

# Serum bile acids in the diagnosis of hepatobiliary disease

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**SUMMARY** The value of serum bile acids (SBA) in the diagnosis of hepatobiliary disease has been investigated. A modified GLC method was used, with an overall coefficient of variation of  $\pm 11\%$  in the control range. Serum was obtained after a 12 hour fast, and two hours after a fatty meal from 73 patients and 14 control subjects. In controls the total fasting SBA of  $2.17 \pm 0.86 \mu\text{mol/l}$  increased significantly ( $P < 0.001$ ) to  $3.81 \pm 1.14 \mu\text{mol/l}$  after a meal. All icteric patients had raised SBA, but in 23 anicteric patients there was no significant difference in the detection of chronic liver disease by fasting SBA, postprandial SBA, AST, or  $\gamma$  GTP. Compared with controls, serum in patients contained proportionately less deoxycholic acid ( $P < 0.001$ ), there was proportionately more cholic acid in extrahepatic obstruction ( $P < 0.001$ ), and proportionately more chenodeoxycholic acid in patients with cirrhosis, viral hepatitis, and neoplasia ( $P < 0.001$ ). In control subjects, the fasting cholic:chenodeoxycholic acid ratio ranged from 0.5-1.0, and differed significantly ( $P < 0.001$ ) from patients with extrahepatic obstruction 0.96-3.6, and cirrhosis 0.1-0.5. It is concluded that serum bile acids measured by sensitive methods can provide useful diagnostic information.

The concentration of total serum bile acid increases in patients with hepatobiliary disease (Sherlock and Walshe, 1948; Rudman and Kendall, 1957; Makino *et al.*, 1969). It has recently been suggested that the measurement of total serum bile acids in the postprandial state is superior to conventional liver function tests in the detection of hepatobiliary disease (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975; Fausa, 1976). Early claims that the ratio of trihydroxy bile acids to dihydroxy bile acids is useful in differential diagnosis (Rudman and Kendall, 1957; Carey, 1958) have been challenged because of overlap (Neale *et al.*, 1971). More recently, the ratio of cholic:chenodeoxycholic acid has been advocated in the evaluation of patients with primary biliary cirrhosis (Bloomer *et al.*, 1976) and cholestatic syndromes of infancy (Javitt *et al.*, 1973).

The purpose of this study was to evaluate changes in individual and total serum bile acids in the diagnosis of hepatobiliary disease, using a modified GLC method which permitted accurate measurements at control values and more rapid analysis. Particular attention was paid to total serum bile acids, in fasting and postprandial serum, and the cholic:chenodeoxycholic acid ratios.

## Methods

The subjects studied are shown in Table 1. The control group consisted of asymptomatic, clinically and biochemically normal volunteers, who were not receiving any drugs. The patients' diagnoses were based on clinical, biochemical, radiological, and histological grounds, with some exceptions. In two cirrhotic patients and a patient with acute hepatorenal failure coagulation studies did not permit percutaneous liver biopsy. Of six patients with viral hepatitis who were not subjected to liver biopsy, five had the hepatitis associated antigen detected transiently in their serum. Patients with infectious mononucleosis were diagnosed on the basis of the Paul Bunnell test with appropriate differential adsorption. All the patients with extrahepatic obstruction underwent laparotomy, and patients with viral hepatitis were studied within 15 days of the onset of symptoms, with the exception of the patient with prolonged cholestasis. Percutaneous liver biopsies were obtained with the Menghini needle.

Twenty-five millilitres of blood was withdrawn from antecubital veins at 9.00 a.m. after a 12 hour fast. Each subject was then given a standard fatty meal consisting of a 19 g chocolate bar (Damancy

