

# Rotor's Syndrome: A Family Study\*

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## ABSTRACT

A family was studied in which three members in the sibship belonging to the fourth generation were found to have Rotor's syndrome. More detailed examinations including blood studies, liver profiles, oral cholecystograms, and liver biopsies were performed on the affected siblings. The results were related to what is at present known about the features and mechanisms of Rotor's syndrome, *pari passu* the current concept of bilirubin metabolism. It is suggested that the constant finding, and possibly the only characteristic one in Rotor's syndrome, is the absence of abnormal hepatic cell pigmentation. Pedigree analysis of the present family shows that the transmission of this disorder may be conditioned by an autosomal recessive gene.

*Key Words: Rotor's Syndrome, hereditary hyperbilirubinemia, Philippines*

## INTRODUCTION

In 1948 Rotor, Manahan and Florentin<sup>1</sup> first reported a syndrome described as familial non-hemolytic jaundice with direct van den Bergh reaction. The other features noted were compatibility with life and health, and normal liver histology. Subsequent published reports<sup>2-10</sup> of cases exhibiting the classic picture and documented by liver biopsies not only established Rotor's syndrome as a distinct entity but also characterized further its clinical and biochemical spectrum. Locally, aside from the original report, there has been no similar publication in the available literature. A case, however, was seen by Millora<sup>11</sup>, and there are probably others.

In the study done by Schiff, Billing, and Oikawa,<sup>2</sup> it appears that the main abnormality is an inborn error of metabolism expressed as a defect in the ability of the liver cells to excrete bilirubin. Arias' observations<sup>7</sup> suggest a possible interrelationship between Rotor's syndrome and Dubin-Johnson syndrome<sup>12-14</sup>; *i.e.*, that the two may represent varied expressions of a common functional hepatic excretory defect. More recently there has been increasing interest in the genetic studies<sup>10</sup> of this disorder.

The present study deals with a family in which three members in the sibship belonging to the fourth generation were documented to have Rotor's syndrome. The significance of the data obtained is emphasized in the light of current knowledge. As was indicated in the original report of Rotor and associates, it is hoped that future investigations may provide more definite clues to the exact nature of the pathophysiology of this disorder. Much is still not known about the processes involved in bilirubin transport<sup>15,16</sup> from plasma to bile, and it is possible that further studies of patients with Rotor's syndrome should also prove helpful in elucidating those poorly understood mechanisms.

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## MATERIALS AND METHODS

A detailed pedigree of the family was obtained (Figure 1). All the members in the sibship of the propositus (IV-2) with the exception of those who had died in childhood of illness not characterized by jaundice, were examined by us. In addition, we were also able to examine the mother and also the child of the propositus. Clinically two other siblings were found icteric (IV-5 and IV-7). They comprise, together with the propositus, the subjects on whom detailed laboratory examinations and liver biopsies were performed. The serum bilirubin studies were determined by the van den Bergh test of Malloy and Evelyn. The alkaline phosphatase was determined by the King and Armstrong method; the prothrombin time, by the Quick method. The following examinations were done according to the standard of methods; cephalin flocculation, thymol turbidity and flocculation, serum albumin, globulin, cholesterol, and transaminase (SGOT), osmotic fragility, and the  $^{51}\text{Cr}$  study for red blood cell survival time.

The bromsulfalein retention was estimated by drawing blood 45 minutes after the intravenous administration of 5mg of dye (BSP) per Kg of body weight. The oral cholecystogram was taken approximately 12 hours after ingestion of a double dose of iopanic acid (12 tablets of Telepaque®).

The liver specimens were obtained by the Menghini technic<sup>17</sup> using the 1.2 mm needle. The specimens were stained with hematoxylin and eosin and examined under ordinary light microscopy by one of us (A.D.C.).

### Family Members Studied in Detail

#### Case 1

L.C (propositus – IV-2), 25 years old, male, mechanic, from Tondo, Manila, was admitted for the first time on December 24, 1964 for the complaints of cough productive of “rusty” sputum, fever and chills, right subcostal pain, and jaundice. The lung findings at this time showed few basal

crepitant rales on the right side. The acute symptomatology subsided in about 5 days' time and was documented by a chest roentgenogram to be due to a pneumonia of the right inner base. The icterus, however, persisted.

