Biochemical Laboratory Tests in Viral Hepatitis and other Hepatic Diseases

Evaluation and Follow-Up *

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The differential diagnosis between viral hepatitis and other liver diseases (particularly obstructive jaundice) is often difficult on purely clinical grounds. Damage to the liver causes changes in the pattern of the serum enzymes and this has led to the development in recent years of a number of enzyme tests. The authors have amassed evidence to show that the most useful of these is determination of the levels of serum glutamic oxalacetic and serum glutamic pyruvic transaminase (SGOT and SGPT), coupled with calculation of the SGOT/SGPT ratio. It is characteristic of viral hepatitis that both levels are greatly increased, but the SGOT/SGPT ratio, normally greater than one, falls considerably below his figure. In a few cases of obstructive jaundice, the serum transaminase picture may initially resemble that in viral hepatitis, but the differential diagnosis can be established by repeating the determinations at intervals. Other enzyme tests, such as determination of alkaline phosphatase and leucylaminopeptidase, may be used to confirm the biliary obstruction. Flocculation tests and electrophoretic determination of the plasma protein picture, while of limited value in the diagnosis of acute viral hepatitis, are useful in conjunction with the serum transaminase test for assessing the activity of the disease and any tendency to progress towards " active " chronic hepatitis or post-hepatic cirrhosis.

The diagnosis of viral hepatitis cannot easily be confirmed by liver function tests (" loading " tests) or by serum iron determinations. The technical difficulties make these methods unsuitable for routine use and, in addition, they are not sufficiently specific. Moreover, determination of serum bilirubin and of the various bilirubin fractions of the blood does not help to clarify the diagnosis except in very rare cases. For these reasons, attention has to be concentrated on two groups of diagnostic tests:

(a) those that examine the changes in protein levels frequently accompanying viral hepatitis—determination of the plasma protein pattern, flocculation tests, Jirgl test;

(b) those that derive from the concept of the so-called "enzymo-plasmatic syndrome" and consist in determining the level of certain plasma enzyme activities.

PLASMA PROTEIN PATTERN AND RELATED TESTS

Tests related to the existence of hypergammaglobulinaemia

In acute viral hepatitis with reversible hepatic lesions, a moderate fall in plasma albumins with an equally moderate increase in the β- and γ-globulins is noted, whereas in cases of hepatitis with acute irreversible lesions (acute atrophy of the liver), the fall in the albumins is accompanied by a parallel fall in the α- and β-globulins and is associated with an increase in the γ-globulins and with general hypoproteinaemia with hypofibrinogenaemia.

The picture is quite different in the case of obstructive jaundice in which there is an increase in the α- and β-globulins but a normal γ-globulin level, with an increase in fibrinogen. These conditions are

found in relatively recent obstructive jaundice; when the jaundice persists for some time, however, signs of marked functional hepatic damage and of reticular stimulation also appear, and these may be the forerunners of biliary cirrhosis (Viollier, 1957). Consequently, it is only in recent jaundice that the presence of hypergammaglobulinaemia argues in favour of a diagnosis of hepatitis while a normal gammaglobulinaemia is evidence of obstructive jaundice.

However, determination of the protein picture, even by the simplest electrophoretic methods, is not a routine diagnostic test; moreover, it provides only indirect pointers and is not specific. Consequently, the use of this test is not recommended except for hospitalized patients in whom there is a suspicion of a tendency towards chronicity or post-necrotic cirrhosis. The information given by the protein pattern is thus of little practical value for a differential diagnosis between viral hepatitis and obstructive jaundice.

Tests indirectly revealing a change in the protein pattern: flocculation tests

In the past, flocculation tests have played a large part in the diagnosis of anicteric hepatitis and in the identification of clinical pictures tending towards " active " chronic hepatitis or post-necrotic cirrhosis. Nevertheless, they have certain important limitations, such as the subjectivity, and therefore inaccuracy, of the optical determination of serum turbidity and the absence of adequate standards (resulting, for example, in considerable variations in the results of comparative thymol-turbidity tests carried out in different laboratories).

The need to adopt certain technical improvements so as to obtain maximum sensitivity, specificity and reproducibility, leads to the transformation of simple tests into complicated techniques with all the disadvantages this entails.

In addition, it is generally necessary to take into account the limitations resulting from the lack of specificity. The most characteristic example is that of long-standing obstructive jaundice in which the progression of the hepatic lesions renders the flocculation tests positive exactly as in acute or subacute viral hepatitis.

Furthermore, in a fairly large number of cases of icteric or anicteric viral hepatitis, the flocculation tests are negative. Recourse to flocculation tests alone, therefore, has now been superseded and is no longer to be recommended.

When flocculation tests are combined with enzyme tests, they do not add very much to the diagnostic value of the latter in acute cases, but are useful as indicators of the " activity " and progressive nature of apparently cured hepatitis cases.

The Jirgl test

This is a flocculation test for determining plasma fractions that are soluble in sulfosalicylic acid but insoluble in phosphotungstic acid. A positive result is a useful pointer to a diagnosis of obstructive jaundice.

The test gives 25% false positive results in viral hepatitis and 10% false negative results in obstructive jaundice. It is of limited value in differential diagnosis (Jirgl, 1957; Coltorti et al., 1963).

ENZYME TESTS

A new line of attack in diagnosis, the usefulness of which has been confirmed during the past nine years, is based on the utilization of a physiopathological phenomenon characteristic of viral hepatitis, namely, the appearance in the serum of enzyme activities that are practically absent in normal subjects, or an increase—often very considerable—in enzyme activities whose normal rate is extremely low. This has been termed the " enzymo-plasmatic syndrome " (De Ritis et al., 1955a, b).

The number of enzymes present, or pathologically increased, in the plasma during viral hepatitis, and their comparative behaviour in two other types of liver disease (obstructive jaundice and hepatic cirrhosis) are given in Table 1.

The first question to be considered is whether the enzymes studied are etiologically specific. On this point the reply is negative. The nature of the enzymes released into the blood plasma from hepatic cells whose physiological structure has undergone a pathological change (ranging from simple modification of cellular permeability to necrosis) is related not to the etiological agent but to the type of lesion. In hepatitis, the principal lesion is necrosis and a similar release of enzymes occurs in other non-viral types of necrosis (ischaemic, toxic).

