Notes

Sebum: A common contaminant of samples for gas–liquid chromatography*

The greatly increased quantitative sensitivity made possible by gas–liquid chromatography (GLC) has presented a vexing problem in that small amounts of contaminating material previously of little consequence have now become a major limiting factor in the measurement of submicrogram quantities of steroids in biological fluids.

In the course of using GLC for the measurement of plasma progesterone it became apparent that there was a significant amount of non-steroid material in the final extract. Further attempts at purification not only failed to remove this contamination but in some instances actually added further material.

Study of the peaks revealed what appeared to be a regularly recurring pattern and a similar pattern was found to occur in material from different sources. In attempts to localise the source of this contamination, it was found not only in the final purification step, but at other stages in the process. It was also present in all the organic solvents in use. Finally it was shown that the identical pattern was produced by sebum from the fingers. A GLC analysis of the composition of sebum has been reported by BOUGHTON AND WHEATLEY[1].

Fig. 1B illustrates the pattern from 4 ml acetone taken from a contaminated bottle; and above this, Fig. 1A the chromatogram of sebum after handling the dry applicator device[2]. Note the difference in attenuation of the two chromatograms (sebum $\times$ 5, acetone $\times$ 1), and also the exact coincidence of the various peaks present in both.

It was found that sebum from one thumb print could significantly contaminate 500 ml organic solvent. Further it was demonstrated that a fresh bottle of ether in constant use, but handled with the utmost care, showed progressive increase in contamination of its contents.

The implication of these findings was that even with extremely careful use, no organic solvent could be kept free of contamination unless reagent bottles and other apparatus were manipulated with gloved hands. It also became clear that cross contamination even with the use of gloves would be certain to occur in time. The ultimate solution would therefore be to remove traces of sebum from all surfaces in the laboratory and henceforth to allow nothing to come in contact with human skin. These measures were considered to be so impractical that an alternative had to be found.

Resolution of this problem became possible when it was found that retention time of progesterone could be altered in relation to the peaks obtained from the contaminating material. This could be successfully achieved by alteration of column temperature. Fig. 2 illustrates how, with changes in column temperature, it was possible to arrange the retention time of progesterone to coincide with an area in the

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chromatogram which was free of other peaks. It is thus apparent from Fig. 2 that the ideal position for the progesterone peak would be between the stippled peak and the black peak at a column temperature a few degrees below 210°, or at 235° which would place progesterone between the unmarked peak and the broad hatched peak. Calculation of log retention time relative to cholesterol, and plotting of these figures against the reciprocal of absolute temperature shows the slope of the progesterone curve to be opposite in direction to the slope of the other individual peaks (Fig. 3).

An explanation for the behaviour of progesterone in relation to the contaminating material is offered in the generalisation made by LITTLEWOOD[3].

"It is possible for two solutes to have roughly similar retention volumes but different heats of solution, particularly when one solute is of a different chemical type to the other and has a different activity coefficient."

On this basis, it may be valid to predict that the peaks from the contaminating material are not steroidal in nature.