He had yellowish discoloration of the eyes since the age of five. The jaundice fluctuated, noted to be more intense when he had fever during which time he would also have “tea-colored” urine. The stools were normal in color. There was no past history suggestive of biliary colic, or hematologic disorder. He had not indulged in excessive alcoholic intake.

Physical examination showed a well-nourished and well-developed patient with scleral icterus. The heart and lung findings were normal. The liver and spleen were palpable.

The following examinations were performed with normal results: complete blood count, erythrocyte sedimentation rate, reticulocyte count, osmotic fragility,  $^{51}\text{Cr}$  study for red blood cell survival (hair-life was 36 days), fasting blood sugar, non-protein nitrogen, serum amylase and lipase, alkaline phosphatase, cephalin, flocculation, thymol turbidity and flocculation, serum albumin and globulin, cholesterol, glutamic oxalacetic transaminase and prothrombin time. The urine was positive for both bilirubin and urobilinogen; the stool, only for urobilinogen. The total serum bilirubin concentration was 21.4mg/100, of which 15.2 mg/100 ml. (71% of total) gave the direct van den Bergh reaction. A repeat examination of the total serum bilirubin concentration nine days after admission (when the acute symptomatology had already subsided) showed a drop in the concentration to 10.3 mg/100 ml.

The bromsulfalein retention was 28% in 45 min. Oral cholecystography revealed faint visualization of the gall bladder. The histologic examination of the liver biopsy specimen showed essentially normal findings. There was no pigment noted.

Approximately two years later, with the patient in apparent good health, a repeat evaluation of the various laboratory examinations, including the oral cholecystography

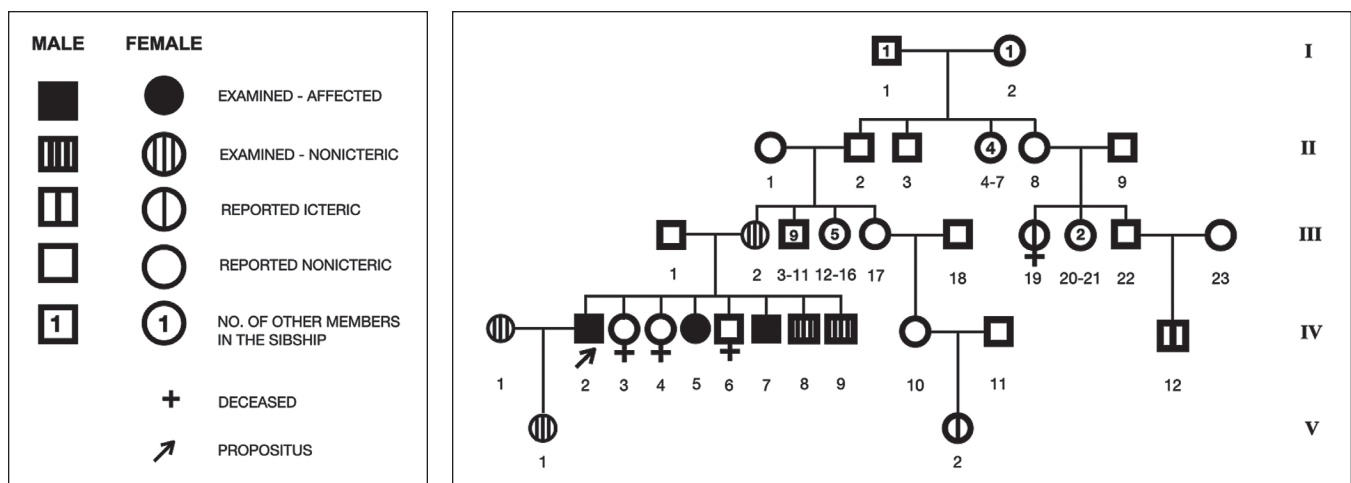


Figure 1. Pedigree of the family studied.

and liver biopsy, was done. The results were practically similar to the previous studies except the BSP retention which had increased from 28% to 55% in 45 min. Histologically, the liver biopsy specimen was still normal.

