirus suspensions are prepared in our own laboratory according to the method of Levintow and Darnell[4]. The experimental results obtained are independent of the virus source.

The solutions for washing the cell monolayers and the RNA solution are incubated in a water bath at the stated temperatures. The cell monolayers are kept at these temperatures for 20 to 30 min before seeding with the RNA.

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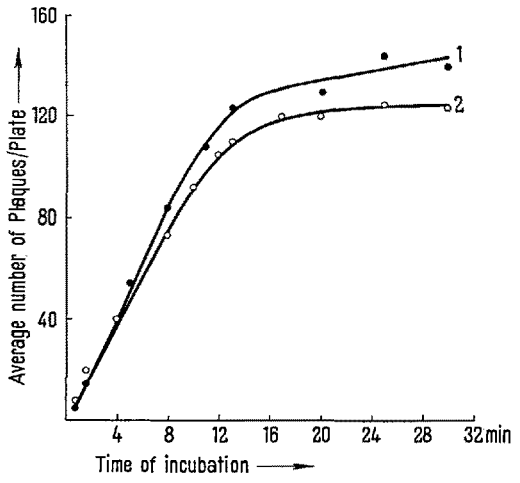

Kinetic of induction of plaque formation in cell monolayers by isolated RNA from poliovirus (average of three experiments), Curve 1 at 36°C, Curve 2 at 20°C.

The interaction of the RNA with the cells is stopped by applying the agar overlay or by washing with physiological saline (Hanks solution). Identical results are obtained with both procedures.

The results of our experiments are shown in the Figure. The interaction of viral RNA with the host cell leads to a rapid initial increase of infected plaque forming units during the first 8 to 10 min after seeding and thereafter to an ever-decreasing rate of further induction of cells to plaque forming units. The rate of plaque induction by RNA does not differ significantly when cell monolayers are incubated at 20° or 36°C after seeding, and an almost identical number of plaques/plate is obtained by a given sample of RNA at these temperatures.

We have also followed the induction of plaque formation by RNA at 0° to 4°C. The results have been erratic from one experiment to another. In all experiments the increase in the number of plaques between 0 and 20 min after seeding was somewhat lower than at 20°C, but otherwise identical with respect to the rate of uptake. The RNA infectivity at 4°C was variable over a wide range from one experiment to another and showed an infectivity of the order of 1 to 70% of its infectivity in parallel experiments at 20°C. No explanation can be offered so far for the erratic results obtained at 4°C.

The time-dependent differences in the rate of induction of plaques by RNA in cell monolayers are understandable by either one of the following assumptions: (1) The cells are sensitive to RNA only for a short interval after exposure to hypertonic saline and become less and less sensitive with time after seeding. Not all potentially infective RNA is taken up by the cells according to this hypothesis. (2) The cell monolayers are not free from RNAase or RNA inhibitors. (3) The isolated RNA from poliovirus is heterogeneous with regard to the ability to interact with cells.

Experiments are being undertaken in our laboratory to prove or disprove these assumptions. Results obtained so far strongly favour hypothesis 1.

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Zusammenfassung

Die Kinetik der Induktion von Plaques in Einschichtenzellkulturen wurde bei 20° und 36°C bestimmt. Es nimmt die Zahl der durch RNS induzierten Plaques in den ersten 8 bis 10 min nach Kulturbeimpfung schnell und dann allmählich langsamer zu. Die möglichen Ursachen dieses Verhaltens werden diskutiert.

Thermische Denaturierung von Kaltblüter-Enzymproteinen

Die Adenosintriphosphatase aus Karpfenmuskulatur zeigt eine bemerkenswerte hohe Hitzelabilität (Partmann[1]). Versuche, eine Hitzedenaturierung bei der Reinigung von Kathepsin aus Dorschmuskulatur anzuwenden, waren ohne Erfolg (Siebert und v. Malortie[2]). Der Dorsch, *Gadus callarias* (= *morrhua*) L., lebt üblicherweise bei einer Wassertemperatur von + 5 bis + 10°C. Es erhebt sich daher die Frage, ob es eine generelle Eigenschaft von Kaltblüter-Enzymproteinen ist, dass sie bereits bei wesentlich niedrigeren Temperaturen denaturiert werden als es der allgemeinen Erfahrung mit Warmblüterenzymen entspricht. Die nachstehend beschriebenen Versuche wurden zur Klärung dieser Frage unternommen.

Methodik. Im allgemeinen werden thermische Denaturierungen von gelösten Proteinen so vorgenommen, dass für den Effekt sowohl die Zeitdauer der Erhitzung als auch die erreichte Temperatur wichtig sind. In den hier zu beschreibenden Versuchen hat es sich bewährt, den Zeitfaktor dadurch zu eliminieren, dass grundsätzlich die gewünschte Temperatur in einer wassergefüllten Durchströmungsapparatur während voller 60 min zur Einwirkung kam. Kontrollversuche haben gezeigt, dass im allgemeinen 10–15 min, höchstens jedoch 30 min ausreichen, um ein bestimmtes Denaturierungsausmass herzustellen, das sich bei Ausdehnung der Erhitzungszeit auf 60 min nicht mehr wesentlich ändert. Die nach so definierter Hitzeeinwirkung messbare Enzymaktivität wurde in % des Ausgangswertes ausgedrückt und gegen die angewandte Temperatur graphisch aufgetragen. Als Kriterium der thermischen Resistenz diente die durch Extrapolation von mindestens 4 – auf einer Geraden liegenden – Messpunkten erhaltene Temperatur, bei der 50% der Anfangsaktivität des betreffenden Enzyms zerstört sind. Als Untersuchungsmaterial wurde in fangfrischem Zustand (auf See) tiefgefrorenes Dorschfilet benutzt, das zur Extraktion der Enzymproteine der Glykolyse mit 1%iger KCl-Lösung, zur Extraktion proteolytischer Enzyme mit 1%iger LiBr-Lösung homogenisiert wurde. Aktivitätsbestimmung der Enzyme: Kathepsin nach Laskowski[3], Glycylglycindipeptidase nach Smith[4], Ninhydrinreaktion nach Moore und Stein[5], Isocitricodehydrogenase nach Siebert *et al.*[6], Milchsäuredehydrogenase nach Bücher

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