The second problem is that of the organo-specificity of the enzymes. The ideal would be to be able to detect in the plasma the appearance of enzyme activities originating exclusively in the liver. There are some enzymes that fulfil this condition but their detection—for reasons that will be referred to later—does not represent any great diagnostic advance. On the other hand, there are certain

TABLE 1

ENZYMES PRESENT OR PATHOLOGICALLY INCREASED IN THE PLASMA IN VIRAL HEPATITIS, OBSTRUCTIVE JAUNDICE AND HEPATIC CIRRHOSIS

Enzyme		Change in plasma level in:		
		Viral hepatitis	Obstructive jaundice	Hepatic cirrhosis
Transaminases	aspartic-ketoglutaric transaminase (SGOT)	considerable increase	moderate increase	slight and irregular increase
	alanine-ketoglutaric transaminase (SGPT)	considerable increase	moderate increase	slight and irregular increase
Glycolysis enzymes	phosphoglycomutase	considerable increase	slight and irregular increase	normal
	phosphohexose isomerase	considerable increase	slight increase	slight increase
	phosphofructose kinase	considerable increase		normal
	fructose-1,6-diphosphate aldolase	considerable increase	slight increase	slight increase
	glyceraldehyde-3-phosphate dehydrogenase	moderate increase	slight and irregular increase	slight increase
	triose phosphate isomerase	large increase	slight and irregular increase	normal
	enolase	moderate and irregular increase	slight and irregular increase	slight and irregular increase
	pyruvic kinase	slight reduction		slight reduction
	lactic dehydrogenase	moderate increase	slight increase	slight increase
Other glucide metabolism enzymes	fructose kinase	moderate increase		slight increase
	fructose-1-phosphate aldolase	large increase	slight increase	slight increase
	sorbitol dehydrogenase	large increase	slight increase	slight increase
	α-glycerophosphate dehydrogenase	moderate increase	slight and irregular increase	slight and irregular increase
	glucose-6-phosphate dehydrogenase	slight and irregular increase	normal	norma
	6-phosphogluconic dehydrogenase	slight increase	normal	normal
	5-phosphoribose isomerase	normal		slight increase
	transketolase	increase	slight increase	
Citric cycle enzymes	isocitric-dehydrogenase	marked increase	slight increase	slight increase
	malic dehydrogenase	marked increase	slight increase	slight increase
	fumarase	marked and irregular increase		
Urea cycle enzymes	arginase	moderate increase		moderate increase
	ornithine-carbamyl transferase	large increase		moderate increase
Peptidases	leucyl aminopeptidase	moderate and irregular increase	large increase	normal
	glycyl tyrosinase	reduction		
	dehydropeptidase	moderate increase		
	aminotripeptidase	slight increase	large increase	
Esterases	alkaline phosphatase	slight and irregular increase	large increase	slight and irregular increase
	5-nucleotidase	slight and irregular increase	large increase	slight and irregular increase
	adenosine triphosphatase	slight and irregular increase		moderate and irregular increase
	cholinesterase	reduction	slight reduction	slight reduction
	cholesterol esterase	slight reduction	normal	slight reduction
	desoxyribonuclease	increase	normal	
Other enzymes	β-glycuronidase	large increase		
	glucose-6-phosphatase	moderate increase	moderate increase	
	glutathione reductase	moderate increase	slight increase	slight increase
	glutamic dehydrogenase	irregular increase		slight and irregular increase

enzymes that have no absolute organo-specificity but whose behaviour in viral hepatitis is nevertheless characteristic.

Determination of serum transaminases

This test was introduced by De Ritis, Coltorti & Giusti (1955a, b) in 1955 and the same authors have since provided, further, exhaustive evidence of its value for the diagnosis of viral hepatitis (De Ritis, Coltorti & Giusti, 1956a,b; 1957; 1961; De Ritis, Ascione et al., 1959).

Now—after eight years of use and extensive studies all over the world—the following conclusions may be reached:

(1) In acute virus hepatitis there is a considerable increase in the activities of serum glutamic oxalacetic and serum glutamic pyruvic transaminase (SGOT and SGPT), an increase which is sometimes of the order of 2000-4000% and which is seldom less than 1000%. The characteristic behaviour of the transaminases in hepatitis is as follows: considerable increase in both SGOT and SGPT activities (up to 40 times the normal figures) with a large relative increase in SGPT with respect to SGOT and a consequent decrease in the SGOT/SGPT ratio to considerably less than one. Normally this ratio is greater than one.

(2) The same behaviour is also seen during anicteric hepatitis (this is why, when the latter is suspected, particularly in communities, it is easy to obtain confirmation by determining the transaminase values).

(3) The increase in the transaminases precedes the appearance of jaundice and a possible change to a positive result in the flocculation tests. In this connexion it may be mentioned that liver biopsies in subjects with a high transaminase level of the hepatic type but without evident symptoms or jaundice have revealed the presence of typical active hepatitis lesions. It is not until 20 to 30 days later that jaundice may appear in such subjects (Tolentino et al., 1957; Delkeskamp et al., 1959).

This finding completely confirms previous ideas in regard to incubation, for it shows:

(*a*) that the disease actually exists, if only in subclinical form, three to five weeks before any clinical manifestation, so that it is hardly correct to speak of incubation during this period;

(*b*) that in certain cases the length of the period when hepatitis is clinically evident may be less than that of the asymptomatic period.

(4) The plasma transaminase values are independent of the degree of bilirubinaemia, the serum protein levels, and the behaviour of the flocculation tests, since all these tests are an expression of various pathogenically independent phenomena.

(5) During viral hepatitis, the plasma transaminase levels may show variations which reflect the subsequent course of disease.

(6) During convalescence while the absolute transaminase values are returning to normal, the SGOT/SGPT ratio may remain low for one or more weeks which sometimes makes possible a retrospective diagnosis of viral hepatitis.

(7) The mere finding of an SGOT/SGPT ratio below one cannot be considered as characteristic of viral hepatitis if the absolute figures for the two enzyme activities are normal or only moderately increased.

In no other liver disease (hepatitis with bacterial hepato-cholangitis, cirrhosis of the liver, neoplasm) or even in diseases not affecting the liver have plasma transaminase patterns been observed similar to those seen in viral hepatitis, apart from a few rare exceptions (in extensive neoplasm of the liver with severe necrosis and in amoebic hepatitis during the early colliquation stage).

In some cases of very recent obstructive jaundice (the cause of the biliary obstruction is immaterial), the sudden biliary stasis is accompanied by a retrograde action of the bile on the liver cells causing damage to the latter which may lead to necrosis. As a consequence, plasma transaminase levels similar to those of viral hepatitis may be found, although the absolute figures are generally much lower. In such special cases, obstructive jaundice may easily be differentiated from viral jaundice by the repetition at close intervals of plasma transaminase tests, even in the absence of clinical criteria or other laboratory data, such as a high alkaline-phosphatase blood level or hypercholesterolaemia. In obstructive jaundice, the initially high transaminase figures tend to decrease and, in particular, the SGOT/SGPT ratio becomes normal; this does not occur in viral jaundice, where the characteristic very low ratio (about 0.5-0.6) persists while the absolute concentrations of the two enzymes remain elevated and sometimes increase further.

Application of these criteria has made it possible, even in subjects known to be suffering from cholelithiasis to demonstrate that the jaundice is due to

hepatitis with initial symptoms characterized by pain in the right hypochondrium and not to the presence of the calculi.

The above-mentioned criteria, based on our personal experience of more than eight years, have been confirmed by many other workers. Further confirmation has come from the evaluation of serum transaminase patterns in a recent series of 263 icteric patients.

The 263 patients were composed of 195 unselected cases of viral hepatitis, 23 cases of bacterial cholangiolitis, and 45 cases of cholestatic jaundice. The collected data are summarized in Fig. 1-6 and are clearly in accordance with the previous conclusions.

To make clear the usefulness in the diagnosis of viral hepatitis of the simultaneous determinations of SGOT, SGPT and the SGOT/SGPT ratio, we constructed the histogram shown in Fig. 7. The serum transaminase values found in the 195 cases of viral hepatitis were initially divided into two main groups. In the first group, we collected all the cases that showed a very large absolute increase in serum transaminase (particularly those with SGPT values $>7\mu$mol/ml of pyruvate formed); in the second group, we collected those cases in which the increase in the serum transaminases, although considerable, was lower than that in the first group. Each of these two groups was then divided into two subgroups, according to the value of the SGOT/SGPT ratio (between 0.99 and 0.70, or less than 0.70). The mean values in each of the four groups are shown below the abscissa in Fig. 7.

