There are other reasons for favoring structure I. It would appear that ethyl groups attached to the barbituric acid ring do not suffer change in the body. For example, diethylbarbituric acid is excreted unchanged (*1, 4, 7*). Increasing the length of one of the chains increases the activity, but the molecules are then more susceptible to chemical change in the liver. Up to now it has not been known whether the change in the barbiturates was due to oxidation, hydrolysis, conjugation, or a combination of these reactions. It now appears likely that direct oxidative attack of side chains containing four or more carbon atoms is an important part of the chemical alteration of such compounds in the body.

The product of biological oxidation of pentobarbital is asymmetric. The isolated barbituric acid is dextrorotatory in acetic acid: $[\alpha]_D^{28} = +26.6°$. It was also found to be without apparent pharmacological action after intraperitoneal injection of a large dose (180 mg/kg) into mice. Further details of this work and other experiments in progress will be reported elsewhere.

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Detection of Radioactive Impurities by the Constant Solubility Test[1]

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In work with radioactive compounds, it is of importance to establish that significant amounts of the radioactivity measured in the labeled materials are not due to impurities. This is especially true in biological experiments in which the metabolism of a labeled compound is followed solely on the basis of radioactivity measurements. Radioactive contaminants in crystalline material often represent an amount by weight much smaller than can be detected by the common criteria of chemical purity. Specific procedures have been developed which take this problem into account (*1*). Recrystallization to constant specific radioactivity, conversion of the product to a derivative without change of specific activity, and determination of the distribution coefficients between two immiscible solvents have been used to prove the purity of radioactive materials.

A modification of the solubility method of analysis[2] has been devised for the detection of minute amounts of radioactive impurities in chemical compounds. This method is especially suited to the estimation of the purity of a radioactive compound because of the high sensitivity inherent in radioactivity measurements and because of the theoretical soundness of the phase rule. The simplicity of the technique and the easy recovery of the compounds used in the tests recommend it when limited amounts of material are available.

TABLE 1

SOLUBILITY TEST OF PURITY OF RADIOACTIVE *S*-BENZYL-D-HOMOCYSTEINE-S^{35}*

Time	Radioactivity of solution†	
hr	counts/sec/ml	
	flask A	flask B
16	24.0	24.4‡
40	23.6	23.4

* The specific radioactivity was 0.43 counts/sec/γS.
† The solvent was water at 29.3° C.
‡ After this measurement 12.6 mg of solid was added.

The principle of the constant solubility test as adapted to the detection of radioactive impurities is illustrated in the following general procedure, which should be applied to a compound judged to be chemically pure: A solvent is selected in which the compound is sparingly soluble; a convenient volume of the solvent is saturated with the compound by equilibration at constant temperature; a sample of the saturated solution is withdrawn and its radioactivity is measured; more of the compound is added to the solution, and after a suitable time interval for equilibration the radioactivity of the supernatant liquid is again determined. If the compound is impure, addition of more solid will increase the concentration of impurities in the liquid phase. Radioactive impurities will increase the radioactive count per unit volume of the solution. In the absence of radioactive impurities, the radioactivity of the two samples of solution will be the same.

The sensitivity of the method is utilized to its fullest extent by employing, in the first equilibration, an amount of solid just sufficient to saturate the solution, and by adding as large an amount of solid as feasible before the second equilibration. Considerable procedural variations within the principle of the solubility test are possible. Two applications of the method are given here for illustration.

The purity of three times recrystallized *S*-benzyl-D-homocysteine-S^{35} was demonstrated as shown in Table 1. This material had attained constant radioactivity in successive recrystallizations and had been converted to *N*-acetyl-*S*-benzyl-D-homocysteine-S^{35} without change in the specific radioactivity of the sulfur. Three mg of the compound was suspended in 5 ml of distilled water in each of

[1] This work was supported by grants from the American Cancer Society, upon recommendation from the Committee on Growth of the National Research Council, and by the Rockefeller Foundation.

[2] For a discussion of the scope and limitations of the solubility method of analysis as a criterion of purity see Herriott, R. M., *Fed. Proc.,* 1948, **7**, 479.

TABLE 2

DETECTION OF RADIOACTIVE IMPURITY IN L-CYSTINE

Time	Radioactivity of solution*
hr	counts/sec/ml
3	2.6
6	2.9†
19	34.9

* The solvent was water at 0° C.
† After this measurement 15.1 mg of compound was added.

two flasks. After equilibration for 16 hr, 0.5-ml samples were withdrawn through pipettes fitted with cotton filters, and the radioactivities of the solutions were determined. To one flask was added 12.6 mg of the compound and equilibration was continued for another 24 hr. At this time measurements showed that the radioactivity of both solutions had remained constant.

