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I. QUANTITATIVE VARIATIONS AND CLINICAL SIGNIFICANCE OF THE ONE-MINUTE AND TOTAL SERUM BILIRUBIN DETERMINATIONS*

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I. Historical and Introductory

The studies of Hijmans Van den Bergh and his associates[1,2] during the period of the first World War aroused a great deal of interest in the serum bilirubin and gave impetus to further studies of the nature of what has generally come to be known as the Van den Bergh reaction[3]. This is actually the diazo reaction first described by Paul Ehrlich[4], and studied later in more detail by Pröscher[5] and by Orndorf and Teeple[6]. It is curious that while this color reaction for bilirubin was known for many years and while the presence of bilirubin in the serum had long been recognized, the application was not made until Van den Bergh's work, first published in detail in 1918.[3]

A brief review may be given at the outset of the important findings of Van den Bergh and his co-workers, since some of their observations appear to have been lost sight of in more recent years. They recognized two distinct types of reaction given by two groups of body fluids or serums. The first group, including diluted bile, or the blood serum from patients with "obstructive jaundice", exhibited a "direct" diazo reaction and, in fact, they emphasized[3] that this was prompt or even immediate in character, "in a few seconds, at most 30". In the second group, on the contrary, the direct reaction was slow in its development. This group included hemorrhagic fluids from body cavities, or blood serums from hemolytic jaundice or pernicious anemia patients[3]. "It required 2, 3, 4 minutes or longer before the reaction began to be definite and still longer before it had reached its greatest intensity. If one repeated the test with the addition of alcohol then the reaction was maximal almost in the same moment." The addition of alcohol constituted the "indirect" reaction. Many serums from patients with hemolytic jaundice and pernicious anemia, as well as normal horse and human serum and hemorrhagic fluids from body cavities exhibited either an indirect reaction only, or a direct reaction that was long-delayed in its development.

Other investigators in the period shortly after Van den Bergh's publications gave emphasis to the so-called "biphasic" reaction by which was meant that some of the color developed promptly or almost at once while some was slow in development[7,8]. The term biphasic, however, has not met with any general acceptance mainly because the earlier belief has not been born out that recognition of the prompt as contrasted with the delayed type of reaction would serve to distinguish between extrahepatic obstructive jaundice on the one hand and parenchymal or diffuse hepatic jaundice on the other. Nevertheless, as will be pointed out in the following, the great majority of jaundiced serums exhibit biphasic reactions in some degree and when the proportion of the delayed reacting component is considerable, the likelihood of some form of intrahepatic jaundice or of hemolytic jaundice, or a combination, is greatly increased.

Soon after Van den Bergh's publications evidence was described suggesting the existence of two chemically separate bilirubins and it was thought for a time that a strict chemical difference was responsible for the different Van den Bergh reactions. These were variously

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spoken of as hemobilirubin and cholebilirubin[9], or as bilirubin I and II,[10] referring in both instances to the concept that the indirect reacting bilirubin had not yet been changed to the prompt reacting type by the liver cells. Somewhat later, however, strong evidence was brought forward against a chemical difference in the bilirubin molecule as the basis for the difference in the Van den Bergh reaction. The "hematoidin" which Virchow[11] had first noted in areas of blood extravasation and whose similarity with bile bilirubin had been pointed out by him, was isolated in crystalline form from old cerebral hemorrhages by H. Fischer and Reindel[12], and from echinococcus cyst fluid by Jenke[13]. Direct comparison of the crystals with crystalline bilirubin isolated from cattle gall stones revealed complete identity.

Nevertheless, a number of differences in behavior between the bilirubins in Van den Bergh's two groups of serums or fluids, have been described and repeatedly confirmed. These have been considered in detail in previous reviews[14,15,16]. One of these is the chloroform solubility of the indirect reacting bilirubin as it is found in blood serum. This has been observed by a number of investigators[17,18,19], one of whom[19] described a quantitative fractional method depending upon chloroform extraction, which was shown subsequently to be incomplete[20].

It is entirely possible that the difference in chloroform solubility is on the basis of some differing relationship to a protein. Previous papers[16,21] from this laboratory relating to this subject have favored the concept of Duesberg[22] and of Polonovski and coworkers[23] that the delayed or indirect reacting bilirubin is still attached to the globin of the hemoglobin molecule from which it was derived; and that of Hunter[24] that the prompt reacting type is the sodium bilirubinate such as found in the bile. Coolidge[25] subsequently studied the physical characteristics of the prompt direct and indirect reacting fractions and suggested that the latter was bound to the plasma albumin by a valence bond, the former existing as an easily dissociable complex with the albumin fraction. His findings were in agreement with the earlier observations of Pederson and Waldenström[26] and of Snapper and Bendien[27], both groups noting that the serum bilirubin was associated with the albumin fraction regardless of the type of Van den Bergh reaction exhibited. This, however, is not incompatible with the concept that the indirect reacting bilirubin is still attached to the original globin. On the basis of the molecular weight and electrophoretic behavior of globin, which is very similar to that of albumin, it is conceivable that the small amount which might be present would travel with the albumin fraction, and because of the great disproportion, would be very difficult to distinguish.

There are, however, certain apparent objections to the bilirubin-globin concept. One of these goes back to an observation of Van den Bergh's[3] which has been confirmed repeatedly, e.g., that the prompt reacting bilirubin is adsorbed to some extent when the serum proteins are precipitated by alcohol, while with serums giving only a delayed or indirect reaction the protein precipitate is essentially colorless[28]. This difference may possibly be correlated with the chloroform solubility of the indirect type. It is not impossible to reconcile these solubility characteristics with a bonding such as Coolidge suggests, particularly if it were with globin rather than albumin, it being quite conceivable that a small amount of globin would remain in solution in the alcohol water mixture. This is extremely difficult to test experimentally because of the lack of a natural globin with which to work.

The possibility has often been considered and in fact was first explored and rejected by Van den Bergh[3], that the bile salts might play a decisive rôle in bringing about the prompt reaction. Studies by Watson and Pass[15] in this

laboratory also failed to provide evidence in favor of this belief. Quite recently Kühn[29] has restudied the whole question and confirms the earlier finding of Van den Bergh that the critical concentration of an artificial mixture of bile salts for an acceleration of the diazo reaction is far greater than that encountered in the blood in cases of jaundice. A further objection to a possible rôle of bile salts is the finding of large amounts of prompt reacting bilirubin in many cases of hepatic necrosis in which the serum bile salt concentration is negligible.

From the foregoing it is clear that the mechanism of the prompt-direct and the indirect Van den Bergh reaction is still unknown and requires further study. One might have assumed, however, both from the clarity of Van den Bergh's monograph and the many subsequent papers attesting to the clinical value of the different reactions qualitatively, that a fractional quantitative procedure would have been both logical and generally acceptable. In 1945 a simple modification of the Malloy-Evelyn technique[30] for the fractional serum bilirubin determination was described from this laboratory[20]. In this paper it was suggested that the prompt direct reacting serum bilirubin be determined one minute after the addition of the diazo reagent to the diluted serum. This was quite in accord with Van den Bergh's emphasis on the difference between the immediate direct, developing mainly within the first 30 seconds and the delayed direct developing slowly after two or three minutes. Later one of us (CJW)[16] studied the direct reaction of diluted serum at one minute intervals from the time of mixture up to fifteen minutes. The character of the reaction curve was believed to be consistent with the presence of two substances or of two components of the same substance, one reacting much more slowly because of some difference in its physical-chemical state. Similar data had already been described by others[31,32,33].

Employing the fractional bilirubin determination in the above manner, data were obtained for a moderate number of normal individuals and for various cases with different types of jaundice[16]. From this it was quite clear that the amount of prompt reacting (1') bilirubin in the normal serum was extremely small, usually less than 0.15 mg. per 100 cc. and in that series always less than 0.2 mg. per 100 cc. It was evident, too, that the amount of each fraction was not necessarily related to the total amount, since in certain instances of pure retention jaundice the increase of the 1' fraction was only slight while that of the total bilirubin was very considerable. Conversely, cases were observed in which the total bilirubin was only mildly increased, the majority being composed of the prompt reacting fraction. The presence of bilirubin in the urine was better correlated with increases of the prompt reacting than of the total bilirubin. Thus, marked increases of the total were seen without bilirubinuria and with the prompt reacting fraction increased but slightly. Conversely, bilirubinuria was observed with but mild increases of the total bilirubin, the increase being represented largely by the 1' fraction. In certain cases of hepatitis without jaundice or of hepatitis in the preicteric stage, it was striking to observe that the 1' bilirubin might be increased two to three fold while the total was normal; and in these instances bilirubin was often observed in the urine.

