Association between hypouricaemia and jaundice

L. SCHLOSSTEIN, I. KIPPEN, R. BLUESTONE, M. W. WHITEHOUSE, AND J. R. KLINENBERG

From the Department of Medicine (Rheumatology Section), Veterans Administration Wadsworth Hospital Center; Department of Medicine (Rheumatology Division), UCLA School of Medicine; and Division of Medicine, Cedars-Sinai Medical Center, Los Angeles, California

During routine biochemical screening of over 4,000 patients admitted to the Veterans Administration Wadsworth Hospital Center, a small number were found to have serum urate concentrations below 2.2 mg/100 ml. Most of these patients had profound illness, including severe hepatic involvement with jaundice.

It is known that circulating bilirubin is highly bound to proteins (Odell, 1959). Previous studies in this laboratory have shown that many protein-bound anions may displace protein-bound urate *in vitro* and may have a uricosuric effect when given to patients with hyperuricaemia or to normal volunteers (Bluestone, Kippen, and Klinenberg, 1969; Klinenberg, Bluestone, Schlosstein, Waisman, and Whitehouse, 1973). Therefore, a study of urate excretion and plasma binding of urate in transiently jaundiced patients was undertaken to investigate any direct relationship between hyperbilirubinaemia and hypouricaemia. Additional experiments were performed with rats that were made jaundiced by ligation of the common bile duct.

Methods and materials

RETROSPECTIVE PATIENT STUDY

A record search of the VA Wadsworth Hospital Center was undertaken to review the charts of all the patients found to have hypouricaemia (defined as two or more consecutive serum urate concentrations of less than 2.2 mg/100 ml, measured by an autoanalyser) during one calendar year. The age, sex, race, and clinical or post-mortem diagnosis were determined. Serum urate concentration and biochemical evidence of liver disease, including serum bilirubin, alkaline phosphatase, and serum proteins, were measured by autoanalyser (Sobrinho-Simoes, 1965). The recent use of cholecystographic agents and drugs which alter the serum urate was also noted. Finally the subsequent course of the patients under review was determined when that information was available.

PROSPECTIVE STUDY OF JAUNDICED PATIENTS

Five patients with hyperbilirubinaemia (2 with Australia

antigen-associated hepatitis, 1 with halothane-sensitivity hepatitis, 1 with alcoholic cirrhosis, and 1 with gallstone obstruction of the common bile duct) were studied while jaundiced and for up to 10 weeks (mean 5 weeks) after complete or partial remission of jaundice. All jaundiced patients who were observed on the wards were considered for inclusion in the study. Fifteen patients were studied with collections of 24-hour urinary uric acid and serum urate. Only in five patients was it possible to obtain a second 24-hour urinary uric acid and serum urate. These were the patients included in this report.

Blood was collected, allowed to clot, then immediately centrifuged, and the serum frozen and stored at -20°C for up to one month before biochemical determinations. Bilirubin concentrations were assayed the day the blood was drawn. Urine was collected for a 24-hour period at room temperature with 3 ml toluene as a preservative. Urine aliquots were taken after measurement of the volume of the total sample and stored at -20°C for periods up to one month before determination of uric acid and creatinine.

Uric acid determinations were performed by an enzymatic spectrophotometric method (Liddle, Seegmiller, and Laster, 1959), total bilirubin was measured by an autoanalyser, total protein by the biuret method (Gornall, Bardawill, and David, 1949), and albumin after separation of serum proteins by the Beckman microzone electrophoresis system. Binding of urate to plasma proteins was determined as described below.

None of these prospectively studied patients was taking drugs, such as salicylate, known to alter serum urate or binding of urate to serum protein (Bluestone and others, 1969). The patient with gallstones was studied by oral cholecystography using iopanoic acid two weeks before obtaining the first urine and serum samples for the present study. This was after a period that is considerably longer than the known uricosuric activity of cholecystographic agents reported (Mudge, 1971; Postlethwaite and Kelley, 1971).

ANIMAL EXPERIMENTS

The common bile ducts of male CFN rats, weighing approximately 250 g, were ligated under ether anaesthesia and the animals were killed after 20 hours when a vivid jaundice was established, manifested by yellowing of the ears. Blood was obtained by intracardiac puncture at the

time of death, withdrawn into heparinized containers, and the plasma separated by centrifugation. Urate binding to plasma proteins was determined by equilibrium dialysis of the jaundiced plasma alone, or of the jaundiced plasma serially diluted (1:1, 1:2, 1:4) with normal rat plasma, and of normal rat plasma to which was added varying amounts of rat bile. Pure bilirubin could not be added due to its low solubility. 3 ml plasma in a semipermeable dialysis bag were dialysed for 16 hours at 4°C against a large volume of phosphate buffer (0.01 mol/l. sodium phosphate buffer pH 7.35, containing 0.015% sodium azide) containing uric acid at a concentration of 15 mg/100 ml (Klinenberg and Kippen, 1970). The excess urate concentration inside the bag at the end of the dialysis, compared to the urate concentration in the dialysing fluid, was considered bound to proteins in the plasma.

