acids, were obtained by acid hydrolysis of yeast nucleic acid (1 hr., 1 *N* hydrochloric acid, 100° C.).

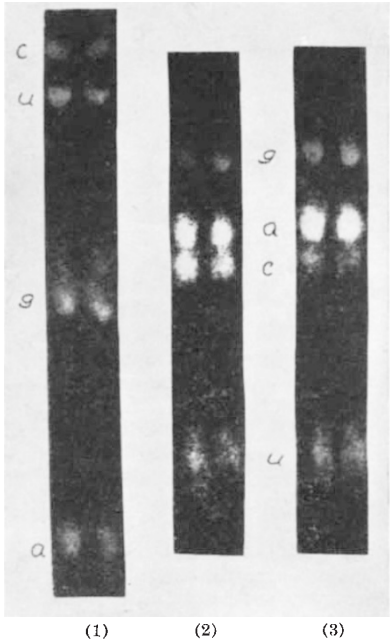
Separation. Spots containing 50–150 γ nucleic acid were prepared on filter paper (Munksell OB) by an Agla micropipette. Butanol was used as a solvent in the following two solutions: (*a*) *N*-butanol (4), dioxane (1) and distilled water (1), the spots on the paper being neutralized by gaseous ammonia; (*b*) *N*-butanol (4), diethylene glycol (1) and 1·5 *N* hydrochloric acid (1). Table 1 shows the R_F values found.

Table 1. R_F VALUES

	Solutions	
	a	*b*
Adenine	0·64	0·29
Guanine	0·37	0·18
Cytidylic acid	0·09	0·35
Uridylic acid	0·17	0·63

A suitable time for separation on paper strips 40 cm. long was found to be 18 hr. After drying the paper strips for two hours at 80–90° C., photography was carried out in the ultra-violet range.

Optical equipment. Monochromatic light of wavelength 257 μμ and 275 μμ was generated between rotating cadmium electrodes of an ultra-violet microscope constructed according to Köhler. A quartz condenser of numerical aperture 0·3 and a 6-mm. monochromat of numerical aperture 0·35 corrected for 257 μμ or 275 μμ (Cooke, Troughton and Simms) was used. 60 cm. from the exit pupil of the objective a drum was fastened to a stand, with its axis perpendicular to the incident light and within the homogeneous field of illumination. The drum is 13 cm. in diameter and 12 cm. wide, provided with a fold at the edges, and it is rotated by a crank. Paper strips 5–10 cm. wide were photographed against a blue-sensitive photographic paper of similar width. Paper and film were braced by two strips of brass, the distance between which could be varied.



(1) (2) (3)

Paper strips 5 cm. broad photographed in the ultra-violet. (1) With butanol–dioxane–distilled water as solvent at 257 μμ, (2) and (3) with butanol–diethyleneglycol–hydrochloric acid at 275 μμ and at 257 μμ. In both instances 65 γ yeast-nucleic acid was subjected to separation. *a*, *g*, *c* and *u* refer to adenine, guanine, cytidylic acid and uridylic acid respectively. Note increase in sensitivity at 275 μμ

Thus, paper up to 40 cm. in length could be photographed continuously. Uniform exposure was obtained by rotation of the drum for 60 sec. at 257 μμ and for 30 sec. at 275 μμ.

Results. Photographs of some chromatograms are reproduced in the accompanying figure. The sensitivity of the method for purines and pyrimidines in γ per cm.[2] can be seen from Table 2.

Table 2

Adenine	0·35
Guanine	0·55
Cytosine	0·75
Uracil	0·45
Thymine	0·60

By using the cadmium band at 275 μμ the value for cytosine can be halved.

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[1] Vischer, E., and Chargaff, E., *J. Biol. Chem.*, **176**, 703 (1948).
[2] Chargaff, E., Magasanik, B., Doniger, R., and Vischer, E., *J. Amer. Chem. Soc.*, **71**, 1513 (1949).
[3] Markham, R., and Smith, J. D., *Nature*, **163**, 250 (1949).
[4] Markham, R., and Smith, J. D., *Biochem. J.*, **45**, 294 (1949).

Identification of Sulphonamides on Paper Chromatograms

THE recent communication on this subject by R. Robinson[1] has prompted me to record some observations made about two years ago on this subject.

Butanol – acetic acid solvent was found the most satisfactory of many solvents tried, being prepared by mixing *n*-butanol, acetic acid and water in proportions 50–15–35 by volume. The mixture is homogeneous and very slightly under-saturated with water at room temperature, it is easily prepared and gives reproducible R_F values which are almost identical with those obtained with the equilibrated solvent suggested by Partridge in 1948.

R_F-values found range from 0·55 (sulphaguanidine) to 0·85 (sulphamerazine). Some good separations are obtained; for example, the three components of sulphatriad can be demonstrated in the urine of a patient on treatment with this drug.

Detection of the sulphonamide spots makes use of the diazo- and coupling reaction carried out in alcoholic solution to minimize spreading of the spots. Nitrous acid solution is sprayed on the dried paper, followed after one minute by a 1 per cent solution of dimethyl-α-naphthylamine or similar coupling reagent. The sulphonamides show as red or pink spots of the azo colour, one microgram being readily detected.

No spreading of the spots occurs and quantitative measurements have been carried out quite successfully using the method described by Fisher *et al.*[2].

The nitrous acid solution is prepared as follows: 0·1 gm. of sodium nitrite in 1 ml. of water is shaken with 10 ml. of *n*-butanol, and 0·3 ml. concentrated hydrochloric acid added with shaking. The mixture is allowed to stand for a few minutes before use; solid sodium chloride may separate. The solution is stable for several days.

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[1] *Nature*, **168**, 512 (1951).
[2] *Nature*, **161**, 764 (1948).