The tetracycline was localized as a yellow spot by spraying the chromatogram with 1N hydrochloric acid followed by heating at 50° C for a few minutes.

Antipyrine-^3H.

150 mg of antipyrine was labelled by the procedure described above at tritium pressure of 3.8 mm Hg (400 mCi). After removing labile tritium by dissolving the product three times in methanol followed by evaporation to dryness under reduced pressure, antipyrine was found to be pure without any further purification.

Yield : 90 mg (60 %).
Melting point : 109-110° C (uncorr.).
Specific activity : 350 μCi/mg (66 mCi/mmole).
Radiochemical yield based on tritium gas : 7.1 %.

Thin-layer chromatography on Eastman-Kodak Silicagel-G sheet, using methanol as developing solvent and Dragendorff sprayreagent to localize antipyrine, showed a single radioactive peak. No decomposition could be observed after keeping the product at room temperature for five months in powder form.

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Preparation of ^3H-Acetyltrypsin

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In response to a need for labelled trypsin, the pancreatic proteolytic enzyme which catalyzes the hydrolysis of peptide bonds between the carboxy group of arginine or lysine and the amino group of another amino acid, its ^3H-acetyl derivative has been prepared. Acetyltrypsin shows similar substrate

specificity to the parent enzyme [1, 2] and it was hoped that use of the ^{3}H labelled acetyl enzyme would serve as a method for its localization in tissue sections by autoradiography.

The buffer solution used throughout the preparation contained sodium chloride (8.0 g), potassium chloride (0.2 g), potassium dihydrogen orthophosphate (0.2 g), *di*-sodium hydrogen orthophosphate (1.14 g) per litre of solution. The pH of this solution was 7.2. The pH of a portion of such a solution was adjusted, by the addition of solid potassium dihydrogen orthophosphate to 6.7 for use in the acetylation.

Bovine pancreatic trypsin (Worthington Biochemical Corporation preparation TRL), 50 mg, was dissolved in buffer (5.0 ml, pH 6.7) at 2-4° C. This solution was employed to transfer acetic anhydride-T (50.4 mg, 200 mC/mmole) from an ampoule to a 25 ml beaker cooled in ice. Several transfers were made to ensure complete removal of the acetic anhydride-T from the ampoule. The resulting mixture was rapidly stirred. Fall in pH of the solution was prevented by the automatic addition of cold 0.1 N sodium hydroxide solution from an autotitrator. After thirty minutes (by which time the pH of the solution had stabilised) the reaction solution was transferred to a dialysis sac and dialysed against buffer (5l, pH 7.2 at 4° C). The sac and contents were transferred at intervals of 24 hours to freshly prepared buffer solutions. In all, twenty litres of buffer solution were required to remove the unbound radio-active products. The volume of the solution contained in the sac after dialysis was 15.5 ml. Aliquots (0.01 ml) of this solution were removed for liquid scintillation counting. The remainder was lyophilized and stored with the buffer salts at −20° C. The specific activity of the ^{3}H-acetyltrypsin was 1.4 C/mmole.

Biological experiments with this labelled material will be reported elsewhere.

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