the viscosity of the cytoplasm is 10 times that of water, the particle weight of the complex is calculated to be of the order of 2000 g/mole. This calculation is presented as the basis for the suggestions that the glucose-carrier complex, if such exists, is not a macromolecule and that the transport does not directly involve organized intracellular particles, such as mitochondria.

In another series of experiments, collection of urine samples was made after injection into the renal artery of glucose uniformly labeled with carbon-14 and of ordinary creatinine; the excretion patterns (*6*) were then determined. After a load of ordinary glucose to produce glucosuria, the excretion patterns of C^{14}-labeled glucose and of creatinine were symmetrical. No displacement of the patterns, such as has been found with urea relative to creatinine (*7*), was noted. This finding suggests that glucose entering the tubule cells does not return to the lumen. After the administration of phlorhizin, the excretion patterns of C^{14}-labeled glucose and of creatinine were essentially identical. It is inferred from these findings that the combination of glucose with the postulated intracellular carrier occurs at the cell barrier rather than within the cell. That glucose may enter the cells from the peritubular fluid side is suggested by the finding, in most experiments of the type illustrated in Fig. 1, of glucose concentration ratios somewhat less than the creatinine concentration ratios in the samples at or preceding the maxima. No marked change in this relationship was noted after phlorhizin.

These findings form the basis for the following tentative conclusions. (i) Glucose is transported by the renal tubule cells without breakdown and resynthesis of the six-carbon chain. (ii) The transport system is of the membrane carrier rather than of the cytoplasm carrier variety. (iii) The postulated glucose-carrier complex is not a macromolecule.

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22 January 1957

Protection of Sulfhydryl Groups against Ionizing Radiation

Sulfhydryl compounds have long been known to be among the most radiosensitive of all organic compounds. A number of them, such as cysteine, glutathione, cysteamine (β-mercapto ethylamine) and 2,3-dimercapto 1-propanol (BAL) have been used as protective agents against the action of ionizing radiation. Their protective action was assumed to be owing to their ability to act as "free radical acceptors"—that is, to compete with other components of the system for the free OH, H, or HO_2 radicals formed as primary products of radiolysis of water.

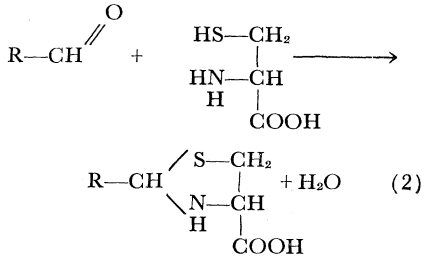
Recently, however, Eldjarn *et al.* (*1*), using "labeled" sulfur components, demonstrated that the protective action of cysteamine and cystamine ($HS—CH_2—CH_2NH_2$ and $NH_2 \cdot CH_2—CH_2—S—S—CH_2—CH_2 \cdot NH_2$) was due not so much to their ability to scavenge free radicals, but rather to a specific chemical reaction of cystamine with free sulfhydryl groups of the protein molecules. By this reaction, cystamine forms a disulfide linkage according to the scheme

$$\text{Protein—S—H} + \text{R—S*—S*—R} \rightarrow \text{protein—S—S*—R} + \text{H—S*—R} \quad (1)$$

where R—S*—S*—R represents the (labeled) cystamine and H—S*—R represents the resulting cysteamine. The resulting disulfide linkage appears to be much more resistant to the action of ionizing radiation than the "unprotected" sulfhydryl group, thus accounting for the effectiveness of these compounds as protective agents. The blocking of the sulfhydryl groups is temporary, and they are eventually restored by normal biological processes.

In the course of investigations of the development of odors on irradiation of food, we became interested in the formation of hydrogen sulfide and mercaptans as contributors to the odor problem, and consequently in methods of prevention of their formation. Because of the extreme radiosensitivity of the sulfur compounds involved, particularly cysteine and glutathione, effective reduction of the hydrogen sulfide formation by addition of free radical acceptors such as ascorbic acid did not appear to be promising.

