

(3) *Chlorine dioxide*. The spectrum of chlorine dioxide, which is mainly diffuse in the glassy solvent, is rapidly destroyed and replaced by a second diffuse spectrum the position of which agrees exactly with that of the ClO radical<sup>3</sup>. It has been shown<sup>4</sup> that the flash photolysis of gaseous chlorine dioxide results in the formation of ClO, the life-time of which at comparable concentrations to ours is only a few milliseconds.

(4) *Aromatic compounds*. Porter and Wright have recently shown that the gas-phase photolysis of many single-ringed aromatic molecules results in the appearance of transient banded spectra with life-times less than  $10^{-4}$  sec., which are attributed to free radicals such as benzyl<sup>5</sup>. We have observed similar spectra from the same series of compounds for several hours after photolysis in rigid solvents. These spectra vanished completely on warming the glass, and were therefore readily distinguished from the spectra of permanent products.

In all these examples the spectra were observed, with undiminished intensity, several hours after photolysis; they were removed by softening the glass and did not reappear on cooling.

This method should have many applications. It may be used for trapping the primary products of photochemical or radiation chemical processes for identification. It makes possible the measurement of properties such as infra-red spectra of free radicals, which has not hitherto been possible owing to the need for rapid recording. Bearing in mind the possibility of using still lower temperatures and other solvents, the method should be applicable to practically all free radicals which can be produced by photolysis.

Full details of our techniques and results will be submitted for publication in the *Transactions of the Faraday Society*. One of us (I. N.) is grateful to the Fulbright Commission for a fellowship during the tenure of which this work was carried out.

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<sup>1</sup> Lewis, G. N., and Lipkin, D., *J. Amer. Chem. Soc.*, **64**, 2801 (1942).

<sup>2</sup> Porter, G., *Proc. Roy. Soc., A*, **200**, 284 (1950).

<sup>3</sup> Porter, G., *Dis. Farad. Soc.*, **9**, 60 (1950).

<sup>4</sup> Lipscomb, F. J., Norrish, R. G. W., and Porter, G., *Nature* (in the press).

<sup>5</sup> Porter, G., and Wright, F. J. (in course of publication).

### Anhydrous Trifluoroacetic Acid as a Solvent for Proteins

It has recently been reported<sup>1</sup> that liquid anhydrous fluoride is a powerful solvent for a wide variety of proteins. It has now been found that the lower perfluoro-aliphatic carboxylic acids possess solvent properties for proteins very similar to those of hydrogen fluoride. Thus it has been observed that trifluoroacetic acid (m.p.  $-15.6^{\circ}\text{C}$ ., b.p.  $72^{\circ}\text{C}$ .) readily dissolves the proteins lysozyme, ribonuclease, trypsin, pepsin, crystallized egg albumin, bovine plasma albumin, bovine plasma globulin, edestin, peanut protein globulin, casein, zein, urease, uricase, blood fibrin, insulin, silk fibroin, hide collagen and rat-tail tendon. Solutions in the concentration-range 20–30 mgm./ml. of the above proteins can easily be prepared at  $25^{\circ}\text{C}$ . Solutions of proteins in trifluoroacetic acid prepared *in vacuo* are clear and colourless; on exposure to air, a purple colour

develops. Cytochrome *c*, haemoglobin and catalase are also soluble; solutions of these iron-containing proteins are browner in colour than are the corresponding solutions in hydrogen fluoride.

The physical properties of trifluoroacetic acid are such that dissolved proteins can be readily recovered either by direct evaporation or by lyophilization. Of the proteins listed above, ribonuclease, bovine plasma albumin, trypsin, lysozyme, insulin, cytochrome *c*, haemoglobin, hide collagen and rat-tail tendon are recovered in a water-soluble condition. Recovered bovine plasma albumin dissolves readily in distilled water; the pH of such a solution can be adjusted to neutrality without precipitation of protein, and the recovered material yields a pattern on ultracentrifugation identical with that of the starting material. Cytochrome *c* recovered from trifluoroacetic acid gives a typical ferricytochrome spectrum in aqueous solution; this solution can readily be reduced to ferrocyanochrome *c* with sodium hydrosulphite. Cytochrome *c* recovered from hydrogen fluoride, on the other hand, although still water-soluble, can neither be oxidized nor reduced.

Perfluoropropionic and perfluorobutyric acids behave like trifluoroacetic acid except that proteins are dissolved at a much slower rate, especially by perfluorobutyric acid. The lower perfluoro-aliphatic carboxylic acids are all reasonably volatile and can be handled in ordinary glass apparatus. They therefore pose much less serious experimental problems than does the manipulation of hydrogen fluoride. The experiments described here were all carried out on a conventional glass vacuum line with the solvents transferred by vacuum distillation.

As in the case of hydrogen fluoride, these media lend themselves to the carrying out of chemical reactions on proteins, and reactions on proteins using nitrogen dioxide, hydrogen peroxide and trifluoroacetic acid anhydride, as well as other reagents have been carried out. It has further been observed that hydrogen fluoride and trifluoroacetic acid are miscible in all proportions with each other and with sulphur dioxide in the liquid phase. While most proteins are substantially insoluble in pure liquid sulphur dioxide, mixtures of sulphur dioxide and hydrogen fluoride, or sulphur dioxide and trifluoroacetic acid are very good solvents, even when the sulphur dioxide is present in large excess. These mixed solvent systems may be of special interest in protein fractionations and in the study of other high polymeric materials.

A detailed description of these experiments is being prepared for publication elsewhere.

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<sup>1</sup> *Nature*, **173**, 265 (1954); *Arch. Biochem. Biophys.* (in the press).

### Histology and Chemistry of Keratin Formation

KERATINIZATION, by the holocrine transformation of epithelial cells, occurs normally in the mammalian epidermis, oesophagus, oestrous vagina, and thymic corpuscles. It accompanies abnormal epithelial transformations after oestrogen administration (in genital organs), in vitamin A deficiency, consequent to trauma (including radiation), and in certain cancers. The etiology of keratinization, which represents differentiation by organized cell deaths,