in turn strengthened in some way associations among subunits. This inference was drawn from the fact that the release was also retarded when the immobilized ferrihemoglobin was treated with the buffer of pH 10.5, which is otherwise very efficient for the dissociation of native hemoglobin[13].

Recently Amiconi et al.[14] has reported that macromolecular polyanions, dextran sulfate and heparin, were strongly bound by hemoglobin and that their complexes existed as octamer. A primary interaction has been described to be electrostatic also in this case, but resulting complexes were quite different from those of hemoglobin-PLL. Thus it may be said that polyanion and polycation produce a distinct effect on molecular assembly of hemoglobin molecule.

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γ-Glutamyltranspeptidase (GGTP) and cytochrome P-450 after portacaval shunt in the rat

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Summary. The increased hepatic activity of γ-glutamyltranspeptidase after portacaval shunt is due to derepression of a fetal enzyme rather than to an induction mechanism.

End to side portacaval shunt (PCS) in the rat leads to an increase of GGTP activity in the liver; under normal conditions it is rather low[1]. High levels of hepatic GGTP activity, similar to those after PCS, have been observed during fetal development[2]. This increase has been interpreted as a sign of functional changes occurring in the liver and leading to a reappearance of the fetal enzyme[2]. It has been speculated that the underlying mechanism may be associated with the derepression of an enzyme normally present only in fetal and neonatal liver.

Since GGTP is located mainly in the microsomal fraction of the liver, it might be stimulated by different pharmacological agents[3]. It has never been shown, however, whether the increase of GGTP after PCS may not be due to enzyme stimulation or induction rather than to derepression of a fetal enzyme. We therefore also examined PCS rat livers for cytochrome P-450, an enzyme known to be induced by many pharmacological agents, as is GGTP. We wanted to see if the functional changes after the shunt operation might have an effect on cytochrome P-450 content similar to that on GGTP activity.

Material and methods. The PCS experiments were carried out on a strain of SPF Sprague-Dawley male rats bred in a closed colony. Free access to standard food (Altromin R 300) and water was allowed. Coprophagy was not prevented. The general conditions for maintenance of the animals and the techniques of portacaval shunting and of sham operation have been described earlier[4]. 10, 20 and 30 days after PCS the animals were killed by decapitation. The liver was removed and processed immediately. The

Activity of γ-glutamyltranspeptidase (GGTP) and amount of cytochrome P-450 in liver of control rats and in 3 groups of animals 10, 20 and 30 days after portacaval shunt (PCS) operation

Enzymes	Sham operated controls (n = 10)	Days after PCS 10 (n = 8)	20 (n = 4)	30 (n = 7)
GGTP				
nmoles/min/g liver	112 ± 29	329 ± 39	386 ± 118	613 ± 188
		p < 0.001	p < 0.001	p < 0.001
nmoles/min/mg microsomal protein	2.3 ± 0.6	6.4 ± 0.8	7.9 ± 2.4	14.3 ± 4.4
		p < 0.001	p < 0.001	p < 0.001
Cytochrome P-450				
nmoles/g liver	27 ± 11	11 ± 6	14 ± 2	13 ± 6
		p < 0.005	p < 0.05	p < 0.01
nmoles/mg microsomal protein	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.06	0.3 ± 0.13
		p < 0.001	p < 0.01	p < 0.005

Each value represents the mean ± SD of the number of animals (n). p-Values indicate significance versus sham-operated controls.

microsomal fraction was prepared according to a method described previously[5].

The activity of GGTP and the amount of cytochrome P-450 were determined in the microsomal fraction. The activity of GGTP was measured using the method of Adolph[6] and cytochrome P-450 was quantified by the method of Omura and Sato[7]. The protein content was determined by the method of Lowry et al.[8].

Results. Compared to the sham operated controls, hepatic GGTP activity increased significantly after PCS (table), when expressed per microsomal protein as well as per g wet liver weight. 10 days after PCS, the amount of cytochrome P-450 was significantly decreased and remained at this low level for the experimental duration of 30 days.

Discussion. Portacaval shunting leads to an increase of GGTP activity in rat liver as has been shown previously[1,2]. No similar elevation, however, could be observed for cytochrome P-450 but rather a decrease which has been attributed to a deprivation of one or several substances normally supplied by portal blood and contributing to the maintenance of the cytochrome level[9].

