MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

January 13, 1966

UNCONJUGATED HYPERBILIRUBINEMIA

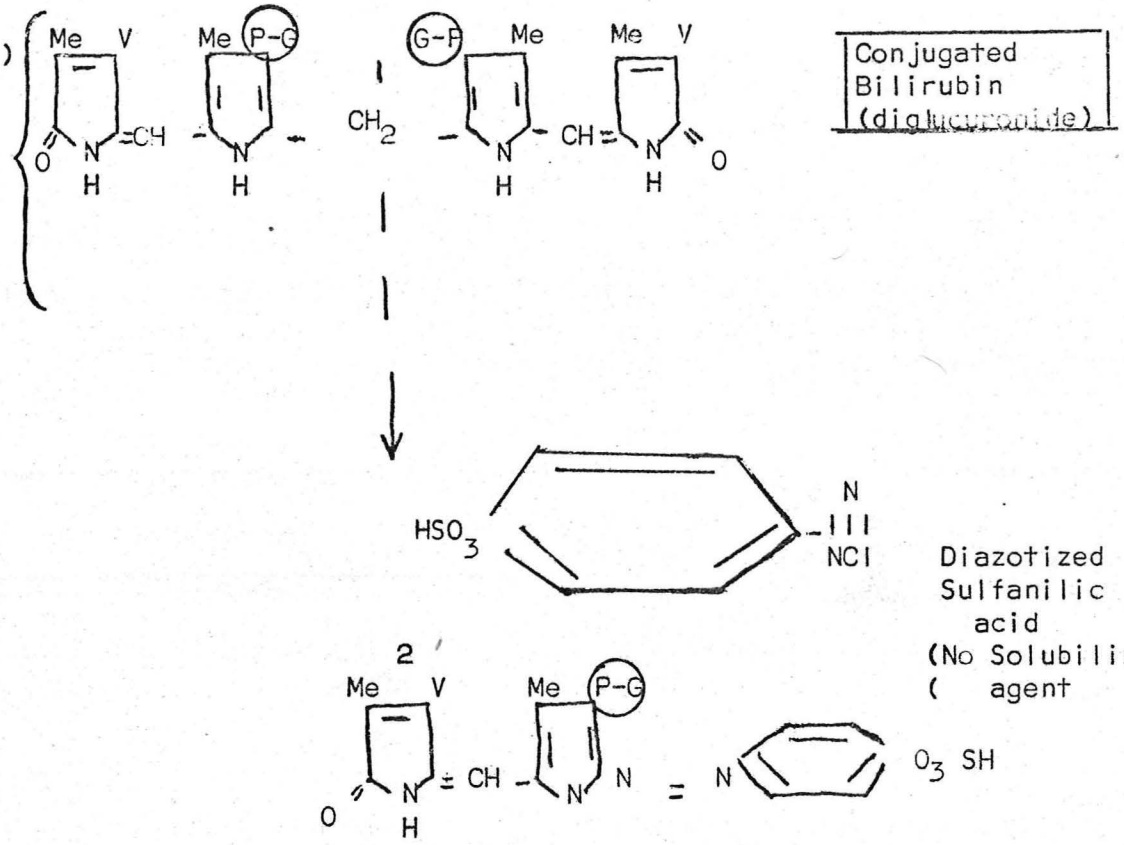
Unconjugated Hyperbilirubinemia

1. Definition
2. Bile Pigment Metabolism
3. Clinical Spectrum (characteristics and mechanism)

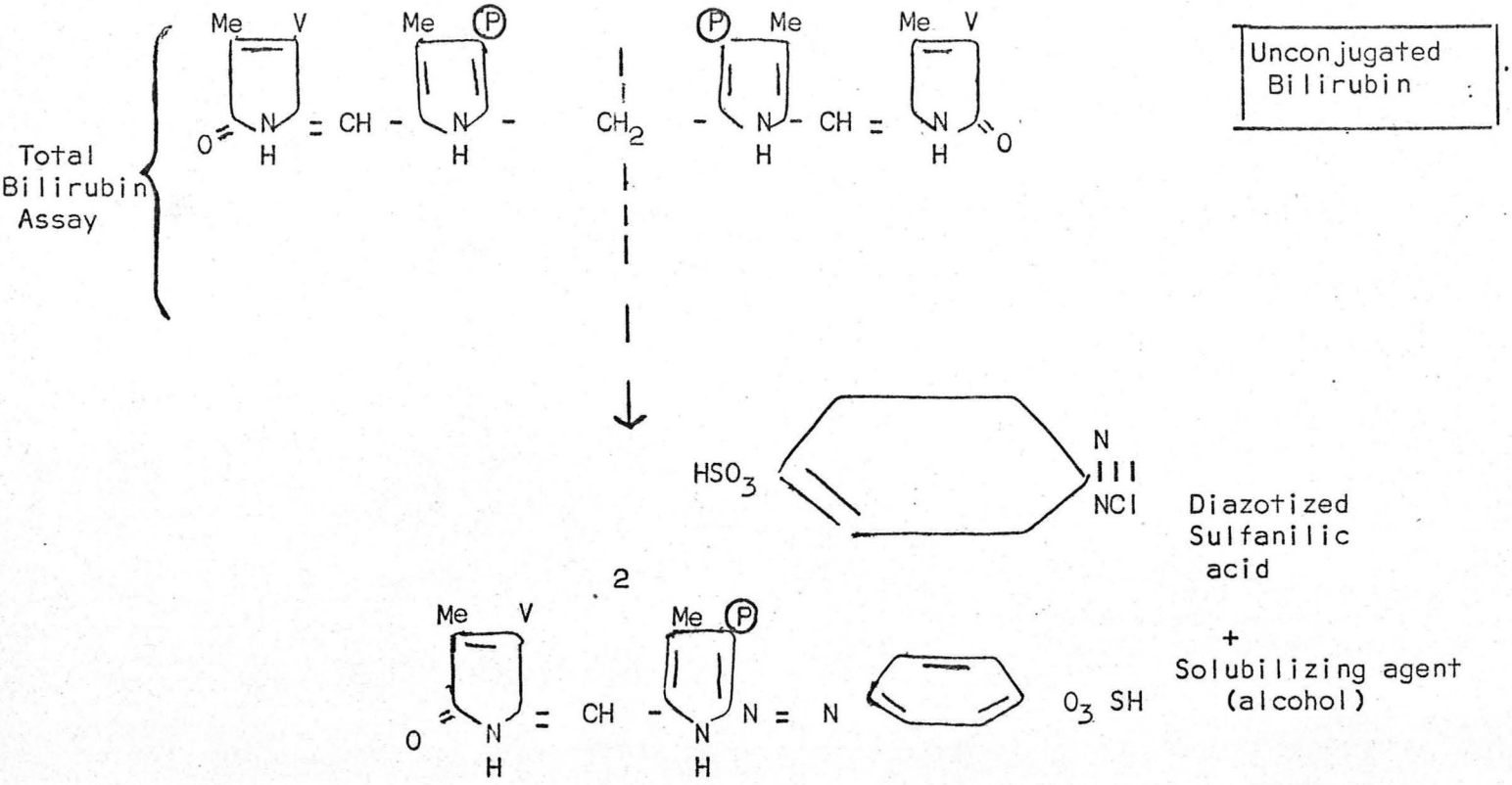
Definition: Serum indirect-reacting bilirubin in excess of 1.2 mg% (1) and direct-reacting fraction less than 20% of total bilirubin (1,2,3,4), as determined by Ducci-Watson modification (5) of the standard diazo reaction (6).

Methodology of bilirubin determination: Diazo Reaction - (Van den Bergh test)

Direct (one-minute) bilirubin assay)



Red color read in 1 minute at 540 μ gives approximate concentration of conjugated bilirubin.



Red color develops over 15-30 minutes. Read at 540 μ. Will give sum of one-minute and indirect bilirubins - accuracy ± 2 %.

Difference between total and direct-reacting fractions gives an approximate measurement of unconjugated bilirubin.

Diazo reaction is specific for bilirubin, only mesobilirubin (bilirubin whose vinyl groups are reduced to ethyl) and monopyrroles react similarily (7).

Caffeine Benzoate } - accurately indirect bilirubin to reset as direct bilirubin

Normal bilirubin values (1) 719 normal male subjects - Age 30.3 ± 5.2 years - Av. wt. 162 ± 24 lbs.