Table 1 Clinical diagnoses in 74 patients studied

| Group and diagnosis                | Nos. |
|------------------------------------|------|
| Control subjects (14)              | 14   |
| Cirrhosis (16)                     |      |
| Alcoholic                          | 10   |
| Chronic active hepatitis           | 4    |
| Primary biliary cirrhosis          | 1    |
| Haemochromatosis                   | 1    |
| Extrahepatic obstruction (15)      |      |
| Gallstones in common bile duct     | 8    |
| Carcinoma of pancreas              | 6    |
| Carcinoma of ampulla               | 1    |
| Viral hepatitis (12)               |      |
| Type A                             | 6    |
| Type B                             | 5    |
| Prolonged cholestasis              | 1    |
| Neoplasia (8)                      |      |
| Carcinoma of bronchus              | 3    |
| Miscellaneous secondary carcinomas | 5    |
| Infectious mononucleosis (7)       |      |
| Miscellaneous (14)                 |      |
| Steatosis                          | 3    |
| Drug hepatotoxicity                | 3    |
| Chronic active hepatitis           | 3    |
| Chronic persistent hepatitis       | 1    |
| Primary biliary cirrhosis          | 1    |
| Acute hepatorenal failure          | 1    |
| Unclassified                       | 2    |

cholecystographic fatty meal) plus 200 ml milk. Thereafter the subjects fasted for a further two hours when a second 10 ml blood sample was obtained. The second sample was omitted in patients with viral hepatitis and some patients with neoplasia who were intolerant of the meal. Fifteen millilitres of blood from the first sample was used for analysis of bilirubin, alkaline phosphatase, aspartate aminotransferase, and  $\gamma$  glutamyl transpeptidase by standard laboratory methods in the Department of Biochemical Medicine. Sera from the remaining 10 ml specimen as well as the 10 ml from the second sample were frozen ( $-20^{\circ}\text{C}$ ) until analysed for bile acids.

A modified GLC method was used which has been described in detail elsewhere (Ross *et al.*, 1977). In brief, an internal standard of 7 ketolithocholic acid was added to 0.5-2.0 ml serum. Ethanol precipitation ( $2 \times 3$  ml), followed by evaporation to dryness, preceded hydrolysis with cholyglycine hydrolase at pH 5.6 for one hour. Free bile acids were extracted from the acidified residue with  $3 \times 2$  ml ethyl acetate. Excess cholesterol was removed by partition between heptane and 70% methanol in water. The dried residue was redissolved in 0.5 ml methanol, methyl esters were formed with excess diazomethane, and trifluoroacetate derivatives produced. The trifluoroacetate methyl esters were injected into columns of 1% OV 210 on gas chrom Q using a flame ionisation detector. Bile acids were identified by relative retention time and peak addition. The overall coefficient of variation was  $\pm 11\%$  in the control range and  $\pm 7\%$  at higher values.

The following statistical methods were employed. Student's *t* test for paired observations was used to evaluate differences between fasting and postprandial total SBA. The *t* test for unpaired observations was used to compare the ratio of the postprandial total SBA:fasting total SBA in control subjects with different patient groups, after log transformation, and similarly to compare the mean proportions of major bile acids in different diagnostic groups with controls, after an arc sine transformation. Differences in sensitivity of total fasting SBA, postprandial SBA, and conventional liver function tests as judged by the number of positive responses in patients with anicteric liver disease were evaluated by Cochran's  $\chi^2$  test. Finally, Scheffe's test was used to assess between-group differences of the cholic:cheno-deoxycholic acid ratio, after log transformation.

## Results

### TOTAL SERUM BILE ACIDS

In control subjects fasting values ranged from 0.96-3.94  $\mu\text{mol/l}$ , and increased significantly ( $P < 0.001$ ) to 2.3-6.2  $\mu\text{mol/l}$  in the postprandial sample. The normal range was taken as  $< 4.5 \mu\text{mol/l}$ , and  $< 6.5 \mu\text{mol/l}$  in the fasting and postprandial state respectively. The fasting total serum bile acids in patients are illustrated in Fig. 1. The highest values were found in viral hepatitis, range 78-405  $\mu\text{mol/l}$ , mean 157  $\mu\text{mol/l}$ . High values were also found in patients with extrahepatic obstruction (mean 106  $\mu\text{mol/l}$ ), but in patients with cirrhosis (mean 32  $\mu\text{mol/l}$ ) and neoplasia (mean 44  $\mu\text{mol/l}$ ) the increase was not so marked. These values frequently but not invariably increased in the postprandial sample. The extent of this change expressed as the postprandial:fasting ratio is shown in Fig. 2. The ratio in control subjects  $1.97 \pm 0.97$  is similar to that in

