CCLXXXVII. THE IMPORTANCE OF "PANTOTHENIC ACID" IN FERMENTATION, RESPIRATION AND GLYCOGEN STORAGE.

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WHILE the primary research dealing with the isolation and chemical characterization of "pantothenic acid" [Williams *et al.* 1933; Williams & Saunders, 1934] has engaged our study for some time, we have also carried out certain experiments which give a clue as to why this substance is universally present in tissues whether of plant, animal or bacterial origin.

If the yeast with which we have been dealing [Williams *et al.* 1933] is seeded sufficiently heavily into a synthetic medium or better into one containing a very little added pantothenic acid, a crop of yeast large enough for experimental use may be obtained. Repeated experiments in which such yeast was grown and extracted have shown that pantothenic acid is not produced as the yeast grows (slowly), but on the contrary the yeast crop and medium after growth together seem to contain somewhat less pantothenic acid than was present before growth took place. Yeast grown under such conditions may have a pantothenic acid content as low as 2% of that present in "normal" yeast.

This yeast is almost lacking in fermenting power as compared with yeast grown under favourable nutrient conditions. It is capable of bringing about a much increased fermentation, however, if a minute amount of a pantothenic acid preparation is added directly to the sugar solution in which the yeast is suspended. Under these conditions yeast multiplication does not take place as determined by our thermocouple method [Williams *et al.* 1929].

The results of a 21-hour fermentation are indicated in Table I. With cruder pantothenic acid preparations similar results were obtained. The largest amount of pantothenic acid added in this particular experiment was evidently an overdose. It is many times that which would be present in 80 mg. of yeast grown under ordinary conditions.

Table I. *Effect of pantothenic acid on fermentation.*

Weight of yeast (mg.)	Sucrose solution (ml.)	Pantothenic acid preparations added (γ)	Volume CO ₂ produced (ml.)
80	25	0	0.1
80	25	1 γ (2 units)	0.45
80	25	10	1.60
80	25	100	0.55

An even more striking increase in the fermenting power of this deficient yeast was obtained when a cold water extract of fresh yeast (Fleischmann's) plus a pantothenic acid preparation were together added to the fermentation mixture. The effect of this addition, which was also noted when glucose was the

substrate used, is not always uniform. It is well known from the researches of Euler and others that at least one agent other than the classical enzyme and coenzyme is involved in the fermentation process. Our experiments clear up this complex situation only to the extent of demonstrating that pantothenic acid is an indispensable factor.

The aerobic respiration of the deficient yeast is also very low as compared with that of normal yeast. The rate is increased only very little when pantothenic acid is added directly to the yeast after it is grown, but yeast grown with a good supply of pantothenic acid respire much more rapidly than the deficient yeast, which is grown under otherwise identical conditions. These experiments seem to point unmistakably to the conclusion that pantothenic acid is necessary for carbohydrate utilization whether aerobic or anaerobic.

We call attention to the fact also that in alfalfa seedlings stimulated in growth by pantothenic acid [McBurney *et al.* 1935] the amount of carbohydrate was about doubled whereas the nitrogenous constituents were not increased at all, again indicating a relationship of pantothenic acid to carbohydrate metabolism.

Finally we cite the recent experiments of McAnally & Smedley-MacLean [1935] in which it was shown that glycogen storage in yeast is greatly aided by maltose. From our previous experience with maltose and from experiments carried out since the above paper was published it is clear to us that maltose as such is probably not the active agent which promotes glycogen storage but that pantothenic acid is at least one of the factors involved. That maltose (as purchased) is always effective, and sometimes exceedingly so, in stimulating yeast growth is shown by the following series of results when the 2% sucrose in our ordinary medium [Williams & Saunders, 1934] is replaced by different brands of maltose.

Table II. *Different samples of maltose as yeast stimulants.*

Sugar used	Yeast crop (duplicates) mg./ml.
Sucrose, Baker's C.P.	0.020 0.019
Maltose, tech. Difco	0.040 0.039
Maltose, tech. Pfanstiehl	0.55 0.57
Maltose, Difco, recrystallized and dialysed	0.0375 0.038
Maltose, Difco, twice recrystallized and dialysed	0.025 0.027
Maltose, Difco, "standardized"	0.0325 0.0330
Maltose, electrically purified (see below)	0.015 0.014

In another experiment Eastman's maltose was shown to be about on a par with the "standardized" Difco brand. The electrically purified sample was purified exhaustively by fractional electrical transport [Williams, 1935] and is a product such as is not obtainable otherwise. Its response shows that maltose itself is somewhat less effective as a carbohydrate source than high grade sucrose.

