Quebec Cooperative Study of
Friedreich's Ataxia

Familial Hyperbilirubinemia in Friedreich's Ataxia

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SUMMARY: *The combined metabolic stresses of fasting and the intravenous injection of 50 mg nicotinic acid in Friedreich's ataxia resulted in the delineation of two sub-groups of responses. High bilirubin ataxics maintained abnormally elevated levels of bilirubin, while normal bilirubin ataxics behaved like the normal control group. It is postulated that this finding infers the possible linkage of the gene for Friedreich's ataxia and that for Gilbert's disease.*

RÉSUMÉ: *L'association des stress métaboliques causés par le jeûne et l'injection intraveineuse de 50 mg d'acide nicotinique dans l'ataxie de Friedreich a résulté en la délinéation de deux sous-groupes de réponses. Les ataxiques "à bilirubine élevée" ont montré un degré anormal de rétention de leur bilirubine, alors que les ataxiques "à bilirubine basse" se sont comportés comme les sujets contrôles normaux. Nous postulons que cette observation est le résultat d'un linkage possible entre le gène de l'ataxie de Friedreich et celui de la maladie de Gilbert.*

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INTRODUCTION

In the course of Phase One of this cooperative study, we discovered that a percentage (circa 30%) of our patients with typical Friedreich's ataxia had values of total and indirect-reacting serum bilirubin above normal (Barbeau et al., 1976). Further investigation revealed normal liver function tests and normal liver biopsies. Serum bilirubin responded to protein deprivation with an increase and values oscillated from day to day. These characteristics were those of familial nonhemolytic jaundice or constitutional hepatic dysfunction, or Gilbert's disease (Gilbert, 1900, 1901, 1902). The main question was the pertinence of this finding to the pathophysiology of Friedreich's ataxia. Gilbert's disease, usually asymptomatic, is not rare in any population and, according to some (Bailey and Robinson, 1977), may reflect the upper limit of the normal range of serum bilirubin concentrations. Since our survey was based on single blood determinations, it was important to ask whether, given the proper metabolic circumstances, all Friedreich's ataxia cases fall outside the usual normal range (0-1.0 mg % total bilirubin) or whether this characterized only a sub-group of patients. The metabolic stresses which we used were fasting and the injection of nicotinic acid according to the technique of Fromke and Miller (1972).

SUBJECTS AND METHODS

Ten patients with typical type Ia Friedreich's ataxia were chosen for the investigation. Seven of these patients were known to have had, on survey day, high total bilirubin values. The remaining 3 patients had bilirubin levels within usual normal limits. Eleven age-matched normal controls were chosen similarly for study. Two had to be eliminated due to active infectious (viral) pathology. All patients and controls gave informed consent to the studies. Blood was withdrawn using the indwelling "butterfly" technique. After withdrawal, each sample was immediately centrifuged and the direct-reacting bilirubin (1 min) and total serum bilirubin were determined according to the technique of Malloy and Evelyn (1937).

The nicotinic acid test was performed in accordance with the method of Fromke and Miller (1972): 50 mg of nicotinic acid were injected intravenously over a period of 30 seconds, and venous blood was withdrawn serially at 0′, 30′, 60′, 90′, 120′, 180′, 240′ and 300′ following injection. In all cases, tests were performed in the morning, with subjects fasting since the previous evening (a minimum of 15 hours).

The effects of nicotinic acid were minimal and without risk to the subjects, who described an internal pricking and burning sensation, with a feeling of warmth followed or accompanied by flushing, mainly of the face and neck, but occasionally of the whole body. These effects disappeared within 5 minutes of injection in all cases. The administration of nicotinic acid had no effect on blood pressure in our subjects.

RESULTS

(A) *Baseline serum bilirubin:* As shown in Table 1 the sub-group of patients previously classified as high bilirubin ataxics were found to have an elevated total serum bilirubin on the morning of the test, after a 15 hour fast. This elevation was statistically significant when compared to the normal bilirubin ataxics or to the

