

# Serum enzyme levels in alcoholism and drug dependency

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**SYNOPSIS** Serum gamma glutamyl transpeptidase (GGTP), isocitrate dehydrogenase (ICD), ornithine carbamoyl transferase (OCT), alanine aminotransferase (AIT), aspartate aminotransferase (AsT), and alkaline phosphatase (ALP) activities were assayed in 67 alcoholics and 40 drug dependent patients. Bilirubin, total protein, albumin, and globulin were also measured.

GGTP elevation was observed in 48% of alcoholics and in 50% of drug dependents. The incidences of elevated levels of other enzymes were: ICD 39 and 38.7%; OCT 23.7 and 36.1%; AIT 30 and 33%; AsT 24.2 and 21.7%; ALP 10.4 and 5% respectively.

Measurement of GGTP is thus more useful as a screening test for involvement of the liver in alcoholics and drug dependent patients than that of the other enzymes.

Gamma glutamyl transpeptidase (GGTP EC2.3.2.1) is an enzyme which catalyses the transfer of the gamma glutamyl group from peptides containing it to other peptides and to L-amino acids. In normal subjects this enzyme is concentrated mainly in the kidney with only one-tenth as much in the liver and less in other tissues. It has been reported to be a sensitive and fairly specific indicator of liver damage (Szczyklik, Orłowski, and Szewczuk, 1961; Rutenburg, Goldburg, and Pineda, 1963; Aronsen, Hägerstrand, Nordén, and Pihl, 1969; Aronsen, Nosslin, and Pihl, 1970; Lum and Gambino, 1972).

Excessive alcohol consumption is associated with liver damage (Goldberg and Watts, 1965; Konttinen, Härtel, and Louhija, 1970; Rollason, Pincherle, and Robinson, 1972; Rosalki and Rau, 1972) and we suspect similar liver injury with long-term drug dependence. Laboratory evidence of such damage is usually derived from determinations of serum aspartate aminotransferase (AsT EC2.6.1.1), alanine aminotransferase (AIT EC2.6.1.2), alkaline phosphatase (ALP EC3.1.3.1), isocitrate dehydrogenase (ICD EC1.1.1.42), and sometimes ornithine carbamoyl transferase (OCT EC2.1.3.3) determinations. In this laboratory the incidence of liver damage revealed by routine liver function tests in these patients was surprisingly low, and this study presents the results of multiple serum enzyme analyses for the detection of liver damage in alcoholics and drug dependent patients.

## Material and Methods

Sixty-seven alcoholic patients with a history of continuous or periodic heavy alcohol abuse were studied. All had been admitted to a special alcoholism department of a psychiatric hospital during or immediately after a heavy bout of drinking. Forty patients with a history of drug dependence were studied; 28 were out-patients attending a special clinic and were maintained on drugs, while the remaining 12 were patients admitted to the psychiatric hospital for detoxification.

The alcoholics admitted to a daily intake which ranged from 5-6 pints of beer to 4-5 bottles of whisky, and some used surgical spirit. The drug addicts used heroin, morphia, cocaine, physostigmine, LSD, amphetamines, barbiturates, and cannabis usually in combination; two of them had begun to take drugs at the age of 13 years.

Bilirubin, ALP, and total protein were measured by routine Technicon AAI methods (Methods N-12b, N-28, and N-14b respectively), and albumin was measured on Technicon AAI by the Bromocresol Green dye binding method. AsT and AIT were measured by colorimetric methods on a Technicon AAI (Methods AAI-10 and AAI-22). ICD was measured by the spectrophotometric method of Wolfson and Williams-Ashman (1957), and OCT by the method of Konttinen (1968).

GGTP was measured by the method of Rosalki, Rau, Lehmann, and Prentice (1970) using L-gamma glutamyl paranitroanilide as substrate, glycyl-

glycine as glutamyl group acceptor, and a temperature of 37°C but modified to use a pH 8.4 buffer (Patel, 1972). The enzymic release of paranitroaniline was followed at 410 nm, at which wavelength the substrate exhibits minimum absorbance. Determinations were carried out on fresh serum if possible, otherwise the serum was stored at +4°C but not for more than 36 hours. The normal range for GGTP is 6-35 IU/litre for males and 6-30 IU/litre for females.