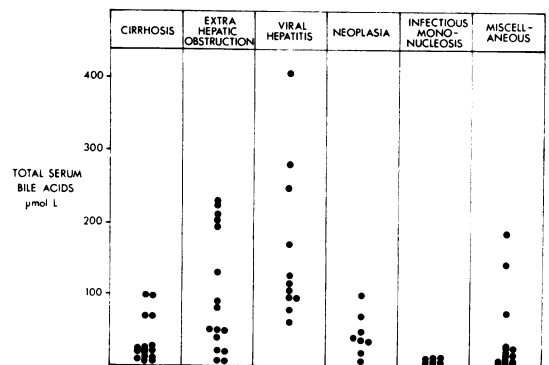


Fig. 1 Fasting total serum bile acids in patients with hepatobiliary disease (normal range  $< 4.5 \mu\text{mol/l}$ )

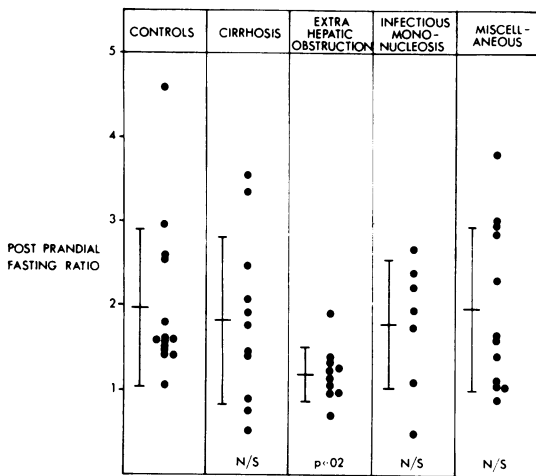


Fig. 2 The postprandial:fasting total serum bile acid ratio, with mean values  $\pm 1$  SD and P values of significance of difference from control data.

cirrhosis  $1.82 \pm 0.99$ , and infectious mononucleosis  $1.79 \pm 0.76$ . In comparison with the control group, patients with extrahepatic obstruction had lower values  $1.19 \pm 0.32$  ( $P < 0.025$ ).

Because it was considered that the main application of measuring the total serum bile acid would lie in the detection of occult liver disease, the measurements of total fasting SBA and total postprandial SBA were compared with each other and with aspartate aminotransferase, alkaline phosphatase, and  $\gamma$  glutamyl transpeptidase, in terms of the relative frequency of abnormal values in 23 patients with histologically established chronic hepatobiliary disease who were anicteric. This group consisted of eight patients with cirrhosis, three with chronic active hepatitis, three with alcoholic steatosis, two drug-induced hepatic damage, two with common bile duct stones, and five with various different abnormalities. The comparative frequency of abnormal values of total SBA and other liver function tests are shown in Fig. 3. The measurement of alkaline phosphatase was significantly inferior ( $P < 0.02$ ) in the detection of these patients. There was no significant difference, however, between the remaining indices, including fasting, and postprandial total SBA, AST, and  $\gamma$  GTP, in the detection of patients with liver disease.

No correlation was noted between total SBA and bilirubin in patients with cirrhosis, extrahepatic obstruction, neoplasia, or infectious mononucleosis. There was however a correlation in patients with viral hepatitis ( $r = 0.6955$ ,  $P < 0.02$ ).

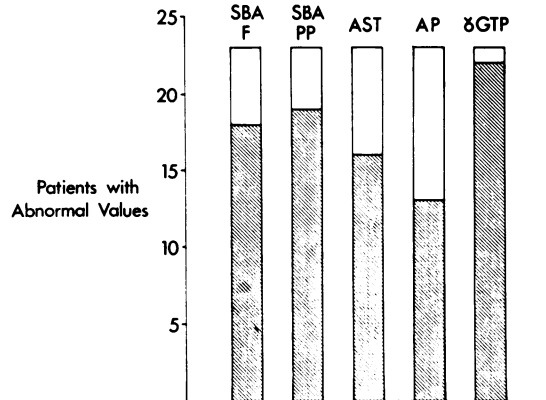


Fig. 3 Comparison of the fasting and postprandial total serum bile acids with other tests of liver function in 23 anicteric subjects. The shaded areas represent the number of patients with abnormalities in the indices shown (AST: aspartate aminotransferase; AP: alkaline phosphatase;  $\gamma$  GTP: gamma glutamyl transpeptidase).

