We have confirmed that aluminium scarcely moves from its starting point, but have found that beryllium moves down the paper giving a clear separation. For these experiments strips of Whatman No. 1 filter paper about 1 in. wide were used, and, using the well-described technique of paper chromatography[3], the spots were eluted with a solvent consisting of 80 per cent by volume of *n*-butanol and 20 per cent of concentrated hydrochloric acid (AnalaR). After elution overnight, the solvent front moves about 8 in. The R_F value of aluminium is about 0·03, whereas that of beryllium is about 0·3. Satisfactory separations were achieved when the following amounts of aluminium and beryllium as a solution of their chlorides in 0·01 ml. of dilute hydrochloric acid were placed on the paper and eluted overnight.

Aluminium	Beryllium
10 γ	20 γ
110 γ	8 γ
11 γ	170 γ
300 γ	2 γ
2 γ	300 γ

This technique provides a valuable method of detecting traces of aluminium in beryllium salts of reagent-grade quality, and also of traces of beryllium in the presence of aluminium. Its application to quantitative estimations should prove useful and work is proceeding along these lines.

During these experiments, a dark band across the width of the paper and about ½ in. deep was obtained at the solvent front. This is probably due to impurities in the paper.

It is worth noting that on occasions using other solvents, we have found impurities carried down the paper which often concentrate at the solvent front. For example, using *N*/100 hydrochloric acid as solvent, a band is obtained at the solvent front which gives a very intense fluorescence with 8-hydroxyquinoline. If this band is cut off and the paper washed and used again in the same way, no concentration of impurities at the solvent front is observed.

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Paper Partition Chromatography of Reducing Sugars with Benzidine as a Spraying Reagent

In the course of investigations into the application of paper chromatography to the identification of reducing substances in urine it was found that, when ammoniacal silver nitrate was used as a developing reagent, urates, normally present in urine, produced a dark brown spot with an R_F value of approximately 0·20. This spot, which generally 'tails', tends to mask the spots produced by other reducing substances with R_F values in the region of 0·20, namely, those of glucose, sorbose, arabinose and fructose.

Demineralization of the urine by means of ion-exchange resins[1], in an attempt to remove the urates, proved unsuccessful. A benzidine reagent has been formulated, however, which, although reacting with reducing sugars, does not react with urates. The reagent is prepared as follows: benzidine AR, 0·5 gm.; glacial acetic acid, 20 ml.; absolute ethanol, 80 ml. The chromatogram, after drying to remove the solvent, is sprayed with this reagent and then heated in an oven at 100°-105° C. for fifteen minutes, observing at five-minute intervals.

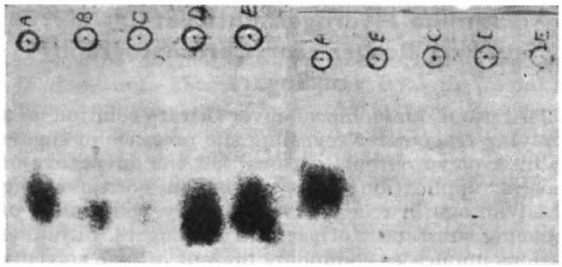

Fig 1

Fig 2

Pentoses produce a characteristic chocolate brown spot within five minutes. Lactose, maltose, galactose, glucose and lævulose form dark brown spots within ten minutes. Ascorbic acid gives a faint brown spot after ten minutes. Urates, in concentration much greater than that normally found in urine, may produce a very faint spot; but even then the colour is not sufficient to interfere with the spots produced by reducing sugars. The reagent will detect 5 μgm. of all the reducing sugars. In particular, it is a more sensitive reagent for lactose and maltose than ammoniacal silver nitrate. Unlike ammoniacal silver nitrate, the benzidine reagent is only slightly affected by light and heat. Even after exposure to daylight for a few days the filter paper background is only coloured a pale brown, in contrast to the dark brown spots produced by reducing sugars.

There is normally no reaction with urines containing pus or blood, but specimens which are grossly contaminated with blood occasionally produce a faint violet-coloured spot with an R_F value of approximately 0·28.

Fig. 1 shows a series of five urine samples. Specimens *A* and *D* were suspected of containing traces of reducing substances by Benedict's qualitative test. Specimens *B*, *C* and *E* are normal urines giving a negative reaction with Benedict's test. The chromatogram was run for 24 hours using *n*-butanol-acetic acid-water[1] as the solvent mixture. The chromatogram was then sprayed with ammoniacal silver nitrate reagent.

Fig. 2 indicates the same urine samples, chromatographed under similar conditions to Fig. 1, but sprayed with the benzidine reagent. In this case only one spot has developed, and urine *A* is the only specimen containing a reducing sugar (which was identified as glucose). The other spots given with ammoniacal silver nitrate (Fig. 1), from urine samples *B*, *C*, *D* and *E*, are due to urates. Urine *D* contained a large quantity of urates, which probably accounts for the doubtful reaction with Benedict's reagent.

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