## The Urinary Excretion of Bilirubin After Increased Plasma Hemoglobin Concentration in Dogs

The circumstances of the formation and excretion of urinary bilirubin in hemolytic conditions are not well known. Bilirubin appears in the urine of the dog about 30 min after artificially induced hemolysis[1]. Other authors found a little or no increased quantity of urinary bilirubin after hemolysis[2]. The contradiction in the literature and our knowledge of this problem obliged us to make new observations. It appeared necessary to investigate at what concentration of hemolysis in the plasma we found an excretion of bilirubin in the urine and to elucidate the mechanism of this process.

In 113 healthy dogs the excretion of bilirubin in the urine (UBV) in mg per unit of time was relatively constant in each individual dog. The total 24 h bilirubin excretion ranging from 0.1 to 0.8 mg/24 h (mean 0.4 ± S.D. 0.25) in all dogs, was also constant in each individual animal. The absolute UBV/24 h values were influenced neither by the total body weight (5–30 kg), nor by the age (1–10 years) nor by the diet[3], nor by the diuresis of the dog (urine volume ranging from control levels of 1 ml up to 8 ml per minute). All fractional determinations of UBV per unit of time were always expressed in values of UBV/24 h. The bilirubin in the urine was determined by a slight modification of the GRIES and GRIES's method[4], by which unconjugated bilirubin could be determined with an accuracy of more than 1% and the conjugated bilirubin with a deficit of 5%. All experiments were performed under light nembutal anaesthesia, with cannulation of the 2 ureters, so that fractions of even small urine volumes could be determined with great accuracy at any time. After a single i.v. injection of hemoglobin solutions, ranging from 300 mg to 6 g, the excretion of bilirubin in the urine of all 56 dogs remained very constant and normal for 24 h if the concentration of hemoglobin in the blood did not exceed the binding capacity of the plasma haptoglobin and also, in 83% of the cases, if the saturation of the binding capacity of plasma haptoglobin did not exceed 50 mg/100 ml. Where the free plasma hemoglobin concentration was higher than 50 mg/100 ml, there was, in 82% of the cases, a marked increase of the UBV, which was sometimes more than 80 mg/24 h. One can follow the course of the urine bilirubin excretion in 5 dogs in Figure 1. The figure of the increased UBV/24 h in correlation with time was characteristic for each animal. After repeated injections of a similar dose of hemoglobin at at least 6 h intervals, we had nearly always an identical line for the same animal (Figure 2). The slope and velocity of the increased excretion demonstrated a certain analogy with the absolute rate of bilirubin excretion in the bile of the rat after administration of hemoglobin solutions[5]. The clearance of conjugated bilirubin, which in controls was always lower than 1 ml/min, and as suggested by FULOP and BRAZEAU[6] at most 2.5% of the clearance of creatinine, increased in certain dogs, so that the clearance of conjugated bilirubin was more than 100% of the creatinine clearance (Figure 3). In icteric dogs we had the same results. Known inhibitors of the tubular secretion, like Probenecid (*p*-dipropylsulfanyl benzoic acid) did not cause a fall in the increased UBV/24 h.

We know that only a small fraction (mean 1.6%) of the plasma conjugated bilirubin is involved in filtration by the glomerulus[6], and there was never a sufficient rise in the serum conjugated bilirubin levels as a result of the increased production of bilirubin from hemoglobin breakdown in other sites. The Table illustrates this by representative data from one of our experiments. We see that the real urinary bilirubin excretion is much higher than the calculated 1.6% of the total filtered load ($C_{Cr} \times P_{DB}$). Our findings could not be explained by a change either in glomerular filtration, or in tubular function, or in total filtered load, or in the dialyzable fraction of plasma conjugated bilirubin. We believe that the increased urine bilirubin excretion could be an auxiliary mechanism, by which the organism is able to change the accumulated plasma hemoglobin in the kidney to bilirubin, which is immediately excreted in the urine.

