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-

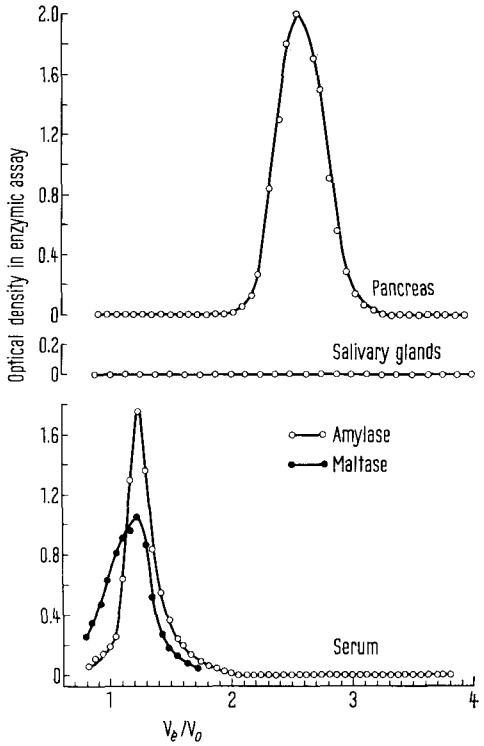

Fig. 1. Elution pattern of maltase and amylase activities from a Sephadex G-100 column, after horse pancreas, salivary glands and serum fractionation. The V_o of the column was the elution volume of blue dextran 2000.

[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).

creas into serum: the tempting speculation that the low molecular weight of the enzyme may play a role in its diffusibility does not agree with present data. The mechanisms of entry of the pancreatic enzyme into blood serum are not clearly understood[9], and may vary from one animal species to another. The contribution of pancreatic to serum amylase has already been challenged, in some animal species, on the basis of different experimental approach[10-12].

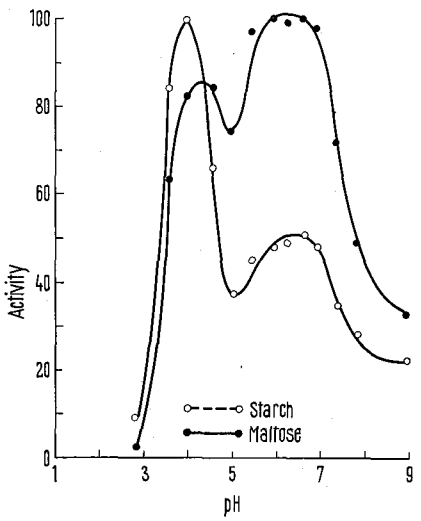

Fig. 2. pH curves, in maltose and starch hydrolysis, of the partially purified enzyme from horse serum.

Riassunto. Mediante gel-cromatografia si è confermata la presenza di α-glucosidasi e l'assenza di α-amilasi nel siero di cavallo. La α-amilasi è anche assente nelle ghiandole salivari ma presente nel pancreas dello stesso animale.

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[9] H. D. JANOWITZ and D. D. DREILING, Am. J. Med. *27*, 924 (1959).
[10] R. L. MCGEACHIN, J. R. GLEASON and M. R. ADAMS, Archs Biochem. Biophys. *75*, 403 (1958).
[11] R. L. MCGEACHIN, J. M. REYNOLDS and J. I. HUDDLESTON JR., Archs Biochem. Biophys. *93*, 387 (1961).
[12] R. L. MCGEACHIN and W. D. JOHNSON JR., Archs Biochem. Biophys. *107*, 534 (1964).
[13] Present address: Ospedale Civile, 27029 Vigevano (Italy).
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A Comparative Study of the Intracellular Ca^{++} Movements in White and Red Muscle

In a recent study on the distribution of in vivo injected $^{45}Ca^{++}$ among the subcellular fractions of the heart, the largest part of the radioactivity was recovered in the mitochondria[1]. The movements of Ca^{++} were more active in mitochondria than in the sarcoplasmic reticulum, a result suggesting a role for mitochondria in the Ca^{++} movements associated with the contraction and relaxation of the heart. Results pointing to the same conclusion have recently been reported by FEHMERS[2,3]. Studies of Ca^{++} transport in the isolated sarcoplasmic reticulum have, on the other hand, led to the view that the movements of Ca^{++} linked to the contraction and relaxation of muscle are under the control of the sarcoplasmic reticulum[4-6]. However, most of these studies have been carried out on white muscles, which contain very few mitochondria and a very well developed sarcoplasmic reticulum. The possibility was thus considered that the intracellular movements of Ca^{++} were under the control of different subcellular organelles in white and red muscles. Sarcoplasmic reticulum would be predominant in the former, and mitochondria in the latter: a similar possibility has been suggested by GERGELY et al.[7] a few years ago.

Rabbit masseter (red) and adductor magnus (white) were used. The rabbits were injected i.v. with 60 μC $^{45}Ca^{++}$ (equal to about 6 μg $CaCl_2$) 15 min before the sacrifice. The masseter and adductor magnus muscles were quickly excised, cut into small pieces, soaked in several changes of 0.4 *M* sucrose, at 0 °C, and squeezed between layers of filter paper to minimize the contamination by highly labelled blood plasma. They were freed from the connective tissue, minced with scissors, and homogenized in 0.1 *M* KCl-0.005 *M* histidine-Cl, pH 7.0 (masseter) or in 0.4 *M* sucrose (adductor magnus): a lucite Potter homogenizer was used for the masseter, and a Waring Blendor for the adductor magnus. Total Ca^{++} and $^{45}Ca^{++}$ were determined on aliquots of the homogenates. The myofibrils, the nuclei, and the cell debris were discarded at 600 g for 10 min, and the mitochondria were separated at 12,000 g for 10 min; the sarcoplasmic reticulum was sedimented at 125,000 g for 45 min, after having discarded an intermediate fraction at 34,000 g for 20 min. The purity of the fractions was checked by determining the total cytochrome oxidase activity and the RNA/protein ratio. The yields were as follows: about 2.8 mg and 0.1 mg of mitochondrial proteins/g of masseter and of adductor magnus, respectively. About 1.1 mg and 1.3 mg of sarcoplasmic reticulum protein/g of masseter and of adductor magnus, respectively. $^{45}Ca^{++}$ was counted on aliquots of the suspensions of the various

[1] P. PATRIARCA and E. CARAFOLI, J. Cell Physiol. *72*, 29 (1968).
[2] M. C. O. FEHMERS, Fedn Europ. biochem. Soc., Oslo, Abstracts *76* (1967).
[3] M. C. O. FEHMERS, Ph. D. Dissertation, Univ. of Amsterdam (1968).
[4] S. EBASHI, Prog. theor. Phys., Suppl. *17*, 35 (1961).
[5] W. HASSELBACH and M. MAKINOSE, Biochem. Z. *333*, 518 (1961).
[6] A. WEBER, R. HERZ and I. REISS, J. gen. Physiol. *46*, 679 (1963).
[7] J. GERGELY, D. PRAGAY, A. F. SCHLOZ, J. C. SEIDEL, F. A. SRETER, M. M. THOMPSON, Molec. Biol. of Muscle Contr., BBA Library *9*, 145 (1965).