Quebec Cooperative Study of Friedreich's Ataxia

Bilirubin Metabolism — Preliminary Investigation

A. BARBEAU, G. BRETON, B. LEMIEUX, AND R. F. BUTTERWORTH

SUMMARY: *In our studies, high total bilirubin values in the plasma were noted in cases of Friedreich's ataxia. A bimodal distribution of the values indicated the possible presence of two subgroups of patients. In these kindred, we demonstrated an elevation in unconjugated bilirubin with features similar to those reported in Gilbert's syndrome: normal liver function tests, elevation after fasting and day to day variability. We also report preliminary experiments indicating that bilirubin levels may be taurine dependent. We postulate that the defect could be a secondary component of the ataxic disease, possibly indicating a defect in membrane transport.*

RÉSUMÉ: *Au cours de nos études, nous avons noté une incidence anormale de valeurs élevées de la bilirubine plasmatique totale chez des sujets atteints d'ataxie de Friedreich. Une distribution bi-modale des valeurs indique la présence possible de deux sous-groupes cliniques. Dans ces familles, nous avons démontré une augmentation de la bilirubine non-conjuguée avec toutes les caractéristiques du syndrome de Gilbert: fonctions hépatiques essentiellement normales, augmentation des taux sous diète calorique basse, variabilité des taux d'un jour à l'autre. Nous décrivons également une série d'expériences préliminaires qui indiquent que le taux de bilirubine est possiblement dépendant de la taurine. Nous postulons que ce trouble apparent est secondaire à la maladie ataxique, indiquant possiblement un défaut du transport membranaire.*

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INTRODUCTION

In the investigation of a large number of cases of ataxia, we were struck by the frequent occurrence of a high total bilirubin in some subjects. The second subject tested, a male of 26 years, had a total bilirubin value of 2.9 mg%. A complete investigation of his liver functions and history failed to reveal any evidence of biochemical liver impairment, obstruction, drug usage, or residual hepatitis. He had often noted intermittent yellowing of the sclerae without jaundice of the skin. On rare occasions, his urine was found to be darker. A liver biopsy was normal. He was thought to have "Gilbert's syndrome" or "constitutional hepatic dysfunction" (also called "idiopathic unconjugated hyperbilirubinemia"), which was probably coincidental with his ataxia. However, in the next few months, we demonstrated the same disturbance in both his sister and brother, equally ataxic, and we found similar patterns in a number of other families. This observation was the reason for the more systematic approach, particularly since a search of the literature failed to reveal a study of bilirubin metabolism in the ataxias.

SUBJECTS AND METHODS

All routine laboratory determinations were done with techniques described in a previous paper. Many of the tests including total bilirubin were carried out with the SMA 15/60 autoanalyser. Conjugated and unconjugated bilirubin were estimated by the method of Malloy and Evelyn (1953). When necessary, and after informed consent, a liver biopsy was carried out by the consultant gastro-enterologist (Drs. L. P. Pichette or R. Clermont). Liver scan was performed in the Isotope Laboratory of the Hôtel-Dieu Hospital (Dr. J. Léveillé).

To evaluate the distribution of total bilirubin in the typical Friedreich's ataxia group of 33 patients (see clinical classification in a previous paper), an age and sex-matched group of 33 hospital control subjects was chosen consecutively from the 425 patients studied in the same laboratory with the SMA 15/60 battery, on two days when ataxia patients were being evaluated. Forty-three subjects were eliminated because of known cardiac, hepatic, renal or infectious pathology before the choice of matching patients was done (blindly from the laboratory results).

Complete liver investigations, including biopsy and scan, were carried out in 6 patients identified as having high total bilirubin (from 2 kindreds). Further metabolic studies were done on some of the latter including low calory intake and dietary supplement with the amino acid taurine. In one patient, levels of taurine in the blood and urine were also measured repeatedly, with a Technicon Sequential Multi-sample Amino Acid Automatic Analyzer (TSM) with the chromobeads type "C" resin following a test dose of the amino acid.

