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Effect of Y-rays on Na^+- K^+- ATPase, enzymatic factor which mediates in the active sodium transport through biological membranes *

by

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During the last years, these laboratories have been engaged on the study of the transport of ions through membranes such as those of erythrocytes, and intestine and skin of amphibious, by using radiotracers. The ions so far studied have been : $SO_4^{=}$, Zn^{++} and Na^+.

This work has been carried out with two aims: To study the type of transport of the ion and to see how this transport is modified by different agents.

The present paper deals with the transport of sodium through skin of frog and the effect of gamma radiation has been used as modifying agent of the transport. The kinetics of the process has been followed, to know the flux ratio through the skin and the changes that appear in the transport due to the radiation.

At the same time, the ATP-ase activity in the membranes has been determined in each case in order to relate the transport variations with the changes in enzyme activity.

Most of the work published on the variation of sodium transport by action of X-rays, has been carried out in erythrocyte membrane : ELLINWOOD (1957), POST (1960), QUASTLER (1962) and (1964), amongst others.

The active transport of any ion through a membrane, takes place as a consequence of a work carried out by the cell. This mechanism has been studied by USSING (1959) in the case of sodium through amphibious skin with the help of radiotracers. The experimental devices used in the present work are based on those described by this author.

CALDWELL (1959) has summarized the hypothesis suggested for the transport mechanism and the participation of ATP on the transport of sodium, potasium and magnesium ions through membranes.

It has been shown that ATP is needed

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tor the enzymatic synthesis of the phosphatidic acids of the membrane and that these acids bind the sodium ions to carry them to the inner part of the membrane, where by the action of a phosphatase, the carrier lets free the sodium ions and diglyceride.

HOKIN and HOKIN (1961) suggest that the sodium transport through the membrane occurs with the help of ATP, according to two different processes that take place at the external and internal part of the membrane. So the cycle of the phosphatidic acid is formulated according to the following reactions:

$$ATP^{4-} + Dg \xrightarrow{\text{Dg-kinase}} PA^{2-} + ADP^{2-} \quad (1)$$

$$PA^{2-} + 2Na^{+} \longrightarrow PA\text{-}Na_2 \quad (2)$$

It is assumed that this mechanism takes place at the internal part of the membrane appearing a liposoluble sodium phosphatidate, undisociated.

This compound passes across the membrane and undergoes the following reaction at the external surface.

$$PA\text{—}Na_2 \xrightarrow{\text{PA-phosphatase}} Dg + P_i^{2-} + 2Na^{+} \quad (3)$$

In this way sodium ions are set free in the external part of the membrane while the metabolic cycle of the carrier is completed.

These authors also suggest that besides the phosphatidic acid, there is also another phosphatide, the phosphoinositic, which can be a carrier of sodium through the membrane.

CHARNOCK and POST (1963), also suggest a two step mechanism for the transport of sodium, according to the following reactions:

$$\text{Enzyme} + ATP \xrightarrow{x\,Na^{+}} (E\text{—}P)Na_x + ADP \quad (4)$$

$$(E\text{—}P)Na_x \xrightarrow{y\,K^{+}} E + P_i + x\ Na^{+} + y\ K^{+} \quad (5)$$

The first reaction depends on the sodium ions and the second on the potasium ions. This hypothesis assumes another step similar to the previous one to account for the two components that appear in the kinetic studies of the outflux of sodium.

In his paper BRESCIANI (1964) indicates that the action of X-rays inhibits the *sodium pump* and that the dose effect curve includes at least two functions suggesting the existence of parallel mechanisms of transport, where ATP-ase have two differently radiosensitive subcomponents of the Na and K-dependent ATP-ase, with different constants of activation by X-rays: one sensitive to 740 rads and other to the 10.700 rads, which coincide with the inhibition of sodium active efflux.

Experimental technique

The membrane studied has been skin from *Rana esculenta* and to follow the kinetics of the process the ringer solutions have been labelled with Na-22.

