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Markers for Detecting Alcoholism and Monitoring for Continued Abuse

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MORGAN, M. Y. *Markers for detecting alcoholism and monitoring for continued abuse.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 1–8, 1980.—Several biochemical and haematological abnormalities are associated with excessive alcohol intake and some are used in the recognition and management of alcoholics. The ideal biological marker for detecting and monitoring alcoholics should be sensitive and highly specific for alcohol abuse; its value should be affected by changes in alcohol intake over relatively short periods of time and it should be quick, simple, convenient and inexpensive to estimate. At the present time no simple reliable marker is available which fulfills these criteria. Measurements of serum aspartate transaminase, serum gamma-glutamyl-transpeptidase and mean corpuscular volume are of proven value however and the majority of alcoholics can be detected and monitored by combining the measurements of these three tests. Blood/breath alcohol measurements are of limited value for detection but are useful for follow up. Measurement of the plasma alpha-amino-n-butyric acid/leucine ratio is of disputed value and not likely to be of great practical use. Measurement of serum alpha-lipoproteins, erythrocyte delta-aminolaevulinic acid dehydrase activity and qualitative estimation of serum transferrin have all been proposed as markers for alcohol abuse and are currently under evaluation.

Alcohol drinking Alcoholic liver disease Blood alcohol Breath alcohol
Erythrocyte delta-aminolaevulinic acid dehydrase Erythrocyte indices Mean corpuscular volume
Plasma alpha-lipoproteins Plasma amino acids Serum aspartate aminotransferase
Serum gamma glutamyl transpeptidase Serum transferrin

ALCOHOL abuse and its consequences pose grave problems for medical and social agencies worldwide. It is often difficult to detect alcoholics within the community and equally difficult to monitor the drinking habits of recognised alcoholics. The ideal biological marker for use in recognition and management of alcoholics should be sensitive and highly specific for alcohol abuse; its values should be affected by changes in alcohol intake over relatively short periods of time and it should be quick, convenient and inexpensive to estimate. One or more biochemical and haematological tests have been advocated and are currently employed for these purposes, but as yet no simple reliable marker is available [6,14].

Markers of Proven Value

Alcohol causes changes in serum enzyme levels by induction, tissue damage or a combination of both. To an extent therefore elevation of enzyme levels provides some indication of the degree of induction and/or magnitude of tissue damage.

The elevated aspartate transaminase (AST) values in alcoholics result from damage to liver, skeletal and cardiac muscle [13]. Skude and Wadstein [26] reported elevated AST values in 73% of 182 chronic alcoholics none of whom had cirrhosis and a similar incidence was reported in a group of alcoholics by Thaler [29]. Konttinen *et al.* [13] performed multiple serum enzyme analyses in 100 chronic alcoholics and found elevated AST values in 64% though no clear indication is given of liver damage in these patients. Galazzi *et al.* [7] found elevated AST values in 64% of a group of alcoholics with biopsy proven minimal liver damage. Because the incidence of elevation of AST varies significantly between populations of alcoholics it is an unreliable marker when used alone.

Serum values of gamma glutamyl transpeptidase (γ-GTP) are perhaps the most widely used biochemical test for screening for alcohol abuse. The rise in γ-GTP values following alcohol occurs as a result of hepatic induction of the enzyme, although hepatocellular damage and cholestasis may also contribute to the increased serum activity. Konttinen *et al.* [13] reported elevated values in 54% of 100 chronic alcoholics; Skude and Wadstein [26] in 69% of 182 chronic alcoholics. Both these groups found a higher incidence of elevated AST values than of γ-GTP values. On the other hand, Rosalki [22] reported elevated γ-GTP values in 75% of hospitalised alcoholics and 90% of outpatient alcoholics and regards γ-GTP as a more sensitive marker than AST [23]. However, long-standing alcohol abusers show a tendency to normalisation of serum γ-GTP values [26] and to an extent elevation of γ-GTP correlates with the degree of alcohol related liver damage [33]. Thus as with AST it is probably unsuitable as a marker when used alone.