**Results**

In the two groups of patients studied the incidence and degree of serum GGTP elevation were compared with those of other serum enzymes and serum bilirubin. The results are shown in fig 1 from which it can be seen that GGTP activity was raised in almost half (48%) of the alcoholic patients. This incidence was higher than that for ICD (39%), OCT (23.7%), AsT (24.2%), or ALT (30%) and very much higher than that for ALP (10.4%) or bilirubin (8.9%).

From fig 1 it can also be seen that GGTP was raised in just one-half (50%) of all drug dependents, a higher incidence of abnormality than that of ICD 38.7%), OCT (36.1%), AsT (21.7%), or ALT (33%).

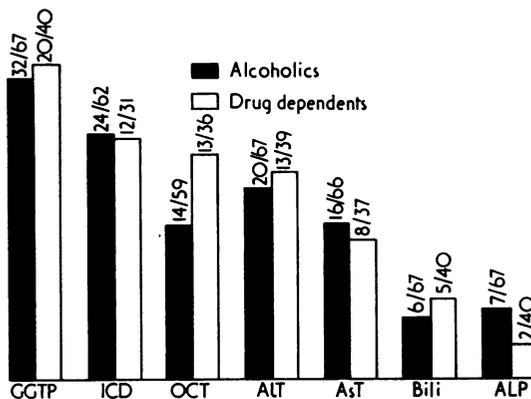


Fig 1 Frequency of abnormal enzyme activities in alcoholics and drug dependents.

ALP and bilirubin were raised much less frequently (5% and 12.5% respectively).

The results of the individual serum assays are given in fig 2 which illustrates the increased sensitivity of GGTP in detecting liver damage.

Where possible, detailed information was obtained

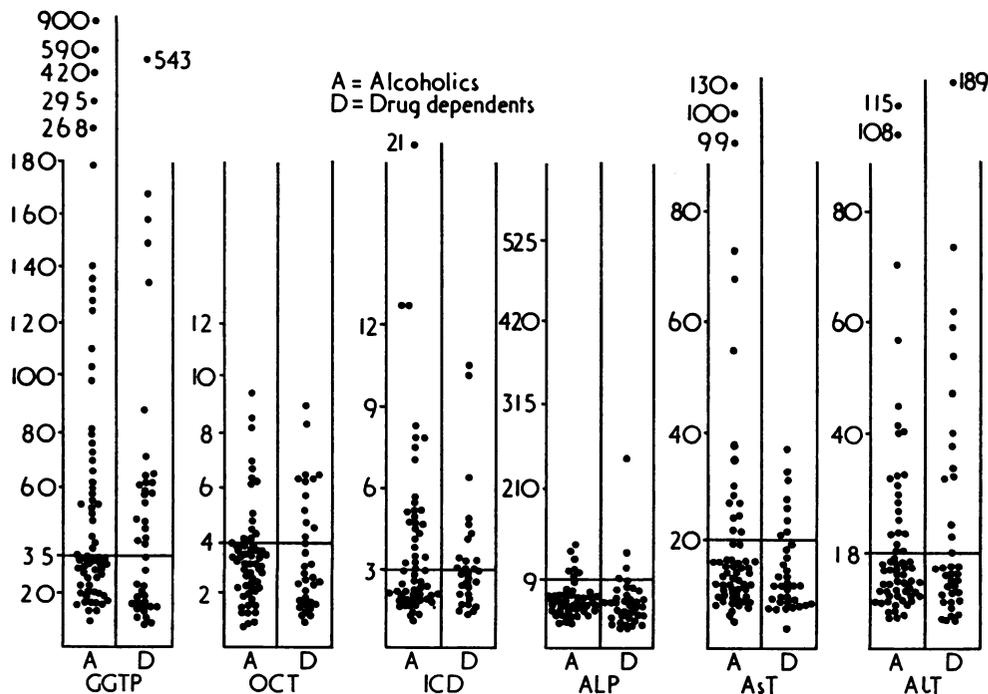


Fig 2 Serum enzyme activities in 67 alcoholics and 40 drug dependents. The upper normal limit is marked by the horizontal line. Activity is expressed as IU/l of serum.

on the amount of alcohol consumed daily, the duration of the last drinking bout, the period of sobriety before the blood sample, the drugs taken, and any other relevant information. No correlation was observed between the serum enzyme activities and the type and amount of alcohol the patient had consumed. Similarly, from drug dependent patients detailed information was obtained as to the age at which the patient first started using drugs upon which he was dependent, when the blood sample was taken, smoking habits, and alcohol consumption. No correlation was observed between the serum enzyme activities and the type of drug used.

