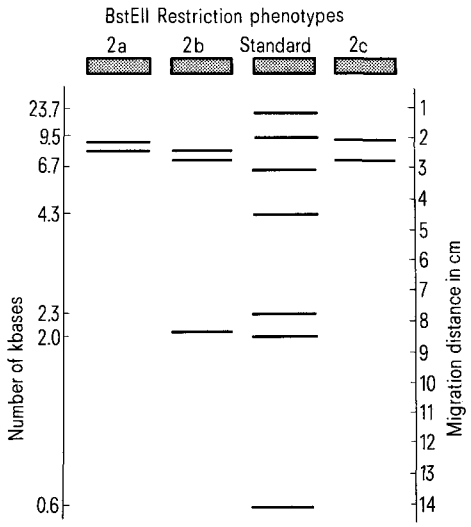
most striking but misleading result of the study is the abnormally high plasma NA level observed in the healthy, unsedated, very young children (age 4 months to 2 years). An age dependency for the level of plasma NA in children could be assumed from this result.

However, this assumption does not hold true, because in the control group of children who were under sedation, no such a difference in the NA levels between very young and older children was observed (fig.). Furthermore, the NA levels of the sedated very young and the sedated older children did not differ from the NA levels obtained from the other healthy, unsedated children above 2 years of age. Undoubtedly, the results from healthy, unmedicated children cannot be compared directly with results from children who were sedated and were under heart catheterization. However, if plasma NA concentration is really age dependent in children, then this must also hold true for the children under sedation during heart catheterization; this was not the case. So, except for the abnormally high plasma NA levels in the unsedated very young children, the normal non-basal plasma NA level in children was in the range of 0.40 ng/ml, without substantial variation between the different age groups (fig.). The plasma NA concentration is not different from the basal plasma levels commonly observed in adults[2-4].



Plasma concentrations of noradrenaline and adrenaline (mean ± SD) in children of various age: Comparison of the results from healthy unsedated children and sedated children.

No differences in the plasma A concentrations were found, either between the young and the older children or between the sedated and unsedated children. Although in most of the children the A levels were as low as in adult subjects (range 0.04–0.14 ng/ml plasma), 4 of the 32 healthy unsedated children and 3 of the 14 sedated children had A levels which were abnormally high (0.41–0.49 ng/ml plasma) and in the same range as their NA concentration. Such abnormally high plasma A levels cannot be observed in adults under resting conditions[5]. An explanation for this finding in children cannot be given; a technical error is excluded.

The plasma CA concentrations given for healthy children in this study must not be taken as basal values. True basal plasma CA levels can be obtained only from unmedicated subjects under defined physical and psychological resting conditions; taking the blood sample for the assay without any pain to the subject by means of an indwelling catheter. In this respect our study is of great practical significance; showing that the child's level of agitation is highly influential in determining the actual plasma NA level in the very young. However, in children older than 2 years of age, the actual plasma NA concentration is obviously not substantially influenced by excitement, hence similar values were obtained in sedated and unsedated children.

Our results suggest that in children older than 2 years of age the plasma CA concentration measured in samples taken from the cubital vein under non-basal conditions is normally sufficiently accurate to exclude a severe pathological derailment of the sympatho-adrenal system. In contrast, in very young children, misleadingly high plasma NA levels must be expected if blood is taken without observing true resting conditions.

1 Da Prada, M., and Zürcher, G., Life Sci. *19* (1976) 1161.
2 Lake, C.R., Ziegler, M.G., and Kopin, I.J., Life Sci. *18* (1976) 1315.
3 Dimsdale, J.E., and Moss, J., J. Am. med. Ass. *243* (1980) 340.
4 Planz, G., and Planz, R., Eur. J. clin. Pharmac. *19* (1981) 83.
5 Planz, G., and Planz, R., Experientia *35* (1979) 207.

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Influence of fasting and of a high-protein diet on the activity of rat liver γ-glutamyl transferase

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Summary. Fasting for 2 and 4 days progressively increases the activity of hepatic γ-glutamyl transferase (γ-GT) in rat. A high-protein diet (with 42.6 energy percent of protein) for 45 and 90 days inhibits it. It seems that liver γ-GT is susceptible to nutritional influences.