RESULTS

a) Results of total bilirubin determinations in the clinical sub-groups of ataxic patients, and in an age and sex-matched control group are given in Table 1. All ataxic groups, except Group 1B, have mean values of total bilirubin higher than the control group. Powell et al. (1967) gave 0.4 ± 0.2 mg% as the mean normal

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value for total serum bilirubin with an upper limit of normal of 0.8 mg. per 100 ml. (95% confidence limit = 0.75 mg per 100 ml). Our control group cannot be said to be normal, since they were in hospital for treatment of a variety of illnesses. The 95% confidence limit, in our own group, would be 0.98 mg per 100 ml (taken as 1.0 mg%). Despite the higher means, all groups of ataxia patients could be said to be within normal ranges. Other indirect tests of liver function (such as alkaline phosphatase, LDH, SGOT) also show higher mean values in ataxic patients.

b) Distribution of total bilirubin values

"Means" can easily mask abnormal distribution of results. This is illustrated in Figure 1, where it is evident that the control patients have a normal bell-shape pattern, but the typical Friedreich's ataxia patients (Group Ia) follow a bi-modal distribution. This was our first hint that there could be two biochemical sub-groups of patients within group Ia: one with normal serum total bilirubin, one with high levels.

c) Characteristics of the high total bilirubin sub-group

It is seen from Figure 1, that 9 patients with ataxia (and none of the controls) have values above the upper limit of normal (1.0 mg%). These 9 patients originate from 7 kindreds with a total of 15 ataxia subjects, of which 13 were included in our prospective survey. Of the 13 patients tested (Table 2), one had elevations of both conjugated (mild, to 0.8 mg%) and unconjugated bilirubin; 9 had elevations only of unconjugated bilirubin. Three patients had normal values. These were the first results. As will be seen, bilirubin values tend to oscillate from day to day. Unfortunately, on the 3 patients with normal values, only one determination had been done.

None of the 13 patients remembered a history of hepatitis, although intermittent jaundice, mainly of the sclerae, was reported by 10. Alcoholism was not a factor although 4 patients admitted to "social drinking". Finally liver biopsies, BSP's and liver scans were within normal limits. In 21 patients, no urinary pigments were found. It can be stated that our patients present some form of idiopathic unconjugated hyperbilirubinemia, of undetermined origin.

d) Investigations of high bilirubin sub-group

The biochemical characteristics of the high and low bilirubin sub-group (13 of 20 patients, respectively) of Group Ia Friedreich's ataxia patients are given in Table 3. One of the striking findings is the consistently lower values for serum cholesterol, serum triglycerides, and all serum enzymes in the high bilirubin sub-group. The other functions tested did not appear to differ between the two sub-groups. However, except for bilirubin and the Danowsky score, both high in sub-group A, all values were within the normal range.

The usual criteria for diagnosis of Gilbert's syndrome are: an unconjugated hyperbilirubinemia of fluctating nature with normal concentrations of conjugated serum bilirubin; scleral icterus as the only abnormal finding; normal serum transaminases and alkaline phosphatase activities, hepatic histology by light microscopy, hemoglobin concentration, peripheral blood smear, white count, reticulocyte count on at least two occasions, and red-cell fragility. As seen in Tables 1 and 2, all these

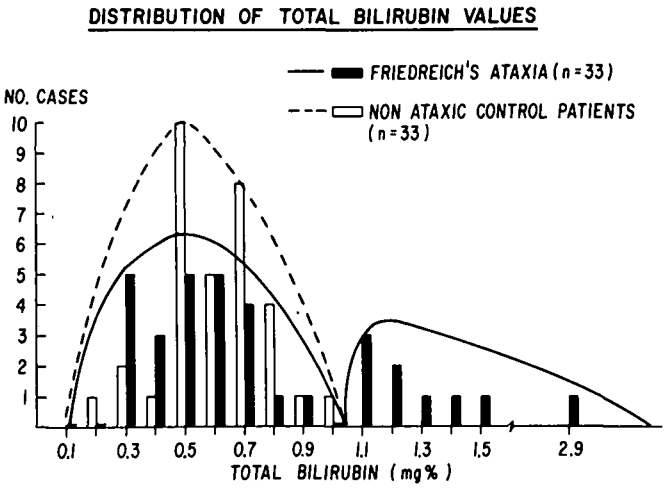

Figure 1—Bimodal distribution of total bilirubin values in Friedreich's ataxia patients.

