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The detection of nitrogenous compounds using a thermionic detector

The excellence of flame ionisation as a general means of detecting organic compounds may be self-defeating when gas chromatography (GC) is applied to extracts of biological material such as urine, for the number of compounds present may be so high that only in rare cases may the homogeneity of peaks be assumed. Hence, the use of more specific means of detection may be increasingly necessitated. We were especially interested in the alkali-flame detector for the detection of nitrogenous substances, but it became obvious to us from the literature (*e.g.* ref. 1) that the use of any given model is a highly empirical art, optimum conditions with respect to variables such as gas flow and the nature of the alkali salt being critically dependent upon design. In addition, we were doubtful as to the effect on performance of deposits of silica produced through the use of silicone liquid phases and, more particularly, of trimethylsilyl derivatives commonly used in GC.

With these problems in mind, we have investigated the behaviour of the Pye thermionic detector (T.D.), a modified flame ionisation detector (F.I.D.) of simple and robust construction. Essentially, it consists of a long metallic probe mounted centrally within a perforated metal cylinder. Into the open end of this cylinder is pushed the salt-tip, a short metal sleeve containing a ring of compressed caesium bromide. This assembly is screwed vertically into the detector body so that the flame impinges upon the salt. If desired, the end of the probe may be moved further from the flame by the insertion of suitable washers, the salt-tip then being withdrawn correspondingly from the cylinder in order to maintain the position of the salt ring with respect to the flame. The detector is designed primarily for the detection of phosphorus; no information regarding its behaviour in the presence of silicon compounds was available to us, but the manufacturer's literature indicated some response to diphenylamine.

Our experiments were carried out with a Pye Series 104 chromatograph, Model 124, in which the column effluent is split equally between a T.D. and an F.I.D., thus permitting a direct comparison between the sensitivities of the two types of detector towards any given compound. Diphenylamine or nitrobenzene was used as a standard nitrogenous compound, depending on the chromatographic conditions. Preliminary experiments were carried out using conditions recommended by the manufacturers as appropriate to phosphorus detection, featuring their caesium bromide tips and high rates of air flow. We were able to obtain T.D. responses to diphenylamine up to twice those of F.I.D. and also to obtain chromatograms from various silylated urine extracts, but results were very erratic, probably due partly to the deposition of silica and partly to a highly critical dependence on the hydrogen flow rate.

Attempts to improve the efficiency of the detector stemmed from the discovery that we could ourselves readily manufacture tips from a variety of salts. Old sleeves were stripped of protruding insulators (these, though desirable, are not essential) and washed free from caesium bromide residues. A simple die was constructed, consisting essentially of a steel block drilled with a hole to contain a sleeve partly filled with a salt and a solid plunger to fit inside the sleeve. Application of pressure to the plunger by means of a hand press produced hard, compact discs from all the salts tried, pro-

vided that they were sufficiently finely powdered initially. Such discs were easily converted into rings by drilling holes of 4 mm diameter. Caesium bromide tips prepared in this way and immediately heated in the flame proved superior to those supplied by the manufacturers. The latter, despite desiccant precautions, were often stained brown, presumably as the result of rusting in the sleeves.

The response of the detector towards nitrogenous compounds was investigated using tips prepared from a selection of the sulphates, carbonates or halides of the alkali metals. With some tips, technical limitations to the rates of gas flow may well have precluded the attainment of conditions approaching those required for optimal performances. Nevertheless, we were able to discern three factors which seemed generally to favour high detector response. Firstly, chromatography was best performed using high rates of carrier gas flow, this presumably being an important factor influencing the temperature of the flame. Secondly, the air flow rate to the flame needed to be the minimum practicable: values below about 85 ml/min produced flames having a tendency to be extinguished by the sudden rush of solvent following injections. Finally, the hydrogen flow rate needed to be as great as possible. However, increasing sensitivity with increasing hydrogen flow rate was limited in value by simultaneously increasing noise level and, ultimately, by unstable base lines. In addition, we found it advantageous to move the collector probe further from the flame. This procedure often appeared to effect some real improvement in the ratio of response to noise level, but the major advantage lay in the increased distance between the tip of the probe and the salt ring, resulting in a greatly decreased likelihood of short-circuiting occurring due to the formation of a bridge between these two of either silica or of "whiskers" derived from the salt.

