RAPID INDUCTION OF ENZYME ACTIVITY DURING FERTILIZATION

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The cytochrome c oxidase activity increases during early development of fish embryos (Neyfakh and Donzova, 1962). A suitable model of this process was proposed (Donzova and Neyfakh, 1964). The cytochrome oxidase activity was shown to increase in vitro during incubation of fish embryo homogenates. This occurs in the heavy fraction of the egg homogenate (600 g, 10 min.- yolk platelets bound with mitochondria) or in isolated mitochondria (12,000 g, 10 min). The increase of enzyme activity in the heavy fraction takes place in isotonic sucrose solution, while the isolated mitochondria require a suitable medium consisting of K^+ and Mg^{++}, substrates and cofactors of oxidative phosphorylation and the complete set of amino acids. The increase of the activity lasts 45-60 minutes and reaches 120-180 % of the initial value. The sensitivity of this process to puromycin or the elimination (in case of mitochondria) of one amino acid (alanine, methionine or tyrosine) shown that the cytochrome oxidase activity increase is related to protein synthesis. If a rise of activity occurs in vivo it can always be obtained in vitro, or vice versa: a complete or a partial inhibition of the

increase of the cytochrome oxidase activity in the embryo (radiation, chemical agents) affects the behaviour of the system in vitro in a similar manner. Such coincidences support the model proposed. The main advantage of this model is that it permits a test of the capacity of intracellular embryonic structures to change the enzyme activity at any time during the development.

After treatment of unfertilized eggs with heavy doses of X-ray (40 kr) their mitochondrial structures lose the capacity to increase the cytochrome oxidase activity. But after fertilization the eggs acquire this capacity again. This stimulating function of spermatozoa and, mainly, the rapid rate at which it occurs is the object of this communication.

The work was carried out on fish eggs (loach, Misgurnus fossilis); cytochrome oxidase activity was determined by the rate of cytochrome C oxidation (Smith, 1955), for the details of egg incubation, X-ray irradiation, media, etc. cf. Donzova & Neyfakh, 1964.

RESULTS

The role of the spermatozoon in the stimulation of the cytochrome oxidase activity increase

Changes in the enzyme activity in homogenates were compared in five variants as follows: 1) normal eggs fertilized by normal sperm (diploid embryos, control), 2) irradiated (40 kr) eggs fertilized by normal sperm (haploid embryos), 3) irradiated eggs fertilized by irradiated sperm (functionally "enucleated" embryos), 4) irra-

diated eggs treated with fresh water without sperm (after such "activation" the vitelline membrane separated and the blastodisc formed but no cleavage occured), 5) irradiated eggs after polyspermic fertilization.[x]

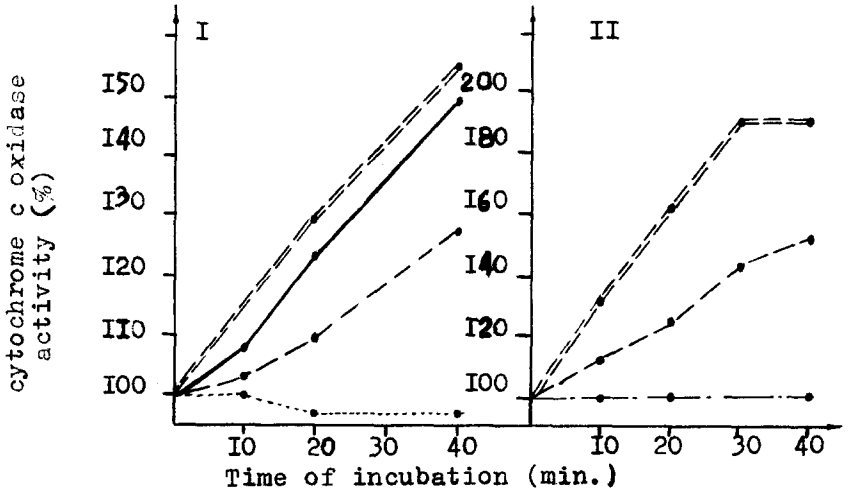

Fig. 1 Changes in cytochrome oxidase activity during incubation of egg homogenates (two experiments).____ diploid -+-------- haploids (irradiated eggs), ... "enucleated" eggs (both gametes irradiated), -.-.-.- irradiated unfertilized eggs, ========== irradiated polyspermic eggs.

