MEDICAL GRAND ROUNDS

PARKLAND MEMORIAL HOSPITAL

April 18, 1968

LIVER "FUNCTION" TESTS - An Appraisal

Indications for liver "function" tests.

Tests considered:

Bile Pigments

a) Serum bilirubin
b) Urine bilirubin
c) Urine urobilinogen
d) Fecal urobilinogen

BSP

Serum Enzymes

a) Alkaline phosphatase
b) 5'-nucleotidase
c) Leucine aminopeptidase
d) SGOT - SGPT

Flocculation Tests

a) Cephalin flocculation
b) Thymol turbidity

Prothrombin Time

Plan of discussion of each test:

a) Physiologic-biochemical considerations
b) Normal values
c) Specificity
d) Sensitivity
e) Selectivity
f) Overall assessment

Optimal test combinations for specific diseases:

a) Hepatic necrosis and other hepatocellular dysfunctions
b) Hepatocellular versus obstructive jaundice
c) Obstructive liver disease
d) Infiltrative liver disease
e) Prognosis in various liver disorders

Indications for Liver "Function" Tests

Establishing the presence of significant liver disease in -

a) Suspected primary liver disease (i.e., hepatitis, cirrhosis), especially in absence of jaundice
b) Suspected involvement of liver in other diseases (i.e., sarcoidosis, metastatic cancer)

Type of Liver Disease

a) Differential diagnosis of jaundice
b) Differential diagnosis of hepatomegaly

tent of Liver Disease: Course of Dysfunction

a) Prognosis in patients with established liver disease
b) Aid in management

Considerations in Assessing Individual Liver Function Tests

1) Specificity - does it reflect only hepatobiliary dysfunction?
2) Sensitivity - how well and how early does it reflect liver injury?
3) Selectivity - how well does it specify the type of liver disease present?

Tests Based on Bile Pigment Metabolism

Serum Bilirubin

a) Physiologic considerations:

Tetrapyrrol pigment. 250-300 mg made per day in adult man. Principally derived from catabolism of hemoglobin in senescent erythrocytes but 10-30% may originate from processes linked to Hgb synthesis in bone marrow, from heme precursors in liver and bone marrow and from nonhemoglobin heme proteins.

In plasma unconjugated bilirubin carried attached to albumin - rendering it water soluble.

Taken up by liver, conjugated to water soluble pigment and excreted into bile.

Differences Between Unconjugated and Conjugated Bile Pigments

Property	Unconjugated Bilirubin	Conjugated Bilirubin
Van den Bergh reaction	Indirect (+ alcohol)	Direct
Solubility in aqueous solution	-	+
Solubility in lipid solvents	+	-
Attachment to plasma albumin	+	+
Presence in icteric urine	-	+
Presence in bile	-*	+
Affinity for brain tissue	+	-
Association with hemolytic jaundice	++	±
Association with obstructive and hepatocellular jaundice	+	+++

* A small quantity of unconjugated bilirubin may be present in common duct bile in the form of bilirubin monoglucuronide. It is unknown whether some unconjugated bilirubin is excreted as such into canalicular bile and subsequently is reabsorbed by the biliary ducts.

A rise in serum bilirubin may arise from a) overproduction of bilirubin (hemolysis), b) decreased uptake and conjugation of bilirubin by the liver, c) decreased excretion of bilirubin by the liver into bile and d) regurgitation of bilirubin from liver and bile into the circulation. Processes a) + b) will result in unconjugated hyperbilirubinemia whereas c) + d) will tend to cause an increase of conjugated bilirubin primarily.

b) Normal values: Based on analysis of 719 normal adults using conventional diazo test (1)

Total bilirubin: Usually 0.3 - 1.0 mg/100 ml

5% - 1.1 mg/100 ml

1% - 1.5 mg/100 ml

Upper limit of normal designated as 1.5 mg/100 ml. However, distribution curve skewed to right and may include persons with actual disturbance of pigment metabolism.

Direct-reacting bilirubin (1): Usually less than 0.15 mg/100 ml

5% - 0.16 mg/100 ml

1% - 0.24 mg/100 ml

Upper limit of normal, based on one-minute Watson diazo test, designated as 0.25 mg/100 ml.

Not known if direct-reacting bilirubin represents actual conjugated bilirubin solubilized by substances such as bile acids, urea, citrate, etc. (2).

Specificity: Major value of test

Red color, maximal spectral absorption at 540 μ with diazotized sulfanilic acid - characteristic for bilirubin (only mesobilirubin and monopyrroles react similarly) (3).

Diazo-documented increase in total serum bilirubin = hepatobiliary disease, overproduction of bilirubin from hemolysis or both.

Sensitivity: Total serum bilirubin not sensitive test of liver damage

a) Functional reserve of human liver at least 2-3 fold greater than normal daily pigment load (250-300 mg) (4,5).

b) Serum bilirubin occasionally and transiently may not reflect intensity of icterus or total body pigment increase due to transient shifts of pigment in and out of circulation (6,7).

Selectivity: As a rule not good test, much overlap in height of total serum bilirubin in various types of liver disease (8). However -

a) In pure hemolysis total serum bilirubin usually < 5 mg% (2)

b) Malignant obstruction of common bile duct on average gives higher values than in parenchymal liver disease (cirrhosis, hepatitis) or in calculous biliary disease.

Fractional Serum Bilirubin Determination (Direct and Indirect-reacting)

a) No value in differentiating parenchymal from obstructive jaundice (2).

b) Distinct value in establishing the presence of unconjugated hyperbilirubinemia which is defined as a serum indirect bilirubin > 1.2 mg% and a direct-reacting fraction (one-minute) < 20% of total bilirubin (2). See Appendix I for list of conditions giving rise to unconjugated hyperbilirubinemia.

c) Distinct value in increasing sensitivity of the test. In various series of patients with liver dysfunction due to cirrhosis, hepatitis (12), congestive heart failure and metastatic liver disease - 30-50% of patients showed increase in direct-reacting bilirubin while total bilirubin was normal (9,10,11). Also direct-reacting bilirubin often positive longer than total serum bilirubin in a variety of hepatobiliary diseases.

TABLE I

Differential Sensitivity of Total and Direct-reacting Serum Bilirubin in Viral Hepatitis (29 cases) (12)

	Positive First	Positive Longer
Total bilirubin	3%	7%
Direct-reacting bilirubin	45%	83%

Serum Bilirubin as Prognostic Test: Few controlled studies but

a) Higher serum bilirubin appears to correlate with histological severity of viral hepatitis and length of disease (13). However, patients may die of fulminant hepatitis with only small elevation of serum bilirubin.

b) Degree of hyperbilirubinemia above 5 mg%, seems to correlate with worse prognosis of patients with acute alcoholic hepatitis (14,15).

Conclusions: Serum bilirubin -

1) Total serum bilirubin: high specificity, low sensitivity, low selectivity.

2) Fractionation: very helpful in diagnosing unconjugated hyperbilirubinemia and increasing sensitivity of the test.

XI

Causes of Unconjugated Hyperbilirubinemia

Pediatric	Mechanism
Physiologic Crigler-Najjar Breast Milk Lucey-Driscoll	Immaturity or inborn or acquired impairment of hepatic glucuronide - conjugating system
Pediatric and Adult	
Intravascular hemolysis	Overproduction of bilirubin
"Shunt" hyperbilirubinemia	
Constitutional Hepatic Dysfunction (Gilbert's)	Impaired uptake and/or conjugation
Post hepatitic	Unknown ? Impaired uptake
Post portacaval shunt	? Overproduction from hemolysis
Miscellaneous (cardiac, hepatobiliary, etc.)	Unspecified

Urine Bilirubin

a) Physiologic Considerations:

Normal urine is free of bilirubin. In liver disease a small fraction of conjugated bilirubin in serum, not attached to serum albumin (< 3%), is filtered via the glomerulus (18). Tubular secretory component, if any, is very small. Much remains unknown about factors which influence renal excretion of bilirubin.

b) Clinical Application:

Routine method for measuring urine bilirubin (diazomat) detects concentrations of 0.05 mg% (16). Test is specific for bilirubin and indicates hepatobiliary disease.