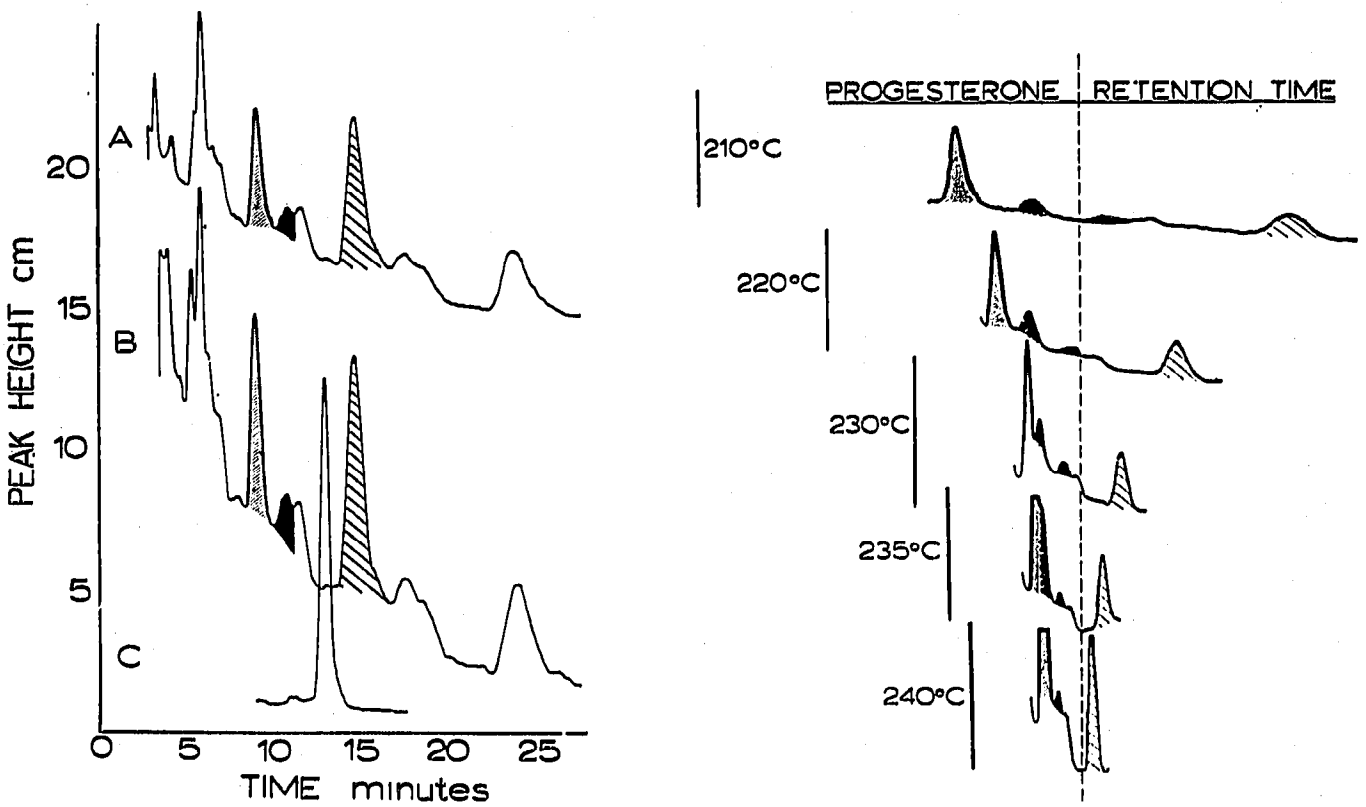

Fig. 1. A shows the peaks obtained when sebum from a finger print is chromatographed. Attenuation × 5 (recorder 1 mV). The four relevant peaks are the close hatched, blackened, unmarked and broad hatched in that order. B shows the chromatogram obtained from a sebum contaminated bottle of acetone. Attenuation in this case × 1. C indicates the height of the peak obtained with 0.1 μg progesterone under the same conditions. Attenuation again × 1. Perkin Elmer Model 801 Gas Chromatograph. 3% XE60. Gas Chrom P 80/100 mesh. Column temp.: 230°. Injector temp.: 280°. Helium 50 p.s.i. Hydrogen flame detector.

Fig. 2. The retention time of progesterone (vertical interrupted line) varies in relation to the peaks obtained from sebum and depends on the column temperature. The carrier gas flow is kept constant. Note that the stippled peak seen in temperatures 210° to 235° is not resolved from the close hatched peak at 240°. Except for variations in column temperature, chromatographic conditions are as for Fig. 1.

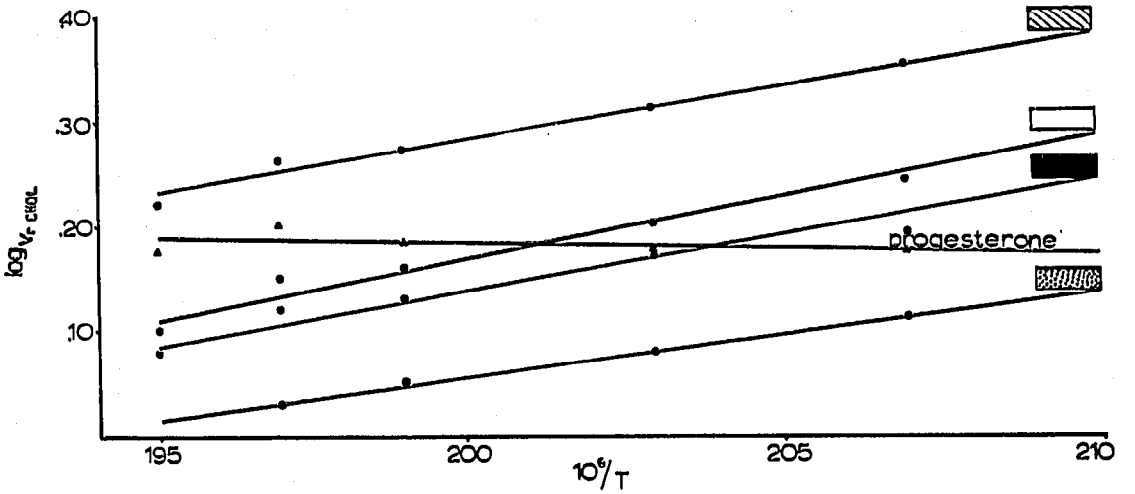

Fig. 3. The logarithm of the retention volume for each peak relative to cholesterol plotted against inverse of the temperature shows that the progesterone line crosses the lines of the blackened peak and the unmarked peak (see Fig. 2). It is possible from a graph of this type to predict the ideal column temperature in order to avoid superimposition of progesterone with contaminating material.

With decreased column efficiency as a result of constant use, the relative retention time of progesterone will gradually change and it is necessary to check the position of the progesterone peak at regular intervals.

A water blank should always be submitted to the same extraction procedures as the biological fluid. This would allow for a blank correction should superimposition of peaks be unavoidable.

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