

# BIOCHEMISTRY AND BIOPHYSICS

## THE ACTION OF X-RAYS ON THE MOTOR FUNCTION AND THE CONTRACTILE PROTEINS OF MOTILE CELLS

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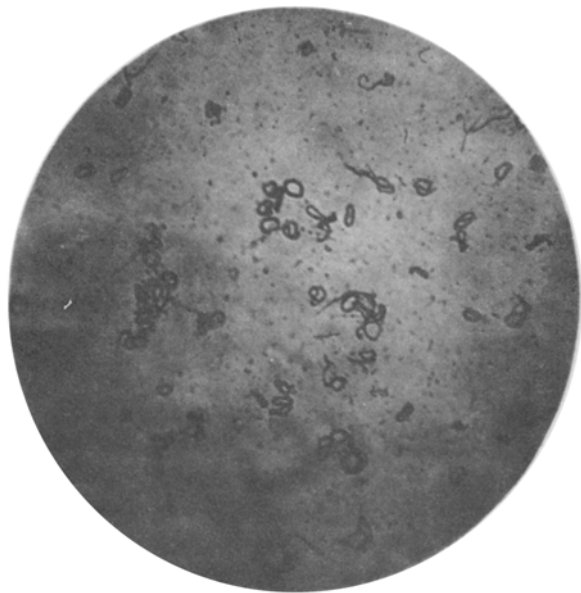
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The study of the action of x-rays on the motor function of cells, and in particular of spermatozoa, is of interest from several points of view. It is possible thereby to obtain in a clearly apparent form data demonstrating that the degree of radiation injury of certain enzyme systems (for example, the thiol enzymes, the system of enzymes of oxidative phosphorylation) is determined primarily by the degree of radiosensitivity of the particular species of cell, and this, in turn, depends in the opinion of certain authors [4,5] on the character of the intracellular localization of these enzymes.

From this point of view it can clearly be understood how marked disturbance of the processes of oxidative phosphorylation can occur in radiation sickness in a number of "radiosensitive" tissues, in the absence of any appreciable changes in these processes in muscle tissue and in other "radioresistant" organs, tissues and cells. Adult spermatozoa are an example of such radioresistant cells.

We know very well that the movement of spermatozoa is inseparably connected with continual resynthesis of adenosinetriphosphate (ATP) at the expense of energy from oxidative processes (respiration) or the anaerobic breakdown of carbohydrates [2, 3, 6, 10, 17, 18]. Any agency connected with the disturbance of the conjugation of respiration or glycolysis and the resynthesis of ATP leads to rapid loss of the motility of the spermatozoa. Thus spermatozoa, like other cells possessing the power of movement (for example the cells of ciliated epithelium), are excellent objects for the study of the association, postulated by several authors, between the development of radiation injury and the inadequate formation of high-energy compounds in the cells of irradiated animals (i.e., a fall in the intensity of oxidative phosphorylation).

The study of the influence of ionizing radiation on the motor function of cells is also of interest in other respects. The biochemical basis of the different forms of cell division is known to be the interaction between ATP and contractile protein, possessing an adenosinetriphosphatase activity and the property of changing its physical state under the influence of ATP. A like mechanism is at the basis of muscular contraction [9, 11], the movements of the flagella of spermatozoa and of trypanosomes [6, 10, 16], the rippling of the cilia of epithelial cells [1] and the movements of the leaves of certain plants [7]. There are indications that the separation of chromosomes and the equatorial division of the protoplasm of the cell in the process of mitosis are also effected by changes in the properties of the specific contractile protein of the cytoplasm during its interaction with ATP [16, 19]. It is also known that the proteins of the actomyosin complex possess low sensitivity to ionizing radiation [5]. Since, according to Hoffmann-Berling's findings [6], the contractile proteins of different species of cell are very close in their physicochemical properties and adenosinetriphosphatase activity to actomyosin, it may be suggested that, like the proteins of the actomyosin complex, they also must possess high resistance to ionizing radiation.



Annular forms of models of spermatozoa (microphotograph).

From considerations such as these it might be possible to explain the high resistance of certain phases of cell division [15], for example the interphase and anaphase, which is due to interaction between the contractile cell proteins and the ATP.

In the present communication we describe the results of research into the effect of massive doses of x-rays on the motor function of living spermatozoa and on the reaction between adenosinetriphosphate and "glycerol models" of myofibrils, spermatozoa and the cells of ciliated epithelium.

#### EXPERIMENTAL METHOD

Experiments were carried out on male spring frogs, which were given a single exposure to x-rays in a dose of 20,000 r. Conditions of irradiation: time of exposure — 61 minutes, voltage — 185 kv, current — 15 ma. Spermatozoa from the testicles of the irradiated frogs were suspended in a 0.1% solution of NaCl. Their motility was examined under the microscope.