(a) Direct-reacting bilirubin

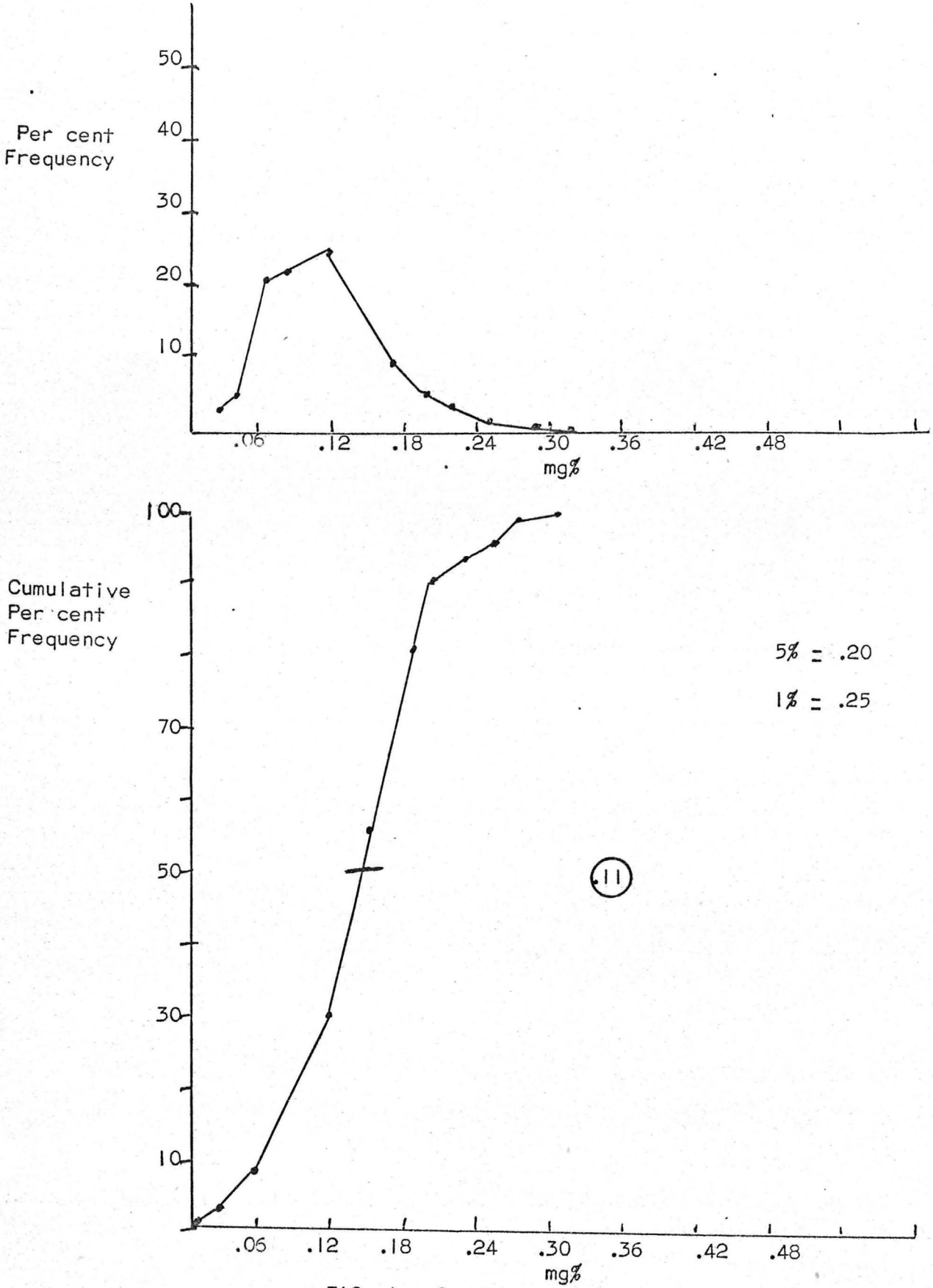


FIG. 1 One-minute (direct-reacting) bilirubin.

Note: This measurement in normal individuals may be artifactual since prior chemical extractio of unconjugated fraction from serum gives no direct-reaction on residual serum (8).

(b) Total bilirubin

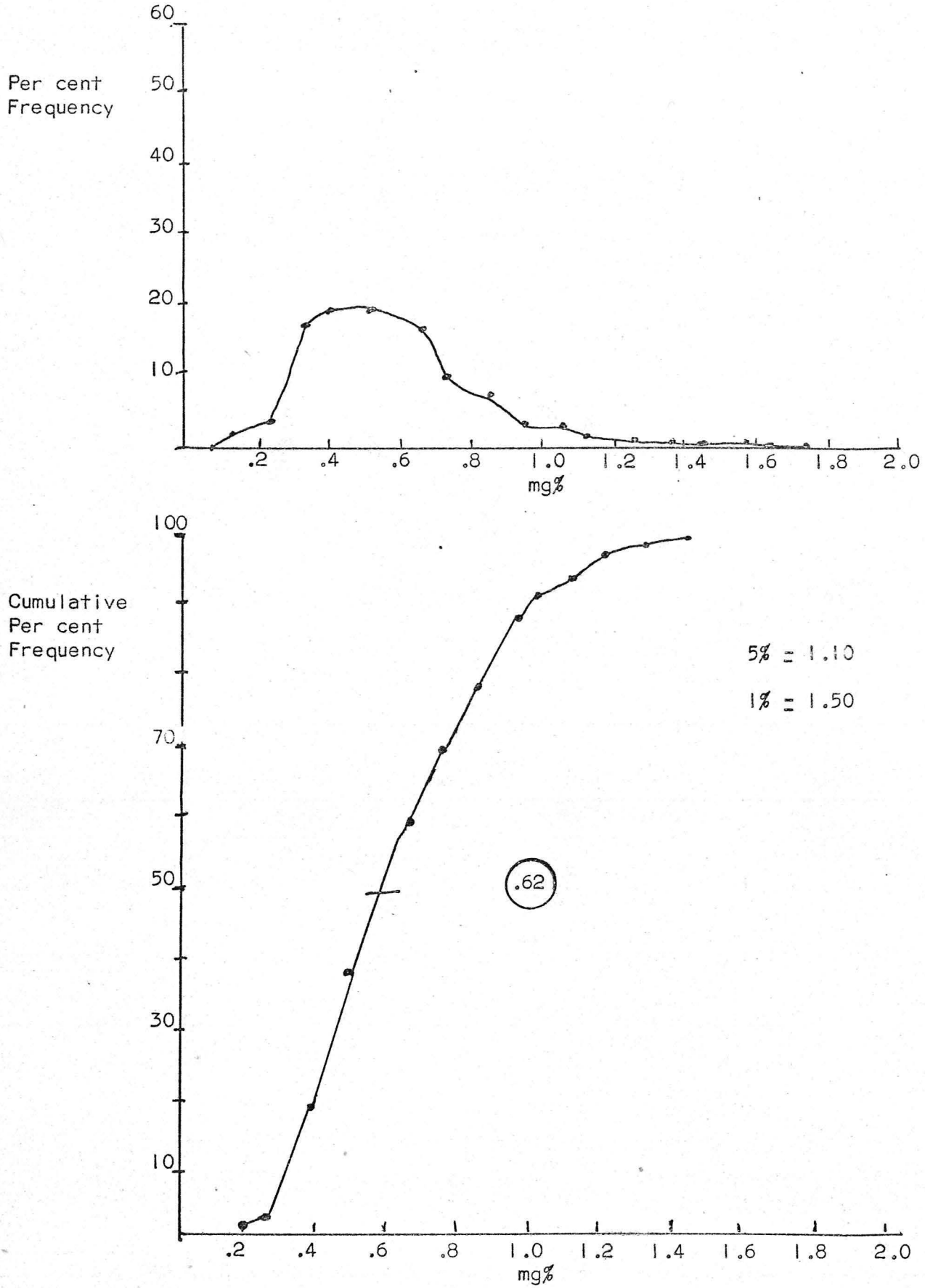


FIG. 2 Total bilirubin

Thus any indirect bilirubin value greater than 1.2 mg% may be considered abnormal.

Direct-reacting fraction in unconjugated hyperbilirubinemia: Pertinent comments

a) Less than 3% of indirect bilirubin diazotizes directly (artifact of method) in absence of alcohol, as shown in vitro (2).

b) Analysis of proven cases of unconjugated hyperbilirubinemia has shown that up to 20% of total bilirubin may react directly, though usually less than 15%.

c) Lowest values, < 5%, are seen in infants with unconjugated hyperbilirubinemia due to defective conjugating apparatus (physiologic jaundice, breast-milk jaundice, Crigler-Najjar, etc.)

d) Highest values, 20%, in hemolysis. With total bilirubin of < 4.0 mg%, the direct fraction may even reach 30% of total. Cause of rise ? - presumably due to regurgitation of the increased pigment load (2).

e) In absence of associated liver disease, hemolysis almost never gives a direct-reacting bilirubin over 1.2 mg%. In presence of such liver disease, direct-reacting fraction may rise substantially both in absolute terms and as per cent of total (2).

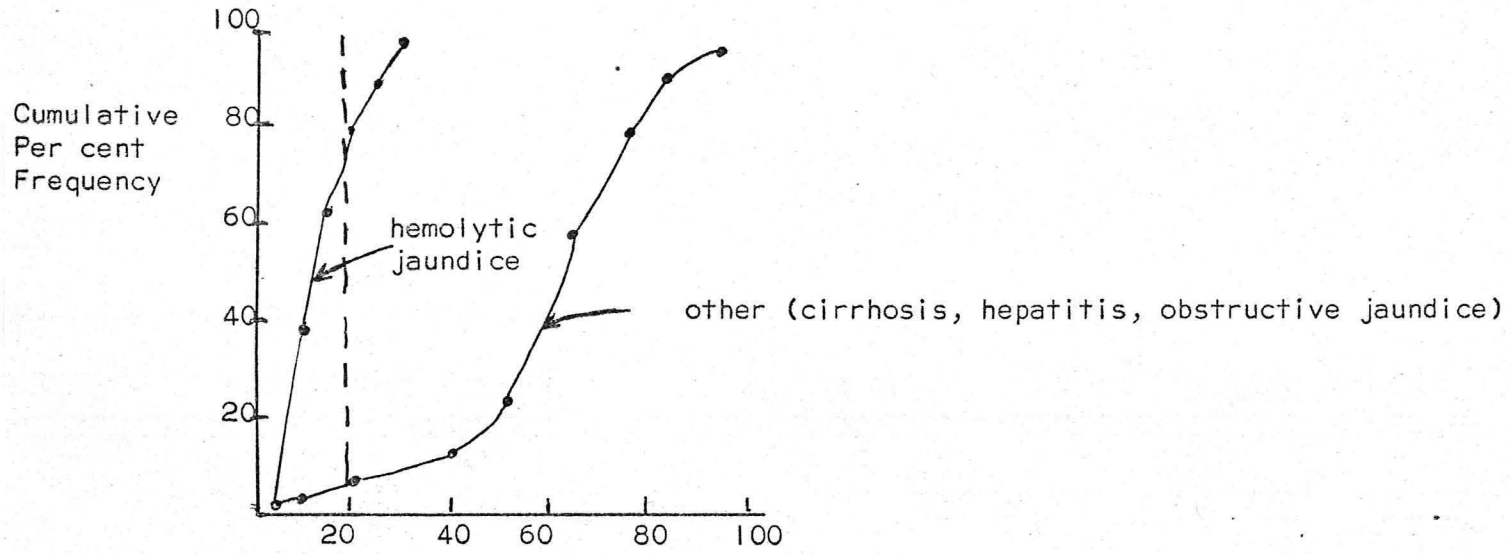


FIG. 3 Direct/Total bilirubin, %, in jaundice states

Hemolytic jaundice - 23 patients
Other jaundice - 326 patients (1)

Conclusion: Unconjugated hyperbilirubinemia:

a) indirect bilirubin > 1.2 mg%
b) direct-reacting fraction < 20% of total bilirubin

Normal Bilirubin Metabolism in Adults

I. Bilirubin Production

Sources

Major: Hemoglobin of circulating senescent erythrocytes. Accounts for about 80% of bilirubin produced.