### Case 2

C.G. (IV-7), 16 years old, high school student, brother of the propositus was admitted for the first time on January 1, 1965 for icteric sclerae and "tea-colored" urine since the age of five. The jaundice also fluctuated becoming more intense when he had fever. Except for the icterus, he had been essentially asymptomatic.

There was no past history suggestive of biliary tract disease, infectious hepatitis, or hematologic disorder. He denied having had pruritus, acholia, or biliary colic.

Physical examination revealed an essentially normal patient except for the icteric sclerae. The liver and spleen were not palpable.

Laboratory examination showed the blood studies (complete blood count, erythrocyte sedimentation rate reticulocyte count, and osmotic fragility test) to be within normal limits. The half-life of the red blood cells as determined by the <sup>51</sup>Cr study was 36 days. Both bilirubin and urobilinogen were present in the urine; only urobilinogen, in the stool. The total serum bilirubin concentration was 10.6 mg/100 ml, of which 6.7 mg/100 ml (66%) gave a direct van den Bergh reaction. The BSP retention was 35% in 45 min. The other liver function tests were within normal limits. There was faint visualization of the gall bladder on oral cholecystography. Histologically, the liver biopsy specimen (Figure 2) was normal.

A repeat of the laboratory examination, and liver biopsy, approximately two years later, showed only an increase in the BDP retention (from 35% to 50%) and non-visualization of the gall bladder on oral cholecystography.

### Case 3

M.G (IV-5), 18 years old, sister of the propositus, had been having yellowish discoloration of the eyes and "tea colored" urine since the age of four. The jaundice deepened whenever she had fever. She had been in apparent good health, except for her icterus.

The past history was essentially non-contributory. She denied any history of biliary colic, pruritus, or acholia.

Physical examination showed icteric sclerae, normal heart and lung findings, and non-palpable liver and spleen.

The hematologic studies were all within normal limits. The <sup>51</sup>Cr study for red blood cell survival showed a half-life of 32 days. The urine was positive for both bilirubin and urobilinogen; the stool, only for urobilinogen. The total hyperbilirubinemia was 6.4 mg/100 ml was mainly of the direct type (67% of total). The BSP retention was 25% in 45 min. The other liver function tests were essentially normal. Oral cholecystography showed normal visualization of the gall bladder. Histologically, the liver biopsy specimen was normal.

## RESULTS AND DISCUSSION

The results of the important studies performed on the affected siblings are summarized in Table 1. As shown only the liver function tests primarily associated with excretory function are abnormal. The total serum bilirubin concentration ranges from 6.4 mg/100 ml. to 21.4 mg/100 ml., of which 66% to 87% are direct-reacting, or conjugated bilirubin.<sup>18-20</sup> The BSP retention ranges from 25% to 55% in 45 minutes. Visualization of the gall bladder varied from normal faint to absent. Histologic examination of the liver biopsy specimens showed normal finding, and failed to reveal any abnormal pigmentation.

The hematologic data reveal absence of anemia, and normal reticulocyte count, osmotic fragility test, and red blood cell survival. These findings together with elevation of the direct-reacting bilirubin indicate that the icterus is not of hemolytic origin. Biliary tract obstruction is unlikely, notwithstanding the faintly visualizing gall bladder in two of our cases, on account of the absence clinically of biliary colic or acholia, and the normal serum alkaline phosphatase and cholesterol values. Also, obstruction of the duration as seen in these patients, should probably show changes in the histologic picture of the liver biopsy specimen, but such changes<sup>11,21</sup> were not noted in these cases.

