interfere with the detection of moderately elevated levels of PHPL. Such levels could be confirmed by chromatography on columns of OV-17 though some caution was required since in some normal urines the PHPL peak was evidently heterogeneous.

In connection with more general studies on the aromatic constituents of urine the behaviour of PHPL under temperature programmed conditions was examined, using columns of OV-1 (10%) of length 1.5 (discarded after preliminary experiments), 2.7, 3.4, 4 and 5.5 m. Results were compatible with the following conclusions. First, overlap between PHPL and peak X (Fig. 1) was considerable on 2.7 m columns but separation between the two peaks became increasingly effective with increasing length of column. Secondly, peak Y (Fig. 1) appeared to contain at least 3 constituents, one of these being the physiologically interesting 4-hydroxy-3-methoxymandelic acid; whilst separation between the main part of this peak and PHPL improved with increasing column length one, usually minor, constituent separated out and overlapped PHPL when 5.5 m columns were used. It thus appeared that optimum separation of PHPL required a 4 m (13 ft.) column; temperature programming from 170° at 1°/min provided suitable experimental conditions.

*RAF Institute of Aviation Medicine,*
*Farnborough, Hants. (Great Britain)*

R. F. COWARD
P. SMITH

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## Use of silicic acid–glass fiber sheets for bioautography of antimicrobial substances

Bioautography of thin-layer chromatograms is used routinely for detection of antimicrobial substances and is the subject of numerous publications. The general technique is to press the developed thin-layer plate face down onto the surface of agar seeded with a microorganism, remove the plate, and after a suitable incubation period, observe zones of inhibition of the separated antibiotics.

To avoid the adherence of the adsorbent to the agar surface, a number of methods have been used. Probably the most common technique is that of MEYERS AND SMITH[1], who inserted a sheet of filter paper between the plate and the agar surface. Another difficulty which sometimes arises is the lack of contact between the entire plate surface and the agar, resulting in poorly defined spots.

Both of these problems can be avoided by use of a silicic acid–glass fiber sheet (ChromAR® Sheet 500, code 2182, Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.). This sheet is composed of approximately 70% silicic acid and 30% micro fiber glass, and can be cut to the desired size with a pair of scissors or a paper cutter.

Although the sheet does not have high tensile strength, if one is careful it is easily handled.

The ChromAR® sheet has several advantages over TLC plates: (1) it conforms entirely to the agar surface, making complete contact; (2) the adsorbent does not adhere to the agar, therefore no paper need be used to separate the sheet from the agar; (3) much lower levels of antibiotic need be spotted (compared to TLC plates), apparently due to a more efficient transfer of material from sheet to agar; and (4) in a number of chromatographic solvent systems, development was up to twice as rapid as with TLC plates.

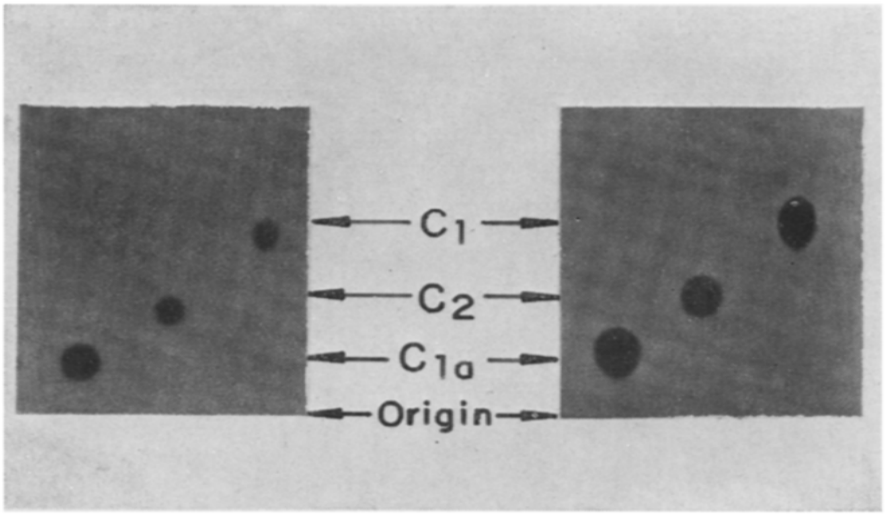


Fig. 1. Comparison of bioautograms of gentamicin C components on a thin-layer plate (left) and a ChromAR® sheet (right). The quantities spotted on the plate for $C_{1a}$, $C_2$, and $C_1$ were 50 μg, 50 μg and 150 μg, respectively; on the thin-layer sheet, 20 μg, 20 μg and 60 μg, respectively. Both chromatograms were bioautographed on the same plate against *Sarcina lutea*. Note the larger zones resulting from plating of the thin-layer sheet, although less material was spotted.

An example of chromatograms of gentamicin components using a TLC plate and a ChromAR® sheet is seen in Fig. 1. Although the quantity of gentamicin spotted on the ChromAR® sheet was less than half that used on the TLC plate, it is obvious that the zones of inhibition are larger and the resolution of components is excellent. Also, using this solvent system on the sheet, the development time of 45 min was about half that required for the TLC plate. With other solvent systems, the ChromAR® sheets were successfully utilized for chromatography of other antibiotics including erythromycin, oleandomycin, megalomicin, everninomicin, actinomycin and chloramphenicol. This thin-layer medium appears to be a very satisfactory tool for use in separation and bioautography of antimicrobial substances.

*Microbiology Department,*
*Research Laboratories,*
*Schering Corporation,*
*Bloomfield, N.J. 07003 (U.S.A.)*

G. H. WAGMAN
J. V. BAILEY

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