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HETEROGENEITY OF RAT HEPATOCYTES IN TRANSPORT AND HEPATIC BINDING OF ASIALO-ALKALINE PHOSPHATASE STUDIED AFTER INDUCTION OF SELECTIVE ACINAR DAMAGE BY *N*-HYDROXY-2-ACETYLAMINOFLUORENE AND CARBON TETRACHLORIDE

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Abstract—In order to investigate rat hepatocyte heterogeneity in asialoglycoprotein transport, rats were pretreated with *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF, 90 μmol/kg, i.v.) to damage zone 1 hepatocytes, or with carbon tetrachloride (CCl_4, 2.1 mmole/kg, p.o.) to damage zone 3 hepatocytes. Twenty-four hours after pretreatment, the asialoglycoprotein dog intestinal alkaline phosphatase (As-ALPase) was injected and plasma disappearance and biliary excretion were measured. In addition, the acinar distribution of the hepatic binding of As-ALPase 10 min after injection *in vivo*, or after incubation of fixed liver sections with As-ALPase *in vitro*, was determined by enzyme histochemistry.

In control rats, a rapid biexponential plasma disappearance was observed and 6.4 ± 1.5% of the dose was excreted into bile after 60 min. Hepatocyte binding occurred predominantly in zone 3, both after administration *in vivo* and after incubation with liver sections *in vitro*.

In rats with zone 1 liver damage, both the half-lives of the first and of the second phase were strongly increased, but biliary excretion did not change significantly. Both *in vivo* and *in vitro* the relatively weak binding of As-ALPase in zone 1 of the liver was abolished, whereas binding to zone 3 cells was normal or only slightly decreased.

After CCl_4-pretreatment histochemically detectable binding to zone 3 cells was completely abolished, leaving only the relatively weak binding in zone 1. Unexpectedly, a normal plasma disappearance and biliary excretion rate were found in these rats.

The discrepancy between the pharmacokinetic results, which point to a predominant involvement of zone 1 cells in As-ALPase transport, and the enzyme histochemical studies, which show preferential binding of As-ALPase in zone 3, is discussed.

The presence of a galactose-specific receptor on the plasma membrane of rat hepatocytes has been firmly established [1–5]. This receptor is involved in the very efficient removal of asialoglycoproteins with a terminal galactose residue from the general circulation by the liver. After binding to the receptor the asialoglycoproteins are taken up by the hepatocytes by absorptive endocytosis, transported in vesicles to the lysosomes and subsequently degraded [6, 7]. Probably a number of vesicles escape from fusion with the lysosomal membrane and either associate with the bile canalicular membrane, resulting in secretion of the intact protein into bile [8, 9], or reassociate with the sinusoidal plasma membrane, resulting in exocytosis from liver into plasma [10–13].

Recently, Hardonk and Scholtens [14] and Scholtens *et al.* [15] studied the distribution of this galactosyl receptor in the liver acinus, using the asialoglycoprotein dog intestinal alkaline phosphatase (As-ALPase). Histochemical detection of the localization of As-ALPase on cryostat sections of rat liver after injection of the enzyme *in vivo*, as well as after binding of the enzyme *in vitro* to fixed liver sections, revealed a substantially higher binding of As-ALPase in the perivenous zone (zone 3 of Rappaport) than in the periportal zone (zone 1 of Rappaport). Moreover, the subsequent appearance of ALPase activity inside the hepatocytes was higher in zone 3 [15]. The histochemically detectable binding to the receptor, as well as the plasma clearance *in vivo* and in the isolated perfused liver, could be inhibited completely by other asialoglycoproteins and various galactosyl sugars, which strongly indicated the involvement of the galactosyl receptor [12, 15]. From these studies it was concluded that As-ALPase is rapidly removed from the circulation by the liver, and it was speculated that the transport of As-ALPase predominantly occurs in zone 3. However, Geuze *et al.* [16] studied the localization of the galactosyl receptor using antibodies against the receptor and found a homogeneous localization in the acinus. In other studies

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where the binding and internalization of asialoglycoproteins were investigated by employing morphological techniques, a preferential localization in the acinus was not mentioned [4, 6, 17–19].