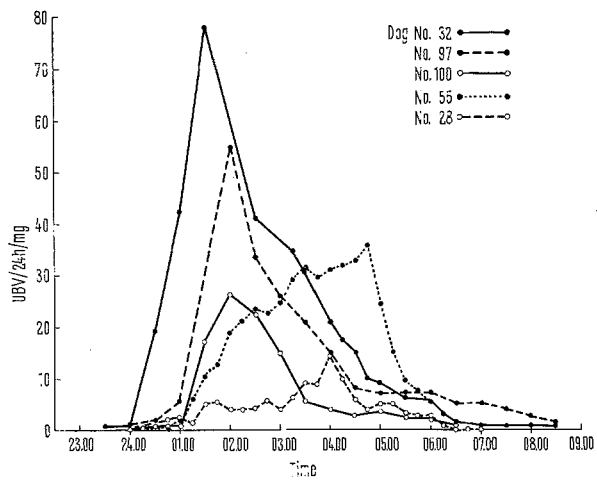


Fig. 1. The excretion of bilirubin in the urine after total saturation of the plasma haptoglobin with hemoglobin and an excess of free plasma hemoglobin > 50 mg/100 ml.

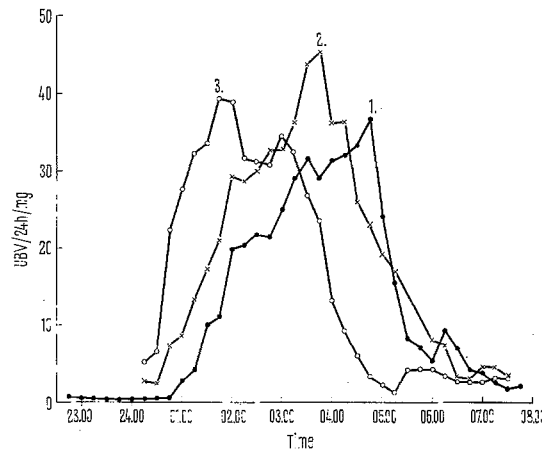


Fig. 2. The excretion of bilirubin in the urine of a dog after repeated i.v. injections of hemoglobin with total saturation of the plasma haptoglobin and an excess of free plasma hemoglobin > 50 mg/100 ml (1:66 mg/100 ml, 2:70 mg/100 ml, 3:66 mg/100 ml).

[1] T. K. WITH, *Biologie der Gallfarbstoffe* (G. Thieme Verlag, Stuttgart 1960), p. 234.
[2] R. SMID, Clin. Chem. *3*, 394 (1957).
[3] A. VERSTRAETE, J. VAN DER STOCK and J. DE SCHEPPER, Vlaams diergeneesk. Tijdschr. *37*, 376 (1968).
[4] F. A. GRIES and G. GRIES, Klin. Wschr. *34*, 1084 (1956).
[5] J. D. OSTROW, J. H. JANDL and R. SCHMID, J. clin. Invest. *41*, 1628 (1962).
[6] M. FULOP and P. BRAZEAU, J. clin. Invest. *43*, 1192 (1964).

By micro agar electrophoresis of blood-free kidney tissue, which was homogenized about 2 h after the administration of the hemoglobin solutions, we found in the kidney a quantity of hemoglobin, which was bound to a tissue protein. This hemoglobin could be released in vitro from this connection by administration of plasma haptoglobin to the kidney tissue homogenate. We observed that the lower hemoglobin concentration of the kidney tissue homogenate (10–1200 mg/100 ml), the higher the maximum increased urine bilirubin excretion (10–22.3 mg/24 h).

In clinical hemolytical conditions in dogs an increased urine bilirubin excretion may be an indication of:

(1) A strong hemolysis, because the increase of the UBV usually does not begin until the free plasma hemoglobin concentration is higher than 50 mg/100 ml.

(2) A slow free plasma hemoglobin clearance, because there is a correlation with the increase of the urine bilirubin excretion[7].

(3) A good kidney function, because there was no increase in the UBV in dogs with renal diseases (our unpublished results).

In these circumstances an increased urine bilirubin excretion is not an indication of a liver disease.