Results

RETROSPECTIVE PATIENT STUDY

Twenty-two out of 4,148 patients, screened by auto-analyser, during the year had hypouricaemia. Table I summarizes the clinical data available for fifteen of these 22 patients. The remaining charts could not be located. The age range of the patients (41 to 75 years) was representative of the overall population of the hospital. There were two women patients and thirteen men patients, reflecting the male predominance expected in the hospital.

Five of the fifteen patients had liver disease and jaundice: either cirrhosis resulting from alcoholism

or metastatic disease. Four of these subsequently died and one was lost to follow-up. Four other patients were taking drugs known to be uricosuric. One of these four was taking both hydrochlorothiazide and high-dosage aspirin (the former drug raises serum urate when given chronically, and the latter lowers serum urate by impairing tubular urate re-absorption). Two others in this group were taking high-dosage aspirin. The last of these four patients (who had adenocarcinoma of the lung as well) was given dexamethasone, a drug reported to be uricosuric (Sprague, Power, Mason, Albert, Mathieson, Hench, Kendall, Slocomb, and Polley, 1950). Of the remaining six patients, three had chronic alcoholism, one with associated maturity onset diabetes mellitus, and another had associated chronic osteomyelitis of the femur and was undergoing extended treatment with tetracycline. The other three patients had carcinoma. One had bronchogenic carcinoma and mild diabetes mellitus controlled without drugs; at necropsy no liver metastases were found. Another had carcinoma of the oropharynx and died of ventilatory obstruction, but showed no evidence of liver involvement at necropsy. The final patient had carcinoma of the retromandibular area and tonsillar pillar without clinical evidence of liver disease.

PROSPECTIVE STUDY OF JAUNDICED PATIENTS
Shown in Table II are data derived from the jaundiced patients studied prospectively. Bilirubin was variably

Table I Details of retrospectively studied patients

Case no.	Sex	Age	Uric acid (mg/100 ml) (n 2.6-8.0)	Bilirubin (mg/100 ml) (n 0.2-1.1)	Alkaline phosphatase (K-A units) (n 2-14)	Albumin/ globulin (g/100 ml) (n 3.5-5/2.5-3)	Comments
1	M	57	2.1	5.3	33	1.3/5.3	Cirrhosis; died
2	M	62	2.1	4.3	27	1.6/4.5	Adenocarcinoma stomach, died; liver metastasis
3	M	49	2.2	12.4	55	2.6/5.4	Adenocarcinoma pancreas, died; liver metastasis
4	M	50	2.0	12.0	18	2.9/3.2	Cirrhosis; recovered
5	M	66	2.2	3.0	64	2.3/3.4	Adenocarcinoma pancreas, died; liver metastasis
6	F	71	2.2	0.92	13	4.2/3.5	Healed pulmonary TB, ASA 2.5 g/day
7	F	74	2.2	0.3	20	3.2/3.7	Peripheral vascular disease, ASA 1.8 g/day
8	M	59	1.6	0.4	7	3.4/3.3	Adenocarcinoma lung, died; no liver metastasis; dexamethasone 16 mg/day
9	M	89	1.5	0.3	12.8	3.2/2.6	ASHD-CHF-pneumonia, died; ASA 2.7 g/day; IHN, hydro- chlorothiazide 50 mg/day
10	M	41	1.9	0.7	11.2	4.5/2.1	Alcoholism
11	M	64	2.2	0.7	10	3.4/4.1	Alcoholism, diabetes mellitus
12	M	46	1.8	0.4	10.4	2.8/3.8	Osteomyelitis, alcoholism, emphysema
13	M	49	2.2	0.7	15	4.8/2.5	Carcinoma, tonsil
14	M	47	1.1	0.7	13.5	3.3/4.0	Carcinoma, oropharynx, died; no liver metastasis
15	M	75	0.8	1.1	9.0	2.9/3.7	Bronchogenic carcinoma, died; no liver metastasis

