# DISTURBANCE OF GLYCOLYTIC OXIDOREDUCTION IN THE LIVER IN EXPERIMENTAL BURNS

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UDC 617-001.17-092.9-07: 616.61-008.931:577.152.11

The intensity of glycolytic oxidoreduction in the rat liver was investigated 3, 6, 12, and 24 days after the production of an experimental burn. Burns were shown to depress glyceraldehyde phosphate dehydrogenase activity and to reduce the formation of 3-phosphoglyceric acid in the liver of the animals, the effect being most marked during the period of burn trauma (6th day). The factor limiting the course of this reaction after burns is NAD deficiency in the cell. Disturbance of the course of this reaction is one of the causes of the decrease in intensity of the whole glycolytic pathway of carbohydrate breakdown and the decrease in the energy-supplying role of glucose in burns.

KEY WORDS: burns; rat liver; glycolysis.

Burns are accompanied by a marked disturbance of several pathways of carbohydrate metabolism in a number of intact organs of the experimental animals [1, 5, 6, 8].

The object of this investigation was to study the reactions of glycolytic oxidoreduction which occupies the central position in glycolysis, one of the main pathways of carbohydrate conversion.

## EXPERIMENTAL METHOD

A burn of the IIIa-IIIb degree was inflicted on noninbred albino rats weighing 180-200 g by applying a burning spirit-soaked cotton swab to the epilated spinal and lateral surfaces of the body. After decapitation of the rats on the 3rd, 6th, 12th, and 24th days after burning, activity of glyceraldehyde phosphate dehydrogenase (GAPD) was determined [11] in the supernatant obtained after centrifugation of the liver homogenates in 0.15 M KCl (12,000g, 15 min, 0-2°C). The composition of the incubation medium was (in mM): Tris-HCl, pH 8.6 40, sodium arsenate 5, NAD 0.4, glyceraldehyde phosphate 0.02; +0.02 ml of supernatant. The protein concentration was determined by Lowry's method [9]. The glyceraldehyde phosphate used for the determination was obtained as a mixture of phosphotrioses by enzymic hydrolysis of fructose diphosphate (FDP) [10].

In a parallel series of special experiments the rate of formation of 3-phosphoglyceric acid (PGA) in the supernatant of the liver obtained by centrifugation of a homogenate made up in 40 mM nicotinamide (12,000g, 15 min, 0-2°C) was studied. Incubation was carried out in phosphate buffer, pH 7.8, containing (in $\mu$moles per sample) FDP 27, sodium pyruvate 18, NaF 50. In some experiments 2.5 $\mu$M NAD or 5 $\mu$M ADP or NAD and ADP together were added to the incubation medium.

## EXPERIMENTAL RESULTS

The experiments showed that GAPD activity in the liver of rats with thermal burns was considerably reduced – to 51% of the initial level on the 6th day after burning (Table 1). However, as Weber [12] rightly points out, a decrease in the activity of any one enzyme does not mean a corresponding decrease in the intensity of the process in which that enzyme takes part, because optimal conditions are created for the manifestation of its activity.

Department of Biochemistry, Ivanovo Medical Institute. Department of Biochemistry, Chelyabinsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 8, pp. 171-173, August, 1977. Original article submitted July 9, 1977.

TABLE 1. GAPD Activity in Liver of Rats with Experimental Burns (in units of increase of optical density at 340 nm per milligram protein per minute)

| Index | Intact rats | Time after burning, days | | | |
|---|---|---|---|---|---|
| | | 3rd | 6th | 12th | 24th |
| $M \pm m$ | 0,38±0,02 | 0,23±0,05* | 0,195±0,06* | 0,30±0,06* | 0,313±0,025* |
| % inhibition | — | 40 | 49 | 21 | 17 |

*Here and in Table 2 $P < 0.05$ compared with control.

