Aspartate Aminotransferase and Carbohydrate Metabolism of Rat Brain

In 1960 JENKINS and SIZER[1] reported spectrophotometric evidence for the reversible interconversion between the 'pyridoxal' and 'pyridoxamine' form of aspartate aminotransferase (L-aspartate-2-oxoglutarate aminotransferase; EC 2.6.1.1.). A few years later evidence for the occurrence in vivo of such an interconversion was also produced by exposure of 7-days-old rats to anoxia (BONAVITA et al.[2]). Though no measurement of single ketoacids participating in the Krebs cycle was performed, an increase of ketoacids participating in the transamination reaction was supposed to be responsible for the increase of the 'pyridoxal' form of the brain enzyme in the anoxic rat. The finding of a significant elevation in the brain of dinitrophenylhydrazine reacting compounds was in agreement with the hypothesis (BONAVITA et al.[2]).

The present communication deals with 2 experimental conditions which are known to modify the rate of flux through the Krebs cycle and also the steady level of ketoacids participating in the reactions of the cycle. GOLDBERG et al.[3] have shown that insulin hypoglycemia which decreases the flux through the cycle causes a drop of pyruvate in the mouse brain (−40%) as well as a decline of all ketoacids of the Krebs cycle with the exception of oxaloacetate whose normal level is extremely low (4 μmoles/kg wet brain). Following these considerations, the attempt to measure changes of the ratio between the 'pyridoxal' and 'pyridoxamine' form of brain aspartate aminotransferase during insulin hypoglycemia or alloxan-induced hyperglycemia has appeared of some interest.

Materials and methods. Insulin coma was obtained in male Wistar rats (160–180 g), kept in separate cages and starved for 12 h, by s.c. injection of zinc insulin (400 U/kg body weight). Diabetes was induced in the same rat strain by a single i.p. injection of alloxan monohydrate (150 mg/kg body weight).

Aspartate aminotransferase from the brain of adult rats was extracted and measured (total activity and activity of the 'pyridoxal' form) under the experimental conditions previously reported by AMORE and BONAVITA[4]. Blood sugar was determined according to HAGEDORN and JENSEN[5]. Total nitrogen of the extracts was measured by a submicrospectro-photometric method (BALLENTINE[6]). L-aspartic acid and 2-oxoglutaric acid were products from Fluka (Buchs, Switzerland). Pyridoxal 5-phosphate was a product of the California Corporation for Biochemical Research (Los Angeles, USA). Zinc insulin was purchased from Lilly & Co. (Indianapolis, USA). All the other compounds were reagent grade.

Results. Figure 1 shows the sudden drop of the 'pyridoxal' form of aspartate aminotransferase from rat brain during insulin hypoglycemia. A significant drop is already noticed by the 15th min, when blood sugar has attained the mean level of 62 ± 10 mg/100 ml. Figure 2 shows the opposite changes of the ratio between the 'pyridoxal' and 'pyridoxamine' form of the enzyme during the alloxan-induced hyperglycemia, which undergoes a progressive increase and a subsequent decline after a single injection of alloxan. The maximal value of the 'pyridoxal' form is measured at the 4th day after alloxan injection, when the blood sugar level has also attained its maximum (370 ± 40 mg/100 ml).

Discussion. The data described in the present article deserve a short comment. Patterns of substrate changes in the brain under conditions of increased or decreased metabolic rates have suggested 'the presence of coordinating controlling steps in the citric acid cycle at the point of isocitrate and succinate oxidation as well as at some step between pyruvate and citrate' (GOLDBERG et al.[3]). The present finding of changes in the 'pyridoxal'/'pyridoxamine' ratio of aspartate aminotransferase under conditions of decreased or increased flux through the Krebs cycle indicates that aspartate aminotransferase is most likely another controlling site which is affected by and may affect the substrate levels. During insulin hypoglycemia, the drop of the 'pyridoxal' form of aspartate aminotransferase is the direct consequence of the lack of metabolites in the Krebs cycle.

