Comparative Structural Analysis of the Liver and Kidneys in Experimental Renal Failure

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UDC 616.61-008.64-036.12-092.9-07:616.36+616.61]-076

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 5, pp. 546-548, May, 1993
Original article submitted December 28, 1992

Key Words: *chronic renal failure; subtotal nephrectomy; liver; kidney; enzyme activity*

The status of the liver and its involvement in the detoxication and compensatory processes of metabolic disturbances in chronic renal failure are of great clinical importance for the course of the disease and the prognosis [3, 5]. However, both clinical and experimental studies of liver function and structural alterations in the liver in chronic renal failure have been scarce. Experimental chronic renal failure is induced in most cases by subtotal nephrectomy [6]. At early stages of the development of chronic renal failure hyperperfusion and hyperfiltration are observed, which result in further damage to the renal glomeruli and tubules [4].

The aim of the present study was to investigate the characteristic structural and metabolic changes in the liver and kidneys at different stages of the development of chronic renal failure.

MATERIALS AND METHODS

The experiments were carried out on 40 male Wistar rats subjected to double-stage subtotal nephrectomy using ether anesthesia. The animals in the control group were subjected to a sham operation under the same conditions. The animals were sacrificed 1, 7, 14, 30, and 60 days after the operation. Renal and hepatic tissue blocks frozen in liquid nitrogen with MK-25 at 18°C were used to prepare 10 μ-thick slices analyzed for succinate, lactate, $NADPH_2$ and $NADH_2$ dehydrogenases by the method of Nakhlass and Zadigman and for acid and alkaline phosphatase by the azo coupling method [1].

The enzyme activity was assessed by cytospectrophotometry using an MTsF-U2 spectrophotometer in the hepatocyte cytoplasm, apical and basal areas of the cytoplasm of the proximal convoluted tubules, as well as in the endothelium of the peritubular capillaries [2]. Parallel tissue pieces were fixed in 10% neutral formalin solution. The paraffin slices were stained with hematoxylin, eosin, and picrofuchsin by Van Gizon's method using Schiff reagents. The data obtained were assessed using standard methods of mathematical statistics with estimation of the means and the significance of the differences using the 95% confidence level.

RESULTS

During the first few months after nephrectomy signs of hemodynamic disturbances such as plethora and sinusoid dilatation are predominant. Albuminoid degeneration, a large number of activated Kupffer's cells, and moderate round cell infiltration are observed. In most cases binucleate hepatocytes are found. Later hepatocyte necrosis develops, both mononuclear and microfocal, and in some areas confluent necrosis is found. In such areas macrophagal response is pronounced. In the periportal zones

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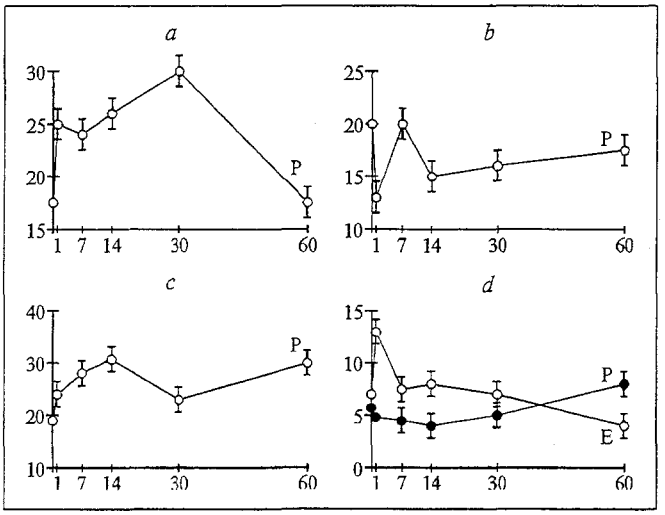

Fig. 1. Activity of succinate dehydrogenase (*a*), lactate dehydrogenase (*b*), $NADPH_2$ dehydrogenase (*c*), and alkaline phosphatase (*d*), expressed in conventional optical density units (U) at different times after nephrectomy (T) in perinuclear (P) areas of hepatocyte cytoplasm and in endothelium (E) of hepatic vessels.

marked lymphomonocytic infiltration is observed against a background of moderate fibrosis. These structural alterations in the liver are accompanied by marked changes in the intracellular metabolism.

As early as one day after nephrectomy the succinate dehydrogenase activity in the perinuclear zone of the hepatic cytoplasm is significantly increased. The highest activity of this enzyme is observed 30 days after the operation. However, two months later the succinate dehydrogenase activity is normalized (Fig. 1, *a*). Unlike SDH, the lactate dehydrogenase activity drops markedly immediately after the operation. By the 7th day its activity has risen virtually to the control level; however, later on, beginning from the 14th day, the LDH activity decreases (Fig. 1, *b*). The alterations in $NADH_2$ and $NADPH_2$ dehydrogenases follow a similar pattern: their activity markedly increases immediately after nephrectomy and is maintained at this level throughout the experiment (Fig. 1, *c*). The alkaline phosphatase activity in the endothelium of the vessels at first increases, then reverts to the control level, and is inhibited by the end of the experiment. The changes in the activity of this enzyme in the cytoplasm follow a different pattern: a slight decrease at the beginning of the experiment is followed by a significant increase 60 days after the operation (Fig. 1, *d*).