Of the three types of familial jaundice occurring in the adult<sup>3</sup>, Gilbert's disease can be excluded because of the findings of abnormal BSP retention and elevated direct-reacting or conjugated bilirubin. On the other hand, the

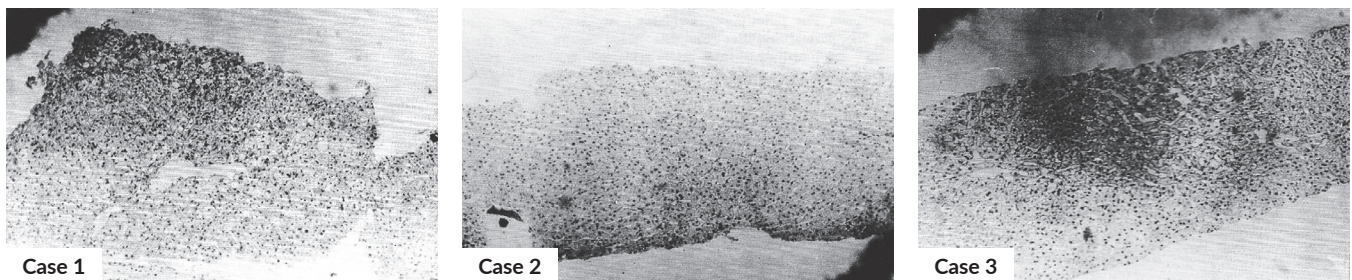


Figure 2. Photomicrographs of the liver biopsies.

Table 1. Pertinent studies done on the three siblings

Patient	Date	Serum Bilirubin mg/100ml (% of total)		Alkaline Phosphatase King-Armstrong units/100 ml	Cephalin Flocculation Test in 24 hr.	Thymol Turbidity Test units	Thymol Flocculation Test in 24 hr.	Serum Albumin gm/100 ml	Serum globulin gm/100 ml	Serum Cholesterol mg/100 ml	SGOT Photo colorimetric units/ml	Prothrombin Time (seconds)		BSP Retention % in 45 min	Oral Cholecystography	Liver Biopsy
		Direct	Indirect									Patients	Control			
L.G	12-10-64	15.2 (71%)	6.2 (29%)	10.1	-	6.2	-	3.5	2.3	170	88	19	17	28	Faint	Normal
	12-29-64	9.1 (84%)	1.7 (16%)	7.9	-	4.4	-	4.9	2.5	142	44	16	15	55	Faint	Normal
C.G	9-7-66	12.5 (74%)	4.6 (26%)	6.6	-	3.8	-	3.4	2.4	170	54	15	17	35	Faint	Normal
	1-1-65	6.7 (66%)	3.9 (34%)	14.0	-	2.9	-	4.6	2.3	174	46	15	17	35	Faint	Normal
M.G	9-21-66	5.6 (87%)	0.8 (13%)	10.5	-	3.5	-	3.3	1.9	156	24	15	17	50	No Visualization	Normal
	1-1-65	4.3 (67%)	2.1 (33%)	5.7	+	4.1	-	4.5	2.7	146	101	16	17	25	Normal	Normal

absence of abnormal hepatic pigmentation on repeated examination rules out Dubin-Johnson syndrome.<sup>12-14</sup>

Case 3 exhibits the more consistent findings<sup>1-10,23</sup> in Rotor's syndrome: hyperbilirubinemia, predominantly of the direct-reacting or conjugated type; abnormal BSP retention, normal cholecystogram; and normal liver biopsy specimen, without any demonstrable pigment. Cases 1 and 2 however, differ slightly in the sense that both showed abnormal visualization of the gall bladder on oral cholecystography, i.e., faint in one and absent in the other. This finding is in striking contrast to what we found in our review of the fifteen published cases that were documented by liver biopsies. Cholecystographic studies were done in twelve cases, and all revealed normal visualization of the gall bladder. On the other hand, BSP retention studies were available in fourteen cases and all showed abnormal results ranging from 15% to 65% in 45 minutes.

Until the present studies, non-visualization of the gall bladder has not been found as a feature of Rotor's syndrome. In 1962, Dubin<sup>24</sup> set the major differences between the two syndromes, as follows: 1) cholecystographic visualization of the gall bladder in Rotor's syndrome and the absence of visualization in chronic idiopathic jaundice i.e., Dubin-Johnson syndrome); 2) presence of an abnormal amount of lipofuscin\* in the liver cells in the latter and absence of this finding in the former. Our present data suggest that cholecystographic visualization of the gall bladder may not hold true in all cases of Rotor's syndrome. It appears that the constant finding, and possibly the only characteristic one, is the absence of abnormal liver cell pigmentation.