The mean values of the SGOT/SGPT ratio are very much less than one in each group. This low value of the ratio makes the diagnosis very easy in the first and second groups of cases (comprising 77% of the total) because it is always associated with very high serum transaminase levels. In the third group (20% of the total) the increase in the transaminases is less pronounced, but the diagnosis is also easy because of the very low values of the ratio (mean=0.49). Only in 2.1% of the cases is the diagnostic value of the test impaired because relatively low values of the SGOT/SGPT ratio (average=0.82) are associated with rather small increases in the serum transaminases.

The usefulness of simultaneous determination of the SGOT and SGPT levels and the SGOT/SGPT ratio in the differentiation of cholestatic jaundice from viral hepatitis is evident from Fig. 8, which shows the mean values found in 45 cases of cholestatic jaundice, divided into four groups.

In the first group—which represents 77.8% of the total—are included cases with normal absolute figures of serum transaminases, or with values only slightly increased, and with an SGOT/SGPT ratio greater than one. In the second group—15.6% of the total—are included cases with the transaminases slightly increased and the ratio lower than one. The third group comprises the cases in which a moderate increase in transaminase levels is associated with an SGOT/SGPT ratio greater than one. In these first three groups, therefore, the differentiation from viral hepatitis is unmistakable.

It is only in the fourth group—4.4% of the total—where greatly increased serum transaminase levels are associated with a ratio decidedly lower than one that no useful diagnostic conclusions can be drawn.

Our observations on cases of bacterial cholangiolitis show in all cases low transaminase figures associated with an SGOT/SGPT ratio near to one. It is therefore easy to make a differential diagnosis between this disease and viral hepatitis, but it is not possible to differentiate it from cholestatic jaundice.

On combining the information from Fig. 5 and 6, it is apparent that simultaneous determination of the SGOT and SGPT levels and of the SGOT/SGPT ratio makes the diagnosis easy in more than 97% of the cases of viral hepatitis and in more than 95% of the cases of cholestatic jaundice.

The differential diagnosis in the small percentage of the cases of cholestatic jaundice in which the behaviour of the serum transaminase levels is not conclusive can be established by means of further repetitions of the SGOT and SGPT tests. In fact, transaminase figures that are initially high show a quick decrease, while bilirubin values remain high or increase further (Fig. 9).

Other plasma enzyme tests studied for diagnosing hepatocellular lesions of the necrotic type

Among the very numerous enzymes that are found in the plasma during viral hepatitis (see Table 1), certain ones are particularly suitable for diagnosing hepatocellular lesions of the necrotic type. These are:

fructose-1,6-diphosphate aldolase, phosphohexose isomerase, phosphoglycomutase, triose-phosphate isomerase, isocritric dehydrogenase (Bruns & Puls, 1954; De Ritis, Giusti & Coltorti, 1957a,b; Giusti, 1962; Wolfson et al., 1958).

FIG. 1

SGOT AND SGPT LEVELS AND SGOT/SGPT RATIOS IN NORMAL SUBJECTS AND IN PATIENTS WITH DIFFERENT HEPATIC DISEASES WITH JAUNDICE
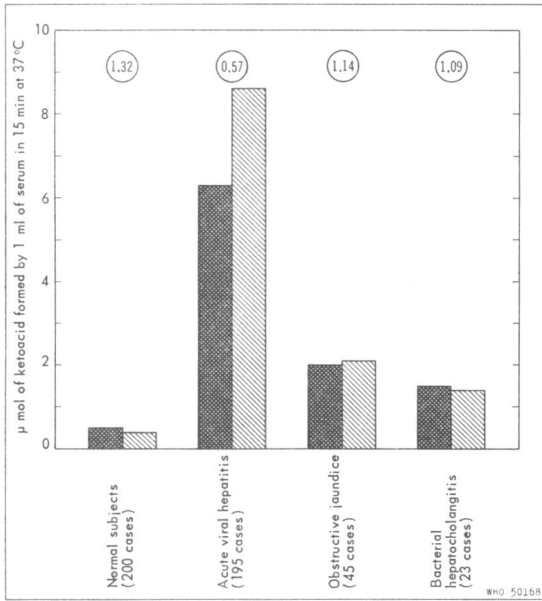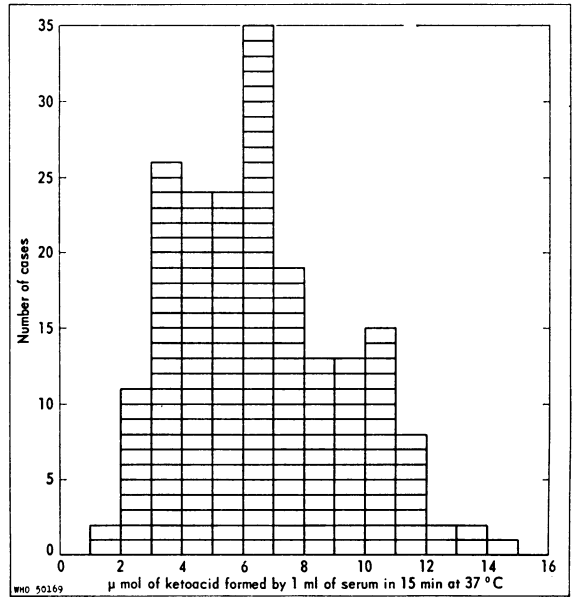
FIG. 2