The "glycerol models" of spermatozoa were obtained by Hoffmann-Berling's [16] method, which we simplified in accordance with the findings of Aleksandrov and Arronet [1], which was essentially as follows: Minced tissue from the testicles of a sacrificed frog was immersed in 2-3 volumes of a 50% aqueous solution of glycerol and placed in the refrigerator for 24 hours; one drop of the glycerol suspension was then mixed with 2-3 drops of a 0.12 M solution of KCl, 0.01 M phosphate buffer (pH = 7.0) and 0.005 M  $MgCl_2$ ; a few minutes later one drop of a neutralized 0.01 M solution of ATP was added to the suspension of spermatozoa thus obtained, on a glass slide, and the action of the ATP on the "cell models" of the spermatozoa, or more accurately, on the "cell cadavers", was studied under the microscope.

The muscle fibers were extracted with a 50% glycerol solution by the method described by Bendall [14].

Preparations of the cilia of the soft palate were made by the method of Aleksandrov and Arronet [1].

#### EXPERIMENTAL RESULTS

Living spermatozoa from frogs irradiated with 20,000 r retained good motility in isotonic saline solutions, and in this respect were not appreciably different from the spermatozoa of unirradiated control animals.

The preservation of the motility of living spermatozoa obtained from the testicles after irradiation of frogs with massive doses of x-rays demonstrated the radioresistance of the mechanisms maintaining the ATP at the level necessary for effecting the motor function. These experiments therefore gave no grounds for postulating in this case any disturbance of oxidative phosphorylation under the influence of ionizing radiation. This in no way implies that if the distribution of the enzymes catalyzing the processes of oxidative phosphorylation (in radio-sensitive tissues) were of a different character, the oxidative resynthesis of ATP would not be disturbed also. On the contrary, from the general considerations outlined above and from the reports in the literature [5], the opposite may be declared.

After the addition of ATP and a solution containing  $MgCl_2$ , the spermatozoa of the frog, extracted with glycerol, often change their shape: 65-70% of the spermatozoa form "annular" structures (see figure); many of them perform energetic oscillatory movements with their tails. In some cases, after extraction with glycerol, solitary motile spermatozoa were found even after the addition of saline without ATP; in these cases, however, the motility disappeared completely after 15-20 minutes and reappeared after addition of a 0.01 M solution of ATP to the suspension. In these cases the reappearance of motility in the spermatozoa extracted with glycerol without the addition of ATP was probably explained by the presence of a small amount of endogenous, protein-

-bound ATP, which was quickly used up after the addition of the saline solution containing  $MgCl_2$  which activated the utilization of ATP by the spermatozoa.

The "glycerol models" of the spermatozoa of animals irradiated with 20,000 r preserved their motility under the influence of ATP and  $MgCl_2$  ions introduced from outside.

The contractility of the muscle fibers of frogs, irradiated with massive doses of x-rays, when treated with glycerol, did not differ appreciably after the addition of ATP from the contractility of the fibers of unirradiated animals. For instance, the myofibrils obtained from the gastrocnemius muscle of the irradiated animals reacted to ATP by a shortening of 50-70% of their length, and the myofibrils of the unirradiated frogs by a shortening of 55-60%. The rate of contraction, too, was roughly the same in both cases.

The experiments with preparations of the soft palate of frogs, extracted with 50% glycerol solution, showed that the cilia of the cells of the ciliated epithelium of animals irradiated with 20,000 r retained the power of rippling movement after the addition of ATP and  $MgCl_2$ .

The results of the experiments on "glycerol models" thus showed that the contractile proteins of cells possess radioresistance and do not lose their power to interact with ATP under the influence of massive doses of ionizing radiation.

The facts which we have described, together with indications in the literature on the common structural and functional features of the different contractile proteins, suggest that the low sensitivity of certain phases of cell division (interphase, anaphase, associated with separation of the chromosomes and equatorial division of the cytoplasm) to ionizing radiation is also explained by the radioresistance of contractile protein, which is responsible for the cytokinesis of the dividing cells.

The preservation of the contractile properties of these proteins and of their power to interact with ATP after irradiation even with massive doses of x-rays indicates the radioresistance of those SH-groups in the protein molecule which give it its adenosinetriphosphatase character [12, 8]. This is evidence against Barron's theory that the primary biological action of ionizing radiation is connected with the inactivation of the thiol enzyme systems and is unconcerned with their intracellular localization [13].

The findings described above are in agreement with direct observations indicating the absence of any essential lowering of the content of SH-groups in the proteins of the actomyosin complex or in the serum proteins in response to the action of minimal lethal doses of x-rays on animals [5].

#### SUMMARY

Experiments showed that there are no significant changes of the motor function in the seminal cells after x-ray irradiation of frogs (20,000 r). This shows that disturbance of oxidative phosphorylation under the action of radiation is not a general biological regularity. The effect of ionizing radiation on the resynthesis of high-energy (ATP) differs in the cells of radiosensitive and radioresistant tissues; evidently it is primarily determined by the different localization of the corresponding enzyme systems therein.

There are no visible disturbances in the ability of "glycerol models" of myofibrils, the cilia of the ciliated epithelium and spermatozoa (obtained from the tissues of irradiated animals), to interact with ATP. This points to a rather low radiosensitivity of the contractile cellular proteins and explains the high radioresistance in some phases of cell division connected with the separation of the chromosomes and equatorial division of the cytoplasm.

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\*See G. B. Translation.