From our data and other observations it can therefore be concluded that PCS does not lead to an induction of cytochrome P-450. It is therefore unlikely that the increased activity of GGTP and its histochemical appearance is due to any induction mechanism. Rather, a derepression of a fetal enzyme, as has been put forward as a hypothesis[2], must be the cause of the increased hepatic GGTP activity after PCS.

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A high molecular weight inhibitor of Ca^{2+}-dependent neutral protease in rat brain

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Summary. An endogenous, heat-stable inhibitor of high mol. wt (approximately 3×10^5) was found to be present in rat brain, which inhibited Ca^{2+}-dependent neutral protease specifically but not due to its binding of Ca^{2+} in the medium.

The occurrence of a Ca^{2+}-dependent neutral protease in the brain was first described by Guroff[1]. We have reported the enhancement of the activity of this protease in human malignant glioma tissues, as well as in methylcholanthrene-induced glioma G203 cells in mice[2]. In the course of purification of the Ca^{2+}-dependent neutral protease from rat brain by chromatographies, we often encountered increase in recovery of total activity of this enzyme over 100%, sometimes even more than twice the activity found before the chromatography. This prompted us to search for an inhibitor or inhibitors which might have been present in the starting material and removed during the purification procedures. We have thus found the presence of a high mol. wt protease inhibitor in the soluble fraction of rat brain homogenate, which inhibits the Ca^{2+}-dependent neutral protease specifically but not due to its binding of Ca^{2+} in the medium.

Materials and methods. Wistar strain rats, weighing 120–180 g, were used. Ca^{2+}-dependent neutral protease in the brain was partially purified as previously described[2]. The preparation contained 1 mg protein per ml. Caseinolytic activity at pH 7.5 (Tris-HCl) was assayed by the method of Kunitz[3], using 5 mM $CaCl_2$ and 10 mM cysteine as the activators[2]. The release of acid-soluble peptides was determined spectrophotometrically at 750 nm using Folin-Ciocalteau reagent.

Results and discussion. Brain tissues were quickly removed and homogenized with 3 vol. of 20 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 5 mM enthylene glycol bis (β-aminoethyl ether) -N,N,N′,N′,-tetraacetic acid (EGTA), and 5 mM 2-mercaptoethanol. The homogenate was centrifuged at 105,000× g for 1 h, and the soluble fraction thus obtained was concentrated by ultrafiltration with Amicon PM-10 membrane. The concentrate was fractionated on a Sephadex G-200 column. An aliquot of each fraction was preincubated with the partially purified preparation of Ca^{2+}-dependent neutral protease, and then the caseinolytic activity of the mixture was assayed. As shown in figure 1, an enhancement of the protease activity over the level for the pre-existent protease can be seen with

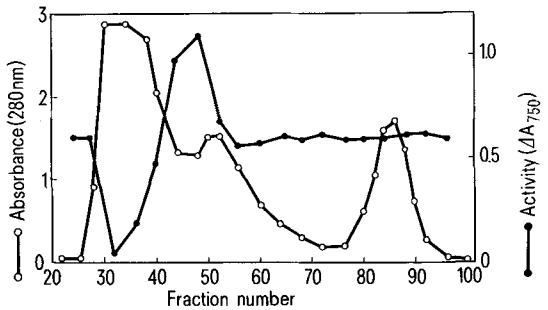

Fig. 1. Resolution of a high mol. wt protease inhibitor and Ca^{2+}-dependent protease in rat brain by gel filtration through Sephadex G-200. The concentrated soluble fraction of rat brain (257 mg protein in 8.0 ml) was applied to a column (3.0× 100 cm) which had been equilibrated with 20 mM EGTA and 5 mM 2-mercaptoethanol in the cold. Elution was carried out with the same buffer, and fractions of 6 ml each were collected at a flow rate of 10 ml/h. A 0.5-ml aliquot of each fraction was preincubated with 50 µl of Ca^{2+}-dependent protease, partially purified from rat brain, at 37 °C for 10 min, and then assayed for caseinolytic activity at 37 °C for 30 min.