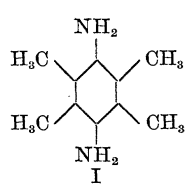
violet). When a solution of the dye in 20 NH_2SO_4 is reduced, say by $TiCl_3$, the free radical appears as intermediate step of reduction. It is yellow and exhibits a series of distinct absorption bands in the blue part of the spectrum, recalling the bands of the triphenylmethyl radical. Analyses of the potentiometric titration curves, obtained by especially adapting the technique to the unusually high acidity of the solutions, showed that this yellow compound is a free radical, not a polymerized compound. The separation of the two steps rapidly diminishes with decreasing acidity but never vanishes entirely. Even in neutral solution an analysis of the potentiometric titration curves reveals that the dye solution in its half-reduced state contains the semiquinone to an extent that may be estimated as between 5 per cent. and 10 per cent. of the total dye. In such solutions the radical can no longer be detected, optically, for two reasons. Firstly, a few per cent. of a light yellow compound escapes optical detection when mixed with the large excess of the deeply colored blue dyestuff itself; secondly, it can be assumed from theoretical arguments that the radical is in a different state of ionization in extremely acid, than in less acid or neutral solution, and that the form existing in neutral solution should exhibit an extremely slight absorption, if any at all, in the visible range of wave-lengths.

The only difference between thionin and methylene blue is that the latter requires still a somewhat higher acidity than thionin in order to obtain the same degree of separation of the two steps of oxidation. The absorption spectrum of the methylene blue radical is quite similar to that of thionin.

Then, these dyestuffs, after all, fit very well into the theory of compulsory univalent oxidation. Any bivalent oxidation reduction system must be capable of forming a semiquinone radical to a measurable extent in order to behave as a reversible system; and any organic dyestuff can act as a catalyst for sluggish oxidations only by intermediation of the radical.

Another experiment should be mentioned with regard to the assertion that the catalytic action of a dye depends on its faculty to form a semiquinone radical. It has been shown recently,[2] that the first oxidation product of diamino durene (I) is a free radical, analogous to those designated as Wurster's dyes. It has been shown furthermore that any methylation at the amino groups destroys the faculty of forming this radical. In the latter case, the benzene ring, the two N atoms and all the atoms attached to the two N atoms are prevented from lying in one plane due to steric hindrance of the voluminous side chains. The steric possibility of such a coplanar arrangement is requisite for establishment of resonance. For this reason the methylated compounds can not form a radical. Now, the unmethylated compound increases respiration of erythrocytes to approximately the same extent as does methylene blue, but the methylated compound has no catalytic effect at all.



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[2] L. Michaelis, M. P. Schubert and S. Granick, *Jour. Am. Chem. Soc.*, 61: 1981, 1939.

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THE METABOLISM OF GLUTATHIONE[1]

INVESTIGATIONS concerned with the fate of glutathione in the living organism have dealt almost exclusively with the behavior of the tripeptide as an SH-carrier. The methods used have been based on determination of the SH-group.

Little attention has been paid to the possibility that the combination of the three amino acids, glycine, cystine and glutamic acid in this peptide, GSH, has some special significance aside from its function as an SH-carrier. It is striking that these three amino acids are the ones which are found combined with compounds other than amino acids in the mammalian body, *e.g.*, bile acids and the products of detoxication. One of us has previously suggested that GSH may act as an amino acid donor in the formation of those compounds.[2] In support of this conception a decrease in the substances titratable with iodine in human blood was found after intake of phenyl acetic acid which is excreted as phenyl acetyl glutamine.[3]

It is now possible by the use of the isotope technique to follow directly the fate of the amino acids of GSH and to estimate its rate of turnover. Glycine containing 1.18 atom per cent. N^{15} excess was administered to rats. After two hours the concentration of N^{15} in the GSH of the liver and intestine, together with the N^{15} content of the protein glycine and NPN of these organs, was determined. The GSH was isolated as follows: The frozen and ground livers were extracted with metaphosphoric acid. The GSH was precipitated as the cuprous compound and purified by reprecipitation. The method of isolation yields about 16 mg of copper glutathione per rat liver, correspond-

[1] This work was made possible through a grant from the Friedsam Fund donated to the Division of Child Neurology, Neurological Institute, New York, N. Y.

[2] H. Waelsch, *Arch. exper. Path. u. Pharm.*, 156: 356, 1930.

[3] H. Waelsch and E. Weinberger, *ibid.*, 156: 370, 1930.

ing to 30 to 40 per cent. of the values given by titration procedures.