Previously we described a method to investigate the acinar heterogeneity in transport functions by induction of selective acinar injury in the rat liver [20, 21]. Zone 1 damage was induced by administration of *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF) and zone 3 damage by administration of carbon tetrachloride (CCl_4). The zonal selectivity of the injury was satisfactorily assessed by histochemistry, transmission electron microscopy and scanning electron microscopy [20]. In the present study we employed this experimental set-up to investigate further the possible zonal heterogeneity in asialoglycoprotein transport. As-ALPase was administered *in vivo* to rats pretreated with either N-OH-AAF or CCl_4, and the plasma disappearance and biliary excretion rate were measured. Furthermore, the binding of As-ALPase was determined *in vivo* and *in vitro* by histochemistry.

MATERIALS AND METHODS

Materials. Alkaline phosphatase from dog intestine (type X) was obtaind from Sigma (St. Louis, MO), Diethanolamine and *p*-nitrophenylphosphate were obtained from Merck (Darmstadt, F.R.G.). Demineralized bovine albumin was obtained from Poviet (Oss, The Netherlands) and DE-11 cellulose from Whatman (U.K.). N-OH-AAF (kindly provided by Dr. J. H. N. Meerman) was synthesized as described by Miller *et al.* [22]. Because of the carcinogenic properties of N-OH-AAF, appropriate protective measures were taken by the investigators (mask, gloves and lab. coat) and pretreated rats were housed in separate cages with a controlled air flow through a filter.

Purification of dog intestinal alkaline phosphatase. The commercial preparation of dog intestinal ALPase was purified by column chromatography on DE-11 cellulose according to Saini and Done [23] as described by Scholtens *et al.* [12].

Pretreatment of rats. Male Wistar rats weighing 280–300 g, which had free access to food and water, were pretreated with 90 μmole/kg *N*-hydroxy-2-acetylaminofluorene (N-OH-AAF, i.v., dissolved in saline) or with 2.1 mmole/kg carbon tetrachloride (CCl_4, p.o., diluted 10-fold with rape oil) between 9 and 10 a.m. as described before [20]. All experiments were performed 24 hr later. Controls received the solvent only.

Plasma disappearance and biliary excretion of As-ALPase in vivo. Rats were anaesthetized with sodium pentobarbital (Nembutal®, 60 mg/kg, i.p.) and were artificially respirated through a trachea cannula during the experiments. The body temperature was measured rectally and maintained at 38° by placing the animals on an electrically heated mattress. The carotid artery was cannulated with polyethylene tubing filled with heparin (10 U/ml in 0.9% NaCl solution) to prevent blood clotting. Blood samples (0.1 ml) were taken in heparinized tubes (Sherwood Medical Industries Inc., St. Louis, MO) and stored on ice. Plasma was obtained after centrifugation for 20 min at 1300 *g*. During the experiments blood pressure was measured using the same cannula to check the general condition of the rats. Bile was collected by cannulating the bile duct in preweighed tubes in 5 min fractions during the first 20 min after the injection of As-ALPase, and afterwards in 10 min fractions up to 60 min. As-ALPase (20 U, dissolved in 1 ml 0.9% w/v NaCl solution containing 2% w/v albumin, pH 7.4) was administered by injection into a cannula in the jugular vein. During the experiments an infusion of 1.9 ml/min of 0.9% w/v NaCl solution containing 2% w/v albumin, pH 7.4, was given into the cannula in the jugular vein to compensate for the loss of body fluid due to the blood samples taken. The ALPase activity in plasma and bile was determined the same day.

Determination of ALPase activity. ALPase activity was determined by incubating 0.1 ml of plasma or bile (diluted in 0.9% w/v NaCl solution) in 0.1 ml of incubation buffer containing 1.0 M diethanolamine, 1.0 mM $MgCl_2$ and 5.4 mM *p*-nitrophenylphosphate, pH 10.1, for 30 min at 37°. The reaction was stopped by the addition of 2.5 ml of ice-cold 1 N NaOH and the extinction was measured at 405 nm.

Kinetic analysis. The plasma disappearance curves were fitted using a computerized programme, yielding straight regression lines of log plasma concentration vs time after balanced iterative pealing of the curves using a least squares method. The clearance (*Cl*) was calculated from the dose and the area under the plasma disappearance vs time curve.

Histological assessment of damage. After each experiment pieces of liver were frozen in liquid freon (−96°). The extent of the damage was assessed histochemically on cryostat section (10 μm) as described previously [20]. The sections were stained with haematoxylin and eosin, and were used to demonstrate the presence of fat and glycogen, and the activities of NADH-reductase, ATPase and endogenous ALPase.

