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EFFECT OF DISTURBANCE OF THE INNERVATION OF THE LIVER AND LOSS OF BILE ON BILIARY AND HEPATIC ENZYME ACTIVITY

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Continuous loss of bile from rats with a bile reservoir connected to the common bile duct led to an increase in specific activity of malate, lactate, glutamate, and glucose-6-phosphate dehydrogenases, alkaline and acid phosphatases, urokinase, and histidase in liver homogenates by the seventh day. By the tenth day their specific activity had fallen. After disturbance of the innervation of the rats' livers the ATP concentration fell sharply and the specific activity of the above-mentioned enzymes in the liver was considerably inhibited. During continuous loss of bile, fluctuating changes took place in the specific activity of these enzymes and also of sorbitol dehydrogenase in the bile, starting from the first and continuing until the tenth day of the experiment. Support for the view that these fluctuations were under the control of the nervous system was given by the considerable changes in their character following disturbance of the hepatic innervation.

KEY WORDS: enzymes; liver; disturbance of innervation; loss of bile.

The trophic influence of the nervous system is often manifested more clearly and distinctively when the function of an organ is disturbed. For the liver, one such model of this state is the continuous and prolonged loss of bile. Whereas biochemical indices of the liver have been studied in sufficient depth during the action of various factors of the nervous system, despite the theoretical and clinical importance of prolonged loss of bile and disturbance of the innervation of the liver, no information could be found on the biliary enzymes in these states. Moreover, the biliary enzymes have received little study even in healthy man and animals. Enzymes of the liver likewise have not been studied during loss of bile when the innervation of the organ is disturbed.

The object of this investigation was to determine the enzymes of the liver and bile during a disturbance of innervation of the liver and during loss of bile.

EXPERIMENTAL METHOD

The experiments of series I (duration 10 days) were carried out on 63 Wistar rats with a reservoir connected to the common bile duct for the continuous collection of bile [4]. The experiments of series II (the same duration) were carried out on 24 Wistar rats in which a receiver was connected to the bile duct immediately after disturbance of the innervation of the liver [6]. The specific activity of the following enzymes was determined daily in the bile and on the first, third, fourth, fifth, seventh, and tenth days of the experiments in liver homogenates during loss of bile and on the first, third, and seventh days after disturbance of innervation, by means of the SF-4A spectrophotometer: lactate dehydrogenase (LD), malate dehydrogenase (MD), glucose-6-phosphate dehydrogenase (G6PD), as described previously [7], glutamate dehydrogenase (GD) [14], sorbitol dehydrogenase (SD) [12], alkaline phosphatase

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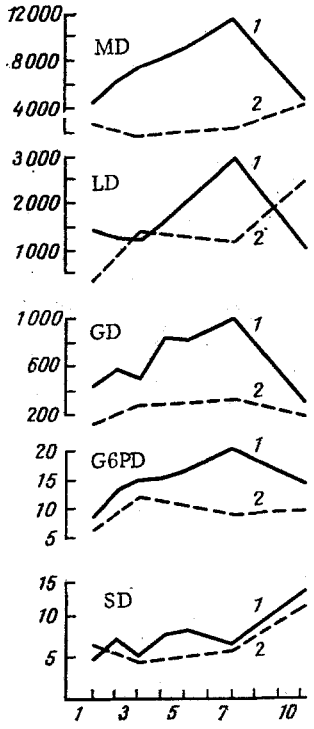

Fig. 1

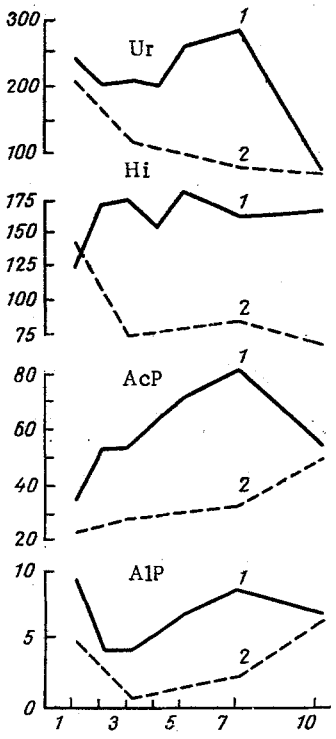

Fig. 2

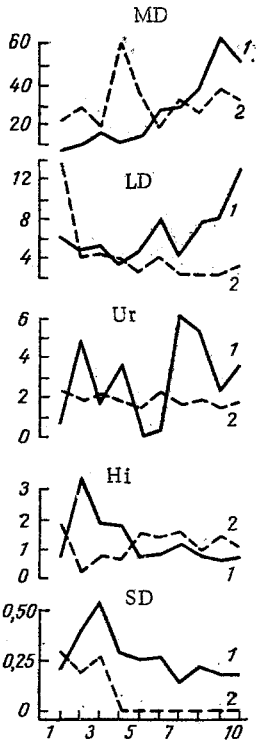

Fig. 3

Fig. 1. Specific activity of dehydrogenases (in μmoles•min^{-1}•g^{-1} protein) of the rat liver after disturbance of its innervation and during loss of bile. In this and subsequent figures: 1) loss of bile; 2) disturbance of innervation with loss of bile. Abscissa, days of experiment; ordinate, specific activity.

