phane, on retrouve de 50 à 60 % de la quantité initialement contenue dans la protéine. Quant à l'ammoniac, sa récupération est irrégulière; en trouve des valeurs oscillant entre 1.7 et 2.0, avec une moyenne de 1.8 % de lysozyme.

TABLEAU I

RÉCUPÉRATION DES ACIDES AMINÉS BASIQUES, EN % DU LYSOZYME

Expérience	*Histidine*	*Lysine*	*Arginine*
1	—	—	13.4
2	0.83	5.94	12.8
3	1.54	6.00	13.1
4	1.12	6.16	12.9
Moyenne:	1.16	6.03	13.1
Résidus:	1.1	6.1	11.0

BIBLIOGRAPHIE

[1] H. L. Fevold, *Adv. in Protein Chemistry*, 6 (1951) 188.

[2] S. Moore et W. Stein, *J. Biol. Chem.*, 192 (1951) 663.

[3] A. T. James, A. J. P. Martin et S. S. Randall, *Biochem. J.*, 49 (1951) 293.

[4] J. C. Lewis, N. S. Snell, D. J. Hirschmann et H. Fraenkel-Conrat, *J. Biol. Chem.*, 186 (1950) 23.

Reçu le 6 mars 1952

CO_2-FIXATION IN PIGEON BREAST MUSCLE

by

C. H. MONFOORT

Laboratory for Physiological Chemistry, The University, Utrecht (Netherlands)

Gruber and Meijer[1] found indications of a CO_2-fixation reaction, influenced by the addition of thiamine pyrophosphate (TPP) *in vitro*, occurring under anaerobic conditions in Latapie mince of the muscle of the left ventricle of the pig heart. I succeeded in finding more distinct clues for the occurrence of such a reaction in homogenates of pigeon breast muscle (m. pectoralis major) in 0.1 *M* phosphate buffer, pH 6.2. Working with pigeons offers the great advantage as compared with pig heart that the activity of certain enzymes, capable of metabolizing pyruvic acid under anaerobic conditions, can be studied in several stages of vitamin-B_1 deficiency, so that the role of thiamine pyrophosphate in connection with these reactions can be established more conclusively.

The amounts of acetoin formed from Na pyruvate were compared with the amounts of CO_2 produced simultaneously. In Mn^{++} supplemented homogenates of breast muscle of normally fed pigeons, the amount of CO_2 produced always showed a deficit as compared with the amount calculated according to the reaction:

$$2 \text{ pyruvic acid} \longrightarrow 1 \text{ acetoin} + 2\, CO_2$$

The deficit is increased by adding TPP *in vitro*. This effect is probably due to damage of the tissue by homogenizing. Breast muscle homogenates from pigeons, which had consumed a carbohydrate-rich, thiamine-free diet[2] administered by forced feeding for twelve days, showed no or only a very slight CO_2 deficit, under the same conditions, which was again increased to the values found for homogenates from normal pigeons by addition of TPP *in vitro* (Table I). This CO_2 deficit can in my opinion only be interpreted by assuming a reaction consuming CO_2, *viz.* CO_2-fixation. The presence of Mn^{++} has been shown to be essential in all cases of CO_2-fixation studied by other authors (see Ochoa[3]). Hence my observation, that the described CO_2 deficit is absent when Mn^{++} is omitted or replaced by Mg^{++}, provides further evidence for the presumed TPP-catalyzed CO_2-fixation in pigeon breast muscle homogenate.

In homogenates of the muscle of the left ventricle of the pigeon heart the CO_2 production always exceeded the amount calculated from the acetoin formation, but the surplus decreased upon adding TPP. Hence I believe that the TPP-catalyzed CO_2-fixation also occurs in pigeon heart muscle, though it was obscured in my experiments by other reactions producing CO_2. No evidence was obtained for the occurrence of the reaction in pigeon leg muscle.

Full details and discussions will be published. This work forms part of the investigations by H. G. K. Westenbrink and collaborators on the metabolism and function of thiamine.

TABLE I

ANAEROBIC FORMATION OF CO_2 AND ACETOIN IN PIGEON BREAST MUSCLE HOMOGENATES

200 mg tissue in 2.3 ml 0.1 M K Na phosphate, containing Mn-ions (concentration 0.001 M); 22.7 μM Na pyruvate added. CO_2 and acetoin formation in 3 hrs at 38° C. 8 normal pigeons and 10 twelve days' B_1-deficient pigeons investigated. TPP contents of breast muscle: normal, 7.2 ± 0.33 γ per g; 12 days deficient 3.3 ± 0.08 γ per g. All standard deviations mentioned are standard deviations of the means. P calculated according to Student's method.

	Addition of 6 γ TPP	*Normal pigeons*		*B_1-deficient pigeons*	
		μM	*P*	*μM*	*P*
CO_2 formation	—	6.3 ± 0.15	< 0.001	4.5 ± 0.20	$\ll 0.001$
	+	7.0 ± 0.16		7.4 ± 0.39	
Acetoin formation	—	4.3 ± 0.15	< 0.001	1.9 ± 0.23	$\ll 0.001$
	+	5.2 ± 0.23		5.0 ± 0.16	
CO_2 deficit	—	2.3 ± 0.14	< 0.001	—0.5 ± 0.32	$\ll 0.001$
	+	3.4 ± 0.10		2.6 ± 0.34	

REFERENCES

[1] M. Gruber and F. G. D. Meijer, *Biochim. Biophys. Acta*, 6 (1951) 445.

[2] M. Gruber, *Nature*, 166 (1950) 78.

[3] S. Ochoa, *Physiol. Rev.*, 31 (1951) 56.

Received March 25th, 1952

SUR UN ACIDE AMINÉ DU PHOSPHATIDE DE *MYCOBACTERIUM PHLEI**

par

M. BARBIER et E. LEDERER

Institut de Biologie Physico-Chimique, Paris (France)

Chargaff, Pangborn et Anderson[2] ont décrit en 1931 un phosphatide isolé du Bacille de la phléole (*Mycobacterium phlei*); après l'avoir purifié par plusieurs précipitations de sa solution éthérée par un volume égal d'acétone, ils l'ont obtenu sous forme d'une poudre jaune se ramollissant à 180°, fondant à 190° et contenant 2.80% de phosphore et 0.22% d'azote. La nature de cet azote n'a pas été précisée. Rappelons qu'il n'y a pas de choline, ni de colamine dans les phosphatides des Mycobactéries et que la nature des faibles quantités d'azote qu'ils contiennent est restée fort controversée[3].

Dans la présente note nous rapportons la présence d'***hydroxylysine*** dans le phosphatide de *M. phlei*. Les phosphatides de souches humaines et bovines de *M. tuberculosis* contiennent d'autres acides aminés[4].

Nous avons isolé le phosphatide d'une souche de *M. phlei* cultivée à l'Institut Pasteur, sur milieu de Sauton. Après cinq précipitations par l'acétone, il se présente sous forme d'une poudre jaunâtre, F. 180–190°, contenant 1.1% de P et 0.62% de N (Kjeldahl). Ce phosphatide est insoluble dans

---

* 15ème communication sur les constituants du bacille tuberculeux. 14ème communication[1].