Value of urine bilirubin determination: 1) Rapidly confirms the presence of clinically suspect jaundice and attests to presence of liver disease; 2) May antedate overt icterus, or a rise in total serum bilirubin and thus may serve as <u>early</u> <u>(sensitive)</u> clue to the presence of hepatic disease. This is very common in early viral hepatitis (Table 3)* (12,17). Similar observations noted occasionally with metastatic liver disease (19).

---

* Note that in convalescent hepatitis urine bilirubin may disappear from urine <u>before</u> serum bilirubin is normal. There urine bilirubin is <u>very</u> <u>insensitive</u> (21).

TABLE 2

Relative Sensitivity of Urine Bilirubin and Serum Bilirubin

in Detecting Early Viral Hepatitis (34 cases) (12)

Total serum bilirubin mg%	Patients with positive urine bilirubin test
< 1.0	59%
< 1.5	70%
One-minute direct-reacting bilirubin	
< 0.15	43%
< 0.25	75%

3) Absence of bilirubin from urine in presence of jaundice (acholuric jaundice) - suggests presence of unconjugated hyperbilirubinemia, since only conjugated bilirubin is excreted into urine. (Note - occasionally even with pure hemolysis some bilirubin will spill into urine, because of a small quantity of conjugated bilirubin in serum, - constituting "false positives") (20).

Caution: Renal insufficiency; decomposition of bilirubin in unrefrigerated, light-exposed urine; improper, nonspecific techniques for measuring urine bilirubin - may confuse results.

Conclusion: Urine bilirubin -

a) Simple, inexpensive (1¢/specimen), specific test; b) sensitive in early viral hepatitis and occasionally metastatic liver involvement; c) selective (if absent) for unconjugated hyperbilirubinemia - not (when present) for other types of jaundice.

Urobilinogens - urinary and fecal

a) Physiologic considerations:

In the intestinal lumen, conjugated bilirubin is sequentially reduced by bacterial action to colorless urobilinogens and their colored oxidation products, the urobilins. In normal individuals - the major part of these is excreted in feces. Ten to 15 per cent is absorbed into portal circulation and most of this is re-excreted in bile (enterohepatic circulation).

Normal urinary urobilinogen: 0.2 - 3.3 mg/day. Based on study of 676 normal subjects - 5% excreted more than 3.3 and 1% - 5.5 mg/day (22).

Normal fecal urobilinogen: 40-250 mg/day, average about 100 mg/day (23).

TABLE 3

Factors Affecting Urinary Urobilinogen Excretion (24)

Increase	Decrease
Excess bilirubin formation (hemolysis)	1. Decreased bilirubin entry into intestinal tract (intra or extra-hepatic obstruction)
Prolonged intestinal transit time (constipation)	2. Bowel sterilization (antibiotics)
Bacterial "invasion" of small bowel (increased intestinal absorption)	3. Increase in intestinal transit time (diarrhea)
Hepatic dysfunction (decreased entero-hepatic circulation)	4. Renal insufficiency
	5. Aregenerative anemia (decreased bilirubin production)
	6. Acid urine pH*
	7. Improper time for urine collection*
	8. Poor urine collection*

See next section

Some aspects of urinary excretion of urobilinogen:

a) Urobilinogen excreted into urine probably by combination of glomerular filtration, tubular secretion and pH-dependent back diffusion (25).

b) Since urobilinogen is a weak lipoid soluble organic acid with pKa of 5.45 it is much more reabsorbed from acid urine by nonionic diffusion of the unionized lipid soluble fraction. So acid urine may "falsely" lower urinary urobilinogen. Also urobilinogen is not stable in acid urine. For quantitative analysis urine should be alkaline, if necessary by administration of sodium bicarbonate.

c) Highest urinary urobilinogen excretion at 12-4 p.m. probably because blood levels highest and urine pH highest then. This is the basis for the 2 hour fractional urobilinogen collection.

Note: This may vary in different individuals. Ideally - 24-hour urine collection.

d) Ehrlich aldehyde reaction not specific for urobilinogen. Details of methodology are listed in References 26,27,28.

Clinical "uses" of urinary urobilinogen determination:

1) Absence or traces (with jaundice) - virtually complete stop of bilirubin delivery into intestine. If persistent, statistically suggests cancer of extrahepatic biliary tree (29).

However, may be occasionally due to intrahepatic (cholangiolitic) hepatitis or transiently with biliary calculi.

2) Increase (with or without jaundice)-

a) Liver disease (in absence of hemolysis) - does not specify cause of liver dysfunction. Sensitivity uncertain, since pH not examined.

b) Hemolysis (in absence of liver disease) - insensitive index of hemolysis versus fecal urobilinogen.

Clinical Uses of Fecal Urobilinogen

Interpretation depends on many of the same factors cited for urinary urobilinogen.

1) Decrease (< 5-6 mg/day) - especially if persistent, statistically suggests cancer of extrahepatic biliary treet.

However: a) Some patients with malignancy excrete > 6 mg/day (20-30%) (30); b) some patients with calculi and cholestatic hepatitis excrete < 6 mg/day (31); c) stool color often a fair and simpler index of quantity of pigment present; d) urine urobilinogen usually equally effective and less tedious test (29).

2) Increase (> 400 mg/day). Signifies overproduction of bilirubin. May be excellent test of hemolysis*. Special application: Shunt hyperbilirubinemia (no intravascular hemolysis).

Conclusion: Urobilinogens

1) Absence (urine, stool) - suggests complete biliary obstruction, and if persistent, statistically cancer.

2) Increase (stool) - hemolysis (intravascular or shunt).

3) Increase (urine) - liver disease or hemolysis or both.

Bromsulphthalein (BSP) Removal from Plasma

a) Physiologic Considerations:

Injected BSP is rapidly removed from the circulation by parenchymal liver cells, the dye is partly conjugated in the liver with glutathione and the free and preferentially the conjugated BSP are excreted into bile.

Advantage is taken of the observation that removal of BSP from blood is delayed in the presence of hepatic dysfunction. Increased retention of BSP may be due to a) impaired delivery of dye to the liver, b) impaired hepatic uptake, c) impaired hepatic conjugation and d) impaired biliary excretion of the dye. It is evident, therefore, that normal removal of BSP from blood depends both on an efficient hepatic circulation and adequate overall hepatic function.

b) Methodology and Normal Values:

Five mg of dye/kg body weight is injected and BSP retention in plasma after 45' is estimated. It is assumed that the dye is injected into a plasma volume of 50 ml/kg body weight and that with instaneous mixing, a concentration of 10 mg/100 ml of plasma will be achieved. This value

---

* Note - Significant liver disease and sterilization of bowel flora will decrease "falsely", and recent release of biliary obstruction (passage of stone) will increase "falsely" fecal urobilinogen and invalidate test for detection of hemolysis.

s considered to represent 100% retention. Concentrations of BSP actually measured in plasma are compared to this value and are expressed as per cent retention.

Five per cent retention at 45' is usually considered as the upper limit of normal. However, Zieve reported that in 718 normal men, 50% had a BSP retention below 4%; 90% below 8% and 99% below 14%. If the value of definite abnormality is taken as the point exceeding 1% of normals then 14% retention is abnormal (22). If the zone of abnormality is taken as the point exceeded by 10% of normals then values above 8% retention are abnormal. The current normal limit of 5% retention at 45' may be falsely low.