Quantitative note: In 60 Kg adult with RBC life span of about 120 days - about 7.5 gms Hgb released/day - resulting in 250 mg unconjugated bilirubin/day, assuming 100% conversion. With large quantities of Hgb (exceeding haptoglobin binding) there may be conversion to bilirubin of only 63-80% (9). Alternate products are not known.

Minor:

a) liver hemes, possibily catalase (10,11,12)
b) heme, or its precursors, of newly formed erythrocytes catabolized in the bone marrow prior to release into circulation or shortly thereafter (10). These account together for about 10-18% of bilirubin produced.
c) ? myoglobin and other hemes - quantity unestablished.

These minor sources add about 50 mg = total 300 mg/day.

Mechanism of Heme Catabolism: Sequential steps for Hgb catabolism are controversial (13). Iron and globin split off and reutilized, ring opened to form bilirubin. Mechanism of conversion of hemes to bilirubin also not certain.

Sites of Hgb Catabolism: Reticuloendothelial tissue of bone marrow, liver and spleen. Relative contribution of each of these sites for catabolism of senescent RBC's *in vivo* is unknown. In rats, infused Hgb is cleared primarily by liver (71%), and the rest by bone marrow (22%) and spleen (7%) (14).

Rate of Hgb Catabolism: Rapid. Mean interval between sequestration of injected labeled Hgb and appearance of C^{14}-bilirubin in bile is about 3 hours (9).

II. Bilirubin Transport from Sites of Formation into Bile.

In plasma unconjugated bilirubin is attached to albumin - maximal binding capacity is 2 moles of bilirubin per mole albumin (15). This corresponds to bilirubin concentrations of 60-80 mg%.

Across liver

Uptake by Liver: Bilirubin probably is dissociated from its albumin carrier prior to uptake by liver (13,16) since albumin:

a) enters liver cells more slowly
b) has a different hepatic subcellular distribution
c) is quantitative insufficient to bind all hepatic bilirubin

It is not known if the uptake process is energy-dependent or even carrier mediated.

Intrahepatic conjugation: Intrahepatic unconjugated bilirubin is rendered water soluble to allow biliary excretion. This is accomplished by complex series of steps wherein glucuronic acid is transferred enzymatically from uridine diphosphate glucuronic acid to bilirubin (80%). In addition, about 10-15% of unconjugated bilirubin may be conjugated with sulfate (17) (although there is disagreement about this for man (18)) and the remainder as other unknown conjugates. Bilirubin conjugation is energy-dependent.

Excretion into bile: Little known about transfer of conjugated bilirubin from liver into bile. Conjugated bilirubin is secreted against a high concentration gradient and exhibits a transfer maximum - features suggestive of an active process.

III. Disposition of Bilirubin Excreted into Intestine

Conjugated bilirubin excreted via bile into intestinal lumen in man does not undergo intestinal absorption to any significant extent and in limited studies is not hydrolyzed to the unconjugated pigment, which is absorbable across biologic membranes (19). Thus conjugation:

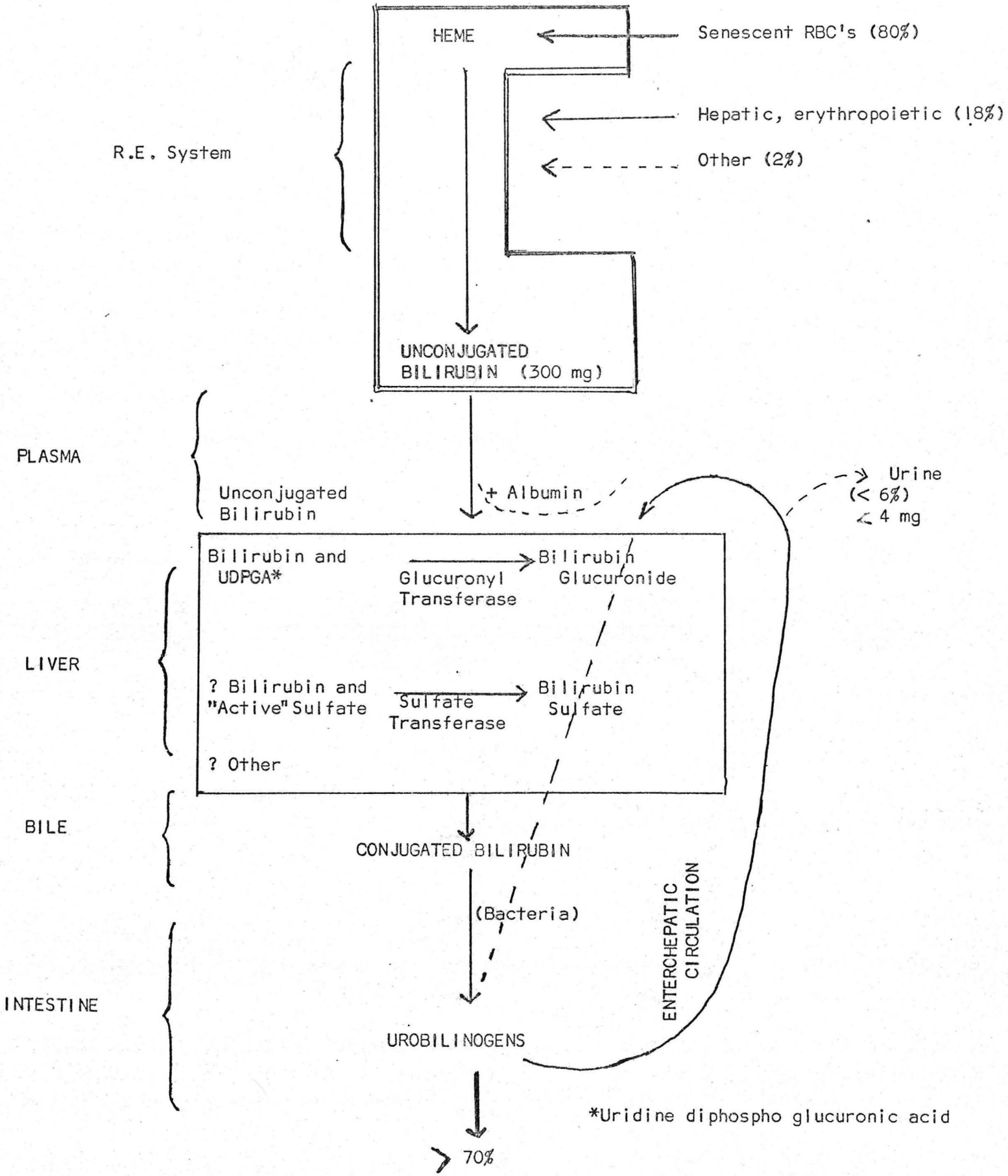
a) allows excretion of bilirubin into bile
b) prevents significant enterohepatic circulation of bilirubin.

In the intestinal lumen conjugated bilirubin is reduced by bacterial action to urobilinogens and their oxidation products, the urobilins. The major part of these are excreted in the stool and are measured as fecal urobilinogens. Less than 30% of the urobilinogen is absorbed across the intestinal mucosa and, in absence of liver disease most is excreted in bile. A small fraction, less than 6%, (< 4 mg) is excreted in urine (2). This urinary fraction rises in presence of liver disease which impedes the enterohepatic circulation of urobilinogen.

Conclusion from above: possible mechanisms of unconjugated hyperbilirubinemia:

1) Increased production of bilirubin
2) Decreased hepatic uptake, decreased transport to conjugating site in liver and/or decreased conjugation of bilirubin.

FIG. 4 Normal Bilirubin Metabolism



CLINICAL SPECTRUM

Pediatric Unconjugated Hyperbilirubinemias

1. "Physiologic", transient, jaundice of newborn.

Definition:

a) Transient hyperbilirubinemia of newborn within pigment values set down below.
b) No obvious cause of jaundice other than hepatic functional "immaturity".

	ONSET		PEAK*†		OFFSET	
	Day	Bilirubin	Days	Bilirubin	Days	Bilirubin
Full Term	Hours	1.8 mg%*	2-5	2-12 mg%	7	< 2 mg%
Premature	Hours	1.8 mg%*	2-6	15 mg%**	7-14	10-5 mg%

* Cord blood. During fetal life bilirubin is cleared via placenta and excreted by mother (28).

** Occasionally much higher and may lead to kernicterus.

*† Bilirubin usually rises no more than 5 mg%/day. Values in excess of these or faster rate of climb should suggest another additional etiology (21).

Incidence: Jaundice: 70-80%
Hyperbilirubinemia - all

Mechanism: Inefficient glucuronide conjugating system in liver (22).

