

Fortuitous Discovery of Urate Nephrolithiasis in Rats Subjected to Portacaval Anastomosis

The rat with a portacaval anastomosis has been described as exhibiting signs of portalsystemic encephalopathy¹. This aspect of the animal model was studied in our laboratory. At the autopsy of one of these animals an ureteral calculus was noted causing a dilated ureter and hydronephrosis. The kidneys and bladders were therefore dissected carefully at autopsy during the subsequent course of the investigation. The systematically collected data confirm the frequency of renal stones in this animal model and form the basis of this preliminary report.

Material and methods. Male Sprague-Dawley rats (strain RACTif)² of 200 to 350 g body weight were used. They were maintained in individual cages and given Altromin Standard rat pellets³ and water ad libitum. Portacaval anastomosis was performed as described by BISMUTH⁴, except for the closure of the abdominal wall which was carried out with interrupted sutures using 4/0 perlon thread⁵. The rats were included in the analysis only if no experiments were performed which significantly influenced the normal living conditions of the animals. Thus, the only experimental procedures in these rats were blood sampling, motility testing and investigations of food intake and fluid balance. Body weight was taken weekly, at operation and at the time of sacrifice. The autopsy included verification of the patency of the shunt, weighing of liver and testes and dissection of both kidneys and the bladder. For the purpose of comparison, similar observations were recorded in all control and sham operated rats.

Weighed samples of calculi were analyzed for the content of uric acid with the uricase method⁶. The inorganic composition of the ash of calculi was dissolved in 1 N hydrochloric acid and assessed for calcium and phosphorus. Calcium was determined in the presence of 1% LaCl₃ on an atomic absorption spectrophotometer Perkin-Elmer 290 B. Phosphorus was measured according to the method of CHEN et al. as modified by BISAZ^{7,8}.

Results. Among the 53 rats prospectively evaluated for the presence of urolithiasis 47% were found to have stones. The calculi in general were of varying size ranging from gravel up to pieces measuring 5 mm in diameter. They were round to oval shaped, layered on cut sections and of yellow to brownish color.

In 51 rats the kidneys and ureters appeared otherwise normal macroscopically. Blockage of the ureteral ostium to the bladder by a calculus with consequent hydroureter

and hydronephrosis was seen in a total of 6 instances (4 of them in another series of rats). Often the bladder was enlarged and firm due to the presence of a large number of calculi. In one case hemorrhage lead to marked distension of the bladder, bilateral hydronephrosis, anemia and death of the animal 80 days after shunting.

The portacaval anastomosis was found to be widely patent in all rats. Liver weight and weight of testes were on the average decreased by 43 and 64%, respectively.

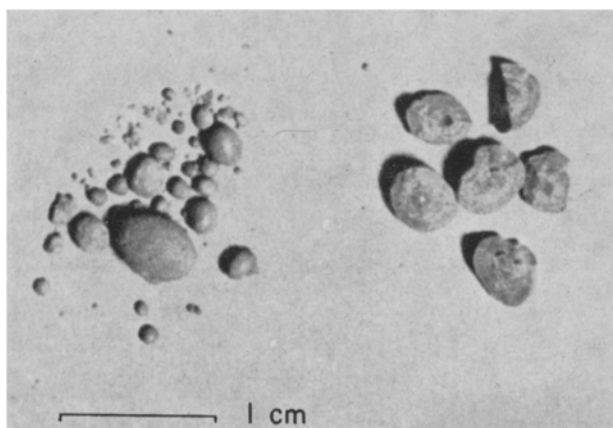
During the same period of time, 92 control and sham operated rats were autopsied. No urolithiasis was found in any of them.

The occurrence of stones appeared to be related to duration of survival after operation and to the changes in body weight (Table). Rats allowed to survive longer than 30 days with a portacaval anastomosis were more likely to exhibit calculi ($P < 0.05$). If the animals did not regain the preoperative weight, they also were more likely to have stones at autopsy ($P < 0.01$). The 17 rats surviving more than 30 days and losing weight had an incidence of urolithiasis of 94%.

Chemical analysis of 2 weighed samples of calculi showed an uric acid content of 70 and 80%, respectively. Examination of the dried ashes for inorganic compounds revealed only traces of phosphate (0.6 mg/100 mg) and calcium (1.2 mg/100 mg).