This experiment (fig.1) showed that the rise of enzyme activity in diploids was almost twice that in haploids, while the enzyme activity of "enucleated" or "activated" eggs showed no changes. In polyspermic irradiated eggs the rate of activity increase was twice that in haploids and equal or somewhat greater than that in normal

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x) Artificial polyspermia was attained by pretreatment with trypsin solution to eliminate the vitelline membrane.

diploid embryos. Therefore it is apparently the sperm--egg interaction which provides the capacity of intracellular structures to increase the cytochrome oxidase activity.

The rate of stimulation provided by the sperm.

Irradiated eggs were fertilized with normal sperm, homogenized after various short intervals and then the changes in cytochrome oxidase activity were determined in the homogenates. Two procedures for quick and exact arrest of the process of fertilization were used. 1) One of the holes in a wide pyrex tube was closed with fine copper net on which unfertilized irradiated eggs were placed. The tube then was plunged into sperm suspension and thereafter into liquid nitrogen. The time between both plunges is the time of sperm-eggs interaction. 2) Eggs were placed on the bottom of a large glass homogenezer, the sperm suspension was poured over them and then all eggs were homogenized by one onward movement of the rotating teflon-pestle. Both procedures gave the same results.

In the first experiment at 21°C (the ordinary temperature for the experiments on Misgurnus fossilis embryos) a time of sperm-egg interaction of ONE SECONDS or more produced the increase of enzyme activity. Further experiments were carried out at a suboptimal temperature, 10°C. The development at this temperature occurs approximately four-five times more slowly than that at 21°C. The results of such experiments (fig.2) show that the enzyme synthesizing system of mitochondria was activated 5 - 6 seconds after placing the eggs in the sperm

suspension. Within the range of accuracy of experiments (± 0,5 sec.) the data for 21°C and 10°C were in good agreement.

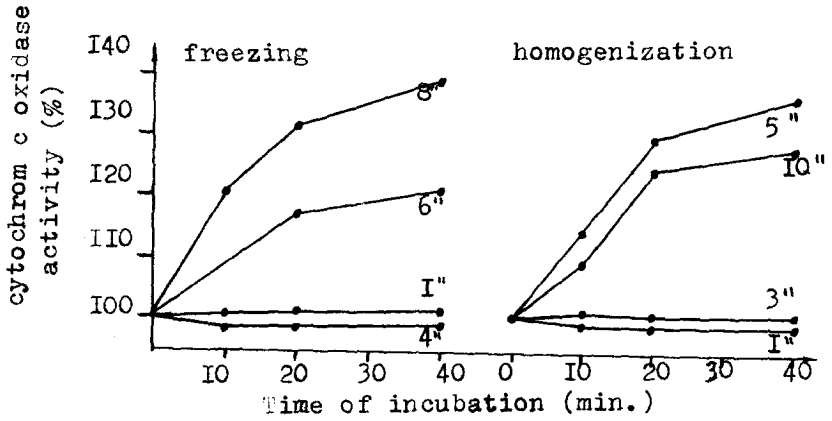

Fig.2. Changes in cytochrome oxidase activity during incubation of egg homogenates obtained at various times of sperm-egg interaction.

D I S C U S S I O N

Usually the initial cytological and biochemical changes in eggs occured during few minutes of sperm-egg interaction. Recently Epel (1964) found out the changes of piridine nucleotides concentrations in sea urchin eggs as soon as 40 seconds after fertilization. As it was shown in our experiments, the stimulation of enzyme activity took place much earlier, namely in a second after fertilization.

The process of stimulation probably consists of

four steps: 1) the penetration of the sperm into the micropyle of the egg, 2) the contact of the spermatozoon with the egg surface, 3) the transfer of the stimulating action from the site of sperm-egg interaction to mitochondrial structures, 4) the effective stimulation of the mitochondrial structures. We cannot determine the fractions of time for each of the processes. However if the rate of the last two processes was limiting the capacity to increase enzyme activity should appear gradually (with the spread of the stimulus over the egg). This capacity appearing suddenly and completely (compare the curves for 3 and 4 sec. with those for 5 and 6 sec. in fig.2). Therefore the slowest steps must be the first two preparative processes, while the spread of the stimulus over the egg lasts a very short time, probably no more than o.1 - o.2 second (at 21°C). Therefore the rate of spreading is of the order of 1 cm/sec. or more. This creates serious difficulties for any theory which would describe the transfer of the stimulus as diffusion of some substances. It would be more natural to speak of some impulse spreading from the site of contact of spermatozoon with the egg surface.

We are now studying the nature of the stimulating action of the sperm. It appears that the spermatozoon does not bring into the egg any stimulating substances.

It would be useful to draw an analogy with the process of interaction of a phage particle or of a colicin molecule with the surface of a bacterial cell. In all these cases one contact is sufficient to stop or to start the synthetic processes in the cell.

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