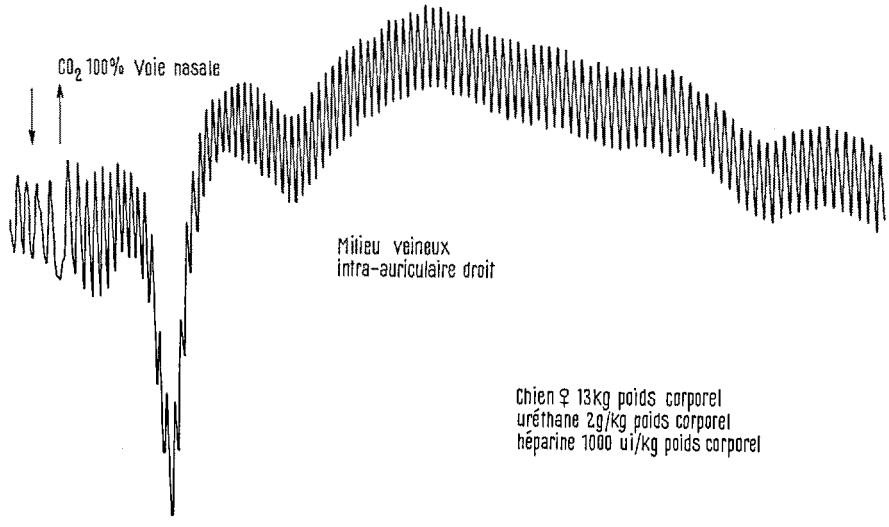

Fig. 5

nique[1,4,5] peut désormais être suivie simultanément en milieux artériel et veineux au niveau de l'hémoglobine même (Figure 4).

En conclusion, une connaissance en continu, in situ, quantitative, instantanée et directe de l'état d'oxygénation de l'hémoglobine érythrocytaire (sans hémolyse) en milieux vasculaires profonds artériel et veineux simultanément est ainsi désormais rendue possible. Cette oxymétrie dans des conditions qualitatives et quantitatives variables d'inhalation gazeuse ne constitue d'ailleurs qu'un aspect de la photométrie intra-vasculaire directe à l'aide de ces sondes-photomètres; la technique entre en effet dans le cadre d'une méthodologie très générale qui sera développée systématiquement dans le proche avenir et qui permet notamment la mesure en continu de la masse sanguine par la technique de dilution d'indicateurs colorés.

Summary. An original instrument, the probe-photometer, permits us to evaluate instantaneously, continuously, in situ and simultaneously into arterial and venous vessels the relative amount of Hb-Hb O_2. The CO_2 reflex described previously can also be fully recorded in terms of hemoglobin.

B. RYBAK

Université de Caen, Zoophysiologie, Faculté des Sciences, Caen (France), 10 juillet 1967.

[5] B. RYBAK, Abstr. XXIII Int. physiol. Congr., Tokyo 240 (1965).

The Microdetermination of L-Glutamic Acid Decarboxylase with Use of ^{14}C-Labelled Substrate

The use of ^{14}C-labelled glutamate evidently facilitates the determination of the activity of L-glutamate decarboxylase (GAD) (EC 4.1.1.15.) and therefore the methods based on this principle are frequently employed. Unfortunately, the method of KONDO[1] is unreliable at low enzyme activities and that of SISKEN and others[2] requires rather complicated reaction vessels, in addition to a relatively large amount of enzyme. For these reasons, an attempt has been made to work out a simple method which would meet the requirements of serial work on ultramicro scale.

Thus, in the method presented here, the activity of GAD is estimated by measuring the radioactivity of $^{14}CO_2$ which has been formed from labelled glutamate due to enzyme action. The radioactivity itself is determined with use of the principle suggested by DAVIES and COCKING[3].

Method. The device for the determination of GAD consists of (1) glass cylinder, (2) polyethylene reaction vessel; it is supported by an approximately 3 mm long bar. The adjacent wall is pierced with a hole. In the inside of the vessel, opposite to the hole, there is a small diaphragm. Other parts are (3) polyethylene stopper with a hole in its bottom, (4) air-tight lid of the stopper also made from polyethylene and (5) glass fibre paper disc. For other details and dimensions, see Figure 1.

The determination is carried out in the following manner: 0.5 ml of 2*N* sulphuric acid are pipetted into the glass cylinder and the reaction vessel is put inside (see also Figure 1). After this, the vials are preincubated for approximately 20 min at required temperature (usually 37 °C). A glass fibre paper disc is placed on the bottom of the stopper and fixed with the lid; 0.1 ml of 0.1*N* sodium hydroxide are then pipetted onto the disc. The reaction mixture is now placed on the polyethylene reaction vessel

[1] O. KONDO, J. Okayama med. Soc. *74*, 623 (1962), after Chem. Abstr. *60*, 2183 g (1964).