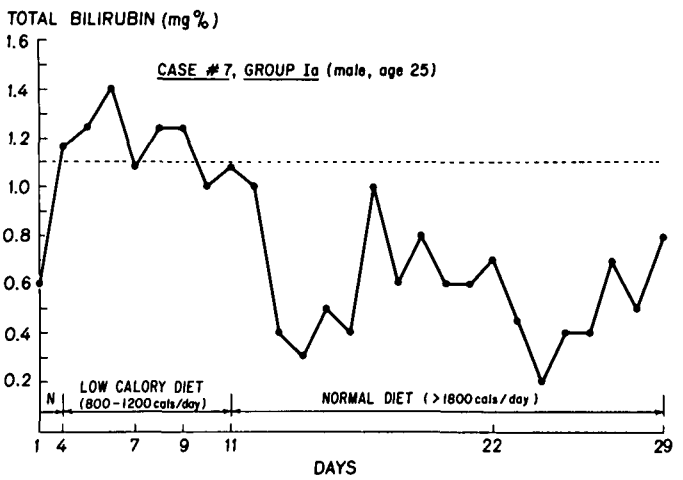

Figure 2—Oscillation in total bilirubin values in a patient with Friedreich's ataxia with dietary variations.

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criteria are fulfilled in every patient where the tests were done.

Gilbert's syndrome is usually inherited as an autosomal dominant trait (Foulk et al., 1959; Smith et al., 1967). The genetics of the elevated unconjugated bilirubin in our families have not been studied. The four parents of only two kindreds had total bilirubin determinations which were all within normal limits (mean 0.6 mg%). It is impossible to conclude on this point until more parents, and all siblings, have been studied.

Two of the constant features of constitutional hepatic dysfunction are the oscillation in bilirubin values from day to day, and the elevation during periods of protein deprivation or fasting (Redeker et al., 1970). These features are well illustrated in a brother and sister whom we have investigated (Figures 2 and 3). It has never been established which constituents of the diet are necessary for the maintenance of normal bilirubin levels. It is known that taurine may play a role in the metabolism of bile acids (Jacobsen and Smith, 1968; Huxtable and Barbeau, 1976; see also infra). For that reason, some preliminary studies with taurine feeding were carried out in one of our patients with high serum total bilirubin.

As seen in Figure 4, a single oral dose of 2 grams of taurine was rapidly absorbed. The concentration of the amino acid increased 13 fold in the plasma and, after a delay of at least 3 hours, also increased in the urine, in both cases with a rapid return to normal. This rise was of a greater magnitude than in normal control patients, and was similar to the situation found in some epileptic patients (Barbeau et al., 1975). Such a rapid increase is compatible with, but not diagnostic of, a total body deficiency in taurine. During the period of high blood levels of taurine, there was a decrease in the concentration of total bilirubin. This observation led to a trial of taurine supplementation (2 grams daily, orally) in the same patient maintained on a restricted caloric diet. As can be seen in Figure 5, there was a gradual return towards normal of

TABLE 1

HEPATIC FUNCTION TESTS

(mean ± S.D.)

	N	BILIRUBIN (mg%)	ALK. PHOSPH. (i.u.)	LDH (i.u.)	SGOT (i.u.)
1) GROUP Ia					
A) Males					
Controls	12	0.73 ± 0.10	77.92 ± 18.98	142.08 ± 18.39	31.67 ± 7.78
Friedreich	12	0.97 ± 0.68	99.58 ± 60.17	218.17 ± 65.11	42.92 ± 16.98
B) Females					
Controls	21	0.52 ± 0.14	68.33 ± 23.36	148.57 ± 22.36	33.33 ± 11.21
Friedreich	21	0.63 ± 0.34	94.04 ± 60.94	199.55 ± 66.50	37.33 ± 12.79
C) Both sexes					
Controls	33	0.60 ± 0.20	70.91 ± 16.22	146.21 ± 30.51	32.73 ± 10.29
Friedreich	33	0.77 ± 0.20	96.06 ± 22.05	206.53 ± 51.48	38.45 ± 14.32
2) GROUP Ib (3F)	3	0.60 ± 0.20	120.67 ± 50.52	232.33 ± 63.68	96.67 ± 68.64
3) GROUP IIa (5F, 1M)	6	0.69 ± 0.26	58.83 ± 24.77	153.0 ± 33.88	25.00 ± 5.62
4) GROUP IIb (1F, 7M)	8	0.71 ± 0.26	106.5 ± 33.45	222.0 ± 36.50	36.25 ± 12.65

