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PANTOTHENIC ACID AND THE NODULE BACTERIA-LEGUME SYMBIOSIS

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The elementary facts regarding the symbiotic relationship between leguminous plants and the rhizobia have been known for many years but the chemical basis upon which this symbiosis rests is as yet imperfectly understood. It is not known with certainty what chemical entities pass from the bacteria to the host and vice versa. The net result of the partnership is nitrogen fixation, and heretofore it has been considered that the primary reaction or series of reactions involving elemental nitrogen are carried on by the bacteria. Fixation has not been demonstrated, however, except in the symbiotic relationship. It is not known definitely whether the fixation of nitrogen should be ascribed to the immediate activities of the bacteria living in the nodules, or to the activities of the host plant resulting from the bacterial infection. Activities of rhizobia other than utilization of atmospheric nitrogen have previously not been considered in explaining the direct benefit of symbiosis to the leguminous plant.

The nutritional need of *Rhizobium meliloti* for an unknown substance which they designate as "coenzyme R" has recently been demonstrated by Allison, Hoover and Burk.[1] Their results on this have been confirmed in our laboratory; moreover, it was found that alfalfa is a very rich source of this unknown substance, which we designate a "nutrilite."[2] One of the chemical entities passing from the alfalfa plant to the rhizobia is thus indicated to be this as yet poorly characterized nutrilite which is necessary for growth of the bacteria.

That one of the significant chemical substances which passes from the bacteria to the host plant is panthothenic acid, and that this substance has a profound effect on the growth of the alfalfa and on carbohydrate anabolism but does not (alone) enable the alfalfa plant to fix atmospheric nitrogen, is indicated by the experimental work herewith presented.

Pantothenic acid[3] has been found in every organic tissue examined whether of animal, bacterial or plant origin. Rhizobia are no exception; in the case of this organism as with certain other bacteria and molds, pantothenic acid is produced as the organism grows. A series of 25 ml. cultures of *Rhizobium meliloti* were grown in a synthetic medium[4] containing ordinary sugar, which carries a small amount of the rhizobium nutrilite. At the end of each successive day a culture was sterilized by heat, the medium filtered clear, using kieselguhr, and its pantothenic acid content determined by its effect on yeast growth under controlled conditions.[5] The original culture medium was practically free from pantothenic acid but after successive days' growth the amount present expressed in arbitrary units was 0.39, 0.78, 1.63, 5.46, respectively. It is therefore evident that the rhizobia are capable of producing pantothenic acid; in the nodule this could be passed on to the alfalfa plant.

Other work from this laboratory (to be published in *Plant Physiology*) has indicated that green plants may be stimulated in their growth by pantothenic acid. The following experiment was performed to check the effect of a pantothenic acid concentrate upon the growth of alfalfa, and to determine whether uninoculated alfalfa seedlings, when furnished with pantothenic acid, are able to fix atmospheric nitrogen.

Alfalfa was grown in three quadruplicate sets in Pyrex test tubes ($2^1/_2'' \times 24''$). Five hundred grams of quartz sand and 150 ml. of modified Crone's[6] solution (nitrogen free) were placed in each tube; these were capped with gauze-enclosed cotton and autoclaved 6 hours at 15 lb. pressure. Four of the tubes served as a control, four were inoculated with *Rhizobium meliloti*, and to each of the other four was added 2 micrograms (6 units) only of a pantothenic acid concentrate in the form of a calcium salt. Each of the twelve tubes was seeded under aseptic conditions with 6–8 alfalfa seeds which had been sterilized with mercuric chloride, and the cultures were placed in the greenhouse. After four days it was apparent that the plants in the tubes with the minute amount of pantothenic acid were uniformly larger than either of the other sets. At the end of two weeks the difference between the pantothenic acid treated plants and the controls was striking, while the inoculated seedlings were almost, but not quite, as vigorous as those which were treated with pantothenic acid and kept sterile. At this time an additional quantity (10 micrograms) of pantothenic acid was added to each of the tubes which had been treated originally. Subsequent results make it doubtful whether this second addition had any beneficial effect. After one month the growth was very slow, perhaps due to the short winter days, and the pantothenic treated plants were a shade lighter green than the inoculated plants. The seedlings were then harvested, measured weighed (air dry) and the nitrogen determined by a micro-Kjeldahl method,[7] with the following results:

