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Effect of Sulfhydryl and Other Reducing Compounds on Decarboxylation of Pyruvic Acid by Alkaline Washed Yeast.*

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Since Grassman¹ first showed that papain and katepsins can be activated by cysteine or by reduced glutathione many papers have appeared in the literature dealing with the influence of sulfhydryl compounds on the activity of hydrolyzing and other enzyme systems. A search of literature has not revealed any researches concerned with the effect of these compounds on carboxylase. This paper is a preliminary report on the effect of sulfhydryl and other reducing compounds on the activity of carboxylase.

The procedure used was a modification of the method of Ochoa and Peters.² As a source of carboxylase commercial baker's yeast which had been air-dried with the aid of an electric fan for 3-4 hours or desiccated over concentrated H₂SO₄ for several days was employed. It was freed from cocarboxylase by rapid washing of the yeast 2-3 times with M/10 Na₂HPO₄ (25-30 cc for 0.5 g yeast), and once with distilled water. The washed yeast was suspended in 5 cc M/10 phosphate buffer pH 6.2. Formation of CO₂ from pyruvic acid was measured manometrically at 28° in air with conventional Warburg manometers attached to 15 cc vessels. Each vessel contained 0.5 cc washed yeast suspension, 0.1 mg Mg as MgSO₄ in 0.1 cc of solution, 0.2 cc sodium pyruvate solution adjusted to pH 6.2 and containing 5 mg pyruvic acid. In each case reagents to be tested or phosphate buffer pH 6.2 were added to bring the total volume of fluid to 2 cc. The pyruvate solution was tipped into the vessel after 8-12 minutes aeration. Merck's synthetic cocarboxylase and crystalline vitamin B₁ were used throughout the experiments.†

The results of 5 experiments dealing with the activation of alkaline washed yeast by cysteine hydrochloride, reduced glutathione, sodium bisulfite and phenylhydrazine hydrochloride are listed in Table I.

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¹ Grassman, W., Dyeherhoff, H., and v. Schoenebeck, O., *Z. f. Physiol. Chem.*, 1929, **196**, 183.

² Ochoa, S., and Peters, R. A., *Biochem. J.*, 1938, **32**, 1501.

† Our thanks are due to Merck and Company for a supply of synthetic cocarboxylase and to Squibb and Company for a supply of crystalline thiamine.

TABLE I.
Effect of Sulfhydryl and Other Reducing Agents on CO₂ Production from Pyruvic Acid by Alkaline Washed Yeast.

Exp. No.	System Tested	Micro-liters CO ₂ liberated
1		in 30 min
	10 γ B ₁	— 11
	10 γ B ₁ + 0.2 γ cocarboxylase	23
	10 γ B ₁ + 2 mg cysteine HCL	130
	10 γ B ₁ + 2 mg cysteine HCL + 0.2 γ cocarboxylase	177
	No B ₁ , 2 mg cysteine HCL	111
	No B ₁ , 2 mg cysteine HCL + 0.2 γ cocarboxylase	110
2		in 45 min
	10 γ B ₁	3
	10 γ B ₁ + 0.2 γ cocarboxylase	110
	10 γ B ₁ + 0.2 γ cocarboxylase + 1 mg cysteine HCL	208
	10 γ B ₁ + 0.2 γ cocarboxylase + 0.5 mg cysteine HCL	174
	10 γ B ₁ + 0.2 γ cocarboxylase + 0.25 mg cysteine HCL	228
	10 γ B ₁ + 0.2 γ cocarboxylase + 0.1 mg cysteine HCL	201
3		in 45 min
	10 γ B ₁	— 26
	10 γ B ₁ + 0.2 γ cocarboxylase	154
	10 γ B ₁ + 0.5 mg GSH	82
	10 γ B ₁ + 0.5 mg GSH + 0.2 γ cocarboxylase	205
	10 γ B ₁ + 0.2 mg GSH	34
	10 γ B ₁ + 0.2 γ cocarboxylase	230
4		in 45 min
	10 γ B ₁	— 11
	10 γ B ₁ + 0.2 γ cocarboxylase	76
	No B ₁ , no Mg	1
	No B ₁ , no Mg, 1 mg NaHSO ₃	44
	10 γ B ₁ + 1 mg NaHSO ₃	49
	10 γ B ₁ + 1 mg NaHSO ₃ + 0.2 γ cocarboxylase	154
5		in 45 min
	10 γ B ₁	— 15
	10 γ B ₁ + 0.2 γ cocarboxylase	101
	10 γ B ₁ + 0.8 mg phenylhydrazine HCL	3
	10 γ B ₁ + 0.8 mg phenylhydrazine HCL + 0.2 γ cocarboxylase	151
	10 γ B ₁ + 1.6 mg phenylhydrazine HCL	23
	10 γ B ₁ + 1.6 mg phenylhydrazine HCL + 0.2 γ cocarboxylase	157

From an inspection of the table it can be seen that all these substances markedly stimulate the decarboxylation of pyruvic acid. Similar effects have been obtained with H₂S. These compounds were found to be effective in amounts as low as 0.01 mg for cysteine hydrochloride, 0.1 mg for glutathione, 0.02 mg for sodium bisulfite, and 0.2 mg for phenylhydrazine hydrochloride. There are 3 possible explanations for the effects observed: (1) Free SH-groups are essential for the activity of the enzyme and since all these compounds are reducing agents they may convert SS-linkages to SH-groups. (2) The compounds, particularly cysteine and reduced glutathione, may combine with the substrate to render it more active. Schubert^a has been able to demonstrate compound formation between pyruvic acid

and SH-compounds. (3) The compounds may react with acetaldehyde, the chief reaction product; thus removing its influence. Lohman and Schuster⁴ have shown that acetaldehyde inhibits the system and Schubert has observed compound formation between SH compounds and the former. The first possibility appears to us to be the most likely.

The work is being continued and will be reported in more detail at some future date. On the basis of the present work, the assay of tissues and biological fluids for cocarboxylase by the method of Ochoa and Peters² or Goodhart and Sinclair⁵ may be open to question, since most tissues are rich in SH-compounds. This may offer an explanation for the results obtained by Lipschitz, *et al.*,⁶ who found that livers of polyneuritic chicks sometimes gave values for cocarboxylase which were higher than those found for normal birds.

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Toxic Effect of Human Urine on Fibroblasts Growing *in vitro*.

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It has long been held that urine possesses toxic properties. Bouchard¹ demonstrated the toxicity of urine by injecting it into the ear vein of a rabbit, determined how much was needed to kill the animal, and called quantity required per kg of body weight the urotoxic coefficient. This method came to be employed for determining the toxicity of the urine in various physiological and pathological conditions. Diminished toxicity in certain pathological conditions, especially uremia, was ascribed to the retention of toxic substances in the blood and was held to be the cause of some or all of

³ Schubert, M. P., *J. Biol. Chem.*, 1936, **114**, 341.

⁴ Lohman, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

⁵ Goodhart, R. S., and Sinclair, H. M., *Biochem. J.*, 1939, **33**, 1099.

⁶ Lipschitz, M. A., Potter, V. R., and Elvehjem, C. A., *Biochem. J.*, 1938, **32**, 474.

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¹ Bouchard, quoted by Volhard, *Bergmann und Stachelin's Handbuch der Inneren Medizin*, Berlin, 1931, **6**, 720.