Crigler-Najjar's Syndrome

By

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One of the forms of jaundice due to a congenital defect in bilirubin metabolism is the disease described in 1952 by Crigler and Najjar under the name of „Congenital familial non-hemolytic jaundice with kernicterus" [13]. The characteristic hyperbilirubinaemia develops during the first postnatal days and lasts throughout life. Haemolysis, obstruction can be ruled out. The original report contains seven cases; the patients originated from three families, in each of them the parents were relatives. Six of the patients died of kernicterus within 15 months. Later, Childs and Najjar published a report [9] on the sole survivor, as well as a further patient, the offspring of the same family. Both showed normal physical and mental development, in spite of the persistently high level of bilirubin in serum (mostly over 20 mg per 100 ml). The patient described by Rosenthal et al. [33] developed severe neurological symptoms at the age of 3 years, while kernicterus developed in infancy in the case reported by Whitington [42], and in the one described by Lelong et al. [29].Some patients, however, reach adult age without any significant neurological defects. Sugar [38] observed a case in which the only nervous change was deafness. The patient married at the age of 26, had two children, one of whom also has a defect of bilirubin metabolism, but has not developed kernicterus. The other offspring is normal.

Hepatomegaly occurred in none of the 13 cases described. The only changes found in the liver were slight, non-diffuse periportal fibrosis and the formation of biliary thrombi. In all but one of the cases examined [42] the gall bladder and the duodenal juice contained only traces of bilirubin, there was little bilirubin in the faeces, and the life span of erythrocytes was normal [42]. After a bilirubin load pathologically high retention values were found. The values were always low following the administration of hippuric acid, and the usual liver function tests yielded normal results.

The observation that the water-soluble and excretable bilirubin giving the "direct" diazo reaction is an enzymatically produced metabolite of liver cells, notably a bilirubin metabolite

conjugated with glucuronic acid [11, 1], opened new possibilities for studying the pathomechanism of the Crigler-Najjar syndrome. Excretion tests were performed with aglycone substances, which, like bilirubin, are excreted with urine or bile through conjugation with glucuronic acid, in view of the fact that the glucuronization of menthol [36] N-acetyl-p-aminophenol [4], tetrahydrocortisone [36, 32, 10], salicylates [36, 10] and chloral hydrate [10] are significantly inhibited in the patients suffering from the condition under review. On the basis of these studies it has been accepted that the cause of the disease is a diminished function of the glucuronic acid-conjugating mechanism of the liver, resulting from a hereditary weakness of the activity of the enzyme glucuronyl-transferase. In the pathomechanism of neonatal jaundice a temporary weakness of the activity of the same enzyme plays the decisive role; however, while this physiological weakness is soon over, in the Crigler-Najjar syndrome the enzyme adaptation cannot take place because, presumably as a result of a hereditary gene abnormality, the protein molecule performing the special metabolic function is abnormal.

Childs [10] tested the parents and other members of the family of his two cases for salicyl excretion. His results, as well as the observation that the condition occurs mostly when the parents are relatives tend to point to recessive heredity; the number of those carrying the heterozygous gene must be small.

In the following we shall report on two cases. The patients were siblings born from the first and the second pregnancy. The parents were not relatives. Both patients had hepatomegaly, accompanied in the first case by phenomena of thesaurosis. Both patients developed bilirubin encephalopathy and died in infancy.

Case Reports

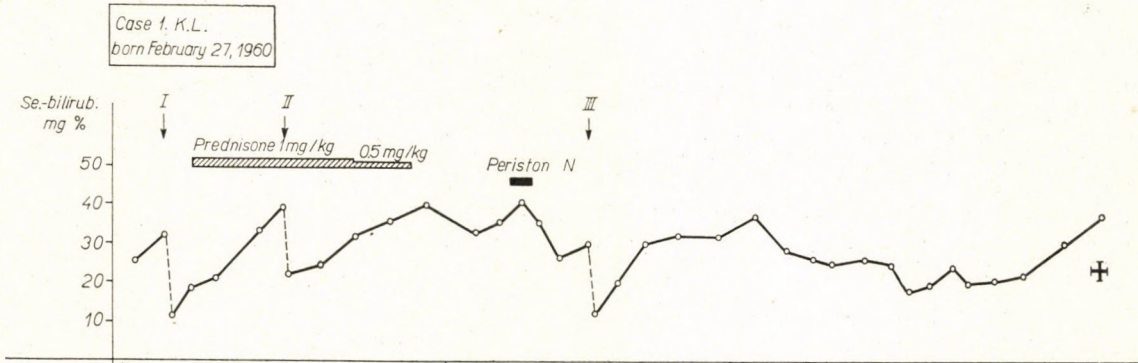
Case 1.

K. L., a male infant born in February, 1960, weighed at birth 3500 g. Jaundice developed on the third day of extrauterine life and the baby was referred to us on the 6th day with the diagnosis of isoimmunization jaundice. On admission he was orange-coloured, in a good condition. He was not anaemic. The liver was palpable. The serum bilirubin concentration was 32 mg per 100 ml, all indirect. The urinary lugol test was negative, the faeces were pigmented. The blood type was B, Rh positive, the mother's A, Rh negative, and the fathers AB, Rh positive. A direct Coombs test was negative; the mother's antibody test during pregnancy had been negative. An exchange transfusion was carried out without delay with 600 ml of B Rh negative blood. On completion of the transfusion the serum bilirubin level was 12.3 mg per 100 ml, on the next day 18 mg, and it continued to increase gradually. On the 11th day it was 38.2 mg per 100 ml. Then another exchange transfusion was carried out, with O Rh negative erythrocytes suspended in AB plasma. Without any significant change in the jaundice, the serum bilirubin level remained 20 mg per 100 ml for a few days, then increased again and for weeks it varied between 30 and 35 mg per 100 ml. During the sixth week the patient became restless, the upper extremities became more and more hypertonic and there were episodes of subfebrility. In spite of all these, the

baby continued to thrive. An attempt was made to lower the serum bilirubin level by administering 2 × 60 ml of polyvidon N daily for 5 days; there was some decrease in the serum bilirubin concentration, but the same was observed after the administration of polyvidon N had been discontinued. Then we gave 3 g of glucuronolactone on each of 3 consecutive days, with no result. Since by the end of the second month the symptoms of kernicterus were more and more marked, the extremities were spastic, the head was held rigidly backward, a third blood exchange was carried out, this time with 900 ml of B Rh positive blood. At the end of this 2 ½-hour procedure the serum bilirubin level was 10.8 mg per 100 ml. Within a few hours jaundice visibly decreased. Three days later, however, the baby was again orange-coloured. In the third month development stopped, there were nystagmus, marked opisthotonus and spasticity, as well as restlessness and ample salivation. During the fourth month tonic-clonic convulsions and tremor were observed in the face and extremities, and often the baby could not be nursed and had to be fed through a tube. The liver was enlarged and dyspepsia appeared when cow's milk had been added to the diet. After the fifth month the baby, who now was in a very poor condition, had repeatedly bronchopneumonia, accompanied by bronchial spasm. The liver continued to increase in size and was palpable three fingers below the right costal arc in the sixth month. It was extremely difficult to feed and nurse the baby because of the excessive opisthotonus, spasticity, torsion movements of the trunk and extremities and episodes of eclampsia. After reaching a maximum weight of 5900 g the baby developed severe enterocolitis and staphylococcal enteritis. His condition deteriorated, meanwhile the serum bilirubin level had become stabilized for several months, at values between 16.5 and 21 mg per 100 ml. An episode of pneumonia was treated with broad-spectrum antibiotics (other than chloramphenicol). Restlessness and fits were controlled by barbiturates, chlorpromazine or scopolamine, with poorer and poorer results. The baby was vomiting frequently, became decebrated, kept thrusting out his tongue. During the last week of his life he developed grave bilateral pneumonia, the serum bilirubin level increased significantly and he died suddenly at the age of nearly 9 months, as a result of aspiration pneumonia. The two lower incisors cutting through during the last month of life were greenish in colour. The lowest serum bilirubin level our patient ever had (except for the values measurable for a time following blood exchange transfusions) was 16.5 mg, the highest 40 mg per 100 ml (Fig. 1).

Case 2.