A high incidence of macrocytosis has been noted in alcoholics [1, 3, 19, 30, 32]. The mechanism responsible has not been established although it seems probable that it is a direct effect of alcohol on the bone marrow [28]. Wu *et al.* [32] noted macrocytosis in 89% of a group of alcoholics with liver damage of varying severity. Buffet *et al.* [1] observed mac-

TABLE 1

NUMBER AND PERCENTAGE OF PATIENTS WHO WOULD HAVE BEEN DETECTED IF ONE, TWO OR ALL THREE MARKERS WERE USED FOR SCREENING

Marker and Test Combination	Patients with Elevated Values n	%
AST	17	85
γGTP	17	85
MCV	17	85
AST ± γGTP	19	95
AST ± MCV	20	100
γGTP ± MCV	18	90
AST ± γGTP ± MCV	20	100

TABLE 2

NUMBER AND PERCENTAGE OF PATIENTS WITH ABNORMALLY ELEVATED VALUES FOR AST, γGTP AND MCV CONSIDERED SINGLY OR IN COMBINATION AT THE END OF THREE MONTHS; THE NUMBER AND PERCENTAGE OF PATIENTS WITH ABNORMAL RESULTS WHO CONTINUED TO DRINK ARE INDICATED

Marker	Patients with Elevated Values n (%)	Patients with Elevated Values Still Drinking n (%)
AST	12 (60)	11 (91)
γGTP	13 (65)	10 (77)
MCV	9 (45)	8 (88)
AST ± γGTP	16 (80)	13 (81)
AST ± MCV	16 (80)	15 (94)
γGTP ± MCV	16 (80)	13 (81)
AST ± γGTP ± MCV	18 (90)	16 (89)

rocytosis in 82% of patients with alcoholic hepatitis, 89% of alcoholic cirrhotics and in 100% of alcoholics with normal livers. Carney and Sheffield [3] observed macrocytosis in 67% of alcoholics admitted to a psychiatric unit. More recently Morgan *et al.* [19] showed a significantly higher incidence of macrocytosis in female (86.3%) than in male (63.0%) alcoholics. As with estimations of serum enzyme values, measurement of MCV is probably unsuitable as a marker if used alone.

Much less information is available on the usefulness of AST, γ-GTP and MCV for monitoring alcoholics for continued abuse. Rosalki [21] observed that γ-GTP values show a 50% reduction in two weeks and become near normal within five weeks following abstinence and Lamy *et al.* [15] suggest that a rapid fall in γ-GTP with abstinence provides useful confirmation of suspected chronic alcohol excess. Wu *et al.* [32] followed a small number of alcoholics with macrocytosis for three months; three stopped drinking and their MCV values returned to normal while four continued to drink and showed no change in MCV values. Buffet *et al.* [1] noted that elevated MCVs returned to normal within 15 days of hospitalisation and remained normal in patients who subsequently abstained but rose again in those who resumed drinking. Morgan *et al.* [14] found that MCVs returned to normal in certain alcoholics over a three month period provided alcohol intake was reduced though not necessarily stopped.

AST, γ-GTP and MCV are estimated by readily available, automated techniques and their measurement widely used for the recognition and management of alcoholics. However, no formal assessment of their usefulness for this purpose had been made until recently when a study with this aim was completed at the Royal Free Hospital, London (Morgan, Colman and Sherlock, unpublished observations).

The study group comprised 20 patients (14 males:6 females) of mean age (±ISD) 48.8 ± 9.9 yr who had been referred because of alcohol abuse and suspected liver disease. All admitted to an alcohol intake in excess of 1 g/kg body weight/day for five or more years and were drinking up to the time of investigation. All patients had precirrhotic liver disease defined as any combination of fatty infiltration, alcoholic hepatitis or fibrosis without nodule formation. Patients were assessed initially as inpatients and then attended as outpatients at two-weekly intervals when their physical condition was reviewed and biochemical and haematological screening repeated. Alcohol intake was assessed from the patients own reports and drinking records, from reports by family members or work contacts and by random breath alcohol estimations. Patients were readmitted at three months for further assessment.

Laboratory reference ranges were AST 3–15 IU/l, γ-GTP male 10–48, female 7–30 IU/l and MCV 85–95 fl. Values above the upper limit of the reference ranges were considered abnormal.

At first presentation mean values for the group as a whole for AST (30.1 ± 16.8 IU/l), γ-GTP (236.6 ± 277.2 IU/l) and MCV (101.0 ± 7.0 fl) were elevated. There was no correlation between alcohol intake and initial values of AST, γ-GTP or MCV.

Table 1 shows the number of patients who would have been suspected of alcohol abuse if each marker had been used singly or in combination. All patients had elevated values for one or more marker and all twenty would have been detected by combining the results of the three tests.

During the three month study period the mean alcohol intake for the group decreased significantly from 258.3 ± 142.5 to 51.9 ± 11.6 g/day ($p<0.001$). Three patients abstained throughout, ten reduced their intake to <50 g alcohol/day while seven continued to drink >50 g/day.