The second point one should consider is the physiological significance of the reported shift in the equilibrium between the 'pyridoxal' and 'pyridoxamine' form of aspartate aminotransferase in diabetic rats. The finding of

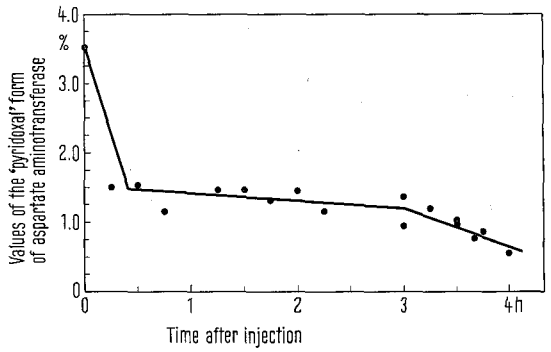

Fig. 1. Percent changes of the 'pyridoxal' form of aspartate aminotransferase from the adult rat brain during insulin coma. Each value represents the mean of at least 3 determinations in triplicate. Specific activity (see AMORE and BONAVITA[4]) did not show any change. For experimental details, see text.

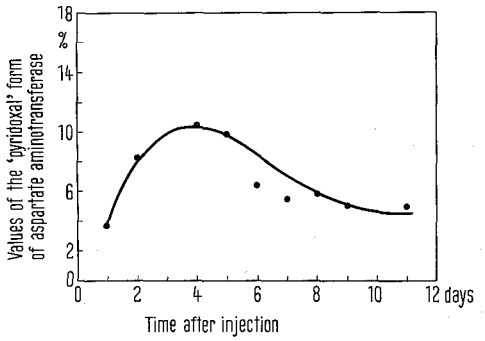

Fig. 2. Percent changes of the 'pyridoxal' form of aspartate aminotransferase from the adult rat brain after alloxan treatment. For other details, see text and Figure 1.

[1] W. T. JENKINS and I. W. SIZER, J. biol. Chem. *235*, 620 (1960).
[2] V. BONAVITA, R. GUARNERI and V. SCARDI, Life Sci. *3*, 889 (1964).
[3] N. D. GOLDBERG, J. V. PASSONNEAU and O. H. LOWRY, in *Control of Energy Metabolism* (Ed. B. CHANCE, R. W. ESTABROOK and J. R. WILLIAMSON; Academic Press, New York 1965), p. 321.
[4] G. AMORE and V. BONAVITA, Life Sci. *4*, 2417 (1965).
[5] H. G. HAGEDORN and B. N. JENSEN, Biochem. Z. *92*, 137 (1923).
[6] R. BALLENTINE, in *Methods in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1957), vol. 3, p. 984.

higher concentrations of the 'pyridoxal' enzyme in proliferating nervous tissues with a predominance of glycolysis versus respiration (AMORE and BONAVITA[1]; PICCOLI et al.[7]) would suggest that at the brain level hyperglycemia stimulates glycolysis more than oxygen consumption[8].

Riassunto. È stato determinato il rapporto tra forma «piridossalica» e forma «piridossaminica» dell'aspartato aminotransferasi nell'encefalo totale di ratti albini durante l'ipoglicemia da insulina e l'iperglicemia da allossana. Il rapporto tra le due forme catalitiche dell'enzima è stato considerato un indice dell'efficienza del ciclo degli acidi tricarbossilici, ed è stata prospettata la possibilità che l'aspartato aminotransferasi abbia una funzione regolativa nei riguardi del ciclo.

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[7] F. PICCOLI, G. AMORE and V. BONAVITA, J. Neurochem., in press.
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Lack of α-Amylase in Horse Serum

Preliminary data were obtained in our laboratory to show that α-glucosidase, rather than α-amylase, accounted for the amylolytic activity of unfractionated horse serum[1]. Further experiments were therefore planned in order to achieve conclusive evidence of the lack of α-amylase in horse serum, since the enzyme has been reported to occur in all mammalian sera[2]; in an attempt to separate α-amylase from α-glucosidase the technique of gel-chromatography was resorted to, since it proved successful with other biological fluids[3–5]. Furthermore, in search for a possible explanation for the lack of the enzyme in horse serum, the gel-chromatographic behaviour of amylases from pancreas and salivary glands was also studied.