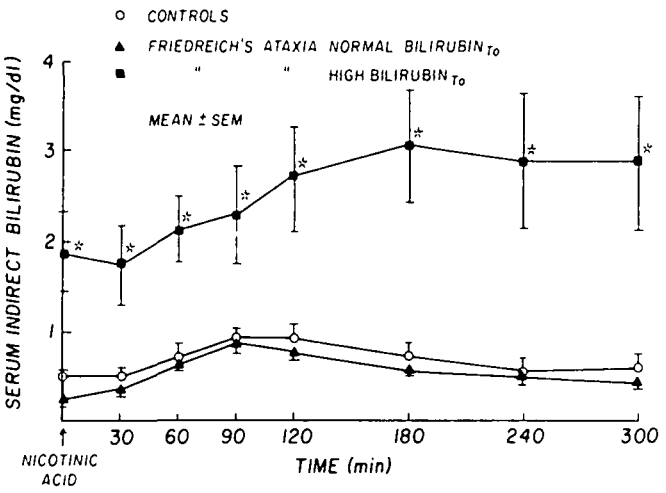

Figure 1—Response of serum indirect-reacting bilirubin to the i.v. injection of 50 mg nicotinic acid in two sub-groups of Friedreich's ataxia and in normal control subjects.

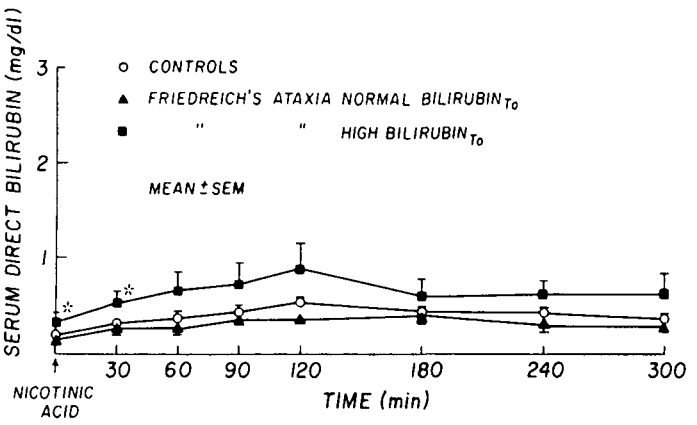

Figure 2—Response of serum direct-reacting bilirubin to the i.v. injection of 50 mg nicotinic acid in two sub-groups of Friedreich's ataxia and in normal control subjects.

normal control group. This relationship held for direct and indirect-reacting bilirubin when studied separately.

(B) Serum bilirubin during nicotinic acid/fasting test

The bilirubin response to nicotinic acid (50 mg i.v.) in fasting subjects is illustrated in Figures 1 and 2. Both total (not shown) and indirect-reacting bilirubin (Figure 1) remained significantly higher in the high bilirubin ataxics than in either the normal control group or the normal bilirubin ataxics at all times up to 300 min after the injection. Direct-reacting bilirubin (Figure 2) was different only during the first 30 minutes after the injection of nicotinic acid.

The most striking behaviour occurred in the high bilirubin ataxics whose indirect-reacting bilirubin failed to return to initial (T_0) values after 5 hours, a phenomen observed in both other groups. Such "retention" of bilirubin can be quantitated as follows:

$$\% \text{ Retention} = \left(\frac{C_{300'} - C_{0'}}{C_{120'} - C_{0'}} \right) \times 100$$

where C_t = concentration (mg/100ml) of indirect-reacting bilirubin at time t min after injection, and t120′ is the normal peak.

This coefficient of retention has been measured for each experimental subject. As shown in Fig. 3, The percentage retention in the high bilirubin ataxics was found to be 114.4 ± 19.4%, a figure significantly higher than in the normal control group (23.9 ± 11.4%) or in the normal bilirubin ataxics (24.3 ± 14.3%). The range of values, as indicated on the histogram, was markedly different between the groups, with only partial overlap.

TABLE 1 BASELINE BILIRUBIN VALUES (To)

(mg % ± S.E.M.)

GROUP	N	TOTAL BILIRUBIN	INDIRECT-REACTING BILIRUBIN	DIRECT-REACTING BILIRUBIN
Normal controls	9	0.65 ± 0.07	0.49 ± 0.06	0.16 ± 0.01
"Normal bilirubin" ataxic patients	3	0.38 ± 0.10	0.25 ± 0.07	0.13 ± 0.02
"High bilirubin" ataxic patients	7	2.17 ± 0.46*	1.85 ± 0.42*	0.32 ± 0.05*

* $p < 0.01$ when compared to other 2 groups

DISCUSSION

A heterogeneous group of patients with chronic acholuric jaundice occurring in families was first described by Gilbert et al. (1900, 1901, 1902). The condition, generally asymptomatic and occurring in young adults, is characterized by low-grade variable jaundice, but is accompanied by normal liver architecture and hepatic function. Biochemically, the disease is marked by an elevation of unconjugated (indirect-reacting) bilirubin. It has been referred to as familial nonhemolytic jaundice (Dameshek and Singer, 1969), congenital hyperbilirubinemia (Billing et al., 1964) and constitutional hepatic dysfunction or Gilbert's disease (Fromke and Miller, 1972). The low grade, mainly unconjugated hyperbilirubinemia never reaches the level

