notes on methodology

Citrate, pyruvate, and lactate contaminants of commercial serum albumin

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SUMMARY Commercial serum albumin was found to contain lactate, pyruvate, and especially citrate in addition to fatty acids. Glucose, aspartate, and α-ketoglutarate were also present but at lower concentrations. Charcoal treatment followed by prolonged dialysis was effective in removing most of these contaminants.

KEY WORDS contaminants · citrate · pyruvate · lactate · free fatty acids · charcoal

IN PRELIMINARY EXPERIMENTS in which intermediates of the tricarboxylic acid cycle were measured in adipose tissue cells that had been isolated in a medium containing serum albumin, we noticed large and variable concentrations of citrate. Control experiments demonstrated that this citrate was derived not from the adipose tissue, but from the serum albumin used in the incubations. This report lists several of the contaminants found in 11 different batches of serum albumin and suggests procedures for eliminating them.

The source and lot numbers of serum albumins used in this study are given in Table 1. Malate dehydrogenase (EC 1.1.1.37), lactate dehydrogenase (EC 1.1.1.27), citrate lyase (EC 4.1.3.6), and aspartate aminotransferase, (EC 2.6.1.1) were obtained from Boehringer Mannheim Corp., New York. As the last-named enzyme is usually obtained as a suspension containing α-ketoglutarate as a contaminant, this sample was dialyzed against 0.01 M sodium phosphate, pH 7.4, the dialyzed solution was adjusted to 90% saturation with ammonium sulfate, and the enzyme was stored at 4° until used. NAD and NADH were obtained from P-L Biochemicals Inc., Milwaukee, Wis. Darco activated charcoal, type G-60 (Atlas Chemical Industries Inc.) was purchased from the Arthur H. Thomas Co., Philadelphia, Pa. The charcoal was suspended in water and centrifuged at 13,000 *g* for 10 min, and the fine particles were removed by decantation.

Analysis of Contaminants in Albumin. To 4 ml of a solution of albumin (3% or 10%) we added 1 ml of 25% perchloric acid. The resultant precipitate was centrifuged, reextracted once with 1 ml of 6% perchloric acid, and centrifuged. Later analysis showed that this procedure effectively removes the contaminants bound to the albumin. The pH of the combined supernatant solutions was adjusted to approximately 2 with 4 N KOH and then to 6 with 2 N $KHCO_3$. This produced a suspension which we cooled in ice and centrifuged to remove the potassium perchlorate precipitate. The supernatant solution was used for the determination of lactate, pyruvate, citrate, oxaloacetate, aspartate, glutamate, glucose, and α-ketoglutarate.

Determination of Contaminants. Lactate (1), glutamate (2), and glucose (3) were measured spectrophotometrically in extracts prepared from 10% solutions of serum albumin. Citrate, oxaloacetate, and pyruvate were measured sequentially by a fluorometric modification of the method of Dagley (4). Aspartate was assayed fluorometrically by the oxidation of NADH in the presence of malate dehydrogenase, aspartate aminotransferase, and α-ketoglutarate; or α-ketoglutarate was assayed similarly in the presence of the two enzymes and aspartate (5).

Free Fatty Acid Determination. The free fatty acid content of four of the noncrystalline albumin samples was determined by the method of Dole and Meinertz (6).

Treatment of Albumin. Charcoal, washed with water to remove fine particles, was added to a 10% solution of serum albumin to give a charcoal concentration of 5%. The pH of the solution was reduced to 3 as described by Chen (7) for the removal of fatty acids. After the charcoal had been removed by centrifugation the solution was neutralized and lyophilized. Either a 3% or 10% solution of albumin was then dialyzed for 3 days against 0.9% NaCl at 2°C. The 0.9% NaCl was changed three times daily with a final 6 hr dialysis against distilled water to remove NaCl. The dialyzed solution was lyophilized and the dry powder refrigerated before the subsequent determination of intermediates.

