is also recommended for caesarean section, thyroidectomy, and patients exhibiting partial respiratory obstruction.

As was noted with the former methods of administering steroid anaesthesia, presuren minimizes the usual post-operative fatigue. It produces a sense of well-being during the recovery period, which enables patients to disregard most of the discomforts following operation.

We thank those registrars, house officers, and nurses, who assisted us in making clinical observations incorporated in this report. Our thanks are also due to Dr. med. J. Friebe and Mr. G. Stuart Blackie, of the Medical Scientific Department, Schering A.G. Berlin, for their co-operation throughout this trial; and Schering A.G. Berlin and their subsidiary company, Pharmethicals (London) Limited, for the generous supplies of presuren. The respiratory tracings were recorded on an apparatus constructed in the physics department, King's College Hospital, with the aid of grants to one of us (A. H. G.) from the University of London research grants committee and the King's College Hospital and Medical School joint research committee.

REFERENCES

- Burstein, C. L. (1956). *Curr. Res. Anesth.*, **35**, 476.
 Dauri, A. (1955). *Gazz. int. Med. Chir.*, **19**, 1516.
 Deligné, P., and David, M. (1956). *Anesth. et Analg.*, **13**, 667.
 Dent, S. J., Wilson, W. P., and Stephen, C. R. (1956). *Anesthesiology*, **17**, 672.
 Galley, A. H., and Rooms, M. (1956). *Lancet*, **1**, 990.
 Gordan, G. S., Guadagni, N., Picchi, J., and Adams, J. E. (1955). *Méd. et Hyg. (Genève)*, **13**, No. 297, p. 251.
 ——— (1956). *J. int. Coll. Surg.*, **25**, 9.
 Grewe, H. E., Piechowski, U., and Zuckert, D. (1957). *Zbl. Chir.*, **82**, 1671.
 ——— (1958). *Chirurg*, **29**, 121.
 Harbord, R. P., and Wild, W. N. (1956). *Proc. roy. Soc. Med.*, **49**, 487.
 Howland, W. S., Boyan, C. P., and Kuo-Chen Wang (1956). *Anesthesiology*, **17**, 1.
 Laborit, H., Huguenard, P., Douzon, C., Weber, B., and Guittard, R. (1956). *Arch. int. Pharmacodyn.*, **107**, 159.
 Landau, E. (1958). *Anaesthesia*, **13**, 147.
 Laubach, G. D., P'an, S. Y., and Rudel, H. W. (1955). *Science*, **122**, 78.
 Lerman, L. H. (1956). *Brit. med. J.*, **2**, 129.
 Murphy, F. J., Guadagni, N. P., and DeBon, F. (1955). *J. Amer. med. Ass.*, **158**, 1412.
 O'Flanagan, P. M., and McGuinness, O. L. (1958). *Lancet*, **1**, 196.
 Opderbecke, H. W. (1957). *Thoraxchirurgie*, **5**, 53.
 Selye, H. (1941a). *J. Immunol.*, **41**, 259.
 ——— (1941b). *J. Pharmacol. exp. Ther.*, **73**, 127.
 ——— (1942a). *Endocrinology*, **30**, 437.
 ——— (1942b). *Curr. Res. Anesth.*, **21**, 41.
 ——— (1943). *Ibid.*, **22**, 105.
 Stedtfeld, G. (1957). *Anaesthesist*, **6**, 140.
 Taylor, N., and Shearer, W. M. (1956). *Brit. J. Anaesth.*, **28**, 67.

Only 213 cases of plague were officially recorded in 1958, the lowest number since the beginning of the century, according to the World Health Organization. This continues the downward trend of the incidence of plague observed in recent years, especially since 1951, when 15,399 cases were reported (of which 8,230 were in India). The distribution of the 213 cases in 1958 was as follows. In continental Africa 6 cases were reported in the north-eastern part of Belgian Congo and 19 cases in the Central Province of Kenya. In Madagascar 21 sporadic cases were recorded in several local areas, most of which were situated in Tananarivo Province. In America active foci were located in Ecuador, Peru, and in the state of Bahia in Brazil. In Asia (outside continental China and the U.S.S.R.) a new focus (11 cases) was discovered in the Kurdistan-Miandoab area of Iran. The 26 cases reported in India were observed in three districts in the southern part of the country. Eighteen cases were reported in Burma, and 15 in Viet-Nam.

