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Carbohydrate Nature of Pantothenic Acid (Williams).

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Williams *et al.*[1] found that extracts from diverse tissues, representing different biological groups, contained material which had a very striking stimulation on the growth of Gebrüde Mayer yeast. From a similarity of biological and chemical reactions it was concluded that this stimulation was due to a single substance, which they called pantothenic acid, and which appeared "to have a molecular weight of about 150, to be distinctly acid without amphoteric properties, to have several hydroxyl groups in its structure, but no olefin double bond, aldehyde, ketone, sulfhydryl, basic nitrogen, aromatic or sugar groups."

Following the discovery of this acid, experiments were conducted in this laboratory to determine the effect, if any, of this substance upon bacterial growth. The organism which seemed most suitable for this work was *Escherichia coli,* since it was readily cultured and possessed considerable carbohydrate fermentative ability. Rice bran was employed because it was readily obtained and conveniently extracted.

Preliminary experiments with extracts of rice bran which were prepared by the method of Williams, refluxing the bran with a 60% methyl alcohol solution, etc., showed considerable stimulation of the organism with small amounts of the extract, but much less stimulation than observed by Williams with yeast. The results in many cases were inconsistent, due principally to the low pH of the extract and to the variations of the inoculum.

To overcome these inconsistencies and to standardize the procedure, all the extracts were first adjusted to a pH between 6.9 and 7.3. Inoculations were made with a constant amount of a broth suspension of the organisms, generally 0.2 cc. No means were attempted to standardize the infusion media, no 2 batches of which were the same.

In later experiments a different procedure was used for the extraction of the rice bran. This procedure, which was used for the experiment to be reported, was as follows: 800 grams of rice bran were mixed with 3 liters of 25% aqueous ethyl alcohol solution and

[1] Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.

allowed to stand, with frequent stirring, in an incubator at 40°C. for 48 hours. The liquid was then filtered off, the yield being about 1850 cc. This solution was then evaporated on a steam bath to about 250 cc., filtered, and the volume made up to 500 cc. A portion of this solution was then adjusted to pH 7.1 and allowed to set for 30 minutes in the ice box. A slight precipitate was observed. Filtration was made through a Seitz filter, while still cold, which removed the precipitate and rendered the solution sterile. This extract was then kept in the ice box until ready to be used.

In the data reported each growth determination was made in duplicate. Inoculations were made with 18-hour cultures of *Escherichia coli* or *Alcaligenes fecalis*. In every case 0.2 cc. of a 0.1% broth suspension was used. In addition to the various amounts of solutions reported in the tables, 5.0 cc. of infusion medium from the same batch were added to each tube, making the total volume in each case 12.2 cc. Growth was determined by centrifuging 10.0 cc. of each culture in a Hopkins tube for 1 hour at a standard speed, and recording the growth in cubic centimeters. All tubes, including the control tubes for the original pH, were incubated together at 37°C. for 8 hours. The hydrogen ion concentrations were determined with the hydrogen electrode. The buffer employed was McIlvaine's Na_2HPO_4—Citric acid[2] of pH 7.0. The glucose solution was prepared so that 1.0 cc. gave a 1% solution. The saline used was a 0.9% sodium chloride solution.

From the results of the extract in various dilutions upon the growth of *Escherichia coli* in unbuffered media (Table I) it will be

TABLE I.
Effect of Extract on *E. coli* in Unbuffered Medium.

Extract cc.	Saline cc.	Growth (cc.)			Gas	pH	
		1st tube	2nd tube	Aver.		Original	Final
0.0	7.0	.007	.007	.007	—	7.18	6.64
0.1	6.9	.012	.013	.012	+	7.19	6.09
0.5	6.5	.016	.017	.016	+	7.17	4.92
1.0	6.0	.023	.022	.022	+	7.16	4.82

observed that there is a steady increase in growth with increasing amounts of the extract, but that the maximum stimulation has not been reached with the amounts of extract used. On the other hand, with varying amounts of glucose, under like conditions, (Table II) there is a constant stimulation which does not increase with increasing amounts of glucose. The decrease in pH in the latter case

[2] Clark, W. M., "The determination of hydrogen ions." Williams and Wilkins, Baltimore, Md. 1928. p. 214.

is much greater than in the former, and is probably the limiting factor of the growth stimulation.

TABLE II.
Effect of Glucose on *E. coli* in Unbuffered Medium.

Glucose cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	7.0	.007	.007	.007	—	7.18	6.64
0.1	6.9	.013	.013	.013	+	7.18	5.71
0.3	6.7	.012	.013	.012	+	7.18	4.78
0.5	6.5	.013	.013	.013	+	7.18	4.74
1.0	6.0	.012	.013	.012	+	7.18	4.69

TABLE III.
Effect of Extract on *E. coli* in Buffered Medium.

Extract cc.	Buffer cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	6.0	1.0	.010	.008	.009	—	6.88	6.83
0.1	6.0	0.9	.016	.018	.017	—	6.87	6.70
0.5	6.0	0.5	.046	.048	.047	+	6.88	6.17
1.0	6.0	0.0	.058	.058	.058	+	6.88	5.69

TABLE IV.
Effect of Glucose on *E. coli* in Buffered Medium.

