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LIVER MICROSOMAL BILIRUBIN UDP-GLUCURONYLTRANSFERASE DISTURBANCES IN BILE DUCT LIGATED RATS

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The activity of bilirubin UDP-Glucuronyltransferase was determined in microsomes from normal and bile duct ligated rats. It was measured after 2 and 8 days following bile duct ligation and compared with normal rats. A decrease of 33% in the total enzyme activity was observed on day 2 ; a fall of 70% was founded on day 8. Bilirubin diglucuronide represented approximately 20% of total conjugates in both groups of cholestatic rats, as compared with 65% found in normals. It was concluded that bilirubin microsomal conjugating capacity is markedly altered during cholestasis. This can be attributed to microsomal membrane damage produced by stagnant bile.

UDP-Glucuronyltransferase (UDP-GT) (EC 2.4.1.17) is a membrane-bound enzyme concentrate in hepatic microsomes that catalyses glucuronic acid transfer from the UDP glucuronic acid to a variety of internal metabolites including bilirubin. During the first step of this pathway BMG is formed, in a second step the major polar product, BDG is produced, and finally it is excreted in bile (1) . BDG formation at this point remains still unclear.

It has been shown that catalytic properties of bilirubin UDP-GT, as occurs with other membrane embehded enzymes depends critically on a subtle interplay between the enzyme and its membrane

Abbreviations used are: BMG, bilirubin monoglucuronide; BDG, bilirubin diglucuronide; cholestasis means our experimental model of extrahepatic cholestasis.

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environment (2) .Bilirubin UDP-GT activity in intact microsomes is very low.Studies carried out using different perturbing agents indicated that total enzyme activity can be modified "in vitro", as well as BDG/BMG ratio.An increase of bilirubin UDP-GT activity with formation of BDG was reported when liver microsomes were treated with digitonin (3) .

The aim of the present work was to study in what extent ex perimental cholestasis in rat produces disturbances in bilirub in UDP-GT activity.

METHODS: Male Wistar rats (body wt.180-250 g) were used.Food (Purina Chow) and water were given ad libitum.Normal rat livers were processed to establish our control values.Cholestasis was produced by surgical ligation and secondary section of the common bile duct (proximal to bifurcation).In a first group of experiments,cholestasis was mantained during a 2 days period, in a second group it was mantained during 8 days.Timely,under light ether anaesthesia,livers were quickly excised and a bit (10 mm diam.) was resected.Each piece was placed in ice cold Sucrose-EDTA medium (3) and immediately homogenates were prepared as describe by de Duve et al. (4) .Microsomal preparations were obtained by ultracentrifugation in a Spinco Model L 50,according to Heirwegh et al. (3) .Activated microsomes were obtained by treatment with digitonin.Digitonin suspensions contained 2.25 - 6.88 mg/ml when microsomal concentration was 0.2 g wet wt. equiv.of liver / ml of suspension,containing 2.14 - 5.98 mg of microsomal protein / ml.Bilirubin as substrate was prepared using 0.25 mg/ml,in bovine serum albumin solution (3) .Duplicate assay incubation mixtures were prepared according to Billing et al. (5) .A control sample was taken for each determination (3) .Incubation at 37°C was achieved in a shaking incubator during 30 min.;following,tubes were transferred on ice,and glycine-HCL buffer was added (5). Extraction of conjugated bilirubin was performed by diazotization and secondary derived azopigments were extracted in an organic phase (5) .Tests and controls were determined reading absorbance at 530 nm in a Beckman DB Spectrophotometer.The difference between these two readings was converted in nmoles of bilirubin conjugate.Molar extintion coefficient for azopigments using this system was determined ($\epsilon \frac{530}{1cm}$: 44,12 . 10^3 litre . mol^{-1} . cm^{-1}).This data was applied in the convertion mentioned above.Analyses of azopigments were performed using T.L. C. method (6) .Resulting azopigments were eluated in methanol (7) .Percentages of azopigments were determined and BDG/BMG ratio calculated (8) .Azopigments structures were established by a series of chemical tests (9) .Microsomal protein concentration was determined (10) using serum albumin as standard. For statistical analysis Student's Test was used to compare groups; $p < 0,05$ or less were considered to be statistically significant.

RESULTS:

Validity of the methods employed: In the concentration adopted by us digitonin proved to be an excellent agent to increase procedures sensitivity (3). Azopigments obtained were identified as δ and α_0, with well known structures (9).