URINARY GLUCURONIC ACID EXCRETION IN LIVER DISEASE AND THE EFFECT OF A SALICYLAMIDE LOAD

BY

H. T. F. BARNIVILLE, M.D., M.R.C.P.I.

Research Assistant

R. MISK, M.D.

British Council Scholar

Department of Medicine, Postgraduate Medical School
of London, W.12

Bilirubin is liberated from the breakdown of haemoglobin in the reticulo-endothelial system and gives the indirect van den Bergh reaction (Lemberg, 1956). It is transformed to direct-reacting bilirubin by conjugation with one or two glucuronic acid molecules to form bilirubin mono- or di-glucuronide, and this occurs at the site of the carboxyl groups of the bilirubin molecule (Billing and Lathe, 1956; Schmid, 1956; Talafant, 1956; Schachter, 1957). The diglucuronide is almost certainly formed exclusively in the liver, the monoglucuronide in other sites also. Bilirubin diglucuronide is present in the bile in much greater amounts than monoglucuronide, and Bollman (1956) has shown that in hepatectomized dogs monoglucuronides of bilirubin can be found in the blood, while the diglucuronide disappears completely.

It has been suggested that some forms of severe congenital, non-obstructive, non-haemolytic jaundice are due to an inability to conjugate bilirubin with glucuronic acid (Arias and London, 1957; Schmid *et al.*, 1957; Axelrod *et al.*, 1957; Schmid, 1958; Peterson and Schmid, 1957).

Our purpose was to investigate the ability of the liver to perform this conjugation in congenital non-obstructive, non-haemolytic jaundice of the milder adult type (Gilbert's disease) (Gilbert and Lereboullet, 1902), in the Dubin-Johnson type of pigmentary hyperbilirubinaemia (Dubin and Johnson, 1954), and in various diseases of the liver. Salicylamide was chosen as the test substance because it is carried in the blood, like bilirubin bound to albumin, and is largely excreted conjugated with glucuronic acid (Goodman and Gilman, 1955); it is also non-toxic.

Material and Methods

Of the 40 subjects studied (see Table), 12 had no evidence of liver disease. Of the others, 14 suffered from portal cirrhosis; 3 from primary biliary cirrhosis; 6 from extrahepatic obstruction to the biliary passages—in one of these (Case 19) the obstruction was intermittent so that he was not jaundiced at the time of study; 4 had intermittent jaundice due to Gilbert's type of hyperbilirubinaemia—in every instance haemolysis had been excluded as the cause of jaundice, and an aspiration liver biopsy showed normal histology; and 1 suffered from the Dubin-Johnson type of hyperbilirubinaemia confirmed by the presence of large granules of unidentified brown pigment in liver biopsy sections.

The subjects received the general hospital diet—those with ascites were excluded, as significant amounts of conjugated substances, such as tetrahydrocortisone, can be found in ascitic fluid, and a falsely low urinary excretion might result (Englert *et al.*, 1957). Patients

with glycosuria were excluded, as glucose may interfere with the urinary estimation of glucuronic acid. Peptic ulcer or a history of sensitivity to salicylates was regarded as a contraindication to the giving of salicylamide.

In all the subjects a 24-hour urine was obtained, and the collection checked by creatinine estimations.

In 25 instances 4 g. of salicylamide was given by mouth and the urine collected 4 and 24 hours later. In two instances a further 24-hour urinary collection was made.

The glucuronic acid content of each specimen was estimated by the method of Fishman and Green (1955). Biochemical tests of liver function were performed by standard procedures.

The results of the investigation are given in the Table.

