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# Congenital familial nonhemolytic jaundice (CFNJ)

# CONGENITAL FAMILIAL NONHEMOLYTIC JAUNDICE

# (CFNJ)

by

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## INTRODUCTION

Since 1952, when Crigler and Najjar reported seven patients with "Congenital Familial Nonhemolytic Jaundice (CFNJ) with Kernicterus"[1], not described in previous literature, with the possible exception of Micheli and Dominici's patients reported in 1932[2], there has been a continued interest in this disease entity, for its contribution toward the understanding of inborn errors of metabolism, the metabolism of bilirubin, and the pathogenesis of kernicterus. To date, there are only twenty cases notable from the literature, and although much has been done toward clarifying the etiology of this disease, not all aspects of its pathogenesis are understood. In this paper, I shall attempt to summarize the present status regarding the Crigler-Najjar syndrome, CFNJ, and its relationship to bilirubin metabolism.

## CLINICAL DESCRIPTION

Crigler and Najjar classically described seven patients with jaundice which appeared within the first three days after birth and persisted throughout life[1]. The onset of jaundice was followed by a short period without any other signs or symptoms. Then, nonspecific symptoms of anorexia, irritability or a mild infection set in. These were followed by symptoms of central nervous system disturbance (excepting one patient), which progressed till death, from two or three weeks up to slightly over one year thereafter.

As is common experience when a new disease is described that patients involved show considerable homogeneity of signs and symptoms, subsequent reports show variations of the original theme. The composite clinical findings are summarized in the following.

Jaundice, consistent with the original description of the disease, has been found in all patients, and in all clearly recorded incidences, was noted in the first few days of life. Total serum bilirubin levels were about 15-25 mg/100ml higher than that seen in "Gilbert's disease", with a range of 12.6 to 50.4. This hyperbilirubinemia in CFNJ patients persisted throughout their lifetime.

There are no other signs or symptoms, except jaundice, for a variable period of time, prior to the development of the manifestation of central nervous system disturbance. In three cases, central nervous disturbances were manifested within the first week of life.[1,3] In the majority of clearly recorded cases, signs and symptoms of kernicterus set in within the first year after birth. These ranged from nonspecific symptoms of

irritability, fever, anorexia, vomiting, sleeplessness to signs of distinct central nervous system disturbance: progressive spasticity, rigidity of the extrapyramidal type, opisthotonoid position, slurring of speech, intention tremor and even convulsions.[1,4,5] Typically, the hands and arms showed frequent involuntary movements of the choreo-athetoid type, slow movements predominating in the fingers and rapid ones in the proximal segments of the arms.[6] The face might become immobile or the site of extensive uncoordinated activity. Occasionally, slow writhing movements of the trunk were observed.[1] These neurological signs can be slowly or rapidly progressive to death.[1,3] In the case of one patient, signs of central nervous system disturbance did not appear until the age of three years[4] and the child died at the age of eleven and a half years, a few days after surgery for diaphragmatic hernia, unrelated to his underlying disease.[7] His neurological status, however, was noted to have deterioted post-operatively.[7] Among the twenty patients known, at this writing, to be afflicted with this disease,[1,2,3,4,5,6,8,9,10] six survived beyond late childhood (five years),[6,8,9,11,12] and three have not yet developed any signs or symptoms of kernicterus.[11,12] (See Table I) The ultimate prognosis of these three patients, however, must be highly guarded.

# TABLE I

| | Patient & Reference | Familial Involvement | Sex | Jaundice (Age Noted) | CNS Disturbance (Age Noted) | Age at Present | Age at Death | Serum Bilirubin Total (mg/100 ml) | Serum Bilirubin Indirect | Urine Bile | Urine Urobilinogen | Feces Bile | Feces Urobilinogen | Hgb (gm/100ml) / HCT % | RbcFragil in Saline | Reticulocyte % | Peripheral Smear | BSP Retention | CF | TT | Protime | Alk. P'tase (Bodansky U.) | Source of Histolog. Specimen | Liver Histology Bile Thrombi in c. Canaliculi | Periport. Fibrosis (slight) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | LM[1] 5½ | X | F | 2 days | 4 wks | | 21 wks approx. | 27.6-37.2 | 33.0 | 0 | N | X | N | 14.5 | N | 1.0 | N | 7%@30min | Neg. | 4.2 | 100% | 10 | Biopsy | X | X |
| 2. | JRH[1] " | X | M | 2 days | - | | 19days | 25.8 | 23.8 | 0 | - | X | - | 14.5 | - | - | N | - | Neg. | 2.7 | 50% | - | Autopsy | X | 0 |
| 3. | JDH[1] " | X | M | 3 days | None[12] | 12½yrs[12] | | 17.2-27.4 | 27.4 | 0 | 0[15] N | X | Decr[15] N | 16.5 / 52 | N | 0 | N | 0.3 mg/100ml@30m | Neg. | 0.8 | 100% | 20.6 | Biopsy | X | 0 |
| 4. | XXH[1] " | X | M | X | - | | 26days | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 5. | JLT[1] " | X | M | 2 days | 9 wks approx. | | 69 wks approx. | 19.0-34.0 | 32.0 | 0 | N | X | N. | 12.5 / 31 | N | 0.8 | N | 0.3 mg/100ml@30m | Neg. | 1.3 | 100% | 14.2 | Biopsy | X | 0 |
| 6. | LT[1] " | X | M | 2 days | 17 days | | 29 wks | 12.2-44.8 | 44.8 | 0 | N | X | N. | 15.0 / 51 | - | 0.1 | N | 0.1 mg/100ml@30m | Neg. | 1.4 | - | 16.4 | Biopsy | X | X |
| 7. | JJT[1] " | X | M | at birth | shortly postnatal | | 47 wks | icteric index 50 | X | 0 | N | X | N | 9.0 | N | - | N | - | - | - | - | - | Autopsy | X | X |
| 8. | MEH[8] 5½ | X | F | 1 day | None[12] | 9yrs.[12] | | 25.0 | 23.4 | 0 | 0 | - | - | 8 / 29 | - | 1.0 | N** | - | Neg. | 2.8 | - | 13.6 | - | - | - |
| 9. | JD[4] " | X | M | 2 days | 3 yrs | | 11½yrs[7] | 20.3-35.9 | 19.9-33.9 | 0 | 0[15] N | - | Decr[15] | 11.8-12.4 / 35 | N | 1.0 | N** | 6%@45min | Neg. | 3.8 | 15.5sec/13.0sec | 28±3.6 | Laparotomy | 0 | 0 |
| 10. | R[4] " | X | - | X | X | | 4 wks | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 11. | J[6] 5½ | ? | F | before ½ yr | before ½ yr | | 44 yrs | 16.0-22 | 0.2-0.4 (Direct) | 0 | - | - | - | 12.4-14 | N | 1.0 | N | N | Neg. | N | - | N | Autopsy | 0 | 0 |
| 12. | K[2] " | - | - | X | - | | | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 13. | WJH[10] 4½ | X | M | 3 days | X | | 1 yr | 15.0-35.0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | Autopsy | 0 | 0 |
| 14. | JH[10] " | X | F | 3 days | X | | 1 yr | 29.0 | 29.0[15] | 0[15] | 0[15] | - | Decr[15] | - | - | - | - | - | - | - | - | - | - | 0 | 0 |
| 15. | RS[9] " | - | F | 1 day | - | | 13½yrs[5,7,8] | 16 - 20 | - | - | - | - | 16.9 | - | - | - | - | - | - | - | - | - | Laparotomy | 0 | 0 |
| 16. | Jd'E[11] | * | M | 3 days | None[11] | 6yrs.[11] | | 15-23 | 05-0.67 (Direct) | 0 | 0 | - | Decr | 9-11.5 / 34 | N | 0 | N | 2.5%@45 min. | - | - | 17sec/14sec | - | - | - | - |
| 17. | W[5] " | 0 | F | 14 days | 4 wks | | | 14.0-25.7 | 13.8-23.4 | 0 | 0 | Brown | 5.0 | 9.5-13.0 / 40 | N | 0.5-1.0 | - | - | Neg. | - | - | 3.9-5.0 | Laparotomy | 0 | 0 |
| 18. | RMB[3] | X | M | 2 days | 6 days | | 14days | 50.4 | 48.8 | X | - | Yellow-Orange | | 19.7 | - | 0.3 | N | - | - | - | - | - | Autopsy | - | - |
| 19. | RPB[3] | X | M | X | 6 days | | 11mons. | 25-34.4 | 32.8 | 0 | - | - | - | 13.0 | - | - | N | - | - | - | - | - | Autopsy | - | - |
| 20. | TJB[3] | X | F | before 3 days | about 1 yr | | after 1 yr | 13-27.8 | 13-26.5 | 0 | - | - | - | 9-18.6 | - | 1.1-8 | N | - | - | - | - | - | - | - | - |

\- Data not available
X Positive evidence
? Paternal identity uncertain
* Father's brother has "Gilbert's Disease"
** Slight anisocytosis and moderate hypochromia
Source of data is noted under "Patients & Reference", unless otherwise noted.