TABLE 2

CHARACTERISTICS OF HIGH BILIRUBIN SUB-GROUP

1) Number kindreds		7
2) Number of tested ataxic subjects in these kindreds	- in study	13
	- in others	2
3) Number with total bilirubin > 1.0 mg%		9 (5M, 4F)
4) Elevation of conjugated bilirubin		1
5) Elevation of unconjugated bilirubin		10
6) Bilirubin values: (mean ± S.E.M.)	Total 1.11 ± 0.2 mg% (n = 13) Conjugated 0.13 ± 0.1 mg% (n = 13) Unconjugated 1.03 ± 0.3 mg% (n = 13)	
7) Liver biopsies	- done	5
	- normal histologically	5
8) History of hepatitis		0
9) Intermittent jaundice (No. of subjects)		10
10) Alcoholism		0
11) B.S.P.	- done	6
	- normal results	6
12) Liver Scan	- done	6
	- normal	6
13) Peripheral blood smears	- done	13
	- normal	13
14) Reticulocyte counts	- done	6
	- normal	6
15) Red cell osmotic fragility	- done	4
	- normal	4

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bilirubin values (mean of 1.74 during the control period to mean of 1.29 under taurine).

DISCUSSION

Abnormalities of plasma bilirubin had previously not been noted in association with ataxic syndromes. Our data indicates that 10 of our 50 ataxic patients (20%) have total bilirubin values exceeding the upper limit of variation in our control group (95% confidence limit = 0.98 mg per 100 ml.). If the upper limit of normal is taken to be 0.8 mg per 100 ml as suggested by Powell et al (1967), we have found 14 abnormal values (28%). Most of these are within Group Ia which is the only group with sufficient numbers to permit evaluation of sub-groups. Since bilirubin levels are notoriously variable from day to day, depending partially on the diet, the 20% figure can be taken as a minimum. It is evident that there are at least two populations as regards bilirubin amongst our ataxic patients (Figure 1).

The high bilirubin sub-group (A) of Group Ia patients (typical Friedreich's ataxia) is clustered within 7 of our 20 kindreds in this group. It comprises a total of 15 ataxic siblings, of which 10 have both ataxia and high bilirubin values. None of the asymptomatic siblings have been studied and the 4 parents tested have normal values. However, this association is far above a chance occurrence. In order to evaluate the approximate incidence of "high bilirubin" in the control population of our hospital, we studied the consecutive SMA 15/60 records of 382 patients (without known cardiac, hepatic or infectious pathologies) over a 2 day period. A total of 6 patients (1.6%) had total bilirubin values above 1.0 mg% and 22 above 0.8 mg% (6%). In either case, the incidence in Friedreich's ataxia (20 and 28% respectively) is significantly higher than in a control population (Kornberg, 1942). It is therefore permissible to ask whether the bilirubin abnormality is secondary to the disease "ataxia", or is a coincidental association of Gilbert's syndrome gene with the Friedreich's ataxia gene in the same families.

Gilbert's syndrome (or disease) is a frequent, benign finding (Gilbert and Lereboullet, 1907; Sicot, 1975; Foulk et al., 1959; Powell et al., 1967) of variable expressivity and generally transmitted as an autosomal dominant. It can be differentiated from congenital familial non hemolytic jaundice with kernicterus and its inherited deficiency of hepatic glucoronyl transferase (Crigler and Najjar, 1952), shunt hyperbilirubinemia (Acocella et al., 1965), portocaval-shunt surgery (Da Silva et al., 1960), and the mild hemolytic syndromes following viral hepatitis (Kalk, 1955). Transmission as an autosomal dominant does not seem to apply to all kindreds (Sicot, 1975). Our patients with ataxia and high bilirubin values meet all the criteria of idiopathic unconjugated hyperbilirubinemia listed above (see results) except the genetic pattern (which has not been properly investigated biochemically, although a clinical history of scleral icterus has not been obtained in any of the siblings or parents interviewed). We have not carried out

TABLE 3

DIFFERENCIATION OF HIGH AND LOW BILIRUBIN SUB-GROUPS

(GROUP Ia PATIENTS)

(mean ± S.D.)

