Histochemical Demonstration of γ-Glutamyltranspeptidase in Rat Liver after Portacaval Anastomosis

As already described, γ-glutamyltranspeptidase (GGTP, γ-glutamyltransferase, E.C.2.3.2.1) is barely measurable in adult rat liver[1]. By histochemical demonstration, the enzyme has been detected in Kupffer cells, endothelium of periportal vessels and bile duct epithelium[2]. High activity of GGTP has been reported in liver of fetal and normal neonatal rats[3,4], in chemically induced rat hepatomas[5] and in rats after portacaval shunt (PCS[4]). These findings were interpreted as a reaquirement of a biochemical feature, which dominates in the fetus but is repressed in adult liver.

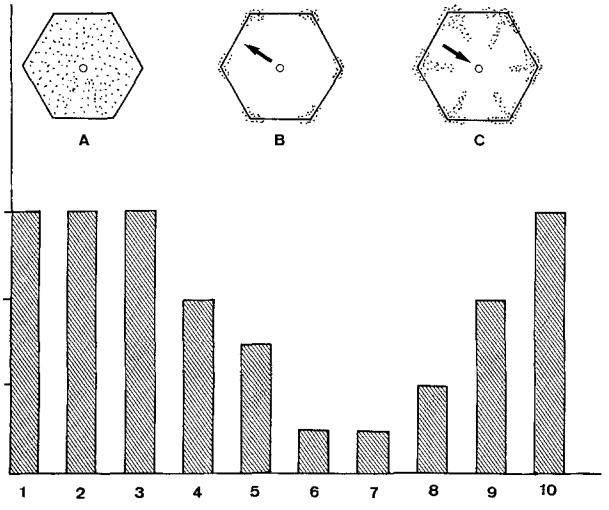

Fig. 1. The striped bars represent the relative evaluation of GGTP-activity in the rat liver by histochemical demonstration: 1. 15th day of gestation; 2. 18th day of gestation; 3. newborn; 4. 3rd day after birth; 5. 5th day after birth; 6. 10th day after birth; 7. adult rat; 8. 10 days after portacaval shunt (PCS); 9. 20 days after PCS and 10. 30 days after PCS. The hexagonal scheme show in A) the diffuse distribution, in B) the flux into the periphery and in C) the reappearance of the enzyme in a liver lobulus.

The aim of this study was to demonstrate by histochemical means whether such ontogenic reversion, with its aquired fetal biochemical features after portacaval shunt, also develops the same distribution of enzymatic activity after PCS as could be demonstrated in fetal liver.

The methods applied were the portacaval anastomosis after HERZ et al.[6] with subsequent histochemical demonstration of GGTP after RUTENBERG et al.[2] using the substrate N-(γ-L-glutamyl)-4-methoxy-2-naphthylamide (Cyclo Chemical Corp., Los Angeles, Calif., USA).

As Figure 1 shows, the GGTP-distribution in the liver undergoes a characteristic change in postnatal life as well as after portacaval shunt operation. In the fetal and newborn liver, the enzyme is regularly distributed through the whole tissue. All hepatocytes show a fine reaction deposit, and the bile capillaries apparently contain most of the activity.

In the postnatal period, the enzyme gradually becomes localized towards the periportal areas of the lobuli. At the 10th day after birth, the activity was low as in the adult rat, and the localization of the enzyme deposit was the same. These observations are in accordance with the biochemical determinations[4]. In the period after PCS, the hepatocytes around the hepatic lobules show the first signs of enzymatic activity. Subsequently, all liver cells between the periportal areas and the middle of the lobule,

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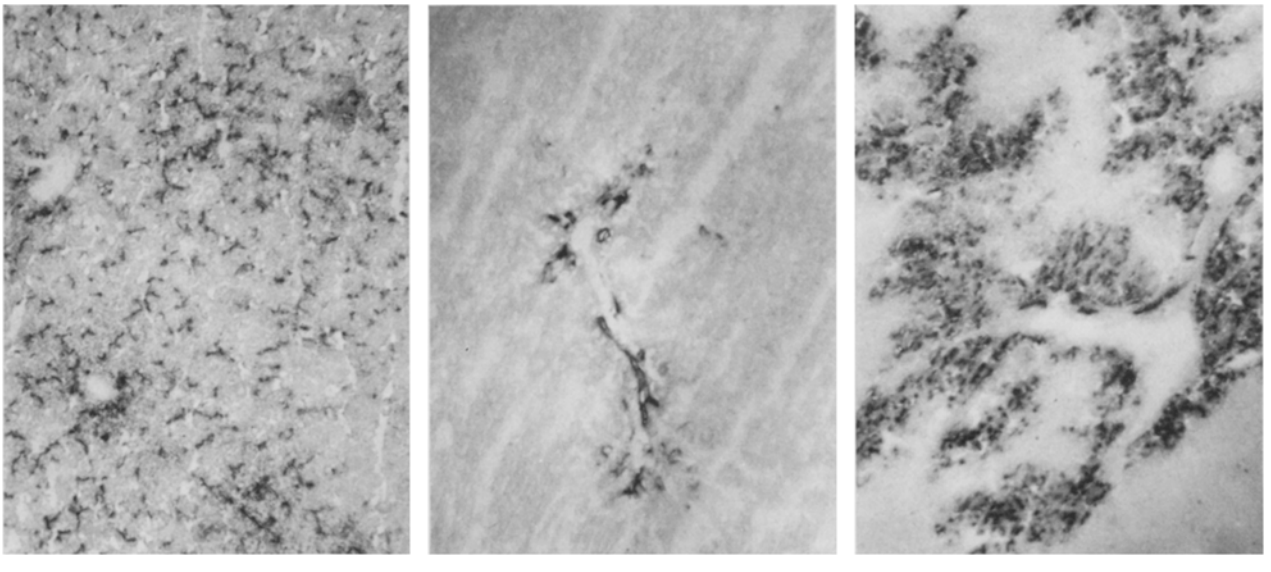

a) b) c)

