phate reabsorption. As in the case of bicarbonate, the end result would be an increase in tubular fluid buffering. Changes in cystosolic calcium could also cause changes in proximal secretory rates of H^+ [12]. As reviewed by Hulter [13], the issue of direct regulation of secretory activity needs additional work before a definitive mechanism can be used to explain these results.

The regression analysis of ammonia excretion as a function of pH shows that control rats have a greater slope and intercept than do chronic alkalotic rats. This result strongly suggests that in alkalosis an adaptive process is produced whereby the kidney excretes less ammonia than the more acidic control animals. This result is complimentary to that found in acidic dogs, wherein more NH_3 is excreted in acidotic animals at identical U_{pH} [1, 2]. Inspection of the data, however, makes it unlikely that this process is of great physiological significance. Over the midrange of U_{pH} (from about U_{pH} of 6 to 7) there is marked overlap of the data in both populations. The quantitative relationship is such that only at low U_{pH} (probably less than 5.5) can the reduced ammonia excretion be observed in alkalotic rats. It is at U_{pH} greater than 7.0 that alkalotic rats seem to have a greater $U_{NH_3}V$ than controls would have at these same U_{pH}.

In conclusion, evidence has been produced to indicate an adaptive decrease in excretion of ammonia in alkalosis. The physiological significance of the process may, however, be questionable.

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Postischemic ATP levels predict hepatic function 24 hours following ischemia in the rat

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Summary. Hepatic function was assessed by the aminopyrine breath test (ABT) in male Sprague Dawley rats 24 h after partial hepatic ischemia. ABT decreased progressively to 26.3 ($p < 0.05$) and 19.7% of dose ($p < 0.05$) after 90 and 120 min of ischemia, respectively. ABT at 24 h after injury was correlated to the concentration of ATP in the ischemic lobes 1 h after the onset of reperfusion ($r^2 = 0.971$) but not to ALT activity in plasma at 1 h ($r^2 = 0.391$). We conclude that postischemic ATP levels are a better index of subsequent hepatic function than ALT.
Key words. Liver ischemia; hepatic function; aminopyrine demethylation; ATP.

Ischemic damage to the liver is a major problem in clinical liver transplantation because it limits the time during which an explanted liver can be preserved without losing its viability [1]. Therefore, the development of protective agents that may prolong the ischemic interval which is still compatible with adequate function of the transplanted organ in the new host has high priority [2]. Putative protective agents are often judged by their effect on biochemical measurements shortly after a period of ischemia [3, 4]. However, the critical test of protective interventions is their effect on hepatic function following recovery from the operation. In order to screen protective agents it would be helpful to be able to predict functional recovery shortly after ischemia. In spite of improved knowledge regarding the pathogenesis of ischemic damage the critical events that mark the transition from reversible to irreversible loss of function in vivo are poorly defined. Many of the biochemical and morphological changes that can be observed immediately following a period of ischemia may be spontaneously reversible and may not predict hepatic function at a later point in time.

The aim of the present study was to correlate biochemical measurements made shortly after ischemia with hepatic function 24 h after injury in a rat model of partial hepatic ischemia, and to assess the predictive value of selected biochemical parameters for subsequent hepatic function.

Material and methods. Male Sprague-Dawley rats (Süddeutsche Versuchstierfarm Tuttlingen, FRG) weighing 250–300 g were kept in a climatized environment with a 12-h dark-light cycle and had free access to food and water until the morning of the study. The blood supply to the left lateral and part of the median lobe of the liver was interrupted by placing a surgical clip around the appropriate branches of the portal vein and the hepatic artery under brief ether anesthesia [5]. The clip was left in place for 45 to 120 min and was then removed under a second brief ether anesthesia. One hour after reperfusion a blood sample was obtained from the retro-orbital plexus under light ether anesthesia for the determination of alanine aminotransferase (ALT). At that time a portion of the liver was removed from some animals with a clamp cooled in liquid nitrogen for the determination of ATP. Sham-operated animals served as controls.