K. M., a female baby, was born from the second, uneventful pregnancy of the same mother, in March, 1961, with a weight of 3300 g. The blood was Rh negative (i. e. identical with that of the mother) and on admission, on the third day of life, there was already marked jaundice of the sclera and of the skin. The serum bilirubin level was 19 mg per 100 ml, indirect. Coombs' test was negative, there was no anaemia, the liver was palpable. Next day the serum bilirubin level was 25.5 mg per 100 ml, all indirect. The baby was crying and sucking vigorously. Between the 4th and 7th days daily, then on the 9th and 10th days, i. e. on a total of six occasions, exchange transfusions were given, with 200 to 250 ml/kg of fresh blood each, in an effort to keep the serum bilirubin level under 20 mg. At first O Rh negative erythrocytes suspended in AB plasma were used, later A Rh negative blood. After the first week the serum bilirubin level could be kept under 20 mg per 100 ml only for a while. It was 48 mg per 100 ml, on the 21st day, then it decreased gradually, while the liver continued to increase in size. Nervous symptoms began to appear in the third week, in the form of



Age	days 4 5 6 7 8 9 10 11 13 15 17	weeks 3 4 5 6 7 8 9 10 11	months 3 3½ 4 5 5½ 6 7 7½ 8 8½ 8¾
Body weight	3500g	4000g 4400g	4700g 5900g 5300g 5300g
Size of liver	palpable	1	2 3 3½
SGOT U.	161	71 42 79 180	247 223 13 56 79 79 145 223 8 174 50 76 45
Alkaline (B.U.) phosphatase		11.7 7.0	4.4 11.0
Pseudocholin-esterase Δ mole		120 90	105 105
Serum cholesterol mg per 100 ml (esterified)		265 mg % 125 188	230 260 (39%) 240 (32%) 290 (43%) 220 (50%)
Total bilirubin mg			

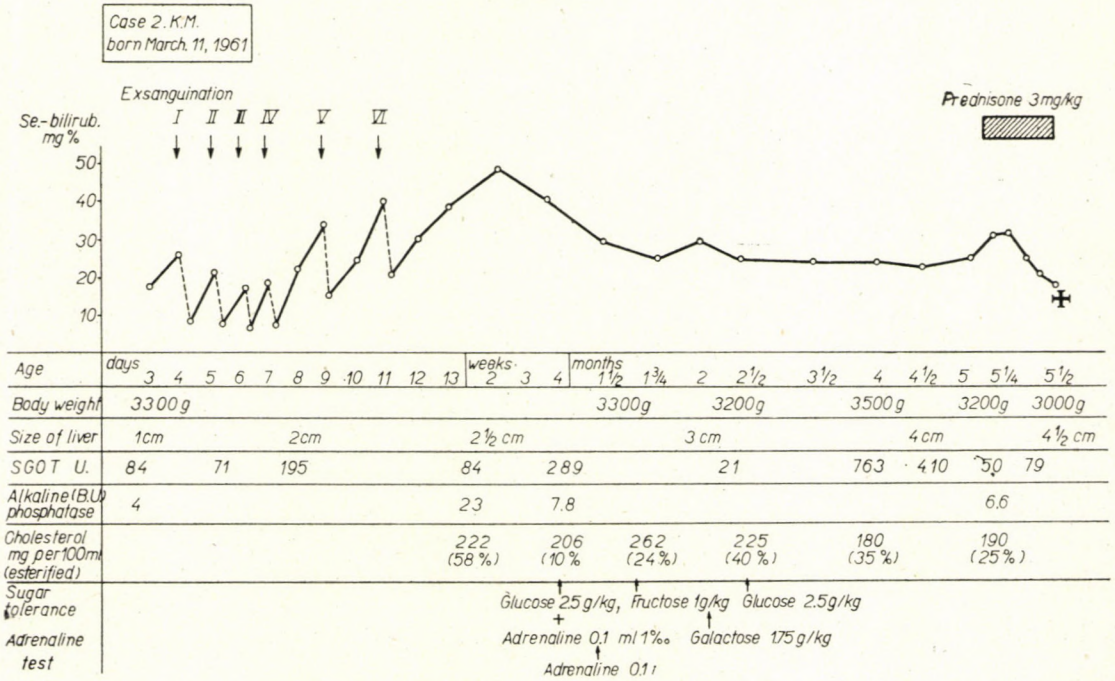
Bilirubin in duodenal juice — Duodenal Tube

Bilirubin in gall bladder — Laparatomy in vitro glucuronyl-transferase activity zero

11.4 mg % direct

10 mg % direct

Autopsy


Case 2. K.M.
born March. 11, 1961
Exsanguination
I II III IV V VI
Prednisone 3mg/kg
Se.-bilirub. mg%
50
40
30
20
10
Age
days 3 4 5 6 7 8 9 10 11 12 13
weeks 2 3 4
months 1½ 1¾ 2 2½ 3½ 4 4½ 5 5¼ 5½
Body weight
3300g
3300g
3200g
3500g
3200g
3000g
Size of liver
1cm
2cm
2½ cm
3 cm
4 cm
4½ cm
SGOT U.
84
71
195
84
289
21
763
410
50
79
Alkaline (B.U.) phosphatase
4
23
7.8
6.6
Cholesterol mg per 100ml (esterified)
222 (58%)
206 (10%
262 (24%)
225 (40%)
180 (35%)
190 (25%)
Sugar tolerance
Glucose 2.5 g/kg, Fructose 1g/kg Glucose 2.5g/kg
+
Adrenaline test
Adrenaline 0.1 ml 1‰ Galactose 1.75 g/kg
Adrenaline 0.1 r

restlessness and spasticity. After two months of age bronchopneumonia was observed repeatedly. After three months the kernicterus became more and more marked, seizures were frequent. The jaundice subsided and the serum bilirubin level became stabilized for months. The baby was often febrile, had an occipital phlegmone and became marasmic. During the last month the liver could be palpated four and a half fingers below the costal arc, there was total decerebration, extreme opisthotonus. The baby died at the age of 5 ½ months, with bronchopneumonia and heart failure. At that time was the lowest serum bilirubin level, 19.4 mg, measured. The highest value was 48 mg per 100 ml.

Haematology

Case 1.

At first, Rh or ABO incompatibility was suspected, but the direct and indirect Coombs tests, the maternal anti B titre and incomplete antibody tests were all negative. There were no autoantibodies in the maternal serum. By the end of the second month moderate normochromic anaemia had developed, with an erythrocyte count of 2.6 million and a Hb of 8.7 g, but after the third blood exchange the erythrocyte count was invariably higher than 3 million and the Hb more than 10 g. For example, from the sixth months on erythrocyte count was 3.5 million, Hb 11.5 g or higher, the haematocrit between 35 and 40. Electrophoretically the haemoglobin did not differ from that of the control infants. The reticulocyte count never exceeded 22 per thousand, the values usually were 8 to 18 per thousand. Bone marrow samples were studied twice, at 1 ½ and 4 months of age. Bone marrow pattern and normoblast count were normal, there was no evidence of an increased erythrocytopoiesis, the bone marrow reticulocyte count was 18 per thousand. Osmotic resistance and mechanical fragility of the erythrocytes were repeatedly normal. Nucleated erythrocytes, Heinz bodies could not be demonstrated, the erythrocytes were normal in size and shape and stained normally. During the first month of life there was 8 to 13 per cent eosinophilia, but subsequently the eosinophil count was 2 to 4 per cent. The thrombocyte count was normal throughout.

Case 2.

In this case blood group incompatibility could be ruled out on the basis of the blood type. On admission Hb 18 g, erythrocytes 4.5 million, leucocytes 8000, eosinophils 10 per cent were found. After the repeated blood exchanges the erythrocyte count was 4.1 million, the Hb concentration 15.6 g. At the age of one month the erythrocyte count was 3 million, the reticulocyte count varied from 6 to 14 per thousand, the eosinophil count from 2 to 4 per cent. Later the number of erythrocytes varied from 3.1 to 3.6 million, the Hb values from 11.5 to 14 g. As determined by the semi-quantitative Motulsky test [30], erythrocytic glucose-6-phosphate dehydrogenase activity was normal, decoloration time 110 minutes. Repeated tests revealed no bilirubin in urine, but on four occasions the With method [45] demonstrated daily outputs of from 0.15 to 0.5 mg of bilirubin, which gave the diazo reaction with caffeine only after shaking out with butanol-ammonium sulphate (i. e. it was indirect).

The thrombocyte count by the end of the blood exchange transfusions invariably decreased, but never below 65.000. After the exchanges the thrombocyte count increased rapidly. The plasma, particularly that obtained after repeated exchange transfusions, caused a significant rise in the number of thrombocytes when injected intravenously into mice. This thrombopoietic activity was the most potent after the fifth and sixth exchange transfusions (18).