Best results were obtained with rubidium and caesium salts, the most satisfactory being caesium bromide tips of our own manufacture. For all experiments carried out so far with these tips, the following standard conditions were found to be satisfactory. T.D.: air flow rate, 85 ml/min; hydrogen flow rate, *ca.* 30 ml/min. F.I.D.: air flow rate, 500 ml/min; hydrogen flow rate, 50 ml/min. Column: carrier gas (argon) flow rate, 100 ml/min. The collector probe was moved an additional 2.5 mm from the flame. The flames were lit afresh each day since the detectors quickly become stable and the hydrogen flow to the T.D. was adjusted until the response to injected diphenylamine or nitrobenzene was 8–12 times that of the F.I.D., the former detector then being employed with a higher attenuation factor when recorded peak heights of the same order were required. With a typical tip, the responses of the T.D. towards dodecane were 1.3, 2.5 and 7% of F.I.D. values under conditions where the responses towards nitrobenzene were 5, 10 and 20 times as great, respectively.

We have obtained satisfactory results using a variety of extracts of urine in which compounds were converted into trimethylsilyl derivatives in the presence of a large excess of reagent, usually hexamethyldisilazane. The potential of the T.D. in producing simpler chromatograms than the F.I.D. is illustrated in Figs. 1–3. Many nitrogenous compounds are naturally present in urine. For instance, ether extracts of acidified urine contain urea, indoles and amides such as hippuric acid. The high excretion of 5-hydroxyindoleacetic acid, characteristic of argentaffinoma, is illustrated in Fig. 1, the T.D. response providing confirmatory evidence concerning the identity of the appropriate peak. It is of interest that ether extracts of some urines, particularly pathological ones such as the above, show occasional peaks in which the T.D.

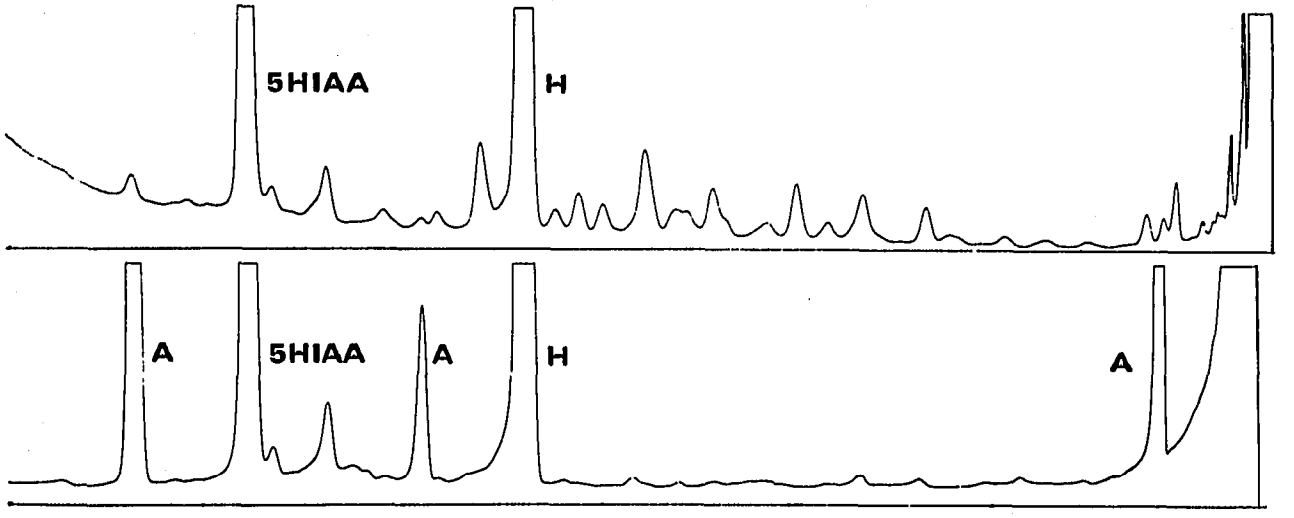

Fig. 1. Chromatograms of a silylated ether extract[2] of urine from a subject with argentaffinoma, simultaneously recorded using an F.I.D. (above) and a T.D. (below). Conditions: temperature programming from 100° at 2°/min on a 1.5 m × 4 mm column of OV-17 (10%). Identified peaks: 5HIAA, 5-hydroxyindoleacetic acid; H, hippuric acid. Note the extreme sensitivity of the T.D. to the compounds marked A and the absence of the rising base line due to column bleed recorded by the F.I.D.