FREQUENCY DISTRIBUTION OF SGOT LEVELS IN 195 CASES OF ACUTE VIRAL HEPATITIS

FIG. 3

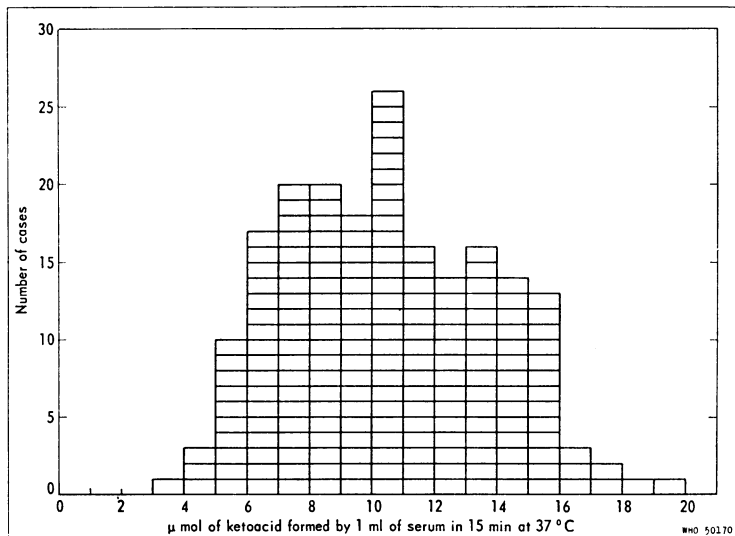FREQUENCY DISTRIBUTION OF SGPT LEVELS IN 195 CASES OF ACUTE VIRAL HEPATITIS

FIG. 4

FREQUENCY DISTRIBUTION OF SGOT/SGPT RATIOS IN
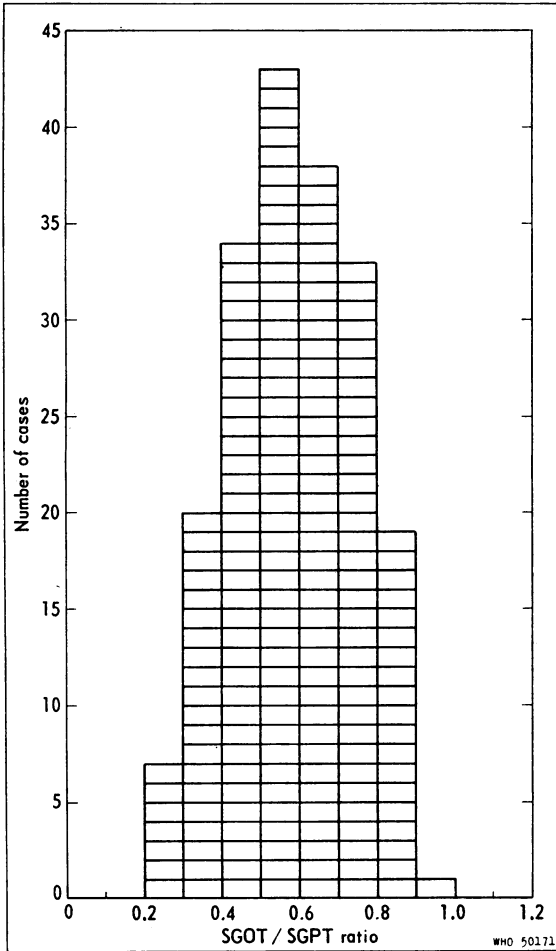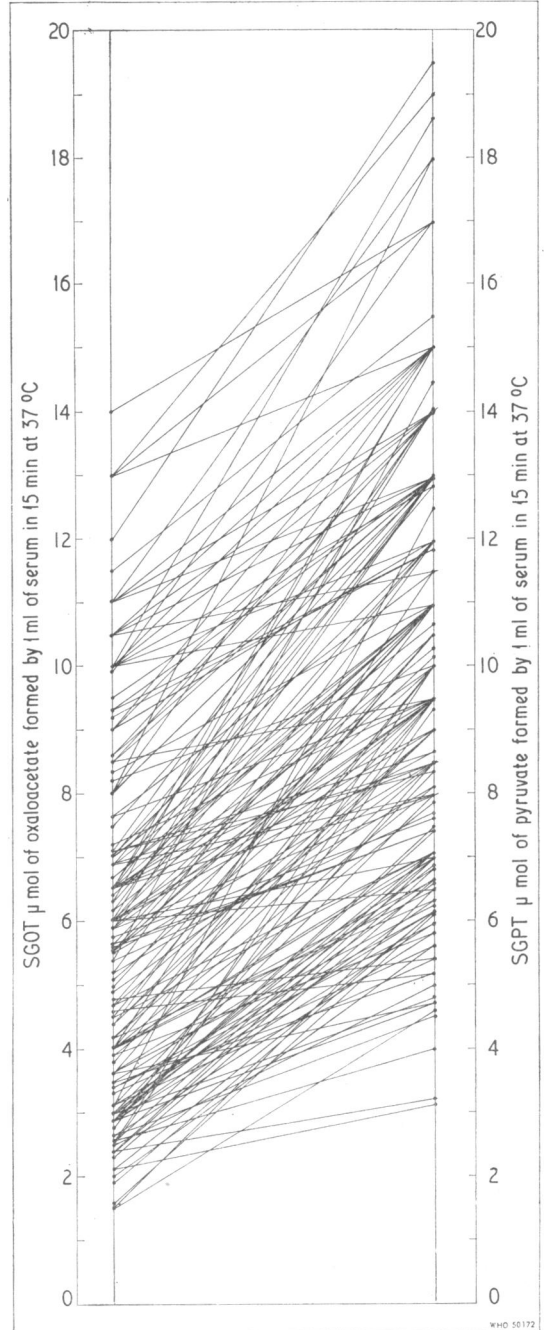195 CASES OF ACUTE VIRAL HEPATITIS

FIG. 5

PAIRED VALUES OF SIMULTANEOUSLY DETERMINED
SGOT AND SGPT LEVELS IN 195 CASES OF ACUTE VIRAL
HEPATITIS

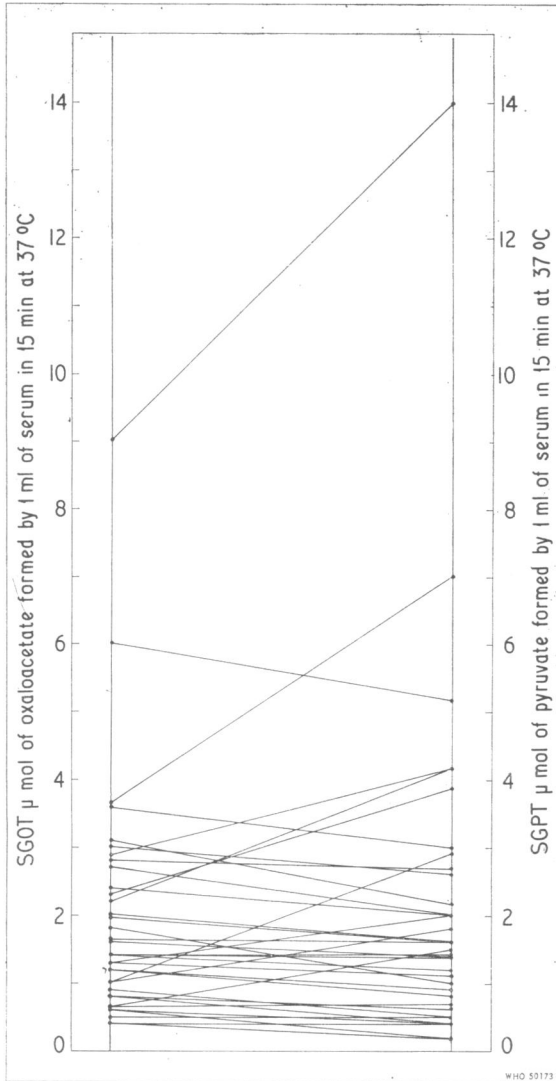Each line •——• joins a pair of values determined in a single patient.

The behaviour of all these enzymes is similar: they show a pronounced and early increase in viral hepatitis (even of the anicteric type) but only a slight or negligible increase in the course of jaundice caused by cholestasis and other liver complaints. The determination of these increases makes it possible in a large number of cases to establish the differential diagnosis of liver diseases with a high degree of probability.

Persistence of high plasma enzymes levels when the bilirubin level has returned to normal and the flocculation test has become negative indicate persistent " activity " of the hepatic disease.