Glucuronyl transferase ↓ 85% (rate limiting)
UDPG dehydrogenase ↓ 70%

Cause of this - unknown. Possibilities:

a) Hormonal: pregnancy hormones inhibit bilirubin conjugation *in vitro* (23) but maternal liver shows no impairment of conjugation (24) and fetal conjugation improves during gestation as hormone concentration rises.
b) Functional "immaturity": more likely explanation. Many other hepatic enzymes also increase with development.

Conjugation defect may be augmented by bilirubin overproduction (hemolysis) or greater impairment of conjugating capacity by factors such as hypoxia, starvation, etc.

Quantitative note: Assuming normal adult RBC life span for the newborn (it is probably somewhat shorter ~ 100 days (25)) one can calculate a daily production of about 17 mg bilirubin/3 Kg infant. Since this quantity leads to jaundice and adult liver can handle about 800-1000 mg/day - the newborn liver capacity for bilirubin metabolism is only about 2.5% of the adult.

Clinical Aspects: Danger of bilirubin encephalopathy (kernicterus) at high levels.

Mechanism of encephalopathy: Lipophilic unconjugated bilirubin penetrates into brain where it is cytotoxic, probably by affecting phosphorylation and intracerebral energy metabolism (26,27).

Prognosis: Excellent, in absence of kernicterus.

Rx: Exchange transfusions in appropriate cases of very severe hyperbilirubinemia.

2. Transient, familial, neonatal hyperbilirubinemia (Lucey-Driscoll Syndrome) (29).

Definition:

a) Serum bilirubin greater than in physiologic jaundice.

b) No obvious precipitating cause for ↑ bilirubin.

c) Transient.

d) Familial.

e) Associated with factor(s) in serum inhibitory to conjugation of bilirubin in vitro.

Clinical Aspects: 24 children - 8 familes; equal sex ratio.

Hyperbilirubinemia

Onset: Hours after birth

Peak: 3-7th day (8.9-65 mg%, mean 25.4 mg%; <5% direct-reacting)

Duration: 7-15 days

Prognosis: Kernicterus 3/24
Cerebral palsy 1/24

Rx: Exchange transfusions

Family History:

a) Syndrome seen in all siblings.

b) Parents, other relatives and their children unaffected.

c) No consanguinity among parents.

Conclusion: Familial but not hereditary.

Mechanism: Increased inhibitor of bilirubin conjugation by factor(s) present in maternal and neonatal serum, as shown by in vitro assay.

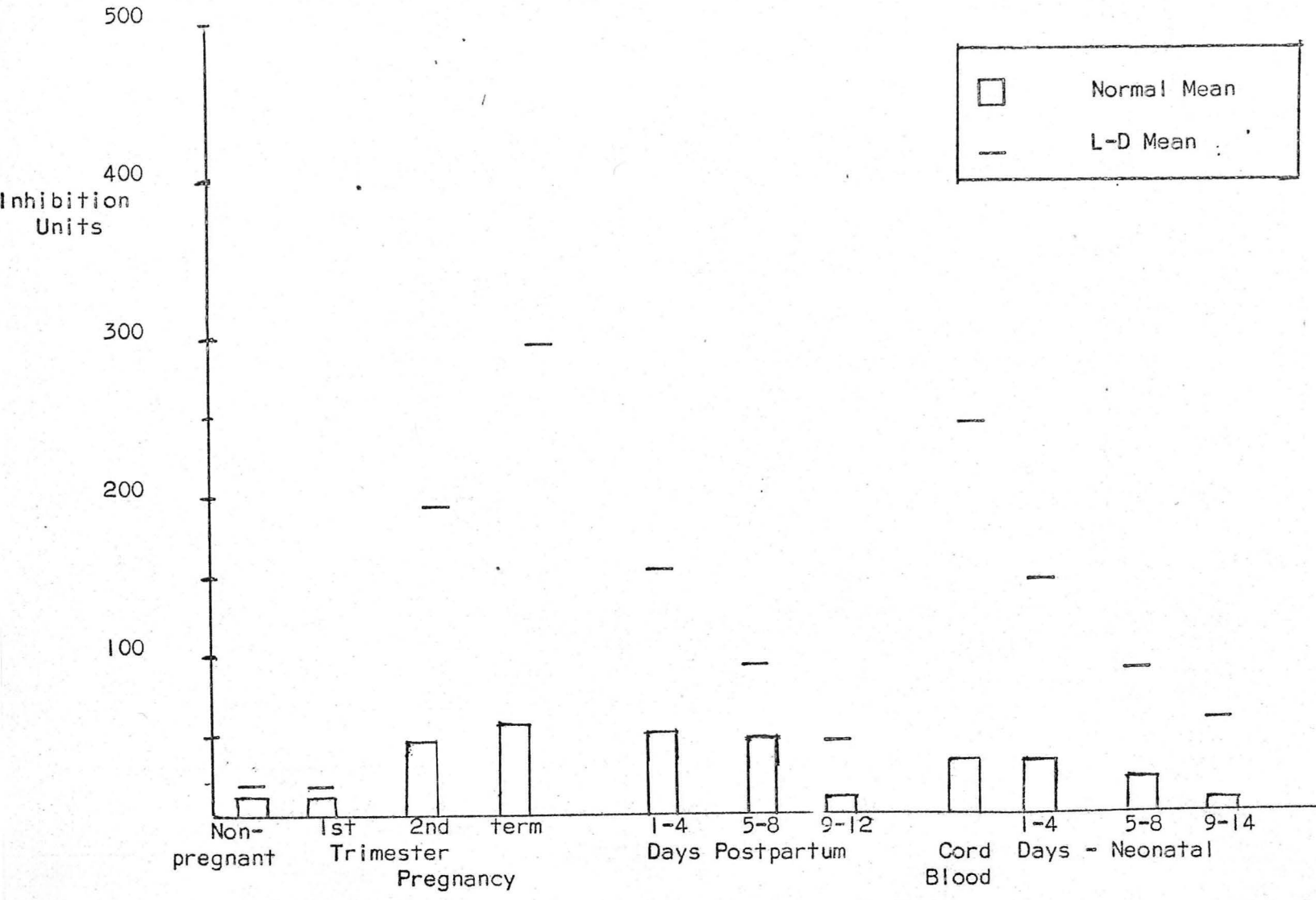


FIG. 5 Inhibition of Bilirubin Conjugation in Lucey-Driscoll Syndrome

Comments about inhibitor(s):

a) Nondialyzable, heat and cold stable and temporarily associated with pregnancy - probably progestational steroid. Hsia isolated pregnane-3(α),20(α)-diol from pregnancy serum and showed competitive inhibition of bilirubin conjugation in vitro with hepatic microsomes (23). This is unconfirmed with human liver slices (30) but has been shown with an unidentified serum steroid.

b) Factor is probably of maternal origin since maternal levels higher than those in cord blood

c) It is not known if the increased inhibitory effect, as compared with normal, is on qualitative or quantitative basis.

3. Breast Milk Hyperbilirubinemia

Definition:

a) Serum bilirubin elevation persists longer than in physiologic jaundice or exhibits 2° rise.

b) No obvious precipitating causes for ↑ bilirubin.

c) Transient (but more prolonged than Lucey-Driscoll)

d) ? Familial

e) Associated with breast feeding and remits gradually on its discontinuation.

f) No increase in serum factor(s) inhibitory to conjugation of bilirubin.

g) Presence in breast milk of factor(s) inhibitory to bilirubin conjugation.

Clinical Aspects: At least 19 instances reported since 1963 (31,32,33). Equal sex incidence.

Hyperbilirubinemia

Onset: 7-14 days

Peak: 10-19 days (14.3-24.5 mg%; < 10% direct-reacting)

Duration: 2-6 weeks with continued breast feeding; 3-6 days with cow's milk

Prognosis: No kernicterus - benign course, ? due to late onset and maturation of blood-brain barrier.

Family History:

a) No single ethnic group

b) Parents, other relatives and their offspring unaffected

c) In one group of 13 siblings - 6 breast-fed and 5 had prolonged jaundice; 6 bottle-fed - none had jaundice.

Conclusion: ? Familial but not hereditary

Mechanism: Inhibitor of bilirubin conjugation demonstrated in breast milk fed to affected children.

Comments about Inhibitor:

a) Average inhibition with affected breast milk (30-70%)

b) Colostrum is not inhibitory

c) Pregnane-3(α), 20(β)-diol isolated from the affected milk and shown to inhibit bilirubin conjugation both in liver microsomes in vitro and on feeding (1 mg/day) to newborn infants (35).

d) Maternal serum is not inhibitory and source of hormonal inhibitor in breast milk is unknown.

Rx: ? Stop breast-feeding

4. Congenital familial nonhemolytic jaundice (Crigler-Najjar Syndrome) (35,36,37,38)

Definition:

a) Severe icterus noted within 3 days of birth

b) Icterus persists for life

c) No obvious cause for jaundice

d) Familial

e) Genetically determined

f) Liver shows persistant inability to form bilirubin glucuronide in vitro and has decreased capacity to form other glucuronides in vitro and in vivo.

g) Absence of serum inhibitor of conjugation

h) Not related to breast feeding

Clinical Aspects:

Hyperbilirubinemia

Onset: Within 3 days of birth

Peak: None - levels of 18-45 mg%, all unconjugated, persist for life.

Prognosis: About 20 cases described in literature. Most died with kernicterus or its sequellae within 15 months. A few survive with severe neurological damage. Two with bilirubin levels of about 20-30 mg% are well at 6 and 36 years.

