DETERMINATION OF STEROIDS BY PAPER STRIP ELUTION CHROMATOGRAPHY

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(Received August 24th, 1960)

Despite the widespread application of paper chromatography to almost every field of biochemistry, the quantitative analysis of the separated compounds presents problems which are still to some extent unsolved. Measurements are usually undertaken either *in situ* on the paper, or in solution after the area containing the compound has been cut out and eluted. The first method, whether it involves visual, densitometric or fluorometric[1] comparison with standards chromatographed at the same time, is subject to greater error than most other laboratory analytical procedures. The second method involves an extra step, with consequent loss of time and material. In addition, other compounds in the near vicinity may be included in the area cut out for elution.

The method of fractional analysis of the eluate, commonly used in column chromatography, has not, to our knowledge, been applied to descending paper chromatography except by SOLMS[2], who employed a rotating paper cylinder provided with saw teeth along its lower border. This arrangement was convenient for preparative purposes. In the present work we used a paper strip, which is preferable for analytical purposes because of the smaller volume of effluent collected. Consideration was also given to the importance of minimizing vapour losses, so that systems employing volatile solvents[3] could be used.

EXPERIMENTAL

The apparatus is shown diagrammatically in Fig. 1. The chromatographic chamber is a glass cylinder 12 in. in height and 6 in. in diameter, and is provided with a hole 1 in. in diameter in the center of the base. This hole is sealed with a hollow polyethylene stopper ("Teflon" would probably be more satisfactory, because it is more resistant to organic solvents). The chromatographic paper is a strip approximately 27 cm long, pointed at its lower end. The distance from origin to tip is 19 cm. Mobile phase drips off the end of the paper and through a glass funnel into the fraction collector. The stem of the funnel passes through a small hole in the polyethylene stopper and is thereby held securely. A small drop of fluid is normally retained at the tip of the funnel, thus minimizing exchange of gases with the outside atmosphere. For equilibration purposes the usual arrangements appropriate for chromatography

with volatile solvents[3] are used. The trough containing mobile phase is supported on a wire basket adapted from a test tube rack. Delivery of mobile phase into the trough may be effected automatically by a time clock, if desired, using the apparatus previously described[4].

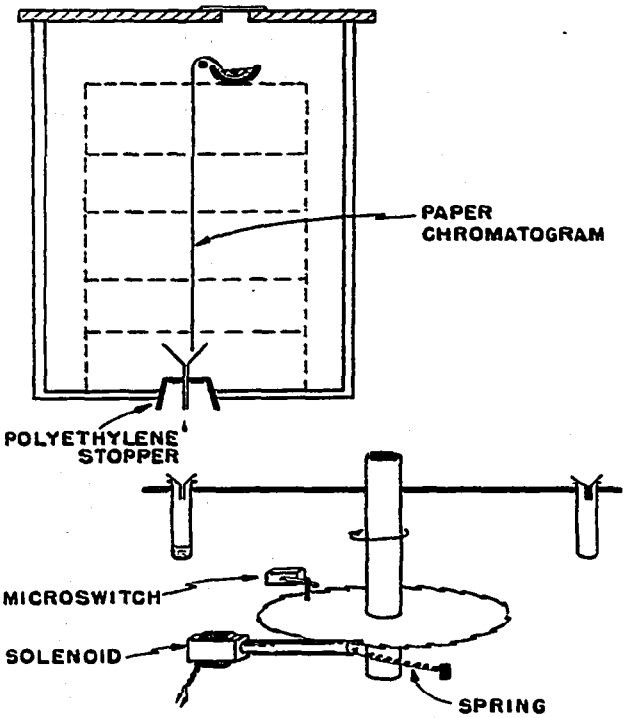

Fig. 1. Apparatus for paper strip elution chromatography.

The fraction collector comprises a circular steel plate 11 in. in diameter, carrying twenty-four 75 × 10 mm Pyrex test tubes. The plate is rotated by a solenoid acting on a toothed wheel at intervals preset by an electronic timer. After tube 24 has been reached, the turning mechanism can be switched off by a lever mounted vertically on the wheel, which actuates a micro-switch.

The parameters of the method were examined using mixtures containing 5 or 10 μg of each of the standard steroids (corticosterone, cortisone and cortisol), chromatographed at 25° in the toluene–75 % methanol system of BUSH[5], after 4 hours' preliminary equilibration. In some experiments the paper was first washed with boiling methanol–ethyl acetate (20:1) for 72 hours in a Nolan extractor.

