saturated $Ba(OH)_2$ solution is added until no more precipitate forms and the solution is just commencing to turn yellow. The precipitate is centrifuged off and the supernatant fluid is decanted. The precipitate is extracted 4 times for 5 minutes with 40 cc of cold water. All 5 supernatant fluids are united and after cooling 3% H_2SO_4 is added to slight blue reaction of congo red paper. The $BaSO_4$ is centrifuged off and discarded. The Ba-free solution is concentrated to 30 cc in vacuum at 25°. It is cooled in ice water and 15 volumes of a mixture of 1 part of absolute alcohol and 2 parts of ether are added which precipitates the cocarboxylase in the form of microscopic needles. Sometimes a gummy mass will form which turns into long macroscopic needles on short standing in the cold. The preparation obtained on 6 recrystallizations from the alcohol-ether mixture, redissolved each time in 10 cc of N/5 HCl, and dried in vacuum is readily soluble in water and is free of inorganic salts.

This synthetic preparation is practically as active as natural cocarboxylase[2] (very kindly furnished by Professor Lohmann). Phosphorus and thiamin content, however, indicate that my cocarboxylase still contains a small amount of impurities. The synthetic cocarboxylase gives a yellow color with the formaldehyde-azo-test of Kinnersley and Peters. Thiamin gives a red color.

I wish to thank Merck and Company, and Winthrop Chemical Company, for generous gifts of synthetic vitamin B_1.

10053 P

New Activators of the Carboxylase System and the Function of Cocarboxylase.

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The enzyme carboxylase which plays an important rôle in carbohydrate metabolism of plants was discovered by Neuberg in 1911. Only 21 years later was it shown by Auhagen[1] that the activity of this enzyme depends on the presence of a specific coenzyme. At the

[2] Lohmann, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

[1] Auhagen, E., *Z. Physiol. Chem.*, 1932, **204**, 149.

same time Auhagen found that for full activity Mg ions are necessary. This was also known to be the case with zymase. Recently Lohmann and Schuster[2] have shown that cocarboxylase is the pyrophosphoric acid ester of thiamin (vitamin B_1 pyrophosphate) and that manganese ions are better activators than magnesium ions. Copper, iron, bismuth, and cadmium salts, as well as sodium fluoride inhibit the activity of the enzyme carboxylase.[3]

Some New Activators. I have tested a series of salts and found that NaCl, Na_2SO_4, KCl, as well as NaCN activate the carboxylase-cocarboxylase system. While Mn and Mg ions, respectively, activate best, the order of activation obtained by NaCN is close to that shown by $MgCl_2$. For example activation by 0.1 mg Mg as $MgCl_2$, is slightly more than by 0.4 mg of Na as NaCN. With 4 mg of Na, of any of the 3 neutral salts, good activation was obtained. Li_2SO_4 and $NaNO_3$ did not activate. The fact that NaCN does not inhibit proves that heavy metals are not involved in this catalysis. NaCN activates, probably, because it forms a more reactive "enol" compound (cyanohydrin) with pyruvic acid. For these salt activation experiments bottom yeast washed alternately with a solution of acid phosphate and alkaline phosphate was employed.[2]

It is believed that cocarboxylase combines with the enzyme carboxylase to form a new compound which in the presence of Mg ions becomes highly active. This compound, however, has not been isolated. Nor has the enzyme carboxylase yet been obtained in pure state. Thus the rôle of cocarboxylase is unknown.

A Specific Function of Cocarboxylase. The following experiments show that one of the functions of cocarboxylase is to protect carboxylase, a very labile enzyme, from destruction. Brewers' bottom yeast was extensively washed with water and dried at room temperature. The dry yeast was then freed of cocarboxylase (by alkaline washing at 30° using a shaking machine)[2] and suspended in 10 volumes of phosphate of pH 6.2.

Experiment 1. One cc of yeast suspension and 30 μg of cocarboxylase* in 1 cc phosphate of pH 6.2 were placed in the main

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[2] Lohmann, K., and Schuster, P., *Biochem. Z.*, 1937, **294**, 188.

[3] Discussed by Oppenheimer, C., *Die Fermente*, Supplement, 1938, **2**, 1421.

* In all experiments described in this paper synthetic cocarboxylase[4] which had been freed of inorganic salts and was 6 times recrystallized,[5] and natural crystalline (yeast) cocarboxylase were employed with identical results.

[4] Tauber, H., *J. Am. Chem. Soc.*, 1938, **60**, 730.

