A Method for Serum Octanoate in Hepatic Cirrhosis and Hepatic Encephalopathy

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We describe a new, more efficient, and more reproducible method for determination of octanoate in serum. This method involves ethanol extraction, followed immediately by alkali addition before concentration of the extract. The concentrate is made acidic only before it is to be steam distilled (in a special all-glass apparatus with an alkali trap). The material is acidified again just before separation by gas–liquid chromatography. The yield is 89–107%. When assayed by mass spectrometry, only octanoate was found in the fraction from chromatography. Previous methods yielded only 30–55% of the expected octanoate value and the recovered materials showed impurities by mass spectrometry. Octanoate concentrations were determined in the serum of 24 fasting controls and that of 85 fasting cirrhotic patients, of whom 50 had encephalopathy. Concentrations in arterial and venous blood were significantly higher in cirrhotic patients in coma than in those not in coma, and arterial concentrations were statistically higher than venous concentrations in the cirrhotic patients.

Additional Keyphrases: gas chromatography of octanoate, arterial and venous octanoate concentrations, normal values, lipid metabolism, cirrhosis, hepatic encephalopathy, mass spectrometry

Several investigators have suggested that octanoate concentrations are increased in hepatic encephalopathies (1–7). Linscheer et al. demonstrated that both mildly and severely cirrhotic patients, on fasting, have higher serum octanoate concentrations than do controls, and that they show abnormally high concentrations of octanoate in their serum after rectal administration of octanoate solution or of a test meal of medium-chain triglycerides; some patients developed encephalopathy during these treatments (5, 6). Sodium octanoate is known to induce a reversible coma in various species of experimental animals, alone or co-administered with ammonium salts (8–11).

A dependable and reproducible method for the assay of octanoate in serum was needed. Various authors (4, 12–16) have reported great difficulties in all of their assays for serum octanoate.

This paper presents a new method for measurement of serum octanoate that takes into consideration the fact that this acid is volatile at low pH values. This method is efficient and reproducible. We give data on 85 patients with cirrhosis of the liver and 24 adult controls.

Materials and Methods

Patients. Eighty-five patients with alcoholic cirrhosis of the liver and 24 adult control patients with proven normal liver function were selected. The diagnosis of cirrhosis was made on the basis of history and clinical and laboratory findings, including liver biopsy. Of the 85 cirrhotic patients, 50 had hepatic encephalopathy. The state of the encephalopathy was graded according to the system of Sherlock (17) immediately before each blood sample was taken. Grade zero corresponds to a cirrhotic patient with normal mental and neurological state. The cirrhotic patients were also categorized as compensated or decompensated (without or with ascites). Blood was collected after an overnight fast. Both arterial and venous blood were obtained from cirrhotic patients, only venous blood from controls.

Apparatus. A specially designed all-glass steam-distillation apparatus was used (Figure 1). The gas chromatograph used in this study was a F & M Biomedical 402 (Hewlett-Packard, Cupertino, Calif. 95014) equipped with a dual-flame detector, operated under the following conditions: glass column, 1.5 m × 3 mm, containing 1% orthophosphoric acid, 10% DEGS Chromosorb W (Supelco, Bellefonte, Pa. 16823). Platinum wire-mesh plugs (A. H. Thomas, Philadelphia, Pa. 19106) were used to retain the support phase within the column. Temperatures: injector, 150 °C; column, 130 °C; detector, 200 °C; flow rates (ml/mm): carrier gas N2, 40; H2, 20; air: 142 kPa (1064 mmHg) pressure; injection, directly into column; chart recorder, Moseley Model 7128 A. The mass spectrometer (Perkin-Elmer Corp., Norwalk, Conn. 06856) was a MS 12 AEI with a 2 m × 3 mm glass column packed with Chromosorb W (80–100 mesh) Carbowax 20 M 10% (Analabs, New Haven,
Conn. 086473); carrier gas, helium; flow rate, 25 ml/min; column temperature, 110 °C; and temperature of the ion source, 195 °C. The electron energy was set to 70 eV and the accelerating voltage was 8 kV. The apparatus was equipped with a mass counter. The apparatus was focused on the ions at m/e 127, 74, and 87, and the peak heights were measured.

**Chemical reagents.** All chemicals were reagent grade (J. T. Baker Chemical Co., Phillipsburg, N.J. 08865) and were used without further purification. These included: absolute ethanol; sodium hydroxide, 0.2 and 0.01 mol/liter solutions; sulfuric acid, 0.5 mol/liter; phosphoric acid, 10 g/liter, in acetone; and heptanoic acid (the internal standard), 2.5 mmol/liter in acetone.

**Procedure for assays.** To 1 ml of serum sample obtained from blood centrifuged immediately after clotting in a glass-stoppered tube, add 20 μl of the heptanoic acid solution (internal standard) and 5 ml of ethanol. Stopper the tubes and vigorously shake by hand for 1 min and centrifuge. Transfer the supernatant fluid to a conical-tip tube, add 0.1 ml of 0.2 mol/liter NaOH, and stir gently with a glass rod. Add a drop of phenolphthalein. Evaporate the mixture in a boiling water bath under a gentle nitrogen stream. Use four 250-μl portions of water consecutively to dissolve and transfer the residue as completely as possible. Place this alkaline solution at the bottom of the central tube of the steam distillator (Figure 1). Add 1 ml of 0.5 mol/liter H₂SO₄, immediately stop the central tube with a Teflon stopper, and heat the flask. Collect the distillate in a conical tube containing 2 ml of 0.01 mol/liter NaOH with one drop of phenolphthalein, keeping the tip of the tube under the surface to avoid loss of the steam-volatile components. Evaporate the distillate under nitrogen and redissolve the dry residue with three 100-μl portions of water, transferring these to a 400-μl Spinco micro-test tube equipped with a stopper (Beckman Instruments, Inc., Irvine, Calif. 92713). Evaporate the solution in a water bath, then add 40 μl of the H₂PO₄ solution and immediately stop the tube and centrifuge. After 30 min, inject an aliquot of the supernatant solution into the chromatograph with a 1-μl microsyringe (Hamilton, Whittier, Calif. 90608).

