The Transport Capacity for Bilirubin in Rat Liver and its Relation to Bile Flow

Many recent investigations have dealt with the transport capacity for bilirubin in normal and pathological rat livers. The normal values were found to be 61 ± 8.0 µg per 100 g body weight per min[1], 69 ± 9.2[2], 65 ± 12[3], 57.1 ± 13.6[4], 57[5], 56[6]. These determinations were performed immediately after the cannulation of the bile duct and using continuous pentobarbital anesthesia. One group of authors[7] maintained the body temperature of the rats at 37 °C and waited 30 min before starting the infusion of bilirubin. They found an apparent T_m of 70 ± 4 µg per 100 g body weight per min. For this reason we thought it necessary to investigate whether monitoring of the body temperature and postoperative recovery could increase the apparent T_m for bilirubin.

Four groups of male Wistar rats were used. The bile duct was cannulated with a PE 10 polyethylene cannula and a second PE 10 tubing was inserted in the femoral vein. All animals received a priming dose of 1–2 ml of a 3–4 mg/ml solution of bilirubin[1], followed by a continuous infusion of the same solution delivered by a Braun Perfusor at 6.7 ml/h. After 20 min a second dose of 0.5–1 ml was given. The bile was collected in tared plastic vessels for 10 min for the first sample and 5 min for the subsequent 9 samples. The bile volume was determined gravimetrically. The bilirubin concentration was estimated by the method of LUCASSEN[8] after dilution to a suitable concentration. In the first group (E_{38}), the animals were operated under ether anesthesia, allowed to recover during 10 h and their body temperatures were monitored at 38 ± 0.05 °C. In the second group (N_{38}), the operation was performed under pentobarbital (Nembutal®) anesthesia, and treated in the same way as the E_{38}. A third group (E_s) was operated on under ether anesthesia; the animals were kept at room temperature and were not allowed to recover, and their temperatures were registered. The bilirubin infusions were performed during continuous anesthesia. In the fourth group (N_s) nembutal was used for anesthesia, and the animals were treated as in the E_s group.

With body temperature monitoring and after a 10 h recovery period, the apparent T_m for bilirubin was 105.2 ± 9.60 µg/100 g body wt./min, if the operation was performed under ether anesthesia; it was 100.0 ± 4.20 µg/100 g body wt./min when Nembutal was used. These values are almost 50% higher than the highest values reported in the literature[7]. If, however, the animals were not allowed to recover, their temperatures were not monitored, and the anesthesia was continued throughout the experiment, the apparent T_m was 28.3 ± 8.93 µg/100 g body wt./min in the E_s group and 63.2 ± 7.95 µg/100 g body wt./min in the N_s group. There was a significant correlation between the apparent T_m and the body temperatures in the E_s group. In the groups the average bilirubinemia at the end of the experiment was over 40 mg/100 ml, and in no animal was it below 30 mg/100 ml.

The bile flow correlated significantly with the apparent T_m in all groups. In the E_{38} group a log-log relationship was found between the bile flow and the bilirubin secretion after saturation of the secretory mechanisms which is given by the equation: $\log Y = 1.757 \pm 0.378 \log X$, where Y indicates the bilirubin output in µg/100 g body wt./min, X the bile flow in µl/100 g body wt./min. A correlation coefficient of 0.88 was obtained from data obtained from 5, 5 min collection periods for 5 rats. The relationships were significantly different when the animals were hypothermic.

The finding of a significant correlation between the apparent T_m and the bile flow agrees with the observations of O'MAILLE[9] et al. for BSP where they showed that the T_m for BSP increased with hydrocholeresis caused by bile salt infusions. To our knowledge no systematic study of the relation between bile flow and bilirubin T_m has been published, although it has been reported[5] that phenobarbital pretreatment increases the bile flow and therefore the T_m for bilirubin and BSP.

These results indicate that comparisons between T_m determinations are hazardous if the conditions of body temperature, recovery and bile flow are not controlled and that mechanisms other than active transport may play an important role in the transport of bilirubin through the canalicular cell membrane of the hepatocyte[9].

	n	Body weight g	Bilirubin infused mg	Bilirubinemia[a] mg/100 ml	Apparent[b] T_m µg/100 g per min	Mean[c] body temperature °C
E_{38}	5	229.8 ± 30.99	27.33 ± 5.16	50.0 ± 13.8	105.2 ± 9.60	38.00
N_{38}	4	248.0 ± 14.81	32.80 ± 3.78	42.4 ± 4.0	100.0 ± 4.20	38.00
E_s	5	228.8 ± 34.02	24.66 ± 5.15	59.9 ± 7.5	28.3 ± 8.93	30.18 ± 1.15
N_s	5	217.2 ± 43.53	26.20 ± 3.44	55.4 ± 7.0	63.2 ± 7.95	33.33 ± 0.25

Results are given as mean values ± standard deviation. [a] At the end of the experiment. [b] Calculated as the mean bilirubin output of the last 25 min of the infusion period. [c] Calculated over the whole infusion period.

Résumé. La capacité de transport de la bilirubine dans le foie de rat a été mesurée par différents auteurs. Dans nos expériments le T_m était variable selon les conditions expérimentales. En stabilisant la température à 38 °C pendant les 10 h qui suivent l'opération, le T_m s'avère 50% plus élevé que les valeurs données dans la littérature. En plus la valeur du T_m était étroitement corrélée au débit biliaire.

B. VAN DAMME and V. DESMET

Laboratorium voor Histochemie en Cytochemie, Dienst Pathologische Ontleedkunde B, Akademisch Ziekenhuis St. Rafaël, Leuven (Belgium), 25 April 1969.

1 K. WEINBREN and B. H. BILLING, Br. J. exp. Path. *37*, 199 (1956).
2 I. M. ARIAS, L. JOHNSON and S. WOLFSON, Am. J. Physiol. *200*, 1091 (1961).
3 J. C. NATZSCHKA and G. B. ODELL, Pediatrics *37*, 51 (1966).
4 H. SHIBATA, M. MIZUTA and B. COMBES, Am. J. Physiol. *211*, 967 (1966).
5 R. J. ROBERTS and G. L. PLAA, Biochem. Pharmac. *16*, 827 (1967).
6 R. J. ROBERTS and G. L. PLAA, J. Pharmac. exp. Ther. *155*, 330 (1967).
7 R. J. ROBERTS and G. L. PLAA, J. Pharmac. exp. Ther. *161*, 382 (1968).
8 J. LUCASSEN, *The Diazo Reaction of Bilirubin and Bilirubin Diglucuronide* (Kemink en Zoon, Utrecht 1961).
9 E. R. L. O'MAILLE, T. G. RICHARDS and A. H. SHORT, J. Physiol. *186*, 424 (1966).