response is very much greater than that on the F.I.D. It is entirely possible that some of these represent phosphorus-containing compounds, though the earliest peak to emerge (Fig. 1) does so before that of trimethyl phosphate and can scarcely be so explained. One field in which the T.D. should be particularly useful is that involving

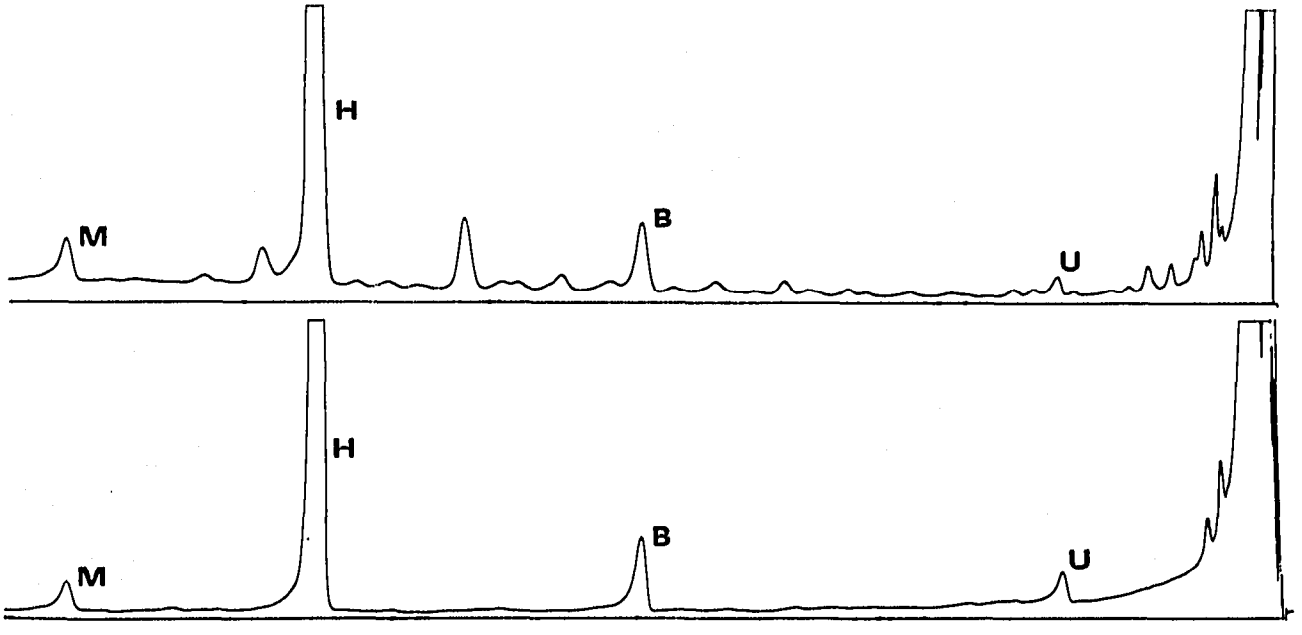

Fig. 2. Chromatograms of a silylated ether extract of urine collected from a normal subject for 8 h following consumption of sodium barbitone (300 mg). Above, F.I.D.; below, T.D. Conditions: as for Fig. 1. Identified peaks: B, barbitone; H, hippuric acid; M, *m*-hydroxyhippuric acid; U, urea.