Results and Discussion. As shown in Table 1, commercially available serum albumin contains not only various amounts of fatty acids but also citrate, lactate, and pyruvate. No oxaloacetate or glutamate was detected in any of the albumin samples listed, whereas the highest concentrations of glucose, aspartate, and α-ketoglutarate detected were 6, 6, and 5 μmoles/mmole of albumin, respectively. Treatment of the albumin with charcoal by the method of Chen (7) followed by a 3 day dialysis effectively removed the fatty acid, citrate, and pyruvate but was less effective in removing lactate.

Albumin is known to bind small anions such as chloride, acetate, and bicarbonate; its affinity for larger ions such as those of azo dyes, pH indicator dyes, fatty acids, and detergents is usually greater (8). The nature and number of binding sites depends on the ion being studied. Association of detergent with albumin to give complexes containing 30% detergent have been demonstrated (9).

TABLE 1 CONTAMINANTS OF SERUM ALBUMIN

Supplier*	Description and Lot No.	Citrate	Pyruvate	Lactate	FFA
			μmoles/mmole albumin		
Armour	Bovine fraction V, B22903	2990 (<10)	<10	91 (75)	280 (30)
Nutritional Biochemicals	Bovine fraction V, 4266	70 (<10)	34 (21)	618 (414)	170 (60)
Nutritional Biochemicals	Bovine fraction V, crystalline, 4710	930	<10	<10	n.d.
Nutritional Biochemicals	Bovine fraction V, crystalline, 5271	835	<10	<10	n.d.
Nutritional Biochemicals	Bovine, "fatty acid-poor" fraction V, 3006	91 (<10)	43 (19)	740 (19)	n.d.
Pentex	Human fraction V, 19	3380 (<10)	<10	77 (45)	760 (20)
Pentex	Bovine fraction V, crystalline, 11	52 (<10)	<10	95 <10	n.d.
Pentex	Bovine fraction V, crystalline, 14	253 (<10)	<10	<10	n.d.
Pentex	Bovine fraction V, crystalline, 15	252 (<10)	<10	<10	n.d.
Pentex	Bovine "fatty acid-poor" fraction V, 17	200 (<10)	37 (43)	746 (618)	1210 (10)
Sigma	Bovine fraction V, crystalline, 37B-0930	940 (<10)	<10	<10	n.d.

Serum albumin samples were extracted with perchloric acid and analyzed for metabolic intermediates as outlined in text. The molecular weight of serum albumin is taken as 69,000 (11). The value in parenthesis is the concentration of the specific intermediate present in the albumin after treatment with charcoal and extended dialysis for 3 days against 0.9% NaCl.

FFA, free fatty acid; n.d., not determined.

* Armour Pharmaceutical Co., Chicago, Ill.; Nutritional Biochemicals Corp., Cleveland, Ohio; Pentex, Inc., Kankakee, Ill.; Sigma Chemical Co., St. Louis, Mo.

Purified albumin usually contains, per millimole, about 2000 μmoles of fatty acids, which may be removed by treatment either with 5% glacial acetic acid in isooctane (10) or with activated charcoal at low pH (7). Apparently neither method results in significant denaturation of the serum albumin and these or similar techniques are often applied routinely before albumin is used for metabolic studies.

No steps are taken, however, to remove smaller ions, and Table 1 shows that serum albumin available from commercial sources is contaminated by citrate, pyruvate, and lactate. This contamination may be of serious consequence when albumin is used in metabolic studies, for example when albumin is added during the incubation of adipose tissue to bind fatty acids released by lipolysis or in the stabilization of enzymes that may be inhibited by fatty acids. Particularly important are the possible errors introduced by the presence of citrate: in two of the albumin samples tested, the concentration of citrate reached 1%. Such high levels are no doubt due to the use of citrate as an anticoagulant during the collection of blood from the animal.

These results indicate the dangers in the indiscriminate use of serum albumin in metabolic studies. Not only do different batches of albumin contain different amounts of impurities, but albumin can also bind substrates and cofactors used in the experiments. Thus it is virtually impossible to calculate the actual concentrations of the reaction constituents in any metabolic experiment. When albumin is essential and when it is also important to ensure that there are no metabolic intermediates present as contaminants, treatment of the albumin with charcoal by the method of Chen (7) followed by prolonged dialysis is recommended.

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