A Rapid Method for Isolation of Mixed Conjugated Bile Acids From Bile.* (21079)

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In the course of studies on the comparative biochemistry of cholesterol metabolism, it became necessary to isolate conjugated bile acids from the bile of several species of animals. Published procedures are based on the use of either single-stage extraction or countercurrent distribution(1). The former method does not readily yield a pure product, while the latter requires elaborate equipment. In this communication, a simple adsorption technic for the rapid isolation of the compounds is presented.

Method. The sample of bile is mixed with 20 volumes of absolute ethanol, and the precipitated salts and proteins are removed by filtration. The filtrate and washings are evaporated to dryness *in vacuo,* with temperature kept below 40°. The brown, gummy residue is taken up in a small volume of methanol, the solution filtered if necessary and applied to an alumina column. (The amount of alumina should be at least 60 times that of the residue.) The column is washed with methanol, and the methanol is collected in fractions. The bile pigments are completely retained on the top of the column, while bile acids, neutral steroids, and fats wash through rapidly. Occasionally the retention of bile pigments is not complete, and when this occurs, larger quantities of alumina in a wide column of high flow rate should be used. The first fraction collected often has a yellow tinge which, however, does not interfere with the subsequent steps. Each fraction is evaporated to a small volume, and excess anhydrous ether is added. The conjugated bile acids precipitate out as a white, flocculent powder, while the neutral steroids, fats, phospholipids, and unconjugated bile acids (if present) remain in solution. (Failure to employ anhydrous ether may result in a gummy, hygroscopic precipitate of bile acids.) Elution of the column with methanol is discontinued when fractions no longer yield precipitates upon the addition of ether. The conjugated bile acids are then filtered off, washed with ether, and dried. It is desirable to reprecipitate the isolated bile acids once or twice from methanol-ethyl ether mixtures as described above.

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TABLE I. Recovery of C^{14}-Labeled Contaminants in Conjugated Bile Acids Isolated from Rat Bile. 10-ml samples of rat bile were used. The column measured 12 mm × 50 mm. Total volume of methanol collected was 150 cc. This was evaporated to about 8 cc and then diluted to 60 cc with ethyl ether to precipitate bile acids.

C^{14}-labeled compound added*	No. of times reprecipitated	% C^{14} recovered	Yield of bile acids, mg
Cholesterol	2	.1	82
Tripalmitin	1	1.5	98
Octanoic acid	2	1.4	134
Glucose	2	.2	87
Succinic acid	2	2.9	81

* Less than one mg of the labeled compound was added.

In order to check the procedure, bile acids were isolated, as described above, from 10-ml samples of rat bile to which had been added trace amounts (less than 1 mg) of a number of C^{14}-labeled compounds which might be found in bile. The extent to which the added C^{14} was recovered in the isolated bile acids is shown in Table I. Since bile ordinarily contains very small amounts of these compounds, the contamination from them can be neglected for most purposes.

Ordinarily most bile acids in bile are conjugated, and since bile is slightly alkaline, these acids will be present as their sodium salts. If for some reason large amounts of free (unconjugated) bile acids are present, these will also be precipitated by ether as sodium salts. These free bile acids can, however, be eliminated by first acidifying the bile sample and then following the procedure as described. The subsequent addition of ethyl ether will then precipitate only the conjugated

TABLE II. Yields of Conjugated Bile Acids from Various Species.

Species	Yield of bile acids per 10 ml bile, mg
Rat	80–100
Rabbit	225
Chick	240
Goose	1800*
Frog	650

* Bile partially dehydrated.

bile acids leaving the free acids in solution.

We have applied this method to the bile of several species (Table II) with uniformly good results. In all cases, the isolated bile acids were chromatographed in collidine-water-NH_3(2), and compared with chromatograms of whole bile. The isolated bile acids were always chromatographically identical with the conjugated bile acids of whole bile. It should be noted, however, that since conjugated bile acids are slightly soluble in methanol-ethyl ether mixtures, this method cannot be used for the quantitative recovery of conjugated bile acids in bile.

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Observations on Effects of X-Irradiation on *Achromobacter fischerii*.* (21080)

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Bacterial luminescence offers a unique opportunity for studying the effect(s) of x-irradiation on the living cell. Due to the interaction of irradiation and cellular substance, an almost immediate visual indication of alteration in the biochemical processes of the cell is observed at proper dosage levels. The most recent review on the effect of x-irradiation on luminescent bacteria [Harvey(1)], has called attention to the need for more extensive investigation of this subject.

Lorenz and Henshaw(2) have studied the survival of *Achromobacter fischerii* after x-irradiation and Whipple(3) has reported some effects of x-rays on bacterial luminescence. Unfortunately, the experimental technics differ, and no correlative evaluation of the relative sensitivities of bacterial luminescence and survival can be made from this material. It has been the purpose of this study to investigate the effect of graded doses of x-irradiation on bacterial luminescence relative to survival of the microorganisms.

Materials and methods. The test organism used for this study was *Achromobacter fischerii*,‡ cultured in Farghaly's basal media (4) supplemented with 200 mg purified casamino acids per liter. The cells were grown at room temperature on a horizontal oscillating machine for a period of 18 hours and then harvested in the cold by centrifugation. The centrifuged cells were washed with a cold NaCl-PO_4 buffer(1), and resuspended in a suitable volume of buffer to give a cell concentration of approximately 5 million cells per milliliter. This cell concentration was used as the standard. Aliquots of the standard cell suspension were pipetted into small round glass cups and exposed to x-irradiation delivered by a 250 kv Westinghouse unit with a 15 ma output at a distance of 14 cm using 1 mm Al and 0.5 mm Cu filters. The dose rate was 545 r per minute as measured with a Victoreen r-meter The liquid depth of the exposed bacterial suspension was 5 mm. After irradiation, samples of both the test and control suspensions were taken to determine the

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