FIG. 6

PAIRED VALUES OF SIMULTANEOUSLY DETERMINED
SGOT AND SGPT LEVELS IN 45 CASES OF OBSTRUCTIVE
JAUNDICE

FIG. 7

SGOT AND SGPT LEVELS AND SGOT/SGPT RATIOS IN
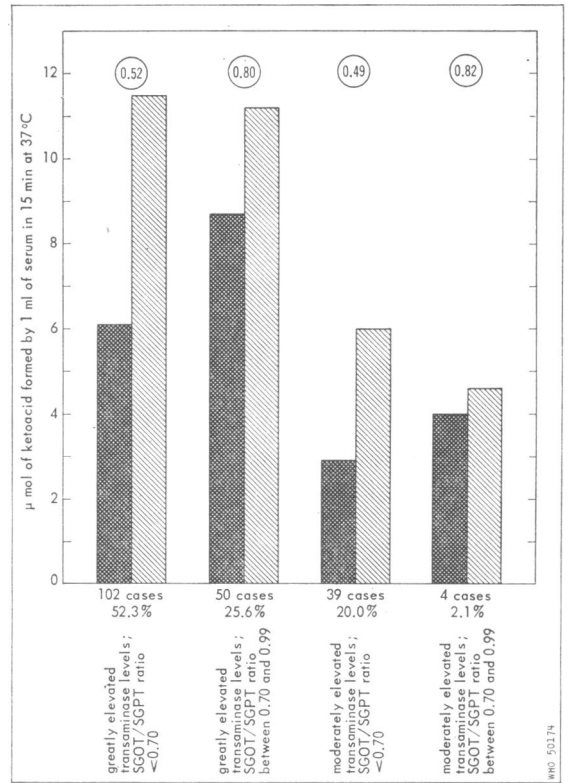195 CASES OF ACUTE VIRAL HEPATITIS

Each line ●——● joins a pair of values determined in a single
patient.

*Hepato-specific enzyme tests: fructose-1-phosphate
aldolase; ornithine-carbamyl transferase; sorbitol
dehydrogenase*

Certain other enzymes that are not present in
any organ except the liver are found in remarkably
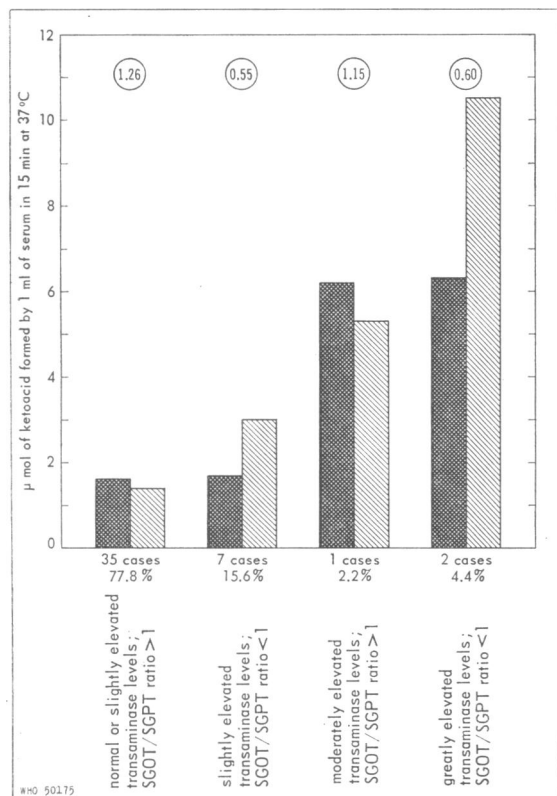increased quantities in the serum during viral
hepatitis, but increase much less in obstructive
jaundice (fructose-1-phosphate aldolase, ornithine-
carbamyl-transferase, sorbitol-dehydrogenase) while
they do not increase at all in extra-hepatic diseases
(Wolf et al., 1957; Giusti et al., 1961; Reichard,
1957).

The hepato-specificity of these enzymes, i.e., their
property of being confined exclusively to the liver,
is a diagnostic advantage only in theory. In practice,
the determination of these enzymes does not offer
any real diagnostic advantage, since the increase in
the transaminases and in fructose-1,6-diphosphate
aldolase during myocardial infarction and in
patients suffering from muscular distrophy does not

FIG. 8

SGOT AND SGPT LEVELS AND SGOT/SGPT RATIOS IN 45 CASES OF OBSTRUCTIVE JAUNDICE

FIG. 9

SGOT, SGPT AND BILIRUBIN LEVELS IN A CASE OF OBSTRUCTIVE JAUNDICE WITH INITIAL TRANSAMINASE VALUES SIMULATING THE VIRAL HEPATITIS PATTERN

Tests on plasma enzymes used for the diagnosis of jaundice of the cholestatic type

A certain number of tests on plasma enzymes are valuable both for the diagnosis of obstructive jaundice (because of the considerable increase of these enzymes in the plasma) and also for the diagnosis of viral hepatis. A comparison between the humoral patterns found in viral hepatitis and in cholestatic jaundice is shown in Table 2.

While these tests are not of any real value in cases where the results of the transaminase test are characteristic, they may be of considerable assistance in the small number of cases where the results of the laboratory tests are not sufficiently typical (e.g., where there is only a slight increase in the levels of the two transaminases, where the flocculation tests are negative, or where there is no appreciable change in the protein pattern). It is difficult to differentiate these cases from obstructive jaundice, particularly if there is high bilirubinaemia and marked decoloration of the stools (hypocholia).

Under such conditions, which are, however, extremely rare, the discovery of a distinct increase in enzyme activity is indicative of obstructive jaundice; on the other hand, the finding of normal figures, or at any rate of figures not significantly
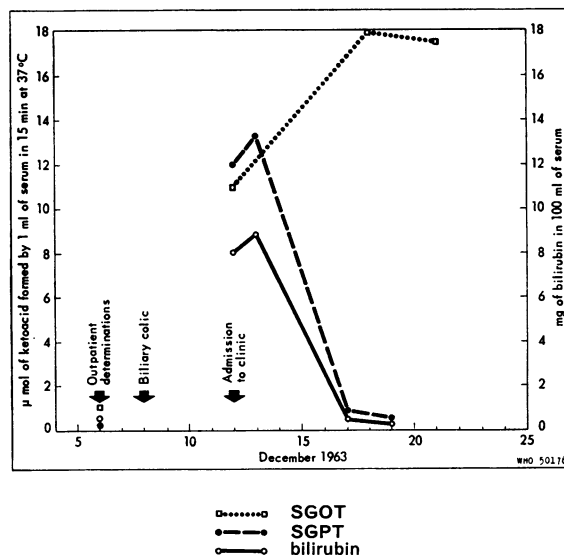
present a problem in the study of the liver diseases.

Determination of the concentration of the organo-specific enzymes is of use only in special cases, e.g., in the diagnosis and follow-up of viral hepatitis (whether icteric or not) associated with muscular dystrophy (where high transaminase or fructose-1,6-diphosphate aldolase levels might be of muscular origin and would not justify a diagnosis of viral hepatitis).

Apart from such extremely rare borderline cases, determination of the hepato-specific enzymes is not advisable in the study of viral hepatitis because of their lower sensitivity and their early decrease in the plasma even while the disease is still in the active phase (jaundice).

