at 4 different temperatures in the presence and in the absence of 1 mM ATP. As may be seen from the figure, the shape of the plots depended in a visible way on the temperature and on the composition of the incubation mixture. In the presence of 1 mM ATP, the plots were hyperbolic at all temperatures tested. In the absence of ATP, the reaction velocities were much lower, particularly in the region of low substrate concentration. The calculated value of cooperativity coefficient was about 1.3 for temperature 30 °C and did not change significantly when the temperature was changed. It may be also noticed from this figure that the range of the substrate concentration in which the activatory effect of ATP was most distinct ('the regulatory region' of the enzyme activity) spreads with the increase of temperature. The table presents calculated values of the substrate concentrations giving the half-maximum velocity ($S_{0.5}$) and maximum velocities (V_{max}) of the reaction catalyzed by rat heart muscle AMP-deaminase for all temperatures tested. As may be seen from this table, both in the presence and in the absence of ATP the increase of temperature caused a rise in V_{max}. The presence of 1 mM ATP did not influence these values significantly. This fact confirms our earlier suggestions that the rat heart AMP-deaminase displays the K_m-type allosteric regulation. On the other hand, at all temperatures tested the values of $S_{0.5}$ calculated for ATP-activated enzyme were much (3–4 times) lower than these calculated for unactivated enzyme. Under both conditions, the $S_{0.5}$ values were lowest at 20 °C and changed only little in the region of 10–30 °C. The rise of temperature from 30 to 40 °C caused a distinct increase of $S_{0.5}$-value for the reaction carried out in the absence of ATP.

The dependence of $S_{0.5}$ and V_{max} on temperature for the reaction catalyzed by purified rat heart AMP-deaminase

Effector added	Temperature (°C)	$S_{0.5}$ (mM)	V_{max} (nmoles/min)
None	10	1.79 (0.30)*	3.69 (0.38)*
	20	1.55 (0.21)*	7.70 (0.53)*
	30	2.85 (0.08)*	17.19 (0.26)*
	40	3.14 (0.28)*	29.75 (0.72)*
1 mM ATP	10	0.42 (0.09)	4.23 (0.21)
	20	0.31 (0.03)	8.12 (0.40)
	30	0.49 (0.01)	17.12 (0.42)
	40	0.86 (0.05)	32.92 (0.66)

The values in the brackets represent SEM or SD*.

Figure 2a presents the Arrhenius plot for the reaction examined in the presence of 1 mM ATP. The value of activation energy (E) calculated from the slope of this dependence being as high as 12140 ± 100 cal/mole of substrate represents the energy which is required for activation of the enzyme-substrate complex[14]. The value of the heat of formation for such complex (ΔH_s) may be calculated from the slopes of the dependence presented in figure 2b. As may be seen from the figure, the line of this dependence is biphasic. If one assumes that $S_{0.5}$ for the ATP-activated reaction represents true thermodynamic dissociation constant K_s, the value of 4280 ± 800 and -9450 ± 300 cal/mole may be obtained from this plot for temperatures below and above 20 °C respectively. This means that at lower temperatures the heat is absorbed during the formation of enzyme-substrate complex, whereas at higher temperatures (above 20 °C) the formation of this complex is evolving the heat[14]. One possible explanation of this peculiarity may be that there are 2 different forms of the enzyme which differs in their thermodynamic properties[15].

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Recovery of renal lactate dehydrogenase (LDH) isoenzyme pattern after obstruction relief in experimental hydronephrosis[1]

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Summary. The release of ureteral occlusion leads to a progressive recovery in LDH isoenzyme pattern with gradual increase of anodic fractions and decrease of middle and cathodic ones. Our findings demonstrate that the recovery is accomplished on the 10–14th day, in agreement with morphological and metabolic observation.

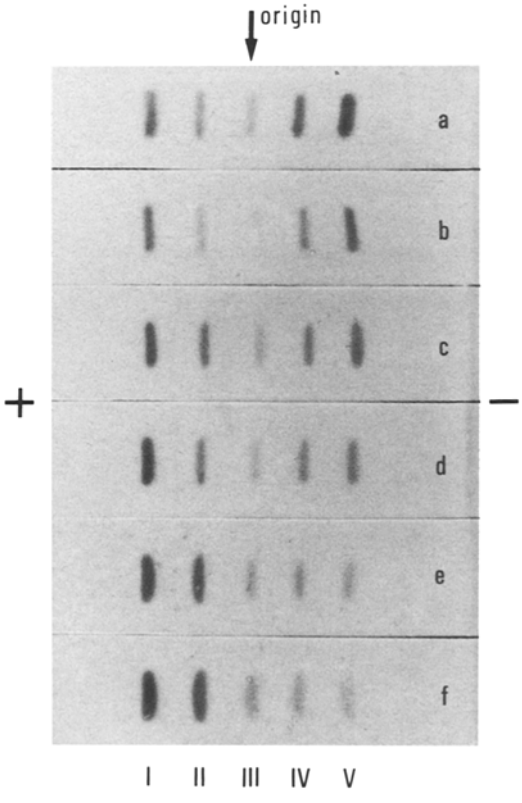
Histochemical and biochemical investigations performed in obstructive nephropathy have demonstrated several alterations in kidney enzyme content[2–4]. In particular, significant changes of renal lactate dehydrogenase (LDH) isoenzymes, as characterized by an increase in middle and cathodic fractions, have been demonstrated after experimental ureteral obstruction[5–7]. Such metabolic alterations have been related both to renal hypoxia and to the presence of a less differentiated cell population as they occur in obstructive nephropathy[5,6].

