polystyrene or nylon, to a thickness of not greater than 0.1 mg/cm². The collecting diameter was defined by a circle scribed in the carbon and was surrounded by a guard ring. The air gap was determined by a capacity measurement for each measurement of ionization current. The ionization per unit volume was determined for several different air gaps, and extrapolated to zero air gap, for each absorber thickness. The measuring volume was at all times surrounded by an ''infinite'' thickness of polystyrene.

The observed ionization due to the thin sources had the functional form

$$\begin{aligned} D(z) &= A - B \ln z \qquad && 0 < z < 200 \text{ mg/cm}^2 \\ D(z) &= C \exp(-\mu z) && 80 < z. \end{aligned}$$

The constants A, B, and the joining point z_1 can be determined in terms of μ by using the conditions that $D(z)$, the point source function $I(r)$, and the first derivative of $I(r)$ must be continuous at the joining point. The remaining constant, C, can be fixed at any convenient value, because of the arbitrary multiplicative constant in $D(z)$. Then the ionization, in arbitrary units, normal to a thin plane source is given by

$$\begin{aligned} D(z) &= 1 - \ln \mu z \qquad && 0 < \mu z \leqq 1 \\ D(z) &= \exp(1 - \mu z) && 1 \leqq \mu z, \end{aligned}$$

where $\mu z_1 = 1$. It is found experimentally that $\mu = 9.10$ cm²/g, and hence $z_1 = 110$ mg/cm² for P^{32}. Since the last equations imply $\kappa = 3/\mu$, we get for the distribution of absorbed energy around a point source of P^{32} in polystyrene

$$I(r) = \frac{\overline{E}_\beta \mu^3}{12\pi} f(\mu r) \qquad \text{(energy/dis)/cc},$$
$$\begin{aligned} \text{where } f(\mu r) &= 1/(\mu r)^2, \qquad && 0 < \mu r \leqq 1 \\ f(\mu r) &= (1/\mu r)\exp(1 - \mu r), && 1 \leqq \mu r. \end{aligned}$$

It is implied that the point source is contained in a block of the absorbing material larger in all directions than the maximum range of the β particles. Then the dose at a distance r from a small volume dV cc containing C μc/g of β emitter is given by

$$0.0608\ \overline{E}_\beta C\, f(\mu r) \mu^3\, dV \quad \text{rep/hr},$$

where now $\overline{E}_\beta$ is the average β energy per disintegration in mev and has the value 0.695 for P^{32} (*3*). The rep (roentgen equivalent physical) has been taken as 93 ergs/g of absorbed energy (*4*), so the resultant dose rate will be 12% lower than in earlier publications, which used the figure 83 ergs/g (*1*). In using this formula for computation, the products μr and $\mu^3 dV$ are dimensionless. With this result, it is a straightforward matter to compute the dose due to any known distribution of β emitter, though only the relatively simple sources such as plane slabs and spheres can be computed in analytic form.

The noteworthy features of the result are these: (1) the dose is given by simple, analytical functions; (2) only two physical parameters are involved in the calculations, $\overline{E}_\beta$ and μ, the first affecting the magnitude and the second the distribution of the dose; (3) the entire dose distribution calculation can be carried out in dimensionless equations if the unit of distance is taken as $z_1 = 1/\mu$; and (4) the initial attenuation around a point source is inverse square.

The experimental value of $D(z)$ is actually not exponential out to the end of the β range, as the equations would imply, but begins to be measurably less than the exponential values for $z \geqq 400$ mg/cm². The intensity at these distances is, however, so small that, at least for biological and medical dose calculations, the equations as stated are entirely adequate.

A few measurements are available on thick sources of other isotopes (*5*). From these it is provisionally concluded that the same type of analysis can be made for other β emitters, and that the product $\mu \overline{E}_\beta$ is approximately constant for all β emitters. Under these circumstances, a calculation of dose distribution for β sources in tissue can be made in dimensionless form, and then applied to any β-emitting isotope for which the mean β energy is known or can be computed. Measurements on other isotopes are now under way. Full details will be published elsewhere.

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Errors of Combustion of Compounds for C^{14} Analysis[1]

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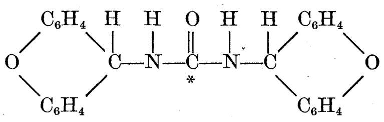
The precipitation of xanthydrol ureide affords a simple method for the isolation of urea from urine and has been used for this purpose in tracer studies with radiocarbon (*5, 13*). Xanthydrol ureide labeled with radiocarbon only in the urea residue, as indicated in the formula below by an asterisk, was prepared in a previous study (*3*) from the urine of a rat.