Factors Causing Increased BSP Retention in Normal Individuals:

1) Age. Over 60 - mean retention 4.6% versus 2.5% under 60. Cause: probably hepatic uptake defect (32,33).

2) Obesity. Mean retention 8.2% in obese versus 3.4% in normal weight group (22,33,34). Cause: ? decrease in relative plasma volume (< 50 ml/kg). Thus, initial BSP concentration in plasma exceeds 10 mg/100 ml. Possible corrections:: a) Measure plasma volume and calculate initial BSP concentration from this. b) Calculation of dose of BSP given on basis of surface area, fat-free weight (35) - not validated yet for clinical testing.

3) Pregnancy. Complex and inconstant interplay of factors.

   a) Increase in plasma volume "lowers" BSP retention.

   b) Calculation of body weight with fetal weight falsely overestimates true distribution of BSP which does not cross placenta. (Similar considerations apply to a large mass of ascites.)

   c) Decrease in hepatic excretory Tm for BSP in late pregnancy - ? hormonal factors.

   Net result - in some normal pregnant individuals there is increased BSP retention.

4) Fever. May increase value of BSP retained. Not certain if this reflects increase in temperature per se, or damage to liver in the course of the disease causing temperature elevation.

5) Other compounds (by competition for transport processes) given concurrently may interfere with hepatic metabolism of BSP and thus may cause a falsely high serum BSP retention. e.g., Telepaque (36). Therefore, BSP test should not be done within 3 days of use of these compounds.

Possible Toxic Effects of BSP Infusion:

   a) Allergy - very rare (inject dye slowly over 5 minutes - > 90% of reactions occur during this time). Reactions more severe in older individuals. Possibly more frequent in the face of allergic history or prior BSP test (37).

   b) Necrosis due to extravasation of dye at site of injection.

Clinical Considerations:

Specificity: Good for liver dysfunction in absence of circulatory disturbances. Fever, thyrotoxicosis, diabetes reported as causing increased BSP retention but this may have been due to concomitant liver disease.

Sensitivity: One of the most sensitive tests available for detecting anicteric cirrhosis, hepatitis, and metastatic liver disease. However, SGOT is probably abnormal earlier viral hepatitis. Good to follow anicteric patients recovering from acute hepatitis. In some instances, one can further enhance sensitivity by measuring BSP disappearance rate (38). This is especially true in presence of increase in plasma volume.

TABLE 4

BSP Retention in Hepatobiliary Disease - Incidence of Abnormal Results*

Diagnosis	% Abnormal
Acute Hepatitis	83
Cirrhosis	87
Extrahepatic Obstruction	79
Metastatic Disease	
with Jaundice	100
without Jaundice	93
Chronic Passive Congestion	88

* 6% retention at 45' considered normal upper limit.

Selectivity: Poor. No value in differentiating hepatocellular from obstructive jaundice or type of liver damage present. Usually test is of no value whatever, therefore, when jaundice is already present.

However, a) if normal in presence of jaundice - suggests either hemolytic jaundice or congenital hyperbilirubinemia of the "Gilbert" type or perhaps posthepatitis jaundice. (These are indirect hyperbilirubinemias.) b) If one gets a delayed (after 60') rise in blood BSP - consider Dubin-Johnson Syndrome. This delayed rise is probably due to regurgitation of BSP as a result of the excretory defect of liver. c) Measurements of S and Tm may further characterize the nature of liver lesion. These are, however, not routine tests.

Conclusion: Main value of BSP retention is its sensitivity in detecting the presence of hepatobiliary disease. The test is not selective for the type of liver disease present.

Serum Alkaline Phosphatase (SAP)

a) Physiologic Considerations:

Definition: Group of enzymes collectively called nonspecific alkaline phosphatase which liberate inorganic P from orthophosphoric esters of a variety of compounds, optimally active at an alkaline pH.

Function: The function of the enzyme in serum, if any, or for that matter in tissues is not known. In tissues it is located primarily in areas concerned with transport - in liver in biliary epithelium.

Metabolism: In normal adult man, SAP believed to originate from bone (40-70%) and liver, sometimes from intestine. The latter especially in individuals who have blood groups O and B (who are secretors) and especially after fatty meal. During growth, rise in SAP due to bone fraction. In pregnancy (second half) rise in SAP due to placental fraction. SAP not believed excreted to any substantial extent via either kidneys or liver but is metabolized as a protein in the body protein pool with half-life of about 7.0 days. SAP at any one time reflects input from bone, liver (biliary epithelium primarily) and sometimes intestine or placenta and degradation by body (39,40,41).

) Normal Values and Methodology:

See Tables 4 and 5 for adult values and conversion factors.

TABLE 5

Range of Normal Values for Serum Alkaline Phosphatase Activity in Adult Man (42,43)

Method*	Substrate	Unit	Normal Range
Bodansky	Beta-Glycerophosphate	1 mg P/100 ml/60'	1.5 - 4.0
King-Armstrong	Phenylphosphate	1 mg phenol/100 ml/30'	3 - 13
Bessey-Lowry	p-Nitrophenylphosphate	1 mM ptnitrophenyl/ 100 ml/30'	0.8 - 3.0

All three methods are equally reliable and correlate well with each other.

TABLE 6

Approximate Conversion Factors for Alkaline Phosphatase*

Conversion from	Conversion to Bessey-Lowry	Conversion to Bodansky	Conversion to King-Armstrong
Bessey-Lowry	-	X 1.3	X 3.5
Bodansky	X 0.79	-	X 2.8
King-Armstrong	X 0.29	X 0.34	-

These are conversion factors based on 215 specimens in various disease states. However, they must be considered as only approximations since 95% confidence limits of duplicate determinations on any one specimen show much scatter (43).

Effect of Age on SAP

a) Correlates well with bone growth. Values of 7-8 B-L units (normal adult values .8 - 3.0 B-L units) may be seen during normal adolescence (46).

b) Past age 60 slightly higher normal values (47).

Effect of Sex on SAP

a) Males on average higher than females < 60 years (45). Females slightly higher than males > 60 years (47).

Note: Much unknown about activators and inhibitors of SAP. Much variation in duplicate assays of a single specimen - caution needed in interpreting small changes.

c) Clinical Aspects

Specificity: Not specific for hepatobiliary disease. Elevated also in bone involvement and pregnancy.

Attempts to enhance specificity:

a) Electrophoresis into isoenzymes. Although some authors report fairly good quantitative separation of the major fractions, the procedure suffers from 1) some overlap in results among various patient groups; 2) lack of agreement among investigators; 3) difficulty in carrying out procedure as practical routine assay (48,49,50,51).

Conclusion: At present not suitable routine procedure.

b) Differential heat inactivation. Placental fraction heat stable (56°C for 30 minutes). Bone fraction very heat sensitive. Bile fraction intermediate. Placental fraction easily identified. Sera with SAP increase due to bone disease and liver disease usually can be separated (52,53). Procedure needs clinical confirmation and may be of real future value.

c) At present, best method of increasing specificity is via LAP or 5'-nucleotidase assays (see below).

Sensitivity: Very sensitive in

a) Intrahepatic and extrahepatic biliary obstruction. Often antecedes development of jaundice and may persist after its disappearance as the only clue of continued partial biliary obstruction.

b) Infiltrative disease of the liver - granulomatous (TBC, sarcoid, fungal) or space-occupying lesions (neoplasms, abscess) - 60-70%. In metastatic cancer - 90% positive.

Mechanism of increase in SAP in a) and b) still in controversy but believed probably due to regurgitation into circulation of increased hepatobiliary phosphatase - not decrease in biliary excretion of bone phosphatase (retention theory of Gutman). Based primarily on a) little obstruction in many of these illnesses - suggesting hepatic (biliary) overproduction of enzyme, b) trivial and equal clearance from plasma of infused alkaline phosphatase in normal patients and those with obstruction of bile duct and c) increased excretion of bile phosphatase in experimental animals (with partial biliary obstruction) showing an increase in the serum enzyme (40).