Fig. 2. Distribution of γ-glutamyltranspeptidase as histochemical reactionproduct in the rat liver of a) newborn, b) adult and c) 30 days after portacaval shunt.

begin to show GGTP synthesis. This enzyme fluctuation in the postnatal period, and after deviation of portal blood supply, can be understood if one considers the fine circulatory situation in the hepatic lobulus. There is a zonal relationship between cells constituting the lobulus and their blood supply. The hepatocytes situated close to the axial terminal branches (vena portae and arteria hepatica propria) are the first to be supplied with fresh blood, rich in oxygen and nutrients. They form the most active and resistant core of the lobulus: they are the last to die and the first to regenerate[7]. In our case, they are the last to stop and the first to start to re-synthesize GGTP. The more distant the cells are from the site where the terminal portal and arterial branches empty into sinusoids, the poorer is the quality of blood that bathes them.

After the portacaval shunt, all blood originating from the intestines, spleen and pancreas (insulin, glucagon) stopped flowing through the liver. The blood is then provided by the arteria hepatica propria coming from the aorta.

Whether it is the change in substrate or oxygen supply, or both ,which is responsible for the high enzyme activity arizing in shunted rats, requires further studies.

Zusammenfassung. Mit Hilfe der histochemischen Methode konnte nachgewiesen werden, dass die Leberzellen nach der portocavalen Shunt-Operation die Fähigkeit der GGTP-Synthese wiedererlangen. Die Enzymabnahme in der postnatalen Periode und ihre Zunahme nach der portocavalen Anastomose stimmt sowohl zeitlich als auch mengenmässig im histochemischen Präparat mit den biochemischen Ergebnissen überein.

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Has Ricin an Enzyme Inductive Effect?

Ricin is a component of the common castor oil seed (*Ricinus communis*, Euphorbiaceae) and is one of the representatives of the socalled phytotoxins. The phytotoxins are very toxic proteinaceous compounds of plant origin with a molecular weight of some 10,000. They play important physiological roles in plants, and they resemble the true bacterial toxins in many respects, especially the diphteria and tetanus toxin.

According to HAUSCHILD[1], ricin is one of the 5 most toxic materials known: tetanus toxin, botulinus toxin, diphteria toxin, gramicidin, ricin. FUHRMAN[2] considers that ricin is the most toxic substance of plant origin.

The signs and symptoms of ricin intoxication vary very much, according to the size of the dose. More than 6% of the cases are fatal (SOLLMANN[3]). Recently BALINT[4] has published an exhaustive review on ricin. It was reported that in late (subacute) ricin intoxication the smooth endoplasmic reticulum of liver cells hypertrophies and the mitochondria are shrunk (BALINT[5,6]).

REMMER[7] showed that in the case of an enzyme induction, caused by different drugs, the hexobarbital sleeping-time is reduced in rats, due to the induction of the drug metabolizing enzyme system in the liver.

In this communication our preliminary results are reported which raise the possibility that ricin also could have an enzyme inductive effect in the liver.

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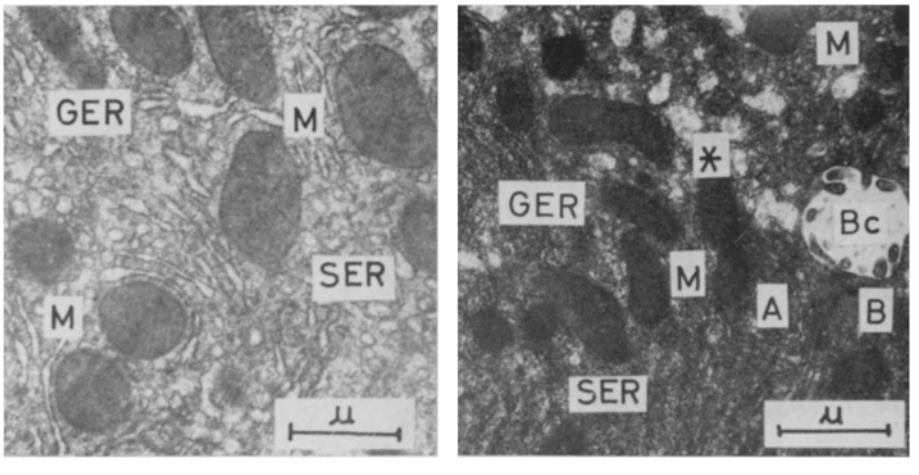

Comparative electron micrograph of a section of rat liver treated with 2×10 μg/kg ricin, taken 1 week after first ricin administration. Glutaraldehyde fixation, araldite embedding. Uranyl-acetate-lead-citrate contrasting. Left side: Control animal. M, mitochondria; SER, smooth endoplasmic reticulum; GER, granulated endoplasmic reticulum. Right side: treated animal. M, mitochondria; SER, smooth endoplasmic reticulum; GER, granulated endoplasmic reticulum; A and B, 2 neighbouring cells; Bc, bile canaliculus; * unidentified material, due to ricin (BALINT[5,6]).