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.

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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-

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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.

crease of the pyruvic acid level and the drop of the ratio P/P point out the insufficient hydrogenation of pyruvic acid, and it should therefore correspond to the change of the redox system NAD/NADH, which is H^+-dependent.

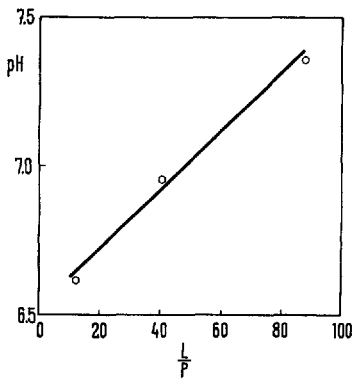

Fig. 1. Relation between pH value and lactate/pyruvate (L/P) ratio in erythrocytes equilibrated at 4 °C.

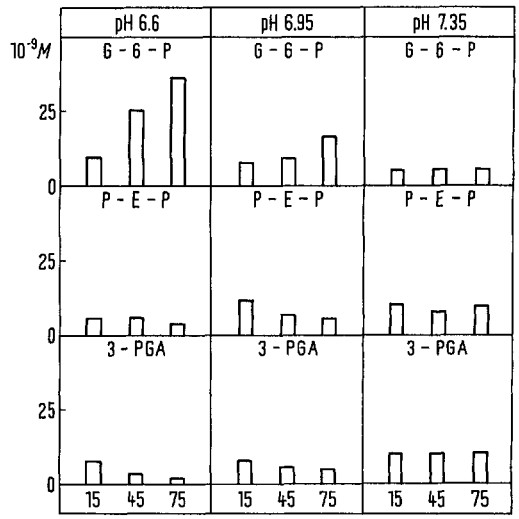

Fig. 2. Levels of Glucose-6-Phosphate (G-6-P), Phosphoenolpyruvic acid (P-E-P) and 3-Phosphoglyceric acid (3-PGA) in erythrocytes incubated in phosphate buffer of different pH values. Incubation time in min at 37 °C, methods according to [7].

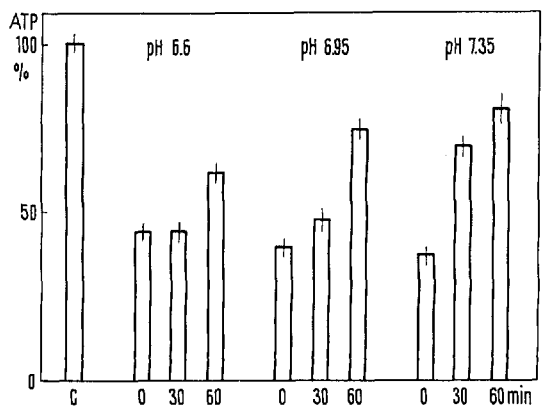

Fig. 3. Re-phosphorylation capacity of washed erythrocytes incubated in buffers of various pH values. Incubation in min at 37 °C. C = ATP level of freshly sampled blood.

The change of this ratio must have caused the accumulation of metabolites before the only one NAD-dependent dehydrogenase of glycolytic cycle. We have therefore determined the glucoso-6-phosphate (G-6-P) level as a metabolite before the triose step as well the level of phosphoenolpyruvate (PEP) and 3-phosphoglycerate (3-PGA). An evident accumulation of G-6-P in acid incubation medium and corresponding decrease of the 3-PGA level occurs (Figure 2).

The influence of pH upon the re-phosphorylation ability of the erythrocytes was followed. Within the time period of washing, the level of ATP drops down to 50% of the initial value of the fresh blood, though the decrease of ATP is smaller than in erythrocytes which were suspended in acid buffer. The lower pH value seems to be the protective factor for keeping the level of ATP within the time period of reaching the equilibrium, while it reduces the glycolysis. The resynthesis of ATP which depends on pH value, occurs within the incubation time (Figure 3). The effect of pH is evidently not of such significance and apparently a compensatory effect of 2, 3-diphosphoglycerate suplies occurs.

The effect of acidosis may be proved almost in the first metabolite of glycolysis by a very significant accumulation of G-6-P. These findings are in full agreement with BRUNS'[4,5] observations. The hexose monophosphate shunt makes only 10% of glucose utilization and is not pH-dependent[6]. The direct affecting of the activity of hexokinase under acidosis[8] is not probable, while there should not occur the accumulation of G-6-P. On the other side it is known that G-6-P regressively inhibits the hexokinase activity[9] and this conception was manifested formerly[10,11]. The increased level of G-6-P and the simultaneous decrease of 3-PGA level and of L/P ratio in erythrocytes incubated in acid medium shows the probable direct change of redox ratio of pyridine nucleotides caused by higher concentration of hydrogen protons.

Zusammenfassung. Der Einfluss der Azidose auf die Glykolyse der Erythrozyten wurde in vitro untersucht. Es resultiert eine Erniedrigung der Milchsäureproduktion bei gleichzeitiger Erhöhung des Glukose-6-Phosphat-Gehalts und des Laktat/Pyruvat/Quotienten. Die Befunde weisen auf eine direkte Beeinflussung der Glykolyse infolge verändertem NAD/NADH-Redox-Zustand durch erhöhte H^+-Konzentration.

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