Relatively insensitive in parenchymal liver disease (cirrhosis, congestive hepatomegaly). Mechanism - probably low concentration of enzyme in parenchymal liver cells.

Selectivity: a) Differentiates fairly well hepatocellular from obstructive jaundice.

TABLE 7

Selectivity of SAP

	Alkaline Phosphatase	
	> 10 B.U.	> 25 B.U.
Cancer of bile duct	80-91%	50%
Calculous obstruction	60-70%	25%
Stricture of bile duct	90%	33%
Viral hepatitis	10-15%	Very rare
Cirrhosis	30%	10%

Therefore, while there is overlap among these groups, values over 10 B.U. and especially over 25 B.U. favor obstruction and values < 10 B.U. even more favor hepatocellular disease. Note that very high values may occur in intrahepatic (cholangiolitic) as well as extrahepatic obstruction, also with infiltrative and space-occupying lesions of liver, biliary cirrhosis and portal cirrhosis accompanied by a fatty-obstructive syndrome. Thus, low values are better diagnostic index of lack of obstruction than high ones are of its presence.

b) Elevated with infiltrative or space-occupying lesions of liver, in absence of jaundice. Note, however, that calculi or early cancer of biliary tree may also present this way.

Conclusions: SAP

1) Nonspecific for liver.

2) Sensitive for biliary obstruction and infiltrative liver disease.

3) Fairly selective for obstructive versus parenchymal jaundice and for presence of infiltrative liver disease in absence of jaundice.

Serum Leucine Aminopeptidase (LAP)

1) Physiologic Considerations:

Definition: Proteolytic enzyme which hydrolyzes amino acids from N-terminal end of various roteins and polypeptides - reacting most rapidly with leucine compounds.

Enzyme present in most human tissues - in liver localized primarily in biliary epithelium. ntracellular function uncertain, possibly hydrolysis of peptide bonds or transfer of L - leucine rom one peptide to another. The metabolism of LAP has not been investigated.

Methodology and Normal Values: Alpha-leucyl-Beta-naphtyl-amide hydrochloride serves substrate with liberated Beta-naphtylamide assayed colorimetrically. Norman values range from

units without significant difference due to sex, age or fasting (54,55). Electrophoresis rmal serum shows one homogenous peak (56).

Clinical Considerations:

Specificity: As for 5-nucleotidase this is the major advantage of this assay versus SAP. P not elevated with bone disease (54,57,58) but rises in pregnancy (59,60). Electrophoresis nnot separate adequately LAP peaks of placental and "hepatic" origin - the latter are not mogenous in contrast to normal serum. Exact sites of origin of LAP peaks present with hepato- liary disease are not known (56).

Sensitivity: At least as sensitive as SAP in detecting obstructive, infiltrative or pace-occupying liver disease. Some feel LAP is better than SAP in anicteric obstruction. AP does not specifically help to diagnose pancreatic disease (54,57,60). Note, however, hat in some patients with known liver disease SAP may rise but not LAP or vice versa.

Selectivity: Controversy if as good as SAP in differentiating parenchymal from obstructive sease, but probably no better (54,57,58,60).

Conclusion: Main proven value is specificity.

Serum-5-Nucleotidase

Physiologic Considerations:

Definition: Phosphatase which specifically hydrolyzes nucleotides with phosphate in 5 sition (adenosine-5-PO_4).

Methodology: Nonspecific SAP first inactivated by incubating serum with EDTA. Rate of drolysis of adenosine-5-PO_4 at pH 7.5 - 0.04 M Mg^{++} carried out. One unit of 5-nucleotidase = mg P liberated/hr/100 ml serum.

Normal Values: 0.3 - 4.0 Bodansky Units. Not influenced by age, sex, or race. Elevated pregnancy (57,61,62,63).

Metabolism: Not known and physiologic function, if any, uncertain. In liver enzyme primarily located in biliary canaliculi and sinusoidal membranes.

Clinical Application:

Specificity: Specific for hepatobiliary disease in absence of pregnancy. Usually not creased in bone disorders (57,61,63). However, occasionally may be slightly elevated in ne disorders (64).

Sensitivity: Either equal to or less than SAP. Note, however, that the two enzymes y not rise proportionately in an individual patient, and in some patients with liver disease, ther enzyme may rise (61).

Selectivity: Controversy if it is as good as SAP for separating parenchymal versus structive jaundice and in picking up infiltrative disease (62,63,64). Majority of evidence: t as selective as SAP.

Conclusion: Proven advantage over SAP - specificity.

Serum Transaminases

Physiologic Considerations:

Definition: Group of enzymes that catalyze transfer of amino groups from an alpha amino :id to an alpha-ketoacid. The enzymes are designated by terms describing to favored products the reaction at equilibrium.

1) Aspartic acid + alpha-ketoglutaric acid $\rightleftarrows$ oxaloacetic acid + glutamic acid
(SGOT: serum glutamic-oxaloacetic transaminase)

2) Alanine + alpha-ketoglutaric acid $\rightleftarrows$ pyruvic acid + glutamic acid
(SGPT: serum glutamic-pyruvic transaminase)

Methodology: Determined by measuring glutamic acid formed or coupling to oxidation of NH. Enzymes stable in serum at room temperature for up to 4 days; 4°C for 2-3 weeks; -)-20° C for 4 months. Spuriously elevated 5-11 X normal i.e., up to about 400 units by evere hemolysis. Little information about minor hemolysis. Variability in duplicates of the der of 7-20% in different laboratories (65).

Normal values: SGOT - 20.0 ± 7.0 (S.E.)
SGPT - 16.0 ± 9.0 (S.E.)

Up to 40 units is considered normal - Men have slightly higher SGOT (↑ 11-30%)

Note: Strenuous excercice may ↑ SGOT by up to 30 units (65).

Metabolism: The source of transaminases present normally in serum is unknown. Damage to issues rich in transaminases (skeletal muscle, heart muscle, liver) leads to release of enzymes nto circulation. The increased transaminases are distributed in interstitial fluid and plasma. isappearance from plasma not primarily via renal or biliary excretion but apparently by catabolism n protein pool. Site and factors affecting rate of the catabolism are not determined (66,67).

Specificity: Not specific for hepatobiliary disease. Both SGOT and SGPT may rise in injury o heart and skeletal muscle (68,69).

In cardiac disease SGOT rises more than SGPT and elevations of transaminase over 400 usually ndicate liver disease (or muscle injury). Levels of 200-300, if due to heart disease, occur ith heart damage of such severity as to be usually obviously related clinically and electro- ardiographically to heart injury. Of course, liver ischemia and congestion due to and in ddition to heart failure and shock may complicate the interpretation and cause a marked rise n SGOT - even up to 2000-3000 units. If SGOT rises markedly ~ 100-150 without significant ise in SGPT - this suggests cardiac disease. However, differential interpretation of pure ardiac versus pure liver injury by SGPT/SGOT ratios (at enzyme levels of - 200 or below) re very uncertain.*

Muscle injury (trauma, surgery, or intrinsic muscle disease such as dermatomyositis nd muscular dystrophy) - may raise enzymes to 300-500 units (68). After surgery rise usually ithin 24 hours and fall by 4 days. Levels of 100-250 units - the closer to biliary tree he higher the levels (in general) - ? due to liver injury concomitant with surgery.

---

* Opiates may cause elevation of SGOT (usually < 250 units) starting within one hour, peak 5-8 ours - last 24-48 hours. Much more common in patients with biliary tract disease or prior holecystectomy; occasional small changes without biliary tree disease. (Mechanism: probably due o increased intrabiliary pressure and release of enzyme from liver). May also see increase in AP.