At the end of the three month period mean values for all three markers had fallen significantly but still remained elevated above the reference ranges: AST 30.1 ± 16.8 to 22.0 ± 15.5 IU/l, $p<0.01$; γ-GTP 236.6 ± 277.2 to 118.2 ± 161.5 IU/l, $p<0.01$ and MCV 101.0 ± 7.0 to 95.3 ± 4.1 fl, $p<0.001$.

Table 2 shows the number of patients with abnormal test results at the end of the three month period together with an indication of those who were still drinking. The markers used singly or in combination did not clearly differentiate patients who had abstained, reduced their intake or continued to abuse alcohol (Fig. 1). However, when values for AST, γ-GTP and MCV were related to alcohol intake over the three month period in individual patients, particular patterns of response emerged (Figs. 2–7). (a) Certain markers reflected alcohol intake more accurately than others; markers varied in sensitivity from patient to patient. (b) In certain patients there appeared to be a threshold of alcohol intake below which values for markers remained normal but above which they changed to accurately reflect intake. (c) Changes

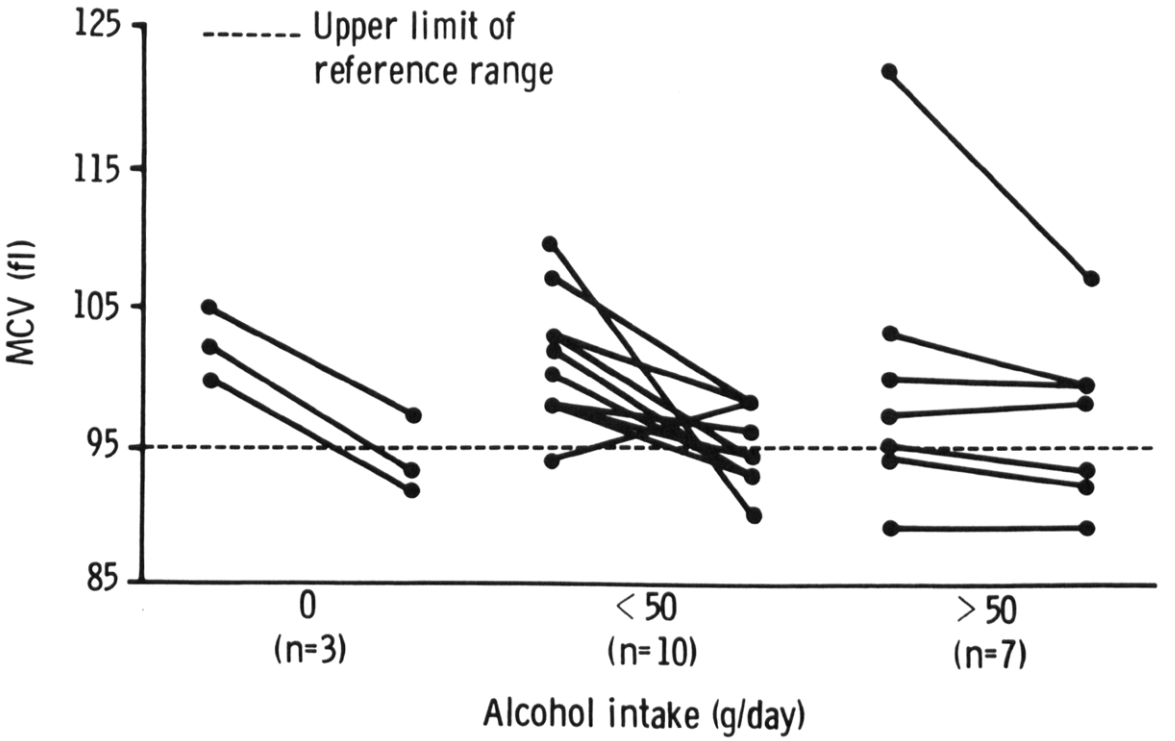

FIG. 1. Changes in MCV and daily alcohol consumption in twenty patients over the three month period.