Materials and methods. Horse serum and proteins extracted from horse pancreas and salivary glands were dialized and, in turn, fractionated on the same column of Sephadex G-100 (Pharmacia, Uppsala): buffered 1 *M* sodium chloride was the eluting fluid. Amylase and maltase activities were monitored in the eluted fractions. Enzyme assay and characterization, and cellulose acetate electrophoresis of enzymatic preparation, were performed as previously described[5].

Results and discussion. Typical chromatographic patterns are shown in Figure 1. Enzymatically active tubes from serum fractionation were pooled and concentrated. The resulting enzymic preparation behaved as an α-glucosidase in starch hydrolysis[5]. Its electrophoretic mobility approached the mobility of horse serum albumin. pH curves in both maltose and starch hydrolysis are shown in Figure 2.

No α-amylase activity was recorded when the tubes corresponding to the V_e/V_o values of 1.6 and 2.5 from serum and salivary glands fractionations were assayed, after concentration, by an amyloclastic method.

The present results may be summarized as follows: (1) An α-glucosidase occurs in horse serum: pH curves would indicate possible inhomogeneity of the enzyme; its identity with the 'amylase' of horse serum[6] is likely on the basis of its electrophoretic mobility. The occurrence of 'maltase' in horse serum has been reported[7]. (2) α-amylase is lacking from horse serum and salivary glands; the lack of the enzyme from salivary glands has been reported, but there is no general agreement[2]. (3) As far as the V_e/V_o values from gel-chromatography can be extrapolated to molecular size, horse pancreatic amylase has the same low molecular size as some amylases from different mammalian sources[5,8].

These findings pose some questions about the mechanisms which favour the diffusion of α-amylase from pan-

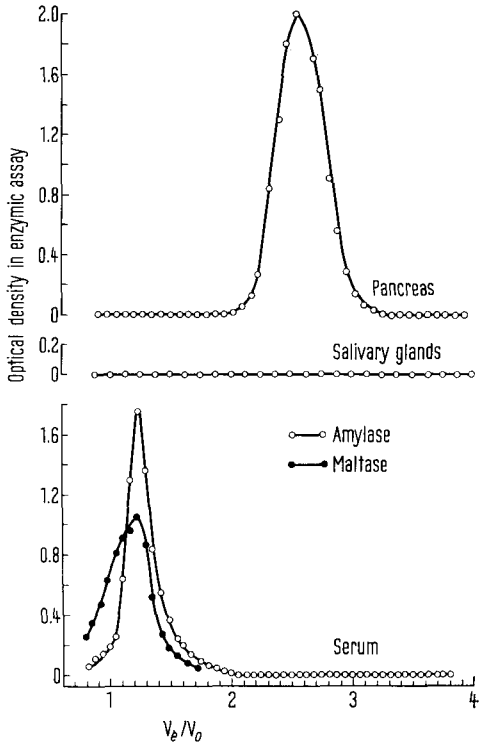

Fig. 1. Elution pattern of maltase and amylase activities from a Sephadex G-100 column, after horse pancreas, salivary glands and serum fractionation. The V_o of the column was the elution volume of blue dextran 2000.

[1] P. A. BONINI and C. FRANZINI, Boll. Soc. ital. Biol. sper. *44*, 1056 (1968).
[2] P. BERNFELD, in *Comparative Biochemistry* (Ed. M. FLORKIN and H. S. MASON; Academic Press, New York and London 1962), vol. 3.
[3] C. FRANZINI and P. A. BONINI, Experientia *23*, 373 (1967).
[4] C. FRANZINI and P. A. BONINI, Clin. chim. Acta *17*, 505 (1967).
[5] C. FRANZINI, P. A. BONINI and M. L. SOLA, Enzymologia *36*, 117 (1969).
[6] R. L. SEARCY, S. HAYASHI, J. E. BERK and H. STERN, Proc. Soc. exp. Biol. Med. *122*, 1291 (1966).
[7] I. LIBERMAN and W. H. ETO, J. biol. Chem. *225*, 899 (1957).
[8] C. FRANZINI, P. A. BONINI and A. ZAPPATA, Clin. chim. Acta *23*, 368 (1969).