Serum bilirubin levels were mildly elevated in six of 67 alcoholic patients and in five of 40 drug dependents; but, except for one alcoholic patient with a serum bilirubin of  $166 \mu\text{mol/l}$  ( $9.7 \text{ mg/100 ml}$ ), levels in five others were less than  $26 \mu\text{mol/l}$  ( $1.5 \text{ mg/100 ml}$ ). Also, the serum bilirubin in five drug dependents was less than  $43 \mu\text{mol/l}$  ( $2.5 \text{ mg/100 ml}$ ). None of the patients showed subnormal levels of total protein less than  $60.0 \text{ g/l}$  or of albumin less than  $30.0 \text{ g/l}$ .

### Discussion

This study has shown a very high incidence of abnormal levels of GGTP in both drug and alcohol dependent patients which probably reflects a greater frequency of liver damage in these patients than has hitherto been suspected on the basis of the more usually applied enzymic tests of liver function. The long-term effects of alcohol upon the liver do suggest that a more sensitive test would reveal damage early in the disease before clinical signs have appeared, and GGTP may be such a test. Similarly, a greater incidence of liver damage will be expected in drug dependency than is commonly observed, for the histological changes of hepatitis with lymphoid infiltration have been reported in 75% of a series of drug addicts (Norris and Potter, 1965).

However, GGTP is not solely of hepatic origin and elevated levels do occur in renal and pancreatic disease as well as in hepatobiliary disease (Szczeplik *et al*, 1961; Rutenberg *et al*, 1963; Goldberg and Watts, 1965) although not in muscle disease (Rosalki and Thomson, 1971). AIT and OCT are highly specific for liver damage, 95% of the total OCT being found in that organ (Goldberg and Watts, 1965), while ICD is less specific (Bell, Shaldon, and Baron, 1962). These enzymes are not as frequently abnormal as is GGTP, although they are more sensitive indices than is AsT, an enzyme distributed widely in the body, including skeletal and cardiac muscle. These findings may depend on the release of enzyme from the damaged cell, which

in turn depends on whether the enzyme is soluble and found in the cytoplasm or is located within the mitochondria. AIT is very largely a cell sap enzyme whereas AsT, OCT, and ICD are all found in both sites, but there is more OCT in the cell sap than in the liver mitochondria and also only the cytoplasmic NAD-linked isoenzyme of ICD is found in serum. GGTP is chiefly in the form of a membrane-bound constituent of the microsomes, but a soluble form is also present in the cytoplasm (Szewczuk, 1966). Release of GGTP into the serum would require damage to cell and microsomal membranes; moreover, cytoplasmic infiltration with structural damage to endoplasmic reticulum and microsomes are known to be early results of excessive alcohol intake (Rubin and Lieber, 1967).

On this basis of intracellular distribution of the enzymes it would be expected that GGTP would be liberated more rapidly than OCT, the release of which might in turn precede that of the transaminases in alcoholic liver damage, and that GGTP would be especially sensitive in its detection. In addition, differences in molecular size and shape of the enzymes and permeability of cell membranes to them might determine the rapidity of elevation of the serum levels.

GGTP was the enzyme raised most frequently and to a higher level above normal in both groups and was the only enzyme of the six assayed to be elevated in 13% of the alcoholics and in 20% of the drug dependent patients. It has been suggested (Rosalki and Rau, 1972) that this could be due to induction of hepatic microsomal enzyme by the alcohol or the drugs, but only the hypnotics and barbiturates out of the large group of drugs on which our patients were dependent had been shown to induce the enzyme, and many of the patients' misuse of the drugs had been prolonged well beyond the time needed for enzyme induction.

Moreover, if the elevated levels we have observed were due to induction, one would have expected a very much higher incidence of abnormal levels even than those we report and also a correlation with the drugs used, which we did not observe. Our findings support the views of other authors (Idéo, de Franchis, del Ninno, and Dioguardi, 1971; Rosalki, Tarlow, and Rau, 1971; Rosalki and Rau, 1972) that elevated GGTP levels reflect liver cell damage and not enzyme induction.

In only one patient was the alkaline phosphatase so raised as to suggest cholestasis, and there was no correlation between levels of that enzyme and GGTP in these patients, and thus no evidence that elevation of the latter is due to cholestasis in either group. We have previously described (Patel and O'Gorman, 1973) a simple method of identification

of isoenzymes of GGTP, further study of which may help to clarify the usefulness of this enzyme in the early diagnosis of hepatic cellular damage.

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