Bilirubin

In Case 1, urinary and faecal bilirubin excretion was determined repeatedly in the 4th, 5th, 7th and 8th months, using urine and faecal samples collected for three days. In the faeces bilirubin was determined by the method of Malloy and Evelyn [20], stercobilin by the method of Schwartz and Watson [37]. The calibration curve was plotted with commercial bilirubin and stercobilin, respectively. Since we could never detect bilirubin in the urine by the lugol, Fouchet, Gmelin and methylene blue reactions, we did not test the urine for bilirubin quantitatively. The controls were three 3 months old premature infants weighing 2800 to 3500 g. Their total bilirubin output ranged from 7.1 to 10 mg daily, with the average at 8.8 mg. Our patient at the above ages had an average output of 3.65, 8.73, 4.62 and 7.6 mg daily, respectively. In the first three tests the faeces contained considerable amounts of bilirubin, while none could be demonstrated in the fourth test. The urinary *v.* fecal stercobilinogen ratio averaged 1 per 50 (see also Fig. 1).

Porphobilinogen could not be detected in the urine. In the 7th month the porphyrin concentration in the faeces was 218.7 μg/100 g, slightly higher than normal. Two weeks later the urine was found to contain 31.5 μg/100 ml porphyrin of the coprophorphyrine type (this is within normal limits). Uroporphyrin was not demonstrable (Dr. G. Kelényi, Institute of Pathology, Pécs).

Bilirubin was determined according to Jendrassik and Cleghorn [26]. All the serum bilirubin of both patients reacted indirectly, except on a few occasions, when the serum bilirubin level was 30 mg per 100 ml, and a fraction of 1 to 2 mg gave a direct reaction. As it is known, beside the bilirubin-diglucuronide produced by the liver there is also a monoglucuronide (bilirubin conjugated with one mol of glucuronic acid) produced extrahepatically in serum [5]. The three bilirubin fractions, the non-conjugated (indirect) the mono- and the diglucuronide were repeatedly assayed by the simple Eberlein method [19], by which the fractions separated on the basis of their solubility can be measured spectrophotometrically. While in a patient suffering from biliary obstruction the total serum bilirubin level determined by the diazo method was repeatedly found to be closely comparable with that determined by the Eberlein method, in our Case 2, as well as in a normal newborn with physiological jaundice, the values obtained with the Eberlein method were only about half of those yielded by the diazo reaction. According to our results, which are thus not absolutely reliable, in our Case 2, 77 per cent of the serum bilirubin was non-conjugated (free), 18 per cent was mono- and 5 per cent was diglucuronide. These values are similar to those obtained with the Eberlein method in cases of physiological neonatal jaundice.

In Case 1, the bilirubin extracted from serum was diazoted and chromatographed according to Schmid [35]. As a control, diazoted and chromatographed bilirubin obtained from newborns with physiological jaundice and commercial bilirubin were used. All these showed the 0.5 Rf value characteristic of free, indirect bilirubin. Moreover, the absorption spectrum of the diazoted bilirubin of our patient was the same as that of the diazoted bilirubin from the control newborn. With both of our patients the bilirubin always migrated in the albumin fraction on protein electrophoresis.

A serum sample containing 30 mg per 100 ml of bilirubin from Case 1 was exposed to strong noon sunshine. As a result, and like in the control serum sample, the bilirubin level decreased rapidly [12]. The value measured 2 hours later was 1.2 mg per 100 ml. During the same period the bilirubin concentration of the control serum decreased from 27.7 mg to 2.6 mg per 100 ml. Twenty-five ml of plasma obtained from the same patient was injected intravenously into a six weeks old baby

weighing 2100 g and suffering from multiple developmental disorders. This meant a load of 4 mg/kg of bilirubin. Within four hours the bilirubin level decreased from the 4.8 mg per 100 ml value measured immediately following the load to the initial 0.6 mg per 100 ml.

Liver Function

The repeated thymol, Takata, $ZnSO_4$ and Weltmann tests yielded normal results for both patients. Repeated tests for total serum protein yielded results ranging from 5.3 to 8.2 g per 100 ml in Case 1, and from 5.6 to 7.5 g per 100 ml in Case 2. Paper electrophoresis showed in both cases an albumin of over 50 per cent and gamma globulin over 10 per cent. Repeated immunoelectrophoretic studies, with suitable controls, always gave normal results in both patients. The erythrocyte sedimentation rate did not exceed 10 mm in 1 hour in either case.

As regards serum enzymes, glutamic acid-oxalacetic acid-transaminase (SGOT) assay was done by the method of Dubach [16], alkaline phosphatase was determined according to Bodansky, and pseudocholesterolase according to De la Huerga [14]. After neonatal age the upper limit of normal of the SGOT values obtained by the above method was considered to be 100 U, taking into account the considerable range of variations in the control values and the limits of error. In both cases SGOT activity was intermittently increased, in Case 2 a very high value was found on one occasion. As determined by a few tests done during the period of normal SGOT activity, the aldolase and isocitrate dehydrogenase activities were normal. In Case 1, alkaline phosphatase activity was increased during the first month, the pseudocholesterolase level was at, or somewhat below, the lower limit of normal.

Fig. 1. and Fig. 2. present the results for serum cholesterol determined by the sulphuric acid ferric chloride method [46] using a 530 mμ colour filter. Our values may be considered higher than real, owing to the determination of bilirubin together with cholesterol [40]. With this method the serum cholestero llevel in Case 1 ranged from 125 to 290 mg per 100 ml, in Case 2 between 180 and 265 mg per 100 ml. In general, the esterified fraction did not amount to more than 50 per cent, it being frequently 10 to 30 per cent.

The results for serum lipid fractions (alpha, beta and neutral lipids) in Case 2 did not differ from those obtained for the control.

As to excretory liver function, in Case 1 two bromosulphophthalein tests gave normal results. In the second month 45 minutes following the administration of 5 mg/kg bromsulphophtalein, 5 per cent retention, in the fourth month 3 per cent retention were found. At the age of five months 10 ml of adipiodone was injected intravenously; by 45 minutes the gall bladder had began to fill and at 120 minutes we saw the shadow of it, 3 cm long, 0.5 cm wide, hook-shaped, contracted, in the projection of the 2nd rib. In the same month normal values were obtained following the administration of 1 g of sodium benzoate; in 4 hours hippuric acid was excreted in an amount corresponding to 51 per cent sodium benzoate.

In Case 1, in the sixth month an intravenous bilirubin excretion test was made with 5 mg/kg of bilirubin. After six hours retention was 30 per cent, instead of the normal 10 per cent [23].

At the end of the sixth month, when the serum bilirubin level showed the lowest value, 16.5 mg per 100 ml, a sample of liver tissue homogenate obtained at laparotomy was tested *in vitro* for glucuronyl-transferase activity by the Grodsky-Carbone method [22]. 0.5 g of liver tissue was homogenized without delay and this was incubated with a 20 per cent solution of bilirubin treated with bovine albumin. Boiled rat liver homogenate served as the source of UDP glucuronic acid. During 45 minutes' incubation no

TABLE I

Aglycone excretion tests

Age	Substrate	Dose		Urine collection period, hour	Conjugated metabolite mg	Per cent of dose administered	Controls
		total mg	mg/Kg				
Case 1. K. L.				24	26	65	4 infants 59 to 66%
6 weeks	Acetanilide	40	10	48	28.7	70	67 to 71%
				24	30	66	2 adults
3 months	Acetanilide	45	10	48	33	73	75 to 80.2%
6 1/2 months	Menthol	180	33	8	18	10	5 infants
7 1/2 months	Menthol	180	35	8	13	7	40 to 54%
Case 2. K. M. 2 1/2 mo.	Acetanilide	50	17	24	33.5	66	—
4 months	Menthol	150	50	8	15	10	—
4 1/2 months	Menthol	120	35	8	18	15	40 to 54%
5 months	Menthol	60	15	8	15	25	—
4 months	N-acetyl-p-aminophenol	100	30	12	14.8	14.8	3 infants 31.2 to 34% in 12 hours
5 months	N-acetyl-p-aminophenol	95	30	12	15.6	17.5	
				24	19.3	21.4	

change was noted in the quantity of direct reacting bilirubin, in other words the liver tissue from our patient did not conjugate bilirubin, whereas during the same period in the control rat liver homogenate the amount of direct bilirubin increased 3.5-fold. This was a direct proof of the defective function of hepatic glucuronyltransferase. The serum contained no inhibitors that would have blocked the glucuronic acid conjugating function of the liver (Mr. J. H. Hilton, Institute of Chemical Pathology, Leeds).

The glucuronic acid conjugating mechanism of the liver was tested also by the administration of acetanilide, N-acetyl-paraaminophenol and menthol. Of acetanilide, 10 mg/kg doses were given on two occasions, and once 17 mg/kg. (We did not give more in view of the risk of aniline formation.) Normally, of the acetanilide 70 to 90 per cent are converted to N-acetyl-paraaminophenol and this metabolite is excreted by means of glucuronization [8]. The determinations were made by the method of BRODIE and AXELROD [7], as

modified by VEST [41]. As seen in Table I, in Case 1 the 10 mg/kg, and in Case 2 the 17 mg/kg, dose of acetanilide was excreted at a normal rate, in the form of paraaminophenol.