Histochemical demonstration of the hepatic binding of As-ALPase in vivo. Under pentobarbital anaesthesia, 100 U of As-ALPase was injected into the tail vein of control rats and of rats pretreated with either CCl_4 or N-OH-AAF. Ten minutes after the injection, pieces of liver were frozen in liquid freon. Cryostat sections (10 μm) were cut and fixed in 4% formaldehyde, 5.4% macrodex, 1% $CaCl_2$, 0.9% NaCl, pH 7.0, for 10 min at 4° and incubated for the demonstration of ALPase activity according to the Gomori method as described by Hardonk *et al.* [24].

Histochemical demonstration of the hepatic binding of As-ALPase in vitro. Cryostat sections (10 μm) of livers of control rats and of rats pretreated with either N-OH-AAF or CCl_4 were fixed in the formaldehyde, macrodex, $CaCl_2$ solution for 10 min at 4° or in a mixture of equal parts of chloroform and acetone for 3 min at 4°. The sections were rinsed in tap water and covered with 5 or 15 U/ml As-ALPase in 0.005 M Tris–HCl buffer, pH 7.2, for 10 min at 37° according to Hardonk *et al.* [24]. Subsequently, the sections were incubated for the demonstration of ALPase activity as described above.

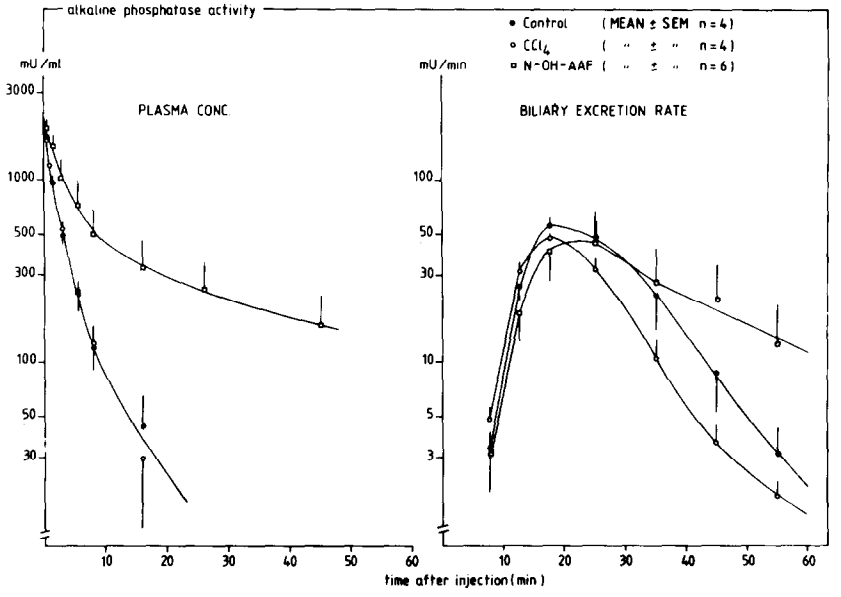

Fig. 1. Plasma disappearance (left panel) and biliary excretion rate (right panel) of As-ALPase in control rats (●) and in rats pretreated with 90 μmole/kg N-OH-AAF (□) and 2.1 mmole/kg CCl_4 (○). Twenty-four hours after pretreatment, As-ALPase (20 U in 1 ml 0.9% NaCl w/v solution, containing 2% albumin, pH 7.4) was injected into the jugular vein of anaesthetized rats. Blood samples were taken from the carotid artery and bile samples were collected in 5 min fractions up to 20 min, and in 10 min fractions from 20 min until 60 min after injection. The ALPase activity in plasma and bile was determined the same day.

RESULTS

Plasma disappearance and biliary excretion of As-ALPase

The influence of the selective zonal liver damage induced by CCl_4 and N-OH-AAF on the plasma disappearance and the biliary excretion rate of 20 U As-ALPase is shown in Fig. 1. Upon analysis of the plasma curves, an open two-compartment model yielded the best fit of these curves and the half-lives of the first ($t_{\frac{1}{2}}\alpha$) and the second ($t_{\frac{1}{2}}\beta$) phase, the volume of the first compartment (V_1) and the total plasma clearance (Cl) were calculated and are given in Table 1.