## LABORATORY FINDINGS

There has been no evidence of blood group incompatibility, except in one case where a mild degree of ABO erythroblastosis was noted.[3] Neither was there any evidence of hemolytic anemia, as shown by normal findings in saline osmotic fragility test, erythrocyte survival time, peripheral smear, hemoglobin, hematocrit, bone marrow morphology and absence of splenomegaly. Low reticulocyte count has been reported uniformly, except in one patient, when reticulocyte count was 8% on one determination, but on subsequent determinations was found to be normal.[3]

Classical liver function tests, including bromsulphalein excretion, cephalin flocculation, thymol turbidity, and alkaline phosphatase, were all within normal limits. Although renal hippuric acid excretion was noted as subnormal after oral sodium benzoate ingestion in one patient.[4] The results of this test were subsequently found to be within normal limits following intravenous sodium benzoate administration.[9]

Normal unobstructed extrahepatic biliary tract was also demonstrated by intravenous cholangiograms in two cases[4,9] and by operative cholangiogram in a third patient.[5] Further evidence for nonobstructive jaundice was obtained by findings of patent biliary tree at laparotomy in the same patients.[4,5,9]

There was no bilirubin in the urine, with one exception.[3] Urine color was variously noted to be normal, light amber, to golden yellow,[1,4,5,6,8,9] and in four cases the color was noted to be significantly different from that of normal urine.[9] The nature of the pigments causing this abnormal coloration was unknown, although they were noted to show similarities to those present in plasma and urine of hepatectomized dogs.[9] It has been noted that nonbilirubin pigments in plasma obtained under conditions of

sustained jaundice, might represent dipyrrol end products of bilirubin degeneration in the tissues.[13]

Stool examination revealed presence of bile and normal values for fecal urobilin excretion, according to Crigler and Najjar's original report on this disease.[1] These results were obtained, however, by a method[14] not in common use, as noted by Schmid.[9] Later determinations for fecal urobilinogen excretion found it to be very low, ranging from 0.23 mg/24 hours (two years old),[9] to 5 mg/24 hours (two years old[5] and seven years old[9]), in spite of serum bilirubin concentrations greater than 20 mg/100ml in all cases. Furthermore, in one child, J.D.H.. reported by Crigler and Najjar as having normal urobilin excretion,[1] fecal urobilinogen excretion was subsequently found to be 1.4 mg/24 hours.[9] At the present time, one cannot say whether this represents a definite reduction in fecal urobilinogen excretion, because there are not enough data on normal fecal pigment excretion in the age group under consideration.[9] Unlike patients with complete biliary obstruction, stool color was normal, ranging from brown to yellow orange.[3,5,15]

Bile from the gall bladder and duodenum was studied in six cases. Direct and indirect reacting bilirubin were found in varying proportions. In three cases, when duodenal bile was obtained before and after stimulation with Decholin or magnesium sulfate, the specimens had a faint lemon juice color and total bilirubin ranged from 0.1-1.5 mg/100ml, with essentially all of the pigment in the indirect reacting form.[9,15] In the fourth case, R.S., the duodenal bile, obtained similarly, contained 4 mg/100ml of total bilirubin, with about one half in the direct reacting form.[9] Bile collected directly from the gall bladder at laparotomy on the same patient contained 70 mg/100ml of total bilirubin, with about one third in the form of glucuronide. In a

fifth case, W., where bile was also obtained from the gall bladder at laparotomy, the color was noted to be dark green, containing 18 mg/100ml of direct reacting bilirubin by the van den Bergh reaction and 35 mg/100ml of total bilirubin.[5] Lastly, bile aspirated in a sixth patient, J., contained virtually only direct reacting bilirubin.[16]

As mentioned earlier, the total serum bilirubin ranged from 12.6-50.4 mg/100ml. The increase of total serum bilirubin was due mainly to the elevation in the indirect reacting type. Direct serum bilirubin was usually not present or negligible, never reaching concentration above 2 mg/100ml.[1] Paper chromatographic studies of the azo derivatives of serum bilirubin on three patients, J.D., J.D.H., J.H"., showed presence of non-conjugated bilirubin only, without any evidence of bilirubin gluduronide.[15] Bilirubin retention was measured spectrophotometrically, by intravenous infusion of 5 mg/kg body weight of commercial bilirubin (crystalline form) dissolved in 0.1 M sterile sodium carbonate.[17] In two patients, J.D.H., L.T., bilirubin retention was 40 and 26% at fifteen weeks of age, while a normal control infant of the same age showed 5% retention.[1]

Studies on the chemical nature of the bile pigments in sera of Crigler-Najjar patients include isolation and crystallization of the pigment. The crystalline bilirubin so isolated had an absorption spectrum same as that of indirect reacting bilirubin from patients with other types of jaundice and moved with the albumin fraction as seen on paper electrophoresis (pH 8.6).[1,4]

In order to determine if the hyperbilirubinemia might be due to an abnormal form of bilirubin, which could not be adequately excreted by the liver, one patient's (J.D.) plasma was infused into a non-jaundiced recipient. Determinations of bilirubin concentration in serum were done on the recipient at intervals after infusion. Within two and a half hours after infusion, the

recipient's serum bilirubin concentration had returned to the pre-infusion value.[4]

## HISTOLOGICAL FINDINGS

Out of the twenty cases of known CFNJ patients, liver tissue was histologically evaluated in fourteen cases; reports of findings on the brain and other organs were available in only three cases out of the seven autopsies performed. There were no significant abnormalities noted in the liver and the pathological findings were primarily limited to the brain.

Microscopic examination of liver tissue from Crigler and Najjar's original patients showed no obvious histologic changes.[1] The only abnormal finding was the presence of bile thrombi in the hepatic canaliculi, and it was noted that the degree of this abnormality varied and was not related to the duration or degree of jaundice. In one child, J.D.H., bile thrombi were also noted in the hepatic ducts and in three other children, L.M., L.T., J.J.T., slight periportal fibrosis was observed in some areas, although the fibrosis was far from being diffuse.[1] Subsequently microscopic evaluations of liver tissue, either by biopsy or at autopsy, were uniformly reported as normal in appearance.[4,6,5,9]

The major pathology in patients with this disease was found in the brain and considered to be consistent with the diagnosis of kernicterus.[18] The brains examined (J.R.H., J., R.M.B.) were stained markedly yellow. Pathologic lesions, varying from fat droplets in the nerve cell to actual loss of nerve cell, were seen in the basal ganglia, subthalamic nuclei, cerebellum, hippocampus, quadrigeminal bodies, and cerebral cortex.[1,3,6]

## REVIEW OF BILIRUBIN METABOLISM

In order to facilitate the understanding and interpretation of the defect and pathogenesis in the CFNJ patients, I shall try to summarize the physiology of bilirubin metabolism, emphasizing some of the recent developments and the phases which are most pertinent to the CFNJ patient.

### I. Sources of Bilirubin :

The principal source of bilirubin is derived from the normal daily destruction of red cells with concomitant degradation of about one per cent of the total hemoglobin mass per day. It is estimated that this accounts for 70-90% of the bilirubin formed.[19,20] In adults hemoglobin is transported in the plasma in the form of a complex with haptoglobins, proteins which have a specifin affinity for hemoglobin.[21] In most newborns, however, it is found that this transport mechanism is lacking.[21]

The precise sequence of events involved in the degradation of hemoglobin is uncertain. The pathway for hemoglobin degradation which is more widely accepted today involves the oxidative removal of the carbon atom in the alpha-methene bridge which opens the porphyrin ring to yield choleglobin. By subsequent removal of iron and globin, choleglobin is converted to biliverdin which, on reduction of its central methene bond, gives bilirubin.[22,23] That this process of red cell degradation to give bilirubin occurs in the reticulo-endothelial system is still generally held to be true, although it was thought that hemoglobin degradation might begin in intact senescent red cells before they were taken up and destroyed by the reticulo-endothelial system.[23] More recently, experiments using labelled red blood cell, hemoglobin in rats, suggested that the metabolic disposition of intracorpuscular hemoglobin and of unbound plasma hemoglobin may differ from that of

the hemoglobin-haptoglobin complex.[24]

Assuming that there is a quantitative conversion of hemoglobin to bilirubin, one gram of the former yields approximately 34 mgms. of the latter. In a three kg. infant, with 300 ml blood volume, total hemoglobin mass of 54 g, 0.5g hemoglobin and 17 mg bilirubin is destroyed daily.[25] In the light of this continuous breakdown of hemoglobin, serum bilirubin is expected to accumulate continuously, if it were not excreted. The serum bilirubin level in the CFNJ patients, however, is maintained at a steady, though elevated level.

Recent studies infusing labelled $C^{14}$ and $Fe^{59}$ erythrocytes, hemoglobin and bilirubin into rats (Sprague-Dawley) with bile fistula and measuring labelled bilirubin in the bile and urine, showed that 20-45% of the administered heme-$C^{14}$ was not recovered as bilirubin-$C^{14}$.[24] Urinary loss of heme pigment never exceeded 4% and although tissue retention was not ruled out, the excretion of unlabelled bilirubin, derived from endogenous erythrocytes, continued unabated. These observations, in addition to the finding that injected bilirubin-$C^{14}$ led to 90% recovery in the bile, would seem to favor the contention that there are alternate pathways which convert heme-$C^{14}$ to excretory products other than bilirubin.[24]

Dipyrrolic compounds, such as mesobilifuscin and propentdyopent, thought earlier to be normally occurring excretory product of heme metabolism,[9] were considered to be unlikely as the non-bilirubin excretory products postulated above,[24] and their identity remained unestablished. That the intravenously injected bilirubin-$C^{14}$ was 90% recovered in the bile would indicate that the postulated non- bilirubin excretory products may only be

derived from heme or perhaps from bilirubin within the reticulo-endothelial system, but cannot be formed from bilirubin in the circulation.[24]

One would, however, be interested to know the amount of fecal bilirubin loss in the above experiments, which were not reported, especially in the light of some recent findings on the transfer of conjugated and unconjugated bilirubin across the intestinal lumen and subsequent excretion in the stool.[26,27]

There are other precursors of bilirubin, among which these is methemalbumin, formed by attachment of the prosthetic heme group of hemoglobin to albumin.[22, 28,29,30] Since this pigment binds albumin, it can compete with bilirubin for binding sites and drive the latter into tissues, thus increasing the likelihood of kernicterus.[25] This will be discussed in greater detail later.

