PAPYROGRAPHIC STUDIES ON THE DEGRADATION PRODUCTS OF NUCLEIC ACIDS

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NUCLEIC acid metabolism has engaged our close attention since 1948 because of their intimate association with (*a*) protein synthesis in yeasts; (*b*) glandular secretions in the silkworm and (*c*) viral proliferations in spiked sandal, subjects in which this laboratory has been interested for some time. We have found periodic fluctuations in basophilic stainability of the contents of the yeast cell, running parallel to the mitotic phases.[1a,b] The basophily has been found to be related to the ribonucleic acid content of the cell; similar observations have recently been made by us[2] with respect to the progressive increase in the ribonucleic acid content of the silk gland as it develops to maturity.[3] In viral proliferations, the first reaction of the host to virus infection, results in an increase in the perinuclear basophilia together with a striking increase in cell size. The virus thus induces a ribonucleic acid synthesis in the infected cells immediately followed by growth and protein synthesis.

In a search for micro-analytical methods for a determination of the nucleic acid constituents, we have explored the possibility of applying the papyrographic method[3,4] pursued in these laboratories. Papyrographic separations possess the further advantage of securing for quantitation constituents of spectro-photometric standards of integrity and purity, which enable us to identify and estimate them with considerable ease, accuracy and elegance.

The application of paper chromatography (papyrography) to problems of nucleic acid chemistry was first reported by Vischer and Chargaff,[5] who extended this technique to a quantitative analysis of purines and purimidines of nucleic acids. The procedure consists in locating the constituents of one-dimensional papyrograms (50 × 2 cm.), developed with solvent mixtures (see Table I), by treating the papyrograms with mercury salts on guide strips and converting this salt to the black sulphide of mercury. These black spots serve as a guide for elution of purines and pyrimidines from the corresponding areas of the untreated papyrogram. The papyrographic procedure described by Hotchkiss for the location and quantitation of the constituent,[6] entails the elution of purines, pyrimidines and nucleosides from successive areas of the papyrogram developed with butanol and a determination of the ultra-

TABLE I

Solvent systems		Constituents separated	Author and Reference
3 : 1 Quinoline collidine	..	Adenine and Guanine	Vischer and Chargaff[5]
n-Butanol (neutral)	..	Adenine, Guanine	
n-Butanol morpholine	..	Hypoxanthine, Xanthine	
do *plus* diethylene glycol		Uracil, Cytosine, Thymine, and corresponding nucleosides	
Collidine			
Butanol-diethylene glycol			
do *plus* HCl	..	(Nucleotides do not move in any of these solvents)	Vischer, *et al.* [10]
Butanol—HCl			
isobutyric acid—Amm. isobutyrate pH 3·6	..	Adenylic acid cytodylic acid and Uridylic acid *plus* Guanylic acid	Vischer, *et al.* [9]
n-Butanol—NH_3	..	Cytosine, Urasil, Adenine, Thymine, Cytidine, Guanosine and Thymidine	Hotchkiss [6]
Butanol—10% Urea	..	Adenine, Guanine, Uracil, Cytosine, Thymine and corresponding nucleosides	Carter [7]
5% Am. citrate pH 3·6 *Iso* amyl alcohol	..	Separates nucleotides	do
do pH 9·6			
Pot. Dihydrogen phosphate—*Iso* amyl alcohol	..	,,	
Disod. Hydrogen Phosphate—*Iso* amyl alcohol	..	,,	
Pot. dihydrogen Phosphate—*Iso* amyl alcohol	..	,,	
Ter. butanol—HCl—H_2O	..	Adenine and guanine and pyrimidine nucleotides	Markham and Smith [8]

violet absorption of the eluates from these areas. Carter[7] has evolved a technique for resolving mixtures of purines, pyrimidines, nucleosides and nucleotides of yeast nucleic acid with solvents indicated in Table I. A special feature of his papyrographic technique, involves the employment of a two-layered solvent system where the filter-paper is introduced so as to pass through both the phases. He locates these compounds on the papyrograms by ultra-violet fluorescence, thus eliminating the necessity of having guide strips. Markham and Smith[8] have developed a photographic technique based on the characteristic absorption of these compounds in the ultra-violet (253·7 and 265 $m\mu$).

A careful study of Table I reveal that among the immiscible alcohols, butyl and *iso*-amyl have been used. Miscible alcohols have not so far, been used as developing solvents for papyrographic separations. N-propanol —0·5 N HCl has, however, been used for the separation of the constituents on a starch column. In our present study, an attempt has been made to compare the relative merits of several solvent mixtures.