#### INDIVIDUAL BILE ACIDS

The major bile acids, cholic acid, chenodeoxycholic acid, and deoxycholic acid were detected in all subjects and accounted for over 90% of the measured bile acids. The mean distribution in fasting serum of the major bile acids in each diagnostic group is illustrated in Fig. 4. In comparison with control subjects, there was a significant reduction ( $P < 0.001$ ) in the proportion of deoxycholic acid in all patients. The cirrhotic group showed a significant ( $P < 0.005$ ) reduction in the proportion of cholic acid, and increase ( $P < 0.001$ ) in the proportion of chenodeoxycholic acid. Patients with hepatitis and neoplasia also showed a significant ( $P < 0.001$ ) increase in the proportion of chenodeoxycholic acid, but in patients with extrahepatic obstruction proportionately more cholic acid was found ( $P < 0.001$ ).

Ursodeoxycholic acid and lithocholic acid were found in both fasting and postprandial samples of control subjects and patients as shown in Table 2.

Table 2 Detection of ursodeoxycholic acid and lithocholic acid in fasting serum

| Diagnostic group         | Nos. | Number in which detected: |                  |
|--------------------------|------|---------------------------|------------------|
|                          |      | ursodeoxycholic acid      | lithocholic acid |
| Controls                 | 14   | 2                         | 4                |
| Cirrhosis                | 16   | 9                         | 6                |
| Extrahepatic obstruction | 15   | 5                         | 9                |
| Viral hepatitis          | 12   | 4                         | 4                |
| Neoplasia                | 8    | 4                         | 5                |
| Infectious mononucleosis | 7    | 1                         | 1                |
| Miscellaneous            | 14   | 5                         | 4                |

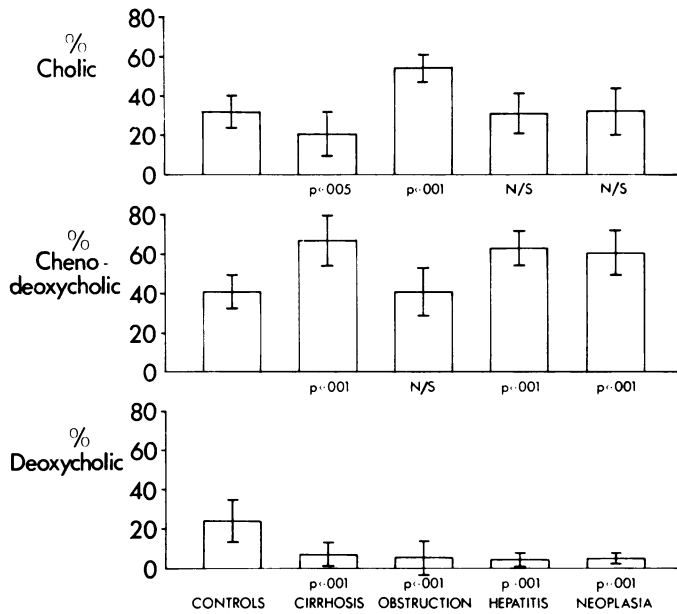


Fig. 4 The mean percentage ( $\pm 1$  SD) of each major bile acid in the main diagnostic groups. P values indicate the significance of the difference from controls.

There was, however, no significant difference ( $\chi^2 = 9.972$ ,  $p < 0.2$ ) in the number of patients within each group from which these bile acids were isolated from fasting serum, and when present they accounted for less than 5% of the total SBA.

**CHOLIC:CHENODEOXYCHOLIC ACID RATIO**

In the fasting state the ratio ranged from 0.5-1.0 in control subjects, 0.96-3.6 in patients with extra-hepatic obstruction, and—with one exception—0.1-0.5 in cirrhosis. The values in viral hepatitis and neoplasia overlapped the normal range, although in these patients the total bile acids were raised. The values in cirrhosis and obstruction were significantly different ( $p < 0.001$ ) from the other groups. Figure 5 illustrates the fasting cholic:chenodeoxycholic acid ratio in the different groups. The significance of the difference between each patient group and the control group is shown. The ratios in the miscellaneous group are documented in Table 3. Increased values were found in steatosis (1.0-1.7), and drug induced hepatotoxicity (2.7-4.74). Two of the three patients with chronic active hepatitis who were receiving prednisolone, and considered to be in remission, had ratios within the normal range, but all showed raised total SBA. In a patient with early primary biliary cirrhosis (stage II) the ratio was 1.46, but was 0.49 in a second patient with established cirrhosis. The patient with acute hepatorenal failure also had a low ratio. The ratios were variable in patients with infectious mononucleosis. In four, they were within the normal range, and in three they

were increased.

