It seems clear, therefore, that the phrenic nerve cells can discharge nerve impulses when afferent connections are blocked—presumably even synapsing ones from the medullary respiratory center.† Similarly, in the isolated olfactory bulb the neurones maintain their rhythm in the absence of impinging nerve impulses, for the rhythm is preserved, actually enhanced and made more regular, even after half an hour's soaking in 0.5% nicotine. Since large regular waves from a cell mass are possible only with considerable synchronization of individual units, this interaction is also possible in the absence of transmitted nerve impulses.

The chemical and physical control of the activity of the single neurone and of the coördination of the group has been discussed[8,9] and additional studies on the frog (Libet and Gerard) and cat (Dubner and Gerard) will soon be published.

We are indebted to Mr. O. Sugar for assistance in some of the cat experiments.

10052 P

Preparation of Cocarboxylase.

HENRY TAUBER.

From the Research Laboratory of the McLeod Infirmary, Florence, South Carolina.

From the reaction mixture which I have recently described[1] the synthetic cocarboxylase (thiaminpyrophosphate) may be obtained in crystalline form by the following procedure: 500 mg sodium pyrophosphate are placed in a Pyrex test-tube and heated until all of the water of crystallization is removed. One cc of orthophosphoric acid (cp 85%) is placed in another large Pyrex tube and heated until a slight amount of solid deposit forms on the side of the tube. Then the pyrophosphate is added and the mixture gently heated until solution takes place. After cooling 500 mg vitamin B_1.HCl are added. The tube is placed in an oil bath of 155°, kept there for 3 min and constantly stirred. Then the tube is removed and after cooling the solid mass is dissolved in 10 cc of cold water. Cold

† The alternate possibility, that these synapses remain functional, is being further explored.

8 Gerard, R. W., *Cold Spring Harbor Symposia*, 1936, **4**, 292.

9 Blake, H., and Gerard, R. W., *Am. J. Physiol.*, 1937, **119**, 692.

1 Tauber, H., *J. Am. Chem. Soc.*, 1938, **60**, 730.

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.