Sensitivity: SGOT and SGPT are sensitive indicators of liver cell damage: a) considered best early index of viral hepatitis (anicteric or preicteric) (65,70) and of relapses in activity during convalescence; b) good guide to continued activity of subacute or chronic hepatitis; c) often good early index of drug-induced liver damage; d) increased in a fair number of patients (37-90% in various series) with metastatic liver disease, congestive hepatomegaly (12-35%), granulomatous diseases, cirrhosis, etc.

Selectivity: a) If values are > 400 units - indicates acute hepatocellular disease; - hepatitis, drug or toxic injury, or ischemia, give highest values - often > 1000. Very rarely levels > 400 occur in obstructive jaundice (65). b) Levels < 300 units are of no help in differential diagnosis of hepatocellular versus obstructive jaundice. Note that early very high values in viral hepatitis fall toward 200 by 1 1/2 - 3 weeks after onset of jaundice (65,71).

SGOT/SGPT Ratio: In diagnosis of type of liver disease present, in individual patients, - not helpful (65).

Prognosis: Degree of elevation of little prognostic value as to eventual outcome of illness in viral hepatitis, alcoholic hepatitis or terminal cirrhosis. Degree of elevation in viral hepatitis does correlate fairly well with severity of cell necrosis (at least at low levels of enzymes in blood). *

Conclusion: 1) Very sensitive index of acute hepatocellular injury; 2) If over 400 - good selectivity for severe acute hepatocellular injury due to hepatitis, toxins and drugs, or ischemia; 3) Not specific for hepatobiliary disease.

*Continued or recurrent elevation of SGOT correlates fairly well with persistence or recurrence of activity in viral hepatitis.

TABLE 8 (68)

Range of SGOT and SGPT Values in Disease States

Disease States	Range of SGOT Activity*	Range of SGPT Activity*
Normal range	8-40	5-35
Transmural myocardial infarction	50-600	5-150
Subendocardial infarction	20-150	5-50
Viral and/or homologous serum hepatitis		
Non-icteric phase	50-300	60-400
Increasing icteric phase	500-2500	600-3500
Toxic hepatitis	50-27,000	50-20,000
Laennec's cirrhosis, progressive	50-250	30-200
Biliary cirrhosis, progressive	50-350	30-300
Extrahepatic biliary tract obstruction	40-300	50-400
Metastatic and primary hepatic carcinoma	40-250	20-150
Skeletal muscle trauma	30-500	20-150
Progressive muscular dystrophy Pseudohypertrophic muscular dystrophy Dermatomyositis	40-250	20-100

* Spectrophotometric units.

Flocculation Tests (cephalin flocculation, thymol turbidity)

Physiologic Basis:

Serum from patients with diffuse liver cell injury on addition of certain reagents often exhibits a precipitate, turbidity or flocculation, in excess of that noted in normal individuals.

Positive test is based on qualitative and/or quantitative changes in various serum protein fractions resulting in increased flocculating or decreased inhibitory activity, or both effects (72,73,74). Main protein fractions involved are γ-globulin and serum albumin-α_1 globulin. Thus γ-globulin from patients with hepatitis and cirrhosis has greater than normal flocculating activity. By contrast, the anti-flocculating activity of albumin-α_1 globulin fractions from these patients is less than normal. Other proteins may also add to or inhibit the flocculating activity of serum and phospholipids participate in the thymol turbidity test. The thymol turbidity test is enhanced by lipemic serum (75). (The zinc sulfate turbidity primarily reflects serum γ-globulin (73).

Abnormal Values (22): Cephalin flocculation 3+ or > at 24 hours.

Thymol turbidity > 7 units (5-7 units:equivocal)

Specificity: Poor. Many diseases that result in altered serum proteins may give positive tests (i.e. SLE). Also a lipemic serum may give a "falsely" positive thymol turbidity.

Sensitivity: In early viral hepatitis these tests often become positive before and sometimes in the absence of a rise in serum bilirubin (12). This is especially true of the cephalin flocculation test which rises first, followed by thymol turbidity (76). A rise of SGOT, however, occurs even earlier and is more sensitive (70).

Selectivity: This is the major role of the flocculation tests.

a) Good for differentiation of active parenchymal versus extrahepatic liver disease (77). Thus, in acute viral hepatitis they are positive in 80-90% of cases (81). (The abnormal thymol turbidity is slightly less common in S.H. hepatitis). In the presence of active cirrhosis incidence is high, though more variable (76). In inactive cirrhosis the percentage of abnormal tests is decreased. By contrast, in obstructive jaundice of short duration the flocculation tests are abnormal in about 25% of cases. With longer obstruction and secondary infection (cholangitis) the incidence of abnormal tests rises.

b) Differentiation of acute viral hepatitis in pregnancy versus cholestatic jaundice of pregnancy and hyperemesis gravidarum. Flocculation tests in the latter two instances are usually normal (78,79) - in viral hepatitis abnormal in about 90% (80).

c) In the jaundiced patient, negative flocculation tests (as well as other liver function tests) suggest the presence of congenital hyperbilirubinemia.

Prognosis:

a) Do not differentiate severe versus mild disease (76).

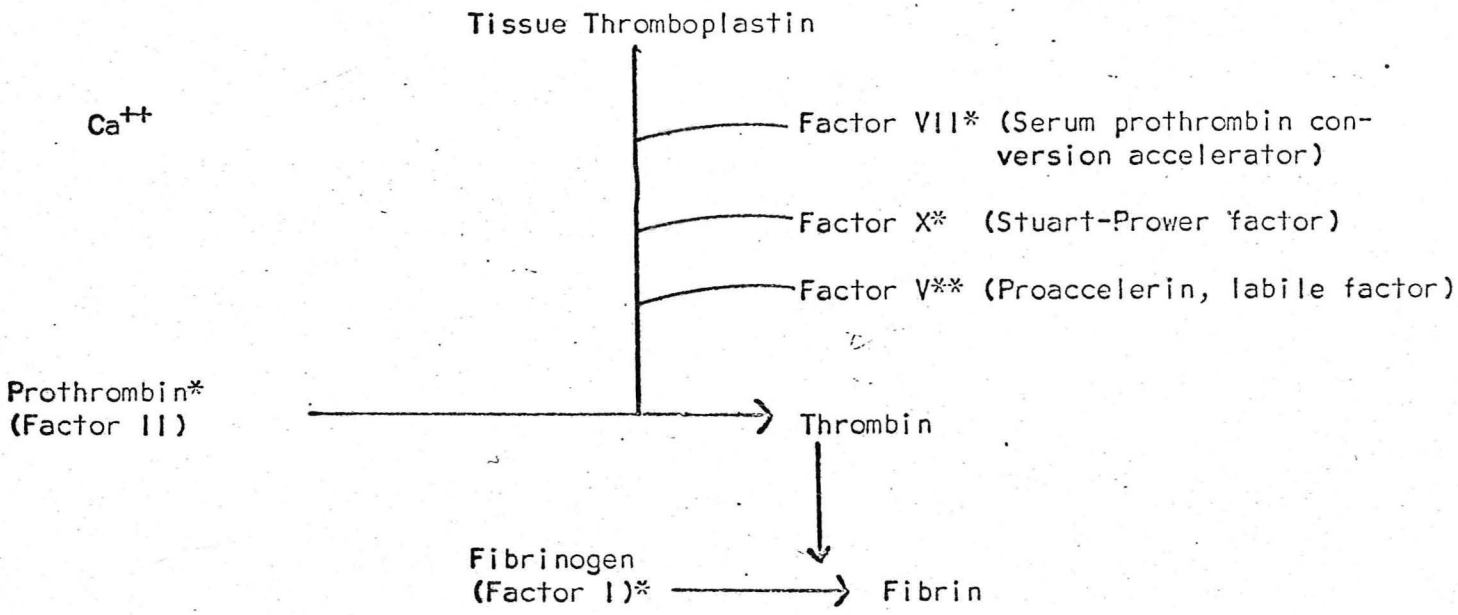
b) May remain abnormal, especially thymol turbidity, for some time after resolution of acute viral hepatitis without indicating a bad prognosis.