Several sources of bilirubin other than the hemoglobin of circulating erythrocytes have been suggested by London[19] and Gray[20] and their associates which include: 1) heme formed in excess of globin during hemoglobin synthesis; 2) intracorpuscular degradation of hemoglobin during the maturation of the early erythrocyte in bone marrow; 3) destruction of newly formed erythrocytes in the bone marrow before they reach the circulation; 4) direct synthesis from a metabolic pool of pyrroles; 5) other heme proteins such as myoglobin, catalase, and the cytochromes. In vitro studies demonstrated formation of bile pigments on coupled oxidation of ascorbic acid and myoglobin, catalase, and peroxidase, although not with cytochrome- c.[31]

II. Transport of Bilirubin in Plasma:

The exact mechanism by which bilirubin is released from the reticulo-endothelial system into the plasma is not clear. The pigment is virtually insoluble in water or blood;[32] and must be carried in plasma by attachment to

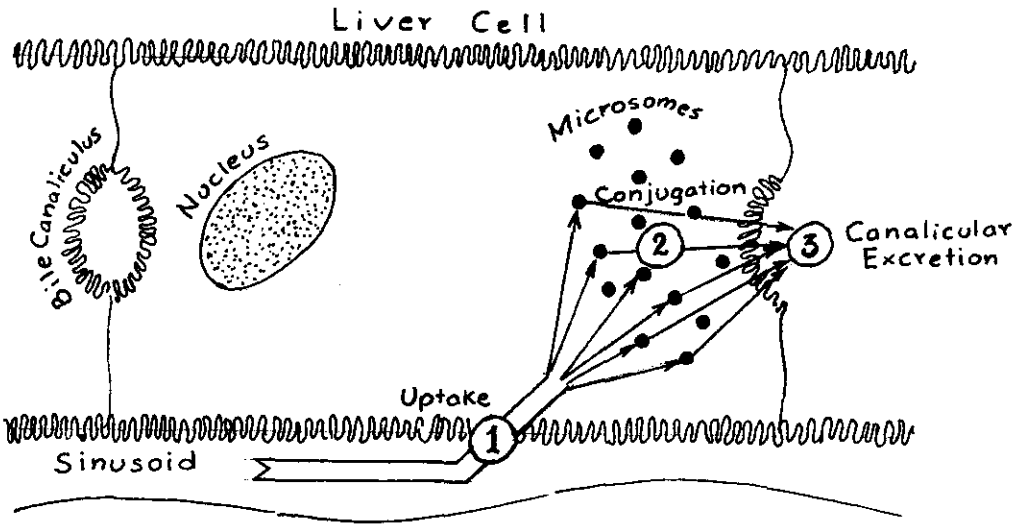
proteins. It is generally agreed that in jaundiced serums most of the bilirubin is bound to albumin,[33] forming a relatively stable complex.[34] Albumin seems to bind bilirubin preferentially in plasma,[39] but other proteins are capable of binding bilirubin.[34] Among these is the alpha$_1$-globulin of fraction V-1,[35] estimated to constitute less than 0.1% of all plasma proteins and regarded as the most stable specific bilirubin-binding plasma protein.[36]

The linkage between bilirubin and albumin is considered sufficiently stable to prevent bilirubin from passing through semipermeable membrane at normal conditions.[37] The binding can be uncoupled by certain organic anions,[38] and by lowering the serum pH below 5.0.[39]

Most of the studies on protein binding of bilirubin in serum were done on icteric serum or with bilirubin added to serum.[9] That under these conditions bilirubin is preferentially bound to albumin does not necessarily indicate that albumin is also the principal carrier of bilirubin under physiologic conditions of much lower serum bilirubin content.[9] Normally the serum bilirubin concentration lies between 0.5 and 1.0 mg/100ml, though the precise upper limit is difficult to define.[40]

III. Biosythesis of Bilirubin Glucuronide:

The individual steps for the biosynthesis of bilirubin glucuronide may be visualized as follows: 1) transport of bilirubin from the plasma across the liver cell membrane facing the sinusoids; 2) conjugation by the microsomal enzyme system; 3) excretion of conjugated bilirubin across the liver cell membrane facing the canaliculi as bile. (See diagram on following page.[41]) I shall however discuss uptake and excretion of bilirubin first before I consider the topic of conjugation.




A. Hepatic Uptake and Excretion of Bilirubin: There is a period of delay between uptake and excretion which appears to depend partly on bilirubin conjugation, and during this delay the bilirubin is stored in the liver.[42] Rate and effectiveness of conjugating mechanism affect the rate of uptake and excretion.[42,43] It is suggested that an active transport system may be required for movement of bilirubin from the cell membrane facing the sinusoids, to the microsomes for conjugation and then to the cell membrane facing the canaliculi for excretion.[40] This contention is supported by Hanzon's direct microscopic studies on uranium excretion, which demonstrated that bilirubin diffuses passively through the sinusoidal endothelial cells, is concentrated close to the endothelial surface of the liver cell by an active process, diffuses passively across the cell as a consequence of the concentration gradiant developed, is then actively concentrated a second time close to the canalicular surface, ultimately to be excreted in the bile.[44] This author stressed the unidirectional nature of the preceding process and also demonstrated that hepatocellular injury or biliary obstruction can reverse the cell polarity, permitting bilirubin to go from the canaliculus to the sinusoid.

The excretion of bilirubin was shown to be proportional to the square of its plasma concentration,[17] and that there is a limit to the excretion capacity of the liver which when exceeded leads to progressive rise in serum bilirubin level.[45,46] Furthermore, it is believed that the limiting factor in the metabolism of bilirubin is not the ability to conjugate but probably involves either the uptake of bilirubin from plasma or the ability to excrete the conjugated product.[42]

B. Conjugation of Bilirubin: The conjugation of bilirubin in liver has been demonstrated by studies using liver preparations from man and various animals,[16,46-48] and shown to involve a series of enzymatic steps, terminating in the enzyme transfer of glucuronic acid (GA) from uridine diphosphate glucuronic acid (UDPGA) to bilirubin,[48a] as outlined below:

1. ATP + UDP (Uridine Diphosphate) — Nucleoside Diphosphokinase → UTP + ADP (Uridine Triphosphate)
2. UTP + G-1-P (Glucose-1-Phosphate) — UDPG Pyrophosphorylase → UDPG + PP (Uridine Diphosphate Glucose)
3. UDPG + 2DPN — UDPG Dehydrogenase (in soluble liver extract) → UDPGA + 2DPNH + 2H
4. UDPGA + Bilirubin — Glucuronyl Transferase (in microsomes) → BiliDG + UDP (Bilirubin Diglucuronide)

The enzyme glucuronyl transferase which accomplishes the transfer is located in the microsomes of the liver cells.[46,47,49-51]

Aside from the bilirubin diglucuronide, indicated above, an intermediary pigment bilirubin monoglucuronide is also found in man.[52] The exact role of the formation of bilirubin monoglucuronide in bilirubin metabolism is not fully understood. Findings of Hoffman et al [53] suggested that bilirubin monoglucuronide might be found extrahepatically or prehepatically which might precede or perhaps facilitate the intrahepatic formation of diglucuronide.[53]

The extent to which this is done under normal circumstances in man has not been determined. Bilirubin monoglucuronide was not found in infants with physiological jaundice,[54] but was demonstrated along with bilirubin diglucuronide by solvent partition technique in serum of infants with erythroblastosis and hepatitis.[55] It is also thought that the toxicity of bilirubin monoglucuronide to tissues is less than that of unconjugated bilirubin on the basis of solubility characteristics.[55] More recently, studies using the preceding technique indicate that there is variability in capacity of premature infants to form bilirubin monoglucuronide and diglucuronide.[56] In the smaller premature and hypoxic infants studied, the formation of monoglucuronide seems to be decreased.[56]

The preponderance of evidence suggests that the major and perhaps the sole mechanism for glucuronide conjugation is the preceding described process, and that glucuronic acid can not be used directly for glucuronide formation.[49,50] That after administration of labelled glucuronic acid, a small fraction of the label may appear in the glucuronides excreted in the urine,[57] is probably explained by the observation that glucuronic acid is an intermediate in one of the glycolytic pathways.[58] Thus, the administered labelled glucuronide, which is partially absorbed and metabolized, may appear in various carbohydrate fractions, one of which appears in that necessary for glucuronidation.

That the bilirubin may be conjugated directly with glucuronic acid by an alternative pathway has been suggested, however. In vivo the rate of glucuronide formation was found to be augmented by administration of glucuronic acid by Danoff et al.[59] Moreover, Brown et al[60] found that liver homogenates of newborn guinea pigs deficient in glucuronyl transferase

were capable of conjugating o-aminophenol when ATP and glucuronic acid, sodium glucuronate or glucuronolactone was added. These authors suggested that the glucuronic acid might enhance the activity of uridine diphosphate glucuronic acid - glucuronyl transferase system by inhibiting beta-glucuronidase, found in high concentration in the newborn and known to decrease the conjugation of o-aminophenol. Hsia[61] gave labelled sodium glucuronate and glucurono lactone to dogs but found no evidence of conjugation with bilirubin. Brown et al's interpretation is further refuted by the finding that 4-methylumbelliferone was conjugated with glucuronic acid when incubated with ATP, UTP, and a soluble fraction of rat liver homogenate in the absence of glucuronyl transferase.[62] But this latter finding was considered as evidence supporting an alternate pathway of glucuronide conjugation.[62] Whether or not an alternate glucuronidation pathway exists in vivo remains to be determined.

It is also not known whether a single or several related microsomal enzymes are involved in the conjugation of bilirubin and other aglycones.[63] Evidence for and against the contention that several, instead of one, specific acting enzyme may exist is discussed under the etiology of this disease.

Isselbacher and Mc Carthy observed that not all of the direct reacting bilirubin is conjugated with glucuronide.[64] Approximately 15% of the direct reacting bilirubin pigment in human bile is conjugated as sulfate. The synthesis of bilirubin sulfate involves the transfer of sulfate from active sulfate to bilirubin by an enzyme, sulfate transferase, found in the soluble fraction of rat liver homogenates.

