proximately some liver lobes may show an irregular yellowish-green mottling on their surfaces. This mottling is generally most marked in the left anterior and posterior lobes and is least in the caudate lobe. At no time was any dye visible in the lymphatics of the gall bladder or the liver hilus. If the animal is now bled to death from the abdominal aorta, the dye may be demonstrated in the serum and the relatively bloodless liver now reveals the dye more clearly than before. If the liver substance is scraped away, scraping from the free border of a lobe toward the hilus, yellow hepatic ducts can be easily demonstrated. These yellowish ducts may often extend practically to the border of the lobe; not all hepatic ducts in any one lobe exhibit the dye, however, and their distribution is irregular.

On the basis of these results it seems probable to us that the chain of conditions described above plays a rôle in producing or maintaining a cholangitis or hepatitis when it happens to a subject with an infected gall bladder. This supposition is strengthened by several observations in rabbit where contraction of the gall bladder occurred at a time when the bile papilla was tonically contracted, so that clamping of the choledochus was not necessary; in these instances the choledochus and bile ducts became markedly distended with the dye-bile solution.

10419

Bacterial Synthesis of Cocarboxylase.*

M. SILVERMAN AND C. H. WERKMAN.

From the Bacteriology Section, Industrial Science Research Institute, Iowa State College, Ames, Iowa.

The authors[1] have submitted evidence indicating that *Propionibacterium pentosaceum* can phosphorylate vitamin B_1 to its pyrophosphoric acid ester (cocarboxylase). This synthesis was carried out in the presence of liver-extract and hexosediphosphate. More recently, Ochoa and Peters[2] have shown that vitamin B_1, or suitable pyrimidine fractions, can in the presence of cocarboxylase, catalyze

* Appreciation is expressed to Merck and Company for synthetic crystalline cocarboxylase, to Winthrop Chemical Company for synthetic vitamin B_1, and to Anheuser-Busch for the bottom-yeast.

1 Silverman, M., and Werkman, C. H., *Enzymologia*, 1939, **5**, 385.

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

cleavage of pyruvic acid. They have also reported that hexosediphosphate in itself increases the rate of breakdown of pyruvic acid by washed yeast in the presence of cozymase and cocarboxylase. This knowledge has cast some doubt on the bacterial synthesis of cocarboxylase reported by us. At present we wish to report on the synthesis of cocarboxylase from vitamin B_1 by *P. pentosaceum* in the absence of any added liver-extract or hexosediphosphate.

Methods. Materials. Bacterial cells. *P. pentosaceum* was transferred from a yeast-extract-peptone broth to the basal medium of Tatum, Wood, and Peterson[3] containing in addition 0.005% cystine and 0.075% hydrolyzed casein. Three transfers were made in the basal medium at 24-hour intervals, the third into 300 cc of medium. The cells were harvested at the end of 72 hours' incubation at 30°C and washed twice in distilled water. The yield from 300 cc of medium amounted to about 0.4 cc of cell-paste. Aqueous extracts showed the presence of only 0.3-0.4 γ of vitamin B_1 per g of dried cells.

Atiozymase. One g of dried brewer's "bottom" yeast was washed rapidly 3 times (*cf.* Ochoa and Peters) in 50 cc M/10 Na_2KPO_4 at 30°C. It was then immediately washed in distilled water and suspended in 10 cc of M/15 phosphate buffer, pH 6.2. One cc was employed in each Warburg vessel in the assay of cocarboxylase.

Chemicals. Magnesium was used as Mg Cl_2 C.P.

Winthrop's Vitamin B_1 and synthetic cocarboxylase (Merck) were employed. The vitamin B_1 solution contained 10 γ per cc.

Synthesis. The following were placed in each of two 50 cc centrifuge cups: 0.2 cc cell paste + 1 cc H_2O + 10 γ vitamin B_1 + 1 cc PO_4 buffer M/15, pH 6.2.

The cups were incubated in air at 30°C with frequent shaking for 4 hours and then placed in boiling water for 5 minutes. The B_1 was added to the second cup at the time of boiling. The cells were then centrifuged from suspension and 1 cc of the supernate was assayed for the presence of cocarboxylase.

Assay. The cocarboxylase assays were carried out in simple Warburg vessels at 30°C under an atmosphere of air. Each assay vessel

TABLE I.

Activator	Supernate		2.3 γ cocarboxylase	Control*
	Cup 1	Cup 2		
mm³ CO_2 in 30 min.†	324	28	236	21

*No added cocarboxylase.
†Values corrected for endogenous CO_2.

[3] Tatum, E. L., Wood, H. G., and Peterson, W. H., *Biochem. J.*, 1936, **30**, 1898.

contained 9 mg pyruvic acid in the side cup and 1 cc of atoizymase and 0.1 mg magnesium in the main vessel. The total volume was 2.3 cc.

