PANTOTHENIC ACID AND THE UTILIZATION OF GLUCOSE BY LIVING AND CELL-FREE SYSTEMS*

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I

INTRODUCTION

Since the discovery of the vitamin properties of pantothenic acid much interest has been aroused as to its biochemical function. Williams and coworkers found that it stimulated the deposition of carbohydrate by green plants without affecting their nitrogenous constituents.[1] The glycogen storage of yeast was also found to be increased[2] by incubating it with pantothenic acid. Yeast grown on a medium deficient in pantothenic acid possessed a low fermenting ability which was greatly increased by the addition of pantothenic acid.[3] Such yeast was found to have a much lower pantothenic acid content than normal yeast. The rate of fermentation of sucrose by dialyzed yeast maceration juice was also observed to be slightly accelerated by the addition of calcium pantothenate. These experiments indicated that pantothenic acid was involved, probably as a coenzyme, in some stage of carbohydrate metabolism.

In the present study these experiments were repeated and extended. Since the tissues of chicks grown on a diet deficient in pantothenic acid are low in pantothenic acid,[4] deficient brain and muscle served as basal tissues on which the effect of added pantothenic acid could be studied. Maceration juice preparations from yeast served as cell-free material on which similar studies could be made. All of the respiration experiments were carried out in a Warburg-Barcroft microrespirometer.

II

Effect on Maceration Juice

Maceration juice was prepared from Fleischmann's baker's yeast. This

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[1] McBurney, C. H., Bollen, W. B., and Williams, R. J., 1935, *Proc. Nat. Acad. Sc.*, **21**, 301.

[2] Williams, R. J., Mosher, Wm. A., and Rohrmann, E., 1936, *Biochem. J.*, London, **30**, 2036.

[3] Pratt, E. F. and Williams, R. J., 1939, *J. Gen. Physiol.*, **22**, 637.

[4] Snell, E. E., Pennington, D., and Williams, R. J., 1940, *J. Biol. Chem.*, **133**, 559.

juice was freed of its major portion of pantothenic acid in one of two ways: (1) by dialysis, (2) by precipitating the active enzymes with acetone and redissolving the precipitate in 1 per cent sodium chloride solution. Either of these procedures gives a preparation containing considerably less pantothenic acid than normal maceration juice as determined by the method of Pennington, Snell, and Williams.[5] However, it was observed that additional pantothenic acid could be liberated by enzymatic action. One such juice, on which the acetone precipitation and re-solution in NaCl solution was repeated twice, contained only 0.45 γ per cc. of "free" pantothenic acid. Enzymatic action liberated from this juice an additional 5–6 γ per cc. of "bound" pantothenic acid. The determination of bound pantothenic acid was carried out by incubating a portion of the juice with homogenized fresh muscle tissue and allowing the autolytic enzymes of the muscle tissue to act on the yeast juice. The difference between the pantothenic acid content of this mixture and of the fresh muscle tissue itself was taken as the sum of the "free" and "bound" pantothenic acid in the yeast juice.

TABLE I

Fermentation of Glucose by Dialyzed Maceration Juice

Calcium pantothenate	0	0	0	0.1γ	0.1γ	0.1γ	10γ	10γ
CO_2 per hr., mm.^{3}	368	379	375	356	358	374	351	379

A number of experiments were performed to determine the effect of pantothenic acid on the fermentation of glucose by the dialyzed and acetone-precipitated maceration juice. No effect due to pantothenic acid was observed. Table I shows a typical run with juice dialyzed $8\frac{3}{4}$ hours at 0–10° C. against 0.9 per cent NaCl solution. A small amount of hexose diphosphate was used in each flask to convert the adenylic acid into adenosine triphosphate. No appreciable fermentation took place unless this was added.

Each flask contained 1 cc. of 0.4 M glucose, 1 cc. of KH_2PO_4 (8 gm. per liter), 10 γ of adenylic acid, 10 γ of coenzyme I, 0.1 cc. of salt solution (0.025 M $MgSO_4$, 0.05 M $MnSO_4$, and 0.3 M $(NH_4)_2SO_4$), and 7 mg. of sodium hexose diphosphate. Total volume, 4 cc.

Although it is generally accepted that no coenzyme, other than cocarboxylase, is required for the decarboxylation of pyruvic acid, several experiments were carried out to test the possibility of pantothenic acid's having some effect on this stage of the fermentation reaction. Pantothenic acid showed no effect on the decarboxylation of pyruvic acid by acetone-precipitated maceration juice.

The effect of pantothenic acid on the rate of phosphorylation of glucose by

[5] Pennington, D., Snell, E. E., and Williams, R. J., 1940, *J. Biol. Chem.*, **135**, 213.

dialyzed maceration juice was determined. The calcium salt of pantothenic acid was used, and controls containing calcium chloride were included since calcium ion can to some extent replace magnesium in the phosphorylation enzyme. Inorganic phosphate was determined according to the method of Briggs[6] with the Evelyn photoelectric colorimeter. The decrease in inorganic phosphate was assumed to be the amount esterified with glucose.

