with ammonium sulphate, dialysis against running water, chromatography (twice) on tricalcium phosphate-'Supercel' columns² and finally, except in the case of C-phycoerythrin, by crystallization from ammonium sulphate solution. Dinitrophenylation of the phycoerythrins was performed by Sanger's method³.

Only one DNP-amino-acid was visible on paper chromatography of ether extracts of diluted hydrolysates of the DNP-phycoerythrins. The chromatograms were run on Whatman No. 1 paper and in a variety of solvent systems, including those described for DNP-amino-acids by Fraenkel-Conrat *et al.*⁴. Freshly prepared DNP-derivatives of the amino-acids which have been reported present in phycoerythrins were also chromatographed in these systems. Of these, only DNP-methionine behaved identically with the DNP-amino-acid from R-phycoerythrin in our six solvent systems. The identity of the two was confirmed by co-chromatography, and by alkaline hydrolysis of the DNP-derivatives⁵. The free amino-acid formed on hydrolysis was identified as methionine by paper chromatography in butanol/acetic acid/water (4 : 1 : 5), followed by development with ninhydrin. A small quantity of methionine sulphone, which was also present on the paper, was assumed to result from oxidation of methionine during hydrolysis. R-phycoerythrin, which had been oxidized with performic acid, yielded DNP-methionine sulphone after dinitrophenylation and hydrolysis; identification was by paper chromatography in four solvent systems with an authentic sample of DNP-methionine sulphone.

These results, which were not altered by further initial purification of the chromoprotein, show that methionine is the N-terminal amino-acid of R-phycoerythrin⁶. We have found by the same methods that methionine is also the N-terminal residue of B- and of C-phycoerythrins.

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Chromatography of Some Phenolic Amines

LITTLE work has, as yet, been published dealing with the paper chromatography of urinary phenolic amines. Solvent systems used have been isopropanol-ammonia¹ and *n*-butanol-ammonia or -methylamine^{2,3} for the first direction, followed by *n*-butanol-acetic acid¹⁻³. Such systems, however, give poor resolutions, particularly in the group of amines derived from phenylethylamine, and have little potential use except for revealing grossly abnormal excretions of the few compounds that can be un-

ambiguously identified: probably the demonstration of increased excretion of metanephrine and normetanephrine in phaeochromocytoma³ or stress represents the limit of their usefulness.

Further investigations in these laboratories indicate that the general separation of phenolic amines thus far identified in human urine (Table 1) from each other and from other diazo-reacting amines requires the use of *tert*-amyl alcohol/17 per cent aqueous methylamine (4 : 1) (solvent A) in the first direction; on Whatman paper No. 1 a 10-in. overnight ascending run must be carried out at about 32°C. Two solvent systems have been found to be particularly suitable for development in the second direction at about 21°C. The first of these, solvent B, is an example of the type *sec*-butanol/aqueous pyridine buffer (4 : 1); the buffer solution, pH 4, has the composition water 100 : pyridine 10, acetic acid 41 by volume. The second, solvent C, has the composition nitroethane/70 per cent aqueous acetic acid (9 : 4). For 10-in. ascending runs about 16 hr. (B) and 3 hr. (C) are required. Approximate distances travelled by authentic amines under the conditions outlined above are indicated in Table 1.

Table 1. MOBILITIES OF AMINES IN SOLVENTS A, B AND C

Amine	A	B	C
Tyramine (ref. 1)	166	100	121
<i>m</i> -Tyramine (ref. 1)	150	103	131
<i>o</i> -Tyramine (ref. 1)	191	125	153
3-Methoxytyramine (refs. 2 and 3)	130	73	152
<i>p</i> -Octopamine (refs. 2 and 4)	69	70	70
<i>p</i> -Sympatol (refs. 2 and 4)	116	88	124
Normetanephrine (refs. 1, 2 and 3)	51	41	89
Metanephrine (refs. 1, 2 and 3)	96	64	145
<i>N</i> -Methylmetanephrine (ref. 3)	142	61	188

Figures indicate typical distances run (mm.) measured to centre of spot. For composition of solvents and approximate conditions, see text. Amines applied to paper as hydrochlorides, except *p*-sympatol (tartrate).

Both the combinations A-B and A-C have been applied to the two-dimensional chromatography of urine extracts. Some anomalies have been noted: thus *m*-tyramine may give rise to multiple spots; absolute and relative movements, particularly in C, are somewhat different from the values recorded in Table 1; extensive decomposition of the 4-hydroxy-3-methoxyphenyl amines in C has been observed on occasion. Any disadvantages appear, however, to be small by comparison with the enormously improved resolutions effected. For example, one of a group of very similar compounds, previously provisionally identified as *p*-sympatol², proved almost impossible to identify with certainty in any given urine. It now seems probable that it may be isolated as a single spot using the A-C combination and that other systems used fail to reveal the presence of a contaminating compound which is rather commonly present in urine extracts and which may sometimes be the major constituent of a single mixed spot. This substance shows no evidence of being an artefact from *p*-sympatol and has an *R_F* value slightly less than has that amine in A and distinctly less so in C. Other spots which could have been confused with *p*-sympatol have been observed less frequently and are completely separated from it in either of the systems A-B or A-C.

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