formation than in the others, slower rate of removal, or the combination of both. With this in view, the exceptionally high concentration of γ-aminobutyric acid in the hypothalamus, for example, might indicate the presence of greater amount of enzyme and the higher rate of α-decarboxilation of the glutamic acid in this highly functional part of the central nervous system. The lowest concentrations of investigated compounds in the cerebral white matter could be accounted for by the improbability, in view of the electron microscopic evidence, that the axoplasm contains the systems responsible for protein synthesis[1]. However, what the functional significance of the highest glutamine contents in the caudate nucleus and cerebellum may be, whether the second highest concentration of γ-aminobutyric acid in the caudate nucleus could be related to the fact that this nucleus represents an integral part of 'supressor system' in the brain[10], remain questions awaiting an answer from further investigations[11].

Résumé. Les auteurs ont déterminé par la méthode de la chromatographie à deux dimensions, la teneur en glutamine et en acide γ-aminobutyrique des différentes parties du cerveau du chat. La concentration la plus élevée de l'acide γ-aminobutyrique a été trouvé dans l'hypothalamus, tandis que celle de la glutamine a été constatée dans le noyau caudé. Les concentrations les plus basses de ces deux protéines apparaissent dans la substance blanche du cerveau.

Lj. Kržalić, V. Mandić, and Lj. Mihailović

Institute of Pathological Physiology, Medical School, University of Belgrade (Yugoslavia), March 11, 1962.

[10] J. G. Dusser de Barenne and W. S. McCulloch, J. Neurophysiol. *3*, 311 (1941).

[11] This work has been supported by a grant from Yugoslav Foundation for Scientific Research, Contract No. 490/1.

Nucleoside Phosphorylase Activity in Guinea Pig Polymorphonuclear Leukocytes

Nucleoside phosphorylase activity has been the subject of a number of investigations in the red cells of man and other mammals[1]. On the other hand, no data are available concerning leukocytes: the present report deals with some studies performed on guinea pig neutrophilic granulocytes.

The cells were obtained from adult female guinea pigs by the peritoneal sterile exudate technique, using 7.2% sodium caseinate, as described by Hirsch[2]; grossly hemorrhagic samples were discarded. The peritoneal fluid was collected with an anticoagulant (1.2% ammonium sulphate in 0.8% potassium oxalate) and centrifuged for 10 min at 1000 × *g*. The sedimented cells were additioned with 1 vol of 0.2% NaCl; after 2 min 1 vol of 1.6% NaCl was added, in order to insure lysis of the rare red cells with minimum damage to the leukocytes. After centrifuging, the slightly pinkish supernatant was discarded, the cells were re-suspended in about 9 vol of water and homogenized in a Potter-Elvehjem apparatus at 0°C. The soluble fraction was obtained from this whole homogenate by centrifuging at 2°C for 1 h at 30000 × *g*. Differential centrifugation was performed in some experiments in 0.25 *M* sucrose according to Montreuil[3].

Nucleoside phosphorylase activity was estimated, with inosine as substrate, by measuring the rate of uric acid formation in the presence of xanthine oxidase[4]. The latter enzyme was prepared from buttermilk according to Horecker and Heppel[5]: a 20–30-fold purification, corresponding to the ammonium sulphate step, was generally satisfactory[6]. For a semi-quantitative estimation of nucleoside phosphorylase, and for purposes of comparison with activity on guanosine, the determination of the disappearance of NaOH-fast pentose[7] has been found suitable, and in good agreement with the spectrophotometric technique. Proteins were determined by Bücher's method[8].

The results obtained with rising concentrations of inosine are shown in Figure 1. When the enzyme is saturated with respect to both substrates (inosine and inorganic phosphate), the activity exhibited corresponds to the splitting of 2 μmoles nucleoside per mg protein in

