ies within the twenty-five square degrees area are fainter than that magnitude.

(*c*) The distribution over the plates has been used to examine the nature of the various systematic errors of discovery and measurement. An error arising from plate tilt is intimated; but there is very little suggestion of error from neglected distance correction (figures 1 and 2).

(*d*) The belt at $+43^{\circ}.6$ crosses the Milky Way twice, and the results obtained are therefore useful in examining the effect, on the distribution of external galaxies, of interstellar space absorption along the Milky Way. Qualitatively the absorption is clearly shown in the last three columns of table 1. This latitude effect and the magnitude-frequency curves will be more closely studied in a following paper.

[1] These PROCEEDINGS, **26**, 166–176 (1940); *Harvard Reprint* 194.

[2] Seares, Kapteyn and van Rhijn, *Mt. Wilson Obs. Papers*, Vol. IV (1930).

[3] *Harv. Ann.*, **105**, No. 10, 226 (1937).

INHIBITION OF CARBOXYLASE BY THIAZOLE PYROPHOSPHATE

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In the course of investigations dealing with vitamin B_1 (thiamin) analogs, and having as their goal the furtherance of our knowledge of the physiological rôle of the vitamin, we have observed that a number of substances having a structure similar to that of cocarboxylase (thiamin pyrophosphate[1]), although not themselves able to replace the latter in the enzymatic decarboxylation of pyruvic acid, are able to inhibit markedly the activity of the carboxylase system. The most active inhibitant studied is the pyrophosphoric acid ester of the thiamin thiazole moiety (the thiazole pyrophosphate portion of the cocarboxylase molecule), which will be referred to as "thiazole pyrophosphate."

The experiments were carried out with the usual Warburg manometric technique. Each vessel contained 0.2 g. dried brewers' yeast which had been washed with alkaline phosphate buffer to remove cocarboxylase. The yeast was suspended in 2.0 ml. 0.2 *M* phosphate (pH 6.2) to which was added 0.5 ml. of 0.1 *M* sodium pyruvate solution (pH 6.2) containing 0.1 mg. Mg^{++} and 0.1 mg. Mn^{++}. In the side arm of each vessel was placed 1.0 ml. aqueous solution containing 4 γ cocarboxylase (synthetic[2])

and varying amounts of thiazole pyrophosphate; this solution was then tipped into the main part of the vessel after temperature equilibrium had been reached. Measurements were made at 25°C.

The thiazole was pyrophosphorylated by the method[3] which Weijlard and Tauber used in the case of thiamin. The crude material was purified by conversion into the silver salt[4] and from this we obtained a crystalline manganese salt which was used in the experiments tabulated below:

TABLE 1

INHIBITION OF CARBOXYLASE BY INCREASING AMOUNTS OF THIAZOLE PYROPHOSPHATE (MANGANESE SALT)

VESSEL NO.	1	2	3	4	5	6
Thiazole pyrophosphate (Mn salt)	0	4 γ	8 γ	16 γ	32 γ	80 γ
Cmm. CO_2 in 30 min.	412	388	385	339	226	41

Free thiazole pyrophosphate, liberated in non-crystalline form from the silver salt, gave results consistent with the above. The inhibition is not due to a change in pH; moreover, pyruvic acid decarboxylation is not affected by free thiazole, thiazole monophosphate or sodium pyrophosphate.

We believe that the observed inhibition phenomenon is to be explained on the basis of a competition between cocarboxylase and thiazole pyrophosphate for the specific carboxylase protein with which the two are similarly able to combine. The introduction, together with cocarboxylase, of a substance also capable of combining with the specific protein but giving an inactive "enzyme analog" results in a lowered rate of pyruvate decarboxylation. This interpretation receives support from experiments in which the thiazole pyrophosphate was not added to the protein simultaneously with the cocarboxylase but at a definite time interval before the addition of the latter. It was found that under these conditions the rate of CO_2 production was initially lower than when coenzyme and inhibitor were added together.

Since it is logical to assume that the protein-inhibitor bond is similar to that between protein and cocarboxylase, this latter must therefore be joined to the protein through the pyrophosphate group,[5] which is common to both it and the inhibitor. Thus we have independent confirmation of the prevailing view[6] regarding the nature of this binding, which view was until now supported almost entirely by analogy with the alloxazine-proteid complex.[7]

We conclude that there has been demonstrated here a not hitherto recognized type of competitive inhibition of enzyme reactions, caused by competition not between substrate and inhibitor but between coenzyme and inhibitor. The importance of this type of inhibition must be emphasized because its study may throw light on structural relationships in protein chemistry and on enzyme reaction kinetics and also because there is the

possibility that such inhibition mechanisms are of significance in the chemistry of the cell.

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[1] Lohmann, K., and Schuster, P., *Biochem. Z.*, **294**, 188 (1937).

[2] Weijlard, J., and Tauber, H., *Jour. Amer. Chem. Soc.*, **60**, 2263 (1938).

[3] We are indebted to Mr. J. Weijlard (Merck and Co., Inc.) who kindly communicated to us the results of unpublished experiments on the pyrophosphorylation of thiazole.

[4] Lohmann and Schuster (ref. 1, page 196) have obtained the silver salt of thiazole pyrophosphate after cleavage of cocarboxylase by sulfite.

[5] A binding through the agency of some other grouping in the cocarboxylase molecule is also to be expected.

[6] See Stern, K. G., and Melnick, J. L., *Jour. Biol. Chem.*, **131**, 610 (1939); Bersin, T., *Kurzes Lehrbuch der Enzymologie*, page 96, Akademische Verlagsgesellschaft, 1938.

[7] Kuhn, R., and Rudy, H., *Ber. Deut. Chem. Ges.*, **69**, 2563 (1936).

ON HOMOTOPY GROUPS

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1. We shall denote by K a finite polyhedron with a fixed cell-decomposition and an orientation attached to each cell. K^m will stand for the subpolyhedron consisting of all cells of K of dimension $\leqslant m$. By definition $K^{-1} = 0$.

Let G be an abelian group and Y a topological space. A triple (K, A^n, f), where A^n is an n-chain in K with coefficients in G and f is a continuous mapping $f(K) \subset Y$, is called a *continuous* n-chain in Y with coefficients in G. Such n-chains and $(n+1)$-chains may serve to define a homology group $\mathfrak{H}^n(Y, G)$.[1]

2. Let y_0 be a fixed point of Y. An n-chain (K, A^n, f) will be called an (n, m)-chain if $f(K^{m-1}) = y_0$ where $0 \leqslant m \leqslant n$. Using the (n, m)-chains and the $(n+1, m)$-chains we can define a new homology group $\mathfrak{H}^{n,m}(Y, G)$.

In the definition of $\mathfrak{H}^{n,m}$ it is essential to specify the point y_0. However, if y_1 is any point which may be joined by an arc to y_0, then the resulting group is isomorphic with the initial group.

3. Let $\pi_i(Y)$ be the ith homotopy group[2] of Y with the point y_0 as origin $(i > 0)$. It is convenient to introduce the notation $\pi_0(Y) = 0$ to indicate that Y is arcwise connected. It is well known that if $\pi_i(Y) = 0$ for $i < m$