On repeated loading with 30 mg/kg doses of acetyl-paraaminophenol only about 50 per cent was excreted in the form of glucuronide metabolite, as compared to the controls.

The doses of menthol ranged from 15 to 50 mg/kg and, using the FISHMANN—GREEN method [21] the measure of menthol-glucuronization was estimated from the increment of conjugated glucuronic acid excretion. In both of our patients only about $^1/_3$ of the amount excreted by the controls was excreted in 8 hours conjugated to glucuronic acid. Menthol was administered after fasting, through a gastric tube, as a colloidal solution in the form of menthol, 2 g; stearinated sorbox, 15 g; distilled water to 1000 g (supplied by Prof. L. KEDVESSY, of the Institute of Pharmacology, Szeged). As determined in 16-hour tests, prior to the administration of menthol the conjugated glucuronic acid output in the urine was 18 to 26.1 mg in our patients, and 21 to 43 mg in the controls.

It has been tried to test chloramphenicol excretion by the FISHMANN—GREEN method, but the oral administration of large doses was not followed by an increase in the urinary conjugated glucuronic acid excretion in the child and adult controls.

The quantity of 17-hydrocorticosteroid and its fractions were determined in both patients. In Case 1, in the third month 0.550 mg was excreted (0.521 mg conjugated and 0.029 mg free). In the fifth month total excretion was 0.714 mg (0.680 mg conjugated and 0.034 mg free). In Case 2, at the age of three months, the total output was 0.39 mg (conjugated, 0.31; free, 0.08 mg) (Dr. I. Faredin, First Department of Medicine, Szeged). These values may be considered normal.

In Case 2, tolerance tests were done with glucose, fructose and galactose. Two hours after the glucose tolerance test we gave adrenaline and on another occasion an adrenaline test was made without the glucose tolerance test (Fig. 2). The results obtained were all normal, the blood sugar level increased significantly in every case.

In Case 2, during the third month the serum and urine were tested over a period of two days for beta-glucuronidase. The results did not differ from those obtained in the controls (serum 130 U, 135 U/100 ml; urine 100 U, 95 U/100 ml). (Dr. B. Rengei, Institute of Forensic Medicine, Szeged).

MORPHOLOGICAL AND HISTOLOGICAL STUDIES

Case 1.

The liver tissue excised at laparatomy was intact in structure. There was slight chronic infiltration around the moderately proliferating bile ducts. No bile casts were visible in the large bile ducts and there was no substance giving bilirubin positive reaction within the liver cells. These were big, polygonal, stained lightly, the cytoplasm was granulated, the nuclei were regular in appearance. Periodic acid-leukofuchsin staining revealed in the cytoplasm a dense network which did not disappear following treatment with diastase and saliva, so that the PAS-positive substance was not normal glycogen. The structure of liver cells resembled the pattern occurring in thesaurosis. (Dr. Z. Mónus, Institute of Pathology, Szeged, and Dr. G. Kelényi, Institute of Pathology, Pécs).

In the non-fixed, frozen material histochemical methods did not detect succinic dehydrogenase (CASCARANO and ZWEIFACH method) and cytochrome oxidase (BURSTONE method). The vascular walls and the larger bile ducts showed ATP-ase activity. Alkaline phosphatase activity was moderate in the biliary capillaries and intense in the vascular walls and bile ducts. The frozen sections showed no

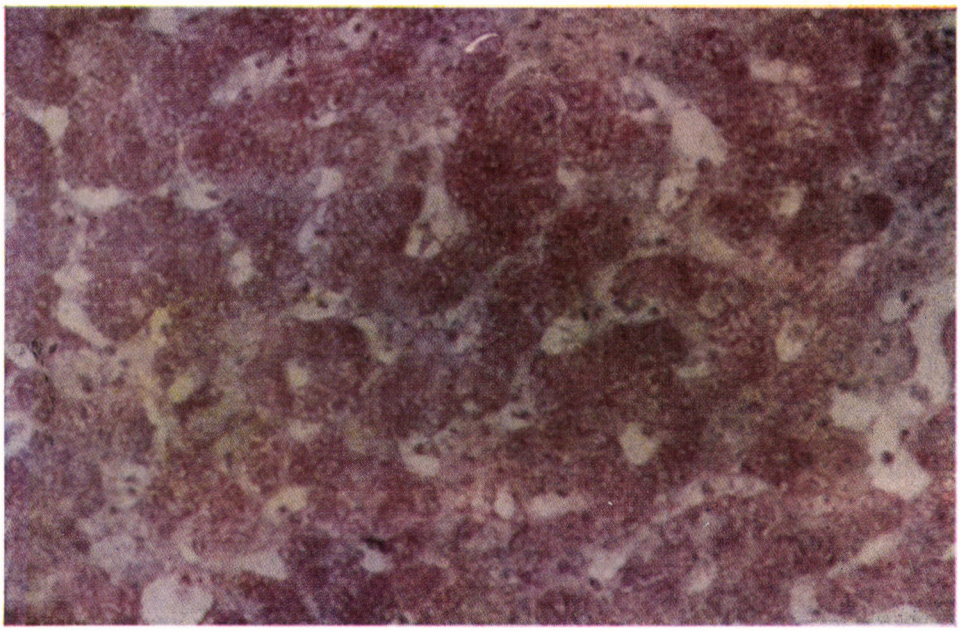


FIG. 1. Case 1 (K. L.) Liver specimen obtained at laparotomy. PAS staining. Magnification, × 286. Note intense network pattern in the cytoplasm after staining for polysaccharide

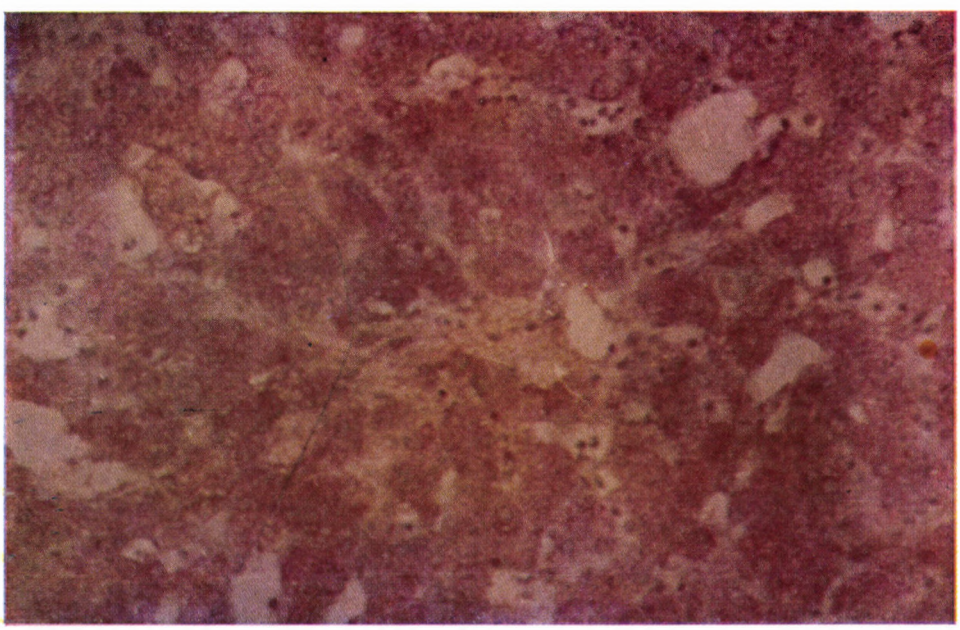


FIG. 2. Case 1 (K. L.) Liver specimen obtained at laparotomy. PAS staining, saliva digestion. Magnification, × 286. The network pattern persists after treatment with saliva