The work of Eldjarn pointed out another approach—namely, masking these sulfhydryl groups by specific chemical reactions, rather than protecting them by competing reactions. Considerable work had been done in this direction. Schubert (*2*) and Vuataz (*3*) found that a number of carbonyl compounds were capable of reacting with sulfhydryl compounds even in dilute aqueous solutions, forming mercaptals, and reported that some of these no longer showed the characteristic sulfhydryl reactions, such as the purple nitroprusside color. The situation seemed to be particularly favorable in the presence of α-amino groups (such as in cysteine), since stable thiazole rings could be formed:



(2)

A number of aldehydes, dialdehydes, keto aldehydes, and keto acids were tried as additives, using approximately 100 percent excess. A nitroprusside test was made first; the mixture was then subjected to the action of atomic hydrogen and γ-rays. The techniques used for the production of hydrogen atoms as well as for treatment with γ-rays from a Co^{60} source have been described elsewhere (*4*). The best protection of cysteine against the action of atomic hydrogen was provided by glyoxal, formaldehyde, pyruvic acid, pyruvic aldehyde diacetyl, and glyceraldehyde, which reduced the amount of H_2S formed to 10 to 15 percent of that formed in unprotected solutions. By contrast, ascorbic acid used in tenfold excess reduced the amount of H_2S only to about one-half. Glutathione was protected best by glyoxal, pyruvic acid, and formaldehyde.

The protection against γ-rays, used at a level of 5×10^6 rep, is even more favorable. No measurable amount of H_2S was formed from cysteine solutions at *p*H 7 when either glyoxal or pyruvic acid was used as an additive in 100 percent excess. This compares favorably with ascorbic acid, which afforded only a 30 percent reduction of H_2S formation. With glutathione at *p*H 7, glyoxal (in 100 percent excess) prevented the formation of H_2S almost completely, pyruvic acid provided a 65 percent reduction; ascorbic acid used for comparison resulted in a 50 percent reduction of H_2S formation. A number of aldoses were tried but found to be ineffective, with the exception of glyceraldehyde. This is consistent with the assumption that sugars containing four or more carbons form cyclic hemiacetals and thus do not possess a reactive aldehyde group.

These results indicate that the masking of radiosensitive groups by specific chemical reactions may be a more effective way of reducing certain effects of ionizing radiation than the addition of free radical acceptors (*5*).

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Effect of Multiple Recrystallization on Derivatives of Mixtures of D- and L-Alanine

Among the problems encountered in tracer studies with biological systems is the difficulty of isolating a compound that is present in minute amounts. To reduce the difficulty of this problem, it is a common practice to add as carrier the non-labeled material sought and then to proceed with the isolation. As a matter of convenience, the DL racemate is sometimes added, although the compound sought may be solely of the D or L configuration. A question arises about the degree of significance of the isomeric nature of the carrier. This is of particular importance if the radioactivity of the isolated material is to be interpreted as representative of the activity of the compound as it exists in the biological preparation.

In the same regard, it is often desirable to determine the amount of the compound in the system. One manner in which this is done involves establishment of the isotope dilution. Again, the significance, if any, of the isomeric form may become an important consideration. This is especially true if the compound is to be isolated as a derivative. It was from interest in the latter case that the present investigation (*1*), which employed a particular system involving the isolation of alanine as the azobenzene sulfonate derivative, was initiated.

Table 1. Radioactivity (count/min) of alanine azobenzene sulfonate.

No. of recrystallization	Source of derivative		
	Parent DL-alanine	Diluted DL-alanine	1/3 D- to L-alanine
1	113	55	52
2	112	56	49
3	110	57	45
4	109	56	42
6	112	56	39

The magnitude of changes in activity during multiple recrystallization of derivatives of alanine having equal activity but differing in the ratios of the D to L isomers has been investigated. Other derivatives prepared from alanine of the same and of unequal amounts of D and L configuration have also been studied.