The enzyme γ-glutamyl transferase (γ-GT, EC 2.3.2.2) attracts attention because of its presence at significantly increased levels in serum in a number of hepatobiliary disorders – cholestasis, hepatomas, alcoholic injuries, porphyria cutanea tarda, and the effects of drugs and chemicals[1-4]. Studies of possible nutritional effects on liver γ-GT are scarce[5,6]. We have measured the activity of hepatic γ-GT during fasting and during a more prolonged high-protein diet.

Material and methods. Female Wistar albino rats, weighing 150–180 g were used. In experiment I the animals were divided into the following groups; nonfasted controls; rats fasted 2 days; rats fasted 4 days; rats re-fed for 2 days after a 4-day fast; rats re-fed for 4 days after a 4-day fast. The animals were housed in individual wire-bottom cages and were allowed free access to a commercial pellet diet except during the fasting periods. No mortality was observed on the 2nd day of food deprivation, but 20% of the animals died on the 4th day of fasting.

Experiment II included 4 groups: rats fed for 45 days on a

Table 1. γ-GT activity in rat liver homogenates in fasting and after refeeding. The animals were fed a commercial pellet food. The results are expressed by mean ± SD; n = 7-8*

Groups γ-GT	Control rats	Rats fasted 2 days	Rats fasted 4 days	Rats refed for 2 days after a 4-day fasting	Rats refed for 4 days after a 4-day fasting
nmoles/min/mg protein	1.18 ± 0.20	1.97 ± 0.68**	2.06 ± 0.41***	1.52 ± 0.44	1.09 ± 0.19
Hepatic activity in μmoles/min corresponding to 1 kg of body mass	12.6 ± 1.9	17.5 ± 5.6**	25.1 ± 4.6***	16.7 ± 3.8**	13.1 ± 2.2

*Comparison with the control rats: **$p < 0.05$ and ***$p < 0.001$.

Table 2. γ-GT activity in liver homogenates of rats fed standard and high-protein diets. The results are expressed by mean ± SD; n = 7-8

Groups γ-GT	Rats fed for 45 days		Rats fed for 90 days	
	Standard diet (21.8 energy percent of casein)	High-protein diet (42.6 energy percent of casein)	Standard diet	High-protein diet
nmoles/min/mg protein	1.14 ± 0.42	0.27 ± 0.10	0.74 ± 0.20	0.40 ± 0.05
	$p < 0.001$		$p < 0.001$	
Hepatic activity in μmoles/min corresponding to 1 kg of body mass	8.8 ± 3.2	2.6 ± 0.8	7.4 ± 2.3	3.8 ± 0.5
	$p < 0.001$		$p < 0.001$	

standard diet - 21.8 energy percent of casein, 69.5% of starch and 8.7% of sunflower oil + butter (2:1); rats fed for 45 days a high-protein diet - 42.6 energy percent of casein, 48.7% of starch and 8.7% of lipids; rats fed for 90 days on a standard diet; rats fed for 90 days on a high-protein diet. Each animal consumed an average of 25 g of food daily. Both diets were isoenergic (369 kJ daily). The mineral and vitamin content corresponded to the energy intake. There was no mortality.

In both experiments 8 animals of each group were sacrificed by decapitation after 16 hours of starvation. Because of this starvation, the refeeding in experiment I lasted 2 or 4 incomplete days.

A piece of liver was homogenized (1:10 - m/v) with a solution containing 5 mmoles/l Tris, 8 mmoles/l $MgCl_2 \cdot 6H_2O$ and 100 mmoles/l KCl, pH 7.5 in a glass Potter-Elvehjem homogenizer with a teflon pestle. The homogenate was mixed with an equal volume of 5 ml/l Triton X-100 in the same solution and after 30 min the activity of γ-GT was determined by the method of Szasz[7]. The total protein content was determined according to Lowry et al.[8].