Prothrombin Time (One-stage Quick) (P.T.)

Physiologic Basis:

Measures the rate of conversion of available prothrombin to thrombin in the presence of thromboplastin, Ca^{++}, and a series of activated coagulation factors, followed by polymerization of fibrinogen to fibrin. This is shown schematically in Table 9.

Factors Involved in Quick, One-stage Prothrombin Time (82)



* Shown to be synthesized in liver.

** Probably synthesized in liver (Factor IX and plasma thromboplastin component also probably synthesized in liver).

The prothrombin time is prolonged if any of the involved factors are deficient, singly or in combination. The basis for decrease in P.T. in patients with liver disease is decreased synthesis of these factors by a diseased liver. Usually prothrombin activity less than 75% of normal is considered abnormal and individuals with values less than 50% of normal are considered to be at risk from bleeding (83).

Specificity: Not specific for liver disease exclusively. Abnormal also in:

a) Various congenital deficiencies of coagulation factors (84,86)

b) Acquired conditions including ingestion of drugs that affect the prothrombin complex (84,86).

c) Low Vitamin K* absorption as seen with steatorrhea (87), obstructive jaundice or use of cholestyramine with decrease in bile salt availability

d) Decreased availability of Vitamin K* as with use of antibiotic which alter the gut bacterial flora (88) or very rarely with dietary deficiency (89).

* The precise mode of action of Vitamin K in the production of prothrombin is not clear yet.

primary hematologic disorder (a and b above) can usually be diagnosed by appropriate hematologic evaluation. Differentiation of decreased Vitamin K absorption (c) and decreased availability of Vitamin K (d) from intrinsic hepatic inability to synthesize prothrombin from Vitamin K is usually accomplished by noting the response in P.T. after one parenteral injection of Vitamin K (90,91). A P.T. rise of at least 30% within 24 hours after one injection of 5-10 mg of Vitamin K_1 - suggests no intrinsic parenchymal liver disease but rather (c) or (d) above. (This is not an invariable differentiation, however, see under Selectivity).

Sensitivity: P.T. not sensitive index of liver disease. Many patients with severe cirrhosis and hepatitis may have a normal P.T. or only a slight prolongation. In a series of 67 patients with "severe" cirrhosis only 22% had a P.T. of less than 50% of normal (82). This is consistent with the concept that normally only 10% of hepatic cells synthesize prothrombin (92,93).

Selectivity: The P.T. does not differentiate among the various hepatocellular disorders (i.e. - hepatitis versus cirrhosis). It may be helpful in differentiation of parenchymal versus obstructive jaundice by response of P.T. to parenteral Vitamin K_1 (See Specificity However, some patients with obstructive extrahepatic disease respond sluggishly and a good and rapid response may sometimes be seen in patients with parenchymal liver disease. Therefore, lack of rapid response of P.T. argues versus obstructive extrahepatic disease, as a sole finding.

Prognostic Value: Most important value of test.

a) Decreased P.T. of less than 40% of normal, if consistent despite parenteral Vitamin K_1, best early index of developing fulminant necrosis in patients with viral hepatitis and drug and toxin-induced hepatic necrosis. A progressive shortening of P.T. conversely suggests improving prognosis.

b) Decreased P.T. also has prognostic value in patients with alcoholic steatonecrosis. A P.T. > 4 seconds over control value occurred six times as often in patients who died (60%) versus group that survived (10%) (94).

c) A P.T. of < 50% of normal and no response to Vitamin K_1 indicates extensive parenchymal liver damage and a poor long-term prognosis (83).

d) P.T., of course, is valuable in deciding as to the relative safety of a liver biopsy and other surgical procedures.

Conclusion: Main value of P.T. is in assessing prognosis in a patient with parenchymal liver disease.

Overall Assessment - Practical Use of Liver "Function" Tests

1. What liver "function" tests should usually be performed?

Total and direct-reacting serum bilirubin
Urine bilirubin
Serum alkaline phosphatase
SGOT
Thymol turbidity; cephalin flocculation
Serum albumin and globulin
Prothrombin time

These usually suffice to detect and characterize a liver dysfunction.

to do a BSP test?

a) BSP test is added only when other tests are normal or not clearly indicative of liver disease. This may be the only abnormal test in some patients with cirrhosis, hepatic metastases, amyloidosis, granulomatous liver disease, etc.

b) In the presence of jaundice - BSP is of no value except perhaps 1) when there is unconjugated hyperbilirubinemia, and a normal BSP confirms the absence of liver disease and 2) in Dubin-Johnson syndrome when a late rise in serum BSP is consistent with that diagnosis.

3. When to do a serum LAP or 5'-Nucleotidase?

a) When it is not clear if increased SAP is derived from liver or bone. A rise in LAP or 5'-nucleotidase indicates that at least part of increased SAP is derived from liver. However, lack of increase in LAP or 5'-nucleotidase does not mean that increased SAP is from bone exclusively.

b) Special application - growing children. In these, SAP increase due normally to osteogenic activity. LAP and 5'-nucleotidase are normal if no liver dysfunction.

c) In late pregnancy - all 3 enzymes normally rise - derived from placenta. Thus, LAP and 5'-nucleotidase are of no differential value.

Possibly, study of heat sensitivity of SAP will help. Normal, placental SAP is apparently heat stable.

4. What are the best (most sensitive) tests for detecting early and/or mild liver dysfunction? (Note overlap (lack of sensitivity) among various disease processes listed below).

a) Acute (anicteric) Viral Hepatitis: SGOT, BSP, urine bilirubin, serum direct-reacting bilirubin.

b) Drug Toxicity: Parenchymal damage - BSP, SGOT
Intrahepatic cholestatis - alkaline phosphatase, direct-reacting serum bilirubin

c) Cirrhosis: BSP

d) Extrahepatic Common Bile Duct Obstruction (Stone, Tumor): Alkaline phosphatase, direct-reacting serum bilirubin.

e) Infiltrative Disease (metastatic disease, granulomatous disease): BSP, alkaline phosphatase (LAP), SGOT, direct-reacting serum bilirubin.

5. What are the best tests for the differential diagnosis of jaundice?

a) To detect the presence of an unconjugated hyperbilirubinemia: Serum bilirubin fractionation and urine bilirubin. In pure unconjugated hyperbilirubinemia: indirect fraction > 1.2 mg%, direct fraction < 20% of total and urine usually free of bilirubin.

If unconjugated hyperbilirubinemia is present - additional tests (fecal urobilinogen etc.) will establish if hemolysis or other disease is the cause. (Post hepatitic versus constitutional dysfunction (Gilbert's) difficult to distinguish but this is probably only of academic importance since prognosis is excellent for both and therapy nil). If not present - serum bilirubin and urine bilirubin of not much value in differential diagnosis.

b) To distinguish obstructive versus parenchymal jaundice: Alkaline phosphatase, SGOT, flocculation tests, P.T. and its response to parenteral Vitamin K.

An alkaline phosphatase over 10 Bodansky (30 K-A) units, and negative flocculation favor obstruction. Even more important - if the alkaline phosphatase is only slightly elevated, this strongly argues versus obstructive jaundice By contrast a rapid (within 24 hours) and substantial response of a prolonged P.T. to Vitamin K given I.M. - is in favor of obstructive jaundice.

Conversely, a phosphatase below 10 Bodansky units and positive flocculations favor hepatocellular jaundice.