The presence of impurities was demonstrated in the following experiment: A sample of chemically pure L-cystine was deliberately contaminated by crystallization from a solution containing a mixture of radioactive sulfur-containing compounds. The apparent specific radioactivity of the recovered cystine was 0.039 counts/sec/γ S. A sample of 1.5 mg of the radioactive compound was suspended in 6 ml of distilled water and samples were withdrawn after equilibration for 3 and 6 hr respectively. After 6 hr, 15.1 mg of the compound was added to the flask and an additional sample was taken after further equilibration for 19 hr. The increase in radioactivity of the solution after addition of more solid (Table 2) proved that the compound contained radioactive contaminants.

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Some Observations on Exchange of CO_2 Between $BaCO_3$ and CO_2 Gas[1]

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Previous studies on the exchange of CO_2 between $BaCO_3$ crystals and atmospheric CO_2 have shown that exchange takes place in the presence of moisture (*1*), and that the amount of exchange can be reduced by heat treatment of the $BaCO_3$ samples (*2*). An attempt has been made to confirm these results, and to obtain additional information on the factors which affect the amount of exchange, using C^{14}.

The original $BaCO_3$ had a specific activity of about 1.2×10^6 cpm/mg.[3] It was attempted to prepare an active $CO_3^=$ solution by heating this material with inactive $0.1N$ Na_2CO_3 for 1 week. However, no exchange occurred. Active $CO_3^=$ was then prepared by mixing active $BaCO_3$ with varying amounts of ordinary $BaCO_3$, evolving the CO_2 by treating with $3N$ HCl in a small glass generating apparatus, and passing the evolved CO_2 in a small stream of N_2 gas through a coiled glass tube filled with $3N$ NaOH solution. After absorption of CO_2, the NaOH solution was removed from the apparatus and diluted about threefold, and $BaCO_3$ was precipitated, in the cold, with $BaCl_2$ solution. This was then filtered and washed with water, alcohol, and finally acetone to hasten drying. The filter paper with the precipitate was pasted to aluminum disks for easier handling and to prevent curling upon drying.

The effect of different amounts of moisture on the amount of exchange was studied first. The method used

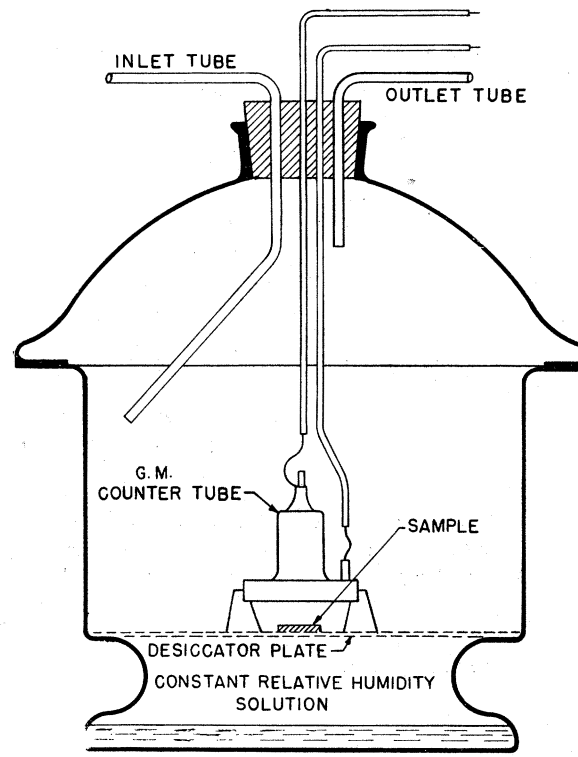

FIG. 1.

was to expose the sample to inactive CO_2 in a static atmosphere of known relative humidity, and to compare the counting rates of the sample before and after exposure. This was performed in a closed desiccator, as shown in Fig. 1. The bottom of the desiccator contained sulfuric acid solution of a density adjusted so that a known constant relative humidity was obtained.

The sample was placed beneath the tube and counted. CO_2 was passed into the desiccator for 15 min and then the vessel was sealed for 2 hr. At the end of this time, air was blown through to sweep out the CO_2, and the sample was recounted.

The addition of CO_2 into the desiccator causes the count to drop 25% to 40% of the count in air, due to increased absorption of β-rays by CO_2. However, all counting was done with air in the desiccator.

[1] Research work done at the Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.
[2] I wish to thank Dr. William Rubinson and Dr. Warren Miller for helpful advice.
[3] Obtained from Oak Ridge.