Activity of Adenosinetriphosphate Following the Incubation of Liver and Brain Homogenates with $KH_2P_{32}O_4$

Source of Homogenate	Time of Incubation	Optical Density[1] at 260 mμ	Counts per Minute[2]	Cts/min Optical Density Units
	2	0·74	202	273
Liver	5	0·66	260	394
(Embryo)	10	0·75	229	305
	20	0·55	180	337
	2	0·74	122	155
Brain	5	0·72	107	149
(Embryo)	10	0·53	122	230
	20	0·64	92	144
	2	0·50	282	564
Liver	5	0·55	303	551
(Hatched	10	0·50	207	414
Chick)	20	0·23	127	540
	2	0·96	111	116
Brain	5	0·94	108	115
(Hatched	10	0·99	110	111
Chick)	20	0·70	102	146

[1] This measurement represents the absorbance of the leached one-fourth inch strip (see text) from the chromatogram.

[2] Following this measurement of radioactivity on the strip, the solute was leached from the strip in order to measure absorbance.

The data expressed, employing a specific activity function, i.e. counts/min/optical density unit, appear in the table. In all cases the specific activity of the ATP is less in brain than in liver. On the basis of the two groups of experiments shown, the difference between the rate of exchange in brain and in liver appears to be greater in the hatched chick than in the embryo incubated for 17 days. Should subsequent experiments support these findings, it seems that this would be of interest in view of recent differences between the hatched chick[1], and the embryo[2], with respect to carboxylations involving certain acids of the Krebs cycle.

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College of Medicine, Howard University, Washington, January 15, 1955.

Zusammenfassung

Der Austausch von P^{32} zwischen anorganischem Phosphat und ATP war grösser in der Leber als im Gehirn des Hühnchens. Dieser Unterschied in den zwei Geweben ist jedoch nach dem Ausbrüten des Embryos grösser.

[1] L. H. Newmann, K. O. Donaldson, and L. M. Marshall, Amer. J. Physiol. *178*, 97 (1954).

[2] L. M. Marshall, K. O. Donaldson, L. H. Newman, and M. H. Kahn, J. Biol. Chem. *209*, 697 (1954).

Propriétés chitinolytiques du liquide exuvial du ver à soie (*Bombyx mori* L.)

Lorsqu'on ligature des vers à soie au dernier âge larvaire, entre la tête et le thorax, 48 h après la dernière défécation, on obtient des nymphes non dépouillées de leur exuvie larvaire et qui n'ont pu déglutir leur liquide exuvial (*moulting fluid*). Dans quelques cas, ce liquide s'accumule rapidement dans la région thoracique entre le tégument nymphal et l'exuvie larvaire: en incisant cette exuvie, le liquide sous-jacent jaillit spontanément; il est jaune clair, transparent, mais noircit rapidement au contact de l'air (type I). Plus fréquemment, le liquide exuvial s'accumule lentement dans la région thoracique et noircit au fur et à mesure de son émission (type II). Le prélèvement, réalisé de la même manière que dans le cas précédent, mais plus tardivement, est particulièrement délicat: en effet, le tégument nymphal est fragilisé par la macération dans le liquide exuvial et l'on doit éviter avec soin toute manipulation brusque de la nymphe qui pourrait provoquer des déchirures du tégument et un écoulement de sang.

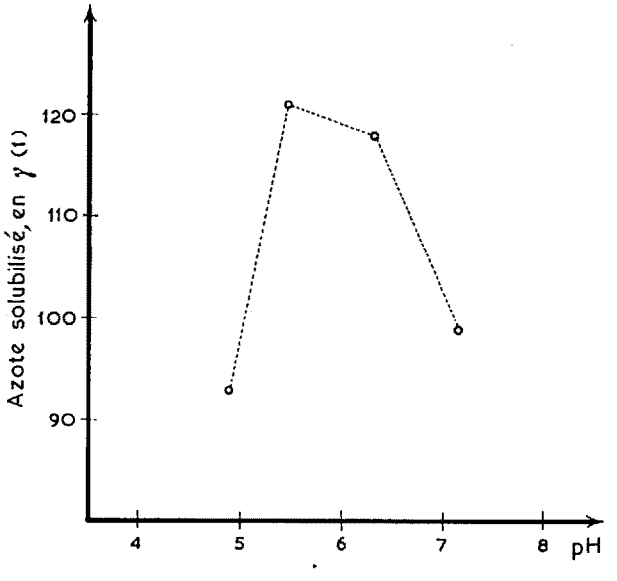

Fig. 1. Activité chitinolytique du liquide exuvial à différents pH. (1): différence entre l'azote-chitine du témoin sans enzyme (139 γ) et l'azote-chitine de chaque test, après 6 h d'incubation.

Conditions expérimentales: 4 ml de liquide exuvial dilué au 1/6; 1 ml de tampon citrate-NaOH 0,6 *M*; 1 ml de suspension de chitine pulvérisée (2,3 mg/ml), à 35°C, en présence de thymol.

Nous avons recherché la présence de chitinase dans le liquide exuvial, par dosage de l'azote-chitine restant après incubation d'une suspension de chitine pure en présence d'une solution de liquide exuvial[1].

Un premier lot de 4 chenilles ligaturées, correspondant au type I décrit ci-dessus, a fourni 4 ml de liquide exuvial. Après dilution au 1/6 par de l'eau bidistillée et centrifugation, on teste l'activité chitinolytique du liquide surnageant. 4 ml de cette solution (soit 0,66 ml de liquide exuvial brut) solubilisent 2,3 mg de chitine, en 14 h d'incubation à 37°C et à pH 6,9. Une station de 2 h à 70°C inactive complètement la chitinase.