The experimental devices, are based on the short-circuit technique described by USSING with some modifications introduced by us.

Figure 1 shows a diagram and figure 2 a photograph of the equipment used in this work.

The membranes were irradiated in a Co-60 source the radiation being applied on the external part of the skin. The doses given in the different experiments were 1000, 5000, 10000 and 20000 rads. As soon as the skin is separated from the animal, it is placed in a plastic frame which suports 7 cm^2 of membrane. This frame is placed in a plastic box containing ringer Rana solution which is in contact with both sides of the skin.

The enzymatic activity of ATP-ase has been studied following a method used by BRESCIANI in the case of red cells. The skin is previously freezed at the

temperature of liquid air, and homogeneized in an Omnimixer (Sorvall) with ice-cold ringer Rana solution. The suspension obtained is incubated, with continuous stirring, at 37° C during 45 minutes.

The basal medium of incubation was: tris (hydroxymethyl-aminomethane) HCl buffer, pH 7.05 (115 mM); ATP neutralized with Tris before addition (1.5 mM); $MgCl_2$ (0.5 mM); cysteine (1 mM).

At the end of the incubation, ice — — cold 6 % perchlorie acid solution, is added to the suspension in a proportion of 1 : 1. Afterwards it is centrifuged and in the supernatant the ortophosphate ion is determined according to the colorimetric method described by KING. Blank experiments with the solvent and the whole suspension without ATP were carried out.

It has to be noted that in this study the effect of potasium has not been considered, its concentration being kept constant, for all the experiments.

Results and discussion

The values obtained for the sodium activity in tests of sodium in and outfluxes at different times with skins irradiated and non-irradiated are shown in figure 3.

With the values from the kinetical measurements, the ratios of the experimental fluxes have been obtained.

The membrane potentials have been

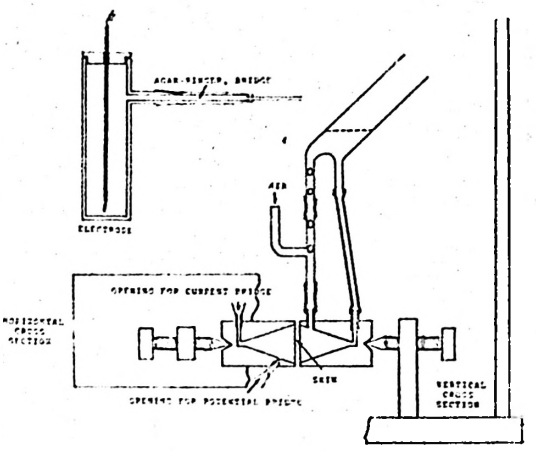


FIG. 1. Diagram of equipment.

measured experimentally and from their mean values, a flux ratio has been calculated by applying the Ussing equation's

$$M_{in}/M_{out} = c_o/c_i \cdot f_o/f_i \cdot e^{zFE/RT}$$

The differences between the measured and calculated ratio, indicate the type of transport and the changes introduced in it when different doses of radiation are applied to the skin.

Table II summarizes the values of phosphate obtained by colorimetric analysis. These values represent ATP-ase activity per mg of skin and hour of incubation, for non-irradiated and irradiated skins with different doses and are shown in figure 5.

A comparison is stablished in each case between the flux ratios experimentally measured using Na-22 and those

TABLE I

Gamma radiation-effect on active sodium transport through frog skin.