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seen in chronic non hemolytic unconjugated hyperbilirubinemia (5-20 mg/100 ml) or in the Crigler-Najjar syndrome (17-43 mg/100 ml) (Arias et al., 1969). Many factors, such as fatique, alcohol, intercurrent infections, stress, may produce changes in total bilirubin levels. In addition, a recent article by Bailey and Robinson (1977) has suggested that some cases of Gilbert's disease may constitute the upper end of the normal range, rather than a disease state. They found that serum bilirubin exhibits a skewed distribution. In a screening of 19,000 healthy individuals, 2% of men and 0.6% of women had serum total bilirubin in excess of 1.5mg/100 ml. However, it should be noted that these individuals were not screened for previous history of jaundice or severe viral infections. Finally, is should be said that most authors agree that Gilbert's disease is genetically determined, usually transmitted as an autosomal dominant trait (Smith et al., 1967).

In this study of Friedreich's ataxia, we found that 7 of 20 kindreds had total bilirubin levels in excess of 1.0 mg/100 ml (Barbeau et al., 1976). This 35% prevalence of the biochemical defect is significantly different from the prevalence in a healthy population mentioned above and in our own control group of 382 hospitalized patients without cardiac, hepatic or infectious pathologies. In the latter group 6 patients (1.6%) had total bilirubin values above 1.0 mg/100 ml. No report of such an association could be found in a search of the literature.

This finding raises important questions: is bilirubin metabolism abnormal in all Friedreich's ataxia patients if the proper metabolic conditions are presented in a standardized way, or do we have two biochemically distinct sub-groups of phenotypically similar ataxics? Could this association be limited to the genetically fairly homogeneous population of French Canada and in fact be a chance meeting of Gilbert's disease gene and the Friedreich's ataxia gene in a population where both genes are frequent? If the observation is not limited to French Canada, are we dealing with genetic linkage or a secondary biochemical manifestation of Friedreich's ataxia?

After publication of our initial observation (Barbeau et al., 1976), we were informed by Dr. Pieter Kark of Los Angeles, that he had found elevated total bilirubin levels in Friedreich's ataxia patients. We also found (see elsewhere in this issue) that similar observations could be made in recessive spastic ataxia (Charlevoix disease), but not in an autosomal dominant olivo-pontocerebellar ataxia (30 cases studied). Thus, the phenomenom observed appears not to be confined to French Canada. An international study team is presently looking into the occurrence of the association in other countries.

In 1954, With noted that diurnal variations of serum bilirubin were associated with increased food intake and fasting in normal individuals. Felsher et al. (1970) have shown