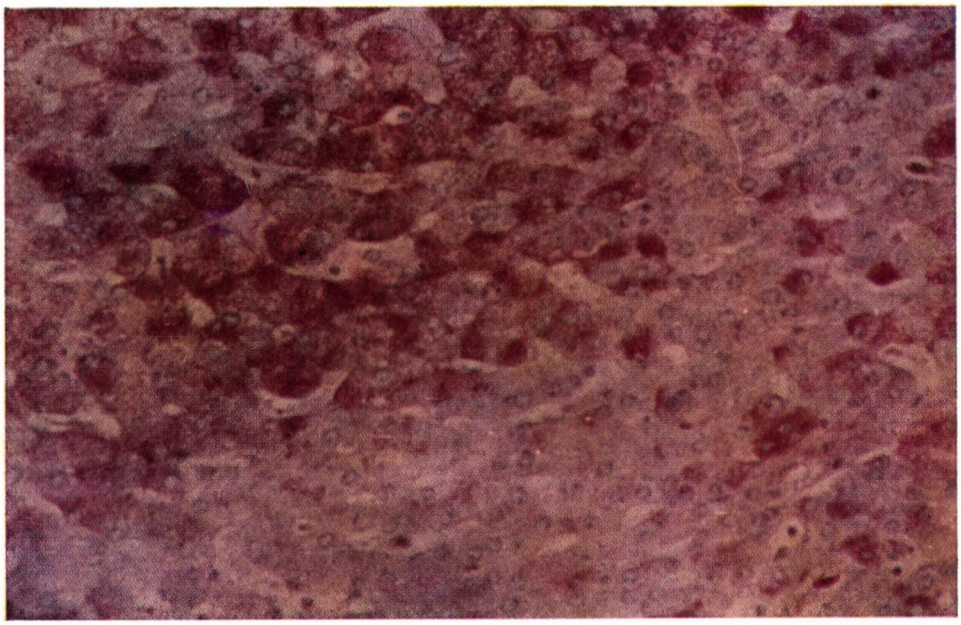


FIG. 3. Case 2 (K. M.) Liver specimen obtained at autopsy. PAS staining. Magnification, × 286. No evidence of storage

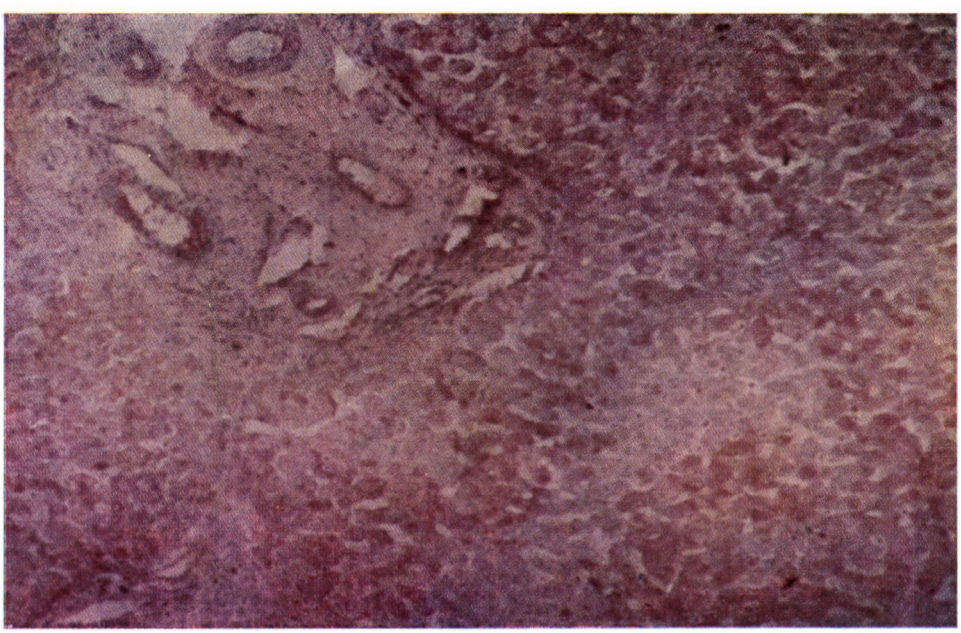


FIG. 4. Case 2 (K. M.) Liver specimen obtained at autopsy. PAS staining. Magnification, × 112. Note uneven, patonydistribution of glycogen

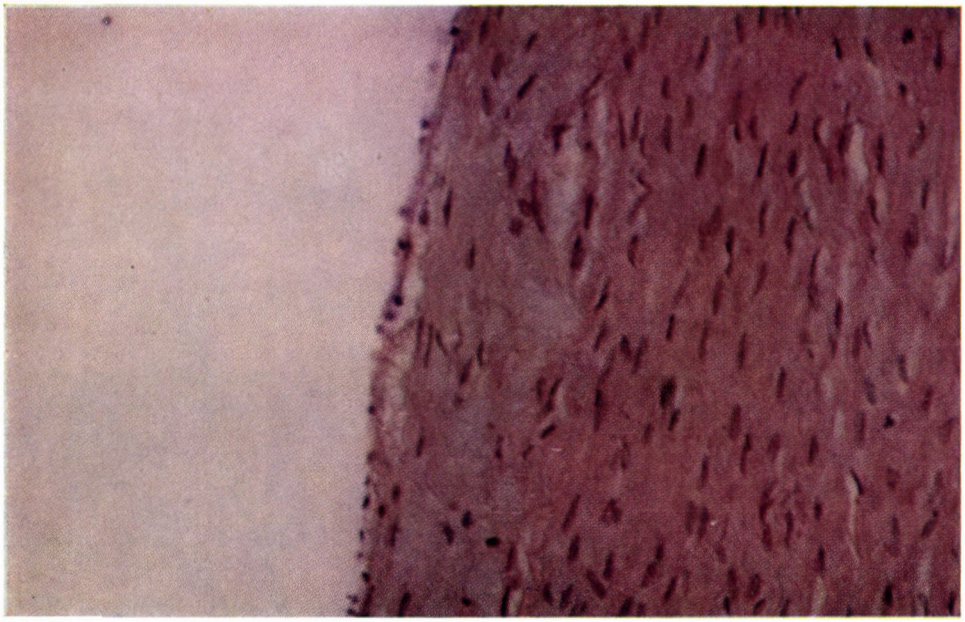


FIG. 5. Case 2 (K. M.) Aorta. Haematoxylin-eosin staining. Magnification, × 286. Small foci of myxomatous appearance in loosened-up areas subintimally

increase of lipids and neutral fats on staining with oil red. Alcian blue demonstrated no acid mucopolysaccharides in the liver cells. (É. Horváth, Institute of Pathology, Szeged.)

At laparotomy, 3 ml of pigmented bile was withdrawn from the gall bladder; it contained 11.4 mg per 100 ml of direct reacting bilirubin. In the third month 5 ml of pale grass-green duodenal juice with a few droplets of orange colour in it were obtained; it contained 5 mg per 100 m bilirubin giving a protracted direct reaction.

Autopsy performed 24 hours after death revealed disseminated focal pneumonia, pulmonary oedema and kernicterus as the causes of death. The body weight was then 5300 g. The liver weighed 320 g, much more than would have corresponded to the age and body weight. In histological pattern the liver was similar to the biopsy specimen obtained earlier; fatty degeneration, biliary pigmentation, accumulation of haemosiderin were not found. Haematoxylin-eosin staining revealed between the liver cells, in and alongside the sinusoids, foci of an intensely basophilic amorphous material. The size of the foci did not exceed that of a few liver cells. The substance could not be identified. The gall bladder contained light bile, with 10 mg per 100 ml of direct reacting bilirubin and 56 mg per 100 ml of glucuronic acid. Owing to a technical error the liver tissue was not tested for glycogen content. The heart was not enlarged (30 g).

Case 2.

One hour after death a piece of liver tissue was excised and two specimens, weighing 1 g each, contained 0.64 and 0.70 per cent glycogen, respectively. These values are at the lower limit of normal. A 2 g specimen of muscle tissue excised at the same time from the thigh contained 0.25 per cent glycogen.

Glucose-6-phosphatase activity was 9 mg P/g liver tissue. (Dr. S. Dán, Second Department of Medicine, Debrecen.) In that Institute the normal values for adults range from 4 to 8 mg P/g liver. The value given in the literature for children ranges from 2.7 to 4.2 mg P/g liver, thus the result obtained in our case indicated an increase of enzymatic activity. The dry liver powder contained 439 mg per 100 ml of hexosamine, calculated for dry acetonic powder. Liver tissue specimens from two infants at the same age who had died of other diseases served as the controls; one specimen contained 355 mg, the other 442 mg per 100 ml of hexosamine (Dr. H. E. Oláh, Institute of Anatomy, Debrecen). The gall bladder contained not more than a few drops of light bile.

Autopsy was performed 6 hours after death. The causes of death were disseminated bronchopneumonia, acute tracheobronchitis, seropurulent bronchiolitis and septicaemia. Histology revealed fresh focal necroses in the spleen. Many parts of the central nervous system were examined. No kernicterus was found. The only changes detected in the specimens stained with haematoxylin-eosin were cortical and subcortical gliosis in the temporal lobes, with circumscribed thickening and adhesion of the pia mater in the adjacent areas. The hypertrophic heart weighed 50 g, and showed no accumulation of glycogen.