Experimental

Inverted bell jars (14 cm. × 26 cm.) with ground edges securely covered with ground glass discs with vaseline, were used as developing vessels. The solvent mixture (30 ml.) was placed at the bottom in a separate (8 cm.) petri-dish. Filter-paper (Whatman No. 1) (10 cm. × 20 cm.) carried 5 spots. Four of them consist of 0·01 ml. of a 1% solution of adenine sulphate (GBI), guanine (BDH), uracil (GBI) and xanthine (GBI), prepared according to the methods given by Gyorgi.[11] The fifth spot represented a mixture of the above constituents.

The solvent systems employed are: (1) 2 : 1 ethanol 0·5 N HCl; (2) ethanol in ammonia atmosphere; (3) 2 : 1 propanol – 0·5 N HCl; (4) propanol in ammonia atmosphere; (5) butanol—5 per cent. aqueous urea; (6) butanol in ammonia atmosphere; (7) *iso*-amyl alcohol—ammonium citrate (two-layer system); (8) *Iso*-butyric acid-ammonium isobutyrate at pH 3·6-3·7. The spotted filter-paper placed in the vessel attained equilibrium with the vapour-phase of the solvent mixture; filter-papers were then lowered into the petri-dish containing the developing solvent. The time taken for developing the papyrogram usually takes about 4 hours, at the room temperature (24 to 25° C.). The filter-paper was then air dried and printed photographically over Ilford document paper No. 50, employing the ultra-violet radiation as recommended by Markham, *et al.*, with slight modifications. The position of the constituents were located on the print as white spots against a black background. The R_F values were determined in the usual way. The R_F values obtained by us are given in Table II along with the values repored by others in brackets.

TABLE II

No.	Solvent system employed	Adenine	Guanine	Uracil	Xanthine
1	2 : 1 Ethanol—HCl	0·59	0·44	0·75	0·0
2	Ethanol—NH_3	0·65	0·54	0·72	0·61
3	Propanol—HCl	0·46	0·30	0·66	0·0
4	Propanol—NH_3	0·67	0·45	0·69	0·52
5	Butanol—5 Urea	0·08 (0·41)	0·12 (0·05)	0·34 (0·35)	0·0 (0·12)
6	Butanol—Ammonia	0·41	0·1	0·27	0·16
7	*Iso* amyl—Am. citrate	0·63 (0·69)	0·0 (0·50)	0·81 (0·72)	0·0 (0·52)
8	*Iso* butyric acid—Am. *Iso* butyrate pH 3·6	0·93	0·70	0·70	0·55

Discussion

From Table II it can be seen that, of all the solvent systems employed, ethyl alcohol-0·5N-HCl 2 : 1 is advantageous over others since, better and more discrete separations of the 3 constituents out of the 4 contained in the mixture, have been secured (see Fig. 1). Further, ethanol is readily available and is less expensive. Equally good separations are obtained with mixtures of *n*-propanol-HCl (2 : 1). Among other solvent systems studied, the R_F values of a pair of the four constituents employed as a mixture overlap each other, resulting in inefficient separations. If the solvent system is too acidic, xanthine (alkaline soluble) gives a streak. If the solvent system is made alkaline by creating an atmosphere of ammonia, the acid-soluble guanine streaks. Isobutyric acids—ammonium isobutyrate at pH 3·6 to 3·7, is the recommended solvent[9] for separating nucleotides. We have used this solvent mixture for separating the bases, because, it is necessary to determine the positions occupied by the various constituents when a mixture of nucleotides and bases are encountered.

Our studies are confined only to the three bases, ordinarily occurring in nucleic acids. Xanthine, however, has been included. Cytosine, thymine and the related nucleosides and nucleotides have not been readily available for our work. We have, however, attempted a separation of a nucleotide mixture obtained from a baryta hydrolysate[12] of yeast nucleic

