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Coprostanol in Severn Estuary Sediments

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The detection of sewage pollution in the environment is of considerable importance for health, aesthetic and ecological reasons. Sewage may contain various pathogenic organisms representative of such conditions as polio, typhoid, dysentery, tetanus and tuberculosis. Although the contamination of potable water supplies with dangerous micro-organisms is an obvious hazard, bathing in polluted waters presents an appreciable, if lesser, risk.

Pathogenic bacteria and viruses usually occur in small numbers in sewage and are extremely difficult to isolate and enumerate (AGG et al. 1978). Thus detection of faecal pollution is generally achieved by the use of an indicator substance, either microbiological or chemical, which is present in sewage in larger amounts. These indicators are not necessarily harmful substances in themselves. Among chemical indicators that have been proposed are the determination of phosphate (MURPHY and RILEY 1962), of ammoniacal nitrogen (DUEDALL et al. 1975), and more recently of siloxanes (PELLENBARG 1979). However, most chemical methods have been shown to be unsatisfactory as they are either non-specific or insufficiently sensitive. Non-chemical indicators such as the concentration of tomato seeds (SHELTON 1971) have also been studied as a possible method for the detection of faecal pollution. However, the method of choice has, in general, been microbiological, involving the enumeration of faecal coliforms in samples (ANON 1969). Whilst this method is simple and sensitive it has been shown that coliform counts can be highly variable. Even at a single sampling point they may range over an order of magnitude in the course of a few minutes (AGG et al. 1978). There are also considerable problems in establishing firm associations between faecal coliforms and pathogenic bacteria. For instance, coliforms may be destroyed under conditions such as variation of temperature, sunlight or salinity in which pathogenic bacteria survive. There is thus considerable scope for the development of a satisfactory chemical indicator of faecal pollution to be used in conjunction with, or alternative to, the present microbiological methods.

Recently coprostanol has been proposed as such a chemical indicator (MURTAUGH and BUNCH 1967) and has been used to detect pollution in the New York Bight (HATCHER et al. 1977), the Clyde Estuary (Scotland) (GOODFELLOW et al. 1977) and the Ariake Sea

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(Japan) (KANAZAWA and TESHIMA 1978).

Coprostanol, formed by the microbiological reduction of cholesterol in the large intestine, is present in the faeces of man and higher animals.

In this paper some results for the coprostanol content of sediments taken from the Severn Estuary are given. The analytical method used requires a simple solvent extraction of the sample, a clean-up via preparative thin layer chromatography and determination after derivatization by capillary gas chromatography.

MATERIALS AND METHODS

Surface sediment samples were collected in clean glass vessels. Samples not immediately processed were stored at -20 ^{o}C. Samples (≈ 50 g) were freeze-dried, ground in a hammer mill to a homogeneous powder and extracted by the Soxhlet method for 24 h using 2:1 (v/v) chloroform/methanol. The crude extracts were purified by preparative TLC (self-prepared plates, silica gel G stationary phase, 0.4mm thickness, activated 1 h/110^{o} prior to use) using chloroform as the mobile phase. Standard sterols were spotted alongside the sample band and visualisation was via Rhodamine 6G. The required region was scraped off the plate, extracted by sonication (2 x 10 mL ethylacetate) and 5α-cholestane added as internal standard. The combined extracts were concentrated and derivatized to give the TMS ethers, (5:1 BSA/TMCS, v/v, 3 h at room temperature). The resultant mixture could be injected into the gas chromatograph without interference to the subsequent determination.

A Carlo Erba Fractovap 2150 Series capillary gas chromatograph fitted with a Grob type split/splitless injection system was used. The column was 20m x 0.3mm i.d. and coated with SE-30 (Ultraphase). Operating parameters were as follows:- injection port 300^{o}, temperature programme 100^{o} to 250^{o} at 6^{o} min^{-1}, carrier gas H_2 (2 mL min^{-1}), injection volume 1 μL (splitless). Detection was by FID. Quantitation was by peak height comparison with the internal standard. Identification of peaks was achieved using a Finnegan 4000 GC-MS instrument coupled to a data system. Secondary confirmation was by co-injection