[5] Tauber, H., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 888.

compartment of a Warburg vessel (of 17 cc capacity and 2 side arms). In one side arm 0.5 cc of sodium pyruvate (5 mg of pyruvic acid containing 0.1 mg of magnesium as $MgCl_2$) was placed. The vessel was connected with a Warburg-Barcroft respirometer and shaken for 105 min at 30°. Then the stopcock was closed and after 15 min of further shaking the pyruvate was washed in from the side arm.

Experiment 2. In another vessel the pyruvate was placed in one side arm and the cocarboxylase was placed in the second side arm while the main compartment of the vessel contained 1 cc of yeast suspension. This vessel also was shaken for 120 min and then the pyruvate and cocarboxylase were added. Cocarboxylase solutions are very stable between pH 4 and 10 at 30°.

Experiment 3. Here 30 μg of synthetic vitamin B_1 (Merck) in 0.1 cc phosphate of pH 6.2 and 1 cc of yeast suspension were placed in the main compartment of the Warburg vessel. The cocarboxylase and pyruvate were added at the end of 120 min.

Experiment 4. The content of this vessel was similar to that of Experiment 1. Cocarboxylase, however, was replaced by 1 cc of H_2O.

It may be seen from Experiment 1, Table I, that when cocarboxylase was immediately added to freshly washed yeast and kept at 30° for 120 min in the presence of air CO_2 formed after the addition of pyruvate very rapidly. If the washed yeast, however, was kept for the same length of time without cocarboxylase the enzyme carboxylase lost almost all of its activity and there was hardly any CO_2 formed (Experiment 2). Thiamin had no protective action on carboxylase and no cocarboxylase was formed during the duration of the experiment from the vitamin (Experiment 3). The yeast suspension without added cocarboxylase did not form CO_2 from sodium pyruvate (Exp. 4). Other experiments (not included in Table I) have shown that the protective function cannot be replaced by Ba-adenosinetriphosphate, Mg-hexosediphosphate, or by reduced glutathione.

TABLE I.

Exp. No.	Content of main compartment during first 120 min.	CO_2* in 10 min.	CO_2* in 20 min.	CO_2* in 40 min.
1	Yeast suspension and cocarboxylase	40	66	102
2	Yeast suspension alone	4	12	24
3	Yeast suspension and thiamin	5	12	24
4	Yeast suspension and water	0	0	0

*After addition of side arm contents.

Cocarboxylase (pyrophosphoric acid ester of thiamin) has a specific protective action upon the enzyme carboxylase.

I am greatly indebted to Professor Lohmann for a sample of natural cocarboxylase, and to J. Ruppert Brewery, through the kindness of Mr. E. Muhlhausen, for furnishing the yeast.

10054 P

Pathologic Changes Produced by Subcutaneous Injection of Rattlesnake (Crotalus) Venom into *Macaca mulatta* Monkeys.*

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Reports of the gross and microscopic lesions in fatal cases of rattlesnake poisoning are rare. Knowledge of the pathologic changes is based mainly upon findings in experimental animals.[1] These changes have been described in detail in dogs.[2,3] Taube and Essex[3] administered large doses of venom (crotalin) intravenously and produced widespread hemorrhagic lesions throughout the body. In nature, however, the venom is usually injected subcutaneously. It is our purpose to report the lesions produced by the subcutaneous injection of a lethal dose of crotalin into the *Macaca mulatta*[4] monkey.

Nine young *Macaca mulatta* monkeys were given rattlesnake *(Crotalus atrox)* venom into the subcutaneous tissues of the lumbar region. The dose, given as a 1% solution in saline, corresponded to 7 to 10 mg of the dried venom per kilo of body weight.

For several hours after injection the animals behaved about as usual, but later became weak, lethargic, and refused to eat. The lethargy and weakness increased in severity until death occurred at an average of 36.5 hr after injection.

* Aided by a grant from the American Association for the Advancement of Science.

1 Noguchi, H., *Snake Venoms*, Carnegie Institution of Washington, 1909.

2 Jackson D., *Southern Med. J.*, 1929, **22**, 605.

3 Taube, H. N., and Essex, H. E., *Arch. Path.*, 1937, **24**, 43.

4 Zuckerman, S., and Fulton, J. F., *The Nomenclature of Primates Commonly Used in Laboratory Work*, Tuttle, Morehouse & Taylor Co., New Haven, 1934.