The results of a typical experiment are given in Table II. The quantities shown are inorganic phosphate, and the difference between the initial reading and the test reading is the decrease in inorganic phosphate. It can be seen from the table that no effect of calcium pantothenate was observed which was not attributable to the calcium ion.

The results of these experiments with maceration juice indicate that pantothenic acid does not function as a dissociable coenzyme for the enzyme systems involved in the fermentation of glucose.

TABLE II

Phosphorylation of Glucose by Dialyzed Maceration Juice

Calcium pantothenate added	Initial	10 min.	30 min.	60 min.	180 min.
0	1.16	1.12	0.89	0.56	0.48
1γ	1.16	1.09	0.92	0.56	0.47
10γ	1.16	1.12	—	0.56	0.48
100γ	1.16	1.10	0.92	0.57	0.50
15γ $CaCl_2$	1.16	1.10	0.91	0.57	0.50

Figures represent milligrams of phosphorus present as inorganic phosphate.

III

Effect on Live Yeast

The large effect of pantothenic acid upon the fermentation of glucose by yeast deficient in pantothenic acid is apparently in contradiction to the fact that pantothenic acid showed no effect on the fermentation by maceration juice from which most of the pantothenic acid had been removed. This apparent contradiction suggested that pantothenic acid may act only in a combined form. If this is the case, one would expect the accelerating action of the pantothenic acid on the deficient yeast to be accompanied by a parallel binding of the pantothenic acid in the yeast. The following experiment was performed to determine if this binding takes place.

[6] Briggs, A. P., 1924, *J. Biol. Chem.*, **59**, 255.

Gebrüder-Mayer yeast was grown from a heavy seeding in a medium containing sucrose, asparagine, salts, thiamin, nicotinic acid, riboflavin, biotin, and inositol, but no pantothenic acid. A twelvefold increase was obtained. These deficient cells were centrifuged off and washed with KH_2PO_4 solution before being used. A preliminary experiment using 2 mg. quantities of this yeast showed that an appreciable increase in CO_2 evolution occurred on the addition of as little as 0.1γ of pantothenic acid.

For the determination of the pantothenic acid bound by the deficient yeast 50 mg. quantities of yeast were used. These were incubated for 4 hours at 30°C. with 5 cc. of KH_2PO_4 solution (4 mg. per liter), 5 cc. of 0.4 M glucose solution, and 1 cc. of the indicated amount of pantothenic acid. The cells were then centrifuged down, washed three times with 1 per cent NaCl solution, and finally suspended in 10 cc. of acetic acid-sodium acetate buffer solution (pH = 4.5). One half of each sample was steamed for 10 minutes, then autoclaved for 15 minutes at 15 pounds pressure. The hot water extract was used for the determination of the free pantothenic acid in the

TABLE III

Pantothenic Acid Bound by Deficient Yeast

Pantothenic acid added	Free pantothenic acid in 50 mg. of deficient yeast	Pantothenic acid bound by 50 mg. of deficient yeast
0	0.08γ	0*
0.5γ	0.06γ	0.30γ
5 γ	0.28γ	0.68γ
50 γ	0.30γ	0.74γ

* Too small to determine.

cells. The other half of each sample was incubated for 36 hours at 37°C. with a mixture of clarase and caroid. This enzyme treatment[7] has been standardized in this laboratory for the liberation of pantothenic acid bound in tissues. This portion was used for the determination of the total pantothenic acid in the cells. The difference between these quantities was then taken as the amount of "bound" pantothenic acid.

The results of this experiment are given in Table III. It is evident from these data that the increase in the rate of fermentation by the deficient yeast is accompanied by a parallel "binding" of the added pantothenic acid in the cell, probably to form an integral part of some enzyme. Since the medium used contained no nitrogen from which proteins might be synthesized, it seems likely that the protein was already preformed in the cell and needed only the addition of pantothenic acid to complete the enzyme system. This would explain the absence of acceleration of fermentation upon the

[7] Unpublished work, Cheldelin *et al.*, Department of Chemistry, The University of Texas.

addition of pantothenic acid to the treated yeast juices. The Fleischmann's yeast is grown in a medium relatively rich in pantothenic acid. In this instance any of the enzyme protein formed would probably be combined with pantothenic acid and none of the pantothenic acid-free protein would be present in the yeast juice. If this were the case, then addition of extra pantothenic acid would be expected to have no effect.

Snell had prepared the sulfonic acid analogue of pantothenic acid, *N*-(α,γ-dihydroxy-β,β-dimethylbutyryl) taurine.[8,9] He has shown that this substance acts antagonistically to pantothenic acid in the latter's effect on the growth of yeast and other microorganisms. Several experiments were carried out to determine if it also acted antagonistically to the effect of pantothenic acid on the fermentation of deficient yeast. No inhibition was observed. On the contrary, when large amounts of the pantoyl taurine were added, fermentation was accelerated. The addition of large quantities of the pantoic lactone also had an accelerating effect. It seems possible, therefore, that this unexpected effect of the pantoyl taurine was at least partially due to the pantoyl radical.