In the kidneys after subtotal nephrectomy pronounced changes are observed on the 30th day, manifested in marked eosinophilia of the cytoplasmic cells of the proximal convoluted tubules, hyalin degeneration, partial destruction of the apical areas, and small areas of low nephron nephrosis with sclerosis and lymphomonocytic infiltration.

The assay of the cellular enzymes revealed an increase of succinate dehydrogenase activity on the first day after nephrectomy, in the apical areas, this increase in activity is observed during one week, after which it decreases to the control values; however, on the 30th day the activity increases again. The activity of this enzyme in the endothelium of the peritubular capillaries is found to be above the control value, especially at the end of the experiment (Fig. 2, *a*).

The $NADPH_2$ dehydrogenase activity in the cytoplasmic apical areas of the proximal convoluted tubules rises only on the 7th day of the experiment and subsequently normalizes. In the basal areas an increase in activity is observed on the first day, and on the 30th day normalization occurs. The alterations in the endothelium of the peritubular capillaries are not marked.

The lactate dehydrogenase activity in the apical areas is found to be within the range of control values during 1 week, and in the basal areas during 2 weeks. On the 30th day the activity of the enzyme is considerably reduced and on the 60th day it is normalized. In the endothelium of the peritubular capillaries the lactate dehydrogenase activity is found to be considerably elevated during two weeks compared with the control level; on the 30th day it becomes normal and then it increases again (Fig. 2, *b*).

The acid phosphatase activity in the apical areas drops on the first day after nephrectomy, while in the basal areas it decreases only on the 7th day. By the

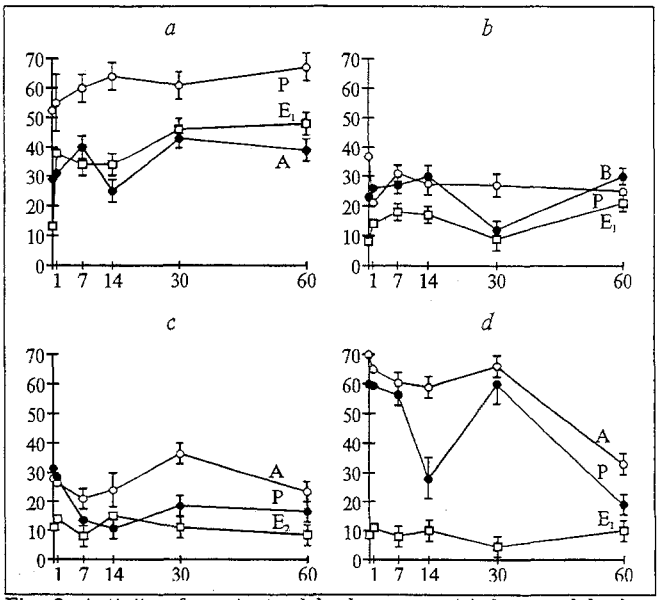

Fig. 2. Activity of succinate dehydrogenase (*a*), lactate dehydrogenase (*b*), acid phosphatase (*c*), and alkaline phosphatase (*d*) in the apical (A) and basal (B) areas of epithelium cytoplasm of the proximal convoluted tubules, perinuclear (P) areas of epithelium cytoplasm, and in the endothelium of the peritubular capillaries of the proximal (E_1) and distal (E_2) nephron.

end of the first month the acid phosphatase activity is found to be normal, whereas in the apical regions this activity is even higher than the control values. However, by the second month the acid phosphatase activity once again decreases (Fig. 2, *c*).

On the 14th day after nephrectomy a steady decrease in the alkaline phosphatase activity in the brush border of the peritubular proximal tubules is observed. By the end of the first month the activity tends to normalize, but later on the decrease in activity becomes still more pronounced. In the basal regions the alkaline phosphatase activity is considerably lower than the control values, especially on the 30th or 31st day of the experiment (Fig. 2, *d*). In the endothelium of the peritubular capillaries cyclic variations of the alkaline phosphatase activity around the control values are observed.

Thus, the development of uremia in subtotally nephrectomized rats results in metabolic changes both in the kidneys and in the liver. The changes in the activity and localization of the enzymes found in the epithelial cells of the proximal convoluted tubules are unlikely to compensate for the disturbances of the excretory renal function. In response to these changes the hepatocytic oxidation-reduction processes related to aerobic glycolysis are activated, as attested to by the high levels of succinate, $NADH_2$, and $NADPH_2$ dehydrogenases. The increase in SDH activity completing the Krebs cycle suggests an increase in the activity of the whole cycle of tricarboxylic acids. The rise in the $NADPH_2$ dehydrogenase activity suggests a rise of the flow in the electron transfer chain and eventually a higher oxygen demand. At the same time, anaerobic glycolysis in the liver drops, as seen in the fall in the lactate dehydrogenase activity. Further development of chronic renal failure is likely to result in energy loss in the hepatocyte membranes, since the succinate dehydrogenase activity is lowered by the end of the experiment. The elevation of the alkaline phosphatase level in the hepatocytes with its simultaneous decrease in the endothelium of the intralobular capillaries in the development of chronic renal failure may indicate disturbances of the transendothelial and intracellular transport.

Thus, the changes in the activity of all the above-described enzymes on the first day after nephrectomy reflect the rate of the metabolic readjustment, whereas the subsequent dehydrogenase activation points to compensatory metabolic changes in the liver occurring during the development of experimental renal failure. The results obtained allow us to conclude that the metabolic and functional disturbances found in experimental chronic renal failure give rise to the activation of the hepatic function and metabolism.

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