Large discrepancies were noted between C^{14} assay of some of the preparations of barium carbonate obtained by wet oxidation of the compound with the Van Slyke-Folch (*12*) solution, when the technique described by Lindenbaum, Schubert, and Armstrong was used (*6*). The investiga-

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TABLE 1

RADIOCARBON ASSAYS OF BARIUM CARBONATE OBTAINED BY FRACTIONAL COLLECTION OF CARBON DIOXIDE DERIVED FROM COMBUSTION OF XANTHYDROL UREIDE

Method of combustion	Fraction No.	% carbon found*	C^{14} specific activity cpm/mg C
Wet	1	6.7	2.4 ± 0.2
	2	18.1	2.2 ± 0.2
	3	25.4	0.6 ± 0.1
	4	14.8	2.1 ± 0.2
	5	12.4	3.2 ± 0.2
	6	22.6	1065.9 ± 4.6
Dry	1	40.8	458.3 ± 3.1
	2	59.2	106.1 ± 1.1

* Percentage of recovered carbon in each fraction.

tion of these discrepancies in radioactivity assay revealed large differences between the rates of conversion to carbon dioxide of carbon atoms occupying the labeled, and those occupying the unlabeled, positions in xanthydrol ureide. These differences, as shown in Table 1, were noted when both wet and dry oxidation methods were used. Because of these findings the work was extended to demonstrate discrimination between carbon isotopes when C^{14}-labeled urea was converted to CO_2 by wet oxidation. Our observations are reported because of their bearing on the methods used for combustion of labeled compounds preparatory to carbon isotope assay, and because they contribute to the general subject of the influence of isotopes on chemical behavior.

No instance has heretofore been noted of differences in the order of conversion of the carbon atoms of a compound to CO_2 on complete oxidation of the compound. However, discrimination between carbon isotopes in chemical reactions other than oxidation has been described. An 8% greater frequency of rupture of the C^{12}—C^{12} bond than of the C^{12}—C^{13} bond was found on partial thermal cracking of propane-1-C^{13} (*11*). Yankwich and Calvin (*14*) found that thermal decarboxylation of malonic acid singly labeled in a carboxyl group with C^{14} resulted in acetic acid with a C^{14} content 12% greater than that of the CO_2 evolved. With the use of the C^{13} naturally occurring in oxalic acid as an indicator, the decomposition of this substance in hot sulphuric acid to CO_2, CO, and H_2O was investigated (*7*). A preference was found for C^{13} in the CO_2 rather than in the CO. A preference was noted also for a higher rate of decomposition of oxalic acid molecules containing only C^{12} than of those containing both C^{12} and C^{13}. In a study (*8*) of the hydrolysis of C^{14}-labeled urea by urease enzyme, it was observed that the first fractions of CO_2 evolved were relatively richer in C^{14} than those produced in later stages of the reaction. The authors indicated that the opposite result is to be expected if the only factor affecting reaction rates is the zero point energy of the C^{12} and C^{14} bonds. We shall report in this paper a preference for the evolution of C^{12} as CO_2 when labeled urea is oxidized by chemical means.

The urea used to test discrimination in the carbon position was prepared by diluting, in alcoholic solution, a few crystals of high C^{14} activity urea with Merck's Reagent Grade urea. The solution was concentrated by evaporation of part of the alcohol, and the crystals that formed on cooling the solution were collected, washed with acetone, and dried. A part of the product was converted to xanthydrol ureide (*9*), giving a compound with one labeled carbon position as indicated in the formula above. The urea employed to determine discrimination in the carbon isotope was prepared in a similar manner, except that it was crystallized three times from alcohol. Further to assure the absence of radiocarbon not present as urea, a portion of the twice-recrystallized material was converted to urea nitrate (*4*).

The wet oxidations of all compounds with the Van Slyke-Folch mixture were carried out using the apparatus of Lindenbaum, Schubert, and Armstrong (*6*). For the fractional collections of the evolved CO_2 this apparatus was modified so that, when a part of the CO_2 had been collected in barium hydroxide solution, the stream of gas could be diverted, by turning a stopcock, into another receiver also containing barium hydroxide solution. In the majority of trials an effort was made to collect approximately half the evolved CO_2, as judged by the amount of precipitated barium carbonate, in each of the two receivers. However, Table 1 gives the results of one experiment in which the CO_2 was collected in 6 fractions. Owing to the vigor of the reaction of the acid-chromic acid oxidizing fluid with xanthydrol ureide, it was necessary to add the digestion fluid dropwise at a slow rate to the ureide contained in the evacuated apparatus. Only after the initial vigorous reaction had subsided was heat applied to the reaction thimble.

The dry combustions were carried out by using an ordinary microcombustion furnace, arranged in the manner described by Rittenberg (*10*), which allowed the emerging gas stream to bubble through barium hydroxide solution. By diverting the gas stream, two or more fractions of barium carbonate were obtained.

The barium carbonate was collected and its radioactivity determined by a previously described method (*1, 2*). A majority of the precipitates exceeded "infinite thickness." In those cases in which the precipitate was of less than saturation thickness, the measured radioactivity was calculated to that at saturation thickness by use of a factor derived from an empirically determined self-absorption curve for C^{14} β rays (*2, 4*). All radioactivity measurements shown in a given table have been made comparable by use of a uranium standard.

