

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 us² 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 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 pyrimidines 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

TABLE I

Solvent systems	Constituents separated	Author and Reference
3 : 1 Quinoline collidine	.. Adenine and Guanine	Vischer and Chargaff ⁵
<i>n</i> -Butanol (neutral)	.. Adenine, Guanine	
<i>n</i> -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, <i>et al.</i> ¹⁰
Butanol—HCl		
isobutyric acid—Amm.	.. Adenylic acid cytidylic acid and Uridylic acid plus Guanylic acid	Vischer, <i>et al.</i> ¹¹
isobutyrate pH 3.6		
<i>n</i> -Butanol—NH ₃	.. Cytosine, Uracil, 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 <i>Iso</i> amyl alcohol	.. Separates nucleotides	do
do pH 9.6		
Pot. Dihydrogen phosphate— <i>Iso</i> amyl alcohol	
Disod. Hydrogen Phosphate— <i>Iso</i> amyl alcohol	
Pot. dihydrogen Phosphate— <i>Iso</i> amyl alcohol	
Ter. butanol—HCl—H ₂ O	.. Adenine and guanine and pyrimidine nucleotides	Markham and Smith ⁸

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

of the constituent,¹⁰ entails the elution of purines, pyrimidines and nucleosides from successive areas of the papyrogram developed with butanol and a determination of the ultra-

violet absorption of the eluates from these areas. Carter⁷ 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 Smith⁸ 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. \times 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. \times 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) Isobutyric 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 reported 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 solvent⁹ 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 hydrolysate¹² of yeast nucleic