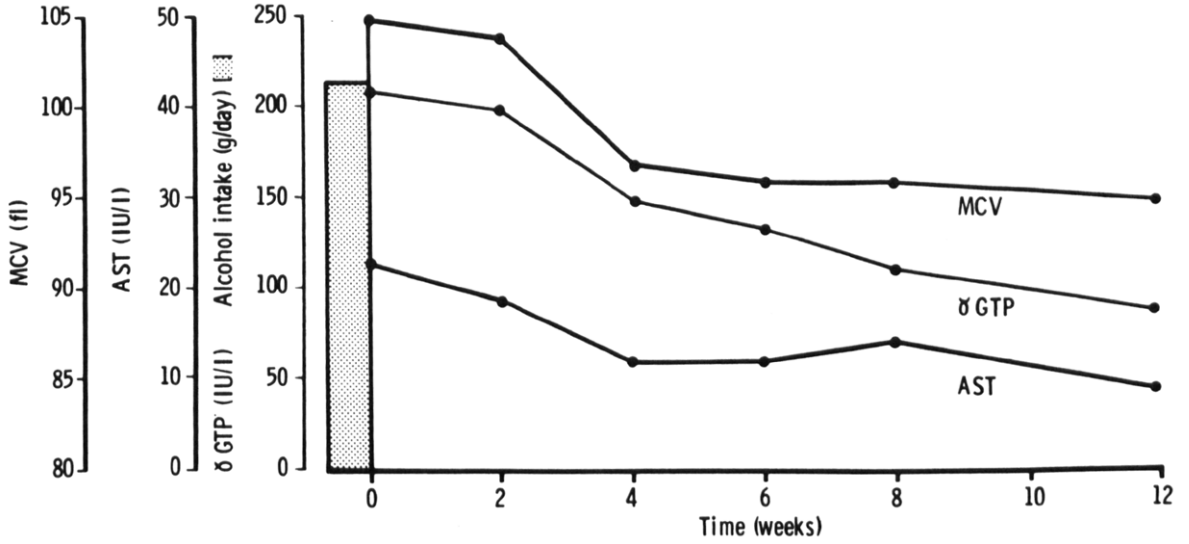

FIG. 2. Changes in AST, γ-GTP and MCV related to alcohol intake in a 34 year old male over three months. Initial liver biopsy mild alcoholic hepatitis and fat; biopsy at three months minimal fat only. Elevated values for all three markers initially. AST normal after four weeks abstinence. γ-GTP and MCV returning towards normal but still elevated after three months abstinence.

in values of markers occur at differing rates in response to changes in alcohol intake; changes in MCV generally take longer than changes in enzyme values. (d) In the presence of liver disease, the γ-GTP though not necessarily the AST may remain elevated even after an extended period of abstinence.

Thus by combining the results of these three tests alcohol abuse can be detected in the majority of patients. Values of AST, γ-GTP and MCV change in response to alterations in alcohol intake in a variable way in individual patients but once these patterns of response have been documented accurate monitoring for continued alcohol abuse becomes possible.