Urinary Glucuronic Acid Excretion

In the control period the daily urinary excretion of glucuronic acid scattered widely, but was rather higher in the presence of jaundice (mean 692 mg.) than in its absence (mean 468 mg.). However, no relationship

FIG. 1.—The 24-hour urinary glucuronic acid excretion related to the serum bilirubin.

could be found between the amount of glucuronic acid excreted and the depth of jaundice (Fig. 1).

The 12 subjects without liver disease showed a mean excretion of 395 mg. daily (211–561 mg., S.D. 111), and in the two patients studied this varied little from day to day. The 13 cirrhotic patients showed a higher excretion (mean 571 mg.), but this was accounted for by increases outside the normal range in the four jaundiced patients—cirrhotic patients without jaundice showed values within the normal range. The three patients with primary biliary cirrhosis excreted amounts in the upper range of normal. Among the six patients with obstruction of the extrahepatic bile ducts, four of the five jaundiced excreted amounts outside the normal range.

The five patients with congenital hyperbilirubinaemia, whether of Gilbert or Dubin-Johnson type, excreted high normal or increased amounts of glucuronic acid.

Effect of Oral Salicylamide (Fig. 2)

After the oral dose of 4 g. of salicylamide the 24-hour urine output of glucuronic acid increased greatly, presumably owing to the conjugation of salicylamide with glucuronic acid. In two patients the second 24-hour urine collected after giving salicylamide contained approximately the same amount of glucuronic acid as in the control period.

Six normal subjects showed a mean increased excretion over the control period of 4,078 mg. The mean increased excretion of the seven cirrhotic patients was less (3,881 mg.). This could be accounted for by the low excretion of the three patients who were jaundiced (Fig. 2). With one exception of a patient with advanced secondary biliary cirrhosis, the three patients with primary biliary cirrhosis and the three with obstructive jaundice showed an increased excretion over the control period within the normal range.

The five patients with congenital hyperbilirubinaemia showed normal or high levels.

Subjects Studied

No.	Sex	Age	Diagnosis	Serum, 100 ml.				Urinary Glucuronic Acid (mg.)		
				Bilirubin mg.	Phosphatase K.A. Units	Albumin g.	Globulin g.	Hours before Salicylamide	Hours after Salicylamide	
								24	4	24
1	F	35	Portal cirrhosis	3.1	60	3.8	4.6	681		
2	F	49	" "	1.2	20	2.3	3.9	463		
3	F	36	" "	0.8	25	2.6	3.8	286		
4	F	11	" "	1.7	20	2.3	3.5	425		
5	M	40	" "	1.0	10	3.3	4.7	410		
6	F	53	" "	0.7	12	3.4	3.7	488		
7	F	14	" "	10.8	11	2.5	8.1	679	248	3,336
8	M	39	" "	0.5	20.6	3.4	3.4	869	2,316	5,406
9	M	31	" "	7.0	29	2.7	6.6	704	2,180	3,808
10	M	41	" "	0.6	8	3.1	4.2	530	3,387	5,000
11	F	27	" "	1.0	8	1.3	2.1	495	2,800	5,177
12	M	18	" "	0.3	5	4.6	4.0	430	2,103	4,963
13	F	13	" "	13.0	15	3.6	5.5		783	4,711
14	M	43	" "	5.0	6.6	2.1	3.7	1,173	718	4,489
15	F	40	Primary biliary cirrhosis	4.3	101	3.2	4.5	494	1,584	4,619
16	F	53	" "	4.0	40	3.9	—	445	1,323	4,765
17	M	47	" "	4.0	40	3.0	3.4	600	2,899	4,819
18	M	49	Carcinoma of bile duct	16.0	54	2.7	3.0	776		
19	F	40	Secondary liver carcinoma	12.0	40	2.9	3.9	601		
20	M	34	Bile-duct stricture	0.5	16	4.4	4.0	423	3,793	5,252
21	F	35	Obstructive jaundice	14.0	61	3.1	3.5	534	2,766	4,595
22	M	56	Carcinoma of bile duct	14.0	182	2.7	3.5	726		
23	F	37	Bile-duct stricture and secondary biliary cirrhosis	16.8	86	3.9	4.5	895	2,711	3,759
24	M	13	Dubin-Johnson disease	1.1	12	4.1	3.2	310	—	4,689
25	M	23	Gilbert's disease	0.6	2.4	3.7	3.2	490	3,727	5,137
26	M	24	" "	1.4	4	4.8	2.5	889	2,072	6,190
27	M	45	" "	0.8	5.7	3.9	3.0	790	3,404	4,688
28	M	30	" "	0.7	7	4.5	3.0	646	3,701	—
			Control	0.5-0.8	4-12	3.5-4.5	2-3	395* (211-561)	2,222† (1,333-3,510)	4,470† (5,004-3,954)