Laboratory Findings:

a) No evidence of liver abnormality chemically, histologically (other than ↑ in bilirubin) or radiographic (cholangiogram)

b) Bile pale yellow and contains little or virtually no conjugated bilirubin

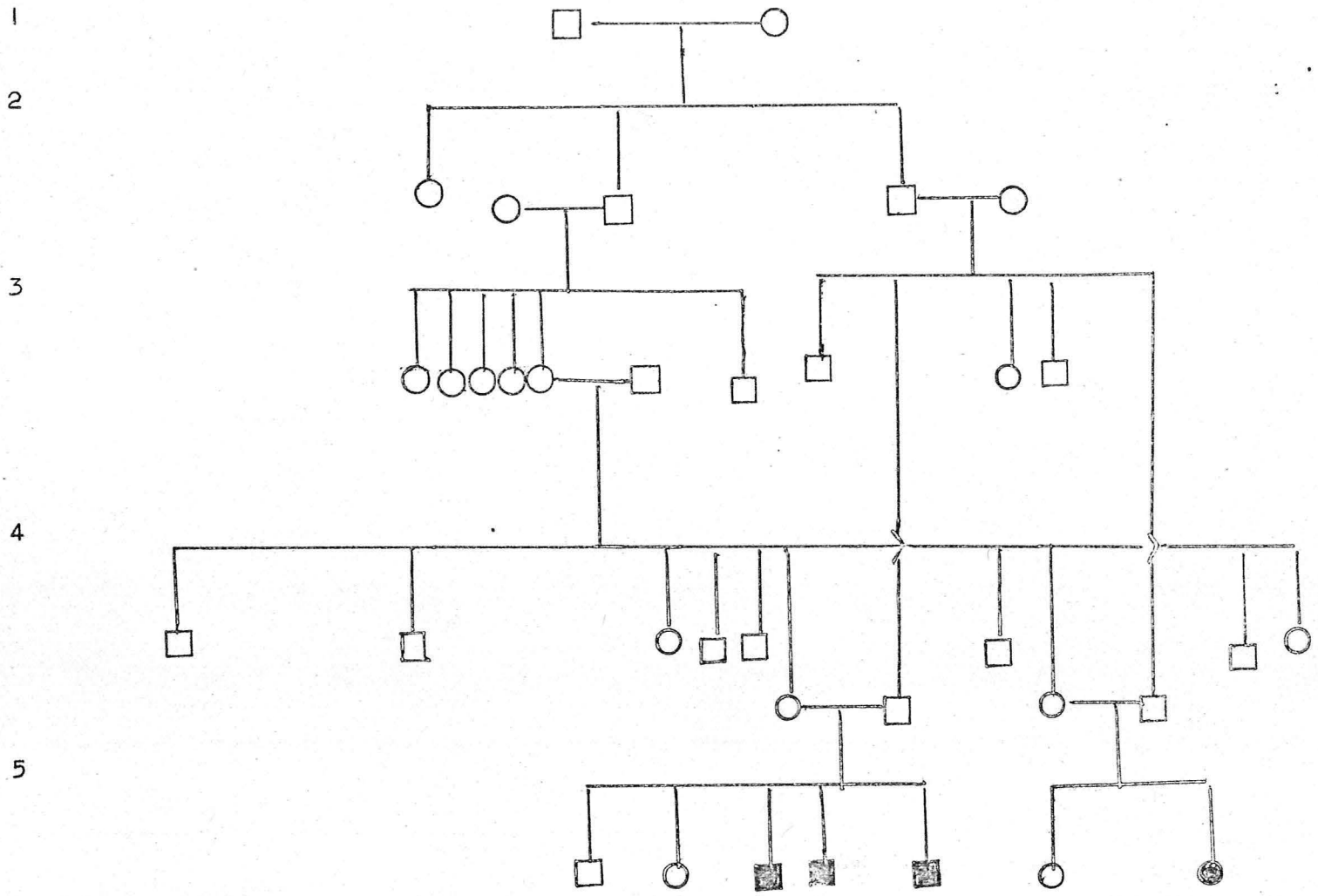
c) Fecal urobilinogen greatly reduced

d) Decreased capacity to conjugate exogenous test substances (menthol, N-acetyl-p-amino-phenol, tetrahydrocortisone) with glucuronide.

Family History:

a) Original 7 children were members of 3 related families stemming from common ancestors. Other cases reported in more than 1 member of a family.

b) Typical family pedigree (39) - (See following page)



c) Since parents exhibit no hyperbilirubinemia - the full defect has been postulated as due to recessive gene with the affected individuals homozygous. However, some parents exhibit a mild abnormality in forming glucuronides suggesting that the single gene (heterozygous) may give incomplete dominance with respect to glucuronide conjugation. This last point is controversial.

Mechanism: Impaired capacity to form glucuronides, especially for bilirubin.

Note: Maintenance of stable serum bilirubin level in patients who survive beyond infancy accomplished by biliary and transintestinal excretion of breakdown products of bilirubin or in latter instance unconjugated bilirubin (40).

Pediatric and Adult Unconjugated Hyperbilirubinemia

I. Hemolysis

Definition:

1) In adults, indirect-reacting bilirubin usually < 5 mg%, unless associated liver disease present. In infants, the associated defect in conjugation causes much higher values, often > 20 mg% (41).

2) Evidence of accelerated RBC destruction:
↓ RBC life span; ↑ fecal urobilinogen, ↑ urinary urobilinogen (in absence of liver disease) ↑ plasma Hgb hemoglobinuria, (+) Coombs test, hemoagglutinins, abnormalities in RBC shape (spherocytes, sickling, etc.); ↑ osmotic and mechanical fragility (42). Splenomegaly may be present.

3) Evidence of accelerated bone marrow activity:
↑ absolute reticulocyte count; ↑ iron turnover and incorporation into RBC's; erythroid hyperplasia of bone marrow (43).

4) Anemia may or may not be present (compensated hemolysis) depending on balance between destruction and production of RBC's (44,45).

Family Hx: May or may not be positive, depending on nature of hemolysis.

Symptoms and Prognosis: Related to degree of disease.

Mechanism: Is it all overproduction of pigment or is there an associated hepatic defect?

1) Over production?

a) Crosby suggests that maximally 45 g Hgb/day → bilirubin 1500 mg/day (48). Based on analysis of blood Hgb and bilirubin values in patients given massive blood transfusions. Data in abstract form - not susceptible to analysis.

b) Bone marrow acutely may increase its effective Hgb production X 3 (approximate) and in chronic states X 6. This latter would release about 1500 mg bilirubin/day, assuming complete conversion of destroyed Hgb → bilirubin. Data derived from measurement of maximal rate of hemolysis which can be compensated for by marrow activity (44). In chronic states theoretically additional bilirubin (? amount) can be released from RBC's, their precursors or heme precursors in bone marrow or liver (shunt).

c) Capacity of normal human liver to metabolize bilirubin

(1) From infusion of bilirubin into man, velocity constants for bilirubin removal from plasma estimated - suggest that threefold rise in bilirubin production will cause slight bilirubin retention (~ 2 mg%). A sixfold rise in bilirubin production (compensated hemolysis - RBC life span 20 days) should raise serum bilirubin to about 3.7 mg% (46).

(2) These calculations supported by bilirubin levels actually obtained in man after infusion of bilirubin (47) or Hgb (48)*. (See following page).

* FOOTNOTE - From Page 14 - 1), c), (2)
Caution: a) Data based on single rapid infusions and not strictly comparable to in vivo bilirubin synthesis.
b) Clearance of bilirubin from blood reflects all net transfer to and from all tissues, not only hepatic excretion.
Data are still valuable as only available index of ability of normal human to handle bilirubin.

Conclusion:

(1) Severe hemolysis alone may result in hyperbilirubinemia.

(2) With mild hemolysis an associated liver abnormality probably has to be implicated. Its nature is speculative, but may involve a ↓ in glucuronyl transferase.

2) Is there associated liver disease?

a) Three reports of decrease (50-65%) in glucuronyl transferase from patients with hemolysis, using bilirubin as acceptor substrate (49,50).

b) Six tested cases, one with bilirubin as substrate, showed no decrease in transferase (49,63,4). No apparent correlation between severity and length of hemolysis, degree of hyperbilirubinemia and any change in transferase. Significance of decrease is therefore questionable.

c) There are cases of hemolysis where ↑ bilirubin is much greater than would be expected as a result of hemolysis alone (45).

d) Generally higher bilirubin levels seen in chronic hemolysis - anoxia may contribute.

2. "Shunt" Hyperbilirubinemia

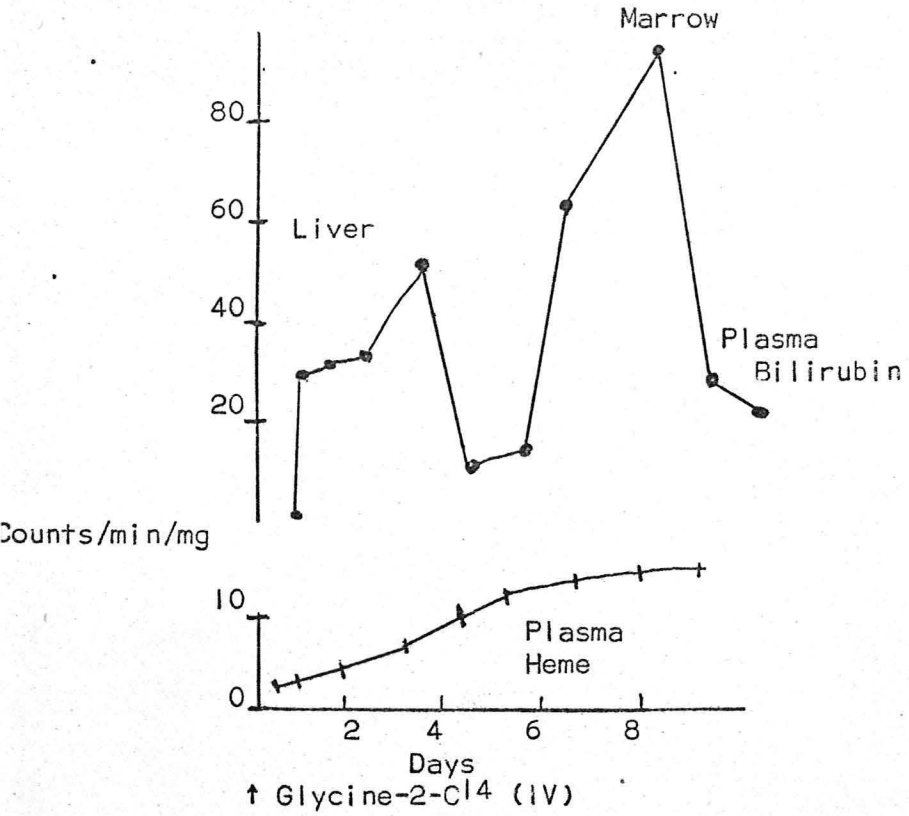
Definition: ↑ Bilirubin formation from sources other than circulating RBC's.