An SGOT under 400 units is of no diagnostic value - it may be seen in both obstructive and parenchymal liver disease, even viral hepatitis. However, an SGOT greater than 400 favors parenchymal disease, - the higher the more significant and the more suggestive of acute liver necrosis. SGOT over 1000 units is essentially confined to viral hepatitis, drug or toxin-induced hepatic necrosis or hepatic congestion and shock. (Flocculations are unnecessary if SGOT is so high).

If SGOT, SAP, and flocculations are normal and conjugated bilirubin is increased in serum - consider a congenital hyperbilirubinemia such as Dubin-Johnson or Rotor Syndrome.

A liver biopsy in these situations can be very helpful.

c) To distinguish acute versus chronic parenchymal liver dysfunction: A very high SGOT favors acute liver disease. High globulins and a low serum albumin favor chronic disease. A liver biopsy is of immense value here.

<u>What are the best tests to distinguish intrahepatic (medical) obstructive jaundice from extrahepatic (surgical) obstructive jaundice?</u>

A very difficult problem. None really very good.

a) Among the "medical" diseases are viral hepatitis, drug-induced cholestasis (Thorazine), alcoholic steatonecrosis with obstructive component, 1° biliary cirrhosis.

If SGOT very high favors hepatitis.

If flocculations positive - against extrahepatic obstruction.

If urinary urobilinogen and fecal urobilinogens <u>consistently</u> and <u>persistently very</u> low - favors extrahepatic obstruction.

b) Ancillary tests are used.

Liver biopsy <u>may</u> confirm hepatocellular disease.

A positive immunofluorescent test in which anticytoplasmic antibodies are detected in serum of patients with 1° biliary cirrhosis but not with extrahepatic obstruction (95,96).

Withdrawal of a suspected drug and observation of patient.

Five-day steroid test (97,98). Rationale: If serum bilirubin falls by 50% or more with prednisolone, 40-60 mg/day, suggests viral hepatitis. However, false positive and false negative cases are observed (99,100).

Percutaneous cholangiography (see Ref. 100 for bibliography).

Rationale: If there is extrahepatic obstruction, hepatic bile ducts will be dilated, will be punctured by this procedure, the diagnosis will be confirmed and patient operated on. False positives apparently don't occur - so if a positive test is obtained - one may operate. False negatives do occur (100) - so if a duct is not punctured, this does not rule out extrahepatic obstruction. Test should be done only if one is willing to operate on the patient.

7. What are the best tests for assessing prognosis?

P.T., total serum bilirubin, serum albumin, SGOT.

a) In viral hepatitis a prolongation of P.T. to less than 40% of normal without response to Vitamin K given parenterally is ominous, especially if persistent.

In cirrhotics this also indicates severe liver disease.

b) A total serum bilirubin > 5 mg% suggests a poor prognosis in patients with alcoholic liver disease.

c) A persistent low serum albumin (< 3 g%) may also be interpreted as indicative of severe liver disease.

d) A recurrence of increased SGOT following a bout of viral hepatitis fairly well reflects the presence of active (continued) liver disease.

. Zieve, L., Hill, E., Hanson, M., Falcone, A.B., and Watson, C.J.: Normal and abnormal variations and clinical significance of the one-minute and total serum bilirubin determinations. J. Lab. Clin. Med. 38:446, 1951.

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

. Gray, C.H. Bile Pigments in Health and Disease, Chas. C. Thomas, Springfield, Ill., 1961.

. Thompson, H.E., and Wyatt, B.L.: Experimentally induced jaundice (hyperbilirubinemia); report of animal experimentation and of physiologic effect of jaundice in patients with atrophic arthritis. Arch. Int. Med. 61:481, 1938.

. Powell, L.W., Billing, B.H., and Williams, H.S.: An assessment of red cell survival in idiopathic unconjugated hyperbilirubinemia (Gilbert's Syndrome) by the use of radioactive diisopropylfluorophosphate and chromium. Australas. Ann. of Med. 16: 221, 1967.

. Diamond, I., and Schmid, R.: Experimental bilirubin encephalopathy. The mode of entry of bilirubin-^{14}C into the central nervous system. J. Clin. Invest. 45:678, 1966.

. Johnson, L.M.: The effect of certain substances on bilirubin levels and occurrence of kernicterus in genetically jaundiced rats. In Kernicterus, Ed., Sass-Kortsak, A., U. Toronto Press, 1961.

. Hoffbauer, F.W., Rames, E.D., and Meinert, J.K.: Limitations and merits of a single serum sample analysis in differential diagnosis of jaundice. J. Lab. Clin. Med. 34:1259, 1949.

. Gambino, S.R., Other, A., and Burns, W.: Direct serum bilirubin and sulfobromophthalein test in occult liver disease. J. Amer. Med. Assoc. 201:171, 1967.

. Schaefer, J., and Schiff, L.: Liver function tests in metastatic tumor of the liver: study of 100 cases. Gastroenterol. 49:360, 1965.

. Watson, C.J.: The importance of the fractional serum bilirubin determination in clinical medicine. Ann. Int. Med. 45:351, 1956.

. Neefe, J.R., and Reinhold, J.G.: Laboratory aids in the diagnosis and management of infections (epidemic). Analysis of results obtained by studies on 34 volunteers during the early and convalescent stages of induced hepatitis. Gastroenterol. 7:393, 1946.

. Sherlock, S.: Biochemical investigations in liver disease; some correlations with hepatic histology. J. Path. and Bact. 58:523, 1946.

. Hardison, W.G., and Lee, F.I.: Prognosis in acute liver disease of the alcoholic patient. New Eng. J. Med. 275:61, 1966.

. Harinasuta, U., Chomet, B., Ishak, K., and Zimmerman, H.J.: Steatonecrosis - Mallory Body type. Medicine 46:141, 1967.

. Klatskin, G., and Bungards, L.: An improved test for bilirubin in urine. New Eng. J. Med. 248:712, 1953.

. Gellis, S.S., and Stokes, J. Jr.: The methylene blue test in infectious (epidemic)

18. Fulop, M., Sandson, J., and Brazeau, P.: Dialyzability, protein binding, and renal excretion of plasma conjugated bilirubin. J. Clin. Invest. 44:666, 1965.

19. Ivy, J.H., and Hurley, J.W.: Routine urine bilirubin determinations. J. Amer. Med. Assoc. 176:689, 1961.

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

21. Watson, C.J., and Hoffbauer, F.W.: Liver function in hepatitis. Ann. Int. Med. 26:813, 1947

22. Zieve, L., and Hill, E.: An evaluation of factors influencing the discriminative effectiveness of a group of liver function tests; normal limits of 11 representative hepatic tests. Gastroenterol. 28:766, 1955.

23. Steigmann, F., and Dyniewicz, J.M.: Studies of urobilinogen; the daily urobilinogen excretion in urine and feces in health and disease; an evaluation of Watson's and Sparkman's methods. Gastroenterol. 1:743, 1943.

24. Lester, R.: The intestinal phase of bile pigment excretion. Gastroenterol. 47:424, 1964.

25. Bourke, E., Milne, M.D., and Stokes, G.S.: Mechanisms of renal excretion of urobilinogen. Brit. Med. J. 2:1510, 1965.

26. Ham, T.H.: A syllabus of laboratory examinations in clinical diagnosis; critical evaluation of laboratory procedures in the study of the patient. Ed. Ham, Harvard U. Press, Cambridge, Mass. 1950, pp. 327-333.

27. Watson, C.J., and Hawkinson, V.: Studies of urobilinogen; further experience with the simple quantitative Ehrlich reaction. Corrected calibration of Evelyn colorimeter with a pontacyl dye mixture in terms of urobilinogen. Amer. J. Clin. Path. 17:108, 1947.

