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.

M. OKADA

Institute of Zoology, Tokyo Kyoiku University, Otsuka 3–29, Bunkyo-ku, Tokyo (Japan), 7 December 1970.

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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

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Percent inhibition data for amino acid uptake

Inhibitor (20 mM)	Inhibition of uptake (% ± S.E.) Substrate (0.8 mM)				
	Alanine	Proline	β-Alanine	Sarcosine	Taurine
Isoleucine	80.4 ± 0.7		20.6 ± 6.7[b]	36.6 ± 2.1	15.1 ± 4.7
Leucine	79.3 ± 1.2	46.3 ± 1.6	17.2 ± 5.3	39.8 ± 2.7	
Methionine	78.8 ± 1.6[a]	43.9 ± 7.0	13.0 ± 3.0		18.2 ± 2.2[c]
Alanine	68.7 ± 1.6[a]	50.6 ± 3.4	34.9 ± 2.7	30.8 ± 6.5	20.3 ± 2.6
Serine	53.3 ± 1.8	21.6 ± 7.1	8.7 ± 2.9	20.7 ± 6.0	22.4 ± 3.9
Threonine	50.9 ± 3.0	36.1 ± 6.1	18.7 ± 5.1	27.8 ± 3.2	26.9 ± 2.2
Glycine	38.5 ± 4.1	38.3 ± 5.2	18.3 ± 2.4	32.0 ± 6.9	30.4 ± 3.4
Hydroxyproline	35.0 ± 4.9	32.1 ± 7.0	13.6 ± 3.2	39.8 ± 5.6	24.6 ± 4.8
Proline	27.4 ± 2 7	35.2 ± 1.9	39.5 ± 3.1[b]	42.9 ± 2.7	30.5 ± 3.1
Taurine	22.8 ± 1 8	−9.3 ± 8.7	19.9 ± 4.1	35.3 ± 4.8	34.2 ± 1.9[c]
Sarcosine	6.5 ± 3.7	31.7 ± 4.5	35.3 ± 3.4	38.4 ± 4.4	26.8 ± 2.3
Betaine	1.5 ± 3.2	2.7 ± 7.1	9.3 ± 2.2		25.3 ± 4.1
β-Alanine	−17.3 ± 2.7	29.8 ± 4.7	32.8 ± 2.6	30.1 ± 2.1	25.0 ± 4.9
D-Proline		10.6 ± 7.7	35.0 ± 2.0		

[a] Methionine vs. alanine, $P < 0.01$. [b] Proline vs. isoleucine, $P < 0.05$. [c] Taurine vs. methionine, $P < 0.001$. Experimental conditions are given in the text. Each experiment was repeated on the average of 5 times.

L-isomers and a nonstereospecific sarcosine system[3,4], and in chicken intestine, a methionine system and a glycine system, both having preference for L-enantiomorphs[2]. Likewise a distinct betaine transport agency has been described for hamster intestine that is shared with sarcosine, proline and hydroxyproline[5]. This report provides evidence for further multiplicity of neutral amino acid transport systems in the chicken small intestine.

Experiments were performed with sections of small intestine employing a modified tissue-accumulation procedure[2]. Briefly, 100 mg sections of tissue on either side of the yolk stalk were incubated 5 min at 37 °C in Krebs-Henseleit buffer (5 ml) containing 0.8 mM ^{14}C-labeled amino acid, alone, or in the presence of 20 mM inhibitory amino acid in an atmosphere of 95% O_2: 5% CO_2 (v/v). Tissues were homogenized in 2.5% trichloroacetic acid (w/v) using 5 ml solution/g tissue. The uptake was determined by assaying a 0.2 ml aliquot of the clarified extract by liquid scintillation techniques.

The initial uptakes of the 5 substrates studied followed Michaelis-Menten kinetics, each having clearly defined v_{max} values and apparent Michaelis constants of 3.6, 10, 14, 20, and 40 mM for alanine, proline, sarcosine, taurine and β-alanine, respectively. Inhibitions of initial flux (from 0.8 mM solution) under anoxic conditions (95% N_2: 5% CO_2 atmosphere) were 61, 38, 34, 23, and 13% for alanine, β-alanine, proline, sarcosine and taurine, respectively. Under conditions of Na^+ depletion (incubation in choline-substituted buffer[2]) the rates were inhibited 80, 64, 62, 48 and 34% for alanine, proline, taurine, β-alanine and sarcosine, respectively.

The order of inhibitions caused by various amino acids to alanine influx (see the Table) indicates that alanine is a substrate for the methionine transport system; its affinity is less than the long-chain aliphatic amino acids in this system; hydroxyproline, proline and taurine are weak substrates, and sarcosine and β-alanine fall outside the specificity limits. The use of proline as substrate suggests that it enters the methionine system to a much lesser degree than alanine as evidenced by the circumscribed nature of the inhibitions produced by methionine and leucine. The relative effectiveness of sarcosine and β-alanine as inhibitors of proline versus their inhibitions of alanine transport implies that another system functions in proline transfer in addition to the methionine agency. To this point, the data on β-alanine entry provide evidence for a route of proline uptake that is shared with sarcosine and β-alanine. We are not certain whether alanine finds transport in this system, because no reciprocal inhibition of alanine transport by β-alanine could be detected. The separate proline mediator discriminates against long-chain neutral amino acids, glycine, betaine and aliphatic-hydroxy amino acids, but lacks stereospecificity in its reactivity towards D-proline.

The inhibitions of sarcosine flux provide additional evidence that this amino acid shares transport with β-alanine and proline, although its inhibitions in the presence of long-chain neutral compounds make it a poor choice for use as a substrate to delineate the new proline system (compare the use of sarcosine to delineate systems in the rat[3]).

Lastly, the observation that substrates such as proline, glycine and taurine inhibit taurine influx to a significantly greater degree than the major substrates for the methionine system is an indication also that amino acids enter across the intestinal membrane by transfer systems independent of the latter system[6].

Zusammenfassung. Die Darmresorption von Prolin, Alanin, β-Alanin, Sarkosin und Taurin bei Hühnern wurde in vitro untersucht und ein spezielles Resorptionssystem für Prolin beschrieben.

J. Lerner and P. Burrill

Department of Biochemistry, University of Maine, Orono (Maine 04473, USA), 18 January 1971.

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