To show that the electrolytic treatment actually removed pantothenic acid from the maltose (Eastman brand 5.0 g.) the results in Table III are cited. It is

Table III. *Electrolytic purification of maltose.*

Sugar used	Yeast crops (mg./ml.)
Maltose, Eastman	0.040 0.039
Same purified electrolytically	0.0125 0.0125
Cell I, electrolytic system pH 3	0.375
1/8 of cell contents used	0.375
Cell II, electrolytic system pH 4	0.475
1/8 of cell contents used	0.475

characteristic of pantothenic acid to migrate to the position where it migrated in this experiment.

We have tried four different samples of maltose and have found that all of them contain pantothenic acid in very appreciable amounts. From a consideration of the sources and the methods of preparation and purification of maltose it is not surprising that this is true. The fact that maltose contains pantothenic acid and that its removal (by electrolytic transport) leaves maltose which is not effective in yeast growth stimulation led us, with other facts, to suspect that maltose itself does not promote glycogen storage as observed by McAnally & Smedley-MacLean but that pantothenic acid is a factor in bringing about this result. In order to investigate this possibility the experiments summarized below were carried out using the methods of the above authors.

These investigators do not give definite information as to the ratio of the weight of yeast used to the volume of the solution in which it was incubated. They do, however, indicate that 2 g. of yeast were incubated in 125 ml. in a fermentation experiment. In order to obtain results which might be on the same basis as theirs we incubated 200 mg. of yeast in 12.5 ml. of solution. The smaller amount was used because of the greater ease involved in making the determinations and the smaller supply of maltose required. The electrolytic purification of large amounts of maltose is time-consuming.

The yeast used was a Munich bottom yeast obtained from the Salem (Oregon) Brewery Association. The yeast was freed from the last trace of wort by suspending in a large volume of water and centrifuging; this procedure was repeated eight times. The sugar solutions were made up of such concentration that, after the addition of the phosphate and pantothenic acid solutions, a 5% concentration of sugar was obtained. The dosage of pantothenic acid was 4 γ per sample indicated. This is about twice the amount contained in 0.6 g. of Eastman's maltose. The phosphate concentration was 0.1%, the same as used by McAnally & Smedley-MacLean. Four incubation tests were run with each sugar; two were used to determine the total carbohydrate of the yeast and two for the glycogen, using the method of Mayer. Bertrand's method was used in all sugar determinations.

All values in Table IV are in g. of sugar as glucose per 10 g. of original moist yeast. The descriptions given of the media used are self-explanatory. The abbreviation P.A. stands for pantothenic acid. The yeast was incubated for 48 hours at 30°.

From the evidence presented it is clear that pantothenic acid is an important factor in the storage of glycogen as well as being effective in increasing the total carbohydrate content of the yeast.

We realize, however, that different strains of yeast, though similar in nature, often present marked differences in behaviour. In order to make our conclusions

Table IV.

No.	Medium	Total carbohydrate (g. glucose per 10 g. original yeast)	Glycogen (g. glucose per 10 g. original yeast)
0	Water (distilled)	0.45	0.214
0	"	0.45	0.214
1	Glucose (Merck)	0.945	0.228
2	"	0.945	0.292
3	Glucose + PO ₄	1.07	0.349
4	"	1.09	0.362
5	Glucose + P.A.	1.06	0.362
6	"	1.045	0.332
7	Glucose + PO ₄ + P.A.	1.15	0.382
8	"	1.11	0.363
9	Maltose (electrolytically purified)	0.935	0.271
10	" "	0.910	0.258
11	Maltose + PO ₄	1.06	0.338
12	"	1.07	0.347
13	Maltose + P.A.	1.02	0.337
14	"	1.045	0.331
15	Maltose + PO ₄ + P.A.	1.08	0.395
16	"	1.08	0.382
17	Maltose (Eastman's)	1.24	0.462
18	"	1.26	0.462
19	Maltose + PO ₄	1.35	0.472
20	"	1.38	0.485
21	Maltose + P.A.	1.36	0.462
22	"	1.33	0.445
23	Maltose + PO ₄ + P.A.	1.40	0.53
24	"	1.44	0.505
25	Maltose (Pfanstiehl's) tech. crys.	1.62	0.585
26	" "	1.66	0.575
	Original yeast	0.86	0.265

more general we have performed similar experiments using Fleischmann's "XR" yeast instead of the brewer's yeast. The results were of the same order and led to identical conclusions. The differences in the values between the two yeasts were accounted for by the different initial glycogen contents.

These results are in line with other direct evidence which we have cited indicating that pantothenic acid plays some fundamental role in carbohydrate anabolism and catabolism, whether the latter is aerobic or anaerobic in nature. It is interesting in this instance to recall that the pantothenic acid concentration in mammalian tissue is the greatest in the liver and red striated muscle [Rohrman *et al.* 1934]. This is a suggestive fact as these tissues are pre-eminently involved in glycogen deposition.

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