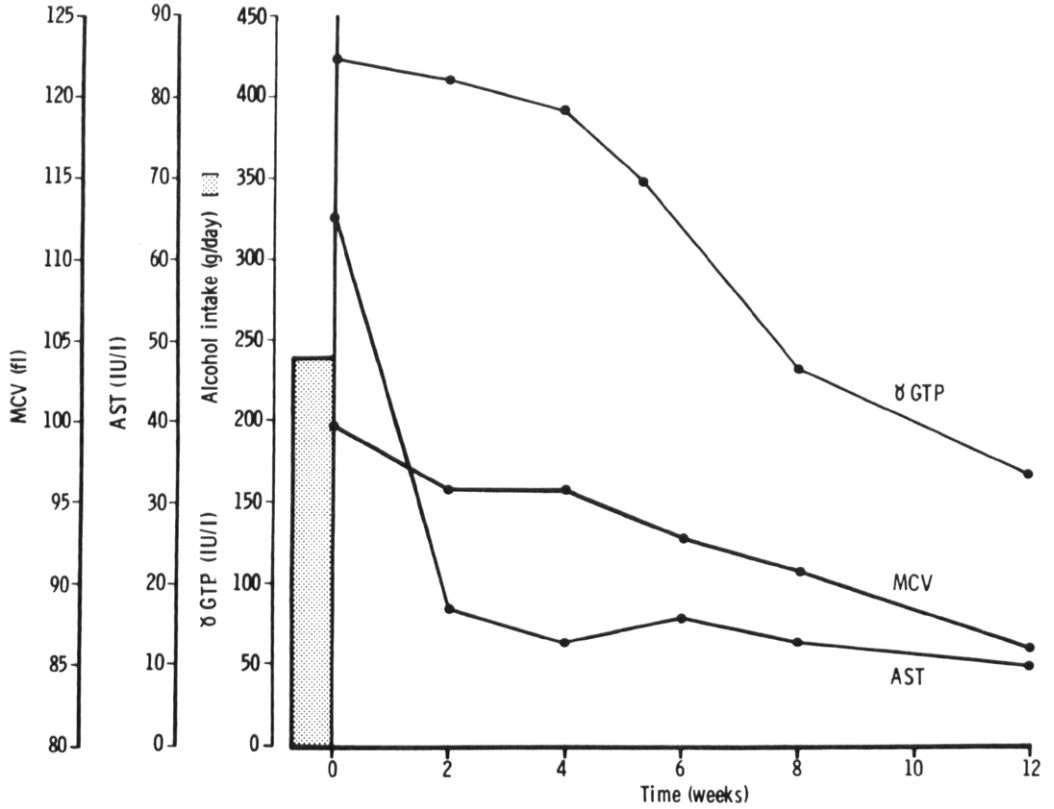

FIG. 3. Changes in AST, γ-GTP and MCV related to alcohol intake in a 45 year old female over three months. Initial biopsy fibrosis and fat; biopsy at three months fat reduced. Elevated values for all three markers initially. AST normal after four weeks abstinence. MCV normal after six weeks abstinence. γ-GTP returning towards normal but still elevated after three months abstinence.

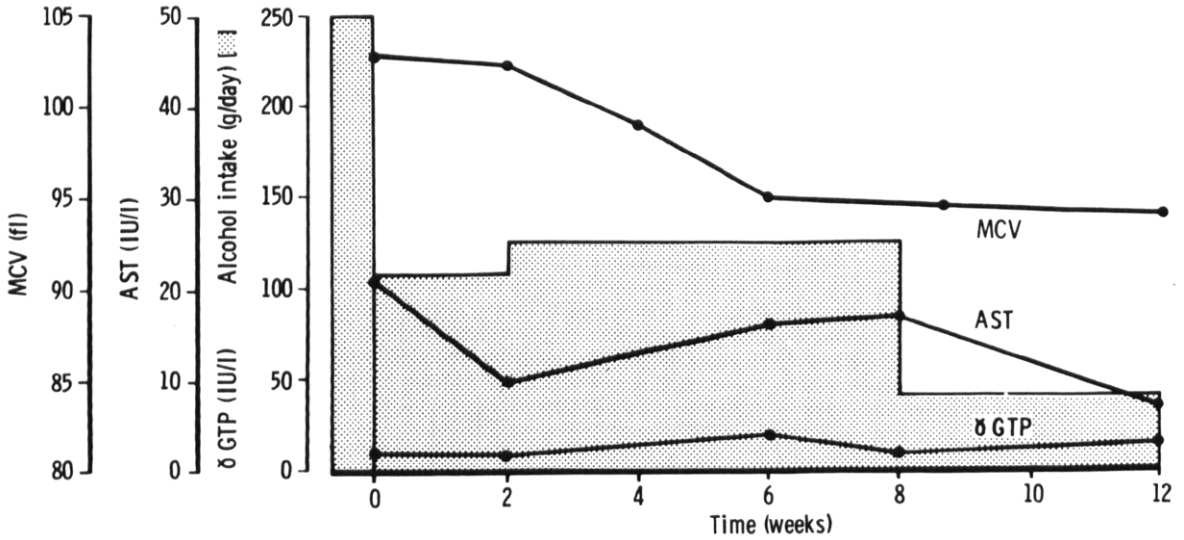

FIG. 4. Changes in AST, γ-GTP and MCV related to alcohol intake in a 37 year old female over three months. Initial biopsy moderate fatty change: biopsy at three months near normal histology. Elevated values for AST and MCV initially. AST normal two weeks after reduction of alcohol intake. MCV normal six weeks after reduction of alcohol intake. γ-GTP normal throughout. NB: With alcohol intakes less than 120 g/day values for all three markers were normal.