Since the assay system was not previously saturated with vitamin B_1, the assay is not quantitative for cocarboxylase, but there is no doubt that synthesis did occur, for where the vitamin was not incubated with the bacterial cells, there is almost no catalysis (cup No. 2).

Additional evidence that vitamin B_1 is converted into cocarboxylase by suspensions of *P. pentosaceum* grown in vitamin B_1 deficient media, may be obtained by comparing the effects of vitamin B_1 and cocarboxylase in the anaërobic pyruvate metabolism of these suspensions. In Table II are presented the data in which equimolar quantities of vitamin B_1 hydrochloride and synthetic cocarboxylase were added to 1 cc portions of 1:20 (by volume) cell-suspensions in M/15 PO_4 buffer, pH 5.6, and tested for its activity on 9 mg of pyruvic acid, as sodium pyruvate, under nitrogen in simple Warburg manometers at 30°C. In cups 3 and 5 the activators were placed in the side arm with the pyruvic acid; in cups 2 and 4, the activators were added directly to the suspensions in the main vessel. The contents were held at room temperature for 15 minutes and then incubated for 30 minutes with agitation in the waterbath. At the end of this time, the substrate and suspensions were mixed and readings were taken.

In Table II we note that in case of cells incubated with the catalysts (cups 2 and 4), the rates of CO_2 production from pyruvic acid are similar. Where cocarboxylase has been added from the side cup (cup 5), a lag of 30 minutes occurs before these cells attain the rates of No. 2 and No. 4. However, where vitamin B_1 is added from the

TABLE II.

Comparison of Vitamin B_1 and Cocarboxylase as Stimulants in the Anaërobic Pyruvate Metabolism of *P. pentosaceum* grown in Vitamin B_1 Deficient Media.

Cup	1	2	3	4	5
Activator	None	0.25 γ vitamin B_1		0.34 γ cocarboxylase	
		Main vessel	Side cup	Main vessel	Side cup
Interval min.					
0-30	28	95	51	95	60
30-60	8	64	35	59	51
60-90	7	54	32	53	50
90-120	7	55	41	56	52
120-150	6	42	37	44	50
150-180	5	46	41	46	47

Results in mm^3 corrected for endogenous CO_2.

side cup (cup 3), there is a lag of fully 120 minutes before this cell-suspension reaches a rate comparable to the others. A reasonable explanation for the existence of this lag is that time is required for the conversion of the vitamin B_1 into cocarboxylase in which form it acts as the catalyst. In any case, however, if no previous incubation with the bacterial cells is permitted, cocarboxylase is a more effective stimulant than is vitamin B_1.

Summary. Evidence is presented that cell-suspensions of *P. pentosaceum* are capable of converting vitamin B_1 into cocarboxylase.

10420

Occurrence and Significance of Oxalacetic Acid in Plant Tissues.*

ORVILLE WYSS, R. H. BURRIS AND P. W. WILSON. (Introduced by E. G. Hastings.)

From the Department of Agricultural Bacteriology, University of Wisconsin, Madison.

The investigations of Szent-Györgyi,[1] Stare and Baumann,[2] and Krebs[3] have established the importance of the 4 carbon dicarboxylic acids in the respiration of animal tissues and several species of bacteria, but extension of their findings to plant tissues has not been made. Such extension might be naturally assumed since succinic, fumaric and malic acids, as well as the corresponding amino acid, aspartic, and its amide, asparagine, are well-known constituents of most plant species. One line of evidence for demonstration of this assumption would be the detection of oxalacetic acid in respiring plant tissues. In connection with our studies on the respiratory enzymes of the root nodules of leguminous plants, examination of these was made for the presence of oxalacetic acid, but none was found.

Both the colorimetric method of Szent-Györgyi and Straub[4] and the manometric method of Ostern[5] were used for detection of the oxalacetic acid. Fresh plant tissue was cooled to 0°C, ground in a Nixtamal mill and the sap expressed in a Carver hydraulic press.

* Herman Frasch Foundation in Agricultural Chemistry, Paper No. 188.

1 Szent-Györgyi, A., *Studies on Biological Oxidations and Some of Its Catalysts*, Budapest, 1937.

2 Stare, F. J., and Baumann, C. A., *Proc. Roy. Soc.* (London), 1936, **121B**, 338.

3 Krebs, H. A., *Biochem. J.*, 1937, **31**, 2095.

4 Straub, F., *Z. Physiol. Chem.*, 1936, **244**, 117.

5 Ostern, P., *Z. Physiol. Chem.*, 1933, **218**, 160.