	HIGH BILIRUBIN Sub-Group A		LOW BILIRUBIN Sub-Group B	
	RESULTS	NO. TESTED (max. 13)	RESULTS	NO. TESTED (max. 20)
1) Bilirubin (mg%)	1.18 ± 0.60	(13)	0.50 ± 0.14	(20)
2) Cholesterol	146.85 ± 26.70	(13)	184.35 ± 21.37	(20)
3) Triglycerides	68.75 ± 22.78	(12)	97.75 ± 37.93	(20)
4) Alk. Phsophatase (i.u.)	93.15 ± 61.97	(13)	91.70 ± 61.15	(20)
5) LDH (N: 105-225) (i.u.)	179.75 ± 52.50	(12)	223 ± 70.40	(20)
6) SGOT (N: 7-50) (i.u.)	33.69 ± 17.7	(13)	40.80 ± 11.48	(20)
7) SGPT (N: 10-30)(i.u.)	15.09 ± 13.92	(11)	26.0 ± 15.47	(10)
8) CPK (N: 0-125) (i.u.)	48.18 ± 26.33	(11)	69.80 ± 55.01	(10)
9) Aldolase (N: 0-8) (i.u.)	1.52 ± 0.71	(11)	2.44 ± 2.14	(10)
10) Fasting glucose (N < 110) (mg%)	98.54 ± 28.40	(13)	106.0 ± 47.88	(16)
11) Fasting insulin (uU/ml)	14.30 ± 6.97	(12)	10.61 ± 8.24	(15)
12) Danowski score (N < 500)	500.54 ±174.13	(11)	488.17 ±190.79	(12)
13) Total proteins (mg%)	7.11 ± 0.58	(13)	7.15 ± 0.50	(20)
14) Albumins (%)	61.99 ± 4.38	(11)	58.85 ± 3.49	(10)
15) Urea (N: 18-44) (mg%)	31.27 ± 6.44	(13)	25.59 ± 5.19	(17)
16) Uric acid (N: 25-80) (mg%)	5.76 ± 1.55	(13)	4.74 ± 1.69	(20)
17) Ceruloplasmin (N: 20-60) (mg%)	28.43 ± 8.76	(12)	30.04 ± 9.59	(20)
18) I.Q. score (N > 90)	97.0 ± 16.13	(10)	92.60 ± 13.44	(10)
19) Hemoglobin	13.81 ± 1.57	(13)	13.66 ± 0.90	(20)
20) Hematocrit	40.27 ± 3.59	(13)	40.28 ± 2.85	(20)
21) White blood count	6520 ± 1060	(13)	6074 ± 840	(20)
22) No. kindreds involved	7		13	

TABLE 4

TRANSFER RATE CONSTANTS IN GILBERT'S DISEASE AND NORMAL SUBJECTS (BILLING ET AL., 1964)

(MEAN ± S.D.)

CONSTANT	GILBERT'S DISEASE (N = 8)	NORMALS (N = 7)	P
a	0.0182 ± 0.0047	0.0352 ± 0.0102	< 0.001
b	0.0243 ± 0.0080	0.0147 ± 0.0063	< 0.02
m	0.0079 ± 0.0049	0.0217 ± 0.0180	< 0.01

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hepatic glucuronyl transferase determinations in our patients, or their parents and siblings. We are unable to decide whether the association of idiopathic unconjugated hyperbilirubinemia and ataxia is fortuitous, the result of the presence of two relatively rare genes, or secondary to the disease.

In most reported series of Gilbert's syndrome, the average levels of serum bilirubin (total) are higher than in our own patients. Thus, Kornberg (1942) found 1.3 to 3.25 mg% in 8 cases; Damashek and Singer (1941) from normal levels to 9.3 mg%. Powell and collaborators (1967) found a mean of 2.36 mg% (range 0.6 to 16 mg%) in a series of 116 cases, 81% of which had values lower than 3 mg% in initial testing. Our 13 patients averaged only 1.11 mg% (range 0.5 to 2.9 mg% on initial testing) which is significantly higher than subgroup B and the 382 control patients (mean $0.62 = 0.02$ mg%; range 0.2 to 1.7 mg%). Although not conclusive, this fact taken in conjunction with the preliminary genetic findings would lead us to postulate that our findings are somehow secondary to the ataxic disease, either as a sign of mild and perhaps specific hepatic damage, or as a manifestation of a transport defect across certain membranes.