TABLE IV

Pantothenic Acid Content of Chick Tissues

Tissue	Deficient	Normal
Brain	23.2γ	46.2γ
Breast muscle	3.2γ	12.1γ

IV

Glycolysis by Chick Tissues Deficient in Pantothenic Acid

Day old chicks were fed a diet consisting of 74.5 per cent yellow corn meal, 18 per cent washed "vitamin-free" casein, and 7.5 per cent salt mixture supplemented by vitamins as follows: liver extract treated with 10 per cent HCl in methanol equivalent to 20 per cent fresh liver in the diet, 0.2 mg. of thiamin per 100 gm. of diet, petroleum ether extract of 1 gm. of alfalfa leaf meal per 100 gm. of diet, and 2 per cent U.S.P. cod liver oil. This feed had a pantothenic acid content of not more than 0.4 mg. per 100 gm. The experiments were carried out on the tissues when the chicks were 3 to 4 months old. The pantothenic acid analyses on the tissues of the deficient chicks and of chicks fed a commercial diet are given in Table IV. As can be seen, the deficient tissues contained considerably lower content of pantothenic acid than the normal tissues. For all the work reported here the tissues were homogenized according to the method of Potter and Elvehjem.[10]

[8] Snell, E. E., 1941, *J. Biol. Chem.*, **139,** 975.

[9] Snell, E. E., 1941, *J. Biol. Chem.*, **141,** 121.

[10] Potter, V. R., and Elvehjem, C. A., 1936, *J. Biol. Chem.*, **114,** 495.

The effect of pantothenic acid on the oxygen absorption of homogenized breast muscle during the utilization of glucose was determined. A control containing no glucose gave considerably less oxygen absorption, showing that glucose was being metabolized. It can be seen from Table V that pantothenic acid had no effect.

Each flask contained 325 mg. (moist weight) of homogenized muscle in 1.5 cc. of phosphate Ringer solution without sodium carbonate or calcium ion, 0.3 cc. of 2.5

TABLE V

Oxygen Consumption of Homogenized Breast Muscle

Calcium pantothenate	Oxygen per mg. of tissue (dry weight)						
	5 min.	10 min.	20 min.	30 min.	45 min.	105 min.	120 min.
	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³	*mm.*³
0*	0.06	0.11	0.18	0.26	0.36	0.73	0.81
0	0.11	0.17	0.25	0.34	0.45	0.93	1.03
0	0.12	0.18	0.24	0.36	0.44	0.88	0.96
10	0.10	0.15	0.22	0.33	0.44	0.91	0.99
10	0.15	0.20	0.25	0.34	0.43	0.85	0.94

* Control, contained no glucose.

TABLE VI

Anaerobic Glycolysis of Homogenized Brain

	CO_2 per mg. of tissue (dry weight)			
	15 min.	45 min.	60 min.	Next 15 min.
	*mm.*³	*mm.*³	*mm.*³	*mm.*³
No pantothenic acid	0.67	2.15	2.56	0.45
10γ calcium pantothenate	0.82	2.13	2.50	0.44
1000γ sodium salt of pantoyl taurine	0.75	2.25	2.25	0.58

per cent glucose, 0.1 cc. containing the indicated amount of calcium pantothenate, and 0.15 cc. of 20 per cent KOH in the center cup. An atmosphere of air was used.

The effect of pantoyl taurine as well as that of pantothenic acid itself upon the anaerobic glycolysis of pantothenic acid deficient homogenized chick brain was determined. The results shown in Table VI indicate that neither pantothenic acid nor its sulfonic acid homologue has any effect.

The gray matter of the brain from a deficient chick was homogenized in phosphate Ringer solution and 2.9 cc. containing 200 mg. (moist weight) were pipetted into each flask. To this was added 0.1 cc. of water containing the test substance. The flasks were then placed in the bath and aerated for 5 minutes with a mixture of 95 per cent

nitrogen and 5 per cent carbon dioxide. The mixture was passed over heated copper to remove all traces of oxygen. The sodium D(+) salt of pantoyl taurine was used. Results are shown in Table VI.

A number of other experiments using deficient chick tissues were performed, but in no case could any effect of pantothenic acid, either on the oxygen consumption or the anaerobic glycolysis, be demonstrated. It therefore seems unlikely that pantothenic acid serves as a *dissociable* coenzyme for the glycolytic system.

SUMMARY

1. Added pantothenic acid was found to have no appreciable effect on the fermentation of glucose when used in conjunction with preparations of dialyzed yeast maceration juice or acetone-precipitated yeast maceration juice.

2. Addition of pantothenic acid failed to affect the rate of phosphorylation of glucose or the rate of decarboxylation of pyruvic acid by yeast maceration juice.

3. Pantothenic acid showed no effect on the rate of glycolysis by homogenized deficient chick tissues.

4. The accelerating effect of pantothenic acid on fermentation by deficient yeast cells was found to be accompanied by a "binding" of pantothenic acid by the yeast cells.