* Mean of 12 observations. † Mean of 5 observations.

FIG. 2.—Control subjects and patients with liver disease. 24-Hour urinary glucuronic acid before and the increase following 4 g. of salicylamide by mouth.

The rate of excretion of glucuronic acid can be expressed as the percentage of the 24-hour output excreted in the first four hours (Fig. 3). The range is very wide in the non-jaundiced, but the deeply icteric do tend to excrete more slowly.

FIG. 3.—Percentage of 24-hour urinary output of glucuronic acid excreted in first four hours after 4 g. of salicylamide orally, related to serum bilirubin level. ●=Normal. ○=Portal cirrhosis. △=Primary biliary cirrhosis. ▲=Obstructive jaundice. ⊙=Congenital hyperbilirubinaemia.

Discussion

Bilirubin is insoluble in water, and when excreted in the urine is conjugated with glucuronic acid as the water-soluble bilirubin glucuronide. The increased urinary glucuronic acid output in the jaundiced compared with the non-jaundiced patients is probably accounted for by this process. All the jaundiced patients studied suffered from hepatocellular or obstructive type of jaundice, in which bilirubin glucuronide would be expected in the urine; patients with haemolytic jaundice were not included.

The four patients who showed a markedly defective ability to increase their urinary glucuronic acid output after salicylamide were suffering from severe hepatocellular jaundice. In three instances the diagnosis was advanced portal cirrhosis and in the fourth decompensated secondary biliary cirrhosis. Patients with acute virus hepatitis were not available for study but might have shown similar findings. It is known that in the presence of severe hepatocellular disease there is failure of conjugation of substances such as benzoic acid (Quick, 1936; Snapper and Saltzman, 1947), cinnamic acid (Saltzman and Caraway, 1953), and also adrenal steroids (Brown *et al.*, 1954).

A form of severe congenital non-haemolytic non-obstructive jaundice has been described by Crigler and Najjar (1952).

It is characterized by a high serum level of indirect bilirubin, by the excretion of almost colourless bile containing only small quantities of bilirubin glucuronide, and by low faecal urobilinogen excretion (Axelrod *et al.*, 1957; Schmid, 1958; Crigler and Najjar, 1952). These patients can excrete such substances as bromsulphthalein and biligradin into the bile, but are unable to conjugate bilirubin (indirect bilirubin) with glucuronic acid to form bilirubin glucuronide (direct bilirubin) (Schmid *et al.*, 1957; Axelrod *et al.*, 1957).

In vitro, when crystalline bilirubin is incubated with uridine diphosphate glucuronic acid (U.D.P.G.A.) and liver microsomes of a normal rat, bilirubin glucuronide is formed (Schmid *et al.*, 1957). Normal amounts are not produced when the liver of a patient with congenital non-haemolytic jaundice is used (Axelrod *et al.*, 1957; Arias and London, 1957). The defect is probably in the enzyme (transferase) transferring the glucuronic acid from U.D.P.G.A. to bilirubin. There is also diminution of conjugating other substances with glucuronic acid; these include N-acetylamino-phenol (Axelrod *et al.*, 1957), tetrahydrocortisone (Axelrod *et al.*, 1957; Peterson and Schmid, 1957), menthol, salicylates, and metabolites of cortisone (Schmid *et al.*, 1957). A similar defect has been reported in a strain of jaundiced Wistar rats (Gunn's strain) (Carbone and Grodsky, 1957; Schmid *et al.*, 1957). The liver of these animals cannot conjugate bilirubin in the presence of U.D.P.G.A.; they also cannot conjugate orally administered *o*-aminobenzoic acid.