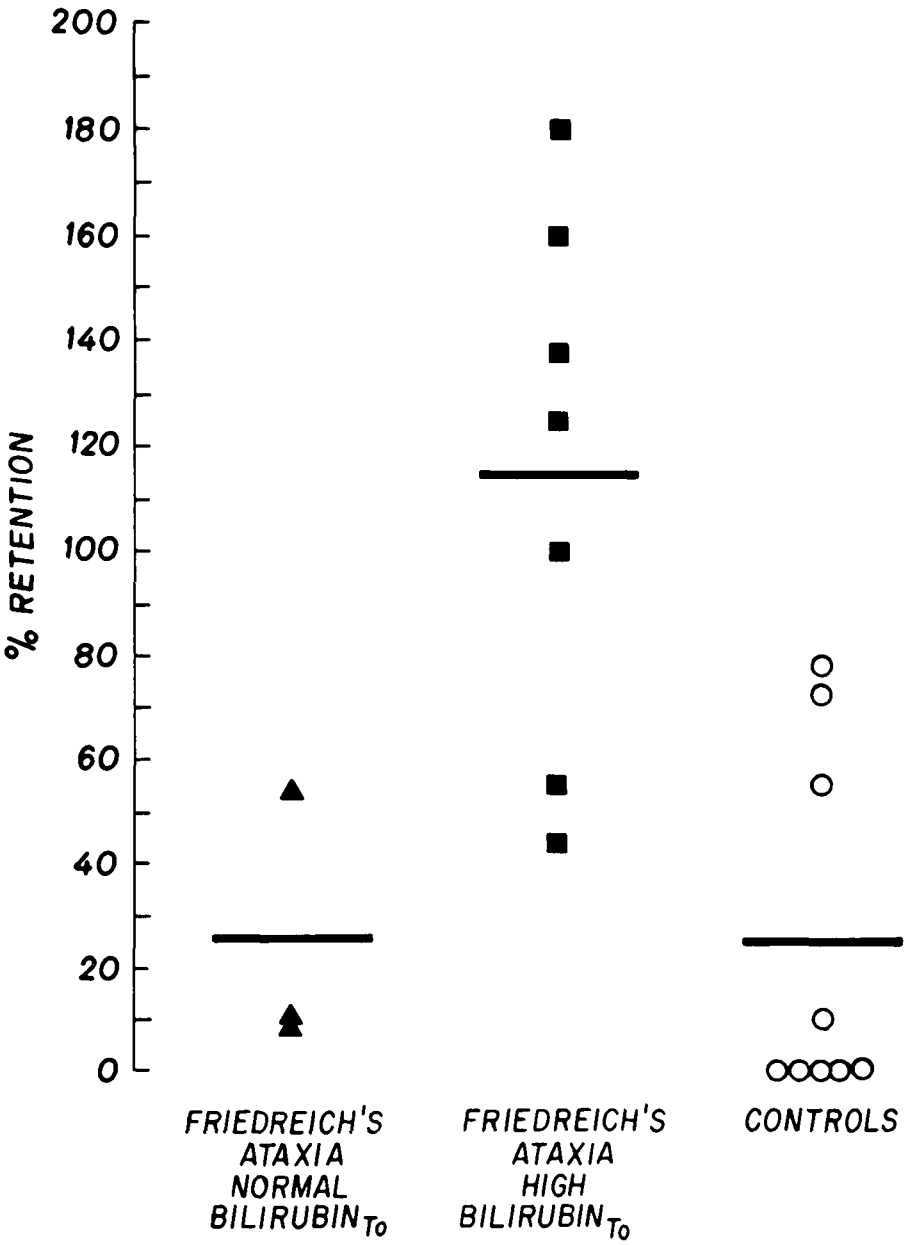

Figure 3—Percentage retention of indirect-reacting bilirubin from 120 min to 300 min after the i.v. injection of 50 mg of nicotinic acid in two sub-groups of Friedreich's ataxia and in normal control subjects.

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an inverse relationship between caloric intake and level of hyperbilirubinemia in seven patients with Gilbert's disease. In our previous study (Barbeau et al., 1976), we had shown that some Friedreich's ataxia patients behaved similarly during caloric restriction, with an increase in total bilirubin, often to abnormal levels. Administration of nicotinic acid has repeatedly been shown (Stefanini, 1949; Gedell, 1959; Fromke and Miller, 1972) to elevate total and indirect reacting bilirubin. This elevation reaches a peak between 90 and 120 minutes after the injection, with a two-fold increase in normal controls and a three-fold increase in Gilbert's disease (Fromke & Miller, 1972). In the latter, as opposed to normal controls, the values do not return to pre-test levels at the end of 5 hours, but remain elevated.

In the present study, 7 patients previously known to be from high bilirubin ataxic families, behaved exactly like patients with Gilbert's disease on nicotinic acid stimulation in a fasting state, while 3 Friedreich's screened as from normal bilirubin ataxic families behaved like the controls (Figs 1-2). Thus, we can state with stronger confidence that we are dealing with two sub-groups of Friedreich's ataxia patients, regarding their bilirubin metabolism. The difference is due not only to their initial value variation, since the two lines lose their parallelism after the peak and the high bilirubin group fails to return to baseline. This indicates a significant degree of retention of the bilirubin, probably in the liver of these patients (Fig. 3). Although the hyperbilirubinemia effect of nicotinic acid is not clearly understood, there is reason to believe that conversion of heme to excess free bilirubin is stimulated by nicotinic acid, mainly in the spleen (Fromke & Miller, 1972).

This retention in these patients could be due to a defect in the transport of bilirubin between liver compartments (Billing, 1964) secondary to a membrane defect or to still undetermined liver disease. Finally, we are unable to state whether our findings correspond to a secondary, but not obligatory, biochemical manifestation of the primary Friedreich's ataxia gene, or to the linkage of two independent diseases possibly on the same chromosome. The fact that, even under metabolic stress, not all Friedreich's patients manifest the trait, and the knowledge that Friedreich's ataxia is transmitted as an autosomal recessive while Gilbert's disease is usually autosomal dominant, would tend to favor the latter hypothesis.

ACKNOWLEDGMENTS

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