dilute solutions should prove of considerable interest in the theoretical interpretation of the Soret effect.

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[1] de Groot, *J. Phys. et le Rad.*, **8**, 129 (1947).
[2] Tyrrell and Hollis, *Trans. Farad. Soc.*, **48**, 893 (1952).
[3] Haase, *Trans. Farad. Soc.*, **49**, 724 (1953).
[4] Colledge, thesis, University of Sheffield (1953).
[5] cf. Tanner, *Trans. Farad. Soc.*, **23**, 75 (1927); **49**, 611 (1953).

Anhydrous Hydrogen Fluoride as a Solvent for Proteins

Anhydrous hydrogen fluoride (m.p., − 83° C.; b.p., 19·5° C.) has been found to be a powerful solvent for a wide variety of proteins. Experiments were carried out in transparent tubes moulded or machined from polychlorotrifluoroethylene polymer; these tubes containing the intensively dried protein samples could be attached to a metal vacuum system, hydrogen fluoride added by distillation, and direct visual observations made in the absence of moisture. Among proteins which are readily soluble in hydrogen fluoride at 0° are ribonuclease, lysozyme, pepsin, trypsin, crystallized egg albumin, bovine plasma albumin, bovine plasma globulin, edestin, peanut protein globulin, casein, zein, silk fibroin, hide collagen, insulin and adrenocorticotrophic hormone. These are all soluble at least to the extent of 20 mgm. per ml., and much more concentrated solutions can in general easily be prepared. Thus, a solution containing 350 mgm. per ml. bovine plasma albumin is still quite fluid at 0° C. The solutions are clear, colourless to faint amber, and are very much less viscous than corresponding aqueous solutions. The globular proteins dissolve rapidly even at − 78° C., whereas silk fibroin and hide collagen undergo strong swelling and require many hours at room temperature for complete solution.

The iron-containing proteins cytochrome *c*, catalase and hæmoglobin are very soluble in hydrogen fluoride. They give pink solutions which have absorption spectra very similar to those exhibited in water. The ability of metal-complex compounds to exist in hydrogen fluoride appears to be a general phenomenon, for it is found that metal phthalocyanines, cobalt(III)amine-complex compounds, and a considerable variety of other metal-complex compounds dissolve in hydrogen fluoride without precipitation of metal fluoride.

Proteins were recovered by evaporation of the solutions on the vacuum line. Films of recovered silk fibroin and collagen gave characteristic polypeptide infra-red spectra. Of the proteins mentioned above, ribonuclease, lysozyme, trypsin, cytochrome *c*, hæmoglobin, hide collagen, insulin and adrenocorticotrophic hormone are recovered in a water-soluble state after remaining in hydrogen fluoride solution for 2 hr. at 0° C. Recovered insulin was found by bioassay on mice to have retained 80 per cent of its initial activity, and similarly adrenocorticotrophic hormone was recovered intact with full retention of its original biological activity. The biological properties of the other water-soluble proteins after recovery remain to be examined. The surprising retention of biological activity by insulin and adrenocorticotrophic hormone after dissolution and recovery from hydrogen fluoride solution suggests that this solvent may have real significance in protein studies.

Because of its low freezing point, liquid hydrogen fluoride may be an interesting medium for carrying out chemical reactions on proteins at very low temperatures. For example, it has been observed that ozone, which appears to be quite soluble in hydrogen fluoride, changes a pink hæmoglobin solution to green. Hydrogen peroxide solutions in hydrogen fluoride (prepared by addition of the 1 : 1 urea–hydrogen peroxide addition compound) change the pink colour of cytochrome *c* and catalase solutions to yellow. Trioxane, which has been found to be highly soluble in hydrogen fluoride, gives a typical formaldehyde reaction when added to a protein dissolved in hydrogen fluoride; the viscosity of the solution increases until a rigid gel results. These and other characteristic protein reactions with many of the usual protein reagents can thus be carried out at much lower temperatures than has been possible in aqueous systems.

Details of these experiments will appear elsewhere.

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Formation of a Mixed Disulphide of Cysteine and Glutathione

In our studies on the synthesis of glutathione by *Saccharomyces cerevisiae*, the yeast was incubated in 0·01 *M* potassium dihydrogen phosphate with cysteine containing sulphur-35. Samples of the mixture were removed at intervals and the component amino-acids and peptides were separated by paper chromatography. The chromatograms were sprayed with a ninhydrin solution and autoradiograms of the chromatograms were made on Kodak No-screen X-ray film.

When a phenol–water mixture was used for preparing a descending chromatogram, a spot which contained a considerable amount of sulphur-35 was observed. This substance (R_F = 0·27) was intermediate between glutathione disulphide (R_F = 0·16) and cystine (R_F = 0·42). It was found in largest amounts in the mixture of yeast and sulphur-35 cysteine immediately after the cysteine had been added to the yeast and before it had been metabolized by the cells to any extent. Since both cysteine and glutathione are oxidized to the disulphides during chromatography under our conditions, it seemed possible that the new substance was a mixed disulphide of cysteine and glutathione formed not by the yeast but by oxidation by the air. The intermediate compound was shown, indeed, to be a disulphide by the use of Toennies's reagent[1].

Further evidence supporting the view that this compound was a mixed disulphide of cysteine and glutathione and that it was formed in the absence of yeast cells was obtained by mixing cysteine and glutathione in aqueous solution in the absence of yeast cells and also by using cysteine or glutathione labelled with radioactive sulphur. A solution of sulphur-35 cysteine was mixed with non-labelled glutathione in the absence of yeast cells, and this mixture was chromatographed immediately after being prepared.