Résumé. L'identification des trois classes majeures d'immunoglobulines humaines peut avoir comme base la détermination des poids moléculaires de ses chaînes lourdes. Ce principe est applicable aux protéines contenues dans des gels de polyacrylamide, bien qu'on puisse les réduire directement à partir des sections de gel qui les renferment, en obtenant un éluat avec des chaînes libres qu'on peut étudier pour déterminer le poids moléculaire des chaînes lourdes.

G. VIRELLA[20] and MARIA MANUEL DE FREITAS[21]

Gulbenkian Institute of Science, Biological Research Centre, Apt. 14, Oeiras (Portugal), 31 July 1972.

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[21] Visiting researcher from the Instituto Nacional de Saúde Dr. R. Jorge, Porto, Portugal.

Erzeugung von Rigor der Rattenextremitäten durch Hemmung der Glyzin-Synthese

Es ist bekannt, dass kurzdauernde Unterbindung der Aorta (40 min) unter den Nierenarterien bei einigen Tieren zur Rigidität der Hinterextremitäten führt[1]. Wir konnten zeigen, dass bei Ratten diese Art von Rigidität durch Glyzin (G) verhindert werden kann[2]. Die Unterbindung der Aorta führt aber auch zum Verschwinden von G und zur Zerstörung der Interneuronen in der medulla spinalis, während die γ-Aminobuttersäure unverändert bleibt[3]. ARNSTEIN und STANKOVIĆ[4] zeigten, dass Mangel an Pteroyl-Glutaminsäure zur Hemmung der G-Synthese führt. Zur Prüfung, ob durch Hemmung der G-Synthese ein Rigor entstehen wird und ob dieser sich auch durch G aufheben lässt, haben wir ca. 150 g schweren, männlichen Wistar-Ratten 2 mg/kg/i.p. den Antagonist der Pteroyl-Glutaminsäure Aminopterin injiziert. 3–5 h später entwickelte sich der Rigor der Hinterextremitäten. Auf der höchsten Schwelle der Rigidität wurden 400 mg/kg/i.p. Glyzin injiziert, worauf der Rigor nach 30 min verschwand. Jede Gruppe bestand aus 10 Ratten und G wurde in jeweilen 2 medulla spinalis nach der Methode von BRENNER und NIEDERWIESER[5] bestimmt.

Glyzin in der Medulla spinalis von Ratten (γ/g)

		P	Bemerkung
1 Kontrolle	$5{,}95 \pm 0{,}19$		
2 Aminopterin	$4{,}13 \pm 0{,}13$	$< 0{,}01$	Signifikanz bezieht sich auf 1
3 Aminopterin + Glyzin	$5{,}08 \pm 0{,}11$	$< 0{,}01$	Signifikanz bezieht sich auf 2

Aus der Tabelle ist ersichtlich, dass es nach Aminopterin zu signifikanter Erniedrigung von G kam, während G nach der Injektion im Rückenmark erhöht war.

Aus diesen Resultaten kann man mit grosser Wahrscheinlichkeit schliessen, dass der durch kurzdauernde Unterbindung der Aorta hervorgerufene Rigor durch das Verschwinden der Interneuronen und die Hemmung der Glycinsynthese in der medulla spinalis entsteht. Zugleich kann dadurch bewiesen werden, dass Glyzin die hemoencephale Barriere durchdringt, was wir schon früher an Mäusen zeigen konnten[6]. Es stellt sich die Frage der eventuellen Glyzin-Anwendung bei gestörter Durchblutung des Rückenmarks in der Humanpathologie.

Summary. Inhibition of glycine synthesis by aminopterine, an antagonist of pteroilglutaminic acid, causes rigidity of hind limbs in rats. This rigidity can be abolished by the injection of glycine.

P. STERN, SABIRA ĆATOVIĆ und N. FILIPOVIĆ

Pharmakologisches Institut der Medizinischen Fakultät, Sarajevo (Jugoslawien), 12. Juni 1972.

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The Incorporation of ^{45}Ca into the Egg of the Medaka, *Oryzias latipes*

Ca may play an important role in the fertilization process of animals[1–3]. It might, therefore, be interesting to trace inter- and intra- cellular movements of calcium upon the fertilization. RUDENBERG[4], HSIAO and BOROUGHS[5] tried to measure the radioactivity of ^{45}Ca which penetrated into the egg of sea urchin by in vitro incubation. It seemed likely that in those experiments the radioactivity of the penetrated ^{45}Ca was not enough to trace the movements of the element. However, there may be another possibility that the ovum accumulates the radioisotope from the body fluid during the period of the egg maturation and subsequently a high level of the intracellular concentration of ^{45}Ca can be expected. The purpose of the present experiment is, therefore, to deal with the incorporation of radioactive calcium into the egg of the medaka which is injected intra-abdominally.

The egg of the medaka, *Oryzias latipes* (orange-red variety) was used as material. Ringer's solution which

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contained $^{45}CaCl_2$ was injected into the abdominal cavity of the female. The radioactivity of the injected ^{45}Ca was 2–4 μCi for each fish. Unfertilized eggs were obtained following Yamamoto's procedures[1] and fertilized eggs were collected from an aquarium every morning. The eggs were washed three times with 100 ml of distilled water and the eggs were homogenized with teflon homogenizer after adding of a certain amount of Ringer's solution. The homogenate was dried with an infra-red lamp and the radioactivity of the dry matter was measured using 2π gas-flow counter. Figure 1 illustrates the change in the radioactivity of the spawned egg after the injection of ^{45}Ca. The radioactivity reached a maximum 2 or 3 days after the injection and gradually decreased.

