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.

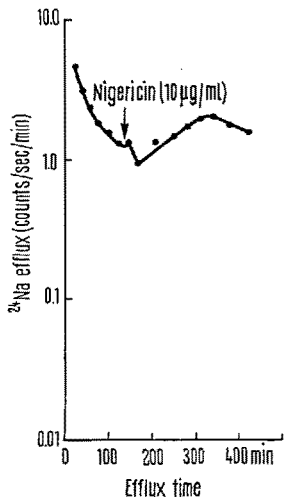

Fig. 4. Delayed stimulation of Na efflux by 10 µg/ml nigericin. The kinetics clearly show that the pump has become partially saturated.

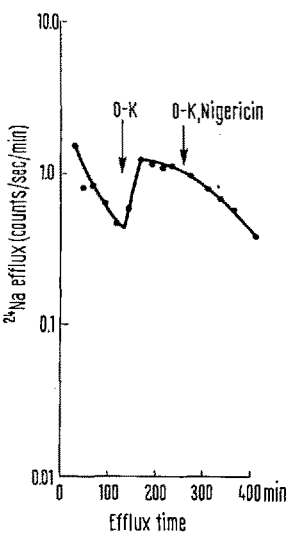

Fig. 5. The lack of effect of 10 µg/ml nigericin on Na efflux into a K-free medium. K removal is shown to stimulate the efflux.

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.

[6] E. J. HARRIS and B. C. PRESSMAN, Nature, Lond. *216*, 918 (1967).
[7] D. J. FRY, D. Phil. Thesis, University of Oxford (1968).
[8] D. A. T. DICK, D. J. FRY, P. N. JOHN and A. W. ROGERS, J. Physiol., Lond. *210*, 305 (1970).
[9] S. GRAVEN, S. ESTRADA-O and H. LARDY, Proc. natn. Acad. Sci., USA *56*, 654 (1966).
[10] Acknowledgment. This work was supported by the Wellcome Trust of London.
[11] Present address: Department of Physiology, University of Wisconsin, Madison (Wisconsin 53706, USA).

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

that injury, which was unavoidable at the operation of removing the chorion, stimulated the egg to terminate the diapause. 60 diapausing eggs were injured in liquid paraffin with a fine needle on the lateral side of the eggs from anterior through posterior pole. After 2 weeks, 20 eggs among them were picked out at random (group 1) and were checked for their development. The remaining eggs were divided into 2 groups. Eggs of group 2 were kept as they had been, while eggs of group 3 were removed out of chorion. The eggs of both groups were reared in liquid paraffin for further 9 days, and were checked for their development. The results are shown in Table II.

Another possibility was that a recovery of exogenous supply of oxygen at removal of the chorion would evoke the egg to terminate the diapause. Liquid paraffin used for these experiments was ascertained to contain 16.6 mg of oxygen per l.

As is evident from Figure 1, when artificial non-diapause eggs were kept in stepwise oxygen-deficient atmosphere, their glycogen content decreased almost at the same rate as that of the diapause eggs[6,7].

Embryos from the artificial non-diapause eggs which had been kept in stepwise oxygen-deficient atmosphere were stained in acridine orange solution as previously described[8]. It was found that the longer the eggs were kept in oxygen-deficient air, the less was the affinity of lysosomes to acridine orange. Finally, the affinity

Table I. Effect of explantation in liquid paraffin on termination of diapause

	No. of eggs explanted[a]	Developed	Not developed	Undiagnosed dead
Diapausing eggs (30 days after oviposition)	40	37[b]	0	3
Post-diapause eggs (chilled for 3 months at 5 °C)	10	9[b]	0	1

[a] Vitelline membrane and serosal cuticle were not removed in this experiment. [b] Larvae were completely formed after 12 days of explantation at 25 °C.

[1] T. Takami, H. Sugiyama, T. Kitazawa and T. Kanda, Jap. J. appl. Ent. Zool. *10*, 197 (1966).
[2] E. H. Slifer, J. exp. Zool. *102*, 333 (1946).
[3] J. J. Matthée, Bull. Univ. S. Afric Dept. agric. Sci. *316*, 83 (1951) (cited from A. D. Lees, 1955).
[4] D. H. Bucklin, *Physiology of Insect Development* (F. L. Campbell, Chicago 1959), p. 94.
[5] T. O. Browning, J. exp. Biol. *43*, 433 (1965).
[6] H. Chino, Nature, Lond. *180*, 606 (1957).
[7] H. Chino, J. Insect Physiol. *2*, 1 (1958).
[8] M. Okada, Zool. Mag. *79*, 204 (1970).

