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.

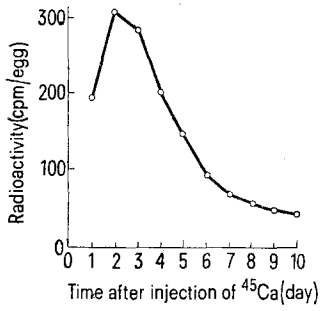

Fig. 1. Radioactivity in spawned eggs of *Oryzias latipes* after injection of ^{45}Ca-contained Ringer's solution.

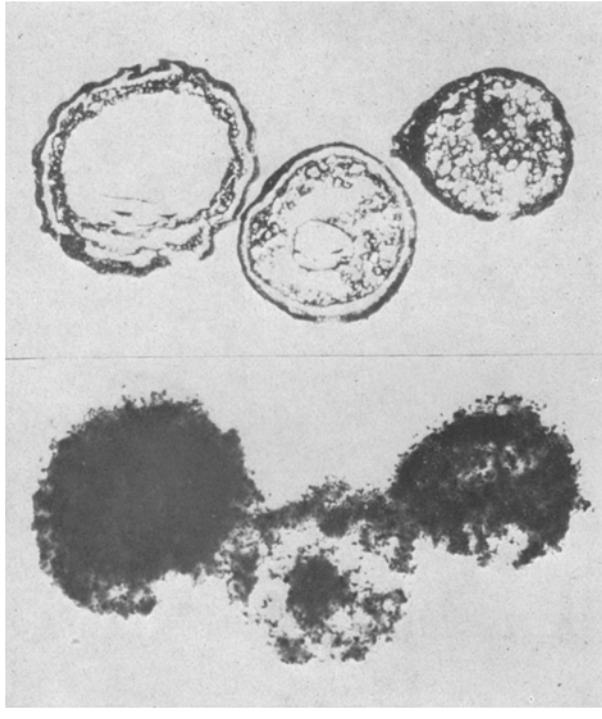

Fig. 2. Microautoradiogram of ova in *Oryzias latipes* (left; mature unfertilized egg, center and right; immature eggs).

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.

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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*)

were decoated and deprived of the inner membrane after 5 h of imbibition at 0 °C, then germinated in Petri dishes at 0 °C or 30 °C, in the dark.

Preparation of the mitochondrial fraction and determination of enzyme activities: the mitochondrial fraction from castor bean endosperm and squash cotyledons was prepared as previously described[3,9].

Succinic dehydrogenase (E.C.1.3.99.1.) activity was measured by the method of HIATT[10]. Cytochrome oxidase (E.C.1.9.3.1.) activity was determined as described by SMITH[11], by measuring in 60 m*M* potassium phosphate buffer (pH 7.2) the oxidation rate of cytochrome c, reduced by sodium dithionyte. Malate dehydrogenase (E.C.1.1.1.37.) activity was determined by the method of OCHOA[12]. Glutamate-oxalacetate transaminase (E.C. 2.6.1.1.) activity was measured as described by SCHWARTZ[13]. Fumarase (E.C. 4.2.1.2.) activity was determined according to RACKER[14] employing 45 m*M tris* buffer (pH 8) instead of phosphate buffer.

For the experiments of in vitro reactivation, the mitochondrial preparations from castor bean were diluted (1:1) with a medium containing $2 \times 10^{-3}M$ ATP, $2 \times 10^{-3}M$ ADP, $2 \times 10^{-2}M$ succinate, $10^{-2}M$ $MgCl_2$, $2 \times 10^{-2}M$ K_2HPO_4 and kept at 0 °C or 27 °C for the periods indicated. The mitochondrial preparations from squash seeds were incubated without dilution at 30°C for the periods indicated.