In spite of the hepatomegaly during life, the liver weighed 125 g and was normal in size. Many foci of fresh necrosis were visible in it. At the margin of the necrotic areas necrobiotic liver cells and at sites leucocytes were found. Like in the blood vessels of other organs, an increased number of neutrophile granulocytes was demonstrated in the sinusoids. Some liver cells showed fatty degeneration and deposits of bile pigment, the latter occurring also in the cells of Kupffer. In the portobiliary areas the only changes found were slight fibrosis and bile thrombosis in a few biliary capillaries. In silver-treated preparations the reticular structure of the liver was destroyed in the areas of focal necrosis. The surviving liver cells

contained, mainly periportally, small quantities of a PAS-positive substance (glycogen) digested by saliva; no glycogen deposits were found. In the aorta haematoxylin-eosin staining revealed pale blue small myxomatous patches subintimally. These patches contained a substance giving a slightly positive reaction with alcian blue (acid mucopolysaccharide). (Dr. Z. Mónus, Institute of Pathology, Szeged.)

The PAS-positive substance occurring mainly around the interlobular veins was neutral polysaccharide, because most of it was digested by saliva. In its place there remained only a diffuse pale pink hue, located in the connective tissue and not in the liver cells. After acetylation most of the liver cells remained red, while this colour disappeared from the connective tissue. Thus, the PAS-positive substance in the liver was most probably glycogen. In addition, the liver cells contained numerous Hale positive haemosiderin granules, which were blue in the acetylated preparations and golden yellow in the saliva-treated ones. (Prof. I. Krompecher, Institute of Anatomy, Debrecen).

Other Tests and Studies

Case 1.

Laboratory. Serum, NPN, 32 mg; Ca, 10 mg; P, 3.5 mg; Cl, 369 mg; Na, 335 mg; K, 18.6 mg, fasting blood sugar, 84 mg; pyruvic acid, 1 mg; glycoprotein, 133 mg, all per 100 ml. Serum Cu, 205 μg; coeruloplasmin, 500 U. (These last two values were higher than normal.) Serum Fe, 68 μg (at the age of eight months).

In urine, urobilinogen was diminished (at the age of two months); indican, 5.4 mg/24 hours; 17-ketosteroid, 1.1 mg/24 hours (at the age of four months).

Bleeding, clotting and prothrombin times were normal (at the age of 6 months).

Cerebrospinal fluid. Sugar, 71 mg; pyruvic acid, 1.1 mg; bilirubin, 1 mg per 100 ml (at the age of nine months).

EEG (at the age of 3 months). Slow wave activity 3 to 6 sec. in duration and 200 to 300 mV in amplitude in the posterior and middle scala leads. No focal activity, no significant deviations from the normal for that age.

EEG. normal tracings repeatedly.

Ophthalmology. Intact papillar, increased pigmentation of fundus, no discolorisation (at the age of six months).

Radiographs. In the periods without pneumonia the diaphragm was at the level of the lower margin of the 9th rib, the marginal areas of the lung were slightly emphysematous. In the periods of the disseminated focal pneumonia there were marked signs of bronchospasm. In serial radiographs taken from 3 months till 8 ½ months of age the size of the liver shadow increased from 4 cm to 6 cm. The enlarged liver was dislocated downward by the deep-lying diaphragm.

Case 2.

Serum. Fasting blood sugar, 60 to 75 mg per 100 ml. The Ca, P, Cu, glycoprotein, and pyruvic acid values were normal.

Urine. Protein, sugar, aceton, phenylhydrazine, and ferrichloride tests were negative; 17-ketosteroid values were normal.

Radiographs. At the age of a few weeks already the lung was emphysematous. This increased when bronchopneumonia and bronchial spasms developed. In those periods the diaphragm as a whole was displaced downward. The lung tissue bulged into the costal interspaces.

At the age of 5 months the chest was asymmetric, the spine showed a slight S-shaped curvature, the diaphragm was at the level of the posterior arc of the 10th rib, the lung pattern was markedly dense, the subpleural zone contained much air. The emphysematous lung tissue bulged into the mediastinum. The heart was small. (Dr. L. Páldy, Department of Radiology, Szeged.)

*

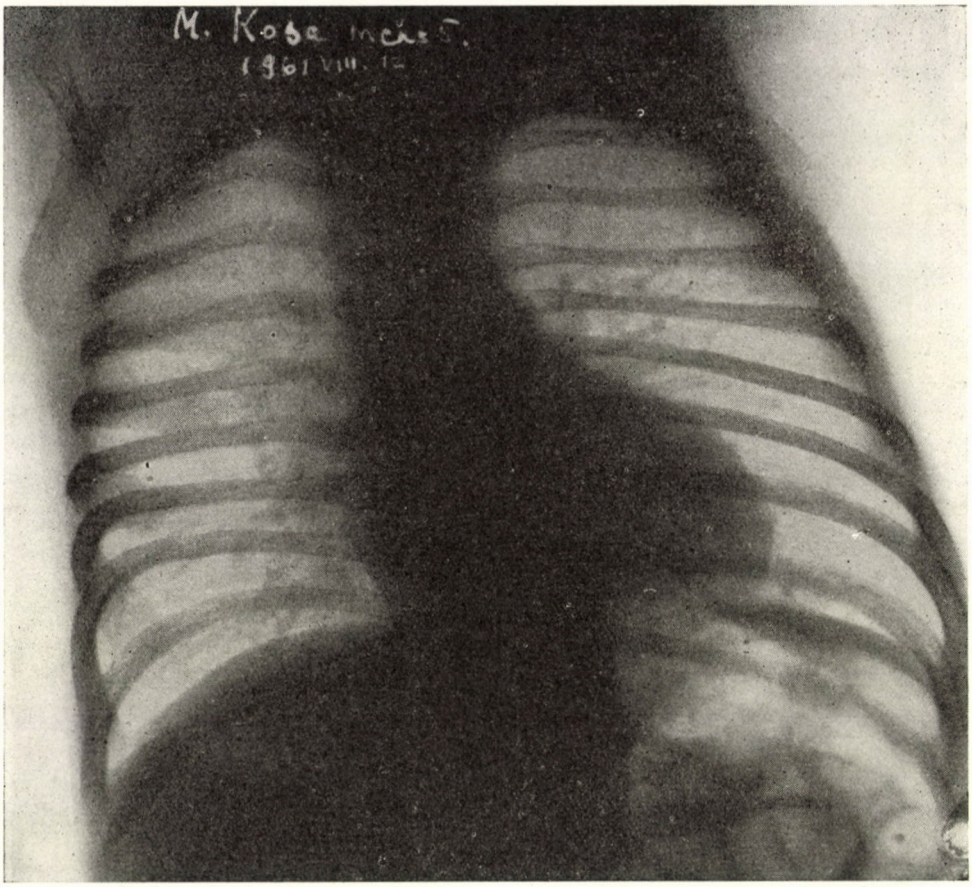


FIG. 6. Case 2 (K. M.) Chest radiograph at 5 months of age. Increased pulmonary pattern, emphysema, deep-positioned diaphragm

Both of our patients have repeatedly been tested for serum and urinary citric acid, using the method of NATELSON, as modified by OTTO [31]. The normal values for infants and children are 2 to 3 mg per 100 ml in serum, and 3.5 to 6 mg/kg in the 24-hour urine [39]. We have obtained the following values in blood samples taken before the exchange transfusions.

Case 1. At two months of age, serum 8.5 mg per 100 ml, urine 6.1 mg/kg; at 3 ½ months of age, serum 2.0 mg per 100 ml, urine 12 mg/kg.

Case 2. At 4 days of age, serum 12 mg per 100 ml; at 11 days of age, serum 15.8 mg per 100 ml; and at 2 ½ months, serum 3.0 mg per 100 ml, urine 6 mg/kg.

