

	${}^4F_{3,2}$	${}^4F_{5,2}$	${}^4F_{7,2}$	${}^4F_{9,2}$
$p^4F_{3,2}$ 2084.6	40622.2 (8)	38766.8 (5)		
${}^4F_{5,2}$ 2405.3	42705.8 (7)	40851.9 (8)	38233.6 (6)	
${}^4F_{7,2}$ 2083.5		43256.9 (10)	40639.1 (5)	37493.9 (2)
${}^4F_{9,2}$			42722.8 (8)	39577.2 (4)
${}^4D_{1,2}$ 726.0	41856.7 (8)			
${}^4D_{3,2}$ 1466.2	42582.7 (6)	40726.2 (9)		
${}^4D_{5,2}$	44047.5 (6)	42193.7 (3)	39577.2 (4)	
		43285.5 (3)	40669.7 (9)	37523.8 (7)

PAPYROGRAPHIC SEPARATION AND LOCATION OF ANTIBIOTICS FROM PLANTS

THE antibiotic activity of the crude aqueous, alcoholic and ether extracts of the leaves of *Toddalia aculeata*, a reputed specific for dysentery, having been first found by one of us (Sirsi, M.) against the gram negative *Bact. flexneri*, it was of interest to determine if a papyrographic separation of the active principle could be accomplished.

A filter-paper strip (400 mm. \times 20 mm. Whatman No. 1) was spotted at one of its ends with a drop of crude ether extract and dried.

A glass tube (450 mm. \times 30 mm.) vertically held, lined with filter-paper moistened with ether-saturated water, was employed as the developing chamber. The tube ground at both ends was provided with wooden corks. The lower cork supported the glass trough which contained the water-saturated ether for developing the papyrogram. The cork at the top had a glass rod, the end of which was drawn into a hook from which the paper strip could be hung vertically by means of a hole punched at the opposite end of the strip. The glass rod was carefully lubricated with vaseline, so that the strip could be raised or lowered within certain limits.

After placing the water-saturated ether in the trough, the cork carrying the glass rod and the paper strip, was carefully inserted into the tubular chamber, so that the strip was kept hanging just a few millimetres above the surface of the developing solvent and without touching the sides of the chamber. The strip was allowed to remain in this position for a couple of hours, so that the filter-paper was equilibrated with the vapour phase in the chamber. By carefully pushing down the glass rod, the strip could be so lowered into the trough, that just a few millimetres of the strip dipped into the solvent, leaving the spotted area clearly above the surface of the solvent. With the capillary rise of the solvent, the papyrogram was developed. The solvent front took about 4 to 5 hours to reach the top. The rise of the solvent front could easily be followed since, it almost agreed with simultaneous ascent of the chlorophyll band. The strip, after development, is air dried and cut lengthwise into two halves. One half was employed for the bioautographic location of the "active" zone,¹ while the other was used for ultraviolet printing of the papyrogram on Illford Reflex Paper.^{2,3} Figs. 1 (a) and 1 (b) give photographs of the clearance on the agar plate seeded with *Staph. aureus* and the ultraviolet print. It will be observed that the zone of clearance is identical with the zone of ultraviolet absorption, suggesting that the antibiotic principle has an absorption in the ultraviolet region (253.7-265 $m\mu$), a fact of considerable significance in further investigations. The circumstance that the antibiotic zone lies adjacent to the chlorophyll band is also helpful in locating the "active" region and for eluting out the antibiotic from the incised portions of the papyro-

gram. The method of combining bioautographic technique with the ultraviolet printing of the papyrogram, which has been successful in the present instance, has immense potentialities of application in a study of antibiotics from natural sources.

1. Venkatesh, D. S. and Sreenivasaya, M., *Curr. Sci.*, 1951, 20, 98. 2. Markham, R. and Smith, T. D., *Biochem. Jr.*, 1949, 46, 509. 3. Bheemeswar, B. and Sreenivasaya, M., *Curr. Sci.*, 1951, 20, 61.

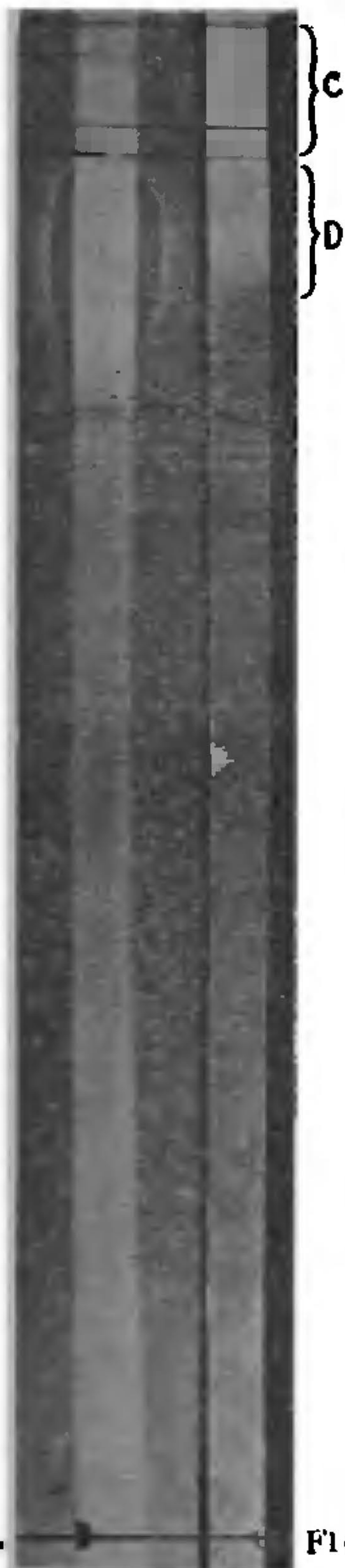


FIG. 1a. Area of clearance. (Bioautography against staph. aureus).

b. Photograph of the Papyrogram of the antibiotic from an Etherial extract of *Toddalia aculeata*, taken in the Ultra Violet region. (253.7 and 265 m μ).

C. Absorption due to Chlorophyll

D. Absorption due to "Active" fraction.

Our grateful thanks are due to Drs. N. N. De, K. P. Menon and A. S. Ramaswamy for their helpful discussions, and to Prof. M. S. Thacker for his kind interest.

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