Concept of Shunt: Normally:

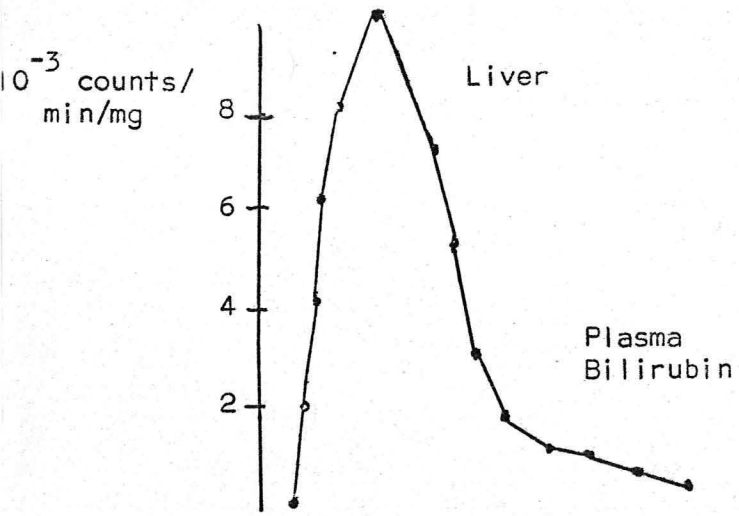
a) About 15% in adult (51) and 22% in infants (25) of bilirubin produced is derived from sources other than senescent erythrocytes.

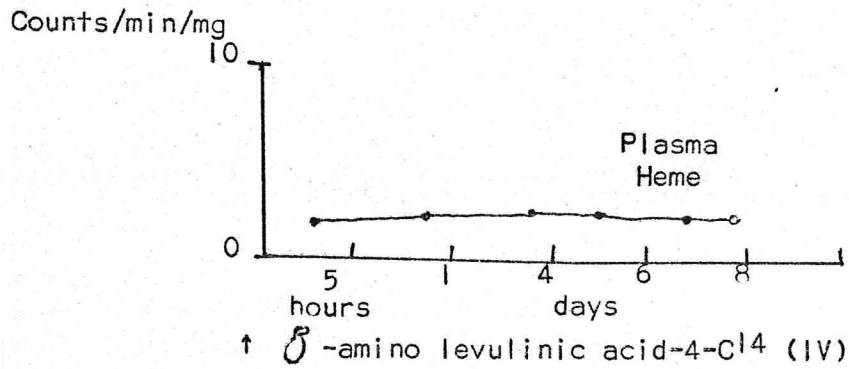
b) There is a dual origin of this bilirubin (called early labeled peak (ELP) since it arises early in the incorporation of exogenous label into circulating erythrocytes. The first part of the early labeled bilirubin (first day after administration of label) comes from heme and its precursors in the liver. The second part (3-5 days after administration of label is derived from heme precursors and red cell heme in bone marrow, i.e., before release of the erythrocytes into circulation (52,53,54,55).

c) The exact proportion of bilirubin derived from each site in man is not known; in the rat most originates from liver (11) and in the dog - 2/3 from marrow (55).



↑ Glycine-2-C^{14} (IV)





↑ δ-amino levulinic acid-4-C^{14} (IV)

FIG. 6 Early Labeled Bilirubin - Site of Origin

In disease states:

Erythropoietic disorders

a) In many primarily hematologic disorders as thalassemia, pernicious anemia, refractory normoblastic anemia, extramedullary hemopoiesis, congenital erythropoietic porphyria, etc., the early labeling peak may be greatly increased (40-80% of total pigment excretion) (62).

b) These diseases in general are characterized by ineffective erythropoiesis, i.e., there is a discrepancy between quantity of RBC's reaching peripheral circulation and quantity of heme synthesized by bone marrow (56,54). In this state, although total erythropoiesis may be increased, most of synthesized heme is destroyed in situ and does not reach peripheral circulation or survives only for hours in peripheral circulation. The 3 characteristics of ineffective erythropoiesis are:

1) Fecal urobilinogen ↑ out of proportion to peripheral RBC survival ↓, which may be only slightly ↓.

2) Active marrow erythropoiesis with low absolute reticulocyte count.

3) Increased plasma iron turnover with poor RBC utilization of iron.

Conclusion: Jaundice mechanism same as in classical hemolysis of circulating RBC's except that heme is degraded to bilirubin before RBC's reach peripheral circulation.

Other disorders

a) Six cases reported in 3 families who manifested: ↑ fecal urobilinogen, normal circulating RBC life span, no anemia, no evidence of liver disease, ↑ indirect-reacting bilirubin 1.3 - 4.5 mg%. In vitro tests (2 cases) and in vivo tests (3 cases) of hepatic glucuronide conjugating capacity were normal in 4 and slightly impaired in 1 (57,58). Extent of ELP was isotopically studied in 2 patients and ranged from 5-8 X normal (57,59). Four of six patients had an atypical spherocytosis (postsplenectomy) with normal fragility studies but a slight reticulocytosis and all had ↑ erythropoiesis on bone marrow smears.

Conclusion: These patients without gross blood disorders apparently also have increased bilirubin production from sources other than circulating erythrocytes.

b) Scattered reports of patients with a similar disorder following recovery from viral hepatitis (60,51,58). Inadequately studied to verify inclusion in this group.

Site and mechanism of shunt:

Site:

a) In pernicious anemia both hepatic and erythropoietic, proportion of each not known (53).

b) In atypical spherocytosis primarily erythropoietic (54).

c) Not known for other diseases.

Mechanism(s):

a) Affects orderly heme and/or hemoglobin synthesis in thalassemia

b) Affects preformed hemoglobin incorporation into RBC's in pernicious anemia (53).

Evidence: Measurement of heme/globin specific activity in labeled hemoglobin.

3. Constitutional Hepatic Dysfunction (C.H.D.); Chronic unconjugated hyperbilirubinemia without hemolysis (Gilbert's Disease - Cholemie Simple Familiale (64).

Definition:

1) Hyperbilirubinemia of indirect-reacting type in absence of clinical, laboratory or histologic evidence of hepatic dysfunction, "extrahepatic" diseases which may cause unconjugated hyperbilirubinemia, hemolysis (compensated or not) or shunt hyperbilirubinemia.

2) Negative data must include RBC life span, fecal urobilinogen studies and probably history of viral hepatitis.

3) Many exclude patients with splenomegaly.

4) A positive family history is helpful evidence, especially spanning several generations

Incidence: Even with above rigid criteria - 58 cases over 11 years (3). Many other smaller series in literature (4,65).

Sex and Age of Onset: Sex ratio equal; onset usually early in life (mean 18 years).

Clinical Aspects (3,4,65):

Symptoms: Nonspecific, subjective discomfort, fatigue, dyspepsia pain in region of liver. ? Psychogenic. Symptoms sometimes apparently aggrevated by concomittant illnesses, especially infections, exercise and ? by alcohol intake.

Signs - Icterus

LAB - ↑ bilirubin, less than 10% direct reacting. Levels of bilirubin usually < 5 mg%, some as high as 12, 18 mg% (individual cases - ? definition). Bilirubin levels fluctuated in the same individual over a broad range and at times were normal. Occasionally appeared to be increased by exercise, infection or alcohol. Steroids do not constantly or significantly decrease the bilirubin level (68) but there is an impression it may fall with advanced age.

Histology - Light microscopy normal. Electron microscopy shows alterations in endoplasmic reticulum (microsomal area) but this was found in instances with and without decrease in hepatic glucuronyl transferase (49,4,67) and in patients with Dubin Johnson Syndrome (67).

Prognosis: In absence of iatrogenic disease, excellent.

Rx: None except for reassurance as to lack of chronic liver disease (hepatitis).

Family History:

a) Positive history is obtained in from 25-60% of cases, often spanning 3 generations (38).

b) The greater incidence is seen in the more jaundiced patients. In some instances their parents are anicteric and evidence of abnormal hepatic metabolism can only be brought out by infusion of bilirubin, menthol or *in vitro* assays of hepatic conjugating capacity (4).

Conclusion: Probably genetically determined defect, most likely dominant with varied expressivity.

Mechanism(s):

a) In all patients with severe hyperbilirubinemia (> 5 mg%) 75% ↓ hepatic glucuronyl transferase was found *in vitro* both with bilirubin and other substrates (4) and a lesser impairment of ability to conjugate menthol and especially tetrahydrocortisone (63). The degree of ↓ in transferase in general correlated with ↑ bilirubin. These patients, however, show an almost normal concentration of conjugated bilirubin in bile.