Fig. 2. Specific activity of phosphatases (μmoles·min^{-1}•g^{-1} protein), Ur, and Hi (μmoles•h^{-1}•g^{-1} protein) of rat liver following disturbance of its innervation and during loss of bile.

Fig. 3. Specific activity of dehydrogenases (in μmoles·min^{-1}•g^{-1} protein), Ur, and Hi (in μmoles•h^{-1}•g^{-1} protein•10^2) in bile of rats following disturbance of innervation of liver and during loss of bile.

(AlP) [11], acid phosphatase (AcP) [10], histidase (Hi), and urokinase (Ur) [13]. The protein content was determined as in [13] and ATP as described previously [7] by the coupled reaction with hexokinase and G6PD. At each time from six to 20 determinations of each enzyme were made. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

During loss of bile the specific activity of hepatic Ur, phosphatases, and dehydrogenases except SD increased until the seventh day and then decreased until the end of the experiment (Figs. 1 and 2). Electron-microscopic study of the hepatocytes revealed [2] evidence of increased mitochondrial function and protein biosynthesis during the first days, but after the seventh day mitochondria with a less dense, or almost empty matrix appeared; in the granular endoplasmic reticulum, marked degranulation was observed, indicating exhaustion of the cells.

After disturbance of the innervation of the liver a definite decrease was observed in the specific activity of the eight enzymes studied, except SD, throughout the experiment; this decrease also correlated with the electron-microscopic picture of separation of the hepatocytes, described by the writers [8], as a result of intercellular edema, disorganization of the biliary capillaries, disturbance of the plasmolemma of the hepatocytes, and destruction of the mitochondria, with severe swelling or condensation of their matrix, lysosomes, cristae, and inner and outer membranes.

The decrease in activity of enzymes of the tricarboxylic acid cycle, of glycolysis, and of the pentose phosphate pathway, and also changes in the ultrastructure of the mitochondria and other organelles after the disturbance of innervation agree well with the significant

($P < 0.001$) decrease in the ATP content in the liver to 1.91 ± 0.16 nmoles/g wet weight of tissue compared with the control value of 3.27 ± 0.06 nmoles/g wet weight of tissue. A decrease in the ATP content has been observed in striated muscles [9] and in the cornea [7] during neurodystrophy.

Analysis of the bile revealed fluctuating changes in the specific activity of all nine enzymes in all experiments. In the course of these experiments, Ur, Hi, and SD were evidently determined for the first time in bile (Fig. 3).

Disturbance of the innervation gave rise to the following changes: a) Starting from the fourth day SD activity was completely inhibited; b) from the first to the end of the second day the specific LD activity was considerably reduced without any subsequent increase, whereas if the innervation remained intact it was increased from the start to the finish of the experiment; c) MD was greatly activated during the first half and inhibited toward the end of the experiment; d) the amplitude of fluctuations in the specific activity of Ur and Hi was reduced. The disturbance of innervation had a much lesser effect on the fluctuating levels of specific activity of AlP, AcP, GD, and G6PD than of MD, LD, Ur, Hi, and SD.

It is interesting to note that these patterns were largely preserved when the activity of the enzymes was calculated relative to the daily volume of bile production.

Loss of bile thus caused a fluctuating increase, with a maximum on the seventh day of the experiment, in the specific activity of most liver enzymes. Disturbance of innervation sharply reduced the ATP content in the liver and considerably inhibited the activity of nearly all the hepatic enzymes studied, whatever their intracellular localization: mitochondrial, lysosomal, cytoplasmic. This is an interesting result in the light of the role of ATP in bile formation [15]. Changes in enzyme activity, in the bioenergetic processes, and in the ultrastructure of the hepatocytes were naturally reflected in the activity of the bile enzymes. The fluctuations observed in the specific activity of the biliary enzymes must be assumed to reflect adaptation of the organism to a stress situation in the form of protracted loss of bile. The view that these fluctuations occurred under the control of the nervous system is supported by the marked changes in their character after disturbance of innervation.

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