Table II Paired values of first and second values for parameters measured

Case no.	Sex	Age	Diagnosis	Bilirubin (mg/100 ml)	Serum albumin (g/100 ml)	Serum urate (mg/100 ml)	Urinary uric acid (mg/24 hrs)	Urate clearance (ml/min)	Urate binding (μg/ml)	μg urate bound/mg albumin
1	M	24	Hepatitis	21.5 2.5	3.4 3.4	5.3 6.7	932 632	12.2 6.5	27 40	0.79 1.18
2	M	29	Hepatitis	11.5 6.7	3.0 3.6	4.9 6.0	970 683	13.7 7.9	20 30	0.68 0.83
3	M	43	Cirrhosis	3.5 0.8	1.9 3.2	3.6 5.0	315 556	6.1 7.7	20 32	1.05 1.00
4	M	48	Extrahepatic obstruction (gallstones)	13.2 1.0	1.9 3.7	3.8 3.7	860 575	15.7 10.8	14 41	0.74 0.90
5	F	59	Halothane hepatitis	6.0 0.7	3.4 3.3	5.6 7.6	484 514	6.0 4.6	25 35	0.74 1.10
Mean difference				8.8 ± 0.3	7 ± 0.4	1.2 ± 0.3	120 ± 109	3.2 ± 1.4	14.4 ± 3.2	0.2 ± 0.07
Paired data analysis				P < 0.05		P < 0.05		P < 0.1	P < 0.01	P < 0.05

but definitely raised at the time of initial study, and returned toward normal levels at the time of the second study. Serum urate concentration rose significantly with remission of the hyperbilirubinaemia, showing a mean rise of 1.2 ± 0.3 mg/100 ml ($P < 0.05$). There was a marked decrease in the renal clearance of urate in four of the five patients with lessening of jaundice. A single patient showed a slight increase in urate clearance. When the four patients who had decreased renal clearance of urate were analysed separately, there was a mean decrease in urate clearance of 4.45 ± 1.0 ml/min ($P < 0.02$). However, when the statistical analysis included the single patient showing a slight increase in renal urate clearance, the mean decrease was not significant at the 0.05 level. Though a less sensitive measure of increased renal clearance, the 24-hour urinary urate decreased in 3 of our patients after reduction of jaundice. Urate binding to serum proteins *in vitro* increased significantly as the serum bilirubin concentration fell, with a mean increase of 14.4 ± 3.2 μg/ml in urate binding ($P < 0.01$). Due to variations in serum albumin concentration in three patients and previous studies showing that most plasma urate binding is dependent upon albumin (Bluestone, Kippen, Klinenberg, and Whitehouse, 1970), we also calculated the protein-bound urate expressed as μg urate/mg serum albumin. There was an 0.2 ± 0.07 μg increase in urate bound/mg serum albumin ($P < 0.05$). Thus, it is clear that as the jaundice resolved there was an increase in the amount of urate bound to serum albumin as measured *in vitro*.

ANIMAL EXPERIMENTS

The urate binding of normal plasma was approximately 40 μg/ml and that of jaundiced plasma was nil. The serial dilution of normal plasma with increasing amounts of jaundiced plasma resulted in a linear decrease in urate bound to proteins. Dilution of rat plasma by 7% with rat bile reduced urate binding by over 30%, and dilution by 18% eliminated urate binding completely. These data include corrections made for the effect of dilution itself. These experiments indicate that bilirubin, or its naturally occurring conjugates, or some other substances which accumu-

late subsequent to bile duct ligation, inhibit the binding of urate to rat plasma proteins.

Discussion

The clinical observation that hypouricaemia is unusual was confirmed by our finding only 22 patients with serum urate below 2.2 mg/100 ml in a review of 4,148 uric acid determinations. Lawee (1969) reviewed 1,000 unsolicited uric acid determinations and found 58 patients with uric acid below 2.6 mg/100 ml. He listed numerous diagnoses associated with hypouricaemia, including neoplasms, diabetes mellitus, arteriosclerotic heart disease, and epilepsy. Bennett, Bond, Singer, and Gottlieb (1972) recently described two patients with Hodgkin's disease with hypouricaemia corrected by successful therapy of the underlying lymphoproliferative disease. They postulated that some products of tumour metabolism enhanced renal tubular urate secretion. In our review of hypouricaemic patients it was noted that one-third were being treated with drugs known to increase urate excretion and the remainder had chronic liver disease, carcinoma with hepatic involvement, or other chronic cachectic disease. We addressed our investigation to the striking relationship between bilirubin and urate apparent in five of the fifteen documented patients. The reasons for the apparent inverse relationship between serum bilirubin and serum urate are unknown, but several possibilities may be considered.