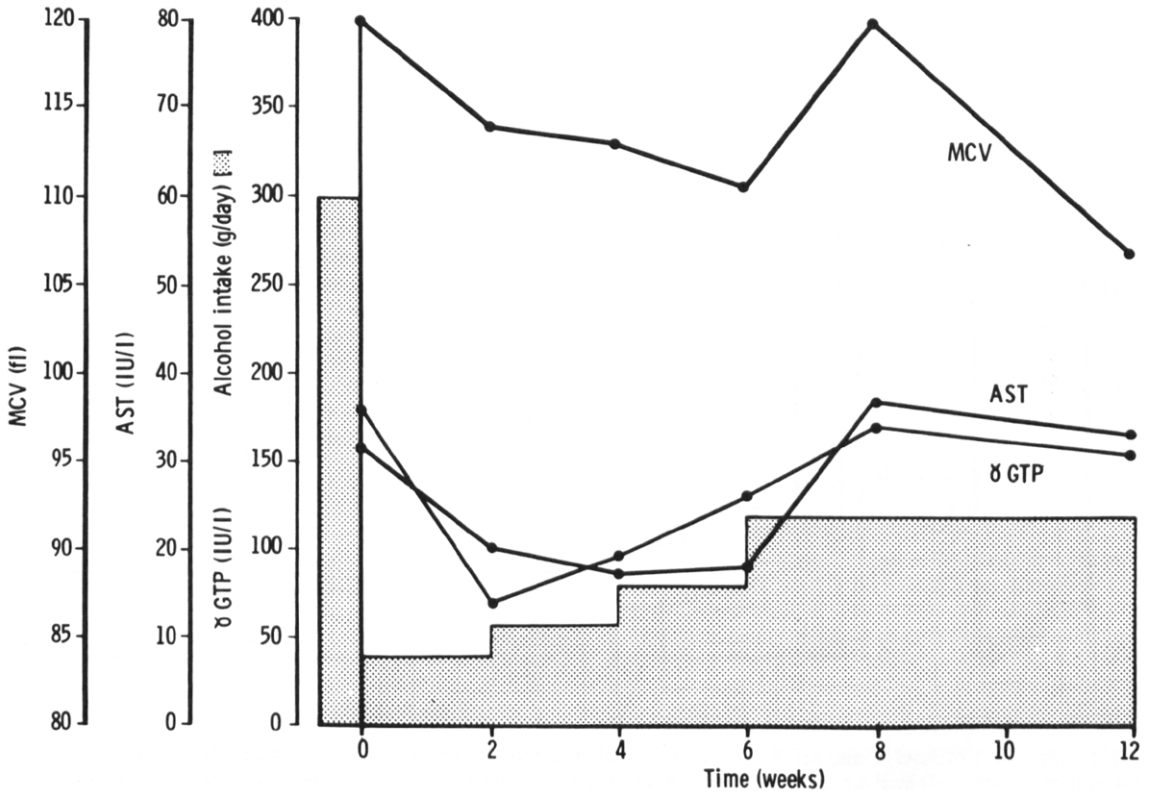

FIG. 5. Changes in AST, γ-GTP and MCV related to alcohol intake in a 51 year old male over three months. Initial biopsy moderate alcoholic hepatitis; biopsy at three months minimal alcoholic hepatitis. Elevated values for all three markers initially. Values for all three markers fall during weeks 0–6 when alcohol intake reduces and rise again when alcohol intake exceeds 80 g/day.

