Altered Composition of Bilirubin Conjugates in Rat Bile after Obstruction of the Common Bile Duct

According to current concepts[1–3], only bilirubin and bilirubin diglucuronide are thought of importance in human and murine bile, and upon diazotisation they give rise to azopigments A (azobilirubin) and B (azobilirubin-β-D-*mono*glucuronide), respectively. The available evidence[4] and recent studies by OSTROW and MURPHY[5] strongly support the existence of bilirubin monoglucuronide. Analysis of azopigments obtained with the diazonium salts of aniline[6] and of ethyl anthranilate[4,7,8] further shows that several other forms of bilirubin conjugates exist.

When human bile, obtained post-operatively by 'T'-tube drainage, was treated with diazotized ethyl anthranilate[7], column chromatography of the azopigments[4,8] yielded 4 dominant azopigment groups, called α, β, γ and δ, which can be further separated by TLC[4] into closely clustered sub-groups. This degree of heterogeneity has also been demonstrated for bile obtained from normal adults by duodenal intubation (FEVERY, unpublished work). The α-group contains mainly azobilirubin, whereas only conjugated azopigments are present in the other groups[4]. The β and γ groups are largely composed of hexuronic acid-containing azobilirubin derivatives, which resist prolonged incubation with β-glucuronidase. Their conjugating moieties contain groups which are easily split off in slightly acid media containing acetone or water-miscible alkohols. The β- and/or γ-azopigments may correspond to the disaccharidic azopigment conjugates described by KUENZLE[6]. The δ-group is mainly composed of azobilirubin-β-D-*mono*glucuronide.

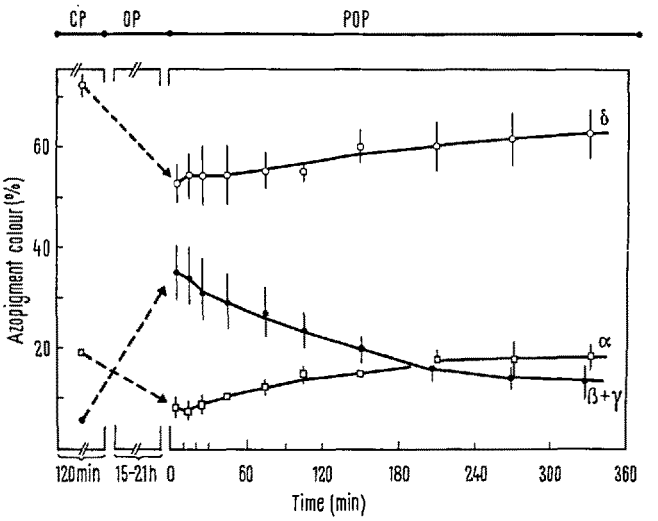
In contrast to this, the azopigment composition derived from normal rat bile is dominated by the α- and δ-azopigments[4,7,8], the $(\beta+\gamma)$-azopigments amounting only to about 6% of total azopigment colour. Experiments were set up to stimulate in rats the biliary excretion of those bile pigments which on azo coupling yield the azopigments β and γ. As described below, this could be achieved by mechanical or hydrostatic obstruction.

For short-term obstruction (15–21 h) normal Wistar rats (R-strain) were anesthetized with ether. A polyethylene catheter (PE 10) was inserted in the common bile duct and bile samples were collected for 6 h at 10 to 60 min intervals. The animals were placed in a restraining cage and then transferred to a thermostated premature-infant incubator to facilitate recovery of 37.8 °C body temperature[9]. Rats had free access to water. Body temperature, which immediately after anesthesia was about 33.5 °C, then gradually rose within 3–4 h to normal values. When the body temperature had stabilized at 37.8 °C (± 0.1 °C) bile was collected for 2 successive one-hour periods to serve as a control. The free end of the catheter was elevated to obtain a hydrostatic pressure (23–24 cm water) sufficient to counteract bile secretion. After 15–21 h the catheter was brought down and bile collected fractionally for 6 h.

For obstruction of longer duration (2 or 4 d) the catheter, after insertion in the common bile duct, was cut at a length of 5 cm and sealed at the end by heating. It was then inserted s.c. in the abdominal wall and the rat allowed to resume normal activities. After 2 or 4 days, the catheter was exteriorized under light ether anesthesia and the rat immobilized in a restraining cage placed in the incubator. After normalisation of the body temperature, the end of the catheter was cut off and bile collection started.

To determine the conjugated bile pigments, aliquots of the bile samples were treated with diazotized ethyl anthranilate[4,7] and the azopigments separated by TLC[4]. The separated azopigments were removed from the plates with methanol and quantitated photometrically[4].

In non-obstructed rats, bile pigment composition gradually changed as the body temperature rose, to reach steady values once the temperature of the animals was normalized: α, 19.4 ± 1.0%; $\beta+\gamma$, 6.0 ± 1.1%; δ, 72.1 ± 2.3% (average values ± S.D.; $n = 4$). As shown in the Figure, short-term obstruction caused a pronounced increase in the percentages of the $(\beta+\gamma)$ azopigment fraction to 35.1 ± 5.7% (average value ± S.D.; $n = 3$) of total azopigment colour immediately after relieving the obstruction. Azopigment composition then gradually returned to pre-obstruction values. In the diagram the β- and γ-azopigments are plotted as their sum, as the changes of both pigment groups paralleled each other very closely. Essentially identical patterns were observed in rats obstructed for 2 or 4 days with the immediate post-obstruction values of the $(\beta+\gamma)$ fraction amounting



Bile pigment composition of the bile of Wistar rats (R-strain) before (CP) and after hydrostatic obstruction (POP) of the common bile duct. Average values ± 1 S.D. obtained with 3 rats are given. During the experiments the body temperature of the rats was controlled at 37.8 ± 0.1 °C. After obtaining two 1-h-control collections (CP; the mean values were averaged to obtain the control points) hydrostatic obstruction was maintained for 15–21 h (OP). After relieving the obstruction (POP) bile was collected for 6 h at 10 to 60 min intervals.