In a thorough study of bilirubin metabolism, Billing et al. (1964) were able to delineate a number of interconnecting compartments (Figure 6). The first is in the plasma, the second the pool of unconjugated bilirubin present in the liver from which some reflux into the plasma occurs, and the third compartment is the pool of conjugated bilirubin into which it passes. A similar, but more complicated compartmental model has recently been proposed by Anwer and Gronwall (1976). An estimate of the rate of transfer of unconjugated bilirubin between these compartments can be obtained from the proportionality constants "a", "b" and "m" (expressed as mg transferred per mg content per minute), where:

"a" indicates the rate of uptake of bilirubin by the liver.

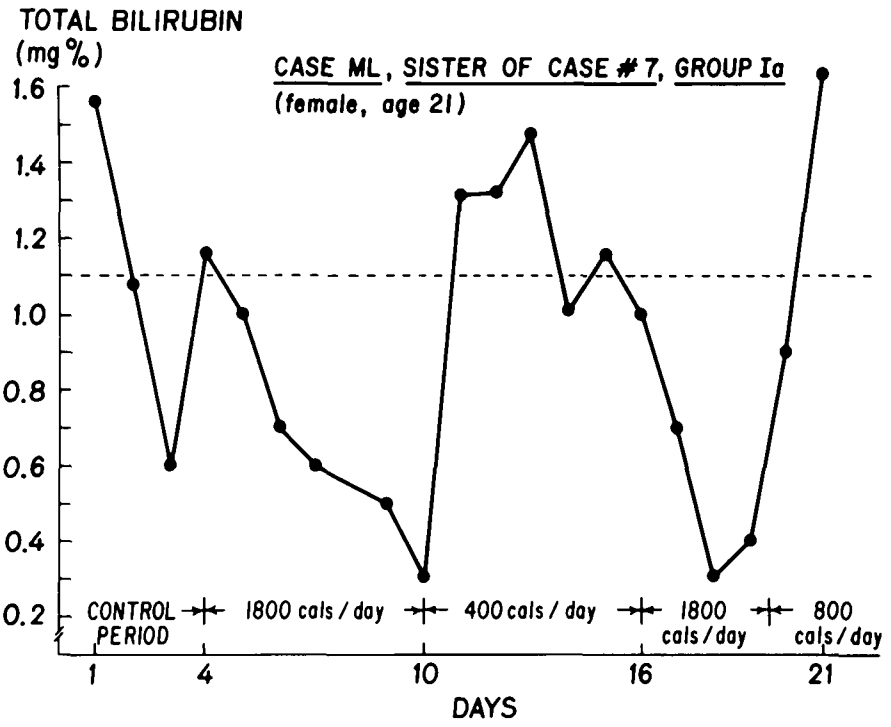

Figure 3—Similar variations in total bilirubin values in an ataxic sibling of the case depicted in Figure 2.

"b" estimates the rate at which the unconjugated bilirubin is returned to the plasma.

"m" represents the rate of transfer of unconjugated bilirubin in the liver to the conjugated bilirubin compartment.

These constants have been calculated for Gilbert's disease (Table 4). The transfer rate constant "a" was reduced below the lower limit of the normal range (mean± 2 S.D.) in all cases, In contrast to the normal subjects, the constant "b" was higher than "a" in 6 of the 8 patients and approximately equal in the other 2 patients. There was also an abnormal percentage retention at 4 hours in all patients (22-50%) as compared to the normal group (0-11%).

From these findings, Billing and her co-workers (1964) conclude that the significant fall in the rate constant "a" in Gilbert's disease indicates a possible defect in cell membrane in these patients which interferes with the normal hepatic uptake of bilirubin. The higher values of "b" may also indicate that the liver has a decreased capacity to hold the bilirubin removed from the plasma. Finally, the inconstant low values of "m" could indicate a diminished rate of conjugation in some cases. In summary, the evidence would favor a defect in hepatic uptake of bilirubin as the most important in Gilbert's syndrome, particularly in cases with total bilirubin values below 3.0 mg%, where glucuronide formation appears to be normal (Arias et al., 1962, 1969; Powell et al. (1967).

Defects in membrane uptake can be genetically determined, be specific or nonspecific, or be secondary to other metabolic alterations. It is possible that a similar mechanism is present in our ataxic patients. This aspect is presently being looked at in our laboratory.