Discussion. The present investigation has documented a highly significant incidence of uric acid nephrolithiasis in rats subjected to portacaval anastomosis. To our knowledge such an association has not been described previously. An allusion only to a dilated right renal pelvis containing some crystals may be found in the careful study by CAVANAGH et al.⁹

The occurrence of uric acid lithiasis in mammals other than primates is unexpected. In these species uric acid is not the main metabolic endproduct of xanthine metabolism, but is broken down to allantoin with the help of the hepatic enzyme uricase. Thus, no ready explanation for the high incidence of nephrolithiasis is apparent. Urate nephropathy has been produced in rats by administration of excessive doses of uric acid¹⁰ or by the combination of the uricase inhibitor oxonic acid with uric acid administration^{11,12}. It appears possible, therefore, to supersaturate the rat urine with uric acid at physiological pH values provided the concentrations of uric acid are raised high enough.



Renal and bladder-calculi found in rats subjected to portacaval anastomosis. (The mark corresponds to 1 cm).

¹ J. D. DEGOS, R. CLOCHE, H. BISMUTH and J. P. BENHAMOU, *Rev. fr. Étud. clin. biol.* 14, 260 (1969).

² Source of supply: Tierfarm AG, Sisseln (Switzerland).

³ Source of supply: Kunath AG, Aarau (Switzerland). Composition: proteins 19.2%, fat 4.0%, fibres 5.3%, ashes 6.5%, water 10.8%, soluble, nitrogen-free substances 54.2%.

⁴ H. BISMUTH, J. P. BENHAMOU and J. LATASTE, *Presse méd.* 39, 1859 (1963).

⁵ Source of supply: Société Steril Catgut, Neuhausen am Rheinfalt (Switzerland) as Supramid®.

⁶ R. RICHTERICH, *Klinische Chemie*, 2. Aufl. (S. Karger, Basel, New York 1968), p. 279.

⁷ P. S. CHEN, T. Y. TORIBARA and A. WARNER, *Analyt. Chem.* 28, 1756 (1956).

⁸ S. BISAZ, R. G. G. RUSSEL and H. FLEISCH, *Arch. oral Biol.* 13, 683 (1968).

⁹ M. H. KYU and J. B. CAVANAGH, *Br. J. exp. Path.* 51, 217 (1970).

¹⁰ H. DUNCAN, K. G. WAKIM and L. E. WARD, *Proc. Staff Meet. Mayo Clin.* 38, 411 (1963).

¹¹ B. STAVRIC, W. J. JOHNSON and H. C. GRICE, *Proc. Soc. exp. Biol. Med.* 130, 512 (1969).

¹² W. J. JOHNSON, B. STAVRIC and A. CHARTRAND, *Proc. Soc. exp. Biol. Med.* 131, 8 (1969).

Incidence of urolithiasis in rats after portacaval anastomosis

	No. of rats	% with lithiasis	P
Whole series	53	47	
Survival after shunting			
7 to 30 days	31	39	<0.05
31 to 80 days	22	73	
Autopsy weight higher than body weight at operation	10	10	<0.01
Autopsy weight lower than body weight at operation	43	63	

In our experiments there are several alternatives to account for supersaturation of the urine with uric acid. The marked atrophy of liver and testes may be associated with increased breakdown of nucleo-proteins and augmented uric acid formation. Since the reduction in weight of liver and testes is completed after 10 and 20 days, respectively, it is difficult to account for the higher incidence of lithiasis 30 or more days after operation. It is possible that an alteration of renal tubular function may lead to a lowered pH in the rats after portacaval anastomosis with a consequent decrease in solubility of uric acid¹³. Alternatively the reduction of the hepatic circulation and of the functioning liver cell mass might be associated with decreased conversion of uric acid to allantoin. This could lead to a significant and sustained

increase in uric acid excretion. Finally the synthesis of purine-metabolites could be accelerated for other reasons.

The incidence of nephrolithiasis in our group of rats is highly significant. It is noteworthy that the observed complications of nephrolithiasis are strikingly similar to those seen in man. It appears, therefore, that the rat with portacaval anastomosis might be a suitable animal model for the study of uric acid stone formation¹⁴.

Zusammenfassung. Nach Anlage eines portocavalen Shunts bei 53 Sprague-Dawley-Ratten wurde in 47% aller Fälle eine Harnsäure-Urolithiasis beobachtet. Das Vorkommen war bei den Ratten am häufigsten (94%), die mehr als einen Monat überlebten und postoperativ an Gewicht verloren.

