

leaves makes it probable that radioactive urea is hydrolysed by urease in the leaves of the *Coffea* tree. A more detailed report of these experiments will be published later².

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Solubilization of Keratins by Reaction with Ethylene Oxide

THE reactions of keratins and other proteins with epoxides have been described by several investigators¹. The chemical modification of wool by reaction with mono- or di-functional epoxides in aqueous or alcoholic solution at or near room temperature has also been studied. In general, the acid and alkali solubility is decreased by reaction with di-functional epoxides, but increased if a monofunctional epoxide is employed². We have recently found, however, that quite different results are obtained if wool is heated with ethylene oxide and a small amount of *N,N*-dimethylaniline with no solvent, as described by Haas *et al.*³ for making hydroxy-ethyl nylon. Under these conditions, the wool fibres are disintegrated and the product is soluble in water. Part of the ethylene oxide does not react and part is converted to polyethylene glycol which can be extracted from the aqueous solution with methylene chloride or toluene, although the latter solvent forms troublesome emulsions which are much more persistent than those formed by methylene chloride. A very small part of the ethylene oxide apparently becomes attached to the solubilized wool.

As an example, 0.960 g wool cloth was sealed in a glass tube with 10 ml. ethylene oxide and 0.1 g *N,N*-dimethylaniline in a nitrogen atmosphere (no special precautions were taken to include or exclude moisture) and heated at 80° C for 25 h. The viscous, syrupy reaction product was washed out of the tube with water, and the resulting aqueous solution was exhaustively extracted in a continuous extractor with methylene chloride. The syrupy extract weighed 6.2 g. The aqueous phase was clarified by centrifuging; the residue in the centrifuge tube, on drying, gave a solid that weighed 2.9 mg. The weight of the water-soluble, methylene chloride-insoluble fraction, estimated by drying an aliquot to constant weight, was 1.724 g. John J. Bartulovich, of this laboratory, found by ultracentrifugation (in a preliminary determination by the Archibald method) that the material not extracted from the aqueous solution by methylene chloride had a weight average molecular weight of about 10,000. Wilfred H. Ward, also of this laboratory, found that the material passed through a dialysis membrane, which indicated that the molecular weight cannot greatly exceed 10,000. The precise extent of degradation of the protein thus remain to be determined.

Other fibrous proteins solubilized by this procedure are: mohair, ovokeratin, cattle horn, and silk fibroin. In one trial with fibroin, complete solubilization was obtained, but in others only one-fifth or one-fourth of the silk was solubilized. The insoluble silk remaining after the reaction with ethylene oxide and *N,N*-dimethylaniline retained the fibrous appearance and structure.

When wool that has received a polyamide treatment applied by the interfacial polymerization procedure⁴ is used, the wool is solubilized and the polyamide sheath is undissolved.

Work is in progress on the nature of the water-soluble reaction product resulting from the reaction of ethylene

oxide with the protein, and the procedure may be useful in fundamental keratin studies.

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Estimation of Urinary Oxalate by the Method of Isotope Dilution

DURING the course of a series of metabolic experiments in which 1-¹⁴C-ascorbic acid and 1-¹⁴C-glycine were fed to a normal adult¹ and hyperoxaluric patients² daily samples of urinary oxalic acid were isolated as calcium oxalate for estimation of the ¹⁴C enrichment. It was found expedient to determine the daily levels of excretion by the method of isotope dilution, using the specific ¹⁴C-activity of the oxalate samples prior to combustion to CO₂ for mass spectrometric analysis. The amounts of ¹⁴C and ¹²C added were low and hence did not interfere with the measurement of ¹²CO₂. Previously reported methods for calcium oxalate estimation have the disadvantage that they depend on the complete recovery of oxalate from a sample of the urine³⁻⁷. Using the method of isotope dilution, however, the only exacting requirement is the purity of the final product, which is checked by repeated reprecipitation to constant specific activity.

Twenty-four-hour urine collections were made in 40 ml. of 6 N HCl, and after the addition of 1 : 2-¹⁴C-oxalic acid (1 μc., 0.2 μg) sufficient concentrated HCl was added to bring the concentration to 2 N and the whole was continuously extracted for 16 h with peroxide-free ether. The ether phase was evaporated in the presence of water (5 ml.) and the aqueous residue was treated with 0.880 NH₄OH (1–2 ml.). Saturated CaCl₂ (0.5 ml.) was added and the precipitated (COO)₂ Ca.H₂O was purified by solution in 2 N HCl (5–7 ml.), decolorization with 'Norit' and precipitation with 0.880 NH₄OH and acetic acid at pH 5.2 (brom-cresol purple). Samples were counted by the liquid scintillation technique (Nuclear Chicago Instrument, model 725) using 100-μl. aliquots (taken from the HCl solution prior to re-precipitation) and a dioxan-based phosphor (10 ml. 'Panax', type T.E.D.). At the concentration used (0.1–0.5 mg of (COO)₂ Ca.H₂O) no quenching due to calcium oxalate was observed, and the counting efficiency was 51 per cent. The disintegrations per min were calculated from an efficiency curve established by adding different volumes (25–300 μl.) of 2 N HCl to a known radioactive standard (Packard, Standard Source Toluene ¹⁴C) in 10 ml. of phosphor and applying the 'Channels Ratio' method⁸. The purification process was repeated until the specific activity remained constant (usually three re-precipitations were adequate). In some cases the purity of the final product was also checked by dissolving 1–2 mg in 2 N H₂SO₄ (5 ml.) and determining the oxalic acid by the method of Hodgkinson and Zarembski⁴. In any isotope dilution analysis the total amount of substance present is inversely proportional to its specific activity and the amount of urinary calcium oxalate was calculated from its specific activity, compared with that of a standard prepared from the same 1 : 2-¹⁴C-oxalic acid.

During metabolic experiments lasting 19 and 15 consecutive days², a normal adult gave daily (COOH)₂.2H₂O excretions of 41.8 mg. (*S.D.* ± 4.6 mg) and 26.7 mg (*S.D.* ± 4.6 mg) respectively, whereas a hyperoxaluric