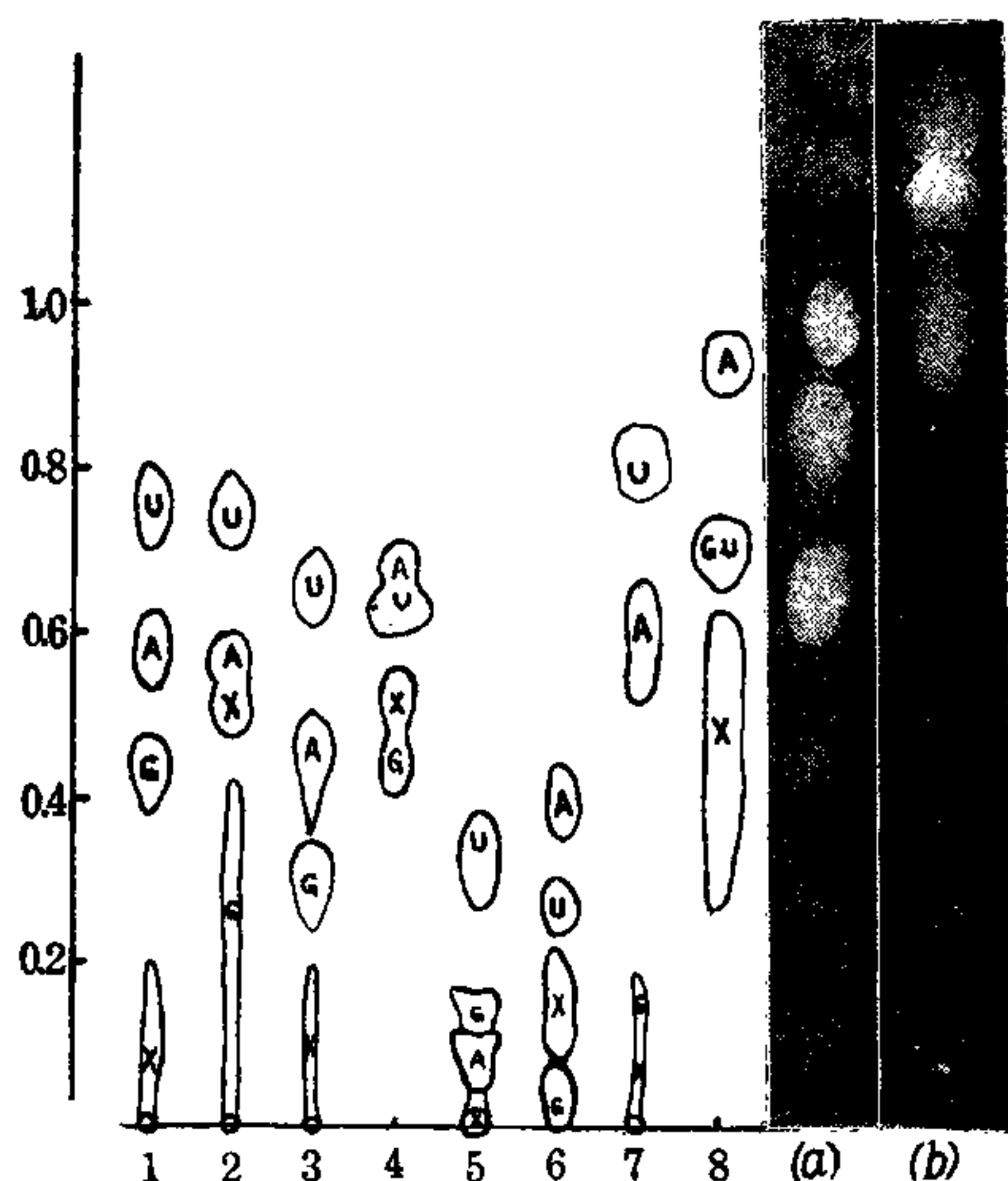


FIG. 1

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

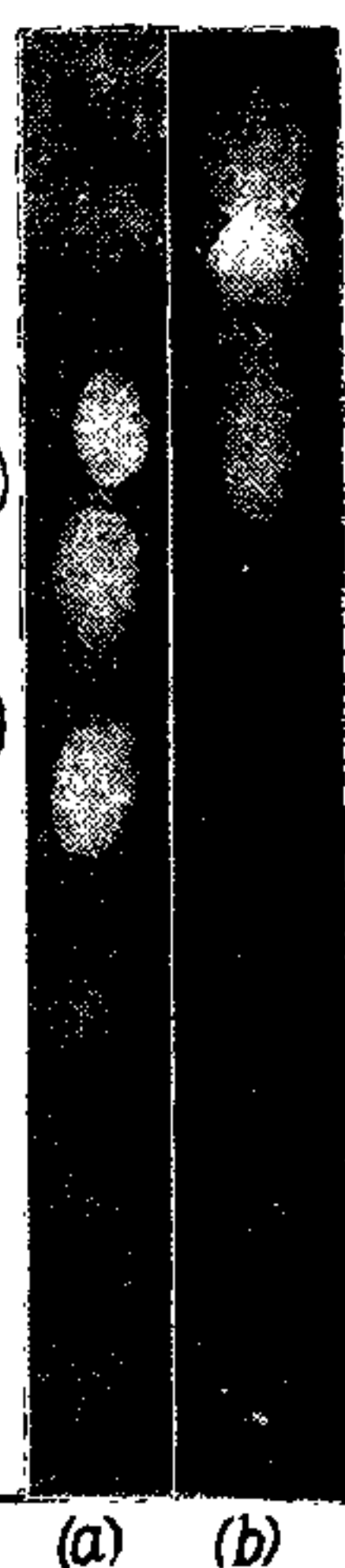


FIG. 2

(a) Isobutyric-Am. butyrate.
Three spots in the descending order are adenylic, cytidylic and (guanylic & uridylic).
(b) Developed 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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SOIL CONSERVATION—A VITAL NEED OF THE NATION*

A SAMPLE survey conducted in 1950 in certain parts of Bombay reveals that 71 per cent. of the land has been denuded partially or totally, of which 32 per cent. is wholly unfit for crops. The danger is on the increase and it is imperative that immediate steps be taken to conserve our soil.

The three guiding factors for conservative farming on level land are: (1) building up of soil structure; (2) improvement in soil permeability; and (3) provision of crop cover during the period of heavy showers.

Measures for mechanical means of erosion control involve a careful study of soil profiles, soil permeability and run-off and field trials to determine, suitable types of terraces for different soil zones, terrace spacing, types of waste weirs, etc.

In areas of low and uncertain rainfall, the major problems associated with dry farming are: (1) selection of crops; (2) moisture conservation; (3) improving soil texture by rotations, tillage and fallows; and (4) supplying deficiencies of plant food by manuring. The needs of

the soil under each of these should be carefully worked out for each zone.

The most common features of deterioration in irrigated soils are water-logging, salinity, loss of soil structure and fertility constituents and adverse microbiological conditions. Well planned soil studies based on a correct classification of the soils and a thorough understanding of their nature are an essential prerequisite for the conservation of the soil fertility over a long period by proper adjustment of water, manure and crop rotations. The genetic method of soil classification based on the entire soil profile as the unit of study and other associated experiments, that are being followed, in Bombay, is worth a detailed study in this connection.

Among unproductive soils, the saline soils deserve a special and detailed study, as the potentialities of obtaining good crops after these soils have been properly reclaimed have been amply demonstrated. These may be naturally occurring saline soils in dry areas or soils rendered saline by irrigation. In the Bombay State successful reclamation methods have been worked out both under irrigation and dry cultivation and proper crop husbandry recommended for right land utilisation.

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* Abstract of Presidential Address, by Dr. J. K. Basu, Ph.D., F.N.I., to the Section of Agricultural Sciences, during the 38th Session of the Indian Science Congress, 1951.