Table 1 presents the data obtained from a wet and dry fractional collection combustion of xanthydrol ureide. A number of similar experiments have given results that are in agreement with those cited. In the case of wet oxidation, it is seen that the first fractions of the collected CO_2 contained far less C^{14} than the later fractions. The first fractions were thus derived mainly from the unlabeled or xanthydrol residue carbons, whereas the later fractions contained most of the carbon from the urea residue. The results of the experiment in which the evolved CO_2 was collected in 6 successive fractions afford a striking demonstration of the relative slowness of conversion, by wet oxidation, of the carbon of the urea residue of xanthydrol ureide to CO_2.

TABLE 2

Discrimination Between Isotopes of Carbon on Wet Oxidation of Urea and Urea Nitrate

Material	Comb. No.	Carbon found, Fractions 1 and 2	Partition total carbon* $\frac{\text{Fraction 2}}{\text{Fraction 1}}$	C^{14} assay
Urea	1	100.40	0.80	1.103
	2	99.75	2.50	1.147
	3	100.40	3.27	1.194
Urea nitrate	1	101.13	0.80	1.035
	2	101.44	1.37	1.142

* Ratio of weights and hence of carbon contents of fractions.

The results of the dry combustions, in contrast to those of the wet oxidations, showed a preponderance of the labeled carbon in the first fractions, indicating a relatively earlier conversion of the urea residue carbon to CO_2 than of some of the xanthydrol residue carbons.

Table 2 presents representative data obtained on the fractional wet combustion of C^{14}-labeled urea and urea nitrate. The radioactivity assays are shown as a ratio. The statistical deviation of the counts does not exceed 0.52% of the net sample count.

The results shown in Table 1 make it clear that it is necessary to oxidize completely xanthydrol ureide and to collect completely the evolved CO_2, if reproducible results are to be obtained on C^{14} assay of barium carbonate derived from this compound by wet oxidation. Since the C^{14} is present in the last portion of the CO_2 to be evolved, undetectably small losses of carbon from this fraction would cause large errors in the C^{14} assay. Such circumstances were undoubtedly the cause of the erratic results that we noted in the examination of the xanthydrol ureide prepared from urine referred to in the introduction. Concordant results with the synthetic xanthydrol ureide were obtained only when the reaction mixture was heated until the appearance of SO_3 (about 8 min) and the collection of the evolved CO_2 prolonged to at least 20 min following the end of the heating period.

Failure to collect completely the CO_2 formed by dry oxidation of xanthydrol ureide would likewise be expected to produce large discrepancies in C^{14} assay, the effect on the specific activity of the barium carbonate being determined by whether the loss occurs from the first or from later portions of the evolved CO_2. In fact, we had found already the counts of thick samples of barium carbonate derived in this manner from the ureide prepared from urine to vary from 215 ± 2.9 to 257 ± 2.4 cpm.

It is possible, in the case of wet combustion, that xanthydrol ureide is first hydrolyzed to xanthydrol and urea, and that the former product is more easily oxidized than the latter. Some support for this viewpoint was obtained from the observation that free xanthydrol, like xanthydrol ureide, begins to react with vigor at room temperature with the combustion fluid, whereas urea alone in the reaction mixture requires heat for initiation of the reaction and the evolution of CO_2. Also related to this point are the results of a fractional wet combustion of a mixture of 2 moles of xanthydrol and 1 mole of labeled urea, which gave the following specific activities of barium carbonate: Fraction 1 (containing 40.4% of the total carbon), 0.6; Fraction 2 (containing 59.2% of the total carbon), 444.5.

The data presented in Table 2 demonstrate that the CO_2 formed late in the chemical oxidation of C^{14}-labeled urea is relatively richer in the label than that which is first produced. Since comparable results were obtained with urea nitrate, added assurance is given that the results were not due to the presence of a radioactive contaminant more resistant than urea to oxidation. Because of the variation of the weights of the 2 fractions among the several combustions and other uncontrolled variations affecting the reaction, no precise statement can be made as to the magnitude of this form of isotope discrimination. However, it appears from an inspection of the results that the last half of the evolved CO_2 contained about 10% more C^{14} than the first half.

It is possible that other isotopic carbon-labeled compounds may exhibit to a degree behaviors similar to that of xanthydrol ureide and urea on oxidation. On this account an important precaution should be observed in the conversion of compounds labeled with either C^{14} or C^{13} to CO_2 when this compound is to be used for isotopic carbon assay. Because of the probable different rates of oxidation of carbons of an organic compound, and isotopic discrimination of identical positions of carbon atoms in a compound, care should be taken to achieve complete oxidation and mixing of the resulting CO_2 before removal of samples for carbon analysis. Dependence should not be placed on a sample of the evolved CO_2 as representative of the C^{14} specific activity or the C^{13} content of the material. The whole of the sample should be burned, and the CO_2 collected completely and precipitated. If an aliquot of the CO_2 is to be used, opportunity should be afforded for mixing before the aliquot is taken.

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