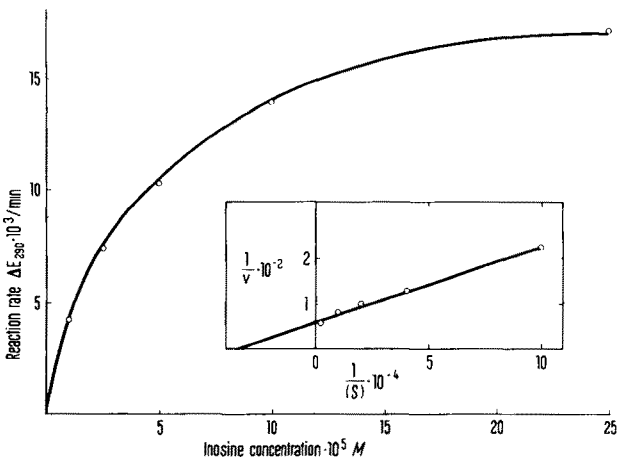

Fig. 1. Inosine phosphorylase activity of guinea pig leukocytes. The reaction mixture was prepared in 1.35 ml 1 cm path spectrophotometric cuvettes and contained 23 μmoles of sodium phosphate buffer, pH 7.6, 30 μl of xanthine oxidase, 10 μl of the leukocyte soluble fraction (corresponding to 0.17 μg of protein), and inosine at the concentrations indicated, in a final volume of 1 ml. Readings were taken every minute at 290 mμ using an Optica CF 4 spectrophotometer. Reaction rate was constant for at least 10 min. In the central part of the Figure, data are plotted according to Lineweaver and Burk[12].

[1] A. A. Sandberg, G. R. Lee, G. E. Cartwright, and M. M. Wintrobe, J. clin. Invest. *34*, 1823 (1955). – K. K. Tsuboi and P. B. Hudson, J. biol. Chem. *224*, 879 (1957). – P. A. Marks, A. B. Johnson, H. Hirschberg, and J. Banks, Ann. N.Y. Acad. Sci. *75*, 95 (1958).

[2] J. G. Hirsch, J. exp. Med. *103*, 589 (1956).

[3] J. Montreuil, Cancérologie *2*, 17 (1955).

[4] V. E. Price, M. Otey, and P. Presner, in *Methods in Enzymology* (S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York 1955), vol. 2, p. 448.

[5] B. L. Horecker and L. A. Heppel, in *Methods in Enzymology* (S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York 1955), vol. 2, p. 482.

[6] We wish to thank Dr. G. Mangiarotti for his kind help in the preparation of this enzyme.

[7] A. Bonsignore, M. Orunesu, C. Ricci, and C. Vergnano, G. Biochim. *2*, 160 (1953).

[8] T. Bücher, Biochim. biophys. Acta *1*, 292 (1947).

10 min. With guanosine the rate is about one tenth of the rate with inosine.

Among the fractions obtained by differential centrifugation, the nuclei and mitochondria were devoid of nucleoside phosphorylase activity. The bulk of the enzyme was found in the soluble fraction, whereas the microsomes had an activity amounting to a few percents of the supernatant (on protein basis), activity which might well result from slight contamination. The intracellular distribution is thus in agreement with that found in other tissues[9].

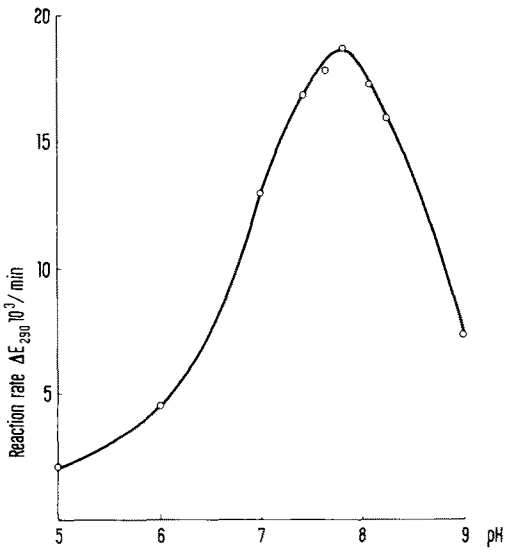

Fig. 2. Effect of pH on leukocyte nucleoside phosphorylase activity. Assay as in Figure 1. The pH 5 reaction mixture was buffered with citric acid maintaining the usual phosphate concentration. An effect of the citrate anion was ruled out by testing at neutral pH.

The pH-dependance is shown in Figure 2. Maximum activity is found at 7.8, which is close to findings on the beef liver enzyme[10]. Also the Michaelis-Menten constant for inosine ($3 \cdot 10^{-5} M$) is of the same order of magnitude with the liver[10] and the leukocyte enzyme.

If the nucleoside phosphorylase activity of white and red blood cells is compared, one finds (on recalculating the data by TSUBOI and HUDSON[11] on human erythrocytes) that the former have a 4-fold activity on a protein basis and an 8–10-fold activity on a cell basis.

