The Action of Nigericin on Sodium Efflux from the Toad Oocyte

Earlier work[1] revealed that the efflux of sodium from the oocyte of *Bufo bufo* is reduced by oligomycin. It also revealed that if the oocyte is pretreated in vivo with tri-iodothyronine, then the behaviour of the efflux toward oligomycin is biphasic, i.e. inhibition is followed 1 h later by stimulation. One reasonable explanation of this biphasic effect is that inhibition of respiration by oligomycin results in the release of mitochondrial sodium, which in turn stimulates a Na–Na or Na–Ca exchange diffusion mechanism. The possibility that sodium is sequestered behind mitochondrial membranes has now been tested by treating the oocyte with nigericin, an antibiotic which is known to inhibit respiration and deplete mitochondria of cations. The following communication brings forward kinetic evidence that nigericin increases the permeability of the oocyte membrane to Na and subsequently liberates the sequestered Na by a mechanism which is K-dependent.

Immature oocytes measuring 600–1200 μm in diameter were obtained from the ovaries of healthy female specimens of *Bufo bufo*. They were then loaded with ^{24}NaCl as described by DICK and LEA[2]. Counting of ^{24}Na activity in the oocyte and effluent was carried out as described by DICK and LEA[2] and BITTAR, DICK and FRY[3]. Nigericin which was a gift from Dr. ROGER L. HARNED, Commercial Solvents Corporation, Terre Haute (Indiana, USA), was used as a solution in ethanol, the final concentration of the alcohol being 0.19% by volume. An equal concentration of the alcohol was present in the control Ringer's solution. The composition of the Ringer's solution was that given by BITTAR, DICK and FRY[3] but its pH was 7 instead of 7.4. The K-free medium had an NaCl concentration of 109 m*M* and a pH of 7.

As first pointed out by DICK and LEA[4], the kinetic results obtained upon stimulation of Na efflux by external agents can have 3 distinct patterns. These patterns, which are depicted in Figure 1, show in the first case that a sudden step-up in the efflux rate is followed by an increase in the efflux rate constant, indicating a raised permeability of the membrane to Na. The second form of behaviour consists of a step-up in the efflux rate, followed by an unchanged rate constant for Na loss, indicating a rise in the rate of exchange between the 2 internal Na fractions. A prime example is the behaviour of the efflux in the absence of external K[5] (see also below). The third form of behaviour consists of a step-up in the efflux rate, followed by a reduced efflux rate constant, indicating partial saturation of the pump by the liberated Na.

The kinetic results with nigericin appear to conform to two of these patterns of behaviour. Figure 2 records an experiment showing that the addition of 10 μg/ml nigericin to an oocyte suspended in Ringer's solution causes a sudden and marked rise in the rate of Na efflux, followed by a rise in the efflux rate constant (1 out of 10 experiments involving oocytes from the same ovary). A more typical result (7 out of the 10 oocytes from this ovary) is that shown in Figure 3, indicating that the response of the pump to nigericin is biphasic. It can be seen that subsequent to a rise in membrane permeability to Na there develops a further rise in the efflux rate. As

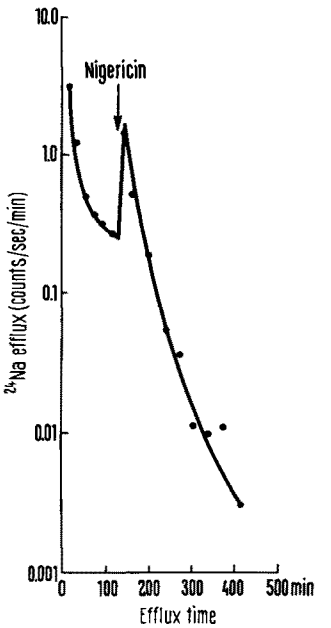

Fig. 2. The effect on Na efflux of applying 10 μg/ml nigericin.

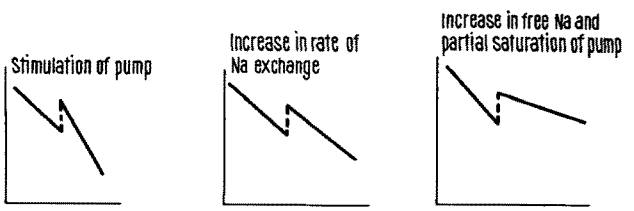

Fig. 1. Stylized behaviour of Na efflux following its stimulation by various means (semi-log plot).

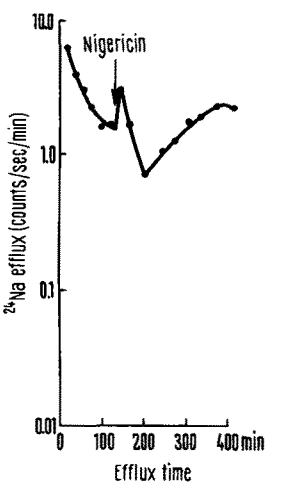

Fig. 3. The biphasic effect on Na efflux of 10 μg/ml nigericin.

