sensitive structures like the dorsal longitudinal ligament[8], the dura and the nerve root could be irritated by the leakage of acid metabolites. It is documented that pain will arise in tissues showing low pH[9–12].

It is thus possible that some cases of lumbar rhizopathy could be due to the demonstrated increase in lactic acid.

Zusammenfassung. An 10 Patienten wurde das während der Operation im Discus gemessene pH mit dem Laktatspiegel des in derselben Zeit entfernten Nucleus pulposus in Korrelation gebracht. Mit absteigenden pH-Werten war der Laktatspiegel höher. Es wird dem erhöhten Laktatspiegel der lumbaren Bandscheiben eine mögliche Rolle in der Pathologie einiger lumbarer Rhizopathien zugeschrieben.

B. DIAMANT, J. KARLSSON and A. NACHEMSON

Department of Pharmacology, Karolinska Institutet, Department of Physiology, Gymnastik och Idrottshögskolan, Stockholm, and Department of Orthopaedic Surgery, University of Göteborg (Sweden), 6 August 1968.

[8] C. HIRSCH, B.-E. INGELMARK and M. MILLER, Acta orthop. scand. *33*, 1 (1963).
[9] H. FRUNDER, Pflügers Arch. ges. Physiol. *251*, 631 (1949).
[10] T. LEWIS, *Pain* (MacMillan, New York 1942).
[11] O. LINDAHL, Acta orthop. scand. *51*, suppl. 179 (1961).
[12] V. MENKIN, *Biochemical Mechanisms in Inflammation* (C. Thomas, Springfield 1956).

Uptake of some Amino Acids by Rat Brain Slices: Effect of Various Substrates

The studies of STERN et al.[1], ABADOM and SCHOLEFIELD[2], and NEAME[3] have established that cerebral slices incubated in a suitable medium are able to concentrate amino acids against a concentration gradient, as do other tissues[4]. Several distinct transport systems have been described for neutral, acid, basic and heterocyclic amino, acids of similar charge and structure[5]. The extent to which rat cerebral slices can actively concentrate glycine has been shown to correlate with concentration of adenosine triphosphate present in the tissue[2].

The present paper examines the effects of glucose and glycolytic and citric acid cycles intermediates on the rate of accumulation of some amino acids. The levels of adenosine triphosphate and phosphocreatine have been measured in the presence of the same substrates; the effects of anoxia and of metabolic inhibitors have also been investigated.

Methods. Cortical brain slices (0.35 mm thick) were prepared from adult Sprague-Dawley rats which were rapidly decapitated without anaesthesia; the slices were incubated in the Krebs-Ringer bicarbonate saline medium previously described[6]. In the anaerobic experiments Krebs-Ringer phosphate buffer was used under 100% N_2 atmosphere with yellow phosphorus in the centre well of the conical Warburg vessels. Substrates were neutralized with *N* NaOH, if necessary, and the corresponding amount of Na^+ was omitted from the saline. After incubation, the slices were picked up with a bent silver wire, drained on glass until no more clear fluid came off, weighed on a torsion balance, and homogenized in 2 ml of 6% cold trichloracetic acid (TCA). They were then dried at 105 °C for 6 h, and the tissue water content calculated. L-tryptophane[7], L-histidine[8], L-arginine[9] and L-proline[10] (Calbiochem, Los Angeles) were analyzed colorimetrically after centrifugation. All results were corrected with blanks of tissues containing no amino acids in the suspending medium. 1-C^{14}-L-glutamic acid, 1-C^{14} glycine, 1-C^{14} γ-amino-butyric acid (Calbiochem) were determined by addition of 0.5 ml deproteinized supernatant to 7 ml of liquid scintillation fluid[11] and counted for 7 min in an Elliot I.D.L. liquid scintillation spectrometer. Internal standards were used to correct for sample quenching. Calculations were based on measurements of total tissue radioactivity, and no correction was made for $^{14}CO_2$ evolution, or the possible presence of other labelled metabolites. As brain slices accumulate water and electrolytes during incubation, results were expressed as mmoles of amino acid/l tissue water, all amino acids being assumed to be evenly distributed throughout the tissue water.