Bilirubin UDP-GT activity: in Table I can be seen the total bilirubin UDP-GT activity as expressed as nmoles of conjugated bilirubin. A clear decrease of total bilirubin UDP-GT activity can be seen comparing normal and both groups of cholestatic rats. In the same Table a similar behavior of microsomal protein content is shown. Figure 1 ilustrates in percentages the behavior of total enzyme activity and microsomal protein during cholestasis, compared with percentages of BDG and BMG formation. Between normal and 8 days post cholestatic rats, a decrease of 70% of total bilirubin UDP-GT activity was observed; in coincidence, the same percentage of decrease in BDG forming capacity was seen. Between normal and 2 days post cholestatic rats, the decrease of total enzyme activity was 33%, and a sim-

TABLE I : Effect of bile duct ligation on bilirubin UDP-GT activity and protein content from rat liver microsomes.(I)

	BILIRUBIN UDP-GT ACTIVITY (Expressed as nmol / 10 min. per g wet wt.equiv of liver)	MICROSOMAL PROTEIN (mg per g wet wt. equiv. of liver)
MICROSOMES	69.8 ± 6 (5)	8.16 ± 0.86 (5)
MICROSOMES 2 DAYS AFTER BILE DUCT LIGATION	46.4 ± 16 (5)	5.3 ± 1.2 (5)
MICROSOMES 8 DAYS AFTER BILE DUCT LIGATION	20.0 ± 11 (5)	2.36 ± 0.63 (5)

(I): Results are expressed as mean values ± S.D. for the number of animals given in parentheses. For each one, the average value of a duplicate assay mixture was considered.

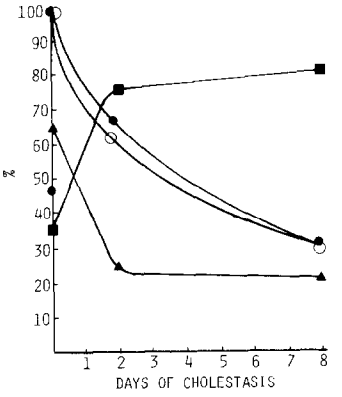

FIGURE 1 : Rat liver microsomes and time of cholestasis. Changes in the final proportions expressed as percentages of normal values. ● bilirubin UDP-GT activity. ▲ BDG formed. ■ BMG formed. ○ content of microsomal protein.

ilar percentage of fall was registered between days 2 and 8 post cholestatic rats (37%).Instead BDG formation capacity diminished a 62% during the first 2 days of cholestasis,while a fall of only 8% was observed during the following days.In both, bilirubin UDP-GT activity and microsomal protein content showed a similar behavior.

DISCUSSION:

Previous studies suggested that extrahepatic cholestasis induces physico-chemical changes in bilirubin molecule (11-12) and different mechanisms were proposed to explain these findings. On the basis that bilirubin UDP-GT is a membrane-bound enzyme, we studied in these experiments the microsomal conjugating capacity in cholestatic rat livers.

According to Goresky et al. (2) BMG represents the substrate for BDG synthesis in a second step of bilirubin conjugation. Bilirubin UDP-GT as many other membrane-bound enzymes,is critically dependant on changes in membrane integrity.Cholestasis produces severe damage on these structures probably by modifying their fluidity and lipoprotein composition.

Treatment with different perturbing agents (2) proved the high lability of BDG formation,altering probably the interaction between catalytic protein and the membrane phospholipids which modifies the affinity for substrate and/or specificity for sugar donnor.

Our results support a close relationship between the period of time that cholestasis is mantained and the decrease of both microsomal protein and bilirubin conjugating capacity.BDG formation,on the contrary,is quickly altered suggesting different membrane conditions for their synthesis.

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REFERENCES:

1. Blanckaert, N., Gollan, J.and Schmid,R. (1979) Proc.Natl. Acad.Sci. 76,2037-2041.
2. Gordon, E., Sommerer, U.and Goresky, K. (1983) J.Biol.Chem. 258,15028-15035.
3. Heirwegh, K.P.M., Van de Vijver, M.and Fevery, J. (1972) Biochem.J. 129,605-618.
4. de Duve, C., Pressman, B.C., Gianetto, R., Wattiaux, R. and Appelmans, F. (1955) Biochem.J. 60,604-617.
5. Black, N., Billing, B.H.and Heirwegh, K.P.M. (1970) Clin Chim.Acta, 29,27-35.
6. Heirwegh, K.P.M., Van Hess, G.P., Leroy, P., Van Roy, F.P. and Hansen, F.H. (1970) Biochem.J. 120,877-890.
7. Blumenthal, S., Taggart, D.B., Ikeda, R.M.and Ruebner, B. H. (1977) Biochem.J. 167,535-548.
8. Fevery, J., Verwilghen, R., Tan, T.G.and De Groote, J. (1980) Eur.J.Clin.Invest. 10,219-226.
9. Blanckaert, N., Fevery, J., Heirwegh, K.P.M.and Compernolle, F. (1977) Biochem.J. 164,237-249.
10. Lowry, O.H., Rosebrough, N.J., Farr, A.L.and Randall, R.J. (1951) J.Biol.Chem. 193,265-275.
11. Compernolle, F., Van Hess, G.P., Blanckaert, N.and Heirwegh, K.P.M. (1978) Biochem.J. 171,185-201.
12. Blanckaert, N., Compernolle, F., Leroy, P., Van Houtte, R., Fevery, J.and Heirwegh, K.P.M. (1978) Biochem.J. 171,203-214.