These authors also observed that an additional 9% of bile pigment, which

gives a direct van den Bergh reaction in human bile is conjugated with neither glucuronic acid nor sulfate. These other conjugates may be of methyl or glycine radicles.[64]

In addition to liver, other tissues have been reported to have ability to form glucuronide in vitro, including kidney[53,65] gastrointestinal tract,[53] especially the mucosa of the upper gastrointestinal tract,[65] and even the brain.[48] Glucuronyl transferase was also demonstrated in serum of animals and humans with hepatic necrosis.[62,40] It was noted, however, that reduction of hepatic transferase activity following liver damage results from a loss of enzyme-containing microsomes rather than from a reduction in their enzyme content,[66] which raises the question of whether the demonstrated serum transferase activity was due to circulating microsomes or to presence of the enzyme in some solubilized form. In vivo studies have demonstrated that Pigment I may be formed in hepatomized, nephrectomized and even complete eviserated animals.[53,67] In none of these experiments was it established that Pigment I was bilirubin monoglucuronide.[40] Furthermore it is believed that extrahepatic conjugation of bilirubin in vivo is probably of little physiological significance.[9]

In the newborn period, during which the onset of jaundice of CFNJ patients occur, it has been established that several of the terminal enzyme reactions involved in glucuronide conjugation are relatively deficient.[46,68,69] The newborn liver in man has much less glucuronyl transferase activity than does the liver of the adult and there is gradual development of this function over the first few weeks of life.[69] It is thought that this relatively low activity of glucuronyl transferase is the basis for accumulation of unconjugated bilirubin in the neonatal period.[25,70] Uridine diphosphate glucuronic

acid is however also decreased in the newborn liver. This is in part accounted for by the limited activity of UDPG dehydrogenase.[69] The synthesis of UDPG is believed not to be the rate limiting step in glucuronide formation.[71]

The developmental capacity for glucuronide conjugation has been found to be different in different organs. Capacity for glucuronide synthesis in the liver and kidney increases slowly in the newborn period, beginning shortly before birth and continuing for several days and even weeks after birth.[68] The capacity for glucuronide synthesis in gastrointestinal tissue, however, decreases after birth, and fetal gastrointestinal mucosa demonstrates more activity of an enzyme which is similar, if not identical with, glucuronyl transferase, as compared to gastrointestinal mucosa of the adult animal.[68]

V. Fate of Bilirubin:

On reaching the intestinal tract, conjugated bilirubin is converted by bacteria to urobilinogens.[72] To a variable degree, these are then oxidized to urobilins, the precise mechanism of this oxidation is uncertain.

The actual conversion of bilirubin to urobilinogen is believed to occur in the colon.[73] Some of this urobilinogen is reabsorbed and returned to the liver ultimately to be re-excreted in the bile,[72] or excreted in the urine.

The possibility that some bilirubin is reabsorbed in the small intestine prior to its reduction to urobilinogen was suggested by early observations that oral administration of bilirubin in dogs led to an increase in its excretion in the bile.[74] The question of enterohepatic

circulation has been studied recently by intraduodenal administration of labelled bilirubin-$C^{14}$ in normal rats and man, and in rats and man with CFNJ.[26,75] 10-30% of either conjugated or unconjugated bilirubin-$C^{14}$ infused into the duodenum was recovered in the bile fistulae of normal rats as conjugated bilirubin-$C^{14}$.[75] This was confirmed by similar studies in mildly icteric, but otherwise normal, human, jaundiced rats (Gunn strain) and human (J.d'E.) with CFNJ which revealed that total intestinal absorption approximated 15% of free or conjugated bilirubin administered into the duodenum.[26] Moreover, irrespective of free or conjugated bilirubin-$C^{14}$ given in the duodenum, there was significant elevation of unconjugated serum bilirubin-$C^{14}$, and in Gunn rats, virtually no bilirubin-$C^{14}$ was recovered in bile, if conjugated bilirubin-$C^{14}$ was administered into the duodenum.[26] These studies indicate that little, if any, conjugated bilirubin is absorbed unaltered, and that a significant fraction is hydrolyzed and absorbed as unconjugated bilirubin.[26]

In similar experiments in man (J.d'E.) with CFNJ and jaundiced rats, it has been found that unconjugated bilirubin is transferred directly across the mucosa into the intestine[27] whereas conjugated bilirubin is believed not to be not excreted via the gastrointestinal mucosa.[78] These studies also demonstrated that derivatives of labelled bilirubin are excreted in bile, in stool uncontaminated with bile, and to a lesser extent in urine.[27] This may explain for the plasma bilirubin levels in patients with CFNJ, to be discussed later. It is thus apparent that, at least in Gunn rats and in man with CFNJ or Crigler-Najjar syndrome, the gut contains a bilirubin pool which exchanges across the intestinal mucosa with the plasma pool.[77]

If it is assumed that the degradation of hemoglobin from senescent

erythrocyte entails its ultimate quantitative conversion to urobilinogen, the amount of pigment actually recovered in the urine and feces falls short of that expected.[40] This descrepancy, yet to be resolved, is closely related to the finding that patients with CFNJ are able to maintain a steady plasma bilirubin level despite continued pigment production, which will be discussed in greater detail under the etiology of this disease.

In this summary of bilirubin metabolism, I have alluded to some of the unsettled problems and the recent developments of interest, including the findings on bilirubin absorption and excretion in the gastrointestinal tract; alternate pathways for glucuronidation; pathways of bilirubin conjugation not involving glucuronidation; pathways for converting hemoglobin to non bilirubin excretory products. These are precisely the same problems, the elucidation of which will give a better understanding of CFNJ. I shall therefore make reference to them again in the discussion of the etiology of this disease.

## THE VAN DEN BERGH TEST FOR BILIRUBIN

In 1916 van den Bergh and Müller [79] introduced the Ehrlich-Proescher bilirubin-diazo reaction for use in clinical medicine. Since then the van den Bergh test has been the major test used to measure conjugated and unconjugated bilirubin levels. Because of its empirical nature,however, its accuracy as a measurement of conjugated and unconjugated bilirubin has been questioned. It is thus desirable to elucidate the advantages and shortcomings in using this test. Moreover, such an elucidation is necessary for the evaluation of bilirubin levels in the CFNJ patients which are mostly measured by the van den Bergh test, and in the experimental work utilizing this test.

Van den Bergh and Muller classically described a direct and an indirect reacting bilirubin with diazotized sulfanilic acid reagent, the former reacting immediately with the reagent in aqueous solution, whereas, the latter required addition of alcohol for reaction. This difference in bilirubin reaction was accounted for by various theories, but the conclusive work was done by Cole and Lathe[80] in 1953 when they separated indirect and direct bilirubin by reverse-phase partition chromatography. The indirect reacting pigment which was soluble in organic solvents, was shown to be indentical with crystalline bilirubin, while the direct reacting pigment proved to be water soluble at acid pH.The direct reacting pigment was subsequently partitioned to yield two fractions, both water soluble and thus reacted directly in the van den Bergh test in aqueous solution. These fractions were designated Pigment I and Pigment II.[80a] The direct reacting PigmentI and II and the indirect reacting bilirubin were subsequently indentified

to be bilirubin conjugated with one molecule of glucuronide, with two molecules of glucuronide and wholly unconjugated bilirubin, respectively.[52, 81, 83-85] Despite the finding that ethanol was necessary for the complete coupling of Pigment I eluted from kieselguhr columns,[81] it was demonstrated later that, at least in plasma, bilirubin monoglucuronide coupled completely in the absence of ethanol.[82] One author, however, still thinks that bilirubin monoglucuronide is measured with the total bilirubin rather than with the one-minute "direct" fraction.[25]

Not all the bile pigments which give the direct van den Bergh reaction are glucuronides.[81] As mentioned earlier, the nonglucuronide fraction constituted 24% of total azopigment derivative of bilirubin in human bile, with about 14% in sulfate form and the remaining 9-10% possibly conjugated with carboxyl-linked methyl or glycine radicals.[64] With and Schmid also found that a small fraction of unconjugated pigment in plasma reacted with the van den Bergh diazo reagent in the absence of alcohol,[33,86] and thought that this fraction was due to the presence of solubilizing substances such as bile acids, urea, and citrate.[33]

In addition to the preceding findings which throw some doubt on the exactness of the van den Bergh reaction as a measurement of the concentration of conjugated and unconjugated bilirubin,[86] other factors can affect the results of the test. These factors include the time allowed for coupling,[87] the concentration of unconjugated bilirubin,[88] the pH, the protein concentration, and the amount of diazotized sulfanilic acid used.[89] Some uncertainty still exists regarding the optimal time for the coupling. Despite the various shortcomings, the van den Bergh test is still widely used and is believed

to provide a reasonable approximation of the total amount of conjugated bilirubin determined by the chromatographic methods,[90] which are yet too cumbersome for routine clinical use.

## ETIOLOGY

A brief review of the clinical and laboratory findings of CFNJ patients will show that this disease is non-hemolytic, non-infectious, and non-obstructive, and that it is probably attributable to failure of conjugation of bilirubin:

(1) There is no hematologic or biochemical evidence of increased hemolysis.

(2) There is no evidence for obstruction.

(3) Classical liver function tests are all within normal limits. The only abnormality found is a prolonged retention of administered crystalline bilirubin.[1]

(4) Serum from patients with CFNJ infused into human control showed normal rate of elimination.[4]

(5) Liver histology shows no significant abnormality.

(6) Paper chromatographic techniques have conclusively shown[15] that the only pigment in the serum is unconjugated. Those who have reported the presence of very small amounts of conjugated pigment have all used the diazo method which is only an approximation, discussed earlier.

(7) No conjugated bilirubin has been found in the bile of patients with CFNJ excepting three cases. In these three cases, J., R.S., W.,[5,9,16] direct bilirubin was found, but as was pointed out previously, presence of solubilizing substances such as bile acids, urea, and citrate will lead to a direct diazo reaction.[33] The presence of these substances has not been ruled out.

Therefore most experimental work has concentrated on the inability of the patients to form glucuronides of different substrates.