The present study has been undertaken to ascertain the extent of the recovery of rabbit renal LDH zymogram through the stages following the relief of a long-lasting unilateral ureteral obstruction. High resolution technique has been utilized to compare morphological features by light microscopy.

Materials and methods. The experiments were performed in male white New Zealand rabbits, weighing 1.5–1.8 kg. Under i.v. sodium pentobarbital and chlorpromazine anesthesia, 10 animals were submitted to occlusion of left ureter close to the proximal end. To obtain a reversible obstruction, we followed the method described by Soer et al.[8] based on the use of a hemostatic clip devised for atraumatic occlusion of small vessels. Ureteral occlusion was maintained for 7 days and then removed. Cortical specimens of the obstructed kidneys were obtained 5, 7, 10, 14 and 21 days after removal of the clip. The ureteral patency was checked by observing the outflow of the urine or with the help of i.v. urography.

Biopsies of the cortical tissue were fixed in 3% glutaraldehyde and embedded in epoxy resin for high resolution light microscopy; usual histological techniques were also performed. Specimens of the cortical tissue were immediately homogenized in 0.066 M phosphate buffer by a refrigerated potter homogenizer and processed as previously described for electrophoretic separation of LDH isoenzymes[6,9].

Results. 7-day ureteral obstruction gave rise to a tubular dilation which mainly involved proximal and collecting ducts. Flattening of tubular epithelium had been diffusely shown also if mainly concerning high cells. In the proximal tubular cells, vacuoles of different size were demonstrable in concomitance to a decrease of intracellular organelles.



LDH isoenzyme pattern of the cortical zone of rabbit kidney: *a* 7-day hydronephrosis; *b–f* 5, 7, 10, 14 and 21 days after obstruction relief. The photogram shows a progressive recovery of the cortical LDH isoenzyme pattern, fully restored at 10–14th day when LDH-1 and LDH-2 are the prominent fractions.

Prompt morphological recovery had been observed in early stages up to 10 days; anyway a slight tubular dilation, the presence of epithelial or jaline casts, scattered widening of interstitial space were found up to 21 days. The LDH zymogram of cortical zone was clearly cathodic after 7-day obstruction (figure). The relief of ureteral obstruction was followed by a progressive absolute increase of the fast-moving anodic fraction. Normal LDH isoenzyme pattern was fully restored on 10–14-day observations.

Discussion. Ureteral obstruction induces a significant shift of the normal anodic LDH isoenzyme pattern of the renal cortex toward the cathodic fractions[5–7]. This finding has been interpreted as a 'cellular adaptation' to abnormal metabolic condition following obstruction[5–7]. In agreement with a decrease of renal O_2 availability and consumption after ureteral ligation, it has been suggested that long-lasting hypoxia may be a factor that contributes to such changes in LDH isoenzyme content. Additional explanation for the observed changes may be the appearance of less differentiated cells, since increased DNA synthesis and ^3H thymidine uptake have been demonstrated in obstructive nephropathy[10,11]. This factor, however, mainly accounts for the increase of the middle fraction, well prominent in the early stages of obstruction. Finally, morphological features of the tubular cells, such as flattening with reduced cellular organelles in obstructed kidney, further indicate the presence of less differentiated cells.

Other different enzymatic activities are altered as previous investigations have demonstrated histochemically[2–4]. From these and our studies, it is clear that ureteral obstruction leads to early metabolic events affecting tubular cells. The extent to which such abnormalities are reversible is known for LDH isoenzyme pattern according to the present investigation. Recovery of the normal pattern requires a suitable time interval, so that functional hemodynamic and regenerating processes may take place in the kidney[12,13]. The patho-physiological implication of this work is that recovery of LDH zymogram well correlates with post-obstructive syndrome characterized by impaired sodium and water handling[14,15].

In conclusion, the changes of LDH isoenzyme pattern in obstructive nephropathy are a marker of a morphological and metabolic damage, fully reversible after relief of obstruction.

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