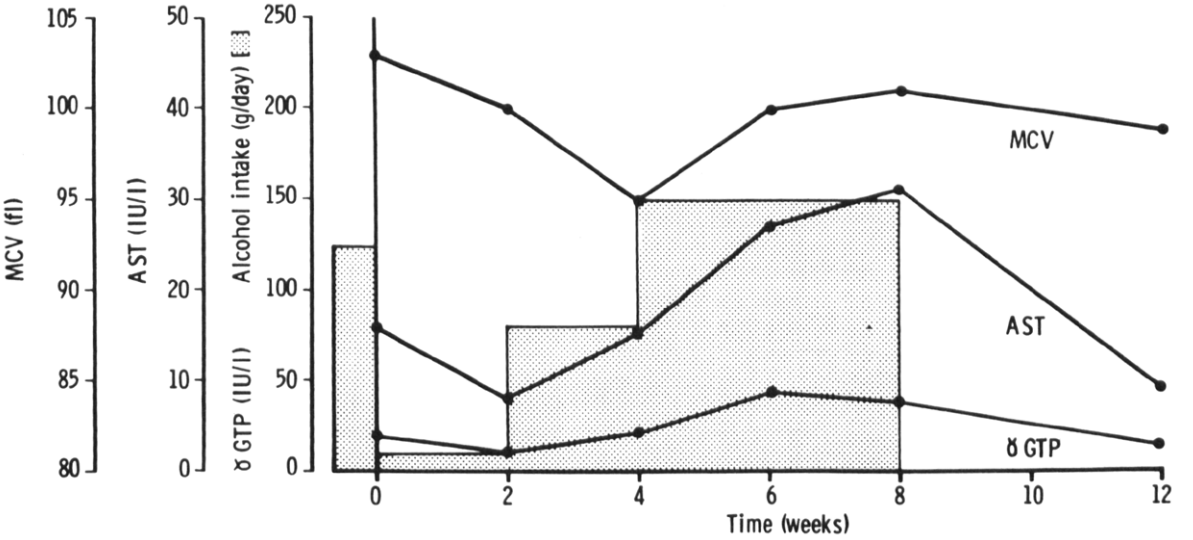

FIG. 6. Changes in AST, γ-GTP and MCV related to alcohol intake in a 49 year old female over three months. Initial biopsy moderate fatty change; biopsy at three months minimal fat. Initially MCV elevated but AST and γ-GTP values normal. Reduction of intake followed by fall in values for AST and MCV. Increase in intake paralleled by increases in values for all three markers. Abstinence followed by prompt reduction in values for all three markers. NB: AST and γ-GTP values normal with intakes of less than 80 g alcohol/day.

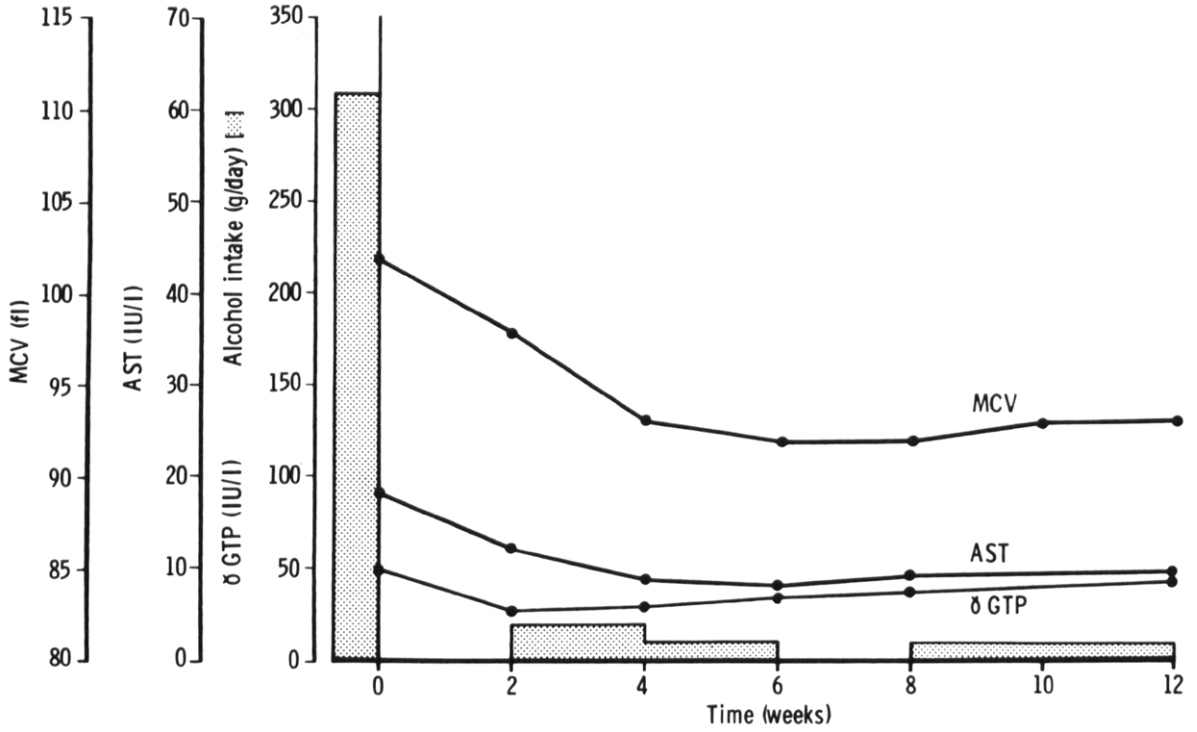

FIG. 7. Changes in AST, γ-GTP and MCV related to alcohol intake in a 42 year old male over three months. Initial biopsy and biopsy at three months minimal fat only. Elevated values for all three markers initially. Reduction of intake followed by return of AST, γ-GTP and MCV values to normal within four weeks. Thereafter values remain normal. NB: Alcohol intakes of 30 g/day are not reflected by changes in values of markers.

Markers of Limited or Disputed Value

Blood alcohol analysis is of limited use for the detection of alcoholism in patients attending clinics for liver disease, since a minimum of three random determinations seem to be required for adequate detection rates and most patients with an elevated blood alcohol readily admit heavy drinking [8]. However, measurement of blood/breath alcohol may be useful in follow up studies as an indicator of non-compliance with abstinence.