After the meal there was considerable variation in the ratios. Control values ranged from 0.25-1.9, and values in cirrhotic patients from 0.19 to 1.6.

**Discussion**

The method (Ross *et al.*, 1977) for SBA measurement was based on procedures previously described

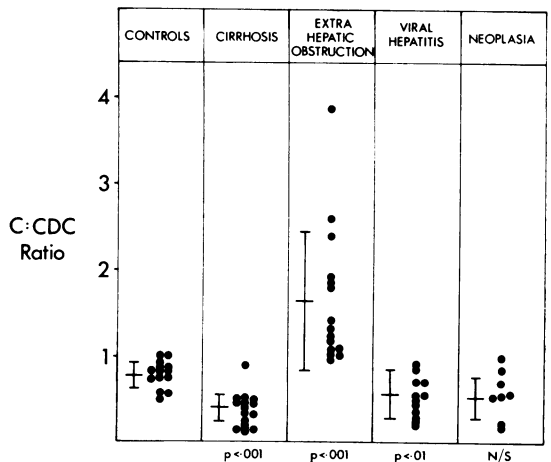


Fig. 5 The cholic: chenodeoxycholic bile acid ratio in control subjects, and patients with hepatobiliary disease. P values indicate the groups that are significantly different from controls.

Table 3 Results of fasting total serum bile acids and fasting cholic:chenodeoxycholic acid ratio in 15 patients with miscellaneous diseases

| Patient | Diagnosis                    | Fasting total serum bile acids ( $\mu\text{mol/l}$ ) | Fasting C/CDC ratio |
|---------|------------------------------|--|---------------------|
| A.L.    | Steatosis                    | 7.33   | 1.77                |
| J.B.*   | Steatosis                    | 1.83   | 1.0                 |
| N.A.    | Steatosis                    | 22.8   | 1.2                 |
| M.A.†   | Drug hepatotoxicity          | 143.7  | 2.7                 |
| S.M.‡   | Drug hepatotoxicity          | 75.3   | 3.9                 |
| T.P.‡   | Drug hepatotoxicity          | 247.0  | 4.74                |
| R.H.§   | Chronic active hepatitis     | 9.2  | 0.6                 |
| A.R.§   | Chronic active hepatitis     | 28.1   | 0.41                |
| C.C.*§  | Chronic active hepatitis     | 3.53   | 0.76                |
| M.A.*   | Chronic persistent hepatitis | 1.58   | 1.9                 |
| J.M.    | Primary biliary cirrhosis    | 14.1   | 1.46                |
| D.B.    | Acute hepatorenal failure    | 186.1  | 0.23                |
| A.A.*   | Unclassified                 | 1.74   | 1.49                |
| F.R.    | Unclassified                 | 17.02  | 0.56                |

\*: normal postprandial total SBA.

†: indomethacin.

‡: cotrimoxazole.

§: prednisolone, in remission, non-cirrhotic.

(Roovers *et al.*, 1968; Henegouwen *et al.*, 1974). It required 0.5-2.0 ml serum, and gave an overall coefficient of variation of  $\pm 11\%$  in the control range, and  $\pm 7\%$  at higher values. The control values were noted to be lower than those previously reported with the enzymatic fluorimetric method (Murphy *et al.*, 1970; Barnes *et al.*, 1975), but similar to those reported with the use of the purified enzyme (Fausa, 1975), which implies non-specificity of the former methods. The method, however, does not measure sulphated bile acids which have been described in serum (Palmer, 1967; Stiehl, 1974; Makino *et al.*, 1975). The cumbersome and, at present, unsatisfactory methodology involved in the measurement of sulphates currently makes their estimation unsuitable in the clinical evaluation of patients with hepatobiliary disease.