TABLE 1

EFFECT OF PANTOTHENIC ACID ON ALFALFA

	TUBE NO.	NUMBER OF PLANTS	AVERAGE LENGTH OF STEMS (MM.)	TOTAL WT. OF ROOTS (MG.)	TOTAL WT. OF STEMS (MG.)	TOTAL WT. OF WHOLE PLANTS (MG.)	TOTAL N OF WHOLE PLANTS (MG.)	N/PLANT (MG.)
Controls	1	6	30	9.5	19.5	29.0	0.882	0.146
	2	6	34	12.5	14.5	27.0	0.750	0.125
	3	8	39	14.5	27.5	42.0	1.382	0.173
	4	5	20	7	12.5	19.5	0.790	0.158
Inoculated	5	4	50	7	22.5	39.5	0.832	0.208
	6	6	54	12	31.0	43.0	1.304	0.218
	7	5	36	8.5	19.5	28.0	0.864	0.173
	8	4	41	8.5	16.0	24.5	0.722	0.180
Pantothenic acid treated	9	6	50	19	35.0	54.0	1.030	0.172
	10	6	55	20	35.5	55.5	1.030	0.172
	11	6	53	13	33.5	47.0	1.076	0.178
	12	6	47	10	27.0	37.0	0.930	0.152

TABLE 2

GRAND AVERAGE OF VALUES FROM TABLE 1

	LENGTH/PLANT	WT./PLANT	TOTAL N/PLANT	% N/PLANT
Controls	31 mm.	4.70 mg.	0.152 mg.	3.23%
Inoculated	45 mm.	7.10 mg.	0.196 mg.	2.76%
Pantothenic acid	51 mm.	8.05 mg.	0.169 mg.	2.10%

Heretofore the favorable effects resulting from inoculating alfalfa and other legumes have been explained solely on the theory that the organisms fix nitrogen. On the basis of our results this position is no longer tenable. Perusal of tables 1 and 2 shows that minute amounts of pantothenic acid exert a striking effect on alfalfa in the early, and often critical, stage of growth. Since it has been shown that *Rhizobium meliloti* produces significant amounts of pantothenic acid, it is therefore likely that a considerable part of the stimulatory effect of the bacteria is due to pantothenic acid synthesized and excreted by the organism in the nodule and passed on to the plant.

From the fact that the weight of the pantothenic acid treated plants is greater than that of either the controls or the inoculated plants, and from the lack of a significant increase in nitrogen over the controls, it is evident that pantothenic acid alone is not the deciding factor in the nitrogen fixation process, but rather that it plays an important part in the carbohydrate anabolism of the plant. This is in keeping with the fact that it has been shown in this laboratory to exert a powerful effect on both the aerobic and anaerobic respiration of yeast. Further experiments are now in progress.

[1] Allison, Hoover and Burke, *Science*, **78**, 217 (1933).

[2] Williams, *Ibid.*, **67**, 607 (1928).

[3] Williams, Lyman, Goodyear, Truesdail and Holaday, *Jour. Am. Chem. Soc.*, **55**, 2912 (1933).

[4] Allison and Hoover, *Jour. Bact.*, **27**, 561 (1934).

[5] Williams, McAlister and Roehm, *Jour. Biol. Chem.*, **38**, 315 (1929).

[6] Fred, Baldwin and McCoy, *Univ. of Wis. Studies in Science*, No. 5, "Root Nodule Bacteria and Leguminous Plants," **266** (1932).

[7] Koch and McMeekin, *Jour. Am. Chem. Soc.*, **46**, 2066 (1924).

LABILITY OF THE BASAL METABOLISM OF THE DAIRY COW

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Approximately fifty years ago Rubner emphasized the concept that all warm-blooded animals give off heat in direct proportion to their surface areas and at an essentially constant rate, irrespective of difference in species, of one thousand calories per square meter of body surface per 24 hours. This theory has stimulated a great deal of research and has been of invaluable service in that it has led to the accumulation of much information with regard to the heat production of various animals. Most writers even at the present day believe in the general applicability of this surface-area law, in spite of the fact that increasing evidence is appearing to show that the heat production per unit of body surface is by no means so uniform with all warm-blooded animals as was originally thought. The production of heat in the animal body is the sum of the endogenous heat production necessary for the maintenance of minimum vital activity and the increases in heat production caused by superimposed factors, primarily the absorption of food and muscular activity. By studying humans and animals in complete muscular repose, after the digestive processes have ceased, measurements can be made that supposedly represent the true basal metabolism and hence are comparable among different animal species. It was soon noted, however, that even within any given species factors other than digestion and muscular activity play an appreciable rôle in the heat production, particularly age, and specific differences due to weight, height and sex have been found.

Study of the basal metabolism of any given species of animal has two specific values. It contributes to the knowledge of the basal metabolism of that given species and the variability existing within the species and