Since the publications from this laboratory relating to the quantitative fractional bilirubin determination, there has been both confirmation and denial of the significance of the 1' or prompt reacting bilirubin[34-39,incl.] Ducci[36] has reported the finding of a generally higher percentage of prompt reacting type in patients with "post hepatic" (extra hepatic obstructive) jaundice than in parenchymal jaundice. Popper[37] found a significant difference in this respect but only in cases of hepatic cirrhosis.

Gray and Whidborne[34] and more recently Klatskin and Drill[38] have described studies, the results of which led them to deny the significance of the 1' or

prompt direct reacting serum bilirubin. Gray and Whidborne[34] interpret the direct reacting curve as a consequence of the failure of Beer's law above concentrations of 1.6 mg. per 100 ml. However, they ignored the fact that the quantitative procedure never involves concentrations of this magnitude.

Klatskin and Drill conclude that the 1' determination is of no value because a) "over a wide range of values there is a more or less direct relationship between the 1' and total serum bilirubin levels," b) "there is no significant difference in either the concentrations of 1' bilirubin or in the 1': total bilirubin ratios in extrahepatic obstruction, cirrhosis, hepatitis, and a variety of miscellaneous conditions," and c) the low 1': total bilirubin ratios of four patients with hemolytic jaundice "fall within the lower limits of the range observed in other types of jaundice, and so are of little value in differentiating this type of jaundice from the others." Aside from the facts that the data on which their conclusions are based appear to be excessively variable, that no tests of significance of differences between disease groups are presented, and that four undefined hemolytic jaundice cases comprise a rather tenuous sample to generalize from; it is not clear how any of the results quoted preclude the utility of the 1' determination.

The present investigation proposes to a) re-examine the validity of the 1' bilirubin as a measure of the prompt direct reacting bilirubin, b) study variations in the color development curves in serums of jaundiced patients, c) evaluate the clinical significance of the 1' bilirubin determination and the bilirubin ratio, and in addition d) measure quantitatively normal and abnormal variation of the 1' and total bilirubin tests.

II. The Modified Malloy-Evelyn Method[20,30]

REAGENTS:

Solution A. 1.0 gm. of sulfanilic acid dissolved in distilled water to which 15 cc. of concentrated hydrochloric acid are added. This is then made up to 1 liter with distilled water.

2. Solution B. 0.5% sodium nitrite.
3. Diazo Reagent is prepared fresh each day by adding 0.3 cc. of solution B to 10 cc. of solution A.
4. Diazo Blank Solution. 15 cc. of concentrated hydrochloric acid diluted to 1 liter with water.
5. Absolute methyl alcohol.

PROCEDURE:

1. 0.5 cc* of serum or plasma is added to 9.5 cc. of water in a colorimeter tube, and after mixing, 5.0 cc. of diluted serum is transferred to a second colorimeter tube. (*If the serum is quite jaundiced, 0.1 cc. of it and 9.9 cc. of water should be used. If the 1:20 dilution has been used and the 1' Evelyn reading is below 40.0, the determination should be repeated with the 1:100 dilution in order to be within the range of accuracy of the colorimeter.)
2. 1.0 cc. of diazo blank solution is added to a tube 1, and 1.0 cc. of diazo reagent to tube 2. Tube 2 is read at exactly 1 minute after the addition of the diazo reagent using the center setting obtained by tube 1 with filter 540 and 6 cc. aperture.
3. 6.0 cc. of methyl alcohol are then added to each of the two tubes. Tube 2 is read again at the end of 15-30 minutes using tube 1 as a blank again.

For directions in preparing a permanent graph of standard concentrations, consult paper of Malloy and Evelyn[30]. Pure bilirubin may be obtained from Armour and Company, Chicago, Illinois.

It should be noted that in the standard solutions used there are varying small amounts of chloroform. Chloroform accelerates the diazo reaction and increases its intensity, but careful study has shown that, in the range of $CHCl_3$ concentration indicated, no difference is observed.

III. Variations in the Color Development Curves of Direct Reacting Bilirubin in Serums of Jaundiced Patients

The serums of 38 consecutive patients with mild to severe degrees of regurgitation jaundice were studied. Direct reacting bilirubin determinations were made at one-half minute, one minute, and every minute thereafter for fifteen minutes, following which the total bilirubin values were measured. The determinations were expressed as percentages of the fifteen minute reading, and color development curves were plotted. The variations in these curves are summarized in Table 1 in which are recorded the means, standard deviations, ranges, and coefficients of variation of the percentage readings at each time interval. Examination of a plot of the average points reveals a curve with a nearly vertical component (prompt reaction) covering the first half-minute, and a largely horizontal component (slow or delayed reaction) covering the last twelve minutes. The slope of the curve changes rapidly in the region of one-half to three minutes, the greatest change occurring in the interval from one-half to two minutes. This interval is most simply represented for this data by the determination at one minute. The relative variation about these means is greatest initially and decreases rapidly. The coefficients of

Table 1

VARIATIONS IN THE DIRECT BILIRUBIN COLOR DEVELOPMENT CURVES

(Values expressed as percentages of fifteen minute reading)

Time Interval	Mean	SD	± 2 SD	Range	CV
½	62.0	9.8	42.4-81.6	32.0-79.1	15.8
1	74.4	8.6	57.2-91.6	42.0-85.7	11.6
2	83.2	8.7	65.8-100.6	50.0-93.8	10.5
3	87.6	7.1	73.4-101.8	59.0-95.3	8.1
4	89.7	7.0	75.7-103.7	64.0-97.1	7.8
5	91.6	5.9	79.8-103.4	69.0-100.0	6.4
6	92.9	4.9	83.1-102.7	76.0-100.0	5.3
7	94.3	4.6	85.1-103.5	81.0-100.0	4.9
8	95.3	3.7	87.9-102.7	84.2-100.0	3.9
9	96.4	3.3	89.8-103.0	84.2-100.0	3.4
10	97.2	2.9	91.4-103.0	87.7-100.0	3.0
11	98.7	1.6	95.5-101.9	93.0-100.0	1.6
12	99.1	1.4	96.3-101.9	93.0-100.0	1.4
13	99.5	0.9	97.7-101.3	96.5-100.0	0.9
14	99.7	0.7	98.3-101.1	96.5-100.0	0.7
15	100.0	0	---	---	0

SD = Standard Deviation CV = Coefficient of Variation

variation at one-half, one, two, and three minutes are, respectively, 15.8, 11.6, 10.5, and 8.1 per cent. Examination of the color development curves for each individual reveals that the rate of change of the slope of every curve is maximal in the region of one-half to two minutes. Analysis of the data presented by Klatskin and Drill[38] in their Tables I and II reveals the same phenomenon. This region can be represented in simplest fashion by the determination at one minute, though a measurement at one and one-half minutes or any intermediate point in the interval would give essentially the same information. The one-minute value thus represents approximately the point of most rapid change from a prompt to a slow reaction and is therefore a useful quantitative measure of the prompt direct reacting serum bilirubin. This use of the 1' bilirubin as a measure of the prompt direct reacting serum bilirubin is based on the actual characteristics of the color development curve, and is independent of any theoretical interpretations of that curve.

The relative amount of prompt direct reacting bilirubin will naturally alter the exact form of such reaction curves. The extremes are well illustrated by curves observed with various specimens of fistula bile and hemorrhagic pleural fluid. With fistula bile the reaction curve rose within a minute to its maximum value and there was no further rise with addition of alcohol, i.e., all of the bilirubin was prompt direct in type. With hemorrhagic (pleural) fluids there was no color development until alcohol was added, indicating that all of the bilirubin was indirect reacting. It is recognized that neither of these results can be translated directly to the situation in blood serum; nevertheless they are compatible with the concept that the prompt reacting, and delayed or indirect reacting components are representative, respectively, of regurgitation and retention factors, as discussed again in the following. The remarkable absence of any direct reaction with hemorrhagic bilirubin containing pleural fluid, is in accord either with a concept of a tighter bond with protein, or absence of some essential catalyst.