TABLE 2. Formation of PGA in Supernatant of Liver at Various Periods of Experimental Burns (in $\mu$moles PGA/g tissue/30 min)*

| Experimental conditions | Index | Intact rats | Time after burning, days | | | |
|---|---|---|---|---|---|---|
| | | | 3rd | 6th | 12th | 24th |
| Normal incubation medium | $M \pm m$ | 28,1±1,7 | 24,8±2,4 | 17,7±2,3* | 19,3±2,6* | 21±1,8* |
| | % inhibition | — | 12 | 37 | 32 | 23 |
| Ditto + ADP | $M \pm m$ | 29,2±1,6 | 24,0±1,8* | 19,6±2,1* | 21,1±1,9* | 22,7±2,2* |
| | % inhibition | — | 18 | 33 | 26 | 20 |
| Ditto + NAD | $M \pm m$ | 33,1±2,2 | 35,0±2,7 | 30,0±2,6 | 29,2±2,1 | 29,1±2,3 |
| | % inhibition | — | ~0 | 10 | 12 | 12 |
| Ditto + ADP + NAD | $M \pm m$ | 40,3±2,6 | 42,0±2,1 | 38,2±2,5 | 39,8±2,8 | 36,7±2,1 |
| | % inhibition | — | ~0 | 6 | 2 | 4 |

*Legend as in Table 1.

Experiments to determine the increase in PGA showed that simultaneously with a decrease in GAPD activity the rate of PGA formation also fell; these changes were most marked also on the 6th day of the experiment and they corresponded on the whole to the degree of inhibition of GAPD (Table 2).

To study the possible causes of the changes and to establish the main limiting factor in the reaction studied, NAD, ADP, or NADP and ADP were added to the incubation medium in a series of experiments. As Table 2 shows, the addition of ADP did not affect the accumulation of PGA in either the control or the experimental samples. This is evidence that the ADP content can hardly be the factor limiting the course of glycolytic oxidoreduction after burns. The results are also confirmed by investigations in which an increase in the ADP content in the liver was found under these conditions [3]. Addition of NAD to the incubation medium led to a considerable increase in PGA formation in the experimental samples, but this index was highest when NAD and ADP were added simultaneously to the incubation medium (Table 2). When the experiments were carried out in this way the most favorable conditions were evidently provided for the acceptance and transport of hydrogen and inorganic phosphate.

It can accordingly be concluded from a comparison of these results that one probable cause of the limitation of PGA formation after burns is a decrease in the NAD content in the cell. This conclusion is also confirmed by the results of the writers' previous investigations, which showed that burns are accompanied by a substantial shift in the ratio between oxidized and reduced forms of pyridine nucleotides toward predominance of the latter [2].

The fact that less PGA is formed in the liver of the burned animals could also be connected with a decrease in phosphotriose formation from FDP, which was used in this investigation as the substrate for their formation. However, this hypothesis can hardly be valid because, as was found in experiments with the addition of NAD, ADP, and NAD + ADP to the supernatant of the liver of the control and experimental animals, equal quantities of PGA were formed. Consequently, in the control and burned rats on incubation of the supernatant of the liver with FDP an equal quantity of phosphotrioses essential for further conversion was formed. The same conclusion was reached by Zaitseva [4], who studied the intensity of glycolytic oxidoreduction in the skeletal muscles in avitaminosis E. It thus seems that aldolase cannot limit the reaction of glycolytic oxidoreduction after burns. This is confirmed by direct investigations of the activity of that enzyme in the liver, which showed no significant changes after burns [5]. Consequently, the results must be attributed to a disturbance of the course of the glycolytic oxidoreduction reaction itself.

This disturbance depends essentially on a change in the activity of GAPD, an enzyme which can undergo transformation and, depending on the conditions, can exhibit either transferase, esterase, phosphatase, or transphosphorylase activity [7]. Burn trauma leads to marked changes in the structure and function of several proteins, including enzymes, and this suggests the possibility of transformation of GAPD; however, this is a problem for special investigation.

Allowing for the central position of glycolytic oxidoreduction in dichotomous oxidation of carbohydrates, it becomes clear that the disturbance of the course of this reaction largely determines the possibility of formation of compounds of lipid nature from carbohydrates and, in addition, it may be one cause of the decrease in the energy-forming role of glucose in burns.

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