Changes in plasma amino acid profiles have been described in patients with acute and chronic liver disease [9,20] and alcohol has been shown to produce acute changes in plasma amino acid levels in control subjects [25]. Recently Shaw *et al.* [24] proposed that the plasma ratio of α-amino-n-butyric acid to leucine (A/L) could serve as a marker for prolonged heavy alcohol consumption. Shaw *et al.* [24] reported that the A/L ratio was: (a) greater than twice normal in chronic alcoholic men. (b) not abnormal after acute alcohol ingestion but became so after prolonged alcohol intake. (c) decreased towards the normal range after one week of alcohol abstinence. (d) normal in non-alcoholics with liver disease. Based on the sensitivity and specificity of the A/L elevations they had demonstrated these workers concluded that this ratio might serve as "an objective, empirical marker for the detection and assessment of alcoholism".

Following this initial report numerous workers tried to confirm the findings of Shaw *et al.* [24] but without success. Morgan *et al.* [18] found that the A/L ratio was raised in alcoholics with significant liver disease irrespective of recent drinking, but was normal in alcoholics with minimal liver disease despite recent heavy alcohol intake. Furthermore, these authors found elevated A/L values in non-alcoholics with liver disease and suggested that the ratio reflected hepato-cellular dysfunction rather than chronic alcohol abuse. Dienstag *et al.* [4] reported that the A/L ratio was not necessarily elevated in chronic alcoholics and that mean values for the A/L ratio did not distinguish between controls, drinking and non-drinking alcoholics and non-alcoholic patients with non-alcoholic liver disease.

Estimation of plasma amino acids is a time consuming procedure requiring technical expertise and expensive equipment not usually available in routine laboratories. While further studies may be required to evaluate the A/L ratio its measurement is unlikely to be of great practical value.

Markers Currently Under Evaluation

Approximately 30% of alcoholics show plasma lipid abnormalities especially hypertriglyceridaemia [5,12]. Recently Johansson and Laurell [10] described a slight to moderate increase in α-lipoproteins in the plasma of alcoholics and Johansson and Medhus [11] in an attempt to replicate this work found increased plasma α-lipoprotein concentrations in 87% of a group of alcoholics who had been drinking recently. Values tended to normalise within two weeks of abstinence from alcohol suggesting a direct effect of recent drinking rather than chronic abuse. The increase in α-lipoprotein did not correlate with other plasma lipoprotein or lipid alterations, nor with the presence of liver damage. It is possible that the α-lipoprotein increase is linked in some way with the altered metabolism of triglycerides and very low density

lipoproteins produced by alcohol [11]. From the clinical point of view estimation of plasma α-lipoproteins might prove useful in the diagnosis of alcohol abuse though similar increases in α-lipiproteins have been found in men exposed to chlorinated hydrocarbon pesticides [2].

Alcohol has an effect on the activity of several enzymes of haem biosynthesis. The activity of erythrocyte δ-aminolaevulinic acid dehydrase (ALA dehydrase) is reduced in over 90% of alcoholics who have been drinking recently [17]. The enzyme also becomes depressed, though transiently, following acute alcohol ingestion in normal subjects [16]. Erythrocyte enzyme activity remains low in alcoholics for about seven days after withdrawal from alcohol whereas in control subjects enzyme activity returns to normal within 24 hr following acute intoxication. The effect of alcohol on ALA dehydrase activity is thought to be mediated by changes in glutathione levels consequent on altered NADH levels [16]. Further research is required to evaluate ALA dehydrase activity as a marker for alcohol abuse; presently the methodology is inconvenient and the lability of the enzyme demands special attention to sample collection and immediate estimation of activity.

Quantitative and qualitative changes occur in the serum proteins in alcoholics especially in the presence of liver disease [10, 27, 31]. Stibler *et al.* [27] have recently described an abnormal serum transferrin component which they found in 81% of drinking alcoholics, 1% of control subjects and in none of a group of patients with non-alcoholic chronic liver disease. This qualitative transferrin abnormality disappeared after 10–14 days of alcohol abstinence. While further studies are required it is possible that this qualitative transferrin abnormality may prove to be a useful and sensitive marker of chronic alcohol consumption.

No simple reliable marker for alcohol abuse exists currently. It is to be hoped, however, that a better understanding of the biochemical and haematological changes associated with alcohol abuse will facilitate development of more sensitive specific tests. Meanwhile measurement of AST, γ-GTP and MCV in combination will serve for routine screening and monitoring of alcoholics.

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