No differences were found between the control rats and the rats pretreated with CCl_4 with respect to both liver uptake and biliary excretion of injected As-ALPase (Fig. 1, Table 1). In the CCL_4-damaged livers, distinct histological and enzyme histochemical alterations were observed in zone 3 of the liver acinus, affecting 30–50% of the cells. These alterations are described earlier [20] and include ballooning, necrosis, fat accumulation, glycogen loss and loss of activity of NADH-reductase and ATPase.

Six experiments were performed with N-OH-AAF-pretreated rats. In two of these experiments the plasma disappearance curve and the kinetic parameters were similar to those found for control rats. Histochemically, these two livers showed only minor damage in zone 1: less than about 5% of the hepatocytes lost NADH-reductase activity and bile canalicular ATPase activity, whereas induction of cytoplasmatic ATPase was limited. In these rats bile flow was normal and clearance of a tracer dose of

Table 1. Kinetic parameters of As-ALPase transport 24 hr after pretreatment with N-OH-AAF and CCl_4

		$t_{\frac{1}{2}}\alpha$ (min^{-1})	$t_{\frac{1}{2}}\beta$ (min^{-1})	V_1 (ml)	Cl (ml/min)	Recovery in bile (% of injected dose)
Control	(4)	1.1 ± 0.2	6.7 ± 0.9	9.1 ± 1.5	3.3 ± 0.6	6.4 ± 1.5
N-OH-AAF	(4)	2.4 ± 0.2*	56 ± 20*	8.0 ± 0.7	0.8 ± 0.2*	5.5 ± 4.8
CCl_4	(4)	1.2 ± 0.3	7.3 ± 3.5	10.4 ± 1.4	3.8 ± 0.7	4.1 ± 0.3

Twenty-four hours after pretreatment with 90 μmole/kg N-OH-AAF and 2.1 mmole/kg CCl_4, rats were anaesthetized and As-ALPase (20 U in 1 ml 0.9% w/v NaCl solution containing 2% albumin, pH 7.4) was injected into the jugular vein. Blood samples were taken from the carotid artery and bile was collected during 60 min. The ALPase activity was determined in plasma and bile. The plasma disappearance curves were analysed using a computerized programme according to an open two-compartment model. The half-lives of the first ($t_{\frac{1}{2}}\alpha$) and second ($t_{\frac{1}{2}}\beta$) phase, the volume of the first compartment (V_1) and the total plasma clearance (Cl) calculated from the ratio of the dose and the area under the plasma disappearance curve are given. All values are mean ± S.E. and the number of experiments are given in parentheses.

* Significantly different from control ($P < 0.05$).

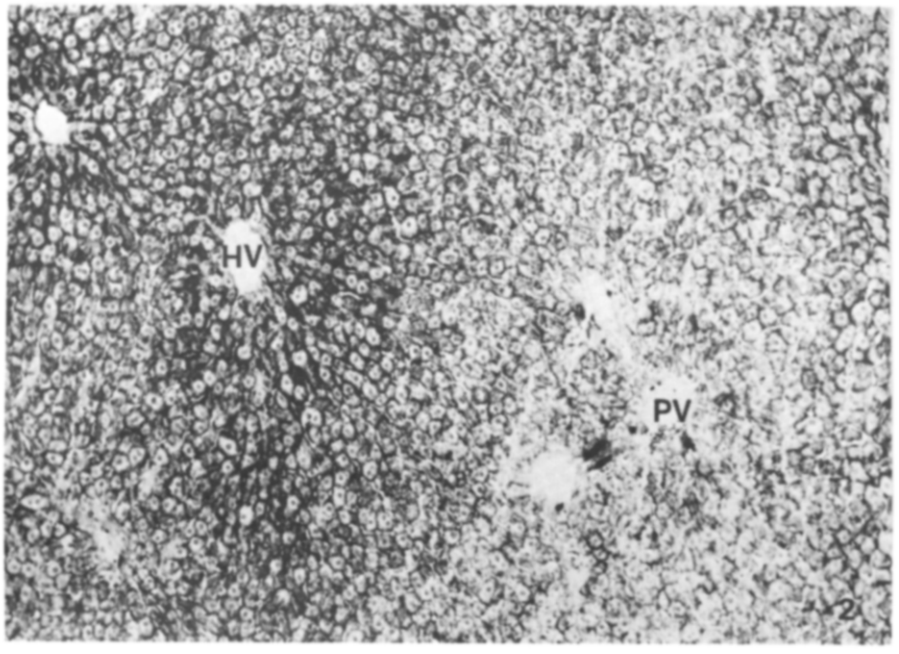

Fig. 2. Alkaline phosphatase activity in the liver of a control rat 10 min after injection of 100 U As-ALPase. Activity is predominantly present along the sinusoids in zone 3. Formaldehyde, macrodex, $CaCl_2$ fixed cryostat section. PV, Portal venule; HV, hepatic venule (90 ×).

