When phenyl magnesium bromide or benzyl magnesium chloride was carbonated with inactive carbon dioxide according to this method at the 1.0 mmole level, almost quantitative yields of benzoic and phenylacetic acid were obtained. Phenylacetic-1-^{14}C acid was prepared in 98 % yield (crude product, m.p. 69—74°) from 1.00 mmole of barium carbonate^{14}C.

1. Murray, III, A. and Williams, D. L. *Organic Syntheses with Isotopes, Part I*, Interscience, New York 1958, p. 86.
2. Ronzio, A. R. in Weissberger, A., Ed., *Technique of Organic Chemistry, Volume VI, Micro and Semimicro Methods*, Interscience, New York 1954, p. 391.
3. Weygand, F. and Simon, H. in *Methoden der Organischen Chemie (Houben-Weyl), Band IV, Teil 2, Allgemeine Chemische Methoden*, Thieme, Stuttgart 1955, p. 643.

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Microdetermination of Protein with Radiocopper and Gel Filtration

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In order to make possible the determination of minute amounts of protein we have developed the following "radiobiuret" techniques:

Sephadex (G-25, coarse) is suspended and allowed to swell in 0.75 N NaOH and packed in the form of a column (diameter up to 1 cm, length about 20 cm). The column is washed overnight with NaOH to remove traces of protein. It is arranged that the protein to be determined is contained in 0.75 N NaOH in a convenient volume (*e.g.* 1—5 ml). To this is added an excess amount (up to 0.5 mg) of radioactive copper with as high specific activity as possible, *e.g.* 1 ml of an aqueous 1:25 dilution of the commercial $^{64}CuCl_2$ solution. All dilutions are made with redistilled water and observing meticulous cleanliness. The mixture is incubated 90 min at room temperature, a measured volume is introduced onto the column followed by NaOH until 25 ml of eluate has been collected. The radioactivity of the eluate is then determined with a scintillation counter. Between samples, the column is washed with NaOH. Known amounts of a standard protein and blanks are run in the same way before and after the unknown. The amount of protein in the unknown sample can be read from the resulting standard curve.

The unbound copper will be retained as the hydroxide in the upper parts of the column, but owing to the low concentration of copper in the original reaction mixture no precipitation will occur there. The determination can also be performed with larger amounts of protein by reading the emerging biuret colour photometrically. However, the free copper will then precipitate, but this can be prevented by complexing the copper ion with citrate as is done in the classic biuret method. When such a mixture is introduced into the column, copper hydroxide will be formed and retained in the upper parts (amino acid complexes are also dissociated). A straight-line relationship *amount of protein/eluted radioactivity* (corrected for radioactive decay) is observable down to at least 1 μg of protein applied onto the column in 5 ml volume, but owing to the obvious advantages of the following method we have made no attempts to reduce further the lower limit of this method.

The reaction may also be performed in another way which enables it to be used for automatic analysis, *e.g.* in continuous registration of protein eluted from chromatographic columns. The chromatographic eluent must first be alkalinized either by the continuous addition of NaOH with a pump or by automatic pH adjustment using a titrator. The mixture is then led to a Sephadex column containing an upper layer of a mixture of radiocopper hydroxide and Sephadex. This can be prepared in a beaker by mixing $^{64}CuCl_2$ with a citrate-carbonate solution (proportions as in Benedict's reagent), pouring the mixture on moist alkaline Sephadex and by shaking until all the copper is adsorbed. The slurry is poured onto the top of a short Sephadex column and the column is then washed with a large volume of NaOH. If desired, the copper hydroxide level can be raised by sucking up acid from below. When the desired level has been reached, NaOH is run in the other direction.

The eluate from the Sephadex column is passed through a glass tube spiral kept in the well of a scintillation crystal fixed in a

counter. The radioactivity is registered with a ratemeter, preferably provided with a pulse-height analyzer, which keeps the background low and makes possible the analysis of the protein content of radioactive eluates, in our case macromolecular ^{57}Co-cyanocobalamin complexes. The readings of the ratemeter are registered graphically with a recorder. Before use, the column is washed with NaOH until the background reading becomes low. Using this system without a pulse-height analyzer we have found 0.45 μg of bovine albumin introduced in 1 ml volume to give noticeable peaks. With the use of better equipment and high-specific activity ^{64}Cu (not available to us due to the distance from the foreign manufacturer) it should be possible to detect still smaller amounts of protein. This system is more sensitive than the preceding one, since by this method the peak radioactivity values are recorded. For practical purposes the method is insensitive to ammonia, since only concentrated solutions elute radioactivity.

The Sephadex functions as a convenient filter and supporting medium, which effectively removes the originally dissolved copper as hydroxide and dissociates small-molecular copper complexes, and which can be prepared rapidly. The main disadvantage of the method is the short half-life of the copper isotope. A longer-lived isotope exists (^{67}Cu), but is not commercially available. On the macro scale our system also works with nickel, but the usual isotope ^{63}Ni emits solely soft beta radiation and is available only in low-specific activity form. Other metals which were tried gave disappointing results.

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Isothiocyanates XLIV *. The Isothiocyanate Glucoside (Glucocapparin) in *Crataeva Roxburghii* R.Br. *(Capparidaceae)*

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The tree *Crataeva Roxburghii* R.Br. (syn. *C. religiosa* Forst.) of the family *Capparidaceae* grows abundantly in the plains of India and affords a popular local remedy in the treatment of several physical and mental disorders. Of particular interest in connexion with current studies in this laboratory is the paper by Lahiri [1] according to which J. S. Chatterjee from "decomposed" bark of *C. Roxburghii* isolated a crystalline compound, $C_{15}H_{16}N_2S$, reported to be obtainable also from *Moringa pterygosperma* and horse-radish on similar treatment. The compound was demonstrated to be of considerable promise in the clinical treatment of cholera [1]. More recently, Chakravarti [2] established its structure as 1,3-dibenzylthiourea on basis of degradation experiments and comparison with a synthetic specimen **. Unfortunately, Chatterjee's isolation procedure never seems to have been published (*cf.* Ref.[2]). Hence, repetition under exactly the same conditions is not possible. In view of the present knowledge, however, it appeared likely that 1,3-dibenzylthiourea might originate from benzyl isothiocyanate, derivable from glucotropaeolin, a glucosidic progenitor widely distributed in the plant kingdom [4].

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** The same thiourea has been previously reported [3] as a constituent of the unsaponifiable matter from the seed fat ("Khakan fat") of *Salvadora oleoides* Den. (*Salvadoraceae*). On steam distillation, the fat gave about 1.5 % of benzyl isothiocyanate [3], and it appears likely that dibenzylthiourea in this case was an artifact produced from the mustard oil during saponification.