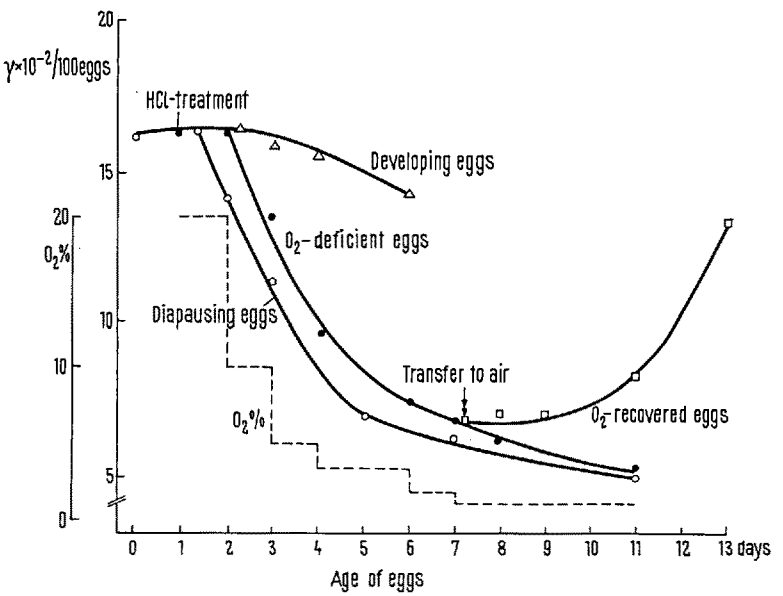

Fig. 1. Glycogen content of artificial non-diapause eggs, being reared either in natural air (△—△) or in stepwise oxygen-deficient atmosphere (●—●), and diapausing eggs (○—○). Diapause eggs of 21 h of age (diapause eggs at this stage have not entered diapause yet) were soaked for 5 min in HCl ($d^{15/4}$ = 1.075) warmed at 46 °C. Those HCl-treated diapause eggs are regarded as artificial non-diapause eggs, since they exhibit no difference as to their development from natural non-diapause eggs. The artificial non-diapause eggs were divided into 2 groups: one was kept in a usual incubator containing natural air; while another was kept in a container in which oxygen was replaced stepwise by nitrogen to decrease its oxygen content stepwise as shown in the figure by a broken line. Some eggs were transferred to natural air after they had been in oxygen-deficient atmosphere for 5 days. The time of transference is shown in the figure by a double arrow. Increase in glycogen content of those eggs (□—□) occurred after a delay of 2 or 3 days. Glycogen content was determined using a method of Kemp et al.[20].

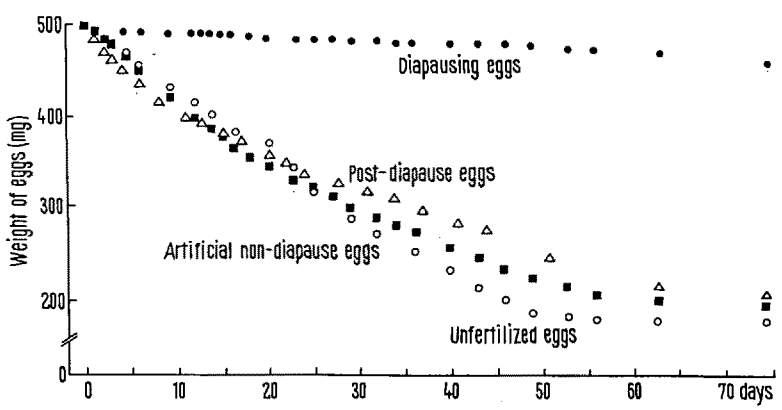

Fig. 2. Decrease in weight of eggs being kept in a desiccator. 500 mg of eggs were weighed out from diapausing eggs, post-diapause eggs that were free of diapause after being kept for 3 months at 5 °C, unfertilized eggs, and artificial non-diapause eggs, respectively. They were kept in a desiccator with silica gel, and weighed at appropriate intervals. To avoid metabolic effect, the eggs were killed by repeating freezing and thawing, preceding the experiment. Diapausing eggs (●) showed almost no decrease in the weight while unfertilized eggs (○), artificial non-diapause eggs (■) and post-diapause eggs (△) exhibited remarkable decrease in weight in the desiccator.

Table II. Effect of injury on the termination of diapause

	No. of eggs	Developed	Not developed	Undiagnosed dead
Group 1[a]	20	0	20	0
Group 2[a]	20	0	15	5
Group 3[a]	20	15[b]	0	5

[a] See text for explanation. [b] Larvae were almost completely formed

was completely lost within 5 days. Loss of the affinity of lysosomes to acridine orange has been shown to characterize the diapausing embryonic cells[8].

These 2 pieces of evidence would seem to show that the anoxiated artificial non-diapause eggs are not different from diapausing eggs, at least for the features investigated. Thus it may be credible to assume that embryos might be anoxiated also in the naturally diapausing eggs, and they might terminate the diapause whenever oxygen supply is recovered. If those conditions actually exist, permeability of the egg membranes to air is expected to be different in diapausing eggs and in non-diapause eggs or developing eggs.