[^{3}H]taurocholate was similar to controls (not shown, [21]). In the other four experiments, a more distinct zone 1 liver damage was seen with 5–20% loss of NADH-reductase activity. The loss of bile canalicular ATPase activity and the induction of cytoplasmatic ATPase activity amounted to 60% of the acinus [20]. In these experiments a distinct inhibition of the plasma disappearance of As-ALPase was found. Upon analysis both the $t_{\frac{1}{2}}\alpha$ as well as the $t_{\frac{1}{2}}\beta$ was significantly increased and the total plasma clearance was reduced. No change was observed in the volume of the central compartment (V_1) (Table 1).

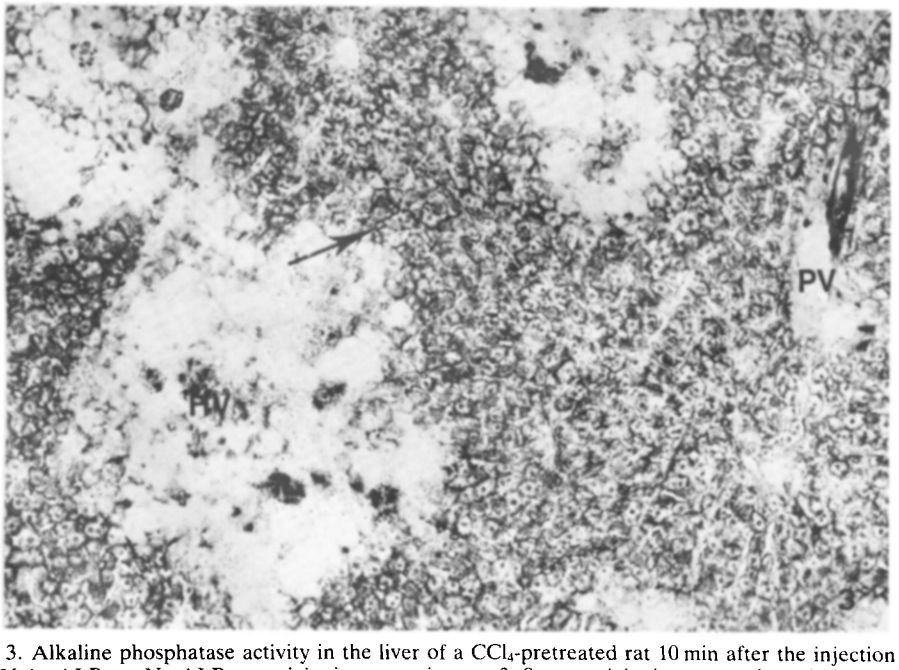

Fig. 3. Alkaline phosphatase activity in the liver of a CCl_4-pretreated rat 10 min after the injection of 100 U As-ALPase. No ALPase activity is present in zone 3. Some activity is present along the sinusoids and in the cells around the injured area (arrow). Formaldehyde, macrodex, $CaCl_2$ fixed cryostat section. PV, Portal venule; HV, hepatic venule (90 ×).

Although in these four rats bile flow was reduced to 44% of control value, the amount of As-ALPase excreted into bile in 60 min was not significantly decreased. However, it should be mentioned here that the amount excreted into bile was highly variable in N-OH-AAF-pretreated rats, ranging from 0.1 to 20% of the administered dose.

Enzyme histochemical localization of As-ALPase binding

Ten minutes after injection of 100 U As-ALPase in control rats, enzyme activity in liver sections was detected predominantly in zone 3 (Fig. 2). After formaldehyde–calcium chloride–macrodex fixation, the binding was found along the borders of the sinusoids. After chloroform–acetone fixation, As-ALPase binding was found along the entire plasma membrane of the hepatocytes and the zonal gradient was less pronounced. The same localization was observed *in vitro* when fixed liver sections were incubated with 5 or 15 U/ml As-ALPase, as was also described by Hardonk and Scholtens [14] and Scholtens *et al.* [15].