Conclusion: This group with bilirubin > 5 mg% apparently has a partial defect in glucuronide conjugation. The presence of other hepatic defects and relationship of this group to Crigler-Najjar is unsettled.

b) A larger group of patients with bilirubins < 5 mg% has variable findings. Most groups have found normal capacity to conjugate bilirubin and other substrates *in vitro* (4,49) and to handle menthol (4) and NAPA (68) but one group (50) has found a consistant reduction (55%) of transferase in 6 patients with bilirubins between 1.9-4.3 mg%. In addition, in this less icteric group a defect in bilirubin uptake and conjugation by the liver has been postulated from elaborate analysis of bilirubin infusion clearance curves (69) and plasma clearance of radioiodinated iodipamide (70). However, the above analyses regarding bilirubin metabolism are open to question and specificity is questionable since similar findings have been noted in 2 patients with posthepatitic hyperbilirubinemia and one of two cases of hemolysis studied.

Conclusion: Nature of hepatic defect in this group is unknown. Perhaps this is a heterogenous group with multiple hepatic defects, of conjugation and/or uptake of bilirubin.

Posthepatitic Hyperbilirubinemia

Definition:

a) As for constitutional dysfunction except:

1) History of viral hepatitis present.

2) Some slight alteration of hepatic histology (round cell infiltration, fat) may be present.

3) Some alterations in liver function tests (BSP).

4) Negative family history of jaundice.

All these are suggestive differential features from constitutional dysfunction. It may be impossible to make the distinction.

Incidence:

a) 7/350 manifested icterus as sole (71) abnormal finding for 12 months after onset of hepatitis.

b) 18 cases over 11 years (Mayo Clinic) (3).

c) 15 cases (65).

Clinical Aspects:

Symptoms: Indistinguishable from constitutional dysfunction.

Signs: Occasionally hepatosplenomegaly and icterus.

Lab: Bilirubin < 5 mg%, almost all indirect-reacting. Occasional abnormalities in BSP test.

Prognosis: Excellent. Jaundice known to persist as long as 12-18 years after onset of hepatitis without other sequellae (3,73). Transition to chronic hepatitis not reported.

Mechanism(s): Unknown. Normal glucuronyl transferase found on in vitro testing in all cases (4,49) and on in vivo studies with menthol (4). Possibility of hepatic uptake defect exists (69) and in some instances there may be associated hemolysis or shunt hyperbilirubinemia (72).

Miscellaneous Causes:

a) Acquired diseases of various origin: 336 cases/15 years (74).

Incidence ↓

1) Cardiac diseases with or without congestive failure.

2) Hepatobiliary

3) Gastrointestinal diseases

4) Miscellanous

5) Infectious

6) Hematologic

7) No apparent abnormality (idiopathic ? - constitutional) 10%

Hyperbilirubinemia: - 1.5 -4.8 mg% - total
< .25 mg% - direct-reacting

Duration: - Few days - 8 years. Often remitted with recovery from associated disease.

Liver function: Hepatomegaly in 12%
1/3 abnormal liver function
Biopsy abnormal 31/40

Comment: In most instances no detailed study of patients (a retrospective study) hence exclusion of hemolysis, shunt, associated liver disease, etc., often impossible.

Conclusion: Various systemic diseases may be associated with unconjugated hyperbilirubinemia . The mechanism of this is unknown.

b) Orabilex-induced (76)

Hyperbilirubinemia occurring only within 1 day after ingestion of dye. Bilirubin levels < 2.5 mg%, essentially all indirect-reacting.

Mechanism: Unknown. BSP clearance also impaired. ? due to inhibition of uptake or conjugation of bilirubin by dye. Telapaque apparently does not cause this.

c) Post portal-caval shunt (75)

Mild hyperbilirubinemia < 5 mg%, < 30% direct-reacting. Observed in patients with and without hepatic parenchymal disease.

Mechanism: Hemolysis demonstrated with labeled RBC study. Alleviated by splenectomy.

TABLE I

PEDIATRIC AND ADULT UNCONJUGATED HYPERBILIRUBINEMIA - DIFFERENTIAL CHARACTERISTICS AND MECHANISM(S)

	Spleno-megaly	Retics ↑	Fecal Urob. ↑	Anemia	RBC Survival ↓	Abnormal Liver Histology	BSP Retention	↑ RBC Fragility ⊕ Coombs	(+)Family Hx	Hepatitis Hx	Mechanism of Hyper-bilirubinemia
Hemolysis	±+	±+	±+	±+	+	-	-	±	±	-/+	1. Severe cases → overproduction of bilirubin 2. Mild cases → over-production and hepatic defect of ? type
Shunt	±	±	+	-/+	-	-	-	-	±	-/+	As above. Over-production of bili-rubin from sources other than circu-lating senescent erythrocytes
Constitutional	-	-	-	-	-	-	-	-	±+	-	1. Severe cases → ↓ hepatic glucuronyl transferase and ? other defects 2. Mild cases → ? as above and/or de-fect in bilirubin uptake
Posthepatitic*	±	-	-	-	-	-/+	-/+	-	-	+	Unknown. ? Defect in uptake of bili-rubin

* Some cases of posthepatic jaundice show evidence of true hemolysis or shunt hyperbilirubinemia. This is considered then a case of hemolysis in this scheme, although a combination of factors may coexist.

TABLE 2

PEDIATRIC UNCONJUGATED HYPERBILIRUBINEMIA - CHARACTERISTICS

	Hyperbilirubinemia						
	Onset	Peak	Duration	Family Hx	Prognosis	Diagnosis	Mechanism(s)
Full Term Physiologic	Hours	2-5 days 2-12 days	7 days	-	Usually good	1. Characteristic bilirubin pattern 2. Transient 3. No associated disease 4. No family hx	Inhibition of hepatic glucuronide conjugating apparatus
Premature		2-6 days 15 mg%*	7-14 days				
(* May rise higher in premature, especially with hemolysis or other disease affecting conjugation.)							
Lucey-Driscoll	Hours	3-7 days 8.9-65 mg% mean 25.4 mg%	7-15 days	Familial, probably not hereditary	Kernicterus often without Rx	1. ↑ in bilirubin over normal pattern above 2. Familial 3. Serum inhibitor 4. No relation to breast feeding	
Breast Milk	7-14 days	10-19 days 14.3-24.5 mg%	2-6 weeks	As above	Good	1. Delayed onset of jaundice 2. Transient 3. Familial 4. No serum inhibitor 5. Related to breast feeding	
Crigler Najjar	Hours	None 18-45 mg%	Permanent	Hereditary ? Recessive	Kernicterus almost invariably	1. ↑ in bilirubin over normal pattern above 2. Permanent 3. Hereditary 4. Not related to serum inhibitor or breast feeding	

REFERENCES

*1. Normal and abnormal variations and clinical significance of the one minute and total serum bilirubin determinations. Zieve, L., et al., J. Lab. Clin. Med. 38:446, 1951.

*2. The significance of the direct-reacting fraction of serum bilirubin in hemolytic jaundice. Tisdale, W.A., Klatskin, G., and Kinsella, E.D. Am. J. Med. 26:214, 1959.

*3. Constitutional hepatic dysfunction (Gilbert's Disease): Its natural history and related syndromes. Foulk, W.T. and Butt, H.R. Medicine 38:25, 1959.

*4. Chronic unconjugated hyperbilirubinemia without overt signs of hemolysis in adolescents and adults. Arias, I. J. Clin. Invest. 41:2233, 1962.

5. The quantitative determination of the serum bilirubin with special reference to the prompt-reacting and the chloroform-soluble types. Ducci, H. and Watson, C.J. J. Lab. Clin. Med. 30:293, 1945.

6. The determination of bilirubin with the photoelectric colorimeter. Malloy, H.T. and Evelyn, K.A. J. Biol. Chem. 119:481, 1937.

7. Bile Pigments in Health and Disease. Gray, C.H., Charles H. Thomas, Springfield, Ill. 1961, pp. 3-18.

8. Quantitative separation and determination of bilirubin and conjugated bilirubin in human serum. Weber, A. Ph. and Schalm, L. Clin. Chim. Acta 7:805, 1962.

9. The formation of bilirubin from hemoglobin in vivo. Ostrow, J.D., Jandl, J.H., and Schmid, R. J. Clin. Invest. 41:1628, 1962.

*10. The early appearing bilirubin: Evidence for two components. Yamamoto, T., Skanderbeg, J., Zipursky, A., and Israels, L.G. J. Clin. Invest. 44:31, 1965.

11. Bilirubin formation in the liver from nonhemoglobin sources. Experiments with isolated perfused rat liver. Robinson, S.H., Owen, C.A., Flock, E.V., and Schmid, R. Blood 26:823, 1965.

12. The continuing challenge of hemoglobin and bile pigment metabolism. Watson, C.J. Ann. Int. Med. 63:931, 1965.

*13. Bilirubin Metabolism - Medical Progress. Lester, R. and Schmid, R. New Eng. J. Med. 270:779, 1964.

14. The sites of hemoglobin catabolism. Keene, W.R. and Jandl, J.H. Blood 26:705, 1965.

15. The protein-binding of C^{14} bilirubin in human and murine serum. Ostrow, J.D. and Schmid, R. J. Clin. Invest. 42:1286, 1963.

16. Intracellular distribution of tritiated bilirubin during hepatic uptake and excretion. Brown, W.R., Grodsky, G.M., and Carbone, J.V. Am. J. Physiol. 207:1237, 1964.

17. Studies on bilirubin sulphate and other non glucuronide conjugates of bilirubin. Isselbacher, K.J. and McCarthy, E.A. J. Clin. Invest. 38:645, 1959.

18. Studies of conjugated bilirubin. II. Problems of sulfates of bilirubin in vivo and in vitro. Gregory, S.H. and Watson, C.J. J. Lab. Clin. Med. 60:17, 1962.

19. Intestinal absorption of bile pigments. II. Bilirubin absorption in man. Lester, R. and Schmid, R. New Eng. J. Med. 269:178, 1963.

20. Intestinal absorption of bile pigments. IV. Urobilinogen absorption in man. Lester, R., Schumer, W., and Schmid, R. New Eng. J. Med. 272:939, 1965.