RESULTS AND DISCUSSION

A number of different techniques were investigated for the determination of sterols in standard samples. Separation of faecal sterols on packed GC columns with detection by FID has been reported (MURTAUGH and BUNCH 1967, HATCHER et al 1977). Sterols have been separated both as the free compounds and as their TMS derivatives. In this study it was found that non-polar (e.g. SE-30 or OV-1) or slightly polar (e.g. SE-52) capillary columns yield the best chromatographic results. Chromatography of the TMS ether derivatives of the sterols rather than the free compounds resulted in improved peak shape, more reliable quantitation and

better sensitivity, i.e. a lower detection limit. Under the conditions used 1 ng of coprostanol may be detected with a signal to noise ratio better than 30 to 1 suggesting a detection limit of about 100 pg. Free sterols may become partially lost on chromatographing either by adsorption on the column or by poor vapourisation efficiency upon injection. The possible use of heptafluorobutyrate derivatives with electron capture detection was investigated. Although absolute detection limits were reduced by a factor of 10 difficulties were encountered in the analysis of real samples (other than standards). Interference due to the response of the ECD to other components of the sample was observed.

The use of capillary columns rather than packed columns allows discrimination between similar compounds. Whereas analysis using packed columns provides a 'total' coprostanol value the high resolution capability of capillary columns allows the discrete quantitation of both coprostanol and epi-coprostanol. The relationship between the levels of coprostanol and epi-coprostanol is complex but appears to be related to the method of sewage treatment prior to discharge into the environment (COOKE et al).

Recovery from sediments spiked with coprostanol averaged 88 %. The reproducibility of the method, determined by 10 replicate analyses of the same sewage sludge sample, was excellent. The relative standard deviation (coefficient of variation x 100) was only 4.3 %.

The extraction and clean-up procedure was designed to isolate free sterols though it was thought likely that some compounds would be present as esters. Sterol esters present would normally be 'lost' at the preparative TLC stage due to their different R_f values. Hence some extracts were saponified (reflux 5 h, 10 % KOH in methanol; w/v). Samples were then purified by TLC. It was found that for all the samples studied the extra coprostanol recovered because of the saponification step amounted to less than 15 % of the total. Due to the length of time for this saponification step it was omitted for the routine analysis of samples.

The locations of the sampling sites are detailed in Figure 1 and the values of coprostanol determined are given in Table 1 (Swansea Bay) and Table 2 (Severn Estuary). Although geographically close together the Swansea Bay sites are important because they represent popular sites for sea-bathing. Site 10 (Watchet) may be similarly classified. A typical capillary chromatogram is shown in Figure 2. The identity of the coprostanol peak in the sediment samples was confirmed by GC-MS. The identity of some other eluates which were carried through the extraction procedure was also established. Both cholesterol and ethyl coprostanol were observed. These two compounds occur in sewage (GOODFELLOW et al. 1977) although cholesterol is non-specific to sewage. Ethyl coprostanol occurs in sewage at lower levels than coprostanol

Figure 1. Location of Sampling Sites.