IV. Normal and Abnormal Variation of the One-Minute and Total Bilirubin Tests

A. Normal limits of the one-minute and total bilirubin tests.

The norm group consisted of 719 healthy males called in for examination during a veteran's follow-up survey. Half had had viral hepatitis approximately five years previously; the others were their controls. None of the subjects had any evidence of liver disease after exhaustive study including careful examination and a battery of eleven liver function tests. The 1' bilirubin and the total bilirubin frequency distributions for these two sub-samples were nearly identical. Their means and standard deviations corresponded closely, and significance tests for differences between the means of these sub-samples yielded critical ratios of zero and 0.46 for the 1' and total bilirubin comparisons, respectively. Being indistinguishable on the basis of their bilirubin values, the two sub-samples were combined to form the norm group. The average age of this group was 30.3 ± 5.2 years; average weight 162 ± 24 pounds; and average surface area 1.86 ± 0.14 square meters.

Frequency and cumulative frequency distributions were plotted for the 1' and total bilirubin data. The distributions are positively skewed. Their means and standard deviations are summarized in Table 2.

In addition to the mean and standard deviation, the upper 5% and 1% points of these empirical distributions were calculated, on the assumption that any future similar samples will have essen-

Table 2

VALUES OF THE ONE-MINUTE AND TOTAL BILIRUBIN TESTS FOR A SAMPLE OF NORMAL SUBJECTS

Variable	Number	Mean	SD	5% Point	1% Point
One-minute Bilirubin	719	0.11	0.05	0.20	0.25
Total Bilirubin	719	0.62	0.25	1.10	1.50

tially the same distributions. Below the 5% and 1% points fall 95% and 99% of the cases, respectively.

The mean of the 1' bilirubin distribution is 0.11 ± 0.05 mg. per cent. The 5% point is 0.20 mg. per cent; the 1% point 0.25 mg. per cent. The mean of the total bilirubin distribution is 0.62 ± 0.25 mg. per cent. The 5% point is 1.10 mg. per cent; the 1% point 1.50 mg. per cent. The upper limit of normal for the 1' bilirubin determination may be taken, with small likelihood of error, as 0.25 mg. per cent; and for the total bilirubin determination as 1.50 mg. per cent.

B. Comparative contributions of technical, physiological, and individual variations to the overall variation of each test.

Test-retest reliability.

These analyses were carried out separately for normal and abnormal subjects. The normal subjects were 32 patients from the neurology and psychiatry wards of the Veterans Administration hospital, who had no evidence of liver disease. The abnormal subjects were consecutive patients with various liver diseases, who had abnormal bilirubin values. Because we were interested primarily in the variation within the abnormal range of these tests, 1' bilirubin values of at least 0.3 mg. per cent and total bilirubin values of at least 1.7 mg. per cent on one determination were required for inclusion in the sample. With the 1' bilirubin test there were 31 cases satisfying the criterion; with the total bilirubin test 23 cases.

For both normal and abnormal groups, tests were performed on each of two consecutive days, and duplicate determinations were made with each day's specimen. There were thus three criteria for subdividing the total variation on these tests into component parts; e.g., variation among individuals, variation from day to day, and variation between duplicates. Analysis of the variation of a given test into these main components together with their interactions takes the following form (using the 1' bilirubin in normal subjects as an example):

Source of variation	df
Days (Da)	1
Individuals (I)	31
Duplicates (Du)	1
Days x Individuals (Da x I)	31
Days x Duplicates (Da x Du)	1
Individuals x Duplicates (I x Du)	31
Days x Individuals x Duplicates (Da x I x Du)	31
Total	127

The second order interaction (Da x I x Du) is used as the estimate of error for initial comparisons. One can combine all degrees of freedom and corresponding sums of squares for comparisons having

no significant effect to form a pooled estimate of the error mean square from which the standard deviation may be obtained.

Test-retest correlations were used as measures of the reliability of the tests. Duplicates for each day were averaged before the correlations were determined. Mean per cent differences between test and retest were also calculated after averaging the duplicates for each day. In calculating per cent differences the larger test score was taken as the reference value.

Table 3 presents a summary of the raw data of each of the four groups for

Table 3

MEAN VALUES OBTAINED ON ONE-MINUTE AND TOTAL BILIRUBIN TESTS IN NORMAL AND ABNORMAL SUBJECTS, BY DAY AND DETERMINATION

	First Day		Second Day	
	Original	Duplicate	Original	Duplicate
1'B, Normal				
Number	32	32	32	32
Mean	0.14	0.12	0.13	0.10
Range	0.06-0.24	0.03-0.19	0.06-0.21	0.02-0.19
1'B, Abnormal				
Number	31	31	31	31
Mean	3.67	3.62	3.63	3.61
Range	0.14-24.95	0.13-23.45	0.30-24.80	0.24-24.80
TB, Normal				
Number	32	32	32	32
Mean	0.53	0.45	0.48	0.47
Range	0.2-1.4	0.1-1.3	0.3-1.4	0.3-1.5
TB, Abnormal				
Number	23	23	23	23
Mean	8.74	8.59	8.42	8.31
Range	1.6-44.5	1.7-43.0	1.6-42.0	1.6-41.5

which analyses of variance were carried out. The results of the analyses of variance are summarized in Tables 4, 5, 6 and 7.

Results with the 1' bilirubin test in normal individuals are summarized in Table 4. The error mean square, which is a measure of the effect of chance variation, is .00067. Comparisons of all other effects with this allows us to test the significance of their contribution to the total variation observed. In this instance each main effect (contribution of each factor taken by itself) is highly significant. However, the interactions of individual with day to day and technical variations are also significant. Comparisons of the main effects with these significant interactions enable us to test the significance of the contribution of each variable uninfluenced by its association with another simultaneously operating variable. Thus comparison of the mean square for Da with that for the interaction Da x I gives an F value of 4.51 instead of 12.09 for the effect of average day to day variation. Similarly comparison of Du with Du x I

Table 4

ANALYSIS OF VARIANCE OF ONE-MINUTE BILIRUBIN IN NORMAL SUBJECTS

Source of Variation	df	Sum of Squares	Mean Square	F
Da	1	.0081	.0081	12.09**
I	31	.0972	.0031	4.63**
Du	1	.0190	.0190	28.36**
Da x I	31	.0571	.0018	2.68**
Da x Du	1	.0001	.0001	0.15
I x Du	31	.0534	.0017	2.54**
Da x I x Du	31	.0209	.00067	
Total	127	.2558		

Additional comparisons: Da/(Da x I), F = 4.51*
Du/(I x Du), F =11.17**
Pooled error mean square with 32 df = .00066. SD = .026

* Significant (5%, but not 1% point)
** Highly significant (1% point or better)

gives an F value of 11.17 instead of 28.36 for the effect of average variations among duplicates. The average daily and duplicate variations are thus significant though far less so than was apparent at first. The scores obtained on individual subjects also varied significantly from day to day and determination to determination. The average absolute difference between values obtained on two successive days was 25.8% of the larger value. One can therefore conclude that in addition to the significant variation among individuals, which one expects, physiological and technical factors contribute significantly to the variability of this test in the normal range. The operation of these factors together with the narrow range over which normal measurements are taken result in the test's being very unreliable if any conclusion is drawn other than that a given value falls within the normal range. The test-retest correlation is only 0.26.

Results with the 1' bilirubin test in abnormal patients are summarized in Table 5. The error mean square is .0227, and the only main effect of significance is that due to average variation among individuals, which one expects to be great. There is also significant variation from day to day between scores of individual patients as evidenced by the significant Da x I interaction. The average absolute difference between the scores obtained on two successive days is 10.6%. Technical factors do not contribute significantly to the overall variation observed in these patients. In this range the test is highly reliable, the test-retest correlation being 0.996.