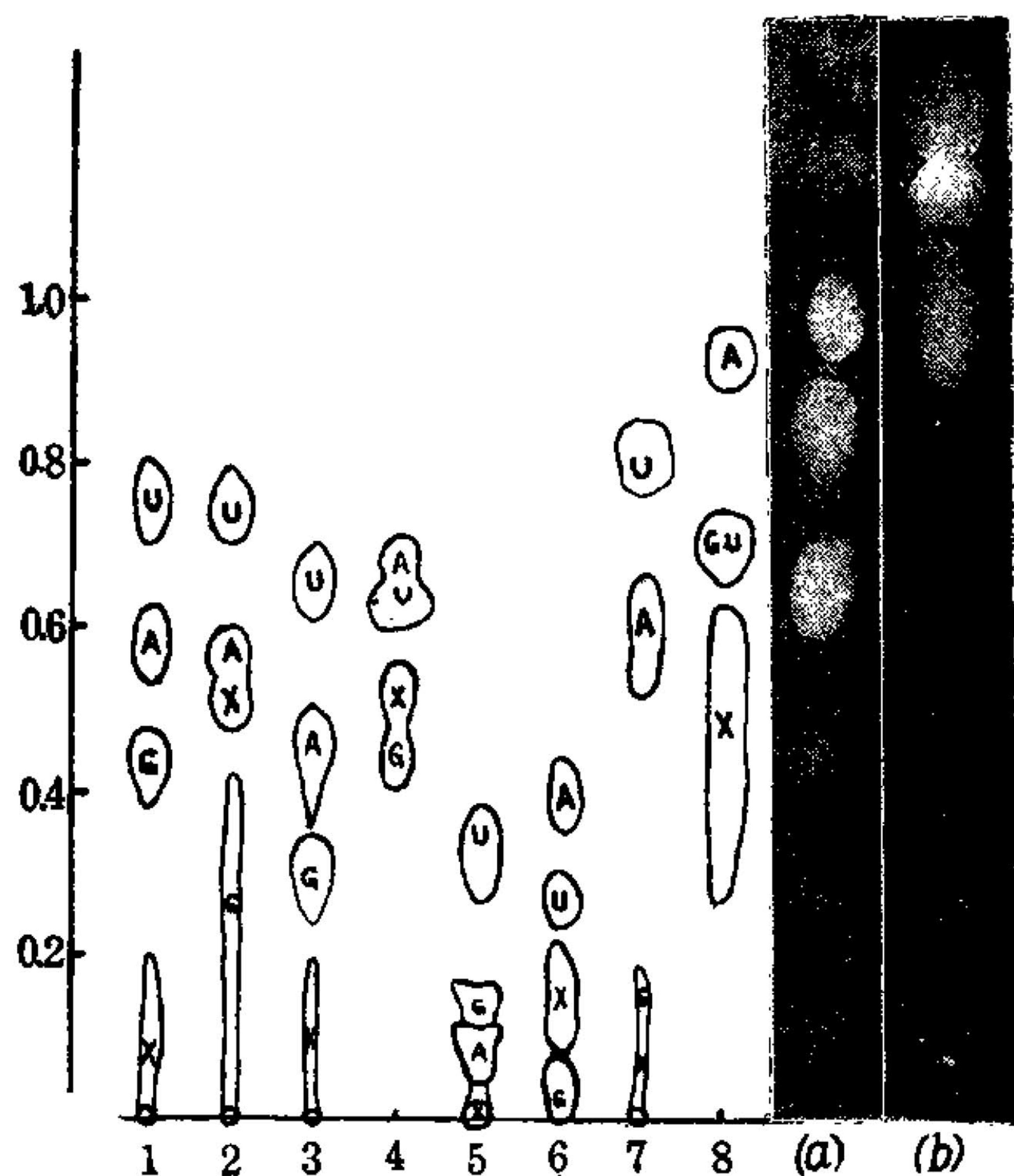

FIG. 1

A—Adenine
G—Guanine
U—Uracil
X—Xanthine

FIG. 2

(*a*) Isobutyric-Am. butyrate. Three spots in the descending order are adenylic, cytidylic and (guanylic & uridylic.
(*b*) Devoloped in Ethanol–Hcl.

acid (BDH). Papyrograms developed with solvent mixtures of ethanol and isobutyric acid are reproduced (Fig. 2). While we are unable to characterise the different constituents on the papyrogram without the reference spots, the results, however, suggest the possibility of securing better separations of the nucleotides on longer strips with suitably buffered ethanol mixtures. Work in this direction is now in progress.

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1. (*a*) Bheemeswar and Somakumar, *J. Sci. and Ind. Res.*, 1948, **7 B**, 131. 1. (*b*) Bheemeswar, B. and Sreenivasaya, M., 1948, Unpublished results. 2. Sreenivasaya, M. and Ramachandra Rao, T. N., 1950, Unpublished results. 3 Govindarajan, V. S. and Sreenivasaya, M., *Curr. Sci.*, 1950, **19**, 269. 4. —, *Ibid.*, 1950, **19**, 39. 5. Vischer, E., and Chargaff, E., *J. Biol. Chem.* 1947, **168**, 781. 6. Hotchkiss, R. D., *Ibid.*, 1948, **175**, 315. 7. Carter, C. E., *J. Am. Chem. Soc.*, 1950, **72**, 1466. 8. Markham, R. and Smith, J. D., *Biochem. J.*, 1949, **46**, 509. 9. Magasanik, *et al.*, *J. Biol. Chem.*, 1950, **186**, 37. 10. Vischer, E. and Chargaff, E., *Ibid.*, 1948, **176**, 704. 11. Gyorgi, P., *Vitamin Methods*, Acad. Press, N. Y., 1950, **1**, 365. 12. Loring, H. E., Roll, P. M. and Pierce, J. C., *J. Biol. Chem.*, 1948, **174**, 729.