When 100 U of As-ALPase was injected in CCl_4-pretreated rats, a severely diminished binding was observed in the liver: no binding was seen in zone 3, only some enzyme activity could be detected along the sinusoids and sometimes diffusely in the cells around the injured area (Fig. 3). Zone 1 binding was low and comparable with zone 1 binding in control livers. Similar results were obtained after *in vitro* binding of As-ALPase to fixed liver sections of CCl_4-damaged livers.

In livers with zone 1 damage after pretreatment with N-OH-AAF, no binding occurred in an area extending to about 50% of the liver acinus in zone 1, and a normal or somewhat decreased binding was observed in zone 3. These results were obtained both after *in vivo* injection and after *in vitro* incubation with the enzyme (Fig. 4). After *in vitro* incubation the area without binding increased with increasing extent of the induced damage, whereas in severely damaged livers a somewhat decreased binding was additionally observed in zone 3.

DISCUSSION

In this study the relative contribution of rat hepatocytes from zones 1 and 3 of the liver acinus to the transport of the asialoglycoprotein As-ALPase was investigated using the induction of selective acinar damage as the experimental approach. Previously, we investigated the selectivity of the induced damage by enzyme histochemistry, scanning electron microscopy and transmission electron microscopy. Both after CCl_4- and N-OH-AAF-pretreatment the injury appeared to be restricted to the hepatocytes of zones 3 and 1, respectively. After CCl_4 administration a greater part of the acinus (20–50%) was affected than after N-OH-AAF administration (5–20% of the acinus). The sinusoidal cells and the bile canaliculi remained intact after both intoxications [20]. Therefore we considered this method useful to study the involvement of zone 1 and zone 3 hepatocytes in the transport of the asialoglycoprotein As-ALPase.

The present results, as depicted in Fig. 1 and Table 1, clearly demonstrate that zone 1 damage resulted in a distinct inhibition of the hepatic clearance of As-ALPase, whereas zone 3 damage had no effect. This might indicate that in undamaged livers zone 1 cells are more involved in the hepatic clearance of As-ALPase than zone 3 cells.

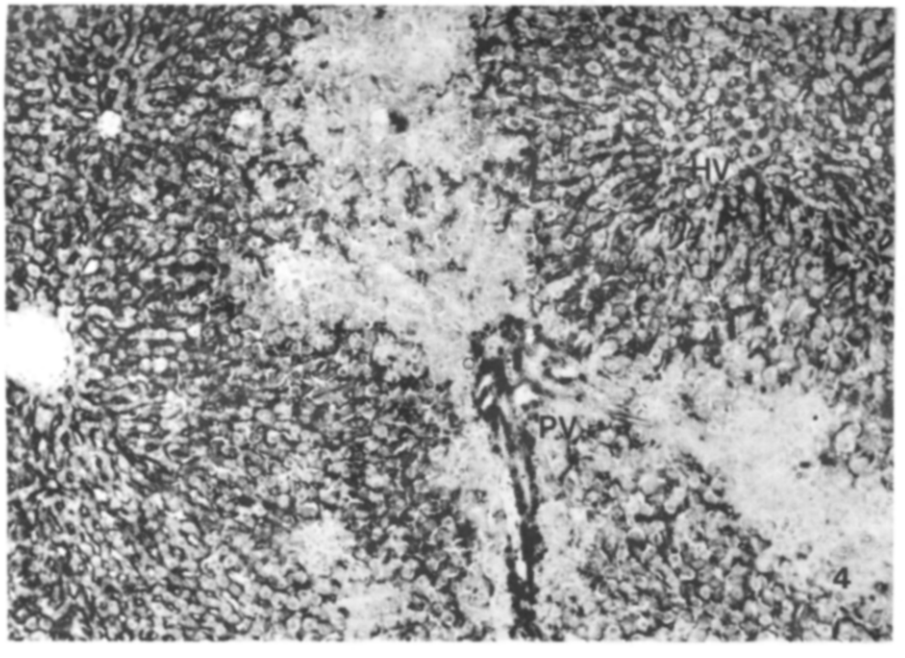

Fig. 4. Alkaline phosphatase activity in the liver of a N-OH-AAF-pretreated rat 10 min after the injection of 100 U As-ALPase. No binding is observed in zone 1, whereas normal binding of the enzyme is evident in zone 3. Formaldehyde, macrodex, $CaCl_2$ fixed cryostat section. PV, Portal venule; HV, hepatic venule (90 ×).

