Organochlorine Pesticides in Gallstones

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A method is described for the determination of organochlorine pesticide residues in human gallstones. The difficulty caused by the presence of large amounts of cholesterol in the stones is overcome by an acetylation step in the “clean-up” procedure, and the method finishes with identification and determination of the individual pesticides by gas-liquid chromatography.

Results are given for 28 samples submitted by 2 hospitals, showing small amounts of dieldrin, p,p'-dichlorodiphenylmethane (pp'-DDE), and benzene hexachloride (BHC) isomers to be commonly present in small amounts in gallstones. Some explanation for the presence of these residues in the stones is given.

A case of suspected poisoning by these compounds showed an unusually large amount of dieldrin in the patient’s stones.

According to Harding-Rains (1), about 10% of all gallstones are composed wholly of bile pigments and are dark lustrous green or black. The commonest form of stone, however, is the “mixed stone,” which varies considerably in shape, size, and color, and consists of radiating crystals of cholesterol with concentric layers of amorphous bile pigments. Pure cholesterol stones are rare and are generally very pale in color; other types of stone are known but are rarer still.

The determination of organochlorine pesticide residues in bile pigment stones was found to present no difficulties when established methods were used. The stones were ground with anhydrous sodium sulfate and extracted with a 1:1 acetone-hexane mixture. The concentrated extracts were then subjected to the “clean-up” method of de Faubert Maunder et al. (2), which includes a dimethylformamide-hexane partition followed by passage through a prepared alumina column. The final solutions were then examined by gas chromatography using silicone and Apiezon columns with electron-capture detectors (3).

The cholesterol present in the other types of stones was found to

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interfere with the method described above, being precipitated as a white mass on concentrating the initial acetone-hexane extracts and subsequently causing intractable emulsions at the partition stages of the clean-up procedure. To overcome this interference, the acetone-hexane extract was acetylated. The temperature during acetylation was kept below 70°, since above this temperature, p,p'-dichlorodiphenyltrichloroethane (pp'-DDT) was rapidly dehydrochlorinated to p,p'-dichlorodiphenyldichloroethane (pp'-DDE) which commonly occurs as a metabolite of DDT in human fat and also in gallstones. Conversion to pp'-DDE at temperatures below 70° appeared to be negligible. The cholesteryl acetate formed caused no interference when the whole of the reaction mixture was subjected to an acetonitrile-hexane clean-up process and was left behind in an aqueous phase. The solution resulting from this clean-up was then passed through a prepared alumina column and injected on to the gas-chromatograph columns in the normal way.

Method

Remove any adhering fluid from the stone (or stones) with a cleaning tissue and weigh. Grind the stones and take not more than 5 gm. for analysis. Further grind the weighed quantity with anhydrous sodium sulfate to give a friable mixture, and extract successively with 200 ml. of a 1:1 mixture of acetone and hexane. Filter the extracts through anhydrous sodium sulfate into a Kuderna-Danish evaporator and reduce the volume almost to dryness on a steam bath to remove all the acetone.

At this point, cholesterol, if present, can be seen as an obvious white mass in the flask. If cholesterol is absent, the dimethylformamide-hexane partition and alumina-column clean-up method referred to above can be used. However, if cholesterol is present, the following procedure is used.

To the concentrated hexane extract add 5 ml. acetic anhydride and 5 ml. pyridine and heat on a water bath at 60–70° for 30 min. Higher temperatures must be avoided. Cool and pour the mixture into a separating funnel containing 200 ml. of water, washing in with 150 ml. of a 1:1 mixture of diethyl ether and hexane. Shake and reject the aqueous layer. Dry the ether-hexane layer by passing it through anhydrous sodium sulfate into a Kuderna-Danish evaporator and then reduce it to a small volume. Take an aliquot of the final volume equivalent to not more than 1 gm. of cholesterol for clean-up. Dilute to 25 ml. with hexane in a 100-ml. separating funnel and extract with 50 ml. and 30 ml. of acetonitrile, running the acetonitrile extracts into a 500-ml. sepa-
rating funnel containing 400 ml. of 2\% (w/v) sodium sulfate solution. Shake and run the aqueous layer into a 1000-ml. separating funnel leaving the hexane layer in the 500-ml. funnel. Extract this hexane layer twice with 20-ml. portions of acetonitrile, adding the extracts to the aqueous layer in the 1000-ml. separating funnel. Extract the total aqueous volume with 150 ml. of a 1:1 mixture of diethyl ether and hexane, and pass the ether-hexane layer through an anhydrous sodium sulfate column into a Kuderna-Danish evaporator. Evaporate nearly to dryness on a water bath at 40° while passing a slow current of dry air. Wash the residue with hexane onto a column of 10 gm. of partially deactivated alumina (5\% water) covered by about 5 gm. of anhydrous sodium sulfate, elute with 100 ml. hexane, and concentrate the eluate to not less than 2 ml. in a Kuderna-Danish evaporator. This solution is then ready for injection on the silicone and Apiezon gas-chromatograph columns.

A blank experiment should be run at the same time.

By this method, recoveries of added organochlorine pesticides at the 0.1- to 1-ppm level were 90–100\% for $\alpha$, $\beta$, and $\gamma$-benzene hexachloride (BHC), dieldrin, endrin, pp'-DDT and pp'-DDE.

**Results and Discussion**

Two hospitals submitted a total of 28 samples of recently removed gallstones, of which 6 were of the bile pigment type and the rest mixed cholesterol stones. Their weights varied between 1 and 14 gm.

Residues of 2 pesticides were detected in most of the stones. These were dieldrin and pp'-DDE, the levels usually being in the ratio of 1:3. Dieldrin levels ranged from 0 to 0.1 ppm, with a mean level of 0.02 ppm, and pp'-DDE levels ranged from 0 to 0.15 ppm, with a mean level of 0.06 ppm. In about half the samples, the $\alpha$ and $\beta$ isomers of BHC were also detected in amounts up to 0.05 ppm. Other organochlorine pesticide residues reported as commonly occurring in human fat in the United Kingdom were heptachlor-epoxide, $\gamma$-BHC, pp'-TDE and pp'-DDT ($\delta$), but none of these was detected in any of the stones.

The accretion of small amounts of these organochlorine pesticide residues in gallstones should not be surprising. It is known that the bile salts maintain cholesterol in solution, and Moss and Hathaway ($\delta$) have demonstrated the ability of sodium tauroglycholate to combine strongly with telodrin, an organochlorine pesticide of the chlorinated norbornene type similar to dieldrin. It would follow that in the course of stone formation, these pesticides would be occluded with the cholesterol.
It has been suggested (1) that an average-sized stone of about 5 mm. diameter would take some 8-12 weeks to grow. Heavy industrial exposure to organochlorine pesticides has been shown to result in relatively high levels of these pesticides in the blood for periods of weeks after exposure has ceased (6, 7). If this period were to be coincident with stone formation, then it is possible that the stone would contain unusually high concentrations of pesticides. This, in fact, appeared to be so in the case of a young woman suspected of suffering from the effects of these compounds, who handled dressed seed in the course of her employment. Two stones totaling 0.2 gm. in weight, removed from this patient and examined immediately prior to this investigation, were found to contain 0.45 ppm of dieldrin.

References