deviation for duplicates and recoveries by this method in all fluids and tissues was 1.3, 1.9 and 2.9% (confidence limits for 96 of 100 analyses) for Na, K and Ca. The internal standard method was found particularly advantageous for estimations in a. bile—containing specimens (bile, intestinal juice), where surface tension affected direct emissions; b. tissue ashes, where iron and phosphates depressed direct intensity measurements; and c. urines containing high protein, glucose or phosphate concentrations.

The detailed investigations will be published.

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SPECTROPHOTOMETRIC MEASUREMENTS OF THE METABOLIC FORMATION AND DEGRADATION OF THIOL ESTERS AND ENEDIOL COMPOUNDS

by

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The role of thiol esters of coenzyme A and of glutathione as intermediates in the oxidative metabolism of aldehydes and keto acids has been recently elucidated[1]. The enzymic oxido-reduction of methylglyoxal to lactic acid consists of 2 steps catalyzed by glyoxalase I and II[2]. In the first step a condensation of glutathione with methylglyoxal gives rise to a thiol ester (lactyl glutathione), which is hydrolyzed in the second step to lactic acid and glutathione. It has now been found that in the presence of glyoxalase I and glutathione, other ketoaldehydes (glyoxal, phenylglyoxal and hydroxypyruvic aldehyde) also give rise to the corresponding thiol esters. These esters all show a high light absorption in the low ultraviolet region and give a characteristic color reaction with hydroxylamine and ferric chloride similar to that given by acyl phosphates[3]. The formation and hydrolysis of the thiol esters was therefore followed spectrophotometrically as well as colorimetrically. A partially purified preparation of glyoxalase II catalyzed the hydrolysis of the lactyl-, glycolyl-, mandelyl-, and glyceryl-esters of glutathione. It also split acetyl glutathione slowly but was apparently inactive with acetyl thioglycolate as substrate. The latter compound as well as acetyl glutathione recently have been shown to be hydrolyzed by large amounts of crystalline glyceraldehyde-3-phosphate dehydrogenase[4]. A non-enzymic ammonolysis of acetyl thioglycolate to acetamide was also observed.

A diphosphopyridine nucleotide linked enzyme is present in yeast which catalyzes the oxidation of lactyl and glycolyl glutathione[2]. Since these thiol esters are presumed to be in equilibrium with their enediol forms, the possibility that they may act as substrates for enzymes which oxidize some enediol compounds was investigated. It was found however that neither ascorbic acid oxidase nor peroxidase catalyzed the oxidation of lactyl glutathione nor did the yeast enzyme which oxidizes the thiol esters have any effect on ascorbic acid or dihydroxymaleic acid.

In the course of these investigations methods were developed to follow the oxidation of enediol compounds spectrophotometrically. At the appropriate wavelengths the enediols absorb light very strongly while the oxidation products do not. Thus the oxidation of ascorbic acid by ascorbic acid

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oxidase* was followed at 265 mμ. Conditions of the test and relationship between enzyme concentration and changes in optical density are recorded in Table I. As can be seen, proportionality is satisfactory for an assay method, and in fact the turnover number calculated from initial velocities is more than three times the value obtained by the manometric test. The purified oxidase has also been used for the spectrophotometric determination of ascorbic acid, by subtracting the small residual absorption at 265 mμ after oxidase action, from the initial absorption.

TABLE I

SPECTROPHOTOMETRIC ASSAY OF ASCORBIC ACID OXIDASE

Enzyme added micrograms of protein	*Log $\frac{I_o}{I}$ (265 mμ) for first minute*
0.060	0.320
0.045	0.260
0.030	0.180
0.015	0.090

Disappearance of dihydroxymaleic acid was measured at 290 mμ in the presence of peroxidase from horse radish or cabbage and 1 micromole of hydrogen peroxide. The tests were carried out in a final volume of 3 ml and 0.1 *M* KH_2PO_4. Proportionality between enzyme concentration and density changes was less satisfactory than in the case of ascorbic acid oxidase. Relatively lower activity values were obtained with increasing enzyme concentration and corrections had to be applied. An enzyme system was found in extracts of cabbage which catalyzes the oxidation of dihydroxymaleic acid without addition of hydrogen peroxide. After fractionation with ammonium sulfate and dialysis, the enzymic activity was lost. Addition of a heat stable factor obtained from cabbage fully restored enzymic activity to the dialyzed preparation.

Into quartz cells (1 cm light path) of a Beckman spectrophotometer the following solutions were pipetted: 2.8 ml of citrate-phosphate buffer pH 5.6 (containing 0.025 *M* citric acid and 0.05 *M* Na_2HPO_4); 0.1 ml of a solution containing 0.05% ascorbic acid and 1% neutralized. Versene: 0.05 ml of 1% bovine serum albumen and 0.05 ml of the ascorbic acid oxidase solution. All dilutions of the enzyme were made in 0.01 *M* phosphate buffer pH 7.4 containing 0.1% bovine serum albumen. Density readings were made at 265 mμ.

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THE PRODUCTS OF THE ACTION OF THROMBIN ON FIBRINOGEN

by

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Analyses for N-terminal residues by the fluorodinitrobenzene (FDNB) method[1] show that the end-groups of bovine fibrinogen are tyrosine and glutamic acid, while the fibrin derived from it after clotting with a small amount of purified thrombin has tyrosine and glycine N-terminal groups.