28. Popper, H., and Schaffner, F.: Liver: Structure and Function, McGraw-Hill, 1957, New York, pp. 359-364.

29. Watson, C.J.: Regurgitation jaundice: clinical differentiation of the common forms, with particular reference to the degree of biliary obstruction. J. Amer. Med. Assoc. 114:2427, 1940.

30. Wollaeger, E.E., and Gross, J.B.: Complete obstruction of the extrahepatic biliary tract due to carcinoma as determined by the fecal urobilinogen test: Incidence and effect on serum bilirubin concentrations. Medicine 45:529, 1966.

31. Gliedman, M.L., Popowitz, L., and Dennis, C.: The preoperative diagnosis of cancerous obstructive jaundice - the quantitative fecal urobilinogen test. Prog. Clin. Cancer 2:68, 1966, Ed. I.M. Ariel, Grune and Stratton, New York, 1966.

32. Thompson, E.N., and Williams, R.: Effect of age on liver function with particular reference to bromsulphalein excretion. Gut 6:266, 1965.

33. Freston, J.W., and Englert, E.: The influence of age and excessive body weight on the distribution and metabolism of bromsulphalein. Clin. Sci. 33:301, 1967.

34. Zelman, S.: The liver in obesity. Arch. Int. Med. 90:141, 1952.

nces

5. Brohult, J.: Standardization of the bromsulphalein test with respect to body composition. Scand. J. Clin. Lab. Invest. 19:67, 1967.

6. Meyer, R.R.: Effect of iopanoic acid on the sulfobromophthalein test. J. Amer. Med. Assoc. 194:343, 1965.

7. Iber, F.L.: Reactions to sulfobromophthalein sodium injection, USP. Bull. Hopkins Hosp. 116:132, 1965.

8. Mendenhall, C.L., and Leevy, C.M.: False-negative bromsulfalein tests. New Eng. J. Med. 264:431, 1961.

9. Clubb, J.S., Neale, F.C., and Posen, S.: The behavior of infused human placental alkaline phosphatase in human subjects. J. Lab. Clin. Med. 66:493, 1965.

0. Posen, S.: Alkaline phosphatase. Ann. Int. Med. 67:183, 1967.

1. Fenster, L.F., and Simon, F.R.: Studies on biliary excretion of alkaline phosphatase in the rat. Yale J. Biol. and Med. 40:57, 1967.

2. Gutman, A.B.: Serum alkaline phosphatase activity in diseases of the skeletal and hepatobiliary systems. Amer. J. Med. 27:875, 1959.

3. Tietz, N.W., Woodrow, D., and Woodrow, B.: A comparative study of the Bodansky and the Bessey-Lowry and Brock methods for alkaline phosphatase in serum. Clin. Chim. Acta. 15:365, 1967.

4. Deren, J.J., Williams, L.A., Muench, H., Chalmers, T., and Zamcheck, N.: Comparative study of four methods of determining alkaline phosphatase. New Eng. J. Med. 270: 1277, 1964.

5. Dent, C.E., and Harper, C.M.: Plasma-alkaline-phosphatase in normal adults and in patients with primary hyperparathyroidism. Lancet 1:559, 1962.

6. Clark, L.C., and Beck, E.: Plasma "alkaline" phosphatase activity. I. Normative data for growing children. J. Pediat. 36:335, 1950.

7. Heino, A.E., and Jokipii, S.G.: Serum alkaline phosphatase levels in the aged. Ann. Med. Intern. Fenn. 51:105, 1962.

8. Taswell, H.F., and Jeffers, D.M.: Isoenzymes of serum alkaline phosphatase in hepatobiliary and skeletal disease. Amer. J. Clin. Path. 40:349, 1963.

9. Newton, M.A.: The clinical application of alkaline phosphatase electrophoresis. Quart. J. Med. 36:17, 1967.

0. Boyer, S.H.: Alkaline phosphatase in human sera and placenta. Sci. 134:1002, 1961.

1. Chiandussi, L., Greene, S.F., and Sherlock, S.: Serum alkaline phosphatase fractions in hepatobiliary and bone diseases. Clin. Sci. 22:425, 1962.

2. McMaster, Y., Tennant, R., Clubb, J.S., Neale, F.C., and Posen, S.: The mechanism of the elevation of serum alkaline phosphatase in pregnancy. J. Obstet. and Gynaec. Brit. Comm. 71:735, 1964.

3. Posen, S., Neale, F.C., and Clubb, J.S.: Heat inactivation in the study of human alkaline phosphatases. Ann. Int. Med. 62:1234, 1965.

Banks, B.M., Pinea, E.P., Goldberg, J.A., and Rutenberg, A.M.: Clinical value of serum leucine aminopeptidase determinations. New Eng. J. Med. 263:1277, 1960.

5. Pruzanski, W., and Fischl, J.: The evaluation of serum leucine-aminopeptidase estimation; the influence of the administration of steroids on S-LAP activity in obstructive diseases of the biliary tract. (Preliminary Report). Amer. J. Med. Sci. 248:581, 1964.

6. Kowlessar, O.D., Haeffner, L.J., Sleisenger, M.H.: Localization of leucine aminopeptidase in serum and body fluids by starch gel electrophoresis. J. Clin. Invest. 39:671, 1960.

7. Kowlessar, O.D., Haeffner, L.J., Riley, E.M., and Sleisenger, M.H.: Comparative study of serum leucine aminopeptidase, 5-nucleotidase and non-specific alkaline phosphatase in diseases affecting the pancreas, hepatobiliary tree and bone. Amer. J. Med. 31:231, 1961.

8. Rutenberg, A.M., Banks, B.M., Pineda, E.P., and Goldbarg, J.A.: A comparison of serum aminopeptidase and alkaline phosphatase in the detection of hepatobiliary disease in anicteric patients. Ann. Int. Med. 61:50, 1964.

9. Bressler, R., and Forsyth, B.R.: Serum leucine aminopeptidase activity in normal pregnancy and in patients with hydatidiform mole. New Eng. J. Med. 261:746, 1959.

50. Bressler, R., Forsyth, B.R., and Klatskin, G.: Serum leucine aminopeptidase activity in hepatobiliary and pancreatic disease. J. Lab. Clin. Med. 56:417, 1960.

51. Hill, P.G., and Sammons, H.G.: An assessment of 5'-nucleotidase as a liver-function test. Quart. J. Med. 36:457, 1967.

62. Bardawill, C., and Chang, C.: Serum lactic dehydrogenase, leucine aminopeptidase and 5-nucleotidase activities: observations in patients with carcinoma of the pancreas and hepatobiliary disease. Canad. Med. Ass. J. 89:755, 1963.

63. Young, I.I.: Serum 5'-nucleotidase: characterization and evaluation in disease states. Ann. N.Y. Acad. Sci. 75:357, 1958.

64. Eshchar, J., Rudzki, C., Zimmerman, H.J.: Serum levels of 5'-nucleotidase in disease. Amer. J. Clin. Path. 47:598, 1967.

65. Clermont, R.J., and Chalmers, T.C.: The transaminase tests in liver disease. Medicine 46:197, 1967.

66. Dunn, M., Martinis, J., and Reissman, K.R.: The disappearance rate of glutamic oxalacetic transaminase from the circulation and its distribution in the body's fluid compartment and secretions. J. Lab. Clin. Med. 51:259, 1958.

67. Frankl, H.D., and Merritt, J.H.: Enzyme activity in the serum and common duct bile of dogs. Amer. J. Gastro. 31:166, 1959.

68. Wroblewski, F.: The clinical significance of transaminase activities of serum. Amer. J. Med. 27:911, 1959.

69. Zimmerman, H.J., and West, M.: Serum enzyme levels in the diagnosis of hepatic disease. Amer. J. Gastroenterol. 40:387, 1963.

70. Krugman, S., and Ward, R.: Infectious Diseases of Children, 2nd Ed., St. Louis, 1960, C.V. Mosby Co.