Doses (rads)	M_{in}	M_{out}	E (mV)	M_{in}/M_{out} found	M_{in}/M_{out} calculated	Inhibition %
	% activity/h					
0	0.400	0.072	20.22	5.556	2.226	0
1000	0.377	0.084	15.87	4.488	1.878	21.6
5000	0.276	0.093	21.59	2.968	2.351	81.4
10000	0.195	0.165	12.12	1.182	1.616	112.9
20000	0.390	0.136	17.22	2.868	1.975	73.3

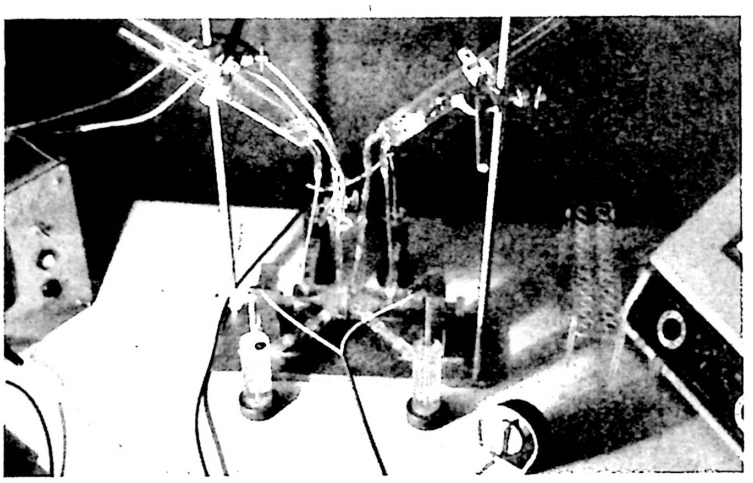


FIG. 2. Photograph of equipment.

TABLE II

Gamma radiation effect on ATP-ase, activity on frog-skin.

Doses (rads)	ATP-ase, activity Y.P/mg skin/h	Inhibition %
0	3.69	0
1000	3.30	11
5000	2.67	28
10000	1.72	54
20000	3.48	6

calculated according to the USSING equation. From the differences obtained the percentage of the inhibition of the active sodium transport is calculated (fig. 6-A)

At the same time the enzyme inhibition is determined from the amount of phosphate measured by the colorimetric analysis. In each case, the enzymatic inhibition of ATP-ase is compared with the inhibition in the active transport of sodium (fig. 6-B).

The curves in figure 3 and figure 4 show that the sodium influx diminishes with increasing radiation doses, until the value of 10000 rads is reached. At 20000 rads the flux increases and the values obtained are similar to those at 1000 rads.

On the contrary, the outflux increases as the radiation dose increases, but as before, at 20000 rads an anomaly is found and the flux is smaller than at 10000 rads.