Two mixtures of alanine were prepared from a parent source of racemic alanine-1-C^{14}. The first was prepared by diluting the radioactive material with an equal amount of DL-alanine. The second mixture contained equal amounts of the radioactive racemate and L-alanine. The mixtures were dissolved in water to insure complete mixing and were then precipitated by the addition of ethanol. In this manner, two alanine mixtures were obtained with equal radioactivity contributed from the D and L isomers, but with different total proportions of the isomers. The first mixture was racemic, while the second contained the D and L isomers in a ratio of 1/3. The alanine salt of azobenzene-*p*-sulfonic acid (*2*) was prepared from each of these mixtures as well as from the parent racemic alanine. The salts were recrystallized once from water, dried, and assayed for radioactivity. Each of the compounds was subsequently recrystallized several times from water and counted following each recrystallization.

The radioactivity was measured on pressed solid mounts of the derivative plated at infinite thickness. Recrystallized samples were mounted in the same aluminum planchet to maintain identical counting-surface area. All counts were made with a thin end-window Geiger-Müller tube. The values reported are expressed as total counts per minute for the sample less background. Since the same derivatives are compared, these values are both proportionate to the activity per milligram and per millimole of derivative. The results of these determinations are shown in Table 1. The activities of the salts of the parent and diluted racemate did not change, while the activity of the 1/3 D- to L-alanine derivative steadily decreased. Based on the mean activity for the salt of the parent alanine, the salt of the racemate after six recrystallizations had 100 percent of the theoretical activity, while the activity of the 1/3 D to L mixture had dropped to 70 percent.

Another alanine mixture was prepared in the manner already described in which the D- and L-isomers were present in a ratio of 2/3, respectively. From this mixture, the phenyl isocyanate (*3*) and *p*-toluenesulfonyl (*4*) derivatives were prepared. After five recrystallizations, neither of these derivatives showed a change in radioactivity. The fact that no apparent shift in activity was observed may be dependent on the nature of the derivatives or the concentrations of the two isomers, which in this case were more nearly equal.

As a final experiment, two alanine mixtures were prepared from a source of racemic alanine-1-C^{14} in such a manner that one contained the isomers in a ratio of 1/3 D to L and the other 1/3 L to D. The mixtures had equal amounts of C^{14} in each isomeric form. Again the azobenzene-*p*-sulfonic acid salt was made from each of the mixtures and recrystallized. In Fig. 1 are shown the changes in radioactivity with subsequent crystallizations. As a control, the same derivative was prepared from a DL-alanine-1-C^{14} source and was likewise subjected to recrystallization. The data are included in Fig. 1. The radioactivity of the 1/3 D to L and the 1/3 L to D derivatives showed a consistent and similar decrease. The results with the alanine azobenzene sulfonates may be explained in each case by an enrichment of the isomer originally present in the larger amount.

The isomeric nature of the carrier appears to be worthy of consideration in the preparation of a derivative from an optically active compound. The degree of importance varies with the derivatives of choice, the number of crystallizations, and the ratio of the D and L isomers. In many situations, analytic results will not be affected to a significant degree by excessive crystallization. However, the im-

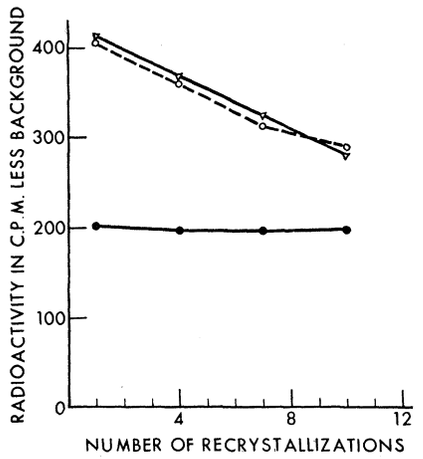

Fig. 1. Effect of successive recrystallization on the radioactivity of alanine azobenzene sulfonates; △, derivative prepared from 1/3 D- to L-alanine; ○, derivative from 1/3 L- to D-alanine; ●, derivative from a racemic mixture.