Similar analyses were made of the total bilirubin data. In Table 6 are listed the results of analyzing the variation in normal subjects. Average differences among duplicates contribute significantly to the total variation as evidenced by the significant variance ratio of

Table 5

ANALYSIS OF VARIANCE OF ONE-MINUTE BILIRUBIN IN ABNORMAL SUBJECTS

Source of Variation	df	Sum of Squares	Mean Square	F
Da	1	.0182	.0182	0.80
I	30	3565.4629	118.8488	5235.63**
Du	1	.0369	.0369	1.63
Da x I	30	7.7141	.2571	11.33**
Da x Du	1	.0057	.0057	0.25
I x Du	30	.9775	.0326	1.44
Da x I x Du	30	.6811	.0227	
Total	123	3574.8964		

Pooled error mean square with 61 df = .0273. SD = .17

** Highly significant

Table 6

ANALYSIS OF VARIANCE OF TOTAL BILIRUBIN IN NORMAL SUBJECTS

Source of Variation	df	Sum of Squares	Mean Square	F
Da	1	.0094	.0094	1.52
I	31	6.4130	.2069	33.37**
Du	1	.0657	.0657	10.60**
Da x I	31	.3681	.0119	1.92*
Da x Du	1	.0345	.0345	5.57*
I x Du	31	.3118	.0101	1.63
Da x I x Du	31	.1930	.0062	
Total	127	7.3955		

Pooled error mean square with 62 df = .0081. SD = .09

* Significant
** Highly significant

10.60. There is also significant individual variation in the scores obtained on successive days (Da x I interaction significant). The average absolute difference between the results obtained on two consecutive days is 16.1%. However, the difference between the means of each day's determinations is not significant. Differences in technique thus contribute significantly to the total variation on this test; average daily differences do not. The test in normal individuals is reasonably reliable, the test-retest correlation being 0.89.

Results of the corresponding analysis in abnormal patients are listed in Table 7. There is marked variation among individuals as expected. The

Table 7

ANALYSIS OF VARIANCE OF TOTAL BILIRUBIN IN ABNORMAL SUBJECTS

Source of Variation	df	Sum of Squares	Mean Square	F
Da	1	2.0104	2.0104	44.28**
I	22	8799.3587	399.9709	8809.93**
Du	1	.3913	.3913	8.62**
Da x I	22	23.6596	1.0754	23.69**
Da x Du	1	.0109	.0109	0.24
I x Du	22	2.2487	.1022	2.25*
Da x I x Du	22	.9991	.0454	
Total	91	8828.6787		

Additional Comparisons: Da/(Da x I), F = 1.87 Insignificant
Du/(I x Du), F = 3.83 Insignificant
Pooled error mean square with 23 df = .0439. SD = .21
* Significant
** Highly significant

initial daily and duplicate comparisons appear significant. When corrected for the individual differences between days and between duplicates, these main effects are insignificant. The overall variation among abnormal patients is thus almost completely due to average differences among the patients or to individual differences from day to day. The average absolute difference between scores of successive days is 7.1%. The test is highly reliable in this range, the test-retest correlation being 0.996.

These analyses have quantitatively defined the major sources of variation on these tests. As was expected, variation among the individual subjects contributes the most to the overall variation on the tests. This is particularly true among abnormal patients where a wide range of scores is possible. The variation of each individual's scores from day to day (physiological variation) contributes significantly to the overall variation in every instance, though the average daily variation is generally insignificant. Technical factors contribute significantly to the variation of both tests in the normal range, but are of no consequence in the abnormal range. Both tests are highly reliable in the abnormal range. The total bilirubin is reasonably reliable also in the normal range. The 1' bilirubin test is unreliable within its narrow normal range for any purpose other than distinguishing a given score as

normal.

C. Correlation of one-minute bilirubin and total bilirubin in regurgitation jaundice.

A plot was made on a log log scale of data from 555 cases with various diseases affecting the liver. Cases with cirrhosis, viral hepatitis, obstructive jaundice, congestive failure, and miscellaneous diseases were represented in the same proportions as in the parent sample of 614 cases considered in Section V-B. The resulting scatter diagram reveals the following facts.

There is no segregation of points according to a given disease group. However, there are relatively few hepatitis or obstructive jaundice cases with total bilirubin values below 2.0 mg. per cent. The scatter of points increases as the bilirubin values decrease below the one-minute bilirubin level of 1.0 mg. per cent. Within the normal range the scatter for patients with liver disease is the same as that for normal subjects. In the region of 0.45 to 1.0 mg. per cent the correlation, based on 71 cases, is 0.62. Above this point there are 349 cases for whom the correlation is 0.96, a nearly perfect relationship. The regression of total bilirubin (y) on 1' bilirubin (x) in this region is $\log y = 0.9 \log x + .3061$ or $y = 2.02x^{0.9}$. There is no marked variation about this curve irrespective of the use of rectangular or logarithmic coordinates, and despite the performance of the tests in four completely independent laboratories. In view of this, the scatter apparent in an analogous correlation diagram presented by Klatskin and Drill[38] seems excessive. The lack of segregation among the various disease types in the correlation plot is consistent with the extensive overlapping found for the same groups when frequency distributions of the bilirubin ratios were compared.

V. Evaluation of the Clinical Significance of the One-Minute Bilirubin Determination

A. Comparative incidence of abnormal values with the one-minute and total bilirubin tests in various liver diseases.

Records of 307 unselected cases from the Veterans and University hospitals were reviewed. The diagnosis in each case was unequivocal. Both sexes and all age groups were represented. However, the sample consisted predominantly of males in the older age groups. Included were 110 cases of hepatic cirrhosis, the majority of which were of the Laennec type; 49 cases of viral hepatitis; 53 cases of obstructive jaundice, including obstructions by neoplasm, stone, or stricture; 19 cases with congestive failure; and 76 cases with approximately forty miscellaneous diseases including lymphomas, metastatic neoplasms, abdominal abscesses, pancreatitis, ulcerative colitis, malaria, empyema of gall bladder, periarteritis nodosa, etc. The information for each disease group is recorded in Table 8. The limits of normal for the 1' and total bilirubin determinations were taken to be 0.25 and 1.5 mg. per cent, respectively, as discussed in the foregoing. In the lower six lines of the table are recorded for each test the frequency of occurrence of values in three selected intervals, listed by disease type. Since the values recorded were generally of the same order of magnitude as the maximal ones listed in the patients' charts, these figures portray approximately the comparative incidence of bilirubin scores at various levels, according to disease grouping. It is apparent that relatively few hospitalized cases of viral hepatitis or obstructive jaundice fail to have moderate or severe jaundice at the height of their illness. Over half the cirrhosis patients on the other

Table 8

COMPARATIVE INCIDENCE OF NORMAL AND ABNORMAL VALUES

ON THE ONE-MINUTE AND TOTAL BILIRUBIN TESTS, BY DISEASE TYPE

Classification	Cirrhosis		Viral Hepatitis		Obstructive Jaundice		Miscellaneous		Congestive Failure	
	No.	%	No.	%	No.	%	No.	%	No.	%
Total cases	110	100	49	100	53	100	76	100	19	100
Abnormal 1'B	78	70.9	48	98.0	52	98.1	44	57.9	13	68.4
Abnormal TB	47	42.7	45	91.8	44	83.0	22	28.9	9	47.4
Normal 1'B, Abnormal TB	0	0	0	0	1	1.9	2	2.6	0	0
Abnormal 1'B, Normal TB	31	28.2	3	6.1	9	17.0	24	31.6	8	42.1
1'B $<$ 0.25 mg. %	32	29.1	1	2.0	1	1.9	32	42.1	6	31.6
0.25 $\leq$ 1'B $<$ 1.00 mg. %	48	43.6	7	14.3	18	34.0	33	43.4	11	57.9
1'B $\geq$ 1.00 mg. %	30	27.3	41	83.7	34	64.2	11	14.5	2	10.5
TB $<$ 1.5 mg. %	63	57.3	4	8.2	9	17.0	54	71.1	10	52.6
1.5 $\leq$ TB $<$ 2.0 mg. %	9	8.2	2	4.1	9	17.0	8	10.5	6	31.6
TB $\geq$ 2.0 mg. %	38	34.5	43	87.8	35	66.0	14	18.4	3	15.8

hand have total bilirubin values falling within normal limits, and only a third have moderate or severe degrees of jaundice. Forty-four per cent have 1' values between 0.25 and 1.0 mg. per cent in contrast to 29 per cent with normal values and 27 per cent with values of 1.0 mg. per cent or greater. The relative incidence in this intermediate 1' bilirubin range for patients with congestive failure and miscellaneous diseases associated with liver dysfunction is similar, being 58 per cent and 43 per cent, respectively.