*

PARENTS

The parents were farmers, had never suffered from liver disease or jaundice and were healthy when examined. (In her adolescence the mother had osteomyelitis of the tibia, and was treated surgically.) They were not relatives. No child

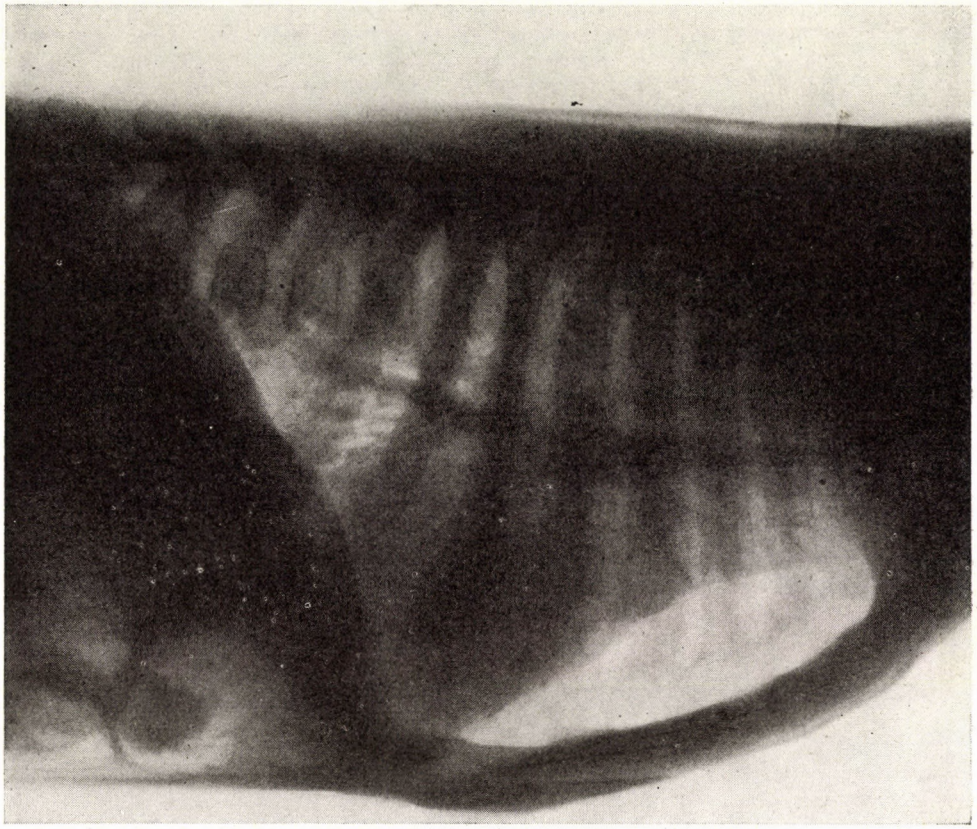


FIG. 7. Case 2 (K. M.) Lateral chest radiograph at 5 months of age. The lung bulges into the mediastinum, the dome of the diaphragm is displaced downward

or adult in the family has ever had jaundice.

Normal results were yielded by both parents for haemoglobin, erythrocyte count, leucocyte count, differential count, haematocrit, reticulocyte count, thrombocyte count, osmotic resistance; thymol, zinc sulphate, SGOT tests; total serum protein, paper and immune electrophoresis, serum bilirubin level, bilirubin excretion test with 50 mg of Homburg bilirubin. Total daily bilirubin output was 270 mg in the mother. Serum cholesterol was 308 mg per 100 ml, (33 per cent esterified) for the mother and 225 mg (45 per cent esterified) for the father. The mother repeatedly showed increased erythrocyte sedimentation rates, ranging from 30 to 115 mm in 1 hour. For this the elevated fibrinogen level (0.53 g per 100 ml with an ESR of 45 mm/l hour) might have been responsible.

DISCUSSION

The role of intravasal haemolysis in the excessive and persistent indirect hyperbilirubinaemia could be ruled out. In Case 2, the haemosiderin deposits in Kupffer's cells were apparently due to the repeated blood exchange transfusions. In Case 1, the

quality of bilirubin was normal, free bilirubin bound to albumin. It may be assumed that the retention of bilirubin was not caused by abnormal bilirubin formation in Case 2, either.

As to differential diagnosis, shunt bilirubinaemia [24] could be ruled out because bilirubin excretion was not increased. As to transient familial hyperbilirubinaemia, in Case 1 no inhibitors could be demonstrated in the serum, and the jaundice persisted without change after one month of age. Finally, Gilbert's disease, intermittent juvenile jaundice could also be ruled out, as the serum bilirubin level was usually above 20 mg per 100 ml [2].

In Case 1, we could prove by incubating the liver homogenate with bilirubin, the weak activity of hepatic glucuronyl-transferase. Since as a source of UDP-gluconate we added no concentrated boiled liver tissue or purified UDP-gluconate, to the control rat liver homogenate, the variations in the UDP-gluconate content of the rat livers could not be potential sources of error. On the other hand, the enzymopathy was not due to inhibitor substances, because no substances inhibiting the bilirubin conjugating activity of the liver have been detected in serum [27].

Glucuronyl-transferase activity has been found greatly diminished also by the menthol and N-acetyl-paraaminophenol excretion tests. Raising the doses of menthol did not increase the amounts excreted in 8 hours in the form conjugated with glucuronic acid.

In both patients about 80 per cent of the endogenous 17-hydrocorticosteroid was conjugated in the 24-hour urine. This value was considered normal on the basis of the data in literature [32], and to indicate that the liver could glucuronize small amounts of the aglycone substances, so that the metabolic block was not complete. The results of the acetanilide excretion tests were remarkable as far as on administering 10 mg/kg in Case 1, and 17 mg/kg in Case 2, conjugation was normal. Following the administration of 30 mg/kg of N-acetyl-paraaminophenol the weakness of conjugation was demonstrable on both occasions.

*

According to present knowledge, the mammalian liver conjugates the endogenous and exogenous aglycone substances differently to the first hemiacetic C atom of the activated glucuronic acid (UDPGA), notably by esteric, ethereal and N-glucuronide bonds [17]. To the carboxyl group of bilirubin, or for example of anthranylic (o-amino-benzoic) acid, glucuronic acid is linked by an esteric (acyl) bond. Conjugation to the aglycone molecules possessing phenolic and alcoholic hydroxyl receptors is of the ethereal type; in this way are conjugated paraaminophenol, phenolphthalein, menthol, tetrahydrocortisone, 4-methyl-umbiliferon, etc. The conjugate of the esteric type is very labile if exposed to bases; beta-glucuronidase breaks up the esteric and ethereal conjugations alike.

The glucuronide forming enzyme glucuronyl transferase (termed recently also UDP transglucuronylase or glucuronosyl transferase), is to be found first of all in the microsomal fraction of the liver. The enzyme is not stable, requires the presence of Mg ions, and has a reaction optimum around pH 7.6. It is unclear whether or not the same glucuronyl transferase enzyme is involved in the formation of the different types of glucuronides in the animal, but especially in the human, liver. Since LATHE [28] reported that bilirubin and o-aminophenol are not conjugated at comparable rates in the different animal species, several enzymes have been found to create glucuronide conjugates [1, 3].

In our cases the persistent weakness of enzymatic activity has been demonstrated *in vivo* and *in vitro* for the esterically conjugated bilirubin and *in vivo* for the ethereally conjugated menthol and N-acetyl-paraaminophenol. VEST showed that a transitory diminution of glucuronyl transferase activity was responsible for neonatal jaundice. He found newborns to glucuronize as little as 20 to 35 per cent of 10 mg/kg of acetanilide; this weakness ceased with the disappearance of jaundice [41]. In our cases no weakness of conjugation could be demonstrated after a 17 mg/kg dose, although at the time of the tests the patients had much higher bilirubin levels than those usual in newborn jaundice.

Our data seem to supply indirect evidence to show that there are at least two kinds of glucuronyl transferase in the human liver. In newborns with physiological jaundice the acetanilide excretion test demonstrates a low activity not of the transferase enzyme conjugating the bilirubin by esteric bond, but of the enzyme which brings about ethereal conjugation and whose function is independent of the former. This would mean that in newborn age there would be a weakness of both types of enzyme, and the two transferases would mature in parallel. It is much less probable that the same enzyme should possess different affinities to different substances at different stages of life.

According to the above, in our cases the disturbance involved protein molecules possessing the same metabolic function, but two types of enzymatic activity. As compared to normal newborns with physiological jaundice, in our patients the enzymatic activity responsible for the ethereal type paraaminophenol conjugation was increased in spite of the grave jaundice, while the activity of transferase producing the esteric bilirubin glucuronide was decreased.

Our assumption that there exist at least two kinds of transferase is supported also by the fact that the bilirubin level did not change in our patients during the excretion tests (bilirubin determinations at two hours intervals were done on three occasions), although enzymatic capacity of the liver was exposed to what is probably a maximum strain, considering the high bilirubin level. More-

over, in five imbecile children the excretion of 5 mg/kg of bilirubin was not influenced by the administration of 30 mg/kg of menthol and 10 mg/kg of acetanilide; acetanilide was excreted normally in glucuronized form. The test made in Case 2 at the age of 2½ months, when 17 mg/kg of acetanilide and 50 mg/kg of menthol were administered simultaneously, may raise the possibility of a multiplicity of the enzyme conjugating with ethereal bond the aglycone molecules possessing phenolic and alcoholic hydroxyl receptors.