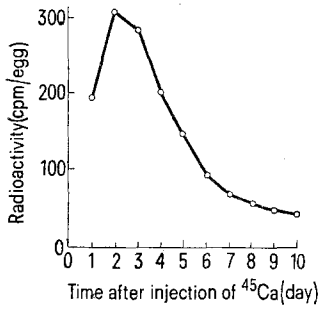

Fig. 1. Radioactivity in spawned eggs of *Oryzias latipes* after injection of ^{45}Ca-contained Ringer's solution.

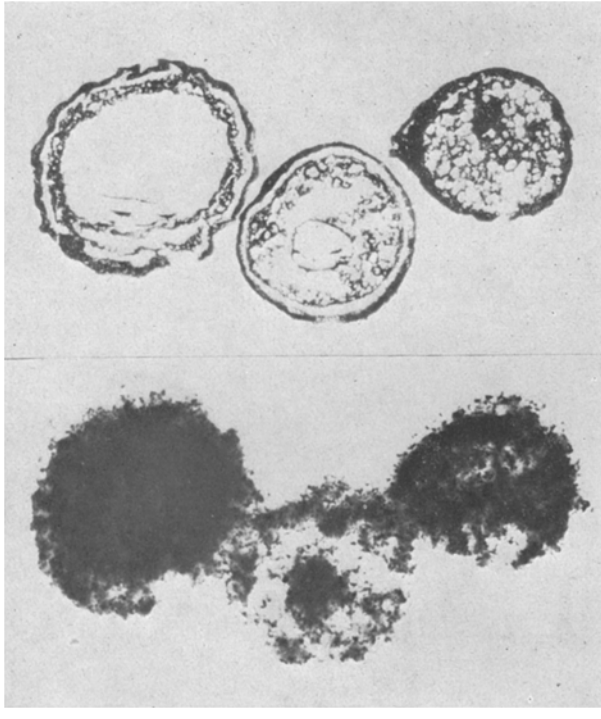

Fig. 2. Microautoradiogram of ova in *Oryzias latipes* (left; mature unfertilized egg, center and right; immature eggs).

In order to check the influence of the injection on spawning, 2 series of the experiments were prepared; one for the injection of Ringer's solution with ^{45}Ca, the other for the injection of Ringer's solution only. The number of the spawned eggs was counted every day after the injection. In both cases, the number was reduced after the injection, and the reduction was remarkable in the former. The recovery to its normal state in the former was slower than that in the latter. From these results, we are inclined to believe that not only ^{45}Ca but also Ringer's solution may induce the inhibition of spawning.

Micro-autoradiography was applied to unfertilized egg, spawned eggs and isolated ovaries, in order to examine the localization of the incorporated ^{45}Ca. After washing with distilled water, the material was fixed with Carnoy's alcohol acetic acid solution, embedded in paraffin, sectioned in the thickness of 10 μm and stuck on a glass slide. The contact method was applied to the slide (Fuji X-ray film, non-screen type No. 200). After the exposure of a certain period, the film was developed and compared with the slide which was stained, afterwards, with haematoxylin and eosine. As shown in Figure 2, ^{45}Ca was incorporated with the cortex and inside of the egg, especially strongly incorporated with the chorion. According to YAMAMOTO[6], the chorion of *Oryzias* egg is charged negative in Ringer's solution and it is easy to stain the chorion with basic dyes such as methylene blue and toluidine blue. From this point of view, it may be assumed that ^{45}Ca can be incorporated, primarily, with the chorion and, secondarily, with the inside of the egg.

Zusammenfassung. Zur Markierung des intrazellulären Ca im Fisch-Ei wurde $^{45}CaCl_2$- enthaltende Ringer-Lösung in die Abdominalhöhle von *Oryzias latipes* injiziert. 2–3 Tage nach der Injektion zeigt die Radioaktivität der abgelegten Eier ihr Maximum und nimmt dann allmählich ab. Aus Mikroautoradiogrammen der abgelegten Eier geht hervor, dass ^{45}Ca im Chorion und innerhalb des Eies eingebaut wird.

R. HORI and K. SATO

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Activation of Mitochondrial Enzymes During the Early Phase of Seed Germination

The increase of activity of many enzymes during the early phase of germination has been shown to correspond to de novo synthesis[1–3]. On the other hand some soluble enzymes, present in an inactive form in the dry seed, are reactivated during the very early phase of germination[4–8]. The possibility that, in the germinating castor bean, mitochondrial enzymes undergo a reactivation process, has been suggested on the basis of the results of experiments with protein synthesis inhibitors[3].

The present work brings further evidence in favour of this view, showing that significant increases of mitochondrial enzyme activities are observed, under conditons of inhibited protein synthesis, both in the whole seeds and (at a lesser extent) in cell free mitochondrial preparations.

Materials and methods. Plant material: Castor bean seeds (*Ricinus communis* var. *sanguineus*) were decoated and germinated in Petri dishes on wet filter paper, in the dark at 0 °C or 27 °C. Squash seeds (*Cucurbita maxima*)