The comparative overall incidence of abnormal values with each test is listed in lines two and three of the table. Of the 110 patients with cirrhosis, 71 per cent had abnormal 1' bilirubin scores and 43 per cent had abnormal total bilirubin scores, an incidence difference that is highly significant (χ^2 = 17.8, P $<$ .001). For the 76 cases with miscellaneous diseases the corresponding figures were: 1' bilirubin, 58 per cent abnormal; total bilirubin, 29 per cent abnormal; again a highly significant difference (χ^2 = 13.0, P $<$ .001). Similar comparisons for the hepatitis and obstructive jaundice groups are of little value since such a large proportion of these cases had severe degrees of jaundice. On the basis of individual observations one may anticipate that in the incipient stages of these diseases findings similar to those in the cirrhotic and miscellaneous disease groups will obtain. The congestive failure group had findings similar to those of the cirrhotic group, however, the number of cases was small and the difference observed could have occurred by chance (χ^2 = 1.73, P = .10-.20).

It is thus apparent that in those diseases in which relatively few cases have severe jaundice the 1' bilirubin will indicate abnormality with greater frequency than the total bilirubin. This is even more apparent when the comparisons in lines four and five of the table

are made. In no disease group are there more than one or two cases having normal 1' bilirubin and abnormal total bilirubin scores. In the case of the important cirrhosis group there were none. In contrast 28 per cent of this group had normal total bilirubin and abnormal 1' bilirubin values. For the miscellaneous and congestive failure groups the corresponding figures were 32 per cent and 42 per cent, respectively. There were 63 cirrhosis patients with normal total bilirubin scores. Of this number 31, or 49 per cent, had abnormal 1' bilirubin values. Similarly, of 54 miscellaneous group patients with normal total bilirubin scores 24, or 44 per cent, had abnormal 1' bilirubin scores. The corresponding figures for the hepatitis, obstructive jaundice, and congestive failure groups were 75 per cent, 100 per cent, and 80 per cent; however, these were based on small numbers of cases.

Though the 1' bilirubin clearly is much more efficient than the total bilirubin in distinguishing an individual with liver disease, the tests are equally efficient in distinguishing a group of abnormal patients from a group of normal subjects. This is illustrated by the following comparison of the cirrhosis patients with a sample of 100 normal males. Tests of the significance of the difference between the means of the two samples were calculated for each bilirubin test. The critical ratio (i.e., difference between means expressed in standard units) for the 1' bilirubin test was 6.59 and for the total bilirubin test was 6.97. There is thus little difference in the efficiency with which these two tests distinguish the two groups, on the average. This is consistent with the finding of a high correlation between the 1' and total bilirubin determinations in the abnormal range. From these results it is evident that among patients with liver disease, total bilirubin values within the normal range, when they occur, are generally higher than those of normal individuals. However such values fall within the limits of normal and in terms of the individual case cannot be used for diagnostic purposes.

B. Discriminative value of the bilirubin ratio (1'/T x 100).

Records of 614 cases with various diseases affecting the liver, including the 307 cases noted above, were reviewed. Readily available records were used; but, in general, cases were selected consecutively starting with the most recent admissions and working backward. Of the total, 338 cases were observed in the Veterans hospital and 276 in the University hospitals. Four different laboratories and at least 15 different technicians were concerned with the bilirubin determinations at one time or another. Included in the sample of 614 patients were 155 cases with cirrhosis, predominantly Laennec's cirrhosis; 110 cases with viral hepatitis; 164 cases of obstructive jaundice including stone, stricture, and neoplasm; 40 cases with congestive failure; and 145 cases with approximately fifty miscellaneous diseases similar to those listed above. No attempt was made to classify these patients with respect to age or sex, but approximately two-thirds were males in the older age groups.

Review was also made of records of 30 patients with hemolytic anemia. Each case was readily classified as such on the basis of the clinical features and the presence of reticulocytosis or significant elevation of the fecal urobilinogen. Half were males, half females. Sixteen were of the congenital type, 7 acquired, and 7 of miscellaneous type.

Bilirubin ratios were calculated for each of the six disease groups, and frequency distributions of these ratios were plotted. It was found that the distributions varied with the level of the total bilirubin, so the groups were subdivided into three levels; TB 0-1.5 mg. per cent, TB 1.6-2.0 mg. per cent, and TB > 2.0 mg. per cent. Distributions of the bilirubin ratio for all cases with regurgitation jaundice subdivided into these three groups are character-

ized by the statistics recorded in Table 9. Corresponding values for the sample of 30 hemolytic jaundice cases are listed for contrast. The bilirubin ratio decreases significantly as the total bilirubin falls below the value of 2.0 mg. per cent. Above this point there is no correlation between the two. The frequency distributions for the various disease groups were plotted for

Table 9

RELATION OF BILIRUBIN RATIO TO DEGREE AND TYPE OF JAUNDICE

	Number	Mean	SD
Regurgitation Jaundice			
TB 0-1.5 mg. %	153	36%	13%
TB 1.6-2.0 mg. %	45	46%	15%
TB > 2.0 mg. %	376	56%	12%
Hemolytic Jaundice			
All cases	30	15%	8%

those cases with total bilirubin > 2.0 mg. per cent, the only classification containing sufficient numbers of cases for such comparisons. The statistics characterizing these distributions are listed in Table 10. The regurgication-type cases overlap nearly completely. However, the hemolytic cases show practically no overlap with any of the other groups. The average for the hemolytic group is 15%, and 96% of the cases have values less than 30%. Of the regurgitation-type cases, the patients with cirrhosis had the lowest mean bilirubin ratio, e.g., 51%. Only 9% of the cases had ratios below 30%. The well known fact that hemolytic jaundice patients have very little prompt direct reacting bilirubin is reemphasized by this comparison, and the value of the bilirubin ratio in differentiating retention from regurgitation jaundice is self-evident.

One cannot differentiate among

Table 10

BILIRUBIN RATIOS IN PATIENTS WITH MODERATE OR MARKED JAUNDICE, BY DISEASE TYPES

All Cases, TB > 2.0 mg. %	Number	Mean	SD
Cirrhosis	77	51%	16%
Hepatitis	101	57%	8%
Obstr. Jaundice	138	57%	10%
Cong. Failure	20	52%	10%
Misc. Reg. J.	60	56%	15%
Hemolytic J.	23	15%	8%

individuals with regurgitation jaundice by means of the bilirubin ratio. However, certain average comparisons reveal significant differences. The mean ratio of 51% for the cirrhotic group is significantly less than the mean of 57% for the hepatitis group (critical ratio = 2.95). A similar comparison is found with the obstructive jaundice sample. A significantly ($\chi^2 = 15$, $P < .001$) greater number of cirrhosis patients also have ratios below 40%. Thus 21% of the cirrhosis cases, 2% of the hepatitis cases, and 4% of the obstructive jaundice cases have ratios below 40%. On the basis of these comparisons, it appears unlikely that a patient having a total bilirubin over 2.0 mg. per cent and a bilirubin ratio less than 40% would be apt to have hepatitis or obstructive jaundice.

The lower average bilirubin ratio in patients with cirrhosis may be a consequence of including some patients having a hemolytic component; or it may be due to relatively greater hepatocellular than cholangiolar impairment. In either event, one would anticipate that the frequency distribution of bilirubin ratios for a group of such patients would fall somewhere between the distributions for the hemolytic and the ordinary regurgitation jaundice groups, as observed.

It should be noted that while there is relatively great overlap among the regurgitation jaundice cases, the variations observed within any disease group are consistent with the concept of varying proportions of retention and regurgitation factors.

VI. Discussion

The results of the present study have fully confirmed previous observations and conclusions as to the validity and usefulness of quantitative measurement of the prompt direct or 1' reacting serum bilirubin. As in the first paper[20] suggesting this measurement it is emphasized again that the time interval of one minute is not regarded as a point of sharp or absolute separation of the prompt and delayed reacting components of the serum bilirubin. It affords, nevertheless, a reliable means of recording in simple quantitative fashion the components of the diazo reaction which Van den Bergh emphasized. In this connection it may be noted that even those who deny the value of the measurement of the prompt reacting bilirubin at 1' readily grant the validity of Van den Bergh's qualitative designations.[34] The present study clearly confirms the view that quantitative measurement of these different components under the conditions specified, enhances the value of the Van den Bergh reaction in the clinical study of jaundice and liver and biliary tract disease.

In the light of the results presented, it is desirable to discuss more specifically the objections which have been raised as to the validity of the prompt reactiong or 1' serum bilirubin. The finding that the diazo color reaction does not obey Beer's law above concentrations of 1.6 mg. of bilirubin per 100 cc. in no way affects results obtained with a photoelectric colorimeter. It is probable that in earlier, simple colorimetric procedures in which great dilution was impossible, error was often introduced because of failure to obey Beer's law in higher concentrations. In other respects the method used by Gray and Whidborne[34] was not comparable to ours, particularly in the use of caffein sodium benzoate which has a pronounced catalytic effect, very similar to that of alcohol itself.