[2] B. SISKEN, K. SANO and E. ROBERTS, J. biol. Chem. *236*, 503 (1961).

[3] J. W. DAVIES and E. C. COCKING, Biochim. biophys. Acta *115*, 511 (1966).

in such a manner that the substrate is initially separated from other constituents by the diaphragm. The total volume is 60 μl and it contains *Tris*-hydroxymethyl-aminomethane (TRIS) phosphate buffer, pH 7.4, 0.033 molar 1-^{14}C-L-glutamate (specific activity 80 μCi/m*M*), 0.000167 molar pyridoxal-5-phosphate and the enzyme.

Finally, the vials are stoppered and the reaction is initiated by mixing the substrate with other ingredients of incubation medium. This is effected by gentle shaking of the vials.

At the end of the incubation period, the reaction is terminated by introducing the reaction mixture into the sulphuric acid through the hole in the wall of the reaction vessel. In order to wash down possible residues of the incubation medium from the polyethylene vessel, the vials are several times vigorously shaken. The $^{14}CO_2$, formed by the enzyme reaction, is liberated by the sulphuric acid and bound to the sodium hydroxide contained in the glass fibre paper disc. In order to achieve quantitative trapping of $^{14}CO_2$, it is necessary to leave the vessels in the shaking incubator for another 60 min at least. The stoppers are then removed and the discs are dried under a stream of hot air.

For the measuring of radioactivity, 0.5 ml of scintillation fluid is pipetted into the counting vial and background count is taken. Dry paper disc is now transferred into the vial and the radioactivity is measured. However, at less strict requirements for the precision and at high enzyme activities, it is reasonable to proceed in a more usual way, i.e. to determine the background for a whole batch of samples. In any case, it is recommendable to start the measuring within 3 h after the stoppers are taken out of the vials, since no measurable isotope exchange with the atmospheric CO_2 occurs during this period. Otherwise it is necessary to protect the samples from the contact with the surrounding atmosphere.

Comments. When a crude homogenate from mouse brains (strain 'H') is used as the enzyme source, optimal

The influence of pyridoxal-5-phosphate and SH-compounds on the activity of L-glutamate decarboxylase

Additions		Relative activity in %[a]
None		56
Pyridoxal-5-phosphate	0.033 m*M*[b]	92
	0.167 m*M*	100
	0.330 m*M*	100
	3.330 m*M*	99
2-Mercaptoethanol	0.330 m*M*	62
1-Cysteine	0.330 m*M*	58
2-Mercaptoethanol plus pyridoxal-5-phosphate	0.330 m*M* 0.167 m*M*	98
1-Cysteine plus pyridoxal-5-phosphate	0.330 m*M* 0.167 m*M*	97

Incubation at 37 °C, 30 min in the standard system (see text). Crude mouse brain homogenate (20 mg prot./ml) in the quantity of 20 μl has been used as the enzyme source. [a] 100%, i.e. the activity in the presence of 0.167 m*M* of pyridoxal-5-phosphate. [b] Final concentrations.

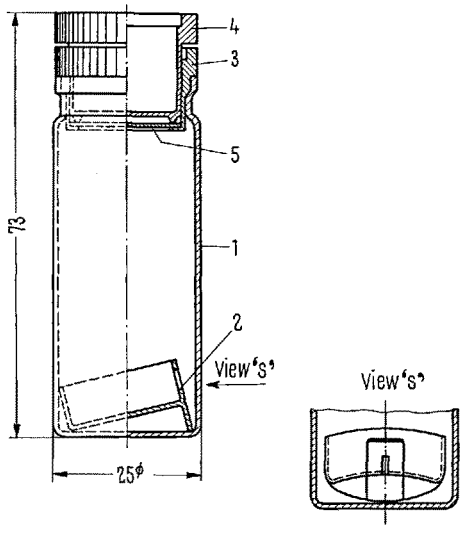

Fig. 1. The device for the determination of GAD. (1) Glass cylinder, (2) polyethylene reaction vessel, (3) stopper, (4) lid of the stopper, (5) glass fibre paper disc.

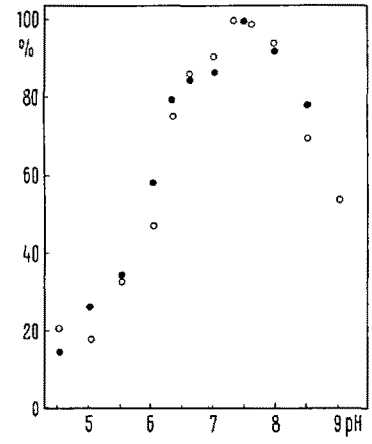

Fig. 2. The optimum of pH. System contained 0.033 *M* L-glutamate, 0.000167 *M* pyridoxal-5-phosphate, 0.02 *M* buffer (*Tris*-phosphate at pH above 7.0 and citrate-phosphate at pH below this limit) and the enzyme (i.e. 20 μl of crude mouse brain homogenate, 20 mg prot./ml). At pH 7.0, the activity was the same with either buffer. Incubation 30 min at 37 °C. ●, experiment 1; o, experiment 2.

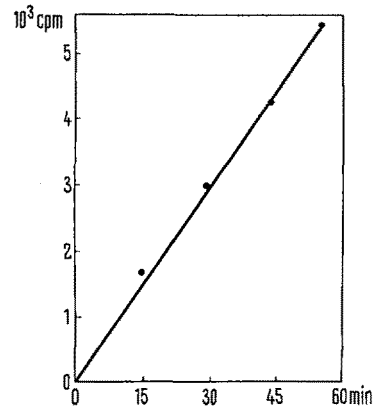

Fig. 3. The decarboxylation of L-glutamate in the relation to time. In this experiment, standard assay conditions were employed. (Se text and the description to the Table).

pH lies between 7.3–7.5 (see Figure 2). SUSZ et al.[4] found similar values for partially purified enzyme.

As may be seen from the Table, the addition of pyridoxal-5-phosphate is necessary for the full activity of the enzyme. Further increase in the coenzyme concentration has no appreciable effect. In contrast to the enzyme contained in the brain acetone powder[5], SH-compounds do not increase the activity of GAD in the crude brain homogenate. In addition, this enzyme preparation does not require anaerobic incubation[2,6].

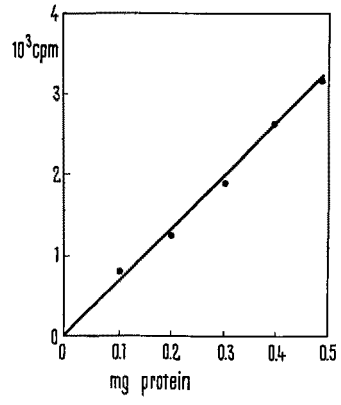

Fig. 4. The decarboxylation of L-glutamate in the relation to the amount of enzyme. The incubation has been carried out under standard conditions. Various concentrations of enzyme were obtained by the dilution of a single stock solution.

Under given conditions, the reactions follows zero order kinetics for at least 1 h (Figure 3) and the measured radioactivity is directly proportional to the amount of the enzyme (Figure 4).

Quenching due to sodium hydroxide does not exceed 5% and it is lower than with 0.1 ml of 1 *M* hyamine hydroxide.

Zusammenfassung. Beschreibung einer Ultramikromethode zur Bestimmung der Glutaminsäure-dekarboxylase, welcher die Aktivitätsmessung des durch die enzymatische Reaktion entstandenen $^{14}CO_2$ zugrunde liegt. Die Reaktion wird in einem Spezialgefäss durchgeführt.

P. KRAUS

Research Institute for Pharmacy and Biochemistry, Praha 3 (Czechoslovakia), 27 July 1967.

[4] J. P. SUSZ, B. HABER and E. ROBERTS, Biochemistry *5*, 2870 (1966).
[5] E. ROBERTS and D. SIMONSEN, Biochem. Pharmac. *12*, 113 (1963).
[6] C. F. BAXTER and E. ROBERTS, J. biol. Chem. *236*, 3287 (1961).

CONGRESSUS

USA

International Symposium on Turbulence of Fluids and Plasmas

Waldorf-Astoria Starlight Roof, New York City, 16–18 April 1968

Turbulence of Fluids and Plasmas is the topic of the eighteenth in the Polytechnic Institute of Brooklyn series of annual international symposia and will be held at the Waldorf-Astoria Hotel in New York City. The following tentative programme has been formulated: introduction to turbulence; theoretical and experimental descriptions of turbulent media; experimental diagnostic techniques and related interactions with turbulent media.
All correspondence should be addressed to: P.I.B. Symposium Committee, 333 Jay Street, Brooklyn, N.Y. 11201, USA, Attn.: Jerome Fox, Executive Secretary.

Israel

Symposium on Permeability Problems

Jerusalem 2–9 July 1968

To be held by the Commission on Cell and Membrane Biophysics of the International Union for Pure and Applied Biophysics. The topics to be discussed will be: transport problems in animals and plants exposed to arid conditions; transport across epithelia; water transport in biological systems; physical chemistry of charged membranes; the theoretical interpretation of tracer fluxes.
Further information can be obtained from each National Committee for Biophysics or Biophysical Society, or from the Secretariat, Symposium on Permeability Problems, Polymer Department, Weizmann Institute of Science, Rehovot (Israel).