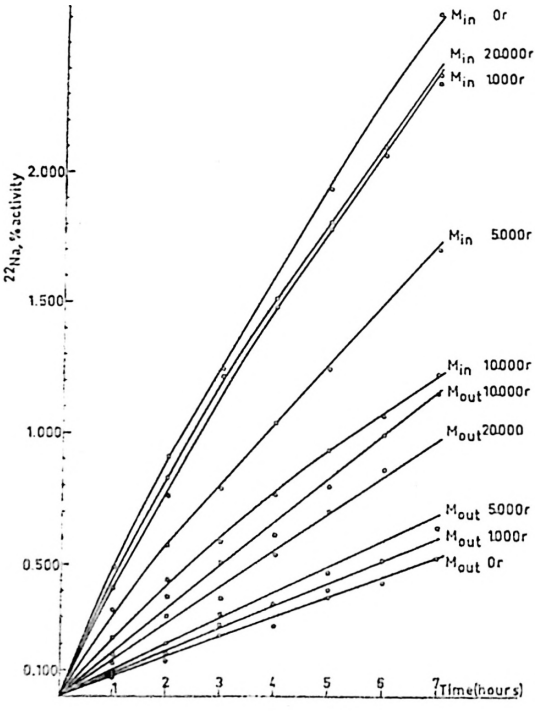


FIG. 3 Kinetic Process curves

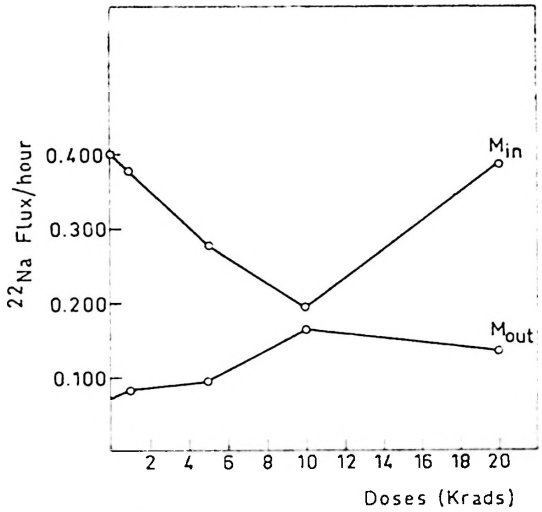


FIG. 4 Dose-effect curve of Kinetic process.

These facts are obviously reflected in the experimental flux ratios, which vary according to the radiation dose applied to the skin.

On the other hand, if the values of the enzymatic activity of the ATP-ase are considered (fig. 5), it can be seen that they also change as a function of the dose applied to the membrane, and as in the case of the influxes of sodium, the activity decreases with increasing radiation dose, but at 20000 rads reached a higher value. The curve obtained is in all parallel to that showing the kinetical process.

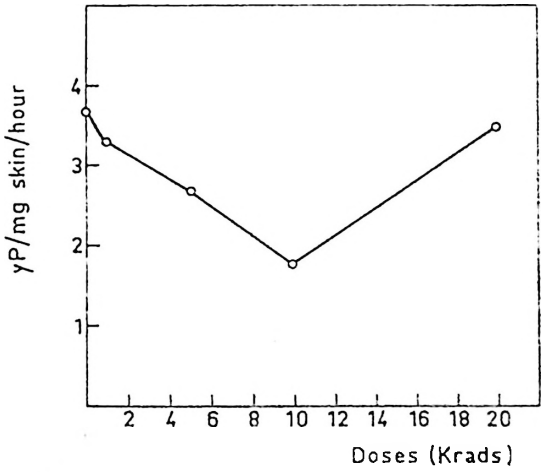
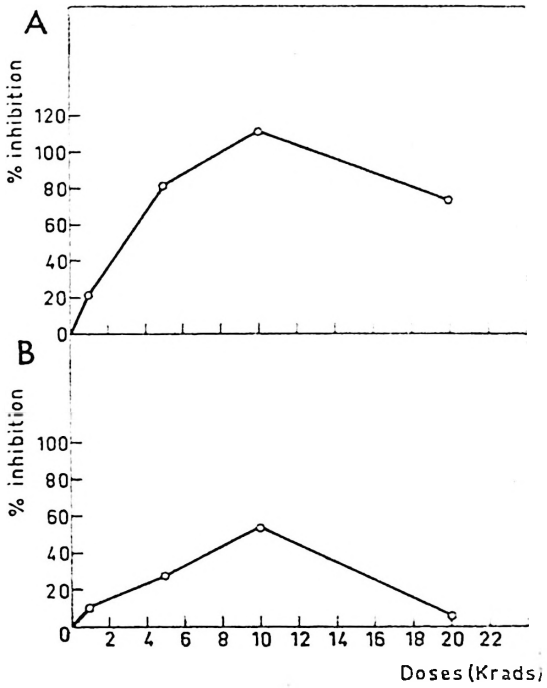


FIG. 5 Dose-effect of ATPase activity in frog skin.

The results described above, suggest:

That there is an evident relationship between ATP-ase activity and active transport of sodium.



A) Transport inhibition by γ-radiation.
B) ATPase inhibition by γ-iradiation.

The phenomenon is extremely complex, as the radiosensitivity of the enzymes intervening in the process is probably different and the doses of applied radiation are very relevant. Furthermore, the mobility of the sodium carries through the membrane can change as a function of the radiation dose. All this can be an explanation of the facts observed.

It is very probably that the change observed in the curve of transport in both the in and outfluxes of sodium, be due to an increase in the passive transport, rather than to an increase of the active transport, which can be still decreasing as an effect of the radiation.

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