I. Studies on Patients, in Vivo

A. Steroid Conjugation: It is known generally that the numerous corticosteroids and 17-ketosteroid metabolites appearing in the urine are conjugated with glucuronic acid. Isselbacher and Axelrod demonstrated that glucuronides of tetrahydrocortisone, a major metabolite of hydrocortisone, as well as other 3 alpha-ol ring A saturated steroids, could be synthesized in vitro by an enzyme present in liver microsomes which transferred glucuronic acid from UDPGA to steroids and other aglycones.[91] It was shown that normally 60% of administered hydrocortisone-4-$C^{14}$ was excreted as metabolites conjugated with glucuronic acid,[92] because hydrocortisone does not have a 3 alpha-ol in ring A, it can not be conjugated directly.[93]

Peterson and Schmid[93] infused hydrocortisone into J.D., four normal subjects and three patients with liver disease (two cirrhotic and one hepatitic). The disappearance of the metabolites, tetrahydrocortisone and therefore presumably hydrocortisone, from the plasma was significantly delayed as compared with the normal subjects and other liver disease patients. Concurrently, the appearance of the conjugated tetrahydrocortisone was also slower. The total amount of glucuronic acid conjugated metabolites appearing in the urine after the infusion of hydrocortisone was only one half of that found in normal subjects. Similar studies of Childs et al[2] also showed glucuronide conjugates of hydrocortisone-4-$C^{14}$ was much decreased in a patient with CFNJ, J.D.H. Here they found that free hydrocortisone and its unconjugated metabolites were slightly but not significantly delayed in leaving the blood, while the conjugated metabolites were present in the blood in slightly reduced amount. They

also infused tetrahydrocortisone in a separate experiment into the same patient to eliminate the possibility that the reduction of hydrocortisone to tetrahydrocortisone was impaired and not the glucuronide conjugation system. The unconjugated compound was slightly delayed in its disappearance but the amount of the conjugated form in the serum was markedly lower as compared to that in the normal subjects.

Thus the formation of steroid glucuronide was found to be impaired by both studies. But as Peterson and Schmid[93] pointed out, the metabolism of endogenously produced biologically active steroids is apparently not disturbed, and the defect in steroid glucuronide formation can be demonstrated only by administration of a loading dose of ring A reduced steroids. Both studies observed that the total steroid excreted in the urine in these patients were normal. This indicated that these patients have an alternative path of hydrocortisone excretion. In normal adults approximately 25% of the dose of administered hydrocortisone appears in the urine in an unknown form.[2] J.D.H. had an unknown fraction greater than 25%.[2] This may suggest that a relatively larger portion of the steroid metabolites was excreted as sulfates.[15]

More recently, Holman and Golubóff[94] reported three siblings with CFNJ. One child (R.P.B. or Twin B) showed definite defect in glucuronidation of hydrocortison and tetrahydrocortisone, in contrast to the earlier findings. But another child (T.J.B.) showed normal metabolism of tetrahydrocortisone.

Thus the ability of CFNJ patients to conjugate steroids seems less than normal; but the question is not yet settled.

B. Salicylate Conjugation: Salicylate conjugation is another means

of studying the defect in conjugation of patients with CFNJ. Salicylate conjugation, however, has the following features. The metabolic fate of ingested salicylate has been relatively well worked out.[95,96,97] The largest fraction to appear in urine is that conjugated with glycine, the second largest fraction with glucuronide, and the lesser fraction remained unconjugated. Two kinds of salicyl glucuronide are formed: the acyl and the phenolic types.[98] This provides an opportunity to test the extent to which patients and their relatives are able to make each type, but also makes interpretion of results more difficult when discrepancies occur. The conjugation with glucuronic acid appears to be characteristic of the individual,[2] and thus lessens fluctuations of data in any one individual. One of the major drawbacks, however, is that , of the administered dose, only 10-15% is excreted as glucuronides in the normal individual.

Childs,Sidbury and Migeon[2] administered sodium salicylate to J.D.H., M.E.H., K., and their relatives. The data showed that, depending upon the dose, the affected patients performed at a level of 1/6 to 1/3 that of the controls, indicating a marked impairment. The relatives showed impairment intermediate between that of the patients and the controls. These findings were confirmed by Holman and Goluboff in T.J.B. and her relatives.[94]

Childs et al also observed that the patients and some of the relatives produced more of the acyl glucuronide than the phenolic while the controls produced the two glucuronides in roughly equal quantities.[2] As bilirubin is presumed to be conjugated exclusively through its carboxyl groups,[85,99] it was anticipated that the amounts of glucuronides formed by the patients would be either generally depressed, or else the

depression would be manifested principally in the acyl fraction. Yet it appears as if the phenolic fraction is depressed more than the acyl in the patients, while in the relatives who show any depression at all, it is the phenolic fraction which is wanting. No explanation is yet available for this phenomenon.

C. Conjugation of Other Non-Bilirubin Aglycones: Other compounds have been used to study the conjugation in CFNJ patients. By giving, orally, N-acetyl-p-aminophenol (NAPA), which forms a glucuronide, it was found that the rate and magnitude of glucuronide formation in these patients is much less than in normal.[100] In addition the rate of disappearance of the free compound from the plasma is delayed.[100]

Later, Schmid[9] administered NAPA to three patients (J.D., J.d'E., R.S.) and normal subjects of the same age and sex. The concentration of NAPA glucuronide in the plasma was much less in the icteric child and the disappearance of the free compound from the plasma was delayed. The total recovery of NAPA was comparable to that in control subjects but the glucuronic acid conjugated fraction was reduced in the patients.

Chloral hydrate and its reduced product, trichloroethanol, have also been used in these conjugation studies. In the body the latter compound is either oxidized to trichloroacetic acid or excreted as the glucuronide in urine and bile,[101] of which only negligible amounts are in the unconjugated form. In J.D.H. and his parents, the urine excretion of trichloroethanol was much less than that of normal controls, after ingestion of either chloral hydrate or trichloroethanol.[2] Similar results were obtained by using menthol.[9] Excretion of hippuric acid and of salicyluric

acid was found, however, to be unimpaired.[15] Thus a defect in the conjugation of glycine can be eliminated. No work has been reported on the state of sulfate conjugation in these patients.

II. Studies on Patients, in Vitro

In vitro studies of the liver homogenates of several patients were done. The liver tissue of R.S. was studied by Schmid.[9] Bilirubin and o-aminophenol were incubated with liver homogenates and microsomal preparations, respectively. It was observed that liver homogenates failed to form direct reacting bilirubin,[46] but the microsomal preparations synthesized o-aminophenyl glucuronide at a rate which was about one half of that found with similar preparations of normal liver.[43]

The transferase activity of liver homogenates of J. was also tested by combining the liver homogenates with bilirubin and either rat or human liver extract containing UDPGA.[16] No direct bilirubin was detected from this system. If, however, the patient's liver homogenate was substituted by the normal rat liver homogenate, direct bilirubin was detected. This implied that the patient's liver homogenate was defective in glucuronyl transferase activity. Conjugation of o-aminophenol was also found to be impaired when it was used instead of bilirubin in the above microsomal system. However, the authors pointed out that the bile aspirated from the patient's gall bladder contained only direct reacting bilirubin, which indicates that either the above transferase test was not sensitive enough or there is an alternative pathway for glucuronide formation. It can be pointed out again that the presence of any solubilizing substance such as bile acid, urea or citrate will lead to a direct diazo reaction.[33] It must also be noted that this patient was first reported as one with

"Gilbert's disease",[6] but later classified as a Crigler-Najjar.[9]

In vitro studies of liver tissue was also in two patients who originally were believed to have "Gilbert's disease",[16] was then thought to have a defect similar to CFNJ.[102] This controversy will be discussed in detail, later. In these two patients liver homogenate was found to lack the ability to form glucuronide both with bilirubin and with o-aminophenol.[16]

III. Studies on Gunn Rats

Another valuable means of studying the CFNJ patients has been the use of a mutant strain of Wistar rats, described by Gunn[103] and Malloy and Loewenstein[104]. These rats are strikingly similar to the patients with CFNJ, thus providing an excellent laboratory tool for studying the etiology of the defect in bilirubin conjugation. But before I go into the results of work done on Gunn rats, I would like to describe them briefly.

Gunn rats, from the time of birth, have elevated bilirubin levels from 5 mg/100ml to 20 mg/100ml, all plasma plasma bilirubin is of the unconjugated type, and bilirubinuria is absent.[43] Similar to patients with CFNJ, these rats have no evidence of increased hemolysis,[43,104] and have reduced fecal urobilinogen.[43] That the jaundice is not obstructive in nature was shown by the rapid excretion of bromsulphalein and cholografin, contrast medium.[43] Conjugated bilirubin injected intravenously is rapidly and completely excreted in the bile, increasing the biliary pigment by 100%.[42,43] Maximal clearance for conjugated bilirubin by jaundiced rats is similar to maximal clearance for unconjugated bilirubin by normal rats.[45] The bile of the jaundiced rats contains much less bilirubin than that of normal rats and the small amount of pigment present is in the non-glucuronide form.[43] The defect in these rats is thought to be inherited by an autosomal recessive

gene because breeding of non-jaundiced litter mates, known to be carriers, produced 25% jaundiced offsprings, usually.[103] Rats with bilirubin levels exceeding 12-15 mg/100ml showed functional impairment and central nervous system pigmentation identical with kernicterus.[105,106]

A. Studies in Vitro: Liver slices and liver homogenates of Gunn rats did not conjugate bilirubin when incubated with liver extract as a source of UDPG and bilirubin.[46,107] Nor did kidney tissue incubation with bilirubin and UDPG produce any conjugated bilirubin.[107] These findings suggested that the defect in bilirubin metabolism is in part caused by impaired activity of glucuronyl transferase, the enzyme responsible for transfer of glucuronic acid from UDPG to the aglycone. Carbone and Grodsky thought that the finding of impaired formation of glucuronyl transferase can be interpreted as (1) glucuronyl transferase activity may include several unknown enzymatic steps, any of which could be defective, (2) the defect may not be enzymatic, but may be caused instead by the presence of an inhibitor or absence of some co-factor, (3) defect in other enzymatic steps further removed in the chain of glucuronide metabolism.[107]

Using Gunn rat liver slices, the conjugation of aminophenol was found to be about one half that of normal Wistar rats and about the same as that of heterozygous rats which were not jaundiced.[46] Liver homogenate of Gunn rat was found to have even less aminophenol conjugation ability than liver slices[43] or no conjugation at all.[107]

Kidney tissue studies showed that there was no conjugation of aminophenol in the affected homozygous rats[43,107] and reduced amount of conjugation in non-jaundiced heterozygous rats.[43]

That the glucuronidation is defective for both bilirubin and o-aminophenol

led to Carbone et al's suggestion that there is an impairment of the mechanism of glucuronidation rather than a specific defect of the metabolism of bilirubin.[107] But the question, why can the Gunn rat's liver tissue conjugate some aminophenols but no bilirubin at all, is not answered.

