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Preparation of Cyanates-^{14}C by an Exchange Reaction with $^{14}CO_2$

The existing methods of preparation of cyanates-^{14}C proceed from various types of chemical transformation of the starting ^{14}C-labelled derivatives, such as oxidation of cyanides-^{14}C ([1-3]) and, synthesis from urea-^{14}C and potassium carbonate ([4-6]).

All these methods have the experimental drawback in requiring isolating procedures for purification of the product, these being frequently quite laborious and resulting in a decrease of the final yield. In addition, one must use aqueous solutions in which the cyanate are unstable.

Therefore, we applied the previously described method for preparing salts of aromatic carboxylic acids labelled with ^{14}C in the carboxyl groups by an exchange reaction with $^{14}CO_2$ ([7-10]) to the preparation of cyanates labelled with ^{14}C ([11, 12]). It was found that during the reaction the ^{14}C is randomly distributed between the molecules of cyanate and carbon dioxide in the reaction atmosphere and, at the same time, that no chemical interaction between the various components of the reaction mixture takes place as might give rise to new products so that it is simple to isolate the two components, viz. the solid cyanate-^{14}C and the gaseous $^{14}CO_2$ with remaining activity. The chemical purity of both radioactive products is the same as that of the reactants as was confirmed by chemical analysis, comparison of IR spectra of cyanate before and after reaction and by determination of radioactivity of incorporated ^{14}C. Carbon dioxide-^{14}C was frozen out after reaction with liquid nitrogen and its amount as determined volumetrically agreed with the amount used for the reaction. The sum of radioactivities of cyanate and CO_2 after reaction was equal to the radioactivity of carbon dioxide used for the reaction.

EXPERIMENTAL

Exchange Reaction between KCNO and $^{14}CO_2$

A thick-walled glass ampoule of 5 ml volume contained 40.5 mg (0.5 mmol) potassium cyanate and the sample was dried at 230° C in vacuo (0.01 mm Hg) for 2 h. After drying, 0.5 mmol $^{14}CO_2$ (8.4 nCi) was introduced into the ampoule by the freezing-out method[8] and the ampoule was heat-sealed. It was then heated in an electrically heated quartz tube to 325-440° C for 0.5 to 120 min (Table I). The reaction time was measured from the moment of melting of cyanate. After cooling, the ampoule was connected to a vacuum line; carbon dioxide-^{14}C with the remaining activity was regenerated

TABLE I. Exchange reaction between KCNO and $^{14}CO_2$ [a].

Expt. No.	Reaction temperature °C	Reaction time min	Radioactivity, counts/min			
			Initial as $^{14}CO_2$	After reaction		
				$^{14}CO_3''$ [b]	K^{14}CNO [b]	$^{14}CO_2$
1	325	0.5	17,950	200	2,800	14,800
2	350	1	16,450	160	3,310	12,980
3	390	7	16,200	250	7,010	9,220
4	420	30	13,600	170	6,705	6,820
5	440	45	17,400	260	7,490	9,400
6	440	120	13,950	190	6,610	7,210

[a] Equimolar quantities of reacting components were used.
[b] Radioactivity determined in the form of $^{14}CO_2$.

by freezing out in liquid nitrogen and radioactivity counted in an internal gas-flow proportional counter [13] (A).

Chemical Analysis of K^{14}CNO and Determination of Its Radioactivity. A 40.5 mg sample of potassium cyanate-^{14}C was dissolved in 5 ml distilled water free of CO_2, alkalinized with 5 ml 0.1N NaOH and the carbonate ions were precipitated with a solution of $Ba(NO_3)_2$. The precipitate of $Ba^{14}CO_3$ was filtred and its radioactivity estimated in the form of $^{14}CO_2$ (B). Its value in all the experiments varied about 2%. After removing the $Ba^{14}CO_3$ by filtration, the filtrate was placed in an apparatus connected through a drying column to a freeze-out finger submerged in liquid nitrogen and was acidified with dilute $HClO_4$. The $^{14}CO_2$ released from the cyanate was carried by the passing nitrogen to the freeze-out finger, its amount determined volumetrically and its radioactivity counted (C). After expelling $^{14}CO_2$, the amount of NH_3 was determined as was formed after decomposition of cyanate-^{14}C by an acid, this providing a control value for the content of cyanate in the reaction sample. This value agreed well with that of incorporated ^{14}C and with that of cyanate used for the reaction. For the standard deviation of determination of the initial radioactivity and of values of A, B and C less than $\pm 1\%$, the sum of these values agreed with the initial radioactivity of $^{14}CO_2$ with a deviation of less than $\pm 3\%$.

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Isolement et purification d'acide désoxyribonucléique de radioactivité spécifique élevée obtenu par incorporation de thymine tritiée par *escherichia coli*

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Parmi les systèmes vivants susceptibles d'incorporer un précurseur porteur d'un radioélément dans leur acide désoxyribonucléique (DNA), les cellules de mammifères (tumeur d'Ehrlich, intestin ou rate de souris : Paoletti, Lamonthezie [4]) présentent l'avantage de permettre d'extraire aisément des quantités assez importantes de DNA. Mais, avec ces systèmes, seule une petite partie du précurseur est incorporée et la radioactivité spécifique du DNA est faible; de plus, on n'est pas assuré d'obtenir un polymère marqué de façon homogène. On peut éviter ces inconvénients en s'adressant à des souches bactériennes mutantes incapables d'effectuer la synthèse de la thymine. En cultivant un tel mutant dans des milieux contenant une quantité limitée de thymine méthyle (^{3}H), on obtient une incorporation rapide et presque totale de ce précurseur spécifique du DNA.

Les méthodes, données par la littérature, d'isolement et de purification du DNA des bactéries ne nous ont pas conduit à des résultats satisfaisants et nous inspirant de travaux antérieurs effectués dans notre laboratoire [1, 2], nous avons mis au point une technique où l'on traite successivement par la papaïne et par le phénol les corps bactériens.

Les bactéries (*E. Coli*, souche CR 34) sont obtenues par culture à 37 °C dans un milieu liquide contenant 1 mg/l de thymine de radioactivité spécifique