TABLE 2

COMPARISON BETWEEN THE HUMORAL PATTERNS CHARACTERISTIC OF VIRAL HEPATITIS AND OF CHOLESTATIC JAUNDICE

	Humoral pattern characteristic of:	
	Viral hepatitis	Cholestatic jaundice
Transaminases	marked increase with low SGOT/SGPT ratio	slight increase with high SGOT/SGPT ratio
Alkaline phosphatase and leucyl amino-peptidase	slight increase	marked increase
Serum iron	marked increase	normal
Serum cholesterol	normal or slight reduction	marked increase
Flocculation tests	positive	negative [a]
Serum globulins	increase	normal [a]

[a] In long-standing cholestatic jaundice, an increase of serum globulins with positive flocculation tests can be observed.

different from normal, may indicate a diagnosis of viral hepatitis.

The following enzymes belong to this group:

(a) *Alkaline phosphatase.* In cholestatic jaundice, serum alkaline phosphatase shows a marked increase, chiefly in complete and long-standing obstruction (Bodansky, 1933; Roberts, 1930; Coltorti et al., 1963); the increases are much less in viral hepatitis and do not occur in anicteric hepatitis.

The following figures show the data found in this condition in our observations:

Normal subjects	1.01 ± 0.54 units
Viral hepatitis	3.25 ± 1.22 units
Cholestatic jaundice	16.30 ± 9.35 units

Determinations were carried out by the method of Bessey, Lowry & Brock (1946) and the results were reported as Bessey-Lowry units (1 unit = 1 μmol/litre/hour of split substrate).

(b) *5-nucleotidase.* The behaviour of this enzyme is similar to that of alkaline phosphatase but it is more specific, since no increase is seen in skeletal diseases (Dixon & Purdom, 1954; Young, 1958).

(c) *Leucylaminopeptidase.* The activity of this enzyme also increases considerably in cholestatic

jaundice, particularly if cholestasis is complete and has existed for a long time. On the other hand, the increase is much less in viral hepatitis (Goldbarg & Rutenburg, 1958; Banks et al., 1960; Giusti & Piccinino, 1963).

Determination of the various bilirubin fractions of the blood is of practically no diagnostic assistance. The pattern given for the normal forms of viral hepatitis, whether icteric or anicteric, holds also for the forms with cholangiolitis or cholestasis which sometimes can hardly be differentiated clinically from cholestatic jaundice (acholia).

It would seem, according to the present literature —and this is also our conviction after experience extending over nearly 10 years—that it is essential in studying liver disease to determine the activities of both transaminases (SGOT and SGPT, as well as the SGOT/SGPT ratio), for if only one were measured this would give much more limited diagnostic information (particularly in the case of the SGOT) and might thus become a source of error.

EPIDEMIOLOGICAL AND CLINICAL APPLICATIONS OF DETERMINATIONS OF PLASMA TRANSAMINASE LEVELS

Identification of cases of anicteric viral hepatitis, or of the pre-icteric stages of viral hepatitis

The determination of the serum transaminase levels in subjects living in closed environments in which cases of icteric viral hepatitis have been reported, or in subjects otherwise exposed to the infection, allows the identification of anicteric cases of the disease as well as of the pre-icteric stages. By conducting an investigation along these lines, we were able to demonstrate that in some environments the cases of anicteric viral hepatitis greatly outnumber the icteric cases, the relative frequency ranging from 3:1 to 30:1; this is especially so in communities of children (De Ritis, Mallucci et al., 1959; Giusti et al., 1959; Coltorti et al., 1961).

The identification of the cases of anicteric hepatitis in the community also permits isolation of virus carriers. These are the subjects most likely to be responsible for the spread of the disease, particularly as they are often subclinically ill.

Identification of suspected carriers of viral hepatitis among blood donors

When determination of transaminase levels in prospective blood donors reveals moderately increased figures with persistent lowering of the SGOT/SGPT ratio, this calls attention to a possible

hepatitis. Such subjects can then be rejected as blood donors.

A transaminase response of this type does not by itself show whether the subject has had viral hepatitis in the past or whether the disease is incipient. The problem can easily be resolved by repeated determinations which, in the latter case, will reveal increasing values for SGOT and SGPT with the SGOT/SGPT ratio falling well below 1.

SGOT and SGPT determinations should be compulsory for all blood donors.

There are not sufficient data regarding the behaviour of the transaminases in unrecognized viral hepatitis carriers.

Evaluation of the drug treatment of viral hepatitis

SGOT and SGPT determinations repeated every four or five days in clinically selected subjects who have been suffering from recent jaundice and who are found initially to have similar serum transaminase and bilirubin levels may supply valuable information on the effectiveness of drugs in the treatment of viral hepatitis. The great clinical variability of the disease makes it impossible to reach a decision solely on a clinical basis.

Fig. 10-12 show the results of a controlled experiment on the effectiveness of prednisone (Δ^1-cortisone) in viral hepatitis (De Ritis et al., 1964).

The results of this study are not statistically significant although the mean values of the serum transaminase and bilirubin levels are somewhat lower for the prednisone group than for the placebo group. The lack of statistical significance is due to the fact that the prednisone group includes a remarkable number of cases that seem to be completely unaffected by the therapy. On the other hand, the placebo group includes a certain number of cases that show an early, spontaneous improvement indistinguishable from that observed in the cases apparently responding to prednisone treatment.

Transaminase determinations in the assessment of the activity of apparently cured viral hepatitis

In most cases, the pathological transaminase figures become normal 3-6 weeks after the appearance of jaundice, whereas the SGOT/SGPT ratio may remain considerably lower than 1 for a period varying from one to several weeks and may persist for some time after the individual levels have returned to normal.

The discovery of normal transaminase levels in successive tests in a subject who has suffered from

icteric hepatitis but no longer shows jaundice may be taken as evidence of cure, even if the SGOT/SGPT ratio remains less than 1, provided that the clinical findings also point in the same direction (return of the liver size to normal, disappearance of splenomegaly, normalization of the protein picture, etc.).

In other cases, the fall in the transaminase levels does not proceed smoothly but is interrupted by fresh rises, probably corresponding to new outbreaks of hepatic necrosis; these fresh rises in the figures for the two enzyme activities are frequently associated in our experience with a decrease in the SGOT/SGPT ratio, whereas a fall in the absolute figures is associated with an increase in this ratio.

In a limited number of cases, while the jaundice disappears and the general condition improves— partly because of better intestinal absorption due to the re-establishment of normal bile excretion into the intestine—the transaminase values remain pathologically high and their ratio below 1. This may occur either in the presence of dysproteinaemia with positive flocculation tests or in absence of other
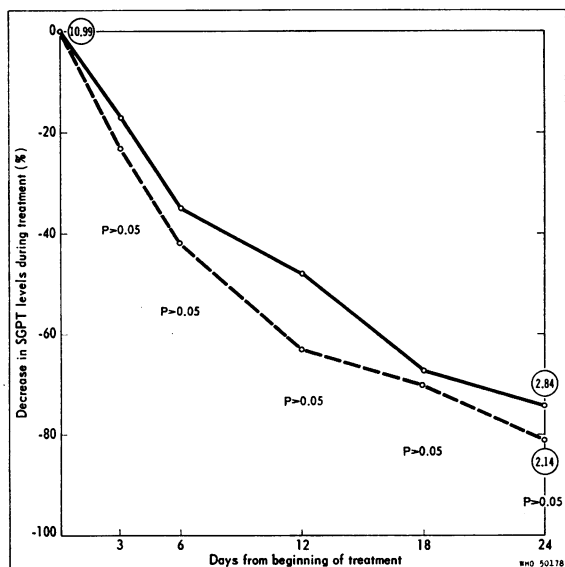
FIG. 10