The results (Table I) show that within 2 hours an

TABLE I

RATS GIVEN 75 MG. GLYCINE (1.18 ATOM PER CENT. N^{15} EXCESS)

Liver	N^{15} excess atom per cent.	Dilution factor of labeled N	Dilution factor of labeled glycine
Cu glutathione	0.09	1:13	1:4.3
NPN	0.04	1:29	
Glycine (protein)	0.019	1:62	1:62
Intestine:			
Cu glutathione	0.038	1:31	1:10.3
NPN	0.014	1:84	
Glycine (protein)	0.017	1:70	1:70

appreciable part (7.6 per cent.) of the nitrogen of the liver GSH was derived from the administered glycine. By contrast the nitrogen of the protein glycine and of the NPN of the liver represented only 1.6 and 3.4 per cent. respectively. A similar relationship was found in the intestine. In view of the short period of the experiment and of the finding by Braunstein and Kritzmann,[4] Bach[5] and others that the speed of deamination of glycine is slow it is probable that all the labeled N is in the glycine portion of the GSH. If this assumption is correct the concentration of N^{15} in the glycine of the liver GSH would be 3×0.09 atom per cent. and the minimum rate of turnover of GSH would be 22.3 per cent. (intestine 9.6 per cent.) in two hours (Table I, column 4). By contrast in the same period only 1.6 per cent. (intestine 1.5 per cent.) of the protein-glycine of the liver was exchanged. This experiment establishes the fact that GSH is a very unstable and reactive compound in the living organism.

Rittenberg and Schoenheimer[6] administered benzoic acid and glycine labeled with N^{15} to rats. One third of the glycine excreted as hippuric acid during the following 24 hours came from the glycine administered. To obtain further information concerning the synthesis of hippuric acid in relation to the turnover of GSH, benzoic acid and glycine containing 1.98 per cent. of N^{15} excess were injected subcutaneously into rats. After five hours GSH was isolated from the liver. The GSH contained 0.151 atom per cent. N^{15} excess. Assuming again that it represents an uptake of labeled glycine by the GSH the minimum rate of turnover of the GSH from the liver would be 23 per cent.

Hippuric acid isolated from the urine collected during the five-hour period contained 1.08 per cent. N^{15} excess, indicating that 55 per cent. of the glycine of the hippuric acid was derived from administered glycine. Since the liver GSH glycine contained less N^{15} (0.453 atom per cent. excess) at the time it was measured than did the excreted hippuric acid (1.08 atom per cent. excess) the experiment offers no support for the hypothesis that GSH furnishes glycine for hippuric acid formation.

Further work is in progress to determine whether the rapid turnover of GSH is indicative of its role as an intermediary in the metabolism of proteins.

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SYNTHETIC ALPHA-TOCOPHEROL AND NUTRITIONAL MUSCULAR DYSTROPHY[1]

RECENTLY Goettsch and Ritzman[2] found that alpha-tocopherol prevented the development of muscular dystrophy in young rats when alpha-tocopherol was fed from the tenth to the twenty-fifth day after birth. Control rats under similar conditions but without the supplement of alpha-tocopherol showed symptoms of muscular dystrophy at the end of the above test period. Their own criticism of the results was that it was conceivable that the alpha-tocopherol employed might have contained impurities that were active in preventing the muscular disease. The alpha-tocopherol was a natural product isolated according to the method of Evans, Emerson and Emerson.[3]

Mackenzie and McCollum[4] later showed that the natural alpha-tocopherol was effective in curing muscular dystrophy of rabbits on the Goettsch and Pappenheimer diet no. 13 plus 10 per cent. of ether extracted wheat germ.

In the present work with muscular dystrophy of rabbits, the writer has used a diet consisting of ground U. S. No. 3 grade alfalfa hay ad libitum, and 20 grams of a grain mixture daily per rabbit. The grain mixture is made up of 40 parts of whole yellow corn meal, 30 parts of wheat bran, 20 parts of soybean meal and 10 parts of linseed meal. One part of sodium chloride is added to the mixture. Each animal received daily 1 cc of a good grade cod liver oil, either placed upon

[1] This research was supported by an appropriation from Bankhead-Jones funds (the Bankhead-Jones Act of June 29, 1935).

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[3] H. M. Evans, O. H. Emerson and G. A. Emerson, *Jour. of Biol. Chem.*, 113: 319, 1936.

[4] C. G. Mackenzie and E. V. McCollum, SCIENCE, 2312, 370, 1939.

[4] D. F. Braunstein and M. G. Kritzmann, *Biokhimiya*, 3: 590, 1938, C.A. 33, 2916, 1939.

[5] S. J. Bach, *Biochem. Jour.*, 33: 90, 1939.

[6] D. Rittenberg and R. Schoenheimer, *Jour. Biol. Chem.*, 127: 329, 1939.