One line of investigation initiated is a possible taurine deficiency in this disorder. It is known that taurine conjugates with bile acids in the liver of many animals (Jacobsen and Smith, 1968; Huxtable and Barbeau, 1976). This conjugation mechanism contributes to the intestinal absorption of lipids by affecting the processes of lipolysis, micelle formation and re-esterification of

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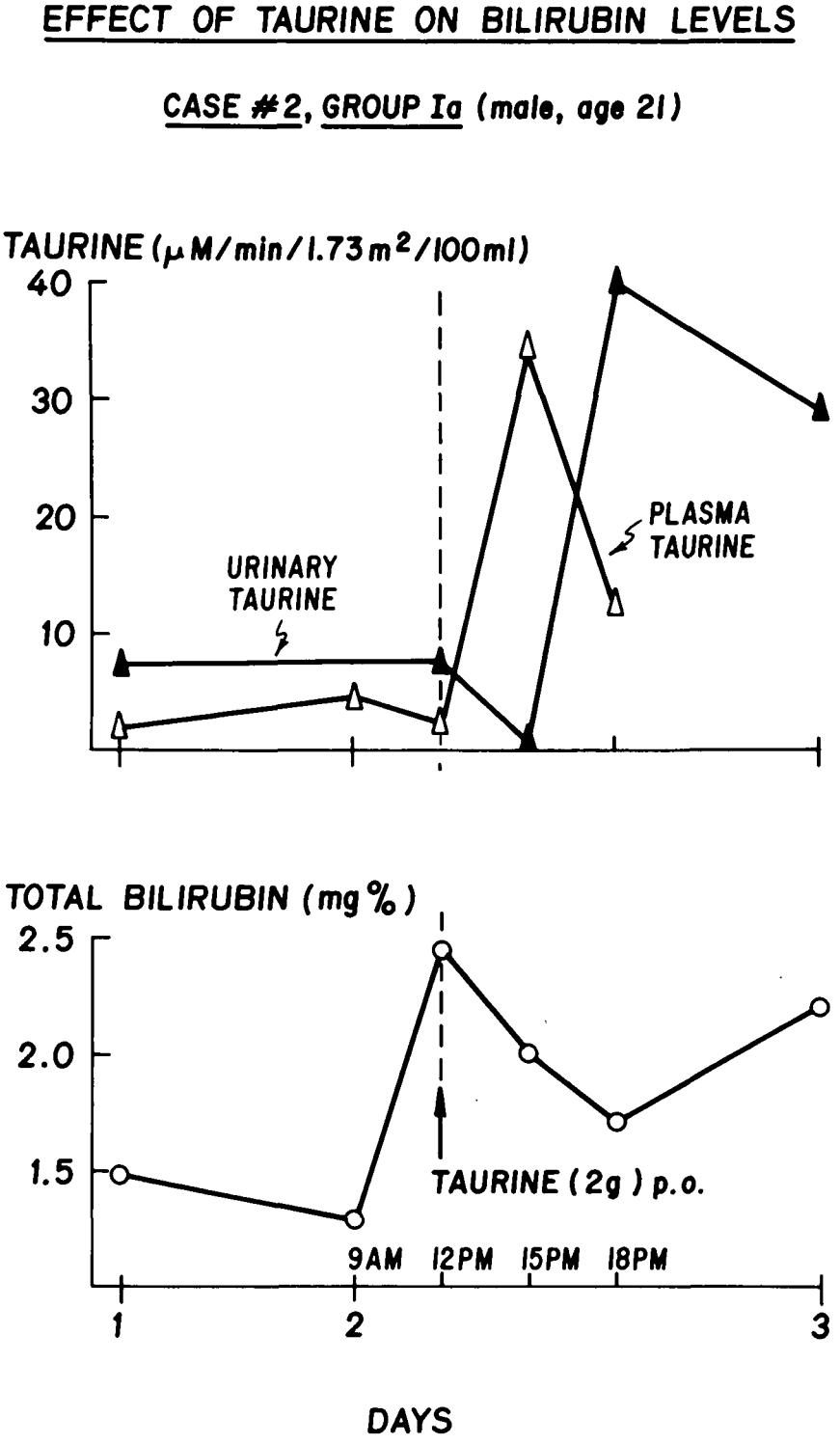

Figure 4—Modifications in plasma and urine taurine levels as well as plasma total bilirubin values after oral ingestion of 2 grams of taurine. Values for plasma taurine are in M/100 ml; for urine in M/min/1.73 m²/100 ml.