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Bile pigment composition and flow characteristics of bile of Wistar R-strain rats before and after obstruction of the common bile duct

Rat No.	Interval of bile collection (min)	Bile flow (µl/min/100 g body wt.)	Concentration of CB/(mM)	Biliary output (nmol/min/100 g body wt.)			
				CB	Azopigments α	$\beta+\gamma$	δ
1 (body wt. 343 g; liver wt. 12.2 g)							
Non-obstructed	0–120	3.50	0.385	1.35	0.269	0.093	0.94
After obstruction	0– 10	8.8	0.98	8.6	0.89	2.51	4.96
	10– 22	9.8	0.91	8.9	0.80	2.60	5.30
	22– 30	7.3	0.81	5.91	0.61	1.53	3.60
	30– 60	3.90	0.92	3.59	0.408	0.87	2.21
	60– 90	2.90	0.443	1.29	0.182	0.313	0.74
	90–120	2.90	0.282	0.82	0.140	0.188	0.452
	120–180	2.90	0.230	0.67	0.106	0.132	0.395
	180–240	2.90	0.168	0.487	0.091	0.086	0.279
	240–300	2.90	0.144	0.418	0.076	0.077	0.238
	300–360	2.40	0.130	0.312	0.055	0.058	0.186
2 (body wt. 379 g; liver wt. 12.8 g)							
Non-obstructed	0–120	4.00	0.308	1.23	0.230	0.060	0.92
After obstruction	0– 10	13.2	1.03	13.6	0.85	5.49	6.9
	10– 20	9.3	1.64	15.3	0.94	6.1	7.9
	20– 30	6.6	1.71	11.3	0.82	4.22	5.95
	30– 60	5.10	1.11	5.66	0.538	2.04	2.92
	60– 90	3.50	0.93	3.26	0.406	1.09	1.66
	90–120	3.50	0.418	1.46	0.213	0.414	0.78
	120–180	3.50	0.400	1.40	0.217	0.325	0.80
	180–240	4.40	0.338	1.49	0.295	0.292	0.85
	240–300	4.00	0.352	1.41	0.309	0.216	0.85
	300–360	3.50	0.366	1.28	0.293	0.181	0.77
3 (body wt. 392 g; liver wt. 13.0 g)							
Non-obstructed	0–120	3.70	0.298	1.10	0.218	0.053	0.81
After obstruction	0– 10	12.8	0.87	11.1	0.99	3.99	5.58
	10– 20	10.2	1.15	11.7	0.95	3.85	6.07
	20– 30	5.10	1.72	8.8	0.86	2.66	4.39
	30– 60	4.30	1.09	4.69	0.536	1.32	2.33
	60– 90	4.30	0.83	3.57	0.406	0.85	2.04
	90–120	3.40	0.366	1.24	0.162	0.254	0.71
	120–180	4.30	0.297	1.28	0.182	0.238	0.81
	180–240	4.00	0.347	1.39	0.230	0.204	0.91
	240-300	3.80	0.300	1.14	0.189	0.142	0.77
	300–360	3.80	0.277	1.05	0.176	0.123	0.72

Obstruction was for 15–21 h. CB denotes conjugated bilirubin.

to $28.9 \pm 3.1\%$ (average value $\pm$ S.D.; $n = 4$) and 28.5–29.6% ($n = 2$), respectively. Return to normal occurred at a slightly lower rate than in the obstruction experiments of shorter duration.

As shown in the Table both the volume flow and bile pigment output are markedly increased after relieving the obstruction. Obviously, considering the body weight of the animals tested (Table), a fluid volume about equal to the total expected biliary tree volume[10] has been released over the first 4–5 min after relieving the obstruction.

Separate cannulation of the 2 left-hand-side liver lobes was tried in 2 rats and successfully achieved in one rat only. The cannula was tied off and inserted s.c. After 72 h, obstruction was relieved and bile collected separately from the obstructed and non-obstructed lobes. As demonstrated by TLC the azopigment patterns from the lobes which had been obstructed showed a relative increase of the $(\beta+\gamma)$ azopigment fraction, whereas bile obtained from the other lobes did not. This experiment suggests that the bile pigments which yield β- and γ-azopigments under the conditions defined in the present work, are produced in the liver and not in extrahepatic tissue.

Temporary obstruction of the bile duct in rats is therefore an easy method for stimulating the formation of those bile pigments which upon azo coupling yield the β- and γ-azopigments. Their mechanism of secretion is at present under investigation. It is expected that such studies may lead to a better understanding of the pathology of obstructive jaundice in man.

Résumé. Changement de la composition des pigments biliaires de la bile de rat après obstruction. L'obstruction des voies biliaires chez le rat Wistar mène à la formation de pigments biliaires donnant par diazo-réaction des azopigments (jusqu'à 35% de la couleur totale) qui diffèrent de l'azobilirubine et de l'azobilirubin-β-D-*mono*glucuronide.

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