21. Neonatal jaundice - a review. Zuelzer, W.W. and Brown, A.K. Am. J. Dis. Child. 101:87, 1961.

22. Studies on neonatal development of glucuronide conjugating system. Brown, A.K. and Zuelzer, W.W. J. Clin. Invest. 37:332, 1958.

23. Inhibition of glucuronosyl transferase by steroid hormones. Hsia, D.Y., Riabov, S., and Dowben, R.M. Arch. Biochem. 103:181, 1963.

24. Hepatic glucuronyl transferase activity and bilirubin Tm in pregnancy in the rat. Shibata, H., Mizuta, M., and Combes, B. Amer. J. Physiol. (In press).

25. The extent of 'shunt' bilirubin and erythrocyte survival in the newborn infant measured by the administration of N^{15} glycine. Vest, M., Strebel, L., and Hauenstein, D. Biochem. J. 95:11c, 1965.

26. Bilirubin, uncoupler of oxidative phosphorylation in isolated mitochondria. Zetterström, R. and Ernster. Nature 178:1335, 1956.

27. Studies of cytotoxic effect of unconjugated bilirubin on brain in vivo. Schenker, S., McCandless, D.W., and Zollman, P.E. (In preparation).

28. Bilirubin metabolism in the fetus. Schenker, S., Dawber, N.H., and Schmid, R. J. Clin. Invest. 43:32, 1964.

*29. Transient familial neonatal hyperbilirubinemia. Arias, I.M., Wolfson, S., Lucey, J.F., and McKay, R.J. Jr. J. Clin. Invest. 44:1442, 1965.

30. Inhibitors of bilirubin conjugation in newborn infant serum and male urine. Holton, J.B. and Lathe, G.H. Clin. Sci. 25:499, 1963.

*31. Prolonged neonatal unconjugated hyperbilirubinemia associated with breast feeding and a steroid, pregnane-3(α), 20(β)-diol in maternal milk that inhibits glucuronide formation in vitro. Arias, I.M., Gartner, L.M., Seifter, S., and Furman, M. J. Clin. Invest. 43:2037, 1964.

32. Hyperbilirubinemia in breast-fed infants. Newman, A.J. and Gross, S. Pediatrics 32:995, 1963.

33. Breast-milk hyperbilirubinemia. Katz, H.P. and Robinson, T.A. New Eng. J. Med. 273:546, 1965.

34. Production of unconjugated hyperbilirubinemia in full-term newborn infants following administration of pregnane-3(α), 20(β)-diol. Arias, I.M. and Gartner, L.M. Nature 203:1292, 1964.

35. Congenital familial nonhemolytic jaundice with kernicterus. Crigler, J.F. and Najjar, V.A. Pediatrics 10:169, 1952.

36. Constitutional nonhemolytic hyperbilirubinemia with findings resembling kernicterus. Jervis, G.A. Amer. Med. Assn. Arch. Neurol. & Psych. 81:55, 1959.

37. Familial nonhemolytic jaundice. Sugar, P. Arch. Int. Med. 108:2, 1961.

*38. Hyperbilirubinemia. Schmid, R. in The Metabolic Basis of Inherited Disease, ed., Stanbury, et al., McGraw Hill, 1960.

39. Glucuronic acid conjugation by patients with familial non-hemolytic jaundice and their relatives. Childs, B., Sidbury, J.B., and Migeon, C.J. Pediatrics 23:903, 1959.

*40. Metabolism and disposition of C^{14}-bilirubin in congenital nonhemolytic jaundice. Schmid, R. and Hammaker, L. J. Clin. Invest. 42:1720, 1963.

41. Bile pigment metabolism. Klatskin, G. Ann. Rev. of Med. 12:211, 1961.

42. The hemolytic states. Crosby, W.H. Bull. N.Y. Acad. Sci. 30:27, 1954.

*43. Erythrokinetics: Quantitative measurements of red cell production and destruction in normal subjects and patients with anemia. Giblett, E.R., Coleman, D.H., Pirzio-Biroli, G., Donohue, D.M., Motulsky, A.G., and Finch, C.A. Blood 11:291, 1956.

44. The limit of hemoglobin synthesis in hereditary hemolytic anemia. Crosby, W.H. and Akeroyd, J.H. Am. J. Med. 13:273, 1952.

45. Hereditary non-spherocytic hemolytic disease. Conrad, M.E., Crosby, W.H., and Howie, D.L. Am. J. Med. 29:811, 1960.

46. The clearance of bilirubin from the plasma. A measure of the excreting power of the liver. Weech, A.A., Vanan, D., and Grillo, R.A. J. Clin. Invest. 20:323, 1941.

47. Experimentally induced jaundice (hyperbilirubinemia). Thompson, H.E. and Wyatt, B.L. Arch. Int. Med. 61:481, 1938.

48. The capacity for bilirubin production as reflected by the concentration of plasma bilirubin. Crosby, W.H. J. Clin. Invest. 37:887, 1958 (Abstract).

*49. Clinical and enzymological observations on cases with Gilbert's Disease. Wakisaka, G. et al., Jap. Arch. Int. Med. 8:634, 1961.

50. Bilirubin glucuronyl transferase activity in liver disease. Metge, W.R., Owen, C. A. Jr., Foulk, W.T., and Hoffman, H.M. II. J. Lab. Clin. Med. 64:89, 1964.

51. On origin of bile pigment in normal men. London, M., West, R., and Sherman, D. J. Biol. Chem. 184:351, 1950.

52. Shunt bilirubin: Evidence for two components. Israels, L.G., Yamamoto, T., Skanderbeg, J., Zipursky, A. Science 139:1, 1963.

53. The early appearing bilirubin: Evidence for two components. Yamamoto, T., Skanderbeg, J., Zipursky, A., and Israels, L.G. J. Clin. Invest. 44:31, 1965.

54. The relationship of erythropoiesis to bile pigment formation. Robinson, S.H. and Schmid, R. Medicine 43:667, 1964.

55. The contribution of non hemoglobin hemes to the early labeling of bile bilirubin. Schwartz, S., Ibrahim, G., and Watson, C.J. J. Lab. Clin. Med. 64:1003, 1964 (Abstract).

56. Ineffective erythropoiesis. Haurani, F.I. and Tocantins, L.M. Am. J. Med. 31:519, 1961.

57. Chronic unconjugated hyperbilirubinemia with increased production of bile pigment not derived from the hemoglobin of mature circulating erythrocytes. Arias, I.M.

J. Clin. Invest. 41:1341, 1962.

*58. Hyperbilirubinemia due to an alternate path of bilirubin production. Israels, L. G., Suderman, H.J., and Ritzmann, S.E. Am. J. Med. 27:693, 1959.

59. Primary shunt hyperbilirubinemia. Israels, L.G., and Zipursky, A. Nature 193:73, 1962.

60. Die post hepatitische hyperbilirubinoemie. Kalk, H., and Wildhert, E. Ztschr. klin. Med. 153:354, 1955.

61. Die nicht hoemolytische hyperbilirubinoemie ohne direct van den Bergh Reaction. Siede, W. Deutsche med. Wochnschr. 15:504, 1957.

62. Jaundice in thalassemia minor: A consequence of "ineffective erythropoiesis". Robinson, S.H., Vanier, T., Desfarges, J.F., and Schmid, R. New Eng. J. Med. 267:523, 1962.

63. On the rate of formation of steroidal glucuronosides in patients with familial and acquired jaundice. Drucker, W.D., Sjikokis, A., Borkowski, A.J., and Christy, N.P. J. Clin. Invest. 43:1952, 1964.

64. La cholemie simpliale. Gilbert, A. and Lereboullet, P. Semaine Med. 21:241, 1901.

65. Cholemie simple familiale and post hepatic states without fibrosis of the liver. Hult, H. Acta Med. Scand. Suppl. 244, 1950.

66. Constitutional hyperbilirubinemia: Its differential diagnosis and the effect of steroid therapy. Eanet, M.P. and Brick, I.B. New Eng. J. Med. 253:1062, 1955.

67. Some aspects of the ultra structural pathology of the liver. Steiner, J.W. et al., in Progress in Liver Disease, Vol. II. Grune and Stratton, New York, 1965.

68. Glucuronide formation in patients with constitutional hepatic dysfunction. Schmid, R. and Hammaker, L. New Eng. J. Med. 260:1310, 1959.

69. Defects in hepatic transport of bilirubin in congenital hyperbilirubinemia: An analysis of plasma bilirubin disappearance curves. Billing, B.H., Williams, R., and Richards, T.G. Clin. Sci. 27:245, 1964.

70. Hepatic uptake defect in patients with "Gilbert's Disease". Galambos, J.T. and McLaren, J.R. Arch. Int. Med. 111:214, 1963.

71. Chronic liver disease following infectious hepatitis: Abnormal convalescence from initial attack. Kunkel, H.G., Labby, D.H., and Hoagland, C.L. Ann. Int. Med. 27:202, 1947.

72. Die posthepatitische hyperbilirubinaemie etc. Kalk, H. Med. Klin. 53;809, 1934.

73. Residual hepatic damage in catarrhal jaundice as determined by bilirubin excretion test. Soffer, L.J. and Paulson, M. Arch. Int. Med. 53:809, 1934.

74. Unconjugated hyperbilirubinemia in the absence of over hemolysis. Levine, R.A. and Klatskin, G. Am. J. Med. 36:541, 1964.

75. Pathogenesis of indirect reacting hyperbilirubinemia after portacaval anastomasis. Da Silva, L.C., Jamra, M.A., et al., Gastroenterol. 44:117, 1963.

76. Interference with bilirubin excretion by a gall-bladder dye (Bunamiodyl), Bolt, R.J., Dillon, R.S., and Pollard, M.H. New Eng. J. Med. 265:1043, 1961.