The objection that the time reaction curve of the direct Van den Bergh is a variable diphasic curve representative of but a single rather than two differing components of bilirubin, deserves careful consideration. From a physical-chemical standpoint the character of the curve points rather to the presence of two components with different speeds of reaction. This, of course, does not necessarily mean the presence of two bilirubins of differing chemical structure. It could equally well be due to two bilirubins existing in a different physical state, perhaps with respect to another

molecule such as a protein. In essence this is the concept of Coolidge[25], and more recently of Deenstra[35]. The idea that the indirect reacting bilirubin is still attached to the original globin of the hemoglobin molecule while the prompt reacting type is a sodium bilirubinate adsorbed on the serum albumin is compatible with these views and with the interpretation that the time reaction curve is a true diphasic curve indicative of two components* Nevertheless, it is recognized that other possibilities have not been excluded.

Considerable emphasis has been given by others[38] to the observation of the same type of time reaction curve in a solution of crystalline bilirubin in a 1:8:1 mixture of chloroform, methyl alcohol, and water. This does not take into account, however, previous recognition that chloroform greatly accelerates the Van den Bergh reaction. Hunter[24] showed years ago that pure bilirubin suspended in alcohol does not react with the diazo compound but that the addition of as little as 1 per cent of chloroform quickly brings about formation of the azo dye. As pointed out in an earlier publication[16] this indicates "a striking affinity of chloroform for bilirubin, quite possibly a formation of a molecular compound." This might indeed be the explanation of the chloroform solubility of the indirect reacting bilirubin, since it is conceivable that chloroform might readily displace globin or another protein, while it would hardly be expected to affect a sodium bilirubinate adsorbed on protein. It is clear that this ought to be a fruitful field for further study.

The bilirubin ratio is not ordinarily applicable to the individual case in the differential diagnosis of regurgitation jaundice, and objections to its use in this fashion seem justified. In fact the present study reveals clearly that a close correlation exists between the 1' and total bilirubin values in cases with appreciable regurgitation jaundice. However, certain significant group differences do exist. Thus cirrhosis patients show a significantly lower average ratio, and more of them have ratios below 40% than is the case for patients with hepatitis or obstructive jaundice.

It has been surprising to us that doubt has been cast upon the value of the 1' bilirubin in hemolytic and other types of retention jaundice, and in patients with incipient or latent jaundice of any type. We are inclined to believe that this can only be on the basis of lack of adequate experience with both disease groups, since the data attesting to its value appears to us to be unequivocal.

Finally, it is desirable to comment on the relationship of the serum bilirubin to the occurrence of bilirubinuria. In the older literature it was often stated that the renal threshold for bilirubin was 2.0 mg. per 100 cc. As mentioned in the introduction, however, the study of hepatitis cases made it apparent that the threshold is quite variable not only from case to case but at different stages of the disease. Bilirubinuria was often noted in the preicteric or prodromal stage, or in cases of hepatitis without jaundice when the total bilirubin was within normal limits (< 1.5 mg. per cent)[16]. In the convalescent stage, bilirubinuria disappeared as a rule when the 1' bilirubin was between 0.8-1.2 mg. per 100 cc., but occasionally much higher than this. In rare instances bilirubin could not be detected in the urine when the prompt reacting serum bilirubin was from 3.6-5.8 mg. per 100 cc. The latter value was noted in a case of acute atrophy of the liver in which coma and nitrogen retention were prominent. It is probable that renal injury is important

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*In this connection it is well to call attention to the observation of Martin[40] that a small fraction of the serum bilirubin is rather tightly bound to the α globulins rather than the albumin. The exact significance of this finding is not yet clear.

in altering the threshold in such instances.

The examples given in Table 11 show clearly that bilirubinuria is not dependent on the total bilirubin concentration. Nor is it correlated with any given level of the 1' bilirubin. Nevertheless, bilirubinuria has been observed only when the prompt reacting bilirubin is increased, and its appearance is better correlated with increases of this component, than with the elevation of the delayed or indirect reacting bilirubin. This, after all, does nothing more than reemphasize the correlation of "acholuric" jaundice with the finding of a delayed or indirect type of Van den Bergh reaction. It should be noted that "acholuric" jaundice is usually synonymous with retention jaundice, which includes hemolytic jaundice, constitutional hepatic dysfunction (familial nonhemolytic jaundice) and certain cases of isolated hepatocellular injury.

Table 11

CASES ILLUSTRATING RELATIONSHIP OF BILIRUBINURIA TO THE ONE-MINUTE AND TOTAL BILIRUBIN VALUES AND TO THE BILIRUBIN RATIO

Case	Diagnosis	Serum Bilirubin 1'	Serum Bilirubin Total	Bilirubin Ratio (1'/T x 100)	Urine Bilirubin
1	Cirrhosis	0.5	0.7	71.4	0
2	Hepatic Carcinomatosis	0.5	1.0	50.0	+
3	Hepatitis s jaundice	0.8	1.8	44.4	+
4	Hepatitis (subsiding)	1.2	2.6	46.2	+
5	Familial Hem. Jaund.	0.24	3.2	7.5	0
6	Familial Hem. Jaund.	0.3	3.5	8.6	0
7	Cirrhosis	1.9	4.0	47.5	+
8	Familial Hem. Jaund.	0.38	5.2	7.3	0
9	Common Duct Stone	6.4	8.4	76.2	+
10	Ca of Pancreas	6.1	8.7	70.1	+
11	Constitutional Hep. Dysf.	0.96	8.8	10.9	0
12	Familial hem. Jaund.	0.71	9.8	7.2	0
13	F. H. J. and C.D. stone	5.2	11.0	47.3	+
14	Cirrhosis	5.9	11.4	51.8	+
15	Acute Atrophy	5.2	14.7	35.4	0
16	Infantile Cirrhosis (?)	0.59	14.8	4.0	0
17	Common Duct Stone	20.0	29.4	68.0	+
18	Ca of Pancreas	17.8	31.8	56.0	+
19	Ca of Pancreas	27.0	44.5	60.7	+
20	Acute Atrophy	31.9	51.2	62.3	+

VII. Illustrative Cases

In the following several case protocols are included which illustrate the striking differences which may occur in the serum bilirubin partition in disease. Other pertinent observations have been included for the sake of comparison.

1. Hepatic cirrhosis with macrocytic hemolytic anemia

Male 54 Long standing chronic alcoholism; jaundice, ascites, spider nevi; recurrent somnolence, eventual coma and death.
Macrocytic anemia; Reticulocytes 15-20 per cent.
Hematinemia; feces urobilinogen 950 mg./d., urine urobilinogen 28-35 mg./d.
SBl' 5.4 mg./100 cc.
SBT 20.5 mg./100 cc.

2. Infantile cirrhosis (?) with retention jaundice

Female 8 mos. Jaundice from 7th day, slowly deepening 1 month, then less intense but persistent.
Stools consistently light brown.
Prominent supf. abd. veins; liver not palp., dulness reduced; spleen not felt.
Hb. 9.0 to 10.2 gms., no erythroblasts, fragility normal, leukocytes normal.
Serum proteins: 7.2 gms/100 cc., (A = 4.78, G = 2.5)
Total cholesterol: 125 mg./100 cc.
Ester cholesterol: 88 mg./100 cc.
Ceph. cholesterol: 0
Alk. p'tase: 13.5 Bodansky U.
S.B.l': 0.35-1.0; T:10.8-16.3 (ten determinations on different dates)
Urine bilirubin consistently negative by Ba strip test.
Duodenal drainage bilirubin, 1':3.2, T:5.8
Feces Ehrlich units (urobilinogen) 3.6-12.8 per 100 gms. (13 determinations)
Urine urobilinogen qual. neg.; EU 0.46/100 cc.

3. Hepatic cirrhosis with pruritus

Female 64 8-18-49. Back pain, bloating, pruritus, 1 year. Recent hematemesis.
Xanthelasma, liver enl. 8 cm. below costal margin in MCL.
S.B:1' 0.5 T 0.7, Urine bilirubin 0 (Hb. 8.6, RBC 2.4 - recent severe blood loss)
Serum bile acids: 6.8 mg.% (control 0.9 mg.%)
CC: 0 TT: 16 ZT: 17 TC: 237, 65% ester.
Brom.: 44%
Serum Proteins: 6.6 gms/100 cc. (A = 2.7, G = 3.9)
Cholangiogram (local anaesth.) normal
Liver biopsy - portal (non fatty) cirrhosis.