Results and discussion. Compared with the nonfasted controls, the total protein (in mg/g fresh tissue) was significantly changed both in the rats fasted 2 days (decrease by 38%, $p < 0.001$) and in the animals refed for 2 days after a 4-day fasting (decrease by 27%, $p < 0.001$). The total protein in the homogenates was higher in the rats fed a high-protein diet, compared with the animals fed a standard diet (increases by 38%, $p < 0.01$ on the 45th day and by 12%, $p < 0.05$ on the 90th day). Because of these changes, the values of γ-GT are expressed both in terms of specific activity and in μmoles in the liver per kg body mass (tables 1 and 2). Irrespective of the manner of expression, fasting raises hepatic γ-GT and the high-protein diet reduces it.

A possible explanation of the changes in the 2 experiments could be suggested by the relations between γ-GT and its basic natural substrate, reduced glutathione (GSH). The protein deficiency increases not only the degradation of proteins in the liver, but also their synthesis[9]. Probably, an intensive degradation of GSH to its structural amino acids occurs, and the latter are involved in anabolic processes. The initial stage of GSH degradation is carried out by γ-GT[10], which could be induced. The elevated gluconeogenesis in fasting[11] also benefits de novo synthesis of γ-GT, which consists of 2 unequal glucopeptide subunits, containing 19% of carbohydrates[12]. Tateishi et al.[6] make a casual mention of increase of liver γ-GT in fasting. In the high-protein feeding an intensive GSH degradation should not be necessary and thus hepatic γ-GT is reduced.

The possibility that other mechanisms may contribute to these findings cannot be ruled out. Fasting leads to an increased formation of glutamine in liver[13]. This exerts an activating effect on γ-GT[14]. On the other hand, γ-GT not only cleaves the γ-glutamyl group of GSH, but also promotes its oxidation to GSSH[15]. It is a powerful inhibitor of protein synthesis[16]. Probably, the decreased γ-GT activity leads to a lower rate of GSSH formation, which might facilitate the utilization of the increased aminoacid intake.

Whatever the exact mechanisms of the observed changes are, it is obvious that liver γ-GT is susceptible to nutritional influences.

1 Köttgen, E., Internist *21* (1980) 231.
2 Schmidt, E., and Schmidt, F.W., Dtsch. med. Wschr. *105* (1980) 967.
3 Adjarov, D., and Ivanov, E., Br. J. Derm. *102* (1980) 541.
4 Ivanov, E., Krustev, L., Adjarov, D., Chernev, K., Apostolov, I., Drenska, E., Stefanova, M., and Pramatarova, V., Enzyme *21* (1976) 8.
5 Mørland, J., Huseby, N.-E., Sjøblom, M., and Strømme, J., Biochem. biophys. Res. Commun. *77* (1977) 1060.
6 Tateishi, N., Higashi, T., Nakashima, K., and Sakamoto, Y., J. Nutr. *110* (1980) 409.
7 Szasz, G., Clin. Chem. *15* (1969) 124.
8 Lowry, O., Rosebrough, N., Farr, A., and Randall, R., J. biol. Chem. *193* (1951) 265.
9 Garlick, P., Millward, D., James, W., and Waterlow, J., Biochim. biophys. Acta *414* (1975) 71.
10 Curthoys, N., and Hughey, R., Enzyme *24* (1979) 383.
11 Andersen, J., and Zakim, D., Biochim. biophys. Acta *201* (1970) 236.
12 Meister, A., in: Functions of glutathione in liver and kidney, p. 43. Eds H. Sies and A. Wendell. Springer, Berlin 1978.
13 Aikawa, T., Matsutaka, H., Yamamoto, H., Okuda, T., Ishikawa, E., Kawano, T., and Matsumura, E., J. Biochem., Tokyo *74* (1973) 1003.
14 Tate, S., and Meister, A., J. biol. Chem. *249* (1974) 7593.
15 Tate, S., and Orlando, J., J. biol. Chem. *254* (1979) 5573.
16 Nolan, R., and Hoagland, M., Biochem. biophys. Res. Commun. *51* (1973) 444.

0014-4754/83/050498-02$1.50 + 0.20/0