Hardonk and Scholtens [14] and Scholtens *et al.* [15] demonstrated the preferential localization of hepatic binding of As-ALPase in zone 3 by enzyme histochemistry. They also showed that this binding is mediated by the galactose-specific receptor present on the plasma membrane of the hepatocytes. We also determined the binding of As-ALPase in the injured livers both *in vivo* and *in vitro* (Figs. 2, 3 and 4). These enzyme histochemical results show that CCl_4-pretreatment completely reduced the hepatic binding of As-ALPase in zone 3, leaving only the normal, relatively weak binding in zone 1. On the contrary, N-OH-AAF-pretreatment abolished the relatively weak binding of AS-ALPase in zone 1 and hardly diminished the hepatic binding in zone 3.

Several explanations might exist for the preferential localization of asialoglycoprotein transport in zone 1 and the preferential histochemical binding in zone 3. The higher As-ALPase binding in zone 3 might be caused by a relatively high affinity of the zone 3 receptor for As-ALPase, compared to the zone 1 receptor. A relatively high affinity of the receptor for AS-ALPase might lead to a relatively slow release of the ligand from the internalized receptor, resulting in a slower uptake rate in zone 3. This implies a heterogeneous receptor population in the acinus with respect to the binding characteristics of the receptor, and readily explains the results with respect to the clearance and hepatic binding after N-OH-AAF- and CCl_4-pretreatment.

Alternatively, the lower histochemically detectable binding in zone 1 can be caused by a partial loss of enzyme activity due to the binding to the receptor.

Strongly decreased binding to zone 3 cells without significant decrease in hepatic clearance after CCl_4-pretreatment could indicate that zone 1 cells in the CCl_4-damaged livers are adapted to compensate for the loss of transport in zone 3. However, the strongly reduced clearance after zone 1 damage indicates that zone 3 cells are not able to compensate for the loss of zone 1 transport in N-OH-AAF-damaged livers.

An additional explanation for the decreased clearance after zone 1 damage apart from a decreased binding could possibly be found in secondary effects of the induced damage. In N-OH-AAF-pretreated rats, elevated bile salt levels were observed in both the systemic blood ($166 \pm 36\ \mu M$ compared to $21 \pm 3\ \mu M$ in control rats) as well as in the portal blood ($543 \pm 83\ \mu M$ compared to $205 \pm 21\ \mu M$ in control rats) as described earlier [21]. In CCl_4-pretreated rats, bile salt concentrations were only moderately elevated ($40 \pm 6\ \mu M$ in systemic blood and $257 \pm 33\ \mu M$ in portal blood). Russel *et al.* demonstrated in our laboratory that infusion of bile salts into rats decreased the hepatic clearance of AS-ALPase when serum concentrations of bile salts corresponding to those in N-OH-AAF-pretreated rats, were reached [11]. However, in these experiments only the plasma disappearance in the second, slow phase of the curve was abolished whereas the $t_{\frac{1}{2}}\alpha$ of the first phase was not altered. Evidence was obtained that elevated bile salt levels enhance the exocytosis of As-ALPase from liver into plasma. These findings might explain the effects of zone 1 damage on the elongation of the $t_{\frac{1}{2}}$ of the β-phase.

Decrease of hepatic binding with concomitant decrease in clearance of asialoglycoproteins is also reported by others. Sawamura *et al.* [25] detected a decrease of hepatic binding of asialoglycoproteins with accumulation of these proteins in serum of galactosamine-treated rats, which resulted in a decreased clearance of injected [^{125}I]asialo-orosomucoid. Decrease in the hepatic galactoysl receptor was also demonstrated after prolonged feeding of *N*-2-acetylaminofluorene (AAF) [26]. This compound probably exerts its carcinogenic effects through its *N*-hydroxymetabolite, N-OH-AAF, which is used as zone 1 toxin in this study [22]; however, AAF does not possess the acute zone 1 toxicity of N-OH-AAF. Marshall *et al.* [27, 28] demonstrated elevated levels of asialoglycoproteins in serum of patients with hepato-biliary disease and with alcoholic cirrhosis, which could also be the result of decreased hepatic binding. Alternatively, high concentrations of asialoglycoprotein may result from increased formation of these proteins, which may decrease the clearance of injected asialoglycoproteins by competition.