Lathe et al[46] suggested that if bilirubin and aminophenol shared the same process of glucuronidation, then one should be able to demonstrate competition for this simple process of enzymatic conjugation. They found that bilirubin conjugation in the normal rat was inhibited by relatively high concentration of o-aminophenol, but bilirubin failed to inhibit o-aminophenol conjugation.[46] At the same time they pointed out that among different animals there was no parallel between the capacities to conjugate the two substrates bilirubin and o-aminophenol: the cat conjugated bilirubin but not o-aminophenol; the rabbit conjugated o-aminophenol two to three times as fast as bilirubin; the homozygous Gunn rat conjugated o-aminophenol but not bilirubin.[46]

Studies to eliminate defects in UDPG dehydrogenase were done by Lathe and Schmid and their associates.[43,46] They found that UDPG dehydrogenase activity was about equal in the liver of homozygous Gunn rats, their heterozygous litter mates, and normal controls. Addition of UDPGA to microsomal preparations of icteric rats failed to augment glucuronide synthesis.[43,46,107]

Schmid et al showed that an inhibition factor in Gunn rats was not likely to be the etiology of the defect in glucuronide conjugation.[43] They observed that when equal amounts of liver microsomes from Gunn rats and from normal Sprague-Dawley rats were mixed, no reduction in the rate

of glucuronidation of aminophenol was observed.

The possibility that rapid breakdown of bilirubin glucuronide by beta glucuronidase and therefore leading to an accumulation of unconjugated bilirubin was also ruled out. Attempts to inhibit endogenous beta glucuronidase by addition of $10^{-4}$-$10^{-3}$ M concentration of potassium saccharate failed to influence the conjugative activity in the Gunn rat liver tissue.[107]

B. Studies in Vivo: Both ester forming aglycone, o-aminobenzoic acid, and ether forming aglycones such as menthol were used, Oral administration of menthol and o-aminobenzoic acid in jaundiced Gunn rats and non-jaundiced animals showed that glucuronide conjugation in the jaundiced rats was much smaller than that in the non-jaundiced rat.[46] Similar results were obtained with intraperitoneal injection of sodium o-aminobenzoate.

Urinary excretion of glucuronic acid and glucuronides of the Gunn rat was about one half that of normal control rats.[46] The difference in the daily glucuronic acid excretion between the non-jaundiced litter mates and normal control rats was also statistically significant.

An attempt was made to stimulate the activity of the glucuronide forming enzyme system in the Gunn rat by repeated administration of large amounts of an aglyconic substrate other than bilirubin.[9] Sodium o-aminobenzoate was injected but there was not increased activity of glucuronyl transferase as compared with non-stimulated jaundiced rats. In the stimulated rats, the liver showed very concentrated amounts of o-aminobenzoate twenty hours after the last injections, whereas non-icteric rats

conjugated and removed it within a few hours.[9] These findings corroborated the fact that as bilirubin increased in the serum in jaundiced rats, there is no stimulation of bilirubin glucuronidation.

IV. Summary of Studies on Etiology of CFNJ:

The available evidence concerning the pathogenesis of CFNJ has just been reviewed. That this disease is not caused by a defect in the bilirubin transport, i.e. binding to plasma protein and transfer from plasma to conjugation site, uptake and excretion is supported by the following findings: (1) conjugated bilirubin is eliminated rapidly and completely;[42,43] (2) maximal clearance of conjugated bilirubin in Gunn rats is comparable to that of unconjugated bilirubin in normal rats;[45] (3) unpublished work of Schmid[78] showed that conjugated bilirubin is taken up by the Gunn rat liver; (4) bilirubin is found in the bile of all six patients measured[5,9,15,16] and in Gunn rats;[43] (5) the diagnostic pattern of initial fall and subsequent sustained rise of injected bromsulphalein and unconjugated bilirubin, seen in defects of hepatic excretion but normal conjugation, is not found in patients with CFNJ. Defects in the transport, hepatic uptake or excretion, per se, of unconjugated bilirubin have not been definitely eliminated. The only finding here is that indirect bilirubin is found in the bile of five patients.[5,9,15]

That most of the evidence point to a defect in the biosynthesis of bilirubin glucuronide and more specifically, the enzymatic glucuronidation of bilirubin, has already been pointed out by the review of the clinical and laboratory findings on patients with the disease and by analogy of findings in the Gunn rats.

The defect in the enzymatic conjugation of bilirubin with glucuronide has been sought along the following lines: (1) enzymatic steps leading to the production of UDPGA; (2) enzymatic step involving glucuronyl transferase

leading to formation of bilirubin glucuronide; (3) inhibition on the enzymatic system resulting in failure of conjugation; (4) deconjugation of conjugated bilirubin in liver cell or canaliculi; (5) non-glucuronide conjugation systems (sulfate, glycine, etc.). I shall eliminate those factors which seem unlikely in the light of the evidence presented.

The enzymatic steps prior to that involving glucuronyl transferase can be temporarily ruled out on the following basis. It was found that UDPG dehydrogenase activity was normal and failure of glucuronide conjugation was not due to insufficient glucuronic acid.[43,46,107] This favors a defect in an enzymatic step after UDPG dehydrogenase, but does not eliminate a defect in previous steps, in the presence of a defect in glucuronyl transferase. One would expect, however, that any defect prior to or directly involving the formation of UDPGA will lead to symptoms due to defects in normal metabolic processes dependent on UDPGA or its precursors. Thus one would expect some manifestation of symptoms of galactosemia and lack of chondroitin sulfate. Such manifestations have not been reported in patients with CFNJ. It is interesting to note here, that in patients with galactosemia, jaundice is a common occurrence.[108]

Failure of conjugation due to enzymatic inhibition has been ruled out by the findings of Schmid et al showing that when Gunn rat liver was mixed with normal liver, the normal liver maintained the ability to conjugate bilirubin.[43]

Deconjugation of conjugated bilirubin can be ruled out because, as was mentioned earlier, intravenously administered conjugated bilirubin was excreted rapidly by Gunn rats,[42,43] and more specifically, beta glucuronidase in the Gunn rat livers was shown not to breakdown conjugated

bilirubin.[107]

A concomittant defect of conjugation of bilirubin by sulfate, glycine and methyl radicals, along with glucuronidation defect, may give the picture of the severly elevated serum unconjugated bilirubin levels seen in CFNJ patients. Glycine defect has been eliminated.[15] Although assays on sulfate conjugation have not been reported, in view of the total excretion of administered steroids in CFNJ, it has been said that a larger portion of steroid metabolites was, in fact, excreted as sulfate under these conditions.[43] The significance of methyl conjugation in bilirubin excretion is, at best, very small. Thus it seems unlikely to expect any concomittant defect in non-glucuronide conjugation to be an important factor for consideration.

These above considerations, then, leaves one with the most likely defect of this disease: that of the enzymatic step involving glucuronyl transferase leading to formation of bilirubin glucuronide. The evidence supporting this has been presented, and (at least, to date) there is no evidence to the contrary. Before anything definite can be said regarding the etiology of CFNJ, however, more work need be done in some of the areas of uncertainty to which I have alluded.

V. Related Problems

A. Alternative Pathway for Bilirubin Metabolism in CFNJ: Patients with CFNJ maintain a remarkably constant serum bilirubin level, despite their defective glucuronidation. It can thus be presumed that an alternative pathway for bilirubin excretion exists, although the finding that direct reacting bilirubin has been noted in the bile of three patients[5,9,16] and to a very small extent in serum leads to the possibility that

glucuronidation, though defective, does occur to some extent, in vivo. Alternate pathways of bilirubin excretion would include conjugation with sulfate, glycine, and carboxyl-linked methyl.[64] These pathways are favored by the findings that ultimate total excretion of injected steroids are undiminished in CFNJ,[15] and that hippurate excretion following given dose of benzoate was greater in Gunn rats than in normal rats,[43] thus making the compensatory loading of these alternate conjugation routes very suggestive.[43] Moreover, recent studies on amphibia showed that biliary excretion of bile pigment was independent of the presence of functioning mechanism for glucuronide formation.[76]

Bilirubin may be excreted without conjugation, since indirect bilirubin is found in the bile of five patients[5,9,15] and in that of Gunn rats.[43]

One of the most likely pathways of elimination is apparent from the recent studies on gastrointestinal excretion of unconjugated bilirubin, mentioned earlier. That extrahepatic sites may be involved in the conjugation and elimination of bilirubin is also supported by earlier findings on extrahepatic conjugation of salicylate.[97]

Other possible alternatives of bilirubin excretion are based on the finding that urobilinogen recovered in urine and feces of normal man falls far short of that expected from hemoglobin degradation. The possibilities of this discrepancy have been sought in terms of (1) degradation of urobilinogen to dipyrrl-methenes, such as mesobilifuscin, (2) reabsorption of urobilinogen from intestinal tract and re-utilization in porphyrin synthesis, (3) destruction of reabsorbed urobilinogen in liver, (4) an alternative pathway for hemoglobin degradation to colorless compounds not coupling with diazotized sulfanilic acid.[40] The first three hypotheses

have since been refuted, [109, 110] and there is no direct evidence reported to support the last contention.[40]