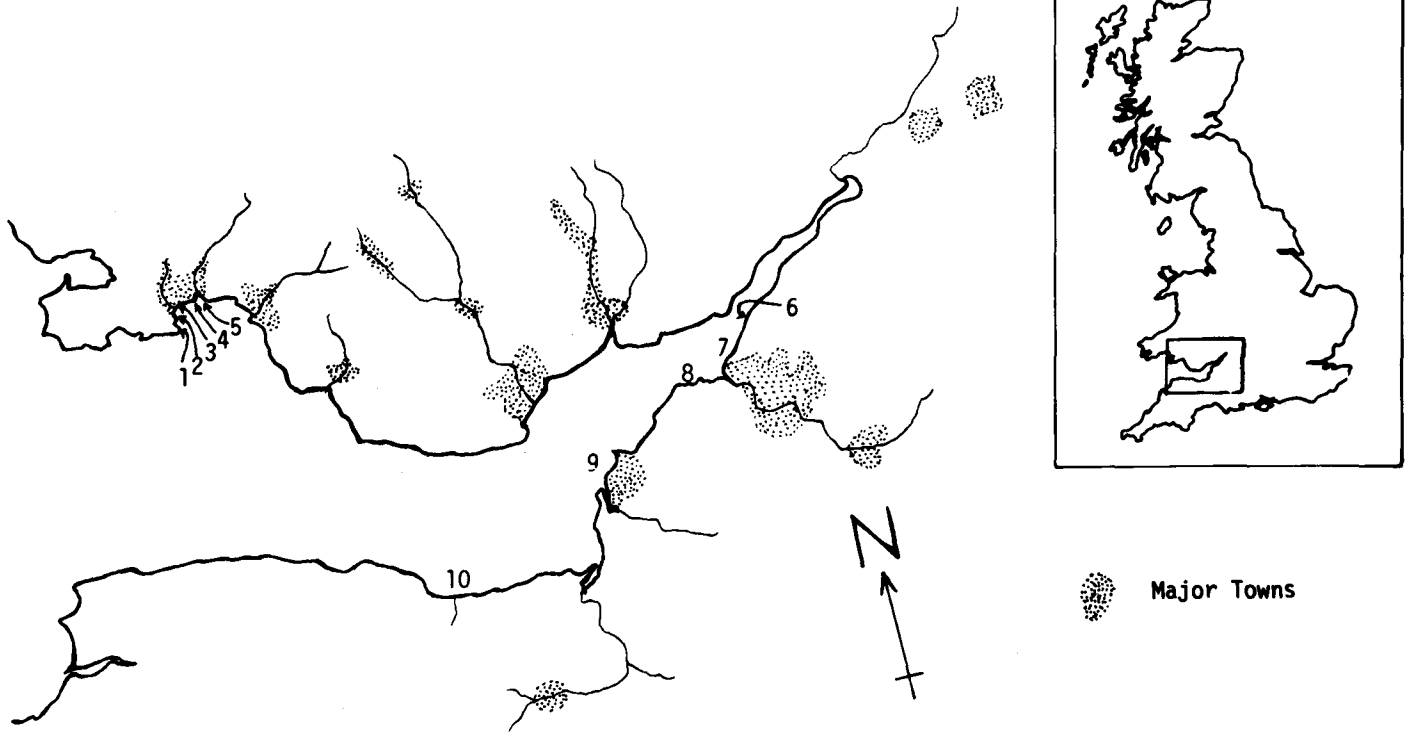
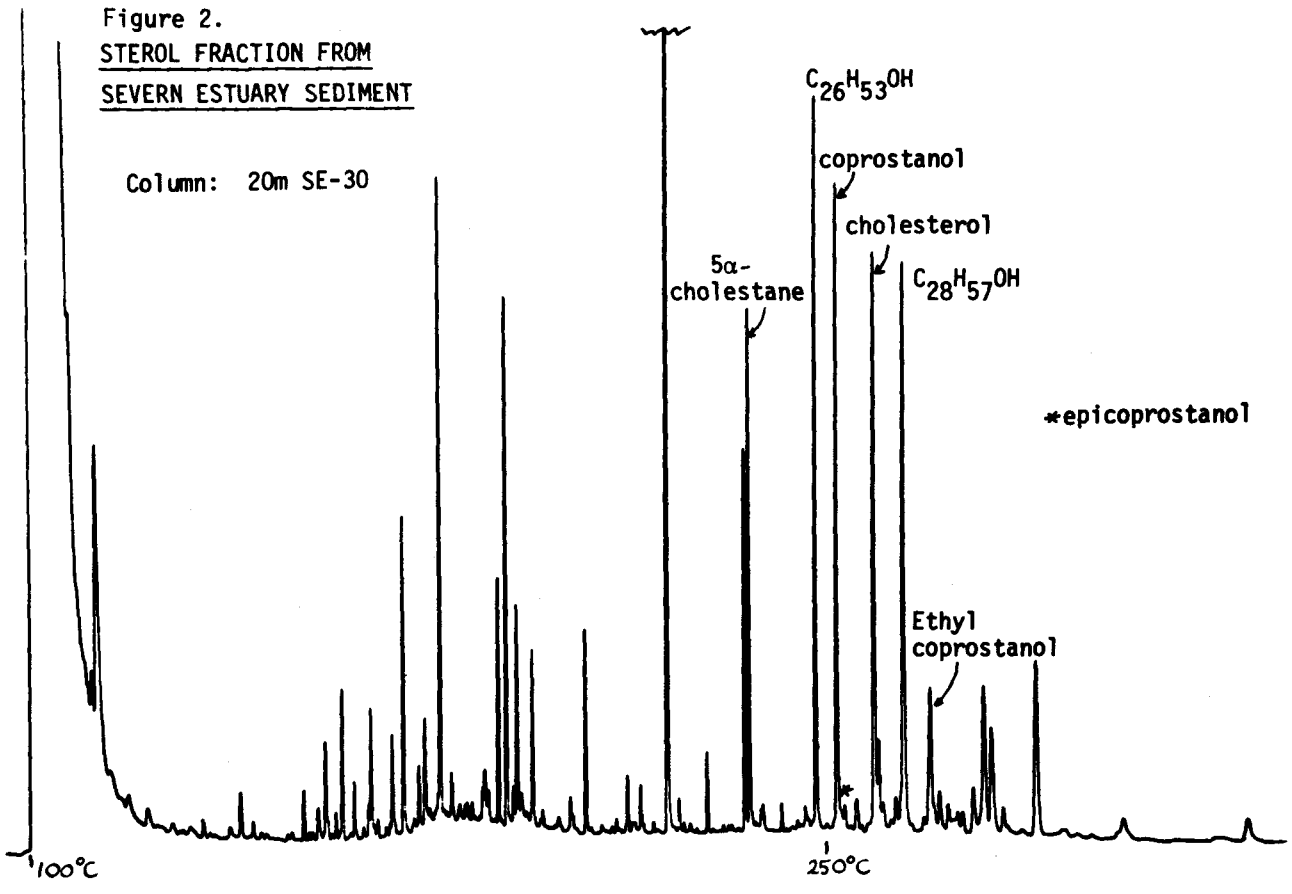