Riassunto. Gli autori dimostrano la presenza di nucleoside fosforilasi nei granulociti neutrofili di essudato peritoneale sterile di cavia. L'attività enzimatica è distribuita quasi totalmente nella frazione solubile, presenta attività 10 volte più elevata sull'inosina che sulla guanosina, ha pH ottimale 7,8 e costante di Michaelis-Menten per l'inosina uguale a $3 \cdot 10^{-5} M$.

P. CALISSANO, G. LEONCINI, and L. LUZZATTO

Centro di Biochimica dei Tumori «Cassa di Risparmio di Genova» presso l'Istituto di Chimica Biologica dell'Università di Genova (Italy), March 15, 1962.

[9] M. DIXON and E. C. WEBB, *Enzymes* (Longmans, London 1958).
[10] J. W. ROWEN and A. KORNBERG, J. biol. Chem. *193*, 497 (1951).
[11] K. K. TSUBOI and P. B. HUDSON, J. biol. Chem. *224*, 889 (1957).
[12] H. LINEWEAVER and D. BURK, J. Amer. chem. Soc. *56*, 658 (1934).

Maltase Activity in Human Semen

It has been demonstrated that mammalian spermatozoa are capable of utilising glucose, fructose, mannose, maltose and glycogen for the maintainance of sperm motility and metabolism (KOELLIKER[1] and MACLEOD[2]). Prior to their utilisation, it is necessary that disaccharides should be converted to monosaccharides. The enzymes responsible for such conversions have not been studied in detail so far. KARASSIK[3] has reported the presence of amylase in dog and human semen. The observation of LANE-ROBERTS et al.[4] that human semen contains an unidentified substance which on addition of amylase gets converted to glucose indicates that glycogen may also be present. It seemed likely that, as in the case of salivary amylase, seminal amylase could then bring about the conversion of seminal glycogen to maltose. It was therefore thought possible that degradation of maltose to glucose would be brought about by an enzyme similar to intestinal maltase. With these observations in view, studies were undertaken to detect and estimate the maltase activity of human and other animal semen.

Semen samples were obtained from fertile donors. Buffalo semen was obtained from the Maharastra Government milk colony. Rabbit semen was collected by means of an artificial vagina and cock semen by abdominal massage. Maltase activity was estimated in terms of glucose liberated. The glucose was estimated by the paper chromatographic method of SHETH and RAO[5]. The spray reagents used for the detection of sugars were the same as described earlier (SHETH and RAO[6]). Protein concentration was determined by the method of LOWRY et al.[7]. All enzyme studies were carried out using veronal acetate phosphate universal buffer.

The maltase activity of semen was estimated as follows. To 0.5 ml of semen were added equal volumes of veronal buffer pH 5.5 and $0.14 M$ maltose. Appropriate control mixtures were also prepared, one without the seminal plasma and the other without the substrate. The mixtures were incubated at 37°C for 24 h. In the reaction as well as the control mixtures, 2 to 3 drops of toluene were added before incubation. After incubation, the sugars present in the mixtures were identified and estimated according to the method of SHETH and RAO[5,6].

The results indicated that two bands developed from the point spotted with the digestion mixture. One of these bands was due to maltase and the second band was identified to be that of glucose. The formation of glucose from maltose indicated that maltase was present in dia-

[1] A. KOELLIKER, Z. wiss. Zool. *7*, 201 (1856) as cited by T. MANN, *Biochemistry of Semen* (Methuen Co., London 1954), p. 55.
[2] J. MACLEOD, Endocrinology *29*, 583 (1941).
[3] W. M. KARASSIK, Z. Ges. exp. Med. *53*, 734 (1927).
[4] C. LANE-ROBERTS, A. SHERMAN, K. WALKER, B. WEISNER, and M. BARTON, *Sterility and Impaired Fertility* (Hamish, Hamilton, London 1948), p. 44.
[5] A. R. SHETH and S. S. RAO, Exper. *15*, 314 (1959).
[6] A. R. SHETH and S. S. RAO, Ind. J. Med. Sci. *15*, 24 (1961).
[7] O. H. LOWRY, N. J. ROSBROUGH, A. L. FARR, and R. J. RANDAL, J. biol. Chem. *193*, 265 (1951).