Results and discussion. It has previously been shown that amino acid concentration reaches a constant level after 40 min incubation in Krebs-Ringer bicarbonate glucose saline[12]. In the present study active concentration can be seen to occur in cerebral slices under similar conditions of L-glutamic, L-histidine, glycine and γ-aminobutyric acid (Table I). This accumulation was much diminished in the absence of substrate (Table I). Substrates could be divided into 2 groups by their effect: (1) maximum accumulation of amino acids occurred in the presence of glucose, pyruvate, lactate and oxaloacetate, (2) succinate and fumarate always gave the lowest amino acid levels not significantly higher than in the absence of substrate. The levels of energy-rich phosphate maintained by rat brain slices during incubation with different substrates are shown (Table II), and there appears to be a correlation between the total amount of labile phosphate and the rate of amino acid accumulation. During hypoxia, and in the presence of metabolic inhibitors, there was a marked decrease or lack of accumulation of amino acids (Table I). The uncoupling agent 2-4 DNP also strongly diminished the uptake. All these experimental conditions are known to reduce the levels of phosphocreatine[13]. These results confirm the dependence of this process on cellular stores of energy-rich com-

[1] J. R. STERN, L. V. EGGLESTON, R. HEMS and H. A. KREBS, Biochem. J. *44*, 410 (1949).
[2] P. N. ABADOM and P. G. SCHOLEFIELD, Can. J. Biochem. *40*, 1575 (1962).
[3] K. D. NEAME, J. Neurochem. *11*, 67 (1964).
[4] S. LAHIRI and A. LAJTHA, J. Neurochem. *11*, 77 (1964).
[5] R. BLASBERG and A. LAJTHA, Brain Res. *1*, 86 (1966).
[6] H. MCILWAIN and P. JOANNY, J. Neurochem. *10*, 313 (1963).
[7] C. DUMAZERT and R. GRANGAUD, Soc. Pharmac., Montpellier *1*, 17 (1954).
[8] H. T. MCPHERSON, Biochem. J. *40*, 470 (1946).
[9] C. DUMAZERT and R. POGGI, Bull. Soc. Chim. biol. *21*, 1381 (1939).
[10] F. P. CHINARD, J. biol. Chem. *199*, 91 (1952).
[11] G. A. BRAY, Anal. Biochem. *1*, 279 (1960).
[12] P. JOANNY and E. BARBOSA, J. Physiol., Paris, in press (1968).
[13] H. MCILWAIN, *Biochemistry and the Central Nervous System* (Churchill, London 1959).

Table I. Amino acid accumulation in cerebral slices

Substrate	Amino acids (mmoles/l tissue water) Glycine	γ Aminobutyric acid	Glutamic acid	Arginine	Histidine	Proline	Trypto-phane
None added	3.86 ± 0.21 (6)	3.50 ± 0.18 (6)	7.62 ± 0.58 (6)	1.36 ± 0.25 (6)	6.05 ± 0.64 (6)	1.62 ± 0.09 (6)	2.76 ± 0.20 (6)
Glucose	9.25 ± 0.85 (12)	7.68 ± 0.42 (10)	10.84 ± 0.76 (10)	2.30 ± 0.17 (12)	9.90 ± 0.72 (12)	4.97 ± 0.2 (12)	3.50 ± 0.18 (12)
Pyruvate	8.39 ± 0.82 (8)	8.70 ± 0.75 (8)	10.34 ± 0.68 (10)	1.45 ± 0.2 (10)	6.7 ± 0.56 (8)	2.85 ± 0.21 (8)	3.16 ± 0.24
Lactate	11.46 ± 0.85 (8)	3.80 ± 0.43 (8)	11.8 ± 0.9 (8)	2.95 ± 0.2 (8)	4.8 ± 0.49 (8)	3.23 ± 0.4 (8)	3.40 ± 0.19 (8)
Oxaloacetate	9.78 ± 0.9 (8)	3.72 ± 0.34 (8)	6.69 ± 0.48 (8)	2.43 ± 0.16 (8)	5.6 ± 0.50 (8)	3.18 ± 0.36 (8)	2.96 ± 0.20 (8)
α-Ketoglutarate	9.26 ± 0.74 (8)	3.35 ± 0.42 (8)	7.15 ± 0.5 (8)	2.40 ± 0.18 (8)	6.8 ± 0.36 (8)	3.10 ± 0.18 (7)	2.50 ± 0.24 (8)
Succinate	3.91 ± 0.43 (8)	2.72 ± 0.27 (8)	6.15 ± 0.5 (8)	0.96 ± 0.008 (8)	4.8 ± 0.25 (8)	1.40 ± 0.08 (8)	2.55 ± 0.19 (8)
Fumarate	5.11 ± 0.36 (8)	2.53 ± 0.25 (8)	4.11 ± 0.4 (8)	0.88 ± 0.03 (8)	5.6 ± 0.43 (8)	1.71 ± 0.08 (8)	2.78 ± 0.16 (8)
Inhibitor							
Anoxia	1.13 ± 0.15 (6)	1.20 ± 0.2 (6)	5.20 ± 0.3 (6)	1.00 ± 0.09 (6)	1.76 ± 0.15 (6)	1.22 ± 0.09 (6)	1.32 ± 0.09 (6)
Na azide	6.26 ± 0.60 (6)	5.99 ± 0.24 (6)	8.69 ± 0.36 (6)	1.33 ± 0.18	4.02 ± 0.2	2.33 ± 0.18 (6)	1.97 ± 0.17 (5)
Na cyanide	7.00 ± 0.45 (6)	7.05 ± 0.62 (6)	8.84 ± 0.50 (6)	1.13 ± 0.08 (6)	5.40 ± 0.35 (6)	3.28 ± 0.17 (6)	1.99 ± 0.12 (6)
2,4-Dinitro-phenol	5.28 ± 0.32 (6)	4.48 ± 0.40 (6)	8.51 ± 0.62 (6)	0.66 ± 0.03 (6)	4.36 ± 0.2 (6)	2.10 ± 0.15 (6)	0.54 ± 0.42 (6)