The suggestion<sup>5,7</sup> that Rotor's and Dubin-Johnson syndrome are interrelated or are variants of the same disease entity is still speculative up to the present time. In the family study reported by Arias concerning two brothers and a paternal aunt, the latter and one of the two siblings were found to have Dubin-Johnson syndrome. Repeated liver biopsies in the other siblings failed to reveal any abnormal hepatic pigmentation on histologic examination. On the other hand, a somewhat analogous observation<sup>25</sup> regarding Rotor's syndrome was noted in 1961 in a follow up study of a patient (D.R.), sister of the proposita (E.R.), belonging to the original family described by Rotor. An open biopsy of the liver was made possible when she underwent an exploratory laparotomy for an intussusception which she had developed. Histologic examination of the liver biopsy specimen\*\* revealed

\* Recent studies of Wegmann and associates suggest that the hepatic pigment differs from lipofuscin structurally and histochemically. It is probably related to melanin and catecholamines (Cited by Scheur and Williams in Popper, H. and F. Schaffne in Progress in Liver Diseases, ed.1. Grune and Stratton, Inc., NY 1965. P. 285)

\*\* The slides were reviewed by Dr. Schiff and Dr. Gall, both from Cincinnati General Hospital, Cincinnati, Ohio, but they did not see any evidence of Dubin-Johnson syndrome. Instead their interpretation was that of chronic, active hepatitis as evidenced particularly by the periportal infiltration. (Schiff, L. Personal communication to Dr. AB Rotor, 1962.

intracellular pigmentation as interpreted by the pathologist, and consequently the diagnosis of Dubin-Johnson syndrome was suggested. Perhaps, it may not be inconceivable that one is probably an earlier stage of the other. But up to now there has not been any report of a case classified originally as Rotor's with subsequent development of abnormal hepatic pigmentation on follow-up studies.

The recent observation<sup>26</sup> that the degree of hepatic pigmentation in Dubin-Johnson syndrome varies considerably and may even be minimal might imply that this criterion for its differentiation from Rotor's syndrome should lose validity. If at all, however, this should be taken to mean that one must undertake great caution in making the diagnosis, so that no case which falls short of the required criteria should be considered acceptable or adequate for classification. We agree with Dubin<sup>24</sup> that until a precise demonstration of the postulated defect or defects in both conditions has been definitely established, distinction of the two as separate entities may be necessary and should be maintained. And at present, the only justifiable point of differentiation between the two syndromes seems to be the presence or absence of abnormal liver cell pigmentation.

Current knowledge of the hepatic aspects of bilirubin metabolism<sup>15,27</sup> (Figure 3) permits visualization of the physiologic steps involved. Thusly: a) the uptake of bilirubin from the plasma into the liver cell, b) the intracellular formation of bilirubin conjugates, primarily bilirubin glucuronide, and c) the excretion of water-soluble conjugates into the bile canaliculi. The mechanism of the hepatic cellular uptake of bilirubin<sup>15,27</sup> is poorly understood, in fact virtually unknown. Conjugation is required prior to excretion of bilirubin<sup>28,29</sup> by the hepatic cell. Little is known about the excretory mechanism itself, but it is assumed to be energy-dependent operating against a concentration gradient, as supported by the work of Hazon.<sup>15</sup> The ability of the hepatic

cell to excrete conjugated bilirubin is rated-limited<sup>20,29</sup>, and normally this capacity limits the overall transfer of bilirubin from blood to bile.

Information obtained from the few cases of Rotor's syndrome studied suggests that the conjugation of bilirubin is not defective. Normal results obtained from salicylamide excretion<sup>2,3</sup> and that of N-acetyl-p-aminophenol<sup>8</sup>, as well as the presence of glucuronide conjugates<sup>2,3,6,7,10</sup> in the plasma, indicate adequate amounts of glucuronyl transferase. In addition, Arias<sup>7</sup> showed normal results for glucuronide formation, both *in vivo* and *in vitro*.

