ISOLATION OF CHOLESTEROL SULFATE FROM HUMAN BLOOD AND GALLSTONES

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Cholesterol sulfate has been isolated from bovine adrenals (Drayer, et al., 1964) but the occurrence of this compound in human tissues has not yet been established. For this reason, blood, as well as gallstones, from humans was analyzed for the presence of this conjugate.

Citrated human blood, which had been stored for more than three weeks, was centrifuged to remove the red cells. To the supernatant, which measured one liter and which, accounting for the citrate solution, is estimated to correspond to about 0.7 l. of plasma, was added 12.2 μg of cholesterol-7α-^{3}H sulfate (401,000 cpm) as tracer and enough concentrated NH_4OH to bring the pH to 11. Three volumes of tetrahydrofuran were added to precipitate the proteins which were removed by filtration through a bed of Celite. The organic solvent was removed from the filtrate by evaporation at 45° in a rotary still. The remaining aqueous phase, after neutralization with hydrochloric acid, was extracted twice with 2.5 volumes of chloroform. The residue, left after removal of the chloroform, was dissolved in 100 ml of a mixture of benzene and chloroform (1:1) and the solution poured onto a column (diam. 5.7 cm)

of 250g of Al_2O_3 (Woelm, neutral, activity III). The column was developed with a further portion of 1.5 l. of the benzene-chloroform (1:1) mixture and then with 1.5 l. of methanol. The radioactive material was eluted with 1.9 l. of a mixture of chloroform-methanol-2 M pyridinium chloride solution-pyridine (3:5:1:0.03). The eluate was concentrated to one tenth its original volume and diluted with an equal volume of water. Extraction twice with two volumes of chloroform removed the pyridinium salts of the steroidal sulfates which, after evaporation of the solvent, were purified by partition chromatography on Celite using, consecutively, the systems: (a) (190g Celite) n-heptane-1-butanol-methanol-0.6 M pyridinium chloride-pyridine (4:1:2:2:0.1), and (b) (30g Celite) n-heptane-1-butanol-methanol-1 M NH_4OH (4:0.7:1.5:2). The bulk of radioactivity was eluted from these columns in the 3rd and 6-7th holdback volumes, respectively. The radioactive product obtained from the second chromatogram was leached twice with 1 ml portions of ether to remove traces of oily material. The remaining amorphous white solid contained 72,600 cpm and weighed 0.5 mg. Its infrared spectrum in KBr was found to be identical with that of synthetic ammonium cholesterol sulfate (Fig. 1). A crystalline product was obtained by allowing a methanol solution of the amorphous residue to evaporate spontaneously. It melted at 197-201°; the melting point of an authentic sample is 197-200°. The isolated ammonium salt was solvolyzed using the previously described technique (Drayer, et al., 1964) and the radioactive product purified by chromatography on alumina. The compound, eluted with benzene, was identified as cholesterol by its infrared spectrum.

