PATHOLOGY

Conjugated Plasma Bilirubin in Jaundice caused by Pigment Overload

WITH overproduction of bilirubin due to haemolytic anaemia, a minor though significant fraction of the plasma bilirubin may exhibit a direct diazo reaction in the absence of detectable liver dysfunction[1]. If the direct-reacting material is in fact conjugated bilirubin[2], this would support the concept[3] that the secretion of conjugated pigment from the hepatic cell into the bile is a rate limiting step in bilirubin transport across the liver. Chemical identification of this direct-reacting pigment fraction as conjugated bilirubin is difficult, however, because the amounts available for analysis are very small, the extraction procedures are semi-quantitative[4,5] and part of the unconjugated pigment may react directly with the diazo reagent[1,6].

The appearance of conjugated bilirubin in the plasma was studied in eight intact male Sprague–Dawley rats infused with unconjugated bilirubin at constant rates for 2–12 h. The infused pigment was bound to human albumin or dissolved in murine serum; similar results were obtained with both vehicles. The rates of infusion were selected so that they were below what has been considered the maximal excretory rate for bilirubin in this species[3,7]. Conjugated and unconjugated bilirubin were estimated by the method of Weber and Schalm[8] on blood samples obtained from the tail at hourly intervals. Biliary obstruction as a cause of retention of conjugated bilirubin was investigated with bromsulphophthalein (BSP); 2 h before the end of each experiment 5 mg BSP were injected intravenously and serum samples were analysed at 5, 10, 20, 30 and 45 min thereafter[9].

Conjugated bilirubin appeared in the plasma within the first hour of infusion, and thereafter the levels of conjugated and total pigment in individual rats remained reasonably constant throughout the experiments (Table 1). In the eight animals the relationship between infused pigment load, total plasma bilirubin and conjugated fraction showed considerable variation (Table 1). In all instances, at least 95 per cent of the injected BSP had disappeared from the plasma in 45 min, indicating unimpaired excretion of the dye[9].

Table 1. SERUM BILIRUBIN CONCENTRATIONS DURING CONTINUOUS INTRAVENOUS INFUSION OF UNCONJUGATED BILIRUBIN IN RATS

No.	Rate of infusion (μg/min/100 g)	Length of infusion (h)	Serum bilirubin concentrations (mg/100 ml.) Total	Conjugated	Conjugated per cent
1	47	12	12·4 ± 1·8*	1·6 ± 0·2*	12·9
2	42	5	10·8 ± 2·3	1·1 ± 0·3	10·2
3	40	12	13·8 ± 1·5	1·2 ± 0·2	8·7
4	31	8	9·0 ± 0·8	0·7 ± 0·2	7·8
5	27	7	10·1 ± 1·0	0·7 ± 0·3	6·9
6	23	8	9·9 ± 0·9	1·3 ± 0·3	13·1
7	17	9	7·3 ± 0·7	0·7 ± 0·1	9·6
8	14	8	3·8 ± 0·6	0·3 ± 0·1	7·9

* Results are expressed as the mean value plus or minus the maximal deviation from the mean for the duration of each experiment.

The polar pigment fraction separated by the method of Weber and Schalm[8] was identified as conjugated bilirubin in the following manner. A rat was infused with ^{14}C-bilirubin (ref. 10) at a constant rate of 26 μg/min/100 g for 2 h and the animal was then exsanguinated. Concentrations of serum ^{14}C-bilirubin per 100 ml. were: total, 5·9 mg; conjugated[8], 0·5 mg (that is, 8·4 per cent of total). An aliquot of this serum (2·5 ml.) was given intravenously to a congenitally icteric Gunn rat with an external bile fistula. Gunn rats lack the apparatus for conjugating bilirubin and therefore cannot transfer unconjugated pigment into the bile[11], but they excrete injected conjugated bilirubin as efficiently as normal rats[3]. During the first hour after injection of the serum, 7·9 per cent of the administered radioactivity was excreted in the bile, and 82 per cent of this label was recovered in ^{14}C-bilirubin crystallized from this bile sample. By contrast, another Gunn rat injected with a comparable dose of unconjugated ^{14}C-bilirubin excreted only 1·3 per cent of the radioactivity in the bile, and most of the label was present in metabolites other than bilirubin[11].

These findings indicate that with chronic bilirubin overload, the appearance in the plasma of direct-reacting material indeed reflects retention of small amounts of conjugated bilirubin. They further suggest that secretion of conjugated pigment into the bile may become rate-limiting at infusion rates below the maximal excretory capacity of the liver[3,7].

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Bile Acids on the Skin of Patients with Pruritic Hepatobiliary Disease

CHOLIC and chenodeoxycholic acids are formed from cholesterol in the liver and are then excreted in the bile to the intestinal tract. There they are broken down to form, for example, deoxycholic and lithocholic acids during an enterohepatic circulation[1].

Bile acids have been implicated in the genesis of pruritus associated with liver disease because: (1) external biliary drainage relieves pruritus in patients with biliary obstruction[2]; (2) the oral administration of ox bile or bile salts increased pruritus in patients with liver disease and caused pruritus to return in those with biliary fistulae[2,3]; (3) Serum bile acids (SBA) are low in normal individuals but are elevated in patients with pruritic hepatobiliary disease[4,5] and elevated in pregnant women with pruritus during the third trimester[6]. (4) The lowering of SBA by cholestyramine, an anion exchange resin which increases the faecal bile acid excretion[7], is associated with the relief of pruritus in most patients with incomplete biliary obstruction[8–11].

Bile acids have not been proved to cause pruritus because: (1) external biliary drainage could as well remove other biliary factors which may be responsible for the itching; (2) commercial preparations of bile salts administered have not been pure and feeding of pure cholic acid produced a high SBA without pruritus[5]; (3) all patients with increased SBA do not have pruritus[5,12]; furthermore, the relief of pruritus by norethandrolone could not be correlated with the level of SBA[12]; (4) cholestyramine might remove other factors which may be responsible for pruritus. Also, relief of itching by cholestyramine without a concomitant decrease in SBA has been reported[9].

Itching is a subjective response to chemical, mechanical, thermal or electrical stimuli acting on nerve fibres in the subepidermal area[13]. If bile acids are related to pruritus they should be present in the area of these sensory nerve endings. There has been no previous report of bile acids on the skin. This communication presents the identifica-