Figure 2.
STEROL FRACTION FROM
SEVERN ESTUARY SEDIMENT



itself and thus is not as suitable as an indicator of faecal pollution. Also shown in the chromatograms are a series of aliphatic alcohols with even numbers of carbon atoms although their exact structures are not deducible from their mass spectra alone.

TABLE 1.

Levels of coprostanol and cholesterol found at various sites in the Swansea Bay area (ppm dry weight).

Site No.	Location	Coprostanol	Cholesterol
1	Oystermouth	2.1	2.9
2	Mumbles Head	1.6	2.1
3	West Cross	1.8	1.0
4	Swansea University	5.2	2.3
5	Brynmill	2.8	3.9

TABLE 2.

Levels of coprostanol and cholesterol found at various sites in the Severn Estuary (ppm dry weight).

Site No.	Location	Coprostanol	Cholesterol
6	Aust	0.9	3.1
7	Severn Beach	1.2	1.8
8	Portishead	3.1	2.7
9	Weston-super-Mare	1.2	2.5
10	Watchet	1.0	2.5

Sediment samples were used for all sites. Some water samples were also analysed but coprostanol levels were found to be very low. Typical values for water were 0.1 to 0.3 ppb. Undoubtedly this reflects the low solubility of coprostanol. Considerably enhanced values may be found if the sample is not filtered prior to analysis. It would seem that the bulk of coprostanol present in 'water' samples is associated with suspended micro-particulate matter. The results obtained for both the Swansea Bay area and the Severn Estuary itself are broadly comparable with those observed for the New York Bight (HATCHER et al. 1977), the Clyde Estuary (GOODFELLOW et al. 1977) and the Ariake Sea (KANAZAWA and TESHIMA 1978). The values for the Swansea Bay area are in general, higher than those for the Estuary itself. Presumably this reflects the longer flushing time of what is essentially a large backwater area compared with the main channel. Although input to

the Bay area is small in relation to that of the main Estuary accumulation occurs because of slow dilution. Incidentally coliform counts for these two areas have been reported, by other workers. The results obtained demonstrate the variation which may be obtained by this indicator method. For the comparatively small Swansea Bay area coliform counts of 600-2500 per 100 mL of sea water were recorded (The Welsh Office 1974). For the larger Severn Estuary region a slightly larger spread of results was found (400-3500 per 100 mL: WARE and ANSON 1978).

The fate of coprostanol in the environment is unknown. However, it seems likely that polyhydroxylation and degradation of the sterol structures both eventually occur. At the present time we have been unable to identify any compounds which may be unambiguously designated as metabolites of coprostanol.

CONCLUSIONS

Coprostanol may be conveniently and reliably determined in environmental samples at the part per billion level by capillary gas chromatography. Typical levels for coprostanol in environmental samples were 0.1 - 0.3 ppb for water samples but 1 - 5 ppm for sediments. For comparative purposes typical values for raw sewage were 2,000 - 5,000 ppm. Sludge which had been 'weathered' for several years contained considerably lower levels (150 - 350 ppm). It is uncertain however what factors contribute to the life time of coprostanol in the environment. It has been reported (TESHIMA and KANAZAWA 1978b) that about 40 % of radiolabelled cholesterol in sediment incubation experiments is degraded to CO_2 over a 30 day period. However, the behaviour of cholesterol in the environment may differ from that of coprostanol. On the other hand, a recent study (LIN et al. 1978) of the faecal steroids from 2,000 year old samples showed values of the same order as in modern man. The lack of degradation has been attributed to the very dry conditions under which the samples were preserved. Obviously the fate of coprostanol in the environment depends very much on the particular conditions to which the sample is subjected.

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