4. Hepatic carcinomatosis without jaundice

Male 71 10-19-50. Rt. upper abd. pain and tenderness 10 wks.
Anorexia, weight loss 30#
Marked tenderness R.U.Q., liver not palp.
SB 1': 0.5 T: 1.0 Urine bilirubin +
Laparotomy: Diffuse hepatic carcinomatosis

VIII. Summary and Conclusions

1. The color development time curves of direct reacting bilirubin consist generally of two components, one prompt, the other slow or delayed. The 1' serum bilirubin is found to be a useful quantitative measure of the amount of prompt direct reacting bilirubin.

2. The mean normal 1' bilirubin is 0.11±0.05 mg. per cent. The mean normal total bilirubin is 0.62±0.25 mg. per cent. The upper limit of normal for the 1' determination is 0.25 mg. per cent; for the total determination, 1.5 mg. per cent.

3. Analyses of the variation on each test in normal and abnormal subjects emphasize the significant contribution of physiological factors to the overall variation in every instance. Technical factors contribute significantly to the variation in both tests in the normal range but are of no consequence in the abnormal range. Both tests are highly reliable in the abnormal range. The total bilirubin is also quite reliable in the normal range. However, the 1' bilirubin is unreliable in the normal range for any purpose other than distinguishing a given score as normal.

4. In regurgitation jaundice the 1' and total bilirubin scores are nearly perfectly correlated above a 1' level of 1.0 mg. per cent. The equation relating the two variables in this region is $(TB) = 2.02\ (1'B)^{0.9}$. There is no segregation of points in the scatter diagram according to disease groups. Below this level the correlation decreases progressively.

5. The 1' bilirubin is more effective than the total bilirubin in detecting certain individuals with liver or biliary tract disease, though the tests are equally efficient in differentiating a normal from an abnormal group of patients, in the aggregate.

6. The bilirubin ratio (1'/T x 100) decreases significantly as the total bilirubin decreases below 2.0 mg. per cent. Above 2.0 mg. per cent there is no correlation between the two.

7. There is nearly complete separation between the bilirubin ratio frequency distributions of patients with hemolytic jaundice and patients with regurgitation jaundice.

8. One cannot differentiate among individuals with regurgitation jaundice by means of the bilirubin ratio because there is relatively great overlapping of the frequency distributions for the various disease groups. Certain group comparisons, however, reveal significantly different average ratios.

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II. MEDICAL SCHOOL NEWS

Coming Events

Jan. 29 - Feb. 10	Continuation Course in Clinical Neurology for General Physicians, Neurologists, and Psychiatrists.
January 30	J. B. Johnston Lecture: "Wakefulness and Sleep." Horace W. Magoun; Auditorium, Museum of Natural History; 8:00 p.m.
February 1	Special Lecture: "The Role of Intestinal Bacterial Flora in Human Nutrition and Disease." F. W. Bernhart; Todd Amphitheater, U. H.; 4:00 p.m.
February 15	E. Starr Judd Lecture: "The Surgical Treatment of Constrictive Pericarditis." Emil Holman; Medical Science Amphitheater; 8:15 p.m.
February 15 - 17	Continuation Course in Cardiovascular Diseases for General Physicians.
March 1	Clarence M. Jackson Lecture: "Fractures About the Hip - Early and Late Therapy." Carl E. Badgley. .

Faculty News

Dr. George E. Moore, Clinical Instructor in Surgery, was recently awarded the Samuel D. Gross prize in surgery. The Gross award is made every five years for the most outstanding contribution to surgery during that period. Dr. Moore's work in the use of radioactive compounds in the diagnosis of brain tumors brought him this coveted honor with the accompanying prize of $1,500. He hopes to use the prize money in helping to meet expenses of publishing the work in book form.

Dr. Donald W. Hastings, Professor and Head of the Department of Psychiatry and Neurology, will give a series of lectures on "Emotional Aspects of Combat Flying" at Randolph Field, San Antonio, Texas, February 12 - 17. Dr. Hastings' lectures will be part of the course for flight surgeons in the Army Air Force's school of aviation medicine.

Dr. Ruth E. Boynton, Director of the Student Health Service and Professor of Public Health, has been awarded a Fulbright Fellowship to study university and college health services in England. Dr. Boynton will also serve as a consultant to university and college administrative officials concerning problems related to health service for students. She will leave early in February and will spend six months in carrying out her study.

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Clinical Neurology Course

A continuation course in Clinical Neurology will be presented at the Center for Continuation Study January 29 - February 10. The course is given under the direction of Dr. A. B. Baker, Professor and Head of the Division of Neurology. Outstanding scientists who will participate as visiting faculty members in the course include Dr. Pearce Bailey, Associate Professor of Neurology, Georgetown University School of Medicine; Dr. Horace W. Magoun, Professor of Microscopic Anatomy, University of California Medical School; Dr. S. B. Wortis, Professor of Psychiatry and Neurology, New York University Medical School; and Dr. Edgar A. Kahn, Professor of Surgery, University of Michigan Medical School. Dr. Magoun will also deliver the annual J. B. Johnston Lecture at 8:00 p.m. on Tuesday, January 30, in the auditorium of the Museum of Natural History. Dr. Magoun's subject on this occasion will be "Wakefulness and Sleep." All interested physicians and the public are invited.

III. UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

Visitors Welcome

January 28 - February 3, 1951

Sunday, January 28

University Hospitals

9:00 - 10:00 Surgery Grand Rounds; Station 22.

10:30 - Surgical Conference; Todd Amphitheater.

Monday, January 29

Medical School and University Hospitals

9:00 - 9:50 Roentgenology-Medicine Conference; L. G. Rigler, C. J. Watson and Staff; Todd Amphitheater, U. H.

9:00 - 10:50 Obstetrics and Gynecology Conference; J. L. McKelvey and Staff; M-109, U. H.

10:00 - 12:00 Neurology Rounds; A. B. Baker and Staff; Station 50, U. H.

11:00 - 11:50 Physical Medicine Seminar; Scoliosis -- Bracing; Glenn Gullickson; E-101, U. H.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Eustis Amphitheater, U. H.

12:00 - 12:50 Physiology Seminar; The Chemistry of Glycogen; Fred Smith; 214 Millard Hall.

12:15 - 1:20 Obstetrics and Gynecology Journal Club; Staff Dining Room, U. H.

1:30 - 2:30 Pediatric-Neurological Rounds; R. Jensen, A. B. Baker and Staff; U. H.

4:00 - Public Health Seminar; 113 Medical Sciences.

4:00 - Pediatric Seminar; Well Baby Care; Dr. Hoeflich; Sixth Floor West, U.H.

4:30 - 5:30 Dermatological Seminar; M-436, U. H.

5:00 - 5:50 Clinical Medical Pathologic Conference; Todd Amphitheater, U. H.

5:00 - 6:00 Urology-Roentgenology Conference; C. D. Creevy, O. J. Baggenstoss, and Staffs; Powell Hall Amphitheater.

Minneapolis General Hospital

9:00 - 10:00 Pediatric Rounds; Dr. Tobin; 5th Floor Annex.

10:00 - 11:00 Pediatric Rounds; Franklin Top; 7th Floor Annex.

Monday, January 29 (Cont.)

Minneapolis General Hospital (Cont.)

1:00 - 2:00 Staff Meeting; Classroom; 4th Floor.

2:00 - 3:00 Journal Club; Classroom, Station I.

Veterans Administration Hospital

9:00 - G. I. Rounds; R. V. Ebert, J. A. Wilson, Norman Shrifter; Bldg. I.

11:30 - X-ray Conference; Conference Room; Bldg. I.

1:00 - Metabolic Disease Rounds; N. E. Jacobson and G. V. Loomis; Bldg. I.

4:00 - Therapeutic Conference; Conference Room, Bldg. I.

Tuesday, January 30

Medical School and University Hospitals

9:00 - 9:50 Roentgenology Pediatric Conference; L. G. Rigler, I. McQuarrie and Staffs; Eustis Amphitheater, U. H.

9:00 - 12:00 Cardiovascular Rounds; Station 30, U. H.

12:30 - 1:20 Pathology Conference; Autopsies; J. R. Dawson and Staff; 102 I. A.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U.H.