Results and discussion. A-Castor bean endosperm. In the castor bean endosperm few mitochondria are present in the dry seed and a rapid synthesis of these organelles occurs during early germination[3,15]. However, the increase of mitochondrial activities in the first 24 h of germination is relatively little affected by protein synthesis inhibitors, thus suggesting the reactivation of preexisting inactive enzyme forms. The results of Figure 1 show that the rapid increase of cytochrome oxidase and of (mitochondrial) malate dehydrogenase in the first 24 h of germination are not significantly inhibited by actinomycin D and by only ca. 50% by low temperature (0 °C). Under the same experimental conditions actinomycin D markedly inhibits RNA and protein synthesis[6,16], and at 0 °C practically no protein synthesis is detectable. Further support to the reactivation hypothesis comes from experiments in which mitochondria isolated from dry seed endosperm were incubated at 0 °C and at 27 °C. In this case, as shown in Figure 2, a variable (from 30% to 100%), but always significant increase of cytochrome oxidase was observed in the preparations from dry seeds. This process is clearly temperature dependent. A parallel study on isolated mitochondria from the same material shows that practically no protein synthesis occurs in the isolated organelles under the same experimental conditions[17]. Moreover in vitro activation of cytochrome oxidase or other mitochondrial enzymes was never observed when the mitochondria were prepared from seeds germinated 60 h. The relatively low values of activation in the cell free extracts compared with the in vivo condition might be easily explained as due in part to activation during the isolation procedure, in part to the obviously different physico-chemical conditions under which the process is occurring.

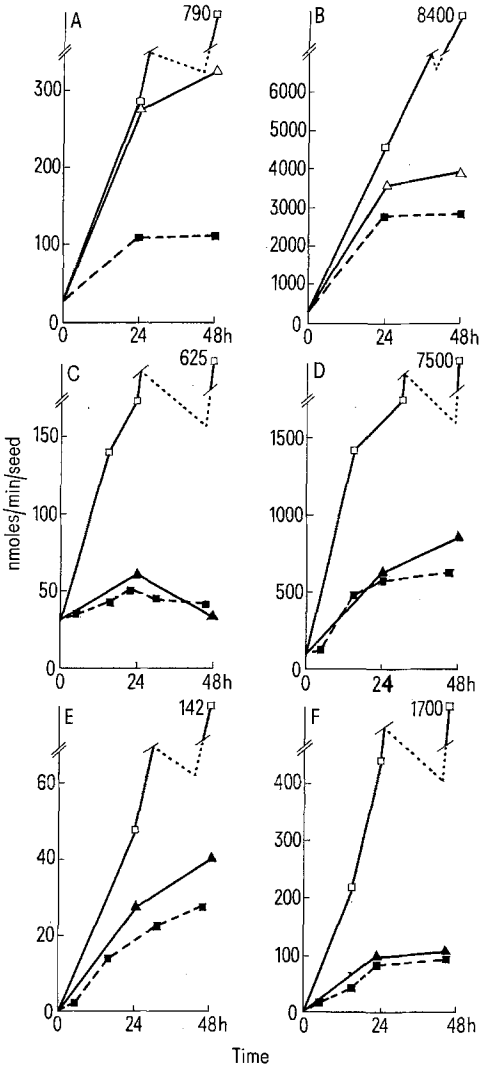

Fig. 1. The effect of RNA and protein synthesis inhibitors and of low temperature on the development of mitochondrial enzyme activities in endosperm from castor bean seeds (A, B) and in cotyledons from squash seeds (C, D, E, F) germinated at 30 °C in water (—□—), in the presence of $10^{-3}M$ cycloheximide (—▲—) or actinomycin D (50 µg/ml.) (—△—) and at 0 °C in water (--■--). The values of enzyme activities are expressed as nmoles substrate consumed/min/seed. Castor bean endosperm: A) cytochrome oxidase; B) malate dehydrogenase. Squash seed cotyledons: C) cytochrome oxidase; D) malate dehydrogenase; E) succinate dehydrogenase; F) glutamate-oxalacetate transaminase.

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B-Cotyledons of squash seeds. The conclusions reached on the basis of the experiments on the castor bean endosperm are confirmed and generalized by the finding of a similar behaviour of some mitochondrial enzyme activities in the cotyledons of germinating squash seeds. Figure 1 shows in this material the effects of cycloheximide or of low temperature (practically no protein synthesis is detectable any more under these conditions) on the development of mitochondrial malate dehydrogenase, succinic dehydrogenase, oxalacetate-glutamate transaminase and cytochrome oxidase activities. In all cases, a significant increase of enzyme activities is observed even under the two conditions of inhibited protein synthesis. This fact is very evident during the first 24 h of germination and much less so in the following 24 h, when the rate of the increase of enzyme activities in the control cotyledons is very high.