In operating the turntable an interval was selected which allowed approximately 0.5 ml of effluent to collect in each tube. Timed operation was found to be simpler than drop-counting and weight- or volume-actuated devices and possessed the additional advantage, when chromatography was carried out in a temperature-controlled room using flammable solvents, that a minimum of electronic equipment was situated in the immediate vicinity of the tank. Such apparatus as was required was located outside the chromatography room and included a revolution counter, which recorded each movement of the turntable and hence the number of tubes filled.

The eluted fractions were dried *in vacuo*, and the Δ^4-3-ketosteroid content estimated in the same tubes by alkaline fluorometry in potassium *tert.*-butoxide[6].

RESULTS AND DISCUSSION

Fig. 2(a) shows the elution profile obtained with a mixture of 10 μg each of corticosterone, cortisone and cortisol, using a strip of unwashed Whatman No. 3MM paper 2 in. wide. Fig. 2(b), (c) and (d) shows the results of experiments using mixtures of

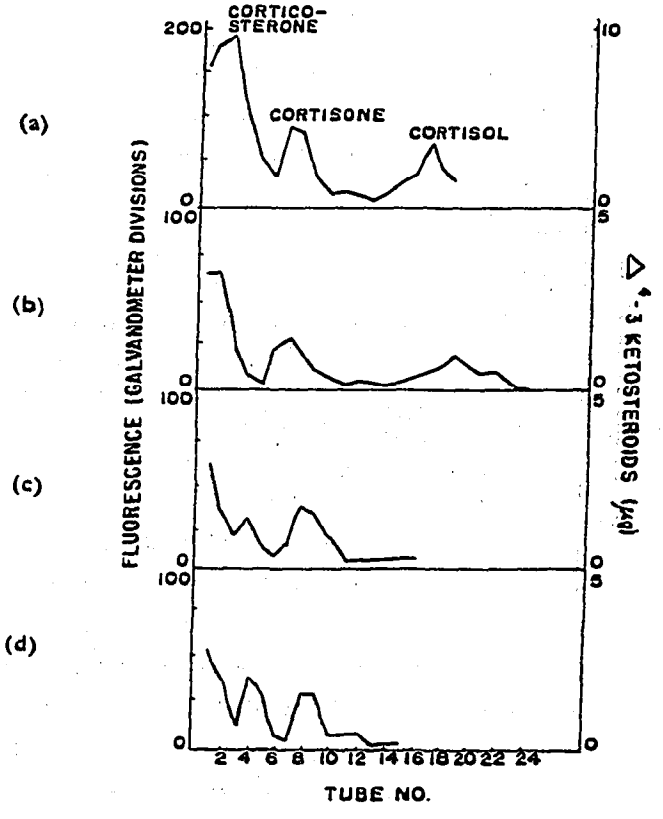

Fig. 2. Elution profiles of mixtures of corticosterone, cortisone and cortisol.

5 μg of each steroid chromatographed on washed paper. In (b) a 2-in. strip of No. 3MM paper was used, in (c) a 1-in. strip of No. 3MM paper, and in (d) a 2-in. strip of Whatman No. 1 paper.

It will be seen that the separation and measurement of steroids in amounts as small as 5 μg are readily possible. At these levels the use of washed paper is recommended, for with the fluorometric technique employed the blank was reduced to negligible proportions (approximately 0.15 μg). The recovery of cortisol in the four experiments was 88 %, 74 %, 104 % and 102 % respectively.

When 2-in. No. 1 paper was substituted for 2-in. 3MM paper, the steroids were eluted in smaller volume. A similar effect was produced when 1-in. 3MM paper was substituted for 2-in. 3MM. In both instances the effect can be attributed to alteration of the cross-sectional area of the strips.

The technique is readily adaptable to other solvent systems, groups of compounds, or detection methods (including liquid scintillation counting).

ACKNOWLEDGEMENTS

The work was aided by a grant from the James Hudson Brown Memorial Fund of the Yale School of Medicine, and by a U. S. Public Health Grant, A-254.

SUMMARY

A technique is described for the separation and quantitative analysis of Δ^4-3-keto-steroids using paper strip elution chromatography. The method is sensitive to quantities of steroid of the order of 5 μg.

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