Thus the addition of sodium azide to the tetrazolium solution resulted in depression of the reduction of the dye. Typical results are indicated in Table 3, which presents

TABLE 3

EFFECT OF NaN_3 ON REDUCTION OF TRIPHENYLTETRAZOLIUM CHLORIDE BY TISSUE SLICES

Tissue	R_0*	% Change 10^{-3}M	NaN_3† 10^{-6}M
Liver	44	−45	−11
Kidney	55	−36	− 2

* R_0 = Colorimeter reading/mg tissue, dry wt, 30-min incubation.

† Sodium azide, 0.5×10^{-3}M and 0.5×10^{-6}M added to 3 ml of 1% tetraphenyltetrazolium solution.

the mean values for tissues from six animals, the experiments having been done in duplicate.

It appears from the data presented that triphenyltetrazolium chloride may be used in a simple and reproducible manner to study the metabolism of tissue slices and the effects of enzyme inhibitors.

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Electrolytically Induced Reactions

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Recently two examples of a novel type of induced reaction have been observed in this laboratory. Both reactions are unique in being electrolytically induced. The first is the electrolytically induced air oxidation of trivalent arsenic, As^{III} in alkaline solution. It has been observed (*1*) that air oxidation of As^{III} in a solution 1*N* in sodium hydroxide is slight. But if electrolytic oxidation of the arsenic is carried on in the same solution, oxidation occurs up to 50% in excess of that corresponding to the current used. This excess oxidation is attributed to oxygen of the air, since it is entirely eliminated if the surrounding air is displaced with nitrogen. The electrolytic process, however, seems essential.

The second reaction is the electrolytically induced precipitation of iridium with rhodium. It was observed by MacNevin and Tuthill (*2*) that iridium could not be deposited from an ammonium chloride solution at cathode potentials as great as − 1.0 volt. But if rhodium is also present, then not only does the rhodium precipitate quantitatively at a cathode potential of − 0.3 volts, but the major part of the iridium does also.

These two reactions are considered to be examples of a new type of induced reaction and it is proposed to call them "electrolytically induced" reactions.

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The Isolation of a Metabolite of Pentobarbital[1]

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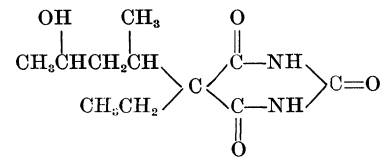
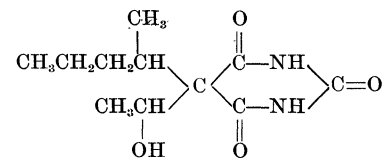
There is general agreement that pentobarbital is excreted only in trace amounts in the urine (*2, 6, 8, 9*), but up to the present no metabolic products of the drug have been reported. It has now been found possible to isolate a metabolite of pentobarbital from the urine of dogs after anesthetic doses of the drug. The compound melts at 209–210° C and has an elementary composition corresponding to pentobarbital with one additional oxygen atom ($C_{11}H_{18}O_4N_2$). In sodium hydroxide solution it has the characteristic ultraviolet absorption spectrum of the dialkylbarbituric acids (*5*). In the range of 225–290 mμ the absorption curve has exactly the same shape as that of pentobarbital, but the extinction is about 7% lower. This would imply a molecular weight of about 242 for the new barbiturate. Further evidence for the barbituric acid ring was obtained by the preparation of a di-*p*-nitrobenzyl derivative (*3*).

The presence of a hydroxyl group was demonstrated by the reaction of the metabolite with acetic anhydride. A new compound was formed which crystallized from aqueous ethanol in the form of blunt needles; mp 147–148° C.



(I)



(II)

It had the elementary composition of the corresponding acetate ($C_{13}H_{20}O_5N_2$). On treatment with sodium hypoiodite in dioxane solution the metabolite yielded iodoform. It would thus appear that the compound could be either I or II. Structure II would not seem likely, however, because such a compound would probably react with sodium hypoiodite to yield acetic acid, not iodoform.

[1] This work was supported by a grant from the U. S. Public Health Service.

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.