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B-Vitamins Stimulatory to Cellulose Digestion by Washed Suspensions of Rumen Microorganisms*

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Numerous investigators have reported the presence of unidentified factors in certain feedstuffs and other materials which stimulate rumen microbial activity in vitro (1, 2, 3, 6, 8, 9, 10). Recently Ruf et al. (11) showed that extracts of yeast, cow manure and several feedstuffs stimulated the rate of cellulose digestion when they were added to a purified medium used for the in vitro culture of rumen microorganisms. Since many of the sources of the unidentified factors are known to contain several vitamins of the B group, it appears desirable to study the influence of certain of these vitamins on the rate of in vitro cellulose digestion. Preliminary reports of Hall et al. (7) and of Bentley et al. (1) seem to indicate that biotin, vitamin B_{12} and possibly other vitamins exert favorable influences on the rate of cellulose digestion by rumen microorganisms. The present investigation is an attempt to find out the stimulatory effects, if any, of each of the B-vitamins or the combination of them on cellulose digestion by washed suspensions of rumen microorganisms.

Experimental

To study the influence of vitamins on the cellulytic activity of rumen microorganisms it is essential that the medium as well as the inoculum used is free from such vitamins. For this purpose the washed suspension technique of Cheng et al. (4) was used. Briefly, this technique consists of separating the rumen microorganisms from the rumen fluid and partially digested feed debris by a process of differential centrifugation. Rumen contents obtained from a fistulated steer were strained through four layers of cheese cloth which constituted the first step in obtaining the rumen inoculum in liquid form. In a typical experiment a sample of 1200 ml. of rumen fluid was taken. After several processes of centrifugation and washing, the bacterial cells thus obtained were suspended in 600 ml. of a nutrient solution prepared according to the formula presented in Table 1. Three grams of Solka-Floc, a commercial

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Table 1

Composition of Basal Medium

Constituent	gm/2 liters	Constituent	gm/2 liters
Cellulose[a]	12.00	$MgSO_4$	0.15
Urea	2.00	$FeSO_4.7H_2O$	0.075
$Na_2HPO_4.H_2O$	1.20	$CuSO_4.5H_2O$	0.002
KH_2PO_4	0.60	$CoCl_2$	0.002
$NaHCO_3$	3.50	$MnSO_4$	0.0004
KCl	0.75	$ZnSO_4$	0.00008
NaCl	0.75		

[a]Solka-Floc—Brown Company, Berlin, New Hampshire

preparation of cellulose, were added to the suspension making the concentration of this insoluble cellulose 0.5 percent. The pH was adjusted to 7.0 with a saturated solution of sodium carbonate. Aliquots of 20 ml. each were pipetted into 75 ml. centrifuge tubes. The suspension was maintained anaerobic by passing through a constant stream of carbon dioxide gas. These tubes were placed in a constant temperature water bath at 39° C. At the end of 24 hours, the fermentation was terminated and the cellulose was determined on the entire contents of each tube using the procedure of Crampton and Maynard (5) with slight modifications.

Each of the B-vitamins was added to the rumen fermentation tubes at levels ranging from 0.1 to 100 micrograms. