The Tryptophan Pyrrolase Activity of Human Liver

Liver tryptophan pyrrolase[1] has been thoroughly investigated in different animal species and in various experimental conditions[2,3].

The enzyme however had not been detected in human liver, its presence having been inferred until now from indirect data only. The accumulation of tryptophan metabolites in the urine of humans after administration of tryptophan has been taken as evidence that tryptophan pyrrolase is present in human liver, as this enzyme catalyses the first metabolic step of the tryptophan→nicotinic acid pathway.

Before starting the study of the tryptophan pyrrolase activity in human liver homogenates, we determined the effect of different storage conditions on this enzyme.

These experiments, carried out on rat livers, showed that the tryptophan pyrrolase activity increased if the animals, after death, were kept at room temperature (18–20 °C) or at 2 °C and the liver was left in situ. The increase was greater and faster in the experiments carried out at room temperature; the % variation is given in Table I.

However, if the liver homogenates, prepared as soon as possible after death, were stored at 2 °C, the values of the enzyme activity became lower than those determined at zero time. The variations after 2 h lie between − 9 and − 22% and after 5 h between − 34 and − 42%.

Finally, if the liver was frozen in dry ice, the changes in activity were not important: the range of the variations being − 11 and + 0.5% after 1 h and − 23 and − 2% after 24 h.

A rapid release of enzymatic activator from hepatic tissue after death is an interpretation of the conspicuous changes seen at room temperature.

On the basis of these results, it was assumed that the best way of storing human liver for a study of tryptophan pyrrolase activity was the freezing of the organ in dry ice. 12 liver samples stored in this way were examined. They were removed from 6 immature or premature new-born babies and 2 new-born babies at term a few h after death and from 3 adults and 1 new-born at term during an abdominal surgical operation.

The cases submitted to abdominal surgery were anaesthetized with sodium penthotal. No evidence of hepatic disfunction was observed in the adult patients. The livers did not show any macroscopic change; histological examinations were not carried out.

The evaluation of the enzyme activity was carried out on the liver homogenate with the method routinely used in our laboratory[2]. In most cases the homogenate was fractionated, first by centrifuging at 20,000 *g* for 30 min to remove tissue debris and mitochondria and by centrifuging this supernatant in a Spinco ultracentrifuge at 105,000 *g* for 60 min to obtain the particle free supernatant (cell sap) and the microsomal fraction[2,4]. Tryptophan pyrrolase activity was estimated in the cell sap and in the cell sap upon addition of microsomes since a heme activator has been located in this fraction[5].

Table II summarizes the data obtained on human liver. The tryptophan pyrrolase activity was never detectable in the homogenate nor in the supernatant of immature new-born babies; it was found in 5 out of 6 new-born babies at term and adults. The values ranged from 0.37 to 2.63 μmoles of kynurenine/h/100 mg N.

The presence of the enzyme activity in human liver therewith demonstrated, was confirmed in a series of activation experiments carried out by adding the supernatant (apoenzyme) both to its own microsomes (activating coenzyme) and to microsomes from other livers, including rat liver.

The tryptophan pyrrolase was activated in all experiments with one exception, namely the experiments in which the supernatant from immature new-born liver was present. In these cases the activity was not detected even after addition of 3 microsome equivalents, obtained from rat liver, which increase the activity of their own

Table I.

Temperature	Control values[a]	h after death		
		2	4	18
2 °C	3.83 ± 0.47	+ 44	+ 18	
18–20 °C	4.98 ± 0.37	+ 156	+ 181	+ 190

[a] Kynurenine μmoles/h/100 mg N.

Table II. Tryptophan pyrrolase activity in human liver

No.	Name, sex	Age	Weight g	Time h[a]	Tryptophan pyrrolase kynurenine μmoles/h/100 mg N		Notes
					homogenate[b]	supernatant[b]	
1	X	Immature new-born			n.d.	n.d.	[c]
2	Y	Immature new-born			n.d.	n.d.	[d]
3	G.L. m	Immature 6th month	650	3	n.d.	n.d.	
4	G.E. m	Monochorioid twin of No. 3	600	2	n.d.	n.d.	
5	G.Bi. f	Premature 7th month	1200	1	n.d.	n.d.	[e]
6	G.Br. m	twin of No. 5	1150	1	n.d.	n.d.	[e]
7	Z.R. m	New-born at term	3200		0.37	1.37	[f]
8	S.A. m	3 days	3350	1	2.63	1.37	[g]
9	G.L. m	4 days	3900	1	0.50	2.26	[h]
10	M.E. m	25 years			n.d.		[i]
11	L.F. m	28 years			1.01		[k]
12	C.M. m	40 years			1.37		[l]

[a] Time elapsed between the death and the removal of the liver sample. [b] n.d., not detectable. [c,d] Sex, weight (foetal) age and time of liver removal not reported. [e] Born normally, died 10 h after birth. [f] Born normally, operated 5 h after birth for congenital aplasia of the abdominal wall. [g] Died from iso-immunization. [h] Died from hemiencephalia. [i,l] Operated for duodenal ulcer. [k] Operated for gastric ulcer.

1 W. E. Knox and A. H. Mehler, J. biol. Chem. *187*, 419 (1950).
2 F. M. Chiancone, *Enzymes of Tryptophan → nicotinic Acid Pathway* in *Newer Methods of Nutritional Biochemistry* (Academic Press, New York 1965), vol. 2, p. 250.
3 F. M. Chiancone, Acta Vitaminol., Milano *17*, 183 (1963).
4 P. Feigelson and O. Greengard, J. biol. Chem. *236*, 153 (1961).
5 O. Greengard, *Advances in Enzyme Regulation* (Pergamon Press, London 1964), vol. 2, p. 277.

supernatant from two- to fourfold. On the other hand, the microsomes of immature new-born activate the supernatants of human and rat liver. Therefore, even if the tryptophan pyrrolase activity is not detectable, the activator is present in immature new-born liver.

The problem arises whether tryptophan pyrrolase is synthetized by liver cells of human foetus at some stage of the development. A similar pattern has been observed in other mammals[6,7].

Riassunto. Viene dimostrata la presenza di attività triptofano pirrolasica nell'omogenato e nel sopranatante ottenuti da campioni di fegato di neonati a termine e di adulti. Nel fegato di neonati immaturi questa attività enzimatica è risultata assente anche nelle prove di attivazione mediante aggiunta di microsomi di fegato.

F. M. CHIANCONE, E. GINOULHIAC, U. BONOMI, L. T. TENCONI and P. CAREDDU

Medical Department and Clinical Research Laboratory Lepetit S.p.A. and Pediatric Clinic of the University, Milan (Italy), 24th February 1967.

[6] A. M. NEMETH, J. biol. Chem. *234*, 2921 (1959).
[7] V. H. AUERBACH and H. A. WAISMANN, J. biol. Chem. *234*, 307 (1959).

On the Question of the Mechanism of Inhibitory Effect of Acidosis on Anaerobic Glycolysis of Erythrocytes

A suitable model for following the effects of acidosis on the glucose utilization is the erythrocyte just for its several metabolic simplifications. It is known from the literature that the glycolysis of a normal mature erythrocyte depends on the pH value[1,2]. Acidosis which is developed in an experimental shock also reduces the utilization of glucose[3].

The effect of acidosis in vitro on the anaerobic glycolysis was followed in freshly sampled blood of clinically healthy donors. The erythrocytes washed in ice cold saline were resuspended in isotonic phosphate buffer of various pH and enriched with glucose to the final concentration of 100 mg %. The buffer capacity of used buffer solutions was sufficient enough to keep the constant pH value of resuspended blood within the time period of 75 min incubation. The erythrocytes were incubated always in 3 buffer solutions of pH 6.1, 6.7 and 7.4. After 15 min lasting incubation at 4 °C occurred the equalization of pH value between the erythrocyte and buffer solution up to the value of 6.6, 6.95 and 7.35 respectively.

The erythrocytes incubated in the buffer of pH 6.1 are producing about 2.5 times less lactic acid. The level of pyruvic acid in acid buffer is simultaneously increased. In that way the ratio lactate/pyruvate (L/P) is further decreased. The difference in the ratio L/P in alkaline and acid buffer after 75 min incubation is twenty-fold (Table).

The dependence of the ratio L/P on the value pH in incubated resuspended erythrocytes was estimated under these suppositions: (1) the pH value inside the erythrocyte and in the incubation medium after 15 min equilibration was the same, (2) within the time period of equilibration, which occurs at 4 °C, practically no glycolysis process exists and therefore no accumulation of lactic acid in the medium outside the erythrocyte occurs. The results are stated in Figure 1 and show the direct dependence of the ratio L/P upon the pH value. The in-

[1] U. HINTERBERGER, W. GERISCHER-MOTHES, D. SUCKROW and S. RAPOPORT, Acta biol. med. germ. *7*, 57 (1961).
[2] L. TRINER, M. MRÁZ, J. KYPSON and B. ZÍCHA, Medna Pharmac. exp. *12*, 190 (1965).
[3] L. TRINER, J. KYPSON, M. MRÁZ and B. ZÍCHA, Medna exp. *10*, 103 (1964).

Effect of the acidosis on the glycolytic activity of erythrocytes incubated on phosphate buffer of different pH values

Time of incubation at 37 °C in min	pH of phosphate buffer 6.1			6.7			7.4		
	pH of resuspended erythrocytes in phosphate buffer after 15 min equilibrium time at 0 °C 6.6			6.95			7.35		
	L/P	Lactate	Pyruvate	L/P	Lactate	Pyruvate	L/P	Lactate	Pyruvate
15	11.5	260	22.5	40	365	9.1	87	601	6.9
30	9.5	360	38.0	47	570	12.0	110	880	8.0
45	8.5	520	60.8	63	950	15.0	181	1590	8.0
60	8.7	655	75.0	61	1090	18.0	177	1700	9.6
75	8.7	853	98.3	58	1163	20.3	179	1850	10.3

Metabolites are expressed in 10^{-3} *M*/ml of resuspended erythrocytes and represent the mean value of 6 samples. Incubation procedure: washed erythrocytes were diluted by equal volume of isotonic phosphate medium consisting of 100 parts of isotonic phosphate buffer, 5 parts of 0.155 *M* KCl and 1 part of 0.11 *M* $CaCl_2$. The mixture was enriched by glucose to the final concentration of 100 mg % and equilibrated for 15 min at 4 °C and then incubated aerobically at 37 °C in ultrathermostat without shaking.