Measure the area of each gas-liquid chromatography peak by triangulation and compare this to the area of the internal standard (heptanoate).

To determine if the material represented by the gas-liquid chromatography peak was indeed octanoate, we collected samples and made the methyl ester by adding 40 μl of a saturated solution of diazomethane (Eastman Co., Rochester, N. Y. 14650) in acetone. The methyl ester of an authentic sample of octanoic acid was prepared in the same fashion. The mass spectra of the two samples were identical (Figure 2), and the ratios of their fragments were equal (Figure 3). We also assayed by mass spectrometry the octanoate obtained by use of the other methods (4, 12-14). Although octanoate was
the major peak, many impurities were also present.

Blood ammonia. To prove the hepatic etiology of the encephalopathies, blood ammonia was determined with the Hyland Blood Ammonia Resin Test, in most cases simultaneously with serum octanoate.

Levels of significance. The distribution of the normal samples was gaussian according to the test of David et al. (18). The level of significance between blood samples from normal and from conscious cirrhotic patients, between conscious and comatose, or between compensated and decompensated cirrhotic patients was calculated with the Mann-Whitney U test (19). Its efficiency approaches 95.5% by use of the t-test for both samples from moderate and severely cirrhotic patients without any need for a hypothesis concerning normality and homoscedasticity (equal variability for y values throughout the range for x values) of the samples.

The differences between values for arterial and venous blood were determined for matched pairs. The t-test efficiency gave a reliability of 95% (20).

Results

Mean venous serum octanoate concentration in 24 control subjects was 1.5 µmol/liter. Normal values were therefore considered to be those below 5 µmol/liter (i.e., the mean + 2 SD; level of significance, P = 0.05).

There were statistically significant differences between the concentrations in blood of controls and (grades 0 to 3) cirrhotic patients (Mann-Whitney U test significance level, 0.001) and between conscious cirrhotic patients and comatose (grades 4 and 5) cirrhotic patients (Mann-Whitney U test significance level 0.01) (Figure 4).

The difference was also significant between arterial octanoate concentrations of cirrhotic patients not in coma (grades 0 to 3) and in coma (grades 4 and 5); the Mann-Whitney U test significance level was 0.06 (19, 20).

Significant differences were found with the Mann-Whitney U test between concentrations in venous blood of control and compensated cirrhotic patients (significance level, 0.01) and between compensated and decompensated cirrhotic patients (significance level, 0.05).

The sign test for matched pairs showed that values for arterial serum were higher than for venous serum in 59 simultaneous arterio-venous determinations in cirrhotic patients (significance level, 0.01) (Figure 5).

Discussion

Octanoate assays. The methods used by several investigators (4, 12-16) involved extractions with ace-
Fasting cirrhotic patients had a higher octanoate concentration in serum than did controls, and 30 and 120 min after rectal administration of an octanoate test solution the values were higher in severely cirrhotic patients than in mildly cirrhotic patients. The increased values were found for serum octanoate in severely cirrhotic patients agree with the observations of Zieve et al. (15, 16), who found increased values for volatile free fatty acids (two to six carbon atoms) in the breath of cirrhotic patients and in the blood of dogs after hepatectomy.

Our finding of higher serum octanoate values in comatose cirrhotic patients than in non-comatose cirrhotic patients agrees with the observation (4) that four cirrhotic patients developed hepatic encephalopathy while receiving treatment with medium-chain triglycerides and that sodium octanoate induces a temporary coma in experimental animals, alone or with co-administered ammonium salts or mercaptans (2, 3, 5).

Most of the encephalopathic patients had simultaneously increased values for ammonia and octanoate in their serum, but these values varied independently and they were not correlated in comatose and non-comatose cirrhotic patients. Simultaneous measurement of ammonia and octanoate may be prognostically useful in some borderline cases.

**Significance of patients’ values.** The increased values for octanoic acid in the serum of fasting cirrhotic patients may reflect both portasystemic shunting and hepatocellular impairment with abnormal fat metabolism. After rectal administration of a sodium octanoate solution (11.4 mg/g body weight) to noncirrhotic, mildly or severely cirrhotic, and surgical portacaval-shunted cirrhotic patients, we saw a highly significant difference between the mean peak values for octanoate in serum from the four groups of subjects (6). We confirmed this, using the same rectal dose in five normal and five compensated cirrhotic patients and the assay technique described in this paper. This suggests that values for octanoate in peripheral blood could be related to the efficacy of portasystemic shunts. Short-chain triglycerides, although they represent only 1 to 2% of the alimentary lipids, may be an endogenous source for octanoate.

Values for serum octanoate were noted to be significantly higher in the arterial blood than in the venous blood of the fasting cirrhotic patients. This suggests a possible oxidation of some of the circulating octanoic acid by striated muscle in man; similar to that shown by rat muscle (21).

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**References**