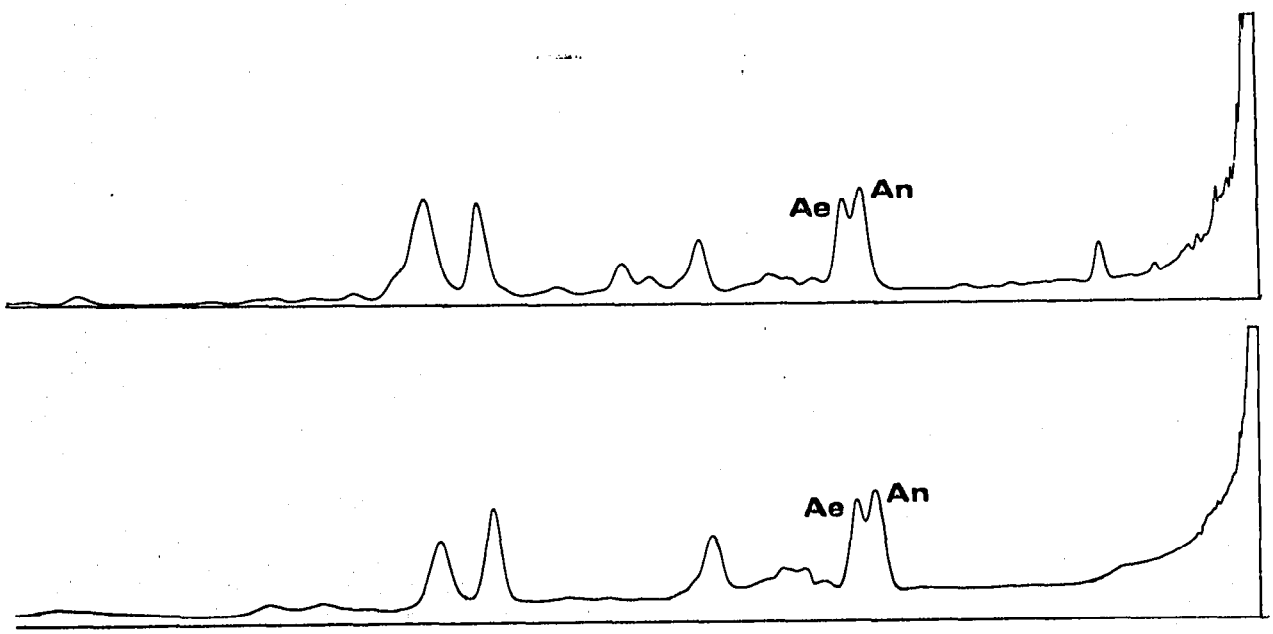

Fig. 3. Chromatograms of a crude steroid extract from a normal urine. Above, F.I.D.; below, T.D. Free and conjugated steroids were extracted[3], treated with glucuronidase–sulphatase, and the steroids isolated by ethyl acetate extraction were then treated successively with methoxylamine hydrochloride in pyridine followed by bis(trimethylsilyl)acetamide. Reactive hydroxyl groups were converted to trimethylsilyl ethers and reactive keto groups in steroids such as androsterone (An) and aetiocholanolone (Ae) to methoximes[4] which were revealed by both detectors. Conditions: temperature programming from 150° at 1.5°/min on a 1.5 m × 4 mm column of OV-1 (1%)

drugs and their metabolites, since so many of these contain nitrogen. The detection of barbitone in a simple extract of urine is illustrated in Fig. 2. The T.D. may also be adapted to the detection of non-nitrogenous substances in cases where these may readily be converted into nitrogenous derivatives. For instance, many steroids contain carbonyl groups which will react with appropriate reagents such as methoxylamine to give detectable derivatives (Fig. 3).

In our experience, the repeated injection of silicon-containing mixtures invariably led to a gradual loss of sensitivity of the T.D. owing to the deposition of silica on the salt-tip and probe. Hence for continued successful use, it was necessary to clean the detector daily. Fortunately, the construction of the tip is such that it could readily be freed from silica by brushing and although this process eventually loosened the salt ring, the effect on performance was negligible. The probe could also be freed mechanically from all but a hard deposit which did not appear to affect performance seriously; this was occasionally removed by soaking in dilute sodium hydroxide. In addition to its effect on sensitivity, accumulated silica also had a tendency to fall from the detector on to the flame jet, resulting in partial or complete blockage and completely unsatisfactory performance. Since cleaning was difficult and often involved dismantling the whole detector unit, precautionary measures proved well worth while. As an additional measure, we found it desirable to inject only small volumes (usually 1.25 μl) of silicon-containing mixtures, although this had the disadvantage that recorded column bleed on the F.I.D. could be undesirably high owing to necessarily low attenuation. When maintained as indicated above, we found that tips remained in good condition for the performance of some

dozens of chromatographs. However, owing to our invariable use of temperature programming, we never injected silylated mixtures more than three or four times each day and doubt the capacity of the detector to handle continuously the numerous injections associated with rapid isothermal analyses.

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