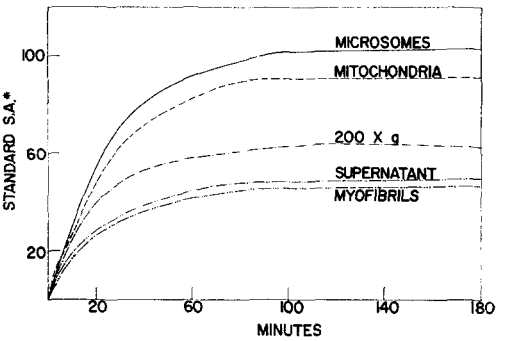


Fig. 1. The rate of uptake of DL-leucine-1-$^{14}$C by the particulate fractions of rat skeletal muscle.

* Standard specific activity. This is the specific activity obtained corrected for the variations in rat weight and in c.p.m. injected, and was calculated as follows:

$$\text{c.p.m. per } \mu\text{mole of protein-bound leucine} \times \frac{\text{rat wt in g}}{200} \times \frac{0.01 \cdot 10^6}{\text{c.p.m. of leucine injected}}.$$

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## Some new solvent systems for the paper chromatography of nucleic acid degradation products

Paper chromatographic methods have proved indispensable for the investigation of the components of nucleic acids[1]. Among the many solvent systems suggested the most generally useful have been the *tert*butanol and *iso*propanol-hydrochloric acid mixtures of MARHAM AND SMITH[2] and of WYATT[3]. These solvent systems have the disadvantage of being somewhat slow in development and it has been found that equally satisfactory results can be achieved with mixtures of methanol, ethanol and hydrochloric acid.

Chromatography has been carried out by upward development on paper strips 16.5 cm × 47 cm. Two holes were punched in each paper and five papers were hung on an all-glass frame which was placed in a glass tank sealed with a plate glass lid.

A mixture of methanol: concentrated HCl: water (70:20:10) (all parts by volume) (Solvent system I) was found to separate the principal bases liberated by complete hydrolysis of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). This solvent system would also separate guanine, adenine, cytidylic and uridylic acids which were liberated when ribonucleic acids were hydrolysed with *N* HCl at 100° C. The chromatograms obtained have been used for quantitative estimations. The solvent rose about 40 cm on Whatman No. 4 paper in 16 hours and Whatman No. 1 gave equally good results.

This solvent system was rather slow in drying and much less hydrochloric acid was needed to separate the products of the hydrolysis of RNA with *N* HCl at 100°. The most suitable system of those tried was a mixture of methanol: ethanol: concentrated HCl: water (50:25:6:19) (Solvent system II). This solvent system moved about the same distance overnight as solvent system I and was the most suitable for quantitative determination. It dried very quickly and gave reproducible and very low blank values. Guanine was eluted with 0.5 *N* HCl, adenine with 0.1 *N* HCl and cytidylic and uridylic acids were eluted with 0.2 *M* sodium acetate. Absorption values were measured at 249, 260, 269.5 and 261 mμ for the four components respectively. The ε values of WYATT[3] were used for guanine (11,000) and adenine (13,000) and values of 9050 and 10,200 were found for cytidylic and uridylic acids at the above wavelengths in 0.2 *M* sodium acetate.

A further series of solvent systems has been investigated and it was found that very little HCl was necessary if a large proportion of acetic acid was present. A mixture of methyl ethyl

ketone: *tert*-butanol: glacial acetic acid: water: concentrated HCl (22.5: 22.5:35:19:1) (Solvent system III) was the most suitable for separating the hydrolytic cleavage products of ribonucleic acids. This mixture moved about 36 cm in 16 hours on Whatman No. 4 paper and had the advantage for 2-way chromatography that the positions of adenine and cytidylic acid were the reverse of those in solvent systems I and II.

The $R_F$ values of bases, nucleosides and nucleotides in solvent systems I, II and III on Whatman No. 4 paper are shown in Table I.

TABLE I

| | *Rf values in the solvent systems* | | |
|---|---|---|---|
| | *I* | *II* | *III* |
| Guanine | 0.18 | 0.20 | 0.19 |
| Adenine | 0.31 | 0.33 | 0.39 |
| Cytosine | 0.42 | 0.30 | 0.39 |
| Uracil | 0.62 | 0.58 | 0.67 |
| Thymine | 0.70 | 0.64 | 0.75 |
| Guanosine | 0.31 | 0.24 | 0.22 |
| Adenosine | 0.36 | 0.31 | Trailed |
| Cytidine | 0.48 | 0.40 | 0.29 |
| Uridine | 0.64 | 0.58 | 0.58 |
| Guanosine-3′-phosphate | 0.43 | 0.38 | 0.22 |
| Adenosine-3′-phosphate | 0.51 | 0.42 | 0.31 |
| Cytidylic acid | 0.60 | 0.53 | 0.26 |
| Uridylic acid | 0.77 | 0.70 | 0.54 |

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## Is foetal haemoglobin present in the blood of normal human adults?

The haemoglobin of the new-born child consists for about 80% of Hb-F and 20% of Hb-A. After four months the foetal component seems to have disappeared. BRINKMAN AND JONXIS[1] used the difference in the rate of alkali-denaturation, but did not succeed in demonstrating the presence of foetal haemoglobin in the blood of older children and adults. The method used, however, was not accurate below 5% of Hb-F present. Later, SINGER *et al.*[2] demonstrated with an altered alkali denaturation technique (starting with $HbO_2$) the presence of 1–3% of a foetal-like component in the blood of adults, while KÜNZER[3] with a similar procedure, but using cyanhaemoglobin, found 0.5–1% of such a component. With immunological methods CHERNOFF[4] demonstrated the presence of 0.05–0.5% of Hb-F, while moreover ROCHE AND DERRIEN[5] with a salting-out technique found small amounts of a component with the same solubility as that of Hb-F from cord blood. The amounts of this component found by these methods are so different that it seemed desirable to repeat the investigation concerning the problem of the possible presence of Hb-F in adult blood. Moreover it may be important to investigate whether the alkali-resistant fraction found was identical with foetal haemoglobin. For this purpose we used in our investigation the estimation of the amino acid composition of this fraction, as there are many differences between Hb-F and the other haemoglobins in this respect[6].

In the present study blood samples of the authors were used. The haemoglobin was prepared and purified by the method described previously[6]. Three samples of $HbO_2$ (no monocarboxyform) with a concentration of 6–8 mg % (total amounts 5320, 4615 and 7670 mg respectively) were exposed to the action of alkali (final pH 12.6) for two minutes. The denaturation process was then interrupted by adding an equimolar amount of hydrochloric acid. The denatured haemoglobin was salted-out by adding a 3.5 *M* phosphate buffer pH 6.5 (= 100%), described by DERRIEN[7], until a final concentration of 80% was reached. After filtration, the unaltered haemoglobin was precipitated by dissolving the calculated amount of pulverized $KH_2PO_4$ and $K_2HPO_4$