Glucose cc.	Buffer cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	6.0	1.0	.010	.008	.009	—	6.88	6.83
0.1	6.0	0.9	.022	.022	.022	—	6.87	6.63
0.3	6.0	0.7	.046	.044	.045	+	6.86	6.15
0.5	6.0	0.5	.045	.047	.046	+	6.87	5.62
1.0	6.0	0.0	.047	.048	.047	+	6.86	5.46

This is borne out more strikingly when buffered media were used (Tables III and IV), for with the extract much greater stimulation was recorded than in the unbuffered media, as was also true with glucose. However, the stimulation due to the glucose reaches a maximum, while that due to the extract does not. It is also observed that the stimulation produced by the extract is greater than that produced by the glucose and that there is a correspondingly smaller decrease in the pH with the former than with the latter, as compared with the growth increase.

When glucose is added together with the extract (Tables V and VI) there is no cumulative stimulation as would be expected if the stimulating agent were similar to glucose, nor is there a constant and unchanging stimulation as was observed with glucose alone (Table II). It will be noted that the final pH does not decrease with

TABLE V.
Effect of Extract on *E. coli* in Presence of 0.5% Glucose.

Extract cc.	Glucose cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	0.5	6.5	.013	.013	.013	+	7.18	4.74
0.1	0.5	6.4	.013	.013	.013	+	7.19	4.77
0.5	0.5	6.0	.016	.018	.017	+	7.17	4.79
1.0	0.5	5.5	.020	.020	.020	+	7.16	4.85

TABLE VI.
Effect of Extract on *E. coli* in Presence of 1.0% Glucose.

Extract cc.	Glucose cc.	Saline cc.	Growth (cc.) 1st tube	2nd tube	Aver.	Gas	pH Original	Final
0.0	1.0	6.0	.012	.013	.012	+	7.18	4.69
0.1	1.0	5.9	.012	.013	.012	+	7.19	4.71
0.5	1.0	5.5	.016	.017	.016	+	7.17	4.76
1.0	1.0	5.0	.023	.023	.023	+	7.16	4.85

increasing amounts of the extract, but on the other hand, increases. The probable explanation of this is that the extract itself contains some buffering salts.

Gas was produced during growth in all cases except with the 0.1 cc. dilution of the extract in buffered medium (Table III) and the 0.1 cc. dilution of glucose under the same conditions (Table IV). In these cases it is probable that the carbonic acid formed was less than the amount required to neutralize the buffer, and with no excess, no gas bubbles were observed to rise and collect at the surface.

To show this more conclusively, another experiment was performed (Table VII). Five cc. of infusion medium were added to

TABLE VII.
The Final pH with Various Amounts of Extract and Buffer. Gas was produced in all tubes except those marked *.

Extract cc.	Buffer 2.0 cc.	4.0 cc.	6.0 cc.	8.0 cc.
0.1	6.28	6.40*	6.61*	6.69*
0.5	5.45	5.75	6.02	6.19
1.0	5.25	5.52	5.89	6.08
2.0	5.29	5.58	5.78	5.97

each tube as listed in Table VII, and the total volume made up to 15.0 cc. with saline. Inoculations were the same as in other experiments. Incubation time was 6 hours. These data show that with smaller amounts of the buffer, gas was observed in all cases where the final pH was less than 6.3, which coincides with the data shown in Tables III and IV.

The effect of the extract upon *Alcaligenes fecalis* is entirely negative. This organism is known to have no carbohydrate-fermenting ability. As would be expected, the pH increases rather than decreases.

From the facts that the extract causes a decrease in the final pH, the production of gas, and has no stimulating effect on the growth of *Alcaligenes fecalis,* the assumption can be made that the stimulating agent, probably pantothenic acid, is related to the carbohydrates. The stimulation observed with the extract, however, can in no way be attributed to glucose or other hexoses which stimulate *E. coli* in a manner similar to glucose.

Conclusion. 1. Rice bran contains a growth-stimulating agent for *Escherichia coli,* but not for *Alcaligenes fecalis.* 2. This stimulating agent is probably related to the sugars. 3. It is not a hexose. 4. The substance is probably identical with the pantothenic acid of Williams.

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Isolation of Glycocyamine from Urine.

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The isolation of glycocyamine (guanido acetic acid) from urine is of interest because of its possible relationship to the origin of creatine. Hunter has critically reviewed the literature on this subject in his monograph on "Creatine and Creatinine".

My interest in this subject began with the finding that an extract of urine prepared with Lloyd's reagent gave a positive Sakaguchi reaction. I also found that the Lloyd's extract of urine from a case of pseudohypertrophic muscular dystrophy gave a stronger Sakaguchi reaction than did urine from normal individuals. Urine from this patient (8-year-old boy) was therefore used in attempting to isolate the substance responsible for the positive reaction. The patient received 15 gm. of glycine daily during the period of urine collection. The urine was collected in 2- or 3-day periods using toluene as a preservative.

The procedure used is briefly as follows: The urine after filtering was made acid to congo red with hydrochloric acid and extracted