The progressive hepatomegaly observed in both of our patients has not been described in the previous case of Crigler-Najjar's syndrome. While in Case 1 the liver was enlarged (320 g) at autopsy and the liver cells stored an unidentified substance of polysaccharide nature, in Case 2 the liver weighed only 125 g at autopsy and no accumulations of any kind were observed. Since in Case 2 the progressive hepatomegaly was detected in neonatal age, sugar tolerance and adrenaline tests were done repeatedly. The results indicated no disturbance in glycogen metabolism. One hour after death specimens of liver and muscle tissue were tested quantitatively for glycogen content; the results obtained were normal, beside an increased glucose-6-phosphatase activity. The hepatomegaly which became more and more clearly observable clinically and the surprising finding at autopsy that the liver weighed only 125 g might find their explanation in the chest radiographs. In Case 1 the lung was emphysematous and the diaphragm displaced downward. This was even more marked in Case 2. This might also explain the fact that during the last month the spleen became palpable. The cause of the pulmonary emphysema in both patients remains unknown.

As to the storage phenomena in the liver, we are reduced to assumptions, since in Case 2 we were unable to carry out tests in that direction. An increase of abnormal glycogen was unlikely, as this would have meant that two kinds of enzymopathy were present simultaneously within the same family. A secondary defect in mucopolysaccharide metabolism and storage might have been at play, in spite of the fact that the deposited material could not be identified histochemically as acid mucopolysaccharide. Still, the following must be taken into account. The glucuronic acid component of mucopolysaccharide formation is supplied by UDP glucuronic acid [6.] and this supplies activated glucuronic acid for the conjugating activity of the liver. In view of the defective activity of glucuronyl-transferase it is conceivable that the UDPG present in excess because of the metabolic block, might have given rise to abnormal mucopolysaccharide formation. It is, however, strange why this should occur in Case 1, when such a phenomenon has not been observed in Case 2, in the cases of Crigler-Najjar's syndrome reported in the literature and in the jaundice of Gunn rats. In our Case 2, slightly increased amounts of a PAS positive

substance not digested by saliva, and disappearing on acetylation, in other words an acid mucopolysaccharide, appeared in the hepatic connective tissue, but the hexosamine content of the liver did not differ from that of the controls. Thus, the possible involvement of a defective mucopolysaccharide metabolism is suggested by the fact that in Case 2 histology showed aortic changes indicative of connective tissue degeneration.

The episodes of increased SGOT activity appeared after the first weeks of life only. This finding cannot be correlated with certainty with a secondary damage to the liver parenchyma, in Case 2 it having been present without any sign of storage, and further, because it might have resulted from the atrophy, since pseudocholinesterase activity may also be low in malnutrition. The serum enzyme values showed no correlation either with the serum bilirubin level, or with the other liver function tests.

It is mainly the cholesterol „Estersturz" that may be interpreted as indicating the occurrence in our patients of more than one defect in hepatic metabolism. In view of the methodical sources of error the hypercholesterolaemia could not be evaluated, but it is worth mentioning that the serum cholesterol levels were increased also in the parents in whose case, too, the esterified fraction was under 50 per cent.

Hypercitraemia occurs in diseases of the bones, kidneys and the liver. Slight hypercitraemia has been observed in Gilbert's disease [34]. It seems likely that hypercitraemia is a sign indicative of a derangement in citric acid metabolism. The serum citric acid level is regulated first of all by the liver, and to a smaller extent by the kidney. When liver function is defective, as in newborn age or in liver disease, repeated transfusions of citrated blood may give rise to citric acid intoxication. Investigations at our Department have shown a marked diminution of urinary citric acid excretion in nephritis, beside normal serum citric acid levels [39]. In the two cases under review, hypercitraemia was observed during the first months of life; urinary citric acid concentration was normal once with an increased serum value, and once it was slightly increased when the serum level was normal. Since these tests had not been preceded by citric acid administration and the patients did not suffer from bone or renal disease, the hypercitraemia should be ascribed to a disturbance in liver function. We cannot explain why the hypercitraemia appeared in Case 2 during the first days of life already and why it disappeared later, just as we cannot explain what connexion, if any, existed between this finding and the defect in bilirubin metabolism.

The stabilisation of serum bilirubin concentration at a high level is a remarkable characteristic of Crigler-Najjar's syndrome. According to the present view the human organism cannot break down further the bilirubin molecule, while in certain animal species the tetrapyrrole biliru-

bin is excreted mainly in the form of dipyrrole metabolites [45]. From the human organism indirect bilirubin can be excreted exclusively after being conjugated in the liver to glucuronic acid in about 75 per cent, to sulphate in 15 per cent, and to unknown substances in 10 per cent [25]. Indirect bilirubin is excreted with urine only at a very high serum bilirubin level and even then in small amounts. Not even in our Case 1 was the bilirubin, obviously produced continuously, excreted in its total amount through the bile with the faeces in the form of the usual tetrapyrrole substances. Although in Case 1 a quantity of direct bilirubin could be detected in the duodenal juice and the gall bladder, in comparison with the amounts of faecal and urinary bile pigment this quantity still does not explain the stabilisation of the serum bilirubin level. In the lack of a beta-glucuronidase preparation of suitable quality, we could not determine whether the bilirubin in the bile of our patient was a glucuronide or eventually some other, e.g. sulphate, conjugate. At any rate, the bile obtained from the gall bladder contained conjugated glucuronic acid (56 mg per 100 ml). What then happens with most of the bilirubin in such patients? According to the recent investigations of WALKER and BILLING [43], in liver disease the steroids lower the bilirubin level by opening up a new metabolic pathway. It is conceivable that in the Crigler-Najjar's syndrome such a mechanism starts to operate after the serum bilirubin level has been high for more time. It is also possible that prednisone has a stimulating action on this mechanism. (The explanation that in response to prednisone the rate of glucuronization increases, does not hold ground.)

In connexion with the above it must be remembered that in Case 2 the serum bilirubin level had reached its lowest value just at a time when — in analogy to Case 1 — a significant increase could have been expected to take place, notably during the week preceding death. This may be brought into correlation with the fact that during the last 10 days the patient was treated with high doses of prednisone. Unfortunately, observations over longer periods were not possible, but it seems justified to give a trial to treatment with large doses of prednisone in the Crigler-Najjar's syndrome.

Some of these patients, however, presumably owing to their peculiar permeability relations, may *abovo* be protected against bilirubin encephalopathy, whereas in others it does not suffice to keep the serum bilirubin level under 20 mg per 100 ml except during neonatal age. In our Case 2, six blood exchange transfusions had been carried out, and yet nervous symptoms developed after two weeks of age, while in Case 1 only two exchange transfusions were done at that age and yet the baby was well until the age of six weeks. None of the patients showed any evidence of changes promoting the development of kernicterus (anoxia, etc). Thus, the problems

of kernicterus prevention could be resolved only by long-lasting treatment continued over months or even years. The administration of large doses of prednisone is apparently unsuitable for this purpose, not even when antibiotic protection is provided for; in our Case 2 the development of septicaemia might be brought into correlation with the large doses of prednisone applied.

In the recently published cases of the Crigler Najjar's syndrome, including our own patients, consanguineous marriage could be ruled out. Moreover, the fact that from the marriage of an afflicted man with a normal woman a child with persistently severe jaundice was born makes it questionable whether the rare gene is inherited recessively in every case.

Summary

Two cases of Crigler-Najjar's syndrome have been observed. The infants were born in the same family. The marriage was not consanguineous. One of the babies developed bilirubin encephalopathy at three weeks of age, the other at six weeks of age. In case 1 an excessive weakness of hepatic glucuronyl transferase activity was proved by tests *in vitro*. No inhibitors were demonstrable in the serum. Of acetanilide 10 mg and 17 mg/kg doses were excreted in the urine normally, in conjugation with glucuronic acid; the urinary excretion of 35 mg/kg of menthol and that of 30 mg/kg of N-acetyl-paraaminophenol was significantly reduced.

It has been concluded from the findings that there must be at least two kinds of glucuronyl transferase in human liver. Of these, in our patients the activity of the enzyme responsible for the ethereal type p-aminophenol conjugation was higher, while that producing the esteric bilirubin glucuronide lower, than in normal newborns with physiologic jaundice.

Both patients showed progressive hepatomegaly. The liver of one of the patients stored an unidentified substance of polysaccharide nature. The gall bladder bile from the same patient contained 10 mg and 11.4 mg per 100 ml, respectively, direct reacting bilirubin. In both cases intermittent increases of SGOT activity, cholesterol „Estersturz", during the first postnatal weeks hypercitraemia and eosinophilia were found. In Case 2 exchange transfusions were carried out 6 times during newborn age. In the same patient the serum bilirubin level decreased during the last week of life in the course of treatment with 3 mg/kg of prednisone.

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