R. HERZ, V. SAUTER and J. BIRCHER¹⁵

Department of Clinical Pharmacology,
University of Berne, CH-3008 Bern (Switzerland),
8 October 1971.

¹³ A. GUTMAN and T. F. YU, *Am. J. Med.* 45, 756 (1968).

¹⁴ Supported by the Swiss National Foundation for Scientific Research.

¹⁵ Acknowledgments. The authors are grateful to Dr. K. LAUBER of the Department of Medical Chemistry and to Prof. H. FLEISCH of the Department of Pathophysiology of the University of Berne for performing the chemical analyses. The technical assistance of Miss M. KAPPELER and Miss B. SCHÜTZ is greatly appreciated.

Über tagesrhythmische Schwankungen der Fettresorption

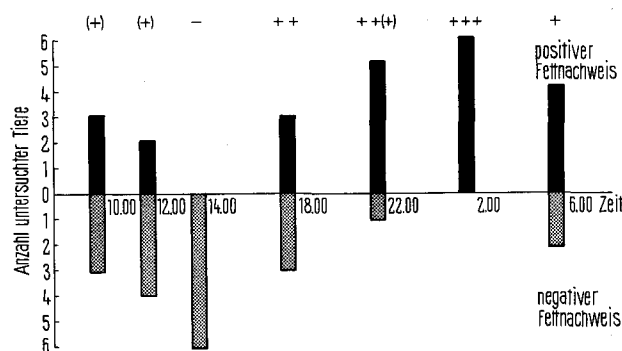
Es gilt als erwiesen, dass der lebende Organismus eine «circadiane Organisation» besitzt, und zahlreiche Organe, Zellen und Zellenorganellen charakteristische tagesrhythmische Veränderungen durchmachen¹. Hier soll geprüft werden, ob sich tagesrhythmische Schwankungen auch bei der Fettresorption im Dünndarm nachweisen lassen.

7 Gruppen von jeweils 6 anästhesierten Ratten (1 ml 10%iges Urethan/100 g Körpergewicht), die 24 h gehungert hatten, wurde zu verschiedenen Tages- und Nachtzeiten jeweils 0,1–0,15 ml Mazola in 3–4 cm lange, abgebundene Abschnitte des oberen Jejunums injiziert. 1 min nach Beginn der Fettinjektion wurde das Darmgewebe entnommen und an 10 µm dicken, mit Sudanschwarz B gefärbten Kryostatschnitten bestimmt, wieviele Tiere einer Gruppe einen positiven bzw. negativen Fettnachweis zeigten. Der Zeitpunkt von 1 min wurde gewählt, weil Vorversuche am Vormittag gezeigt hatten, dass etwa diese Zeitspanne vergeht, bis Fett in den Enterocyten nachweisbar ist.

Die Zahl der Tiere mit einem positiven Fettnachweis ist um 02.00 Uhr am höchsten und nimmt dann sukzessive ab (Figur). Um 14.00 Uhr zeigt kein Tier Fettaufnahme. Danach steigt die Zahl der Tiere mit positivem Fettnachweis wieder charakteristisch an. Nach visuellen Schätzungen der nach 1 min in den Enterocyten vorhandenen Fettmenge enthalten die Enterocyten nachts mehr Fett als am Tage.

Zur Klärung der Frage, ob die tagesrhythmischen Unterschiede der Fettresorption mit der unterschiedlichen

Zusammensetzung des Darmsaftes zu den verschiedenen Zeiten zusammenhängen, injizierten wir 6 Ratten um 14.00 Uhr Mazolachymus, der um 02.00 Uhr gewonnen



Tagesrhythmische Schwankungen der Fettresorption im proximalen Jejunum der Ratte. Dargestellt ist, wie viele Tiere eines Kollektivs 1 min nach Mazolainjektion in das Darmlumen einen positiven bzw. negativen Fettnachweis in den Enterocyten aufweisen. Die Symbole (+) bis ++++ geben die Menge des von den Enterocyten aufgenommenen Fettes an (visuell geschätzt).

¹ E. BÜNNING, in *The Cellular Aspects of Biorhythms* (Ed. H. v. MAYERSBACH; Springer, Berlin, Heidelberg, New York 1967).