4:00 - 5:00 Physiology-Surgery Conference; Todd Amphitheater, U. H.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

5:00 - 6:00 X-ray Conference; Eustis Amphitheater, U. H.

*8:00 p.m. J. B. Johnston Lecture; Wakefulness and Sleep; Horace W. Magoun; Museum of Natural History Auditorium.

Ancker Hospital

1:00 - 2:30 X-ray Surgery Conference; Auditorium.

Minneapolis General Hospital

8:00 - 9:00 Pediatric Rounds; Forrest Adams; 4th Floor Annex.

8:30 - Pediatric Allergy Rounds; Dr. Nelson; 4th Floor Annex.

Veterans Administration Hospital

8:45 - Surgery Journal Club; Conference Room; Bldg. I.

8:30 - 10:20 Surgery Conference; Seminar Conference Room, Bldg. I.

9:30 - Surgery-Pathology Conference; Conference Room, Bldg. I.

Tuesday, January 30 (Cont.)

Veterans Administration Hospital (Cont.)

10:30 - 11:50 Surgical Pathological Conference; Lyle Hay and E. T. Bell.

10:30 - Surgery Tumor Conference; Conference Room, Bldg. I.

1:00 - Chest Surgery Conference; J. Kinsella and Wm. Tucker; Conference Room, Bldg. I.

1:30 - Liver Rounds; Samuel Nesbitt.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III.

3:30 - 4:20 Clinical Pathological Conference; Conference Room, Bldg. I.

Wednesday, January 31

Medical School and University Hospitals

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-109, U. H.

8:00 - 9:00 Roentgenology-Surgical-Pathological Conference; Allen Judd and L. G. Rigler; Todd Amphitheater, U. H.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; Medicine Case; O. H. Wangensteen, C. J. Watson and Staffs; Todd Amphitheater, U. H.

12:00 - 1:00 Radio-Isotope Seminar; Clinical Use of Radio-Isotopes in Present Methods; E. F. Everett; 113 Medical Sciences.

4:00 - 6:00 Ophthalmology Seminar; Todd Room, 5th Floor, U. H.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Eustis Amphitheater.

5:00 - 7:00 Dermatology Clinical Seminar; Dining Room, U. H.

8:00 p.m. Dermatological Pathology Conference; Todd Amphitheater, U. H.

Ancker Hospital

8:30 - 9:30 Clinico-Pathological Conference; Auditorium.

3:30 - 4:30 Journal Club; Surgery Office.

Minneapolis General Hospital

9:00 - 10:00 Pediatric Rounds; Dr. Robin; 5th Floor Annex.

11:00 - 12:00 Pediatric Rounds; Franklin Top; 7th Floor Annex.

12:15 - Staff Meeting; Case Presentation; Harry Orme; 4th Floor Annex.

Wednesday, January 31 (Cont.)

Minneapolis General Hospital (Cont.)

1:30 - Pediatric Rounds; E. J. Huenekens; 4th Floor Annex.

2:00 - 4:00 Infectious Disease Rounds; 8th Floor.

4:00 - 5:00 Infectious Disease Conference; Classroom, 8th Floor.

Veterans Administration Hospital

8:30 - 10:00 Orthopedic-Roentgenologic Conference; Edward T. Evans and Bernard O'Loughlin; Conference Room, Bldg. I

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker.

2:00 - 4:00 Infectious Disease Rounds; Main Conference Room, Bldg. I.

4:00 - 5:00 Infectious Disease Conference; W. Spink; Conference Room, Bldg. I.

7:00 p.m. Lectures in Basic Science of Orthopedics; Conference Room, Bldg. I.

Thursday, February 1

Medical School and University Hospitals

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-109, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

12:00 - Physiological Chemistry Seminar; Chromatography of Nucleic Acids; F. Bollum; 214 Millard Hall.

*4:00 p.m. Special Lecture; The Role of Intestinal Bacterial Flora in Human Nutrition and Disease; F. W. Bernhart; Todd Amphitheater, U. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

4:00 - 5:00 Physiology Seminar on Cardiac Metabolism; 129 Millard Hall.

5:00 - Bacteriology Seminar; Studies on So-Called "Bejel" Treponemes; Garabed Garabedian; 214 Millard Hall.

5:00 - 6:00 X-ray Seminar; Eustis Amphitheater, U. H.

5:00 - 6:00 Radiology Seminar; Functions of Vertebral Venous Circulation; Harry Z. Mellins; Eustis Amphitheater, U. H.

7:30 - 9:30 Pediatrics Cardiology Conference and Journal Club; Review of Current Literature 1st hour and Review of Patients 2nd Hour; 206 Temporary West Hospital.

Thursday, February 1 (Cont.)

Minneapolis General Hospital

8:00 - 9:00 Pediatric Rounds; Forrest Adams; 4th Floor Annex.

11:30 - Pathology Conference; Main Classroom.

1:00 - 2:00 EKG and X-ray Conference; Classroom, 4th Floor Annex.

Veterans Administration Hospital

8:00 - Surgery Ward Rounds; Lyle Hay and Staff.

9:15 - Surgery Grand Rounds; Conference Room, Bldg. I.

11:00 - Surgery Roentgen Conference; Conference Room, Bldg. I.

1:00 - Chest Rounds; William Stead.

Friday, February 2

Medical School and University Hospitals

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:45 - 12:50 University of Minnesota Hospitals Staff Meeting; The Shwartzman Phenomenon as a Model for the Study of Tissue Damage and the Action of Cortisone and ACTH; Lewis Thomas and Robert A. Good; Powell Hall Amphitheater.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

2:00 - 3:00 Dermatology and Syphilology Conference; Presentation of Selected Cases of the Week; H. E. Michelson and Staff; W-312, U. H.

2:00 - 4:00 Physiology Conference; 214 Millard Hall.

3:00 - 4:00 Neuropathology Conference; F. Tichy; Todd Amphitheater, U. H.

4:00 - 5:00 Clinical Pathological Conference; A. B. Baker; Todd Amphitheater, U. H.

4:15 - 5:15 Electrocardiographic Conference; 106 Temp. Bldg., Hospital Court, U. H.

5:00 - 6:00 Urology Seminar; Plastic Operations for Stricture at the Ureteropelvic Junction, with Special Reference to Y-Plasty; F.E.B. Foley; Eustis Amphitheater, U. H.

Ancker Hospital

1:00 - 3:00 Pathology-Surgery Conference; Auditorium.

Friday, February 2 (Cont.)

Minneapolis General Hospital

9:00 - 10:00 Pediatric Rounds; Dr. Tobin; 5th Floor Annex.

9:30 - Surgery-Pediatric Conference; O. S. Wyatt and T. C. Chisholm; 4th Floor Annex.

11:00 - 12:00 Pediatric Rounds; Franklin Top; 7th Floor Annex.

Veterans Administration Hospital

10:30 - 11:20 Medicine Grand Rounds; Conference Room, Bldg. I.

1:00 - Microscopic-Pathology Conference; E. T. Bell; Conference Room, Bldg.I.

1:30 - Chest Conference; Wm. Tucker and J. A. Myers; Ward 62, Day Room.

3:00 - Renal Pathology; E. T. Bell; Conference Room, Bldg. I.

Saturday, February 3

Medical School and University Hospitals

7:45 - 8:50 Orthopedic X-ray Conference; Wallace H. Cole and Staff; M-109, U. H.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; E-221, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amphitheater, U. H.

9:15 - 10:00 Surgery-Roentgenology Conference; J. Friedman, O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

10:00 - 11:30 Surgery Conference; O. H. Wangensteen and Staff; Todd Amphitheater, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

11:00 - Anatomy Seminar; Contributions to Neuro-anatomy During 1950; A. T. Rasmussen; 226 Institute of Anatomy.

Ancker Hospital

8:30 - 9:30 Surgery Conference; Auditorium.

Minneapolis General Hospital

8:00 - 9:00 Pediatric Rounds; Forrest Adams; 4th Floor Annex.
11:00 - 12:00 Pediatric Clinic; Charles May; Classroom, 4th Floor Annex.

Veterans Administration Hospital

8:00 - Proctology Rounds; W. C. Bernstein and Staff; Bldg. III.
8:30 - Hematology Rounds; P. Hagen and E. F. Englund.

*Indicates special meeting. All other meetings occur regularly each week at the same time on the same day. Meeting place may vary from week to week for some conferences.