PERCENTAGE DECREASE IN SGOT LEVELS IN VIRAL HEPATITIS PATIENTS TREATED WITH PREDNISONE AND WITH A PLACEBO
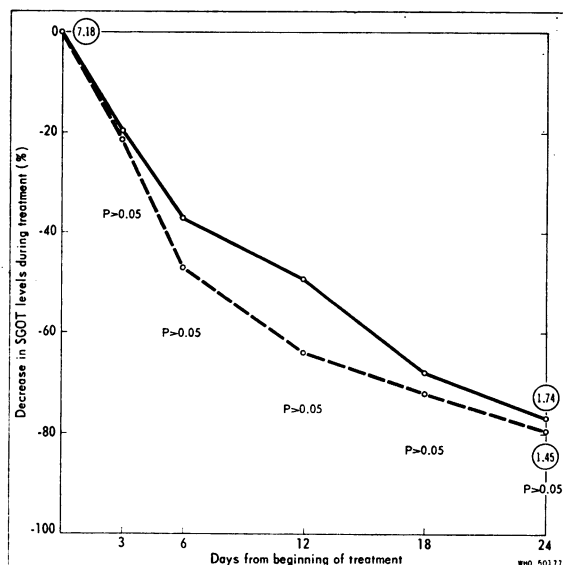
——— mean values in 25 cases treated with placebo
– – – mean values in 37 cases treated with prednisone
◯ SGOT: μ mol of ketoacid formed by 1 ml of serum in 15 min at 37°C (mean of absolute values)

FIG. 11

PERCENTAGE DECREASE IN SGPT LEVELS IN VIRAL
HEPATITIS PATIENTS TREATED WITH PREDNISONE
AND WITH A PLACEBO

FIG. 12

PERCENTAGE DECREASE IN SERUM BILIRUBIN IN VIRAL
HEPATITIS PATIENTS TREATED WITH PREDNISONE
AND WITH A PLACEBO

— mean values in 31 cases treated with placebo
-- mean values in 34 cases treated with prednisone
◯ SGPT: μ mol of ketoacid formed by 1 ml of serum in
15 min at 37°C (mean of absolute values)
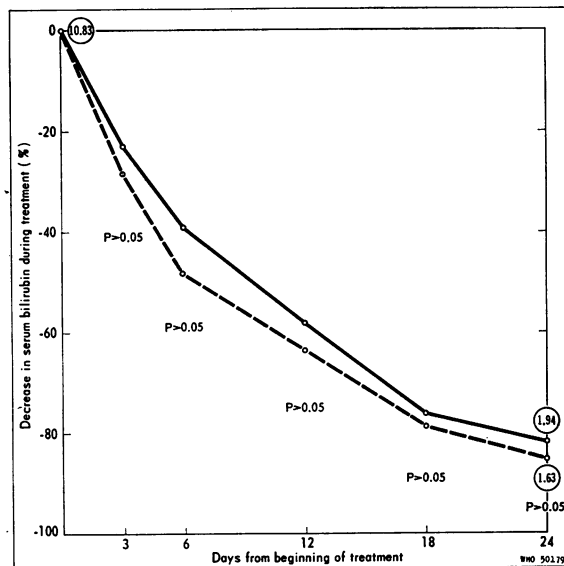
— mean values in 22 cases treated with placebo
-- mean values in 23 cases treated with prednisone
◯ serum bilirubin in mg/100 ml (mean of absolute values)

humoral signs of active disease; these abnormal
figures may then be the prelude to new attacks of
jaundice (relapsing hepatitis). In other cases,
pathological figures may persist, the values fluctua-
ting over fairly long periods without recurrence of
jaundice, while the liver increases once more in size.
Liver biopsies carried out on subjects with these
abnormal enzyme levels have revealed distinct signs
of active and progressing hepatitis. For this reason,
one should reserve judgement of the prognosis, since
it is in such cases that hepatitis most frequently
becomes chronic or that post-necrotic cirrhosis tends
to develop.

In our experience, the transaminase test reflects
better than any other determination the progress
and activity of hepatitis in a case that is apparently
cured and is clinically silent.

CONCLUSIONS

It is often very difficult to differentiate viral
hepatitis clinically from obstructive jaundice. Exten-
sive studies of the many enzyme tests proposed in

various parts of the world during the last 8 years
have led to the conclusion that the " two-trans-
aminase test " proposed by De Ritis, Coltorti &
Giusti in 1955 is still the best, since these serum
enzymes (SGOT and SGPT) show a pattern that is
highly characteristic of viral hepatitis and allows
a diagnosis in as many as 98% of cases.

In acute viral hepatitis, there is a considerable
increase in the activity of the two transaminases—
an increase sometimes of the order of 2000-4000%
and not usually less than 1000%. The characteristic
behaviour of the transaminases in hepatitis is as
follows: considerable increase in both SGOT and
SGPT activities (up to 40 times the normal figures)
with a large relative increase in SGPT as compared
with SGOT and a consequent decrease in the SGOT/
SGPT ratio to figures considerably less than one.

The increase in the transaminase levels precedes
the appearance of jaundice and a possible change in
the flocculation tests to positive. In this connexion,
it has been shown by liver biopsies in subjects with a
high transaminase level of the hepatic type but
without evident symptoms of jaundice that typical

hepatitis lesions are present and fully active. It is not until 20 to 30 days later that jaundice appears in such subjects.

SGOT/SGPT ratios just below 1 cannot be considered as characteristic of viral hepatitis if the absolute figures for the two enzyme activities are normal or only moderately increased.

In no other liver disease (apart from a few rare exceptions), or even in diseases not affecting the liver, are plasma transaminase levels observed similar to those seen in viral hepatitis.

In some cases of very recent obstructive jaundice, sudden biliary stasis is accompanied by a retrograde action of the bile on the liver cells leading to necrosis and a transaminase picture of the viral hepatitis type. In such cases, repetition of the plasma enzyme tests at short intervals will facilitate the differential diagnosis even in the absence of clinical criteria or other laboratory data. In a few doubtful cases, the differential diagnosis between hepatic jaundice and obstructive jaundice calls for additional investigations, e.g., determination of the levels of alkaline phosphatase and leucylaminopeptidase; flocculation tests; or determination of the protein picture, the serum iron level, or the sedimentation rate.

The transaminase test is valuable not only in the clinical study of viral hepatitis—diagnosis and follow-up of cases, evaluation of the " activity " of subacute and chronic hepatitis, effectiveness of drug treatment —but also as an epidemiological aid in detecting anicteric cases of hepatitis by mass screening of population groups and in identifying possible virus carriers among blood donors.

The persistence of an abnormal elevation of the two transaminase levels, or fluctuation of these levels, combined with a lowered SGOT/SGPT ratio is a sensitive index of the " activity " of the disease. Where there are considerable variations in the activity of the disease, association of the transaminase test with flocculation tests and plasma protein determinations is a useful procedure.