In conclusion, probably both the decreased receptor concentration in zone 1 as well as elevated bile salt (and asialoglycoprotein) levels in blood might contribute to the decreased clearance of As-ALPase after N-OH-AAF pretreatment as observed in the present study. However, apparently the decreased binding of AS-ALPase in CCl_4-damaged livers does not have an effect on the hepatic clearance of the asialoglycoprotein.

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REFERENCES

1. G. Ashwell and J. Harford, *A. Rev. Biochem.* **51**, 531 (1982).
2. G. Ashwell and A. G. Morell, *Adv. Enzymol.* **41**, 99 (1974).
3. E. A. Jones, J. M. Vierling, C. J. Steer and J. Reichen, in *Progress in Liver Diseases* (Eds. H. Popper and F. Schaffner), Vol. 6, p. 43. Grune & Stratton, New York (1979).
4. A. G. Morell, R. A. Irvine, J. Sternlieb, I. H. Scheinberg and G. Ashwell, *J. biol. Chem.* **243**, 155 (1968).
5. W. E. Pricer and G. Ashwell, *J. biol. Chem.* **246**, 4825 (1971).
6. A. L. Hubbard, G. Wilson, G. Ashwell and H. Stukenbrok, *J. Cell. Biol.* **83**, 47 (1979).
7. D. A. Wall, G. Wilson and A. L. Hubbard, *Cell* **21**, 79 (1980).
8. D. K. F. Meijer, H. B. Scholtens and M. J. Hardonk, *Pharm. Weekbl. Scient. Ed.* **4**, 57 (1982).
9. P. Thomas and J. W. Summers, *Biochim. biophys. Res. Commun.* **80**, 335 (1978).
10. U. Gärtner, R. J. Stockert, A. G. Morell and A. W. Wolkoff, *Hepatology* **1**, 99 (1981).
11. F. G. M. Russel, J. G. Weitering, R. Oosting, G. M. M. Groothuis, M. J. Hardonk and D. K. F. Meijer, *Gastroenterology* (in press).

12. H. B. Scholtens, M. J. Hardonk and D. K. F. Meijer, *Liver* **2**, 1 (1982).
13. H. Tolleshaug, P. A. Chindemi and E. Regoezi, *J. biol. Chem.* **256**, 6526 (1981).
14. M. J. Hardonk and H. B. Scholtens, *Histochemistry* **69**, 289 (1980).
15. H. B. Scholtens, D. K. F. Meijer and M. J. Hardonk, *Liver* **2**, 14 (1982).
16. H. J. Geuze, J. W. Slot, G. J. A. M. Strous, H. F. Lodish and A. L. Schwartz, *J. Cell Biol.* **92**, 865 (1982).
17. A. V. Lebouton and G. Lippman, *Experientia* **35**, 392 (1979).
18. R. J. Stockert, H. B. Haimes, A. G. Morell, P. M. Novikoff, A. B. Novikoff, N. Quintana and I. Sternlieb, *Lab. Invest.* **43**, 556 (1980).
19. P. P. van Rijk and C. J. A. van den Hamer, *J. Lab. clin. Med.* **88**, 142 (1976).
20. G. M. M. Groothuis, D. K. F. Meijer and M. J. Hardonk, *Naunyn-Schmiedeberg's Archs Pharmac.* **322**, 298 (1983).
21. G. M. M. Groothuis, J. G. Weitering, K. P. T. Keulemans, M. J. Hardonk, D. Mulder and D. K. F. Meijer, *Naunyn-Schmiedeberg's Archs Pharmac.* **322**, 310 (1983).
22. E. C. Miller, J. A. Miller and H. A. Hartmann, *Cancer Res.* **21**, 816 (1961).
23. P. K. Saini and J. Done, *Biochim. biophys. Acta* **258**, 147 (1972).
24. M. J. Hardonk, F. W. J. Dijkhuis, T. J. Haarsma, J. Koudstaal and W. A. R. Huybers, *Histochemistry* **53**, 165 (1977).
25. T. Sawamura, S. Kawasato, Y. Shiozaki, Y. Sameshima, H. Nakada and Y. Tashiro, *Gastroenterology* **81**, 527 (1981).
26. R. J. Stockert and F. F. Becker, *Cancer Res.* **40**, 3632 (1980).
27. J. S. Marshall and S. Williams, *Biochim. biophys. Acta* **543**, 41 (1978).
28. J. S. Marshall, S. Williams, P. Jones and G. W. Hepner, *J. Lab. clin. Med.* **92**, 30 (1978).