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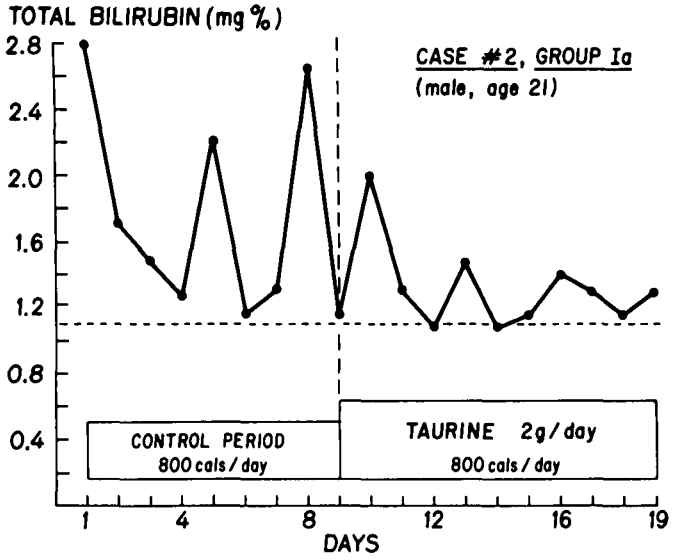

Figure 5—Trial of taurine supplementation (2 grams/day, orally) in a Friedreich's ataxia patient on a restricted caloric diet.

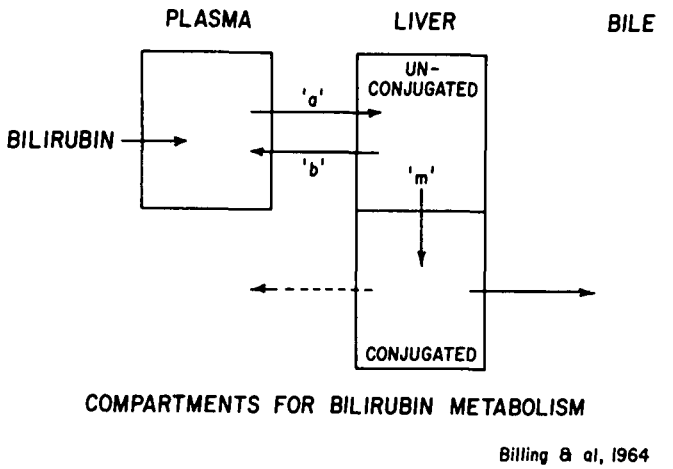

Figure 6—The different bilirubin pools in liver cells. See explanation in text. From Billing et al. (1964).

fatty acids within mucosal cells (Spaeth and Schneider, 1974). Infusion of taurine caused a significant increase in biliary excretion of both total cholesterol and congo red during the perfusion (Oshima and Fujihira, 1971; Fujihira et al., 1970).

Recently, K. Izumi in our laboratory (unpublished observations) has shown that taurine addition clearly modifies intracellular calcium binding, thereby changing the kinetics of many membrane transfer systems. If such a mechanism of action is confirmed for taurine, our preliminary findings (Figures 4 and 5) with the administration of taurine to ataxic patients could be explained. These investigations are being actively pursued in our department.

Animal models for unconjugated hyperbilirubinemias have been recognized. The first of these is a strain of Wistar rats called the Gunn rat where a defect in formation of conjugated bilirubin has been described. Rats with bilirubin levels over 12-15 mg/100 ml exhibited signs resembling kernicterus with ataxia. An associated defect in the kidney, causing polyuria and dehydration, may be due to interference by bilirubin with sodium and urea transport in the renal medulla. The primary defect in these animals seems to involve the glucuronyltransferase system of the liver microsomes.

Another autosomal recessive disorder characterized by congenital hyperbilirubinemia has been identified in Southdown mutant sheep. The disorder appears to be caused by a defect in hepatic uptake of organic anion with a similar defect possibly present in the renal excretory apparatus. These models may be worth detailed studies in relation to the pathogenetis of Friedreich's ataxia.

In conclusion, we have shown that some ataxic kindreds have an elevation in unconjugated bilirubin with features very similar to those reported in Gilbert's syndrome, except for lower mean values than in the latter condition. We postulate that this defect is likely to be a secondary component of the ataxic disease, possibly indicating a defect in membrane transport. Such a transport defect could be related to taurine metabolism. This hypothesis suggests a number of basic and clinical experiments which are being undertaken. Studies with animal models are also under way.

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