The total fasting SBA was found to be raised in all patients who were jaundiced and the majority of patients with chronic anicteric liver disease. There was no significant difference, however, between abnormalities of fasting and postprandial SBA, and the aspartate aminotransferase and  $\gamma$  glutamyl transpeptidase in these patients. This finding is in contrast with a previous report using a GLC method (Kaplowitz *et al.*, 1973), and other reports in which the total value was derived by the enzymatic fluorimetric method (Barnes *et al.*, 1975; Fausa and Gjone, 1976). It is notable, however, that in two of these previous studies the methods used were unable to discriminate between fasting and postprandial values in control subjects (Kaplowitz *et al.*, 1973; Barnes *et al.*, 1975), which suggests lack of sensitivity in their methods. Although claims were made for greater sensitivity of both fasting and postprandial

total serum bile acids in relation to other tests of liver function (Fausa and Gjone, 1976), this applied only to the relative extent to which the various indices increased above normal values. When the same data were analysed to detect the frequency of abnormal values in the patients, the total fasting SBA was found to be significantly inferior to other liver function tests, as well as the total postprandial value. It would be expected, however, that SBA measurement would also have the advantage of specificity.

The cholic:chenodeoxycholic acid ratio fell within a narrow range in fasting control serum, in contrast with previous studies (Carey, 1958; Makino *et al.*, 1969; Neale *et al.*, 1971). Furthermore, it was able to differentiate between control subjects and cirrhotic patients. Earlier reports noted similar values between controls and cirrhotics (Carey, 1958; Makino *et al.*, 1969; Neale *et al.*, 1971). It is also interesting to note that two patients with cirrhosis and extrahepatic obstruction had abnormal ratios with normal fasting total serum bile acids. This is in agreement with previous work (McCormick *et al.*, 1973) which demonstrated that the cholic acid pool declines early in cirrhosis. Patients with extrahepatic obstruction had values greater than one, regardless of the presence or absence of jaundice. The only such patient with a ratio of 0.96 had been jaundiced for four weeks before the study. We have no evidence to suggest that intrahepatic and extrahepatic cholestasis can be differentiated on the basis of this ratio. It is notable that patients with drug-induced liver disease have very high ratios, but the single patient who presented with persistent cholestasis after viral hepatitis provided a ratio of less than 0.5. The variable ratios in postprandial specimens may reflect differences of intestinal transit, and sites of absorption of cholic and chenodeoxycholic acid (Hislop *et al.*, 1967; Angelin, *et al.*, 1976).

The proportionate decrease in deoxycholic acid in patients with cirrhosis, hepatitis, and obstruction has previously been described (Cronholm *et al.*, 1970; Yoshida *et al.*, 1975), and may result from decrease in the formation of deoxycholic acid from cholic acid (Yoshida *et al.*, 1975; Knodell *et al.*, 1976). The increased proportion of chenodeoxycholic acid in cirrhosis has previously been noted (Carey, 1958; Makino *et al.*, 1969), and may reflect 12  $\alpha$  hydroxylase deficiency (Carey *et al.*, 1969) resulting in an early reduction of the cholic acid pool (McCormick *et al.*, 1973). It is also possible that the alternative pathway (Mitropoulos and Myant, 1967), which synthesises relatively more chenodeoxycholic acid (Anderson *et al.*, 1972), may contribute a greater proportion of bile acids in hepatic disease. It has long been known that the proportion of cholic acid increases in extrahepatic obstruction (Carey, 1958;



Makino *et al.*, 1969), and it has been suggested that it reflects an increase in the production of cholic acid operating as a protective mechanism (Greim *et al.*, 1972). The proportionate increase in chenodeoxycholic acid in patients with viral hepatitis may again reflect 12  $\alpha$  hydroxylase deficiency in the presence of hepatic damage. There is evidence, however, that the proportions of bile acids change in different stages of hepatitis (Pennington *et al.*, 1976).

It is concluded that the application of this sensitive GLC method for serum bile acid analysis has for the first time shown that the fasting cholic:chenodeoxycholic acid ratio discriminates between cirrhotics, normal subjects, and patients with extrahepatic obstruction. Furthermore, in this study the total serum bile acids in fasting serum were found to be as sensitive in the detection of hepatobiliary disease as the postprandial value and enzyme assays. Measurement of serum bile acids would therefore appear to be useful in the evaluation of patients with hepatobiliary disease.

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