An aminopyrine breath test was performed 24 h after clamping the liver. (Dimethylamine-^{14}C)-aminopyrine (120 mCi/mmol, Amersham, Buckinghamshire GB) was dissolved in 0.9% NaCl and injected intravenously at a dose of 1 µCi/kg. The animals were then placed in a restraining cage that permits collection of exhaled CO_2 [6]. At the end of the study blood was obtained for determination of ALT, and in some animals the liver was removed for estimation of the extent of necrosis.

Analytical methods. ALT was measured by an automated assay. ATP was measured enzymatically using a kit from SIGMA (St. Louis, MO, USA). $^{14}CO_2$ in breath was collected into hyamine and the trapped radioactivity was measured by liquid scintillation spectrometry [6]. The rate of elimination of $^{14}CO_2$ from breath, k_b, corresponds to the slope of the curve of $^{14}CO_2$ in breath vs time and was calculated by least square regression analysis following logarithmic transformation of the data. The area under the $^{14}CO_2$ exhalation-time curve, AUC, was calculated by the trapezoidal rule.

The relationship between functional impairment and extent of necrosis was studied in 18 rats. Following the aminopyrine breath test the ischemic lobes of the liver were cut into 2 mm thick slices and incubated for 20 min at 37 °C in 1 mM tetranitroblue tetrazolium chloride. The extent of necrosis was estimated by comparing the fraction of unstained, necrotic areas with the total area of the slices [7].

Statistics. All results are given as mean ± SE. The contribution of ischemia and sampling errors to the variation of the values of the measured parameters was determined by one-way analysis of variance. The variation due to ischemia was significantly ($p < 0.001$) larger than the variation due to sampling errors in each experiment. Differences between group means were assessed by the non-parametric test of Wilcoxon.

Results. One hour after 45 min of ischemia the concentration of ATP in the reperfused ischemic lobes of the liver was not significantly different from sham-operated controls. With increasing duration of the period of ischemia, however, there was a progressive depletion of ATP; the values after 60 and 90 min of ischemia were significantly lower ($p < 0.02$) than in controls, and values after 120 min of ischemia were significantly ($p < 0.05$) lower than after 90 min of ischemia (table). The activity of ALT increased significantly with increasing duration of the ischemia (table). Twenty-four hours later the same pattern persisted. Although the plasma transaminases had decreased significantly ($p < 0.01$, paired rank test) in each group compared to 1 h after reperfusion they were still markedly elevated in the two groups subjected to 90 and 120 min of ischemia.

After an initial rise the exhalation of $^{14}CO_2$ in breath declined monoexponentially in all animals. Elevated transaminases at the time of the breath test were associated with decreased demethylation of aminopyrine. The logarithm of the activity of ALT in plasma 24 h after ischemia correlated significantly with the rate of elimination of $^{14}CO_2$ from breath, K_b ($r^2 = 0.795$, $n = 38$, $p < 0.001$; fig. 1), and the AUC ($r^2 = 0.518$, $p < 0.001$). The correlation between ALT activity 1 h after reperfusion and the elimination of $^{14}CO_2$ from breath was not as good ($r^2 = 0.391$), which suggests that the activity of ALT shortly after the ischemic insult is not a very good index of hepatic function 24 h later. On the other hand, the concentration of ATP in the ischemic lobes 1 h after reperfusion was a much better predictor of hepatic function 24 h later ($r^2 = 0.965$, fig. 2). The capacity to demethylate aminopyrine decreased with increasing duration of ischemia (table), the AUC following 90 and 120 min of ischemia being significantly lower ($p < 0.05$) than in control animals and animals subjected to 45 min of ischemia. In the animals where the volume fraction of necrotic tissue was assessed the AUC showed a significant negative linear correlation ($r^2 = 0.752$, $p < 0.001$) with the estimate of the extent of hepatocellular necrosis, which ranged from 0 to 95% of the ischemic lobes.

