

tivity maximum is shifted to a lower temperature, retaining its original magnitude. This explanation, however, would be insufficient to account for the prolonged residual effects observed, if the depression in the permittivity - temperature curve is not taken into account, and without the assumption of a mechanism for the displacement of the Curie point, such as that suggested above.

It is interesting to note in this connexion that the permittivity maximum in this type of material has been reported to occur at lower temperatures in the case of strong a.c. measuring fields than at lower field strengths<sup>7</sup>, and the explanation suggested for the residual effects is consistent also with this result.

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<sup>1</sup> Partington, Planer and Boswell, *Nature*, **160**, 877 (1947).

<sup>2</sup> Roberts, *Phys. Rev.*, **71**, 890 (1947).

<sup>3</sup> Wul, *J. Phys., U.S.S.R.*, **10**, 95 (1946).

<sup>4</sup> Reddish, Plessner and Willis Jackson, *Trans. Farad. Soc.*, **42**, 244 (1946).

<sup>5</sup> Megaw, *Proc. Roy. Soc., A*, **189**, 261 (1947).

<sup>6</sup> Szigeti, B., private communication.

<sup>7</sup> De Bretteville, *J. Amer. Cer. Soc.*, **29**, 303 (1946).

### Chromatography of Dinitrophenyl Amino-acids and Peptides

SANGER<sup>1</sup> has described the use of 1:2:4-fluoro-dinitrobenzene to identify the free amino-groups of several proteins, employing the separation of the N-dinitrophenyl amino-acids on columns of silica gel. We have had only limited success applying this method, owing to variations in different batches of the silica gel, and the difficulty of preparing evenly packed columns, which led to diffuse bands, difficult to separate. Sanger stated that with these derivatives on paper chromatograms<sup>2</sup> long diffuse 'tails' formed, making the method quite inferior to the silica method. We are able to report some success with the two-dimensional method, however, without apparently using any special technique which might account for the improvement. Consden *et al.*<sup>3</sup> have also recently described the identification of  $\alpha$ - $\delta$ -di-dinitrophenyl-ornithine running alone on a paper chromatogram, and Keston *et al.*<sup>4</sup> using the *p*-I<sup>131</sup>-benzenesulphonyl derivatives of amino-acids have been able to estimate them after their partial separation on a one-dimensional paper strip.

We have tried a wide range of alcohols, phenols, amines, esters, ketones and hydrocarbons, all saturated with water, but with most of these 'tailing' was marked. Best results were obtained with *n*-butanol, phenol, collidine and *n*-butanol-acetic acid mixture, each saturated with water. The solution is applied in the usual manner to No. 1 or No. 4 Whatman paper and neutralized with ammonia vapour before the run. No solvent has so far been found which will satisfactorily resolve the group of dinitrophenyl amino-acids, containing large non-polar side-chains; for example, N-dinitrophenyl-leucine, N-dinitrophenyl-phenylalanine,  $\alpha$  $\epsilon$ -di-(dinitrophenyl)-lysine, etc., as the addition of the large

dinitrophenyl-group still further diminishes the differences between these amino-acids. However, provided that only a few of this group are present, identification by running with the added authentic derivative is possible. 5  $\mu$ gm. of a dinitrophenyl acid will give a good spot on a one-dimensional strip, but 20  $\mu$ gm. may be required on a two-dimensional sheet, as the yellow colour becomes difficult to see after two traverses. Ultra-violet examination<sup>5</sup> shows the spots as pale yellow when weak, and as obscuring dark patches in larger quantities. There is no actual fluorescence.

Some months ago, Sanger (private communication) reported that he had separated some dinitrophenyl peptides on silica columns in a similar manner to his dinitrophenyl amino-acid separations. We find that if the hydrolysis of the dinitrophenyl peptide or protein is incomplete, extra yellow spots appear, which do not correspond to the position of the dinitrophenyl amino-acids. Further hydrolysis of such materials after elution has shown them to have been dinitrophenyl peptides, of size up to heptapeptides. This good spot formation with heavy materials extends to those of very large molecular weight. For example, the dinitrophenyl derivative of the 'protein' part of insulin of molecular weight 5,000 left after chymotryptic digestion<sup>6</sup> (before addition of dinitrophenyl groups) runs in phenol-water as a good spot with *R<sub>F</sub>* 0.56. This material and also the mixture of relatively small peptides (average, pentapeptide) from the insulin digest, before treatment with fluorodinitrobenzene, either gave no bands on paper chromatograms in the case of the heavy insulin 'core', or a diffuse overlapping mixture of bands in the case of the peptide mixture.

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<sup>1</sup> Sanger, F., *Biochem. J.*, **39**, 507 (1945).

<sup>2</sup> Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).

<sup>3</sup> Consden, R., Gordon, A. H., Martin, A. J. P., and Synge, R. L. M., *Biochem. J.*, **41**, 596 (1947).

<sup>4</sup> Keston, A. S., Udenfriend, S., and Levy, M., *J. Amer. Chem. Soc.*, **69**, 3151 (1947).

<sup>5</sup> Phillips, D. M. P., *Nature*, **161**, 53 (1948).

<sup>6</sup> Butler, J. A. V., Dodds, E. C., Phillips, D. M. P., and Stephen, J. M. L., *Biochem. J.*, **42**, 116 (1948).

### Influence of Hydrogen Ions and Neutral Salts upon the Hydration of Interstitial Connective Tissue

WHEN a piece of interstitial connective tissue covering the thigh muscles of a rat was placed in water adjusted to pH 4.5, within a few hours it became opaque, white and shrunken. On the other hand, a piece treated at pH 11.0 became translucent, grey and swollen. These changes were mutually reversible and could be repeated several times. Observations with the dark-ground microscope showed that this swelling was not due to any change in the collagen fibrils but to a widening of the spaces between them; shrinkage was accompanied by an obliteration of such spaces and by a condensation and crimping of the collagen bundles.

Clearly, an explanation of these changes must involve a consideration of the nature of the substance which lies between the collagen fibrils. Flemming<sup>2</sup>