B. The Specificity of Glucuronyl Transferase: The findings that the glucuronidation of some compounds are more affected than others, eg. Gunn rats did not conjugate bilirubin at all, but were able to conjugate some o-aminophenol, suggests that perhaps there are different transferase systems for different compounds. It was noted in normal rats that although o-aminophenol inhibited bilirubin excretion, the inhibition was not competitive. Moreover, bilirubin did not inhibit o-aminophenol.[89] There were also no parallel capacities of bilirubin and o-aminophenol conjugation in different animals.[89] Another study showed that infusion of NAPA did not decrease bilirubin conjugation in vivo.[42] Ruptured cell preparations had a markedly different effect on the conjugation systems. In rat and mouse, bilirubin conjugation was increased by using ruptured cells, but in these and other animals, breaking up the cells had a depressing effect on o-aminophenol conjugation.[46]

The findings of Dutton and Axelrod et al suggest that a simple enzyme catalyzes the transfer of glucuronic acid to phenolic, alcoholic, carboxylic and amino acceptors.[42,111] It has been suggested that the time when a compound is not conjugated with glucuronide may be due to operations of steric factors.[91] Thus cortisone is not conjugated, whereas tetrahydrocortisone and testosterone are.[112] Also it is noted that the latter steroids have the same beta configuration as naturally occurring glucuronides.[111] Furthermore, Carbone and Grodsky[107] observed that borneol, which is conjugated by the body only as a glucuronide, competitively

inhibited bilirubin glucuronide formation in vitro. This finding further favors a single enzyme in the transfer of glucuronic acid.

Studies on the Gunn rat and patients with CFNJ also showed that the enzymatic defect in glucuronide formation not only involved synthesis of bilirubin glucuronide but also includes glucuronidation of a variety of compounds. This can also be interpreted as evidence for one enzyme in glucuronidation. It is noted that these same findings have been used to support the contention of multiple enzyme system.

The current viewpoint seems to favor a single enzyme for glucuronidation of bilirubin and non-bilirubin aglycones, since no other enzyme has been demonstrated. A definitive answer, however, must await the purification of the enzyme, glucuronyl transferase.

Furthermore, it has been suggested that the conjugation of non-bilirubin compounds occur primarily outside the liver and thus fail to reflect a possible defect in bilirubin glucuronidation.[102]

## GENETIC CONSIDERATIONS

Familial involvement is an unquestionable feature of patients with this disease ( see Table II ). All,except five of the reported cases, have one or more members of the family group affected by the same disease. In three of the five cases ( J.,J.d'E.,W. ) without reported familial involvement, the following observations are notable. The paternity of J. and her siblings are doubtful because of the obvious sexual promiscuity of her mother.[6] The brother of J.d'E.'s father was noted to have"Gilbert's disease", questionably of hepatitic origin, total serum bilirubin 2mg% and indirect bilirubin 1.7 mg%. [11] W. is the first Negro child reported to have this disease.[5] Unfortunately, there is no available data on the family history of R.S..[9] Consanguinity was noted in one family group only.[1,2] In all, except the case of a negro child, the patients were Caucasians; and Italian parentage was noted in two family groups J.D.,R. and J. d'E..

The familial aspects of this disease suggested that it was genetically determined and the aggregations of patients solely in sibships, never involving parents, suggested that the affected children are homozygous possessors of an abnormal Mendelian recessive trait.[1,2] From the cases with clear family history, which includes all family groups excepting W.J.H., J.H., R.S.,and K.; the ratio of affected children to non affected siblings is 16/50 or 32%. This ratio is not very far from the mode of inheritance of a recessive gene. The number of reported cases are perhaps too few, however, to permit any definitive genetic studies at this time.

Careful geneological investigation has been mostly confined to the

## TABLE II

| Patients | Relationship of Patients | No. of Patients in Family | Total No. of Children in Family | Total No. of Family Group* Members Investigated | Family Group Members Probably Affected by History Only | Heterozygotes as Determined by Glucuronide Excretion Tests: Sibs | Parents | All Other Relatives | Total No. of Heterozygotes in Family Group/ No. Tested in Family Group | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | No. of Heterozygotes/ No. Tested | | | | |
| LM | 6th Generation Descendants of One Couple | 1 | 3 | 505+ | 15 H | - | - | 10/18 | 10/18 | S |
| JRH,JDH XXH | Sibs | 3 | 5 | | | 0/2 | 2/2 | 8/14 | 10/18 | S |
| JLT,LT JJT | Sibs | 3 | 14 | | | 3/6 | 1/2 | 6/10 | 10/18 | S |
| MEH' | | 1 | 3 | | | 0/1 | 2/2 | 8/15 | 10/18 | S |
| JD | Double First Cousins | 1 | 3 | 9 | ** | - | 2/2 | - | 2/2 | N |
| R | | 1 | 2 | | | - | - | 2/2 | 2/2 | N |
| J | | 1 | 8 | 10 | ? | - | - | - | - | |
| K | | 1 | 3+ | 7+ | - | -/2+ | -/2 | -/2 | -/6+ | S |
| WJH";JH" | Sibs | 2 | - | - | - | - | - | - | - | |
| RS | | 1 | - | - | - | - | 0/2 | - | 0/2 | N |
| Jd'E | | 1 | 2 | 5 | *** | - | 0/2 | - | 0/2 | N |
| W | | 1 | 7 | - | 0 | - | - | - | - | |
| RMB,RPB TJB | Twins Sib | 3 | 3 | - | 0 | - | 1/2 | + | 1/2 | S |

*Family Group includes Immediate Family and All Other Relatives;**Brother died few hours after birth, of atelectasis;***Father's Brother has "Gilbert's Disease";S,N means Family Members tested by Salicylate Excretion and NAPA and p-Aminophenol Excretion, respectively;(-) means data not available; H means information obtained by medical family history only.

large family group described by the original authors of this disease.[1] Eight patients from four related families, M.,T.,H.,H'., were the sixth generation descendents of one couple M.. The family pedigree of these patients, including 505 individuals, showed fifteen probably affected individuals,[1] Since the specific criteria used to determine whether any member was"probably affected" were not described, these fifteen individuals were probably discovered from the medical family history. Serum bilirubin concentration of parents and nonaffected siblings were consistently normal.[1,2]

Assuming that glucuronide formation was defective in these patients, Childs et al fed sodium salicylate to eighteen family members ( parents, nonaffected siblings and grandparents ) of related families T.,H.,and H'. and estimated the excretion of salicyl glucuronides and of other salicylate metabolites in urine[2]. In five out of the six parents, a reduction in glucuronide excretion was demonstrated. In three out of nine nonaffected siblings tested, one grandparent, and one great-grandparent, similar findings were noted. This gave a total of ten, out of eighteen, family members tested who showed reduction of salicyl glucuronide excretion.

From these findings, Childs et al postulated that the defect was due to an abnormal gene, recessive with respect to bilirubin, but incompletely dominant for other glucuronide forming compounds.[2]

The parents of J.D., J.d'E. and R.S. were studied by intravenous injection of NAPA followed by estimation of NAPA glucuronide and total aminophenol excreted in the urine[9]. Only two of the six parents showed reduction in glucuronide excretion.

Another study, measuring salicylate conjugation in parents and family

members of R.M.B., R.P.B., and T.J.B. revealed a number of persons who were suspected of being heterozygotic, one parent definitely heterozygotic and the other parent probably heterozygotic also[94].

It is noted, however, that the significance of such family studies is limited by the existing uncertainty with regard to the exact pathogenesis of this disease and by the fact that the extent to which glucuronidation of nonbilirubin aglycones are depressed in this disease is unsettled.

## CFNJ VERSUS "GILBERT'S DISEASE"

The similarity between CFNJ and "Gilbert's disease" was first noted by Crigler and Najjar.[1] It has even been suggested that the two disease entities may represent different degrees of the same defect.[15,114,115] The entity of "Gilbert's disease" is still not clearly defined. If the term is used to include all instances of unconjugated hyperbilirubinemia not due to overt hemolysis, which has been considered reasonable in view of the ill-defined nature of Gilbert's original cases, the entity may include (1) the Crigler-Najjar syndrome, (2) the hyperbilirubinemias seen following viral hepatitis and in associated with other diseases of the hepatobiliary system, caused probably by a defect in the transport mechanism of the hepatic parenchymal cells, (3) compensated hemolytic diseases with probable additional defect in bilirubin transport or hyperplastic bone marrow overproduction of bilirubin.[40] Most of Gilbert's cases probably suffered from the last category.[9]

The overall features of the "Gilbert's syndrome", however, can be distinguished from that of the Crigler-Najjar syndrome.[1] The former is usually a mild, chronic, apprently non-hemolytic, unconjugated hyperbilirubinemia. The question of familial involvement is not settled,[9,40] while familial involvement is the rule in the Crigler-Najjar patients.[1] Onset is usually in late childhood or early adult life, although cases occurring in infancy and later life have been described. The serum bilirubin concentration tends to be 15-25 mg/100ml lower than that in the Crigler-Najjar infants and usually less than 5 mg/100ml. The patients are often asymptomatic and jaundice is discovered during some unrelated

illness or during a routine physical examination, although fatigability, asthenia, and gastrointestinal complaints are reported. Laboratory findings on liver function tests are normal, except for prolonged bilirubin retention. Histologically the liver shows no significant abnormality. Erythrocyte survival time has been found to be slightly reduced, but the reduction is far too little to account for the hyperbilirubinemia.[40] On chromatographic analysis, serum pigment was identified as unconjugated bilirubin, but pigments I and II have been found in bile, the latter predominating.[16,115]