Discussion. Our data indicate that the extent of functional impairment 24 h after an ischemic insult increases in proportion to the duration of ischemia. Except for very short periods of ischemia there does not appear to be a threshold time prior to which all cells recover their function and beyond which most cells are irreversibly damaged. The good correlation between extent of necrosis and functional impairment suggests that even after a relatively short period of ischemia some cells are irreversibly injured and do not recover their

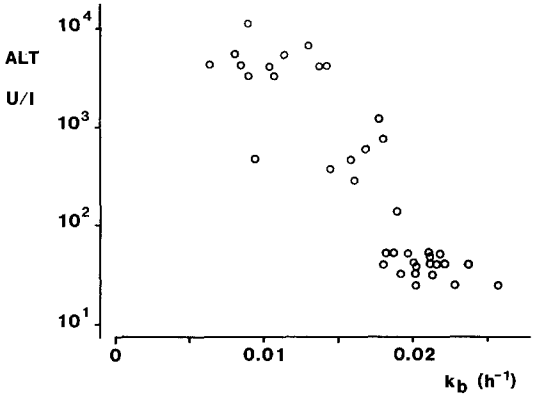

Figure 1. Rate of disappearance of $^{14}CO_2$ from breath (k_b) following the administration of (dimethylamine-^{14}C)-aminopyrine and activity of ALT in plasma 24 h after partial hepatic ischemia of 0 to 120 min duration.

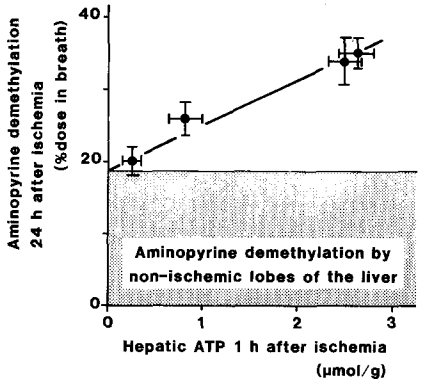

Figure 2. Concentration of ATP in ischemic lobes of the liver 1 h after reperfusion and demethylation of aminopyrine 24 h later in sham-operated controls and in animals that had undergone 45, 90 and 120 min of ischemia. The demethylation of aminopyrine is expressed as % of the administered dose of (dimethylamine-^{14}C)-aminopyrine appearing in breath as $^{14}CO_2$ in 2 h. Mean values for 5–8 animals per group. The shaded area represents the extrapolated contribution to aminopyrine demethylation by the approximately 50% of the liver that was not subjected to ischemia in the chosen experimental model of partial hepatic ischemia.

Concentration of ATP in reperfused ischemic lobes of the liver and activity of ALT in plasma 1 h after reperfusion, and area under the exhalation-time curve of $^{14}CO_2$ in breath (AUC) and fractional rate of disappearance of $^{14}CO_2$ from breath (k_b) following the administration of (dimethylamine-^{14}C)-aminopyrine 24 h after partial hepatic ischemia. Mean ± SE, 5–8 animals per group.

	ATP at 1 h µmol/g	ALT at 1 h U/l	AUC at 24 h % dose	k_b at 24 h h^{-1}
Sham operated	2.61 ± 0.20	51 ± 3	35.1 ± 2.3	1.28 ± 0.03
45 min ischemia	2.50 ± 0.18	1851 ± 369[a]	34.1 ± 3.6	1.22 ± 0.04
90 min ischemia	0.82 ± 0.18[a]	3334 ± 567[a]	26.3 ± 2.3[a]	0.80 ± 0.08[a]
120 min ischemia	0.26 ± 0.11[a]	5117 ± 250[a]	19.7 ± 2.1[a]	0.71 ± 0.07[a]

[a] Significantly ($p < 0.05$) different from sham-operated controls.

function in 24 h. Clearly, a substantial interindividual susceptibility to ischemic injury exists among various hepatocytes in the intact organ.