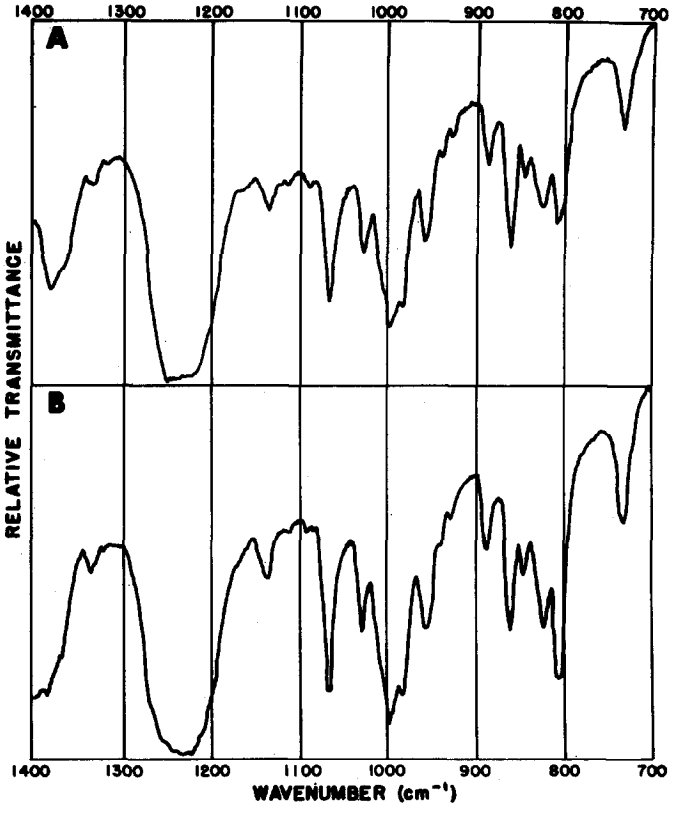

Figure 1. Infrared spectra of ammonium cholesterol sulfate in KBr.
A. Isolated sample B. Synthetic sample

A repeat experiment was performed with 3 l. of supernatant fluid obtained by centrifugation of citrated blood. A trace sample of 4.9 μg of ammonium cholesterol-7-^{3}H sulfate (161,000 cpm) was added. The final radioactive product obtained after extraction and purification procedures similar to those described above contained 62,800 cpm, weighted 2.4 mg and exhibited an infrared spectrum identical with that of synthetic ammonium cholesterol sulfate. Radioactive cholesterol, obtained by solvolysis and chromatography on alumina, was crystallized from a mixture of methanol and acetone. It melted at 143-146° and possessed an infrared spectrum characteristic of authentic cholesterol. As estimated by the

methylene blue reaction (Crepy and Rulleau-Meslin, 1960) and corrected for losses incurred in the method by the amount of radioactivity recovered, human plasma contains cholesterol sulfate in amounts in the order of 0.3 mg per 100 ml.

Gallstones of the cholesterol and mixed type, total weight 130g, were ground in a mortar and extracted 5 times with 0.5 l. of hot absolute ethanol. To the pooled ethanol extract was added 17.9 μg of ammonium cholesterol-7α-^{3}H sulfate (590,000 cpm) as a tracer. The extract was then cooled and filtered to remove precipitated material (mainly cholesterol). When the volume of the filtrate was reduced to 0.9 l., a second crop precipitated and this, too, was removed by filtration. The filtrate was poured on a 200g Al_2O_3 column (5.7 cm diameter), which was further developed with 0.5 l. of ethanol. The radioactive material was eluted with 1.2 l. of a mixture of chloroform-methanol-2 M pyridinium sulfate (3:5:1). After evaporation of the organic solvents, the sulfates left in the aqueous residue were extracted into chloroform. The material left after evaporation of the chloroform was purified by partition chromatography on Celite, using the three systems described in our previous publication (Drayer, et al., 1964). The radioactive material obtained from the third chromatogram was leached twice with 1 ml portions of ether. The infrared spectrum of the remaining white amorphous solid, containing 322,000 cpm and weighing 0.3 mg, was identical in every respect with that of synthetic sodium cholesterol sulfate.* Subsequent solvolysis of the sulfate

* The Celite used as support for the last chromatogram was from the same batch previously shown (Drayer, et al., 1964) to convert pyridinium cholesterol sulfate to its sodium salt.

and purification of the product on alumina yielded cholesterol, identified by its infrared spectrum.

That cholesterol sulfate may serve as a precursor of sulfated products secreted by the adrenals has already been demonstrated (Roberts, et al., 1964). Its isolation from extracts of bovine adrenals adds support to the idea that steroidal sulfates are involved in the biosynthesis of some adrenal steroids. The presence of cholesterol sulfate in human plasma and gallstones now makes it necessary to consider the possibility that this conjugate (and possibly other related sulfates) plays an important role in other metabolic processes.

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