There has been some controversy over two patients reported by Arias and London,[16] with serum bilirubin levels of 8.8 and 18.8 mg/100ml, respectively. The high serum bilirubin levels suggest that these are probably examples of "Gilbert's syndrome" as a consequence of deficiency of glucuronyl transferase in liver.[16] These authors in collaboration with Lowy, subsequently confirmed their contention in six similar patients with serum bilirubin levels ranging from 1.8 to 22.0 mg/100ml,[62] using the conjugation of 4-methyl umbelliferone with glucuronide by homogenates of liver needle biopsy specimens as index of glucuronyl transferase activity. Later studies, however, were not able to demonstrate any defect in the in vivo conjugation of N-acetyl-p-aminophenol (NAPA) and menthol with glucuronide in patients with relatively low levels of serum bilirubin.[102,115] Schmid et al concluded that the defect in "Gilbert's syndrome" is not a deficiency of glucuronyl transferase, but may involve a faulty mechanism for bilirubin transport from plasma to the enzyme conjugation site in the liver cell.[102] Schiff and Billing supported this viewpoint, but they

pointed out that in vivo tests with ethereal forming glucuronide receptors, such as NAPA and menthol, may not be a valid measure of bilirubin glucuronyl transferase activity, since the latter entails the formation of an ester glucuronide.[116] Arias has subsequently attempted to resolve these conflicting viewpoints by suggesting that the controversial patients may have a non-lethal variant of the Crigler-Najjar syndrome without kernicterus and that this entity is included in the heterogeneous group of "Gilbert's syndrome".[117]

There has also been some confusion regarding J. She was first studied[16] and reported[6] as a patient with "Gilbert's disease". Her high serum bilirubin level, 16-22 mg/100ml, early onset of jaundice and kernicterus, severity of central nervous system disturbances, and normal erythrocyte fragility test, however, all point to the Crigler-Najjar syndrome or CFNJ. She has, therefore, been considered as a patient with the latter syndrome.[9]

## PREVENTION OF KERNICTERUS

The majority of the patients died of kernicterus during the neonatal period and the patients who survived this period usually developed signs of central nervous system involvement during their early childhood. A few have not developed any signs of central nervous system damage (J.d.'E, M.E.H. J.D.H.)despite continuously elevated levels of indirect bilirubin around 20 mg/100ml, but they are in constant danger of developing signs of central nervous system involvement.

It is thus evident that the main symptoms of the CFNJ patients are jaundice and central nervous system disturbances, consistent with kernicterus. Although kernicterus is a complex problem and requires a paper of its own, I would like to discuss it very briefly because of its importance as the only outstanding complication of the CFNJ disease. Moreover, why these few children have survived without the development of the full syndrome of kernicterus is still a mystery. The care and treatment of the CFNJ patients is the prevention of kernicterus. Therefore I will limit the discussion to only this aspect of kernicterus.

### I.Factors Which Increase the Occurrence of Kernicterus:

In order to prevent kernicterus one must know the factors which influence and precipitate it. These factors can be divided into three catagories: 1) Those which increase the total body bilirubin; 2) Those which cause bilirubin to move into the tissue, especially the brain; 3) Those which increase the susceptibility of the brain cell to the effects of bilirubin.

Among the factors which increase total body bilirubin are: 1) increased

hemolysis of erythrocytes; 2) large doses of vitamin K (Here the mechanism is still much debated.);[118-123] 3) absorption of hematoma;[124] 4) liver damage due to anoxia.[125-127]

Factors which affects movement of bilirubin into the tissues are: 1) the quantity of serum albumin;[33,37] 2) drugs such as sulfisoxazole and salicylates as well as endogenous substances such as hematin, as mentioned earlier, and nonesterified fatty acids, (These have been shown to compete with bilirubin for albumin binding sites.);[129-133] 3)alterations in pH which affects albumin binding of bilirubin;[34,133] 4) presence of competing anions.[25]

Factors which affect the susceptibility of the brain cell to the effects of bilirubin are:1) the intrinsic susceptibility of the cell, especially the basal ganglion cells;[134,135] 2)the metabolic demands on the cell and therefore the extent to which a cell can continue to function when the basic process of oxidative phosphorylation is impaired;[71] 3) prior damage to brain cells by hypoxia;[126,136] 4) functional maturity of the blood brain barrier;[25] 5) prematurity of the patient,[137,138](Adult animal seem better able than newborn animal to withstand hyperbilirubinemia.[105,139]); 6) infection and trauma,[71]( moribond state of cell tend to take up more bilirubin.)[113]

Knowing these precipitating factors will help us in trying to prevent the occurrence of each in the patients. However, Dr.G.Millichap[15] observed that the purer the bilirubin, the smaller the effect on the depression of oxygen consumption on brain slices. Thus, human kernicterus can not be explained as a single sequalae of bilirubinemia. Also Mores et al[140] believed that hyperbilirubinemia without iso-immunization does not involve risk of brain damage to healthy full term infants. They found no evidence of

any cerebral lesions,irrespective of exchange transfusion or not in the absence of hemolytic disease and in full term vigorous infants. Thus it seems that the factors affecting kernicterus is more complicated than those mentioned above.

II.Therapeutic Measures:

Various therapeutic measures have been tried to treat hyperbilirubinemia, all of which are based on maneuvers which decrease the extracellular bilirubin levels and prevent movement of bilirubin into cells.

Exchange transfusion is the only accepted method of treating hyperbilirubinemia. It removes the albumin bound bilirubin from the circulation and reduces the tissue bilirubin as equilibration takes place between the tissue and the circulation.[141] Bilirubin is not dialyzable because it is bound to albumin.[126,142-144] In CFNJ patients,J.D.H. and L.T., however, exchange transfusion produced lowering of the serum bilirubin for less than 24 hours and is therefore therapeutically of little value.[1]

The evidence on the effectiveness of albumin as a means of treating hyperbilirubinemia has been contradictory. Johnson has shown that when parenteral albumin was given to Gunn rats, the serum bilirubin rose but kernicterus occurred in only 20% compared with the expected 50-60%.[145] Protection was less sure when infection was present.[145] Bowen et al[146] showed that puppies were protected from a toxic dose of bilirubin by simultaneous injection of albumin. Rozdilsky,[147] however, found that when he injected bilirubin-albumin solutions intravenously into rats, rabbits or dogs, high plasma bilirubin levels resulted and three of the 51 animals used died in shock with in 24 hours. Apparently bilirubin may still be toxic even when attached to albumin. There has also been some work done to show

that when albumin is given along with an exchange transfusion the amount of bilirubin removed is increased.[130,148-150] In the face of these contradictory findings, it is difficult to recommend albumin for treatment of hyperbilirubinemia, especially since there is the danger of precipitating heart failure in the loading of albumin.

Steriod treatment is advocated by some,[151] but no systematic studies have yet been done to prove its usefulness. Adequate trials of ACTH in J.D.H. and L.T. produced neither significant changes in the patients nor in their jaundice.[1]

Cremer et al[151a] have recommended blue light exposure as treatment because it may decrease as much as 30% of bilirubin levels in vitro. It is also true that J.d'E.'s jaundice is noticably less in the summer.[11] However clinical trials have had contradicting results. Those by Blondheim et al[152] and Franklin[153] showed no significant effects, whereas those by Ferreira et al[154] showed favorable results. This form of treatment needs also further investigation because of the fear that bilirubin may be converted into an even more dangerous substance.[153] Blondheim et al[155] suggest a more diffusable "bilirubinoid" might be produced which has some of the properties of dissociated bilirubin and therefore potentially more dangerous than albumin bound bliriribin. Thus the use of ultra-violet light is an interesting but questionable method of treatment.

The use of glucuronic acid has been much debated.[156-158] because the role of glucuronic acid intrahepatic or extrahepatic bilirubin conjugation is still under investigation as discussed in section under Review of Bilirubin Metabolism. The results of in vivo administration of glucuronic acid, however has not been encouraging. Infants with unconjugated hyperbilirubinemia due

to erythroblastosis or physiologic jaundice were found to have lowering of serum bilirubin level when glucuronic acid was infused.[159] These findings were confirmed by experiments in the Gunn rats, but it was further noted that the drop in serum bilirubin did not prevent kernicterus and was usually accompanied by increased icterus of body fat.[160] These latter findings suggested that the fall of serum bilirubin observed, might be attributable to a shift of pigment to the tissues, especially in the light of present work on the total miscible bilirubin pool which indicate that it is five to six times plasma bilirubin content.[26] Moreover, Hsia[61] found no evidence of conjugation when glucuronic acid was administered to dogs; and Schwob et al[161] retracted their claims for the therapeutic value of glucuronic acid because they have obtained similar effects for intravenous infusion of glucose-saline.

Other attempts to activate glucuronide conjugating activity by using anthranilate,[162] and N-acetyl-p-aminophenol[163] have been unsuccessful. Triiodothyronine has also been tried but the effect was insufficient to be of therapeutic value.[164,165]

In the light of the recent studies on the absorption and excretion of bilirubin in the gastrointestinal tract, mentioned earlier, a possibly new method to treat hyperbilirubinemia of indirect type may be based on the binding of the unconjugated bilirubin excreted into the gut.[77] If unconjugated bilirubin was removed in the gut from the equilibrium established between the bilirubin pool of the gut and that of the plasma, then plasma bilirubin would move continuously into the gut, until another equilibrium is established. A resin cholestyramine, which was found to exhibit a high affinity for bilirubin was given orally to Gunn rats by Lester, Hammaker, and Schmid.[77] Serum bilirubin concentration decreased abruptly and returned to pretreatment levels on withdrawal of the resin. Similar studies were done on a CFNJ

patient, J.d'E.. So far the results did not indicate that the resin lowered the patient's bilirubin level significantly.[78] More extensive studies are planned in this same patient, and in others.[78]

Thus it is evident that at the present there is no recommendable treatment for patients with this disease, except for prophylactic measures to prevent the occurrence of kernicterus (namely : ? )

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