The postulated defect<sup>2</sup> in Rotor's syndrome is a diminished hepatic cellular uptake of bilirubin from the plasma either due to or superimposed upon a specific defect in the secretion of bilirubin glucuronide. An analysis was made of the plasma disappearance curve of bilirubin<sup>2,30</sup> following the injection of a single intravenous dose of 2 mg bilirubin/kg body weight in an affected patient *vis a vis* another normal subject. The removal of both bilirubin and conjugal bilirubin was shown to be considerably delayed in the patient with Rotor's syndrome as compared with the control. Until the present time, however, direct and unequivocal demonstration of the exact nature of the postulated defect has not yet been possible.

### Hereditary Characteristics

Relevant data were provided by the mother of the propositus about the forty-eight members of the family consisting of five generations. The three affected siblings are off-springs of normal parents not related consanguineously. Three other relatives on the maternal side (III-19, IV-2, and V-2 in the pedigree chart) were reported to be jaundiced or had history of jaundice. (But we cannot assume that this icterus manifested is due to the same disorder which our patients have.) From the available data, however, an

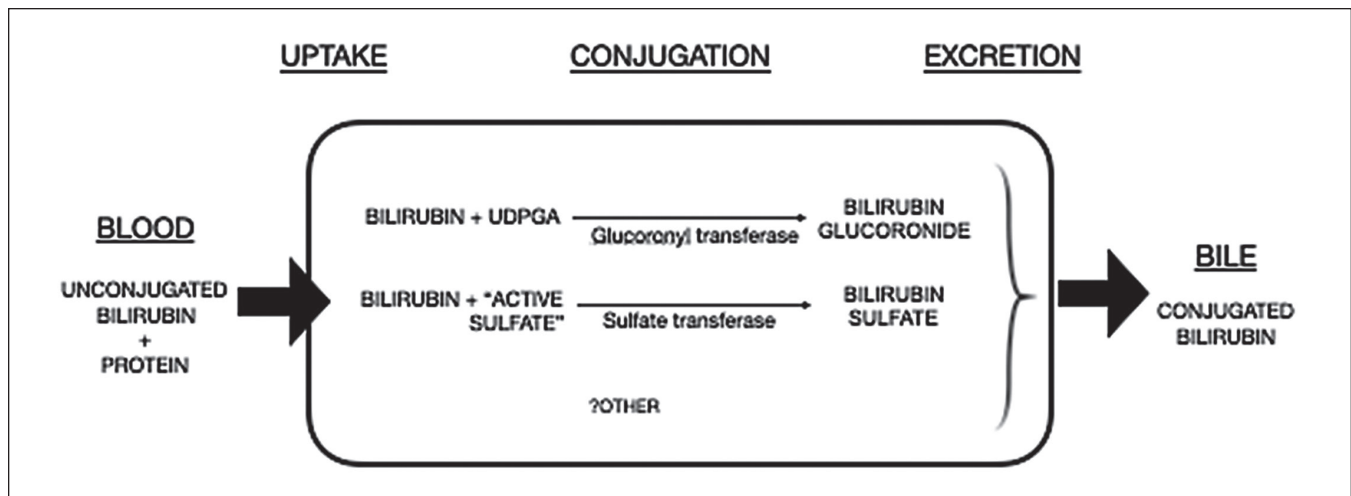


Figure 3. Schematic representation of the hepatic aspects of bilirubin metabolism.

(From Arias IM. *The Medical Clinics of North America*. 44:610, 1960, with permission of the publisher).

autosomal dominant gene with complete penetrance<sup>31</sup> can be excluded. It may be surmised that our present subjects are homozygous to the abnormal gene.

Our findings are in agreement with those of Pereira Lima *et al.*<sup>10</sup> In a well-documented study, they obtained data of about seventy-two relatives in a family consisting of six generations. The results revealed that the affected members were limited only to the sibship of the proposita. The latter's parents were both found to be normal. The authors inferred that transmission of the disorder was conditioned by an autosomal recessive gene. On the other hand, pedigree analysis of the original family described by Rotor, and of those described by Haverback and Wirtschafter, and Peck *et al.* suggest that the disorder is inherited as an autosomal dominant.

It is therefore apparent from the genetic studies<sup>1,5,6,10</sup> thus far recorded, including our present data, that the individual affected with Rotor's syndrome may be either homozygous or heterozygous to the abnormal gene.

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