The concentration of ATP in the ischemic lobes of the liver 1 h after reperfusion proved to be a good predictor of hepatic function 24 h later (fig. 2). A similar strong correlation between postischemic ATP levels and subsequent functional recovery has recently been demonstrated in a model of renal ischemia [8]. In the chosen model of partial hepatic ischemia approximately 50% of the total mass of the liver is subject to ischemia. Thus, non-ischemic parts of the liver contribute one half of the total demethylation capacity, and even with total loss of function in the ischemic lobes one would expect a residual demethylation of 15–20% of the dose. This fraction corresponds to the intercept of the regression line with the ordinate in figure 2 (19% dose appearing in breath as $^{14}CO_2$). The graph indicates that with concentrations of ATP in the ischemic lobes approaching zero 1 h after reperfusion there will be essentially no functional recovery, whereas with ATP levels close to the physiological concentration complete recovery will occur.

The activity of ALT in serum shortly after ischemia was poorly correlated to hepatic function 24 h later. This is not surprising considering the fact that additional ischemic injury may occur much later, as manifested by the release of OCT [9]. In contrast to ALT 1 h after ischemia the activity of ALT at the time of the aminopyrine breath test was strongly correlated with hepatic function (fig. 1). The level of ALT at 24 h is probably related to the total amount of ALT released following the ischemic insult and therefore provides a better index of the extent of hepatic damage than the activity of ALT shortly after ischemia.

In conclusion, our data show that hepatic function decreases with increasing duration of ischemia indicating marked interindividual differences in the susceptibility of hepatocytes to the effects of ischemia in vivo. The concentration of ATP in the ischemic lobes 1 h after reperfusion predicts hepatic function 24 h later. Recent data obtained in man support this conclusion [10]. Measurements of the concentration of free ATP by non-invasive means such as NMR might serve as an index of the subsequent functional capacity of transplanted livers and might be helpful in assessing the effect of agents claimed to give protection against ischemic liver injury.

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Maternal stress alters monoamine metabolites in fetal and neonatal rat brain

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Summary. Heat-restraint stress given rats during the last week of gestation significantly altered dopaminergic dihydroxyphenylacetic acid and homovanillic acid (DOPAC and HVA) and noradrenergic 3-methoxy-4-hydroxy-phenyl-ethylene glycol (MOPEG) forebrain-hypothalamic monoamine (MA) metabolites in female offspring. On gestational day 21, HVA and MOPEG were significantly higher and lower, and on postnatal day 1 all were higher. There were virtually no differences in brain MA concentrations in males. Thus MA metabolic concentrations differ in fetal-neonatal forebrain-hypothalamus as a function of sex differences and maternal stress.
Key words. Maternal stress; monoamine metabolites; perinatal rat brain.

Neuroendocrine-CA systems typically undergo a period of functional organization in parallel with brain development. In the rat, the critical period corresponds to late fetal and early postnatal life. The process is most obvious in the case of the gonadal axis where it results in a number of sexually dimorphic brain functions [1, 2]. Neuroendocrine-neurotransmitter systems are particularly sensitive to environmental influences during this phase of organization. Support for this idea comes from observations that external factors such as alcohol, barbiturates, nicotine and now prenatal stress interfere with the development of the rat gonadal-CA axis [3–8]. With respect to prenatal stress, Moyer, Herrenkohl and Jacobowitz [9] have suggested that maternal stress may modify the neuroanatomical and biochemical organization of the brains of both males and females and turn the direction of male fetal brain development toward that of the female sex. They combined the microdissection procedure of Palkovits for removing individual brain nuclei with sensitive radioisotopic enzymatic assays for norepinephrine (NE) and dopamine (DA). In pregnant rats, Moyer et al. [9] discovered that stress during pregnancy reduced steady-state levels of NE in brain regions associated with gonadotropic secretion. The major noradrenergic pathway that underwent change during stress was the ventral ascending bundle (i.e., the medial preoptic nucleus, anterior hypothalamus, and median forebrain bundle). They also reported that the locations of DA decreased as a function of prepartal stress. Moyer et al. [10] also examined the effects of prenatal stress on CA concentra-