[1] E. E. BITTAR, Life Sci. *9*, 773 (1970).
[2] D. A. T. DICK and E. J. A. LEA, J. Physiol., Lond. *174*, 55 (1964).
[3] E. E. BITTAR, D. A. T. DICK and D. J. FRY, J. Physiol., Lond. *196*, 693 (1968).
[4] D. A. T. DICK and E. J. A. LEA, J. Physiol., Lond. *191*, 289 (1967).
[5] E. E. BITTAR, Life Sci. *9*, 1049 (1970).

illustrated by Figure 4, the delayed step-up change in Na efflux is followed by a reduced efflux rate constant (4 out of 10 experiments involving oocytes from another ovary).

Additional experiments revealed that nigericin can sometimes inhibit the Na efflux. 5 oocytes which were isolated from an ovary responded to 10 µg/ml nigericin at pH 7 by exhibiting in each instance a delayed but small step-down in the rate of Na efflux, followed by an unchanged efflux rate constant. This form of behaviour is not unexpected of a germ cell in view of the observation by HARRIS and PRESSMAN[6] that nigericin reduces the Na content of canine red cells and raises that of human red cells.

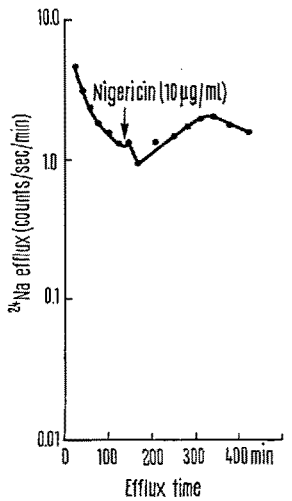

Fig. 4. Delayed stimulation of Na efflux by 10 µg/ml nigericin. The kinetics clearly show that the pump has become partially saturated.

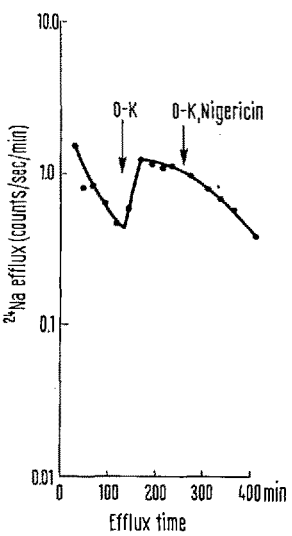

Fig. 5. The lack of effect of 10 µg/ml nigericin on Na efflux into a K-free medium. K removal is shown to stimulate the efflux.

Evidence that the action of nigericin is K-dependent has been obtained by examining the Na efflux into a K-free medium before and after adding nigericin (5 experiments). Shown in Figure 5 is that the removal of K ions from the bathing medium stimulates Na efflux. Also shown is that nigericin is without effect on Na efflux in a K-free medium. This is interpreted to mean 1. that increased outward movement of sodium caused by nigericin in normal Ringer is partly or wholly balanced by an inward movement of potassium, and 2. that the retained fraction of sodium which supposedly nigericin liberates, is mobilized by K removal.

The significance of the biphasic effect of nigericin on Na efflux is clear both from the time-course and the kinetics. Nigericin, it would seem, stimulates the extrusion of sodium in 2 different ways: firstly, by increasing the permeability of the membrane to Na, and secondly, by mobilizing the sodium lying behind the inner membranes. That the sequestered fraction of sodium is not situated in the nucleus is indicated by the studies of FRY[7] who measured the Na content of nuclei isolated from oocytes of *Bufo bufo*, and of DICK, FRY, JOHN and ROGERS[8] who applied an autoradiographic technique and found that the bulk of the sodium in the oocyte is extranuclear. Whether or not the mitochondria of the oocyte are laden with sodium is unknown, but the possibility that they are laden seems very likely not only in the light of the present results but also of the report by GRAVEN, ESTRADA-O, and LARDY[9] that nigericin causes the release of sodium from mitochondria previously treated with gramicidin[10].

Zusammenfassung. Experimente an einzelnen Oozyten von *Bufo bufo* zeigen, dass Nigericin den Natriumefflux anregt, indem gebundenes Natrium freigesetzt wird.

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Department of Investigative Medicine, University of Cambridge, Cambridge (England), 16 November 1970.

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[10] Acknowledgment. This work was supported by the Wellcome Trust of London.
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Role of the Chorion as a Barrier to Oxygen in the Diapause of the Silkworm, *Bombyx mori* L.

It has been shown that embryonic diapause of *Bombyx mori* is brought to an end when the egg is removed out of its chorion and explanted in a hanging drop of physiological saline solution[1].

The present author found that the diapause of *Bombyx* egg terminated also when the diapausing egg was removed out of its chorion and explanted at 25 °C in liquid paraffin as shown in Table I. In liquid paraffin it was improbable that water entered into the egg from outside. Thus, the *Bombyx* egg, differing from some orthopteran eggs[2–5], did not seem to need water-uptake for the termination of diapause and for embryogenesis. Nor was it probable