Slices were incubated for 40 min in 5 ml of Krebs-Ringer bicarbonate saline, at 38 °C, pH 7.4. Values are mean and standard deviation for the number of slices indicated in parentheses. All substrates were added to give a final concentration of 20 mM, except glucose, which was 10 mM. Na azide and Na cyanide: 1 mM. 2,4-Dinitrophenol: $2 \times 10^{-4} M$.

Table II. Concentration of adenosine triphosphate (ATP) and phosphocreatine (PCr) in cerebral slices incubated in different substrates

Substrate	ATP	PCr	ATP+ PCr
None added	0.55 ± 0.06 (8)	0.42 ± 0.1 (8)	0.97
Glucose	1.91 ± 0.09 (8)	1.80 ± 0.15 (10)	3.71
Pyruvate	2.16 ± 0.11 (10)	2.2 ± 0.25 (10)	4.36
Lactate	1.58 ± 0.09 (10)	1.68 ± 0.2 (10)	3.26
Oxaloacetate	1.42 ± 0.06 (8)	1.76 ± 0.15 (8)	3.18
α-Ketoglutarate	1.47 ± 0.12 (8)	1.58 ± 0.15 (8)	3.07
Succinate	1.13 ± 0.1 (12)	0.51 ± 0.1 (12)	1.64
Fumarate	1.14 ± 0.1 (12)	0.46 ± 0.1 (12)	1.60

All values are in μmoles/g fresh weight of tissue. Other conditions are as in Table I.

pounds[2,14]. The inhibition caused by 2-4 DNP suggests that accumulation of amino acids depends directly on the supply of adenosine triphosphate, and not directly on respiration.

Woodman and McIlwain[15] have previously studied the effects of higher amino acid concentrations, (5–25 mM) on inorganic phosphate and phosphocreatine in guinea-pig cerebral cortex respiring in the presence of glucose. These authors reported that amino acids except for glutamate, aspartate and alanine generally did not deplete phosphocreatine. When added as the sole substrate, glutamate supported high respiratory rates but markedly reduced the levels of phosphocreatine and the adenosine triphosphate[16]. Another, but unknown, labile phosphate accumulated to an extent of 0.4 μmoles phosphorus/g wet weight[17]. The exceptional case of glutamic acid, where there did not appear to be a correlation between the rate of accumulation of ATP and phosphocreatine levels suggests possible intervention of an unknown labile phosphate in the active transport mechanism[18].

Résumé. En milieu de Krebs-Ringer oxygéné et glucosé, les coupes de cortex cérébral de Rat accumulent les acides aminés contre un gradient de concentration. L'anoxie, l'exclusion du substrat, et les inhibiteurs métaboliques inhibent cette accumulation. Parmi les substrats de la chaîne glycolytique et du cycle de Krebs étudiés, le succinate et le fumarate donnent les niveaux d'accumulation les plus réduits. On observe très généralement une corrélation entre la teneur des composés phosphorylés riches en énergie, et l'accumulation active des acides aminés.

E. Barbosa, P. Joanny and J. Corriol

Laboratoire de Physiologie, Faculté de Médecine, Marseille 5e (France), 5 August 1968.

[14] P. Joanny and H. Hillman, J. Neurochem. *10*, 655 (1963).
[15] R. J. Woodman and H. McIlwain, Biochem. J. *81*, 83 (1961).
[16] H. McIlwain, L. Buchel and J. Cheshire, Biochem. J. *48*, 12 (1951).
[17] H. McIlwain, Biochem. J. *52*, 289 (1952).
[18] These experiments represent part of the thesis to be submitted for a Doctorat d'Etat of P.J. – E. Barbosa was seconded by a grant from the J. March-Foundation, Spain.