POSTSCRIPT

The WHO Expert Committee on Hepatitis (1964) endorsed the use of the unit recommended by the Commission on Enzymes of the International Union of Biochemistry (1961) and approved by the Clinical Chemistry Commission of the International Union of Pure and Applied Chemistry. This unit (IUB unit) is described as follows:

" One *unit* (U) of any enzyme should be defined as that amount which will catalyse the transformation of 1 micromole of substrate per minute, or, where more than one bond of each substrate molecule is attacked, 1 micro-equivalent of the group concerned per minute, under defined conditions. Where two identical molecules react together, the unit will be the amount which catalyses the transformation of 2 micromoles per minute. The temperature should be stated, and where practicable should be 25°C. The other conditions, including pH and substrate concentration, should be optimal. In order to avoid inconvenient numbers, terms such as milli-unit (mU), kilo-unit (kU), etc., may be used."

Serum enzyme concentrations are usually stated in terms of U/l or mU/ml. For comparision with our previous data, our results have been given in terms of μmol/ml of transformed substrate per 15 min at 37°C. These values may easily be converted into IUB units by means of the following formula:

$$N = \frac{n \times 1000}{15}$$

where N = the number of U/l or mU/ml and n = the number of μmol/ml formed in 15 minutes at 37°C.

RÉSUMÉ

Le diagnostic différentiel entre l'hépatite virale et d'autres affections du foie — l'ictère par rétention en particulier — est souvent difficile d'après les seuls critères cliniques.

Les lésions du foie entraînent des changements des enzymes sériques, ce qui a conduit à la mise au point de tests enzymatiques. Les auteurs, après 10 ans d'expérience, sont arrivés à la conclusion que les tests les plus précis sont ceux qui portent sur les transaminases, oxala-cétique et pyruvique (TGO et TGP) avec calcul du rapport de l'une sur l'autre. L'élévation du niveau des transaminases, qui peut atteindre 40 fois le niveau normal, caractérise l'hépatite virale, tandis que le rapport, normalement de 1, s'abaisse notablement. Ces tests ont permis un diagnostic exact dans 98% des cas. Il arrive que, dans des cas d'ictère par rétention, le taux des transaminases se rapproche, au début, de celui que l'on observe dans l'hépatite virale, mais les différences

s'accentuent si l'on répète les tests à intervalles de temps. D'autres tests enzymatiques, tels que la détermination de la phosphatase alcaline et celle de la leucylaminopeptidase peuvent être utiles pour confirmer l'obstruction des voies biliaires. Les tests de floculation, la détermination par électrophorèse des protéines plasmatiques, bien que d'importance mineure pour le diagnostic de l'hépatite virale aiguë, sont utiles lorsqu'ils sont associés aux tests

des transaminases sériques, pour suivre l'évolution de la maladie et son acheminement vers l'hépatite chronique évolutive ou la cirrhose post-hépatitique.

Les tests des transaminases ont aussi une valeur épidémiologique, en permettant de dépister les cas d'hépatite anictérique par examen de groupes de population et d'éventuels porteurs de virus de l'hépatite parmi les donneurs de sang.

REFERENCES

Banks, B. M., Pineda, E. P., Goldbarg, J. A., Rutenburg, A. M. (1960) *New Engl. J. Med.*, **263**, 1277

Bessey, A. O., Lowry, O. H. & Brock, M. J. (1946) *J. biol. Chem.*, **164**, 321

Bodansky, O. (1933) *J. biol. Chem.*, **101**, 93

Bruns, F. & Puls, W. (1954) *Klin. Wschr.*, **32**, 656

Coltorti, M., Di Simone, A., Barbieri, A. M., del Vecchio Blanco, C. (1963) *Policlinico, Sez. med.*, **70**, 1596

Coltorti, M., Giusti, G. & Cirillo, C. (1961) *Therapeutikon (Pisa)*, **1**, 79

Delkeskamp, A., Schmidt, E. & Schmidt, F. W. (1959) *Dtsch. med. Wschr.*, **84**, 188

De Ritis, F., Ascione, A., Coltorti, M., Giusti, G. & Mallucci, L. (1959) *Epatite virale, Relazione al II Congresso Internazionale di Patologia Infettiva della Società Internazionale per lo studio delle Malattie Infettive e Parassitarie. Milano 6-10 Maggio 1959: G. Mal. infett.*, **11**, Suppl.

De Ritis, F., Coltorti, M. & Giusti, G. (1955a) *Minerva Med.*, **1**, 1207

De Ritis, F., Coltorti, M. & Giusti, G. (1955b) *Boll. Soc. ital. Biol. sper.*, **31**, 394

De Ritis, F., Coltorti, M. & Giusti, G. (1956a) *Minerva Med.*, **1**, 167

De Ritis, F., Coltorti, M. & Giusti, G. (1956b) *Recenti Progr. Med.*, **20**, 533

De Ritis, F., Coltorti, M. & Giusti, G. (1957) *Clin. chim. Acta*, **2**, 70

De Ritis, F., Coltorti, M. & Giusti, G. (1961) In: *Proceedings of the First European Symposium on Medical Enzymology, Milan, 1960*, Basel, Karger, pp. 268-296

De Ritis, F., Giusti, G. & Coltorti, M. (1957a) *Minerva Med.*, **1**, 1958

De Ritis, F., Giusti, G. & Coltorti, M. (1957b) *Experientia*, **13**, 81

De Ritis, F., Giusti, G., Mallucci, L. & Piazza, M. (1964) In: *The Third International Congress of Infectious Pathology, Communications, Bucharest, 8-11 October 1962*, Bucharest, Editura Academiei Republicii Populare Romine, pp. 423-438

De Ritis, F., Mallucci, L., Coltorti, M. & Caldera, M. (1959) *Bull. Wld Hlth Org.*, **20**, 589

Dixon, F. & Purdom, M. (1954) *J. clin. Path.*, **7**, 351

Giusti, G. (1962) *Boll. Soc. ital. Biol. sper.*, **38**, 10

Giusti, G., Coltorti, M. & Cirillo, C. (1959) *Mal. infett.*, **11**, 106

Giusti, G., Di Simone, A. & Coltorti, M. (1961) *Enzymologia*, **23**, 163

Giusti, G. & Piccinino, F. (1963) *Acta hepato-splenol. (Stuttg.)*, **10**, 166

Goldbarg, J. A. & Rutenburg, A. M. (1958) *Cancer*, **11**, 283

International Union of Biochemistry (1961) *Report of the Commission on Enzymes*, London & New York, Pergamon Press, p. 45

Jirgl, V. (1957) *Klin. Wschr.*, **35**, 938

Reichard, H. (1957) *Nord. Med.*, **58**, 1016

Roberts, W. M. (1930) *Brit. J. exp. Pat.*, **11**, 90

Tolentino, P. & Rossi, M. (1957) *G. Mal. infett.*, **9**, 552

Viollier, G. (1957) In: Hartmann, F. W., Lo Grippo, G. A., Mateer, J. G. & Barron, J., *Hepatitis frontiers*, Boston, Little, Brown & Co., pp. 423-438

WHO Expert Committee on Hepatitis (1964) *Wld Hlth Org. techn. Rep. Ser.*, **285**, 18

Wolf, H. P., Forster, G. & Leuthard, F. (1957) *Gastroenterologia*, **87**, 172

Wolfson, S. K., Spencer, J. A., Sterkel, R. L. & Williams-Ashman, H. G. (1958) *Ann. N.Y. Acad. Sci.*, **75**, 260

Young, J. J. (1958) *Ann. N.Y. Acad. Sci.*, **75**, 357