(1) Hepatic cell derangement may result in decreased *de novo* urate synthesis. Four of the prospectively studied patients had primary hepatocellular damage of varying degrees, and it was in these four where the relationship between bilirubin and serum urate was most striking. However, the lowered serum urate at the time of jaundice was associated with measurable increase in renal urate clearance in four patients. Thus, it appears unlikely that a reduction in urate formation by the liver was the sole cause of the lowered serum urate.

(2) Keys, Brozek, Henschel, Mickelsen, and Taylor (1950) showed a drop in serum urate level from 3.9 to

2.8 mg/100 ml in a group of subjects experiencing semistarvation for 24 weeks. While some of our retrospectively studied patients may well have been cachectic, their hypouricaemia was far more pronounced than that produced by Keys and co-workers in semistarvation. Moreover, there was no question of malnutrition in our prospectively studied, transiently jaundiced patients.

(3) The seasonal fluctuations in serum urate (Goldstein, Becker, and Moore, 1972) do not appear to be responsible for the results observed in the present study. The variations we observed were all in a single direction and not like the random seasonal fluctuations which would occur either up or down.

(4) Hypouricaemia and increased urate clearance in twenty jaundiced patients has previously been reported (Pasero and Masini, 1959). They concluded that the increased urate clearance was caused by impaired tubular reabsorption of urate due to damage to the renal tubules. However, the possible biological role of bilirubin as a uricosuric agent has not previously received serious consideration. Previous observations in our laboratories (Bluestone and others, 1969) have shown that the plasma of patients taking uricosuric drugs shows reduced urate binding when measured *in vitro*, due to displacement of urate from binding sites on the plasma proteins. All the uricosuric agents we have tested have shown the ability to displace urate from plasma proteins *in vitro* (Whitehouse, Kippen, and Klinenberg, 1971). Since bilirubin is highly bound to plasma proteins, it could be acting as a uricosuric agent. This is supported by the *in vitro* observations made on the urate binding properties of our patients' plasma. When jaundiced, their plasma urate binding capacity was impaired and returned to normal following the resolution of their jaundice. Further support for this relationship was obtained from the animal studies. In rats made icteric by bile duct ligation, the urate binding to their plasma proteins was completely abolished 20 hours after the surgical procedure. Moreover, dialysis experiments, not described here, using the bile salts of taurocholic and taurochenocholic acid indicated that these compounds had no ability to displace urate from albumin. Thus, the urate-displacing effect of jaundiced plasma resides in the bilirubin or in some other unidentified biliary component fraction.

We have previously postulated that the mechanism of action of uricosuric drugs could be related either to a reduction in *in vivo* urate binding, causing in-

creased glomerular filtration of urate, or to an inhibition of tubular reabsorption of urate. It must be emphasized that the *in vitro* urate binding assay done at 4°C utilized in the present studies does not provide information regarding the *in vivo* handling of urate. This assay showed only that the urate binding to plasma of jaundiced patients is reduced similarly to the urate binding of plasma of patients taking uricosuric drugs.

The hypoalbuminaemia present in three of our prospectively studied patients could conceivably contribute to the diminished urate binding capacity of the plasma by decreasing the number of urate binding sites available. Indeed, unpublished observations in this laboratory have shown impaired urate binding in patients with gross hypoalbuminaemia secondary to nephrotic syndrome. However, when we calculated the μg urate bound per mg albumin present, there was still decreased binding of urate.

Thus, the clinical observation of hypouricaemia in jaundiced patients seems to be a true one and could be related to increased urate clearance caused by bilirubin acting as a uricosuric agent.

Summary

On screening 4,148 patients at a general hospital during one year, only 0.5% had confirmed plasma urate levels of less than 2.2 mg/100 ml. The hypouricaemia was usually associated with severe hepatic disease and jaundice. To determine whether the apparent association between hypouricaemia and hyperbilirubinaemia was a real one, the relationship between plasma urate and bilirubin was studied in five transiently jaundiced patients during their acute illness and later after complete or partial recovery. The mean serum bilirubin fell from 11.1 to 2.3 mg/100 ml as the patients recovered. This was accompanied by (1) a rise in mean serum urate from 4.6 to 5.8 mg/100 ml; (2) a mean decrease in renal urate clearance of 3.2 ml/min; (3) a mean increase of plasma urate binding *in vitro* of 0.2 μg urate bound/mg albumin. Thus, the hypouricaemia associated with hyperbilirubinaemia may be effected in part by increased urate clearance. This increased urate clearance may be caused by bilirubin acting as a uricosuric agent.

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