When the eggs were kept in a desiccator, the rate of decrease in their weight was much higher in artificial non-diapause eggs, post-diapause eggs, and unfertilized eggs, than in diapausing eggs (Figure 2). The decrease in weight of the eggs in the desiccator is probably due to a loss of water by evaporation through the egg membranes. Therefore, the difference in the decrease in weight of the eggs in the desiccator may be ascribed to a difference in permeability of the egg membranes to water vapour. This difference may reasonably be extended to the difference in permeability of the membranes to air. Among 3 egg membranes, i.e., the serosal cuticle, the vitelline membrane, and the chorion, only the chorion seemed to be responsible for the air-tightness in the diapausing egg, because removal of the chorion from diapausing eggs was enough for the embryos to terminate the diapause (Table I).

The process of diapause in *Bombyx* eggs thus could be depicted as follows: The chorion may be permeable to air at the oviposition, thenceforth it may gradually become impermeable to air, which would force the embryo to be situated in an oxygen-deficient environment. The anoxiated embryo would gradually adapt itself to the low oxygenic environment. The presence of adaptation of the embryo to oxygen-deficiency could be inferred from the presence of a latent period for resuming development after transferring the eggs to air, as shown in Figure 1. Initial permeability of the chorion to air recovers after the diapausing egg has been chilled for more than 3 months, and renewed supply of oxygen to the embryo should terminate the diapause.

There have been elaborate experiments with orthopteran eggs on water absorption and its correlation with the changes of the egg membranes[9–18]. Also in *Bombyx* eggs, a preliminary electron microscopic observation has suggested that a structural change of the chorion can be correlated with development and diapause of the embryo. Further, in *Bombyx* eggs, the conversion of glycogen into sorbitol and glycerol in the diapausing egg can well be interpreted if one assumes a mechanism interferring with the oxidation of $NADH_2$ and $NADPH_2$ in the egg[19]. The change in permeability of the chorion could be involved in the mechanism suggested[21].

Zusammenfassung. Dechorionierte Diapause-Eier von *Bombyx mori* L. entwickeln sich in Paraffinöl. Wasseraufnahme kann also für das Brechen der Diapause keine Rolle spielen. Die Diapause könnte durch mangelnde Sauerstoffdurchlässigkeit des Chorions zustande kommen.

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[9] J. E. MCFARLANE, Can. J. Zool. *38*, 231 (1960).
[10] J. E. MCFARLANE, Can. J. Zool. *38*, 1037 (1960).
[11] J. E. MCFARLANE, Can. J. Zool. *39*, 1 (1961).
[12] J. E. MCFARLANE, Can. J. Zool. *40*, 13 (1962).
[13] J. E. MCFARLANE, Can. J. Zool. *41*, 23 (1963).
[14] J. E. MCFARLANE, J. Insect Physiol. *12*, 1567 (1966).
[15] P. J. S. FURNEAUX and J. E. MCFARLANE, J. Insect Physiol. *11*, 591 (1965).
[16] P. J. S. FURNEAUX, C. R. JAMES and S. A. POTTER, J. Cell Sci. *5*, 227 (1969).
[17] T. O. BROWNING and W. W. FORREST, J. exp. Biol. *37*, 213 (1960).
[18] T. O. BROWNING, J. exp. Biol. *51*, 397 (1969).
[19] H. CHINO, J. Insect Physiol. *5*, 1 (1960).
[20] A. KEMP and A. J. M. K. VAN HEIJNINGEN, Biochem. J. *56*, 646 (1954).
[21] Acknowledgment. The author wishes to thank Prof. YUJIRO HAYASHI of Tokyo Kyoiku University for his encouragement and for reading the manuscript.

Multiple Neutral Amino Acid Transport Systems in Chicken Small Intestine: Evidence for a Separate Proline Transfer Agency

We had previously shown that absorption of methionine from the chicken small intestine was inhibitable in the order of increasing amino acid sidechain length, and that if one instead used glycine as substrate, that alanine was its best inhibitor, followed by the long-chain aliphatic amino acids[1,2]. Similarly, in the rat intestine, methionine transfer also was found to be progressively inhibited with increasing chain length, while sarcosine absorption was inhibited less by long-chain compounds, and more by glycine and alanine[3]. Observations such as these have provided the basis for proposing the existence of two separate mechanisms for neutral amino acid transfer in rat intestine, a methionine system stereospecific for

[1] J. LERNER and M. W. TAYLOR, Biochim. biophys. Acta *135*, 991 (1967).
[2] K. M. NELSON and J. LERNER, Biochim. biophys. Acta *203*, 434 (1970).