These patients and these rats were deeply jaundiced. Gilbert's disease is much milder: jaundice is rarely deeper than 2–4 mg./100 ml.; malaise and tiredness are the only symptoms. It may differ from the Crigler-Najjar type merely in degree. Of the four patients with Gilbert's disease that we studied, all had been jaundiced in the past, but only one showed an increased serum bilirubin level when the test was performed. All had normal levels of urinary glucuronic acid and excreted normal amounts of glucuronic acid when

challenged with salicylamide. This does not prove that bilirubin is handled in the same way; it is possible that the enzyme which transfers glucuronic acid from U.D.P.G.A. to salicylamide and to bilirubin may not be the same. It does, however, suggest that the jaundice of Gilbert's disease may not be due to an inability to conjugate bilirubin with glucuronic acid, or that the defect may be intermittent, brought on by minor stresses and strains. Conjugation of salicylamide in these cases may not have occurred only in the liver; other possible sites include the kidney and, in dogs, the mucosa of the gastro-intestinal tract (Hartiala *et al.*, 1958).

The one patient with the Dubin-Johnson syndrome studied also showed a normal glucuronic acid output both before and after salicylamide. This is not surprising, as the defect in this disease is not in conjugation, and most of the bilirubin in the serum is in direct form.

The slower rate of excretion of glucuronic acid in jaundiced as opposed to non-jaundiced subjects may be related to renal function. Renal tubular changes have been reported in the presence of jaundice (Ayer, 1940), and these may interfere with glucuronic acid elimination. The excretion of hippuric acid, for instance, is depressed in jaundiced subjects and in the presence of renal disease (Sherlock, 1946).

Summary

The mean 24-hour urinary excretion of glucuronic acid was greater in 12 patients with jaundice (excluding haemolytic) than in 12 subjects without liver disease. This was attributed to the excretion of bilirubin as glucuronide. Excretion was above or within normal limits in 13 patients with portal cirrhosis, 3 with primary biliary cirrhosis, and 7 with obstruction to the main bile ducts.

Salicylamide is excreted as a conjugate with glucuronic acid, and 4 g. orally was given to test the power of glucuronide conjugation. Impaired conjugation was noted only in three jaundiced patients with advanced portal cirrhosis and in one patient with decompensated biliary cirrhosis.

Patients with cirrhosis without jaundice conjugated the salicylamide normally. The rate of excretion tended to be less in the presence of jaundice.

Five patients with congenital hyperbilirubinaemia (four of Gilbert and one of Dubin-Johnson type) excreted normal amounts of glucuronic acid in the urine and responded normally to a salicylamide load.

We are grateful to Dr. S. Sherlock for advice and encouragement in these studies, and to Dr. B. H. Billing for biochemical advice and assistance.

REFERENCES

- Arias, I. M., and London, I. M. (1957). *Science*, **126**, 563.
 Axelrod, J., Schmid, R., and Hammaker, L. (1957). *Nature (Lond.)*, **180**, 1426.
 Ayer, D. (1940). *Arch. Path. (Chicago)*, **30**, 26.
 Billing, B. H., and Lathe, G. H. (1956). *Biochem. J.*, **63**, 6P.
 Bollman, J. L. (1956). *Proceedings of the 5th Pan-American Congress of Gastroenterology*.
 Brown, H., Willardson, D. G., Samuels, L. T., and Tyler, F. H. (1954). *J. clin. Invest.*, **33**, 1524.
 Carbone, J. V., and Grodsky, G. M. (1957). *Proc. Soc. exp. Biol. (N.Y.)*, **94**, 461.
 Crigler, J. F., and Najjar, V. A. (1952). *Pediatrics*, **10**, 169.
 Dubin, I. N., and Johnson, F. B. (1954). *Medicine (Baltimore)*, **33**, 155.
 Englert, E., Brown, H., Wallach, S., and Simons, E. L. (1957). *J. clin. Endocr.*, **17**, 1395.
 Fishman, W. H., and Green, S. (1955). *J. biol. Chem.*, **215**, 527.
 Gilbert, A., and Lereboullet, P. (1902). *Gaz. hebdom. Méd. Chir.*, **7**, 889.
 Goodman, L. S., and Gilman, A. (1955). *The Pharmacological Basis of Therapeutics*, 2nd ed., p. 300.