These data suggest that a minor, but still significant fraction of the increase of the activities considered depends on the reactivation of preexisting inactive enzyme forms. As in the case of the castor bean this conclusion is confirmed by the data of Figure 2, showing that even in isolated mitochondria obtained from dry seeds a consistent increase of malate dehydrogenase, cytochrome oxidase, and transaminase is observed during the first 2–3 h of incubation. Here again the conditions of incubation are such that no detectable in vitro protein synthesis can occur. Moreover, no activation at all was ever observed in mitochondria isolated from seeds germinated for 24 h or longer.

Conclusions. The present results extend to mitochondrial enzymes the phenomenon of reversible inactivation-reactivation previously reported from some soluble enzymes[4–8]. The contribution of enzyme reactivation to the development of the enzyme pattern of early germination is quantitatively limited. However, its importance is easily understood if one considers that the beginning of rapid de novo enzyme synthesis must be conditioned by some level of metabolism supported by preexisting catalytic activities.

The molecular nature of the enzyme inactivation-reactivation process is largely obscure. The reversible transition from the active to the inactive enzyme form during maturation can be (at least in some cases) markedly speeded up by experimental dehydration[4, 18, 19]. Moreover, the present data, together with those reported by other authors, show that some reactivation can be demonstrated in cell free systems, by simple incubation of the inactive forms in diluted solutions, without drastic treatments[4, 6]. These data suggest that water availability (in this case as in that of the hydration-dependent control of protein synthesis in the germinating seed)[20] is the main factor involved in this process. The problem of the likely interactions between single enzyme and other large or small molecules remains completely open and further work on this interesting point is desirable.

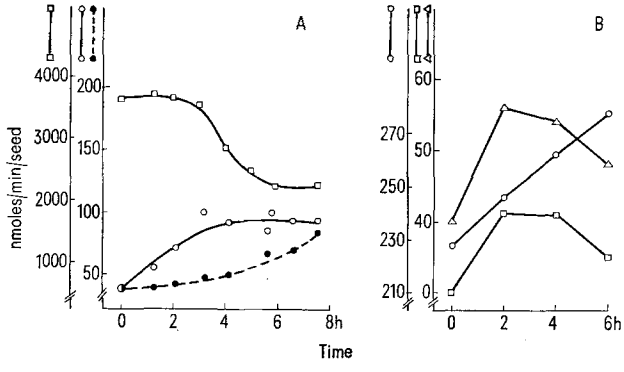

Fig. 2. In vitro time course of mitochondrial enzyme activities of castor bean endosperm and of squash cotyledons. The values are expressed as nmoles substrate consumed/min/seed. A) Castor bean mitochondria were extracted from dry seeds and the preparation incubated at 0 °C (--●--) or at 27 °C (—○—), or from seeds germinated for 60 h at 27 °C and the preparations incubated at 27 °C (—□—). B) Squash mitochondria were extracted from dry seeds and the preparations incubated at 30 °C. —□—, glutamate-oxalacetate transaminase; —○—, malate dehydrogenase; —△—, cytochrome oxidase.

Riassunto. In semi di ricino e di zucca una frazione significativa dell'aumento di attività enzimatiche mitocondriali durante le prime 24 h di germinazione risulta insensibile a inibitori della sintesi proteica. Un significativo aumento delle stesse attività è rivelabile anche in vitro in preparati mitocondriali ottenuti da semi secchi.

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Jodidwirksamkeit auf den Wassergehalt des Lig. nuchae des Rindes als Folge anionischer Abschirmung positiver Ladungsstellen am Strukturprotein[1]

Rahmenthema ist die extrazelluläre Pharmakokinetik des Jodids. Der gegenständliche Beitrag will einen kleinen Teil dieser Wirksamkeit experimentell erfassen, um für deren molekular-biologische Deutung erste Ansätze zu gewinnen. Gegenüber Chlorid scheint Jodid als effektiv (mit Wasserhülle) kleineres Anion vorzugsweise geeignet zu sein, dissoziierte basische Reste der Aminosäureketten (elektropositive AS-Seitengruppen) elektrostatisch abzuschirmen. Folglich werden die salzartigen inter- und intrachenaren Bindungen zwischen den positiven und negativen AS-Seitengruppen geschwächt. Überwiegt, was für die meisten Proteine im biologischen pH-Bereich gültig ist, die Dissoziation der sauren Reste, und trägt demnach das Protein elektronegative Überschussladung, so bedeutet die genannte Abschirmung der nebenbei noch vorhandenen positiven Ladungsstellen (der basischen Reste der

[1] Herrn Professor Dr. Dr. Th. Leipert zum 70. Geburtstag gewidmet.