Un second lot de 12 chenilles ligaturées, correspondant au type II décrit ci-dessus, permet de recueillir 3,1 ml

[1] Ch. Jeuniaux, Arch. int. Physiol. *59*, 242 (1951).

de liquide exuvial. Après dilution au 1/6 et centrifugation, les propriétés chitinolytiques de cette solution sont testées à différents pH (en tampon citrate-NaOH, conc. finale 0,1 *M*) (Fig.).

La cinétique de l'action chitinolytique du liquide exuvial est semblable à celle des autres chitinases connues (liquide intestinal de l'Escargot[1], exochitinase d'un Actinomycète isolé du sol[2]), qui manifestent également une activité optimale entre les pH 4,8 et 5,4. Par contre, nos résultats diffèrent nettement de ceux obtenus par HAMAMURA et al.[3] avec un extrait aqueux d'exuvies larvaires de *Bombyx mori;* le pH optimum pour la chitinase de cet extrait serait de 8,2, tandis que l'activité serait particulièrement faible au dessous du pH 6.

Nos observations confirment la présence d'une chitinase intervenant au cours du phénomène de la mue, hypothèse formulée notamment par WIGGLESWORTH[4]. La dissolution enzymatique de la chitine de l'endocuticule au moment de la mue avait été montrée par RENAUD[5] pour *Maia squinado* et par PASSONNEAU et WILLIAMS[6] pour *Platysamia cecropia.*

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Laboratoires de Biochimie, Université de Liège, et Laboratoire de Biologie animale, Faculté des Sciences de Bordeaux, le 15 janvier 1955.

Summary

The authors add further confirmation to the hypothesis of the role played by a chitinase during the process of moulting. By ligaturing *Bombyx mori*-larvae between head and thorax, accumulation of moulting fluid takes place between the larval and nymphal cuticles, swallowing of this fluid being prevented. The chitinolytic properties of this moulting fluid are demonstrated *in vitro.* The activity of chitinase at different pH is investigated: the optimum pH lying at about 5·4.

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[2] CH. JEUNIAUX, Arch. int. Physiol. *59*, 242 (1951); Mém. Acad. roy. Belg., Classe sci. *28*, fasc. 7 (1954).

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[4] V. B. WIGGLESWORTH, Quart. J. Micr. Sci. *76*, 269 (1933); Biol. Rev. *23*, 408 (1948).

[5] L. RENAUD, Ann. Instit. Océanogr. *24*, 259 (1949).

[6] J. V. PASSONNEAU et C. M. WILLIAMS, J. Exper. Biol. *30*, 545 (1953).

[7] Aspirant du F.N.R.S.

On the Metabolic Significance of Blood Pyruvate in B_1 Avitaminosis

The researches of PETERS and coworkers[1] on the biochemical significance of Aneurin (cocarboxilase) have achieved a firmly established step forward in our knowledge of the mechanism of cellular oxidation. In consequence of their work it has been generally accepted that the hyperpyruvicaemia which occurs during B_1 avitaminosis is the direct expression of the delayed or interrupted oxidation of pyruvate. This opinion is supported by the *Catatorulin Effect in vitro* which was demonstrated by PETERS and coworkers. This phenomenon, which has been especially studied on the pigeons brain, is obtained only when the vitamin content of the tissue (*nervous*) has reached very low values. An analogous result[1] has recently been observed in the living organism of the rat in B_1 avitaminosis with regard to the metabolic response (*increase* of the R.Q. and heat production) after the administration of 1 g of glucose *per os.* This result is not obtained if the Vitamin B_1 content of the tissues is very low.

Since the vitamin B_1 content in the nervous tissue decreases more slowly than in other tissues[2], reaching values of about 0·5 μg per g only in very advanced stages of avitaminosis, it can be presumed that only in this stage, when there is a catatorulin effect *in vitro* and lack of a metabolic response *in vivo* to the administration of glucose, is the oxidative mechanism of the pyruvate sensibly altered (slowed or interrupted).

On the other hand, the blood pyruvate in the rat begins to increase after only 2 weeks of avitaminosis and the increase continues with increasing rapidity for another 2 weeks period during which the animal responds to the administration of glucose *per os* with an increase in the R.Q. and caloric production[3]. It does not seem probable in this period, in which the animal utilizes glucides in the same manner as the controls, that the alteration of the oxidation of pyruvate is such as to justify the already conspicuous increase in blood pyruvate.

The researches of DEANE and SHAW[4] and of SKELTON[5] have shown that the major part of the changes in the organs that result from B_1 avitaminosis in the rat (hypertrophy of the adrenals, involution of the thymus gland, renal hypertrophy, diminution of the ascorbic acid content of the adrenals, diminished secretion of gonadotropin and increased secretion of corticotropin by the prehypophysis) can be interpreted as a manifestation of the general syndrome of adaptation (SELYE). This syndrome is maintained by a hyperactivation of the hypophysis–adrenal system, as a consequence of fasting and the lack of vitamin B_1 in the diet. We are dealing therefore with aspecific symptoms, which are in common with other avitaminosis (B complex, pantothenic acid). Of all these alterations the most important is without doubt the hypertrophy of the adrenals, which was well demonstrated several years ago by MC CARRISON[6].

Recent researches by RINDI, FERRARI, and PERRI[7] have showed that the accumulation of pyruvate in the blood (rat) can be correlated statistically with the adrenal hypertrophy rather than with the values of the Vitamin B_1 content in the tissues.

To control experimentally whether and to what extent, the hypophysis-adrenal system influences the pyruvicaemia in B_1 avitaminosis, we followed the pyruvate variations in hypophysectomized and adrenalectomized rats. In the first case, 5 days after hypophysectomy

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