- Hartiala, K. J. V., Leikkola, P., and Savola, P. (1958). *Acta physiol. scand.*, **42**, 36.
 Lemberg, R. (1956). *Rev. pure appl. Chem.*, **6**, 1.
 Peterson, R. E., and Schmid, R. (1957). *J. clin. Endocr.*, **17**, 1485.
 Quick, A. J. (1936). *Arch. intern. Med.*, **57**, 544.
 Saltzman, A., and Caraway, W. T. (1953). *J. clin. Invest.*, **32**, 711.
 Schachter, D. (1957). *Science*, **126**, 507.
 Schmid, R. (1956). *Ibid.*, **124**, 76.
 — (1958). *A.M.A. Arch. intern. Med.*, **101**, 669.
 —, Axelrod, J., Hammaker, L., and Rosenthal, I. M. (1957). *J. clin. Invest.*, **36**, 927.
 Sherlock, S. (1946). *Lancet*, **1**, 159.
 Snapper, I., and Saltzman, A. (1947). *Amer. J. Med.*, **2**, 327.
 Talafant, E. (1956). *Nature (Lond.)*, **178**, 312.

TRIAIC "STOSS THERAPY" IN SPORADIC GOITROUS CRETINISM

BY

HERMAN ZONDEK, M.D.

HANNAH E. LESZYNSKY, M.D.

AND

GERDA W. ZONDEK, M.D.

Bikur Cholim Hospital, Jerusalem, Israel

Recently we had the opportunity of observing a 21-year-old male sporadic goitrous cretin in whom the administration of 90 mg. of triac (triiodothyroacetic acid) within six days not only brought a very rapid disappearance of the myxoedematous signs and symptoms and of a partly diffuse and partly nodular goitre, but, despite the subsequent cessation of treatment, on two occasions surprisingly also resulted in the maintenance of a perfectly euthyroid and agoitrous state for about seven and six months respectively. The latter effect of the triac "stoss"—which by the way did not produce any ill effects on either occasion—was obtained both during a relapse and during a period of clinical euthyroidism as the result of continuous substitution therapy. This patient required a daily maintenance dose of 0.15 g. of desiccated thyroid or 40 µg. of triiodothyronine or 2–3 mg. of triac. Thyroid function, which during one of these therapy-free remissions was investigated by radio-iodine studies and the serum P.B.I., was shown to be practically normal (Zondek *et al.*, 1958).

Subsequently we had occasion to study this patient's 16-year-old sister, who is afflicted with the same thyroid disorder. The results of these investigations, which throw some new light on the problem, form the subject of the present communication.

Case Report

The patient was severely myxoedematous. She showed the typical outward appearance and behaviour; B.M.R., –21 to –25%; blood cholesterol, 280–310 mg. per 100 ml.; marked bilateral enlargement of the heart shadow; typical myxoedema E.C.G.; etc.; and she also had a large diffuse goitre in which three nodules the size of large plums to large peaches were embedded (Fig. 1). As in her brother, the ¹³¹I uptake over the thyroid was very high after two hours; it then dropped to abnormally low values (first row in Table). This uptake pattern is characteristic of one of the known forms of "inborn errors" of thyroid hormone synthesis (Hutchison and McGirr, 1954, 1956)—namely, an inability of the gland to hold inorganic iodine and to bind it to protein. Serum P.B.I. was correspondingly low (1.8 µg. per 100 ml.). For substitution therapy the patient required 0.2 g. of desiccated thyroid or 50 µg. of triiodothyronine or 3 mg. of triac a day. While this dosage sufficed to keep her euthyroid and to suppress the diffuse goitre, the three nodules never regressed completely, but always remained palpable, varying in size between small cherries and plums.