A typical experiment in a mongrel dog. Hemoglobin binding capacity of the plasma: 215 mg/100 ml.

| Time (min) | $C_{Cr}$ (ml/min) | $P_{CB}$ (mg/100 ml) | $P_{CB} \times C_{Cr}$ (µg/min) | Calculated 1,6% of [6] the filt. load (µg/min) | UBV (µg/min) | UBV/$P_{CB} \times C_{Cr}$ (%) |
|---|---|---|---|---|---|---|
| − 30 | 73 | 0.04 | 29.1 | 0.4 | 0.01 | 0.2 |
| 0 | 86 | 0.03 | 25.8 | 0.4 | 0.2 | 1.0 |
| Hemoglobin: 3.240 g i.v.; hemoglobin plasma concentration: 300 mg/100 ml | | | | | | |
| + 30 | 59 | 0.02 | 11.7 | 0.2 | 0.3 | 2.5 |
| + 60 | 63 | 0.02 | 12.6 | 0.2 | 10.3 | 81.7 |
| + 90 | 55 | 0.03 | 16.6 | 0.3 | 14.2 | 85.5 |
| + 120 | 61 | 0.05 | 30.6 | 0.5 | 18.3 | 59.8 |
| + 150 | 64 | 0.07 | 44.9 | 0.7 | 28.8 | 64.1 |
| + 180 | 68 | 0.05 | 34.4 | 0.6 | 28.3 | 82.2 |
| + 210 | 72 | 0.07 | 50.6 | 0.8 | 37.6 | 74.3 |
| + 240 | 81 | 0.04 | 32.6 | 0.5 | 30.8 | 94.4 |
| + 270 | 83 | 0.05 | 36.5 | 0.6 | 33.4 | 91.5 |
| + 300 | 75 | 0.09 | 67.6 | 1.0 | 34.0 | 50.2 |
| + 330 | 83 | 0.07 | 58.4 | 0.9 | 30.7 | 52.5 |
| + 360 | 82 | 0.05 | 40.8 | 0.7 | 24.8 | 60.7 |
| + 390 | 87 | 0.06 | 51.9 | 0.8 | 35.5 | 68.4 |
| + 420 | 84 | 0.07 | 58.7 | 0.9 | 28.8 | 49.0 |
| + 460 | 80 | 0.05 | 40.1 | 0.6 | 20.7 | 51.6 |
| + 480 | 89 | 0.05 | 44.5 | 0.7 | 21.5 | 47.8 |
| + 510 | 84 | 0.06 | 50.5 | 0.8 | 20.4 | 40.3 |
| + 540 | 83 | 0.05 | 41.5 | 0.7 | 3.8 | 9.1 |

Abbreviations. $C_{Cr}$, creatinine clearance (the method of Bonsnes and Taussky); $P_{CB}$, plasma conjugated bilirubin (the method of Jendrassik and Grof); $P_{CB} \times C_{Cr}$, calculated filtered load of bilirubin.

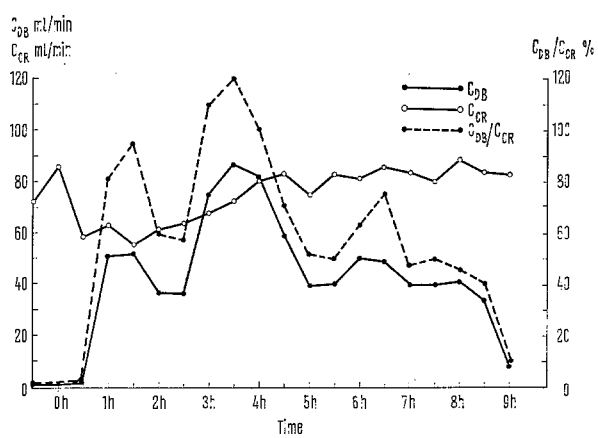


Fig. 3. The changes in the clearance of conjugated bilirubin ($C_{DB}$), the clearance of creatinine ($C_{Cr}$), and the ratio between both ($C_{DB}/C_{Cr}$) after an i.v. injection of hemoglobin with total saturation of the plasma haptoglobin and an excess of 300 mg/100 ml free plasma hemoglobin in a dog.

*Résumé.* L'excrétion rénale de la bilirubine dans l'urine varie entre 0,1 et 0,8 mg par jour dans une série de 113 chiens normaux. Avec une concentration d'hémoglobine plasmatique libre, excédant 50 mg/100 ml, les auteurs ont remarqué une augmentation sensible de l'excrétion urinaire de la bilirubine, sans que les taux sanguin, de la bilirubine ni le clearance de la créatinine soient changés. Les résultats font supposer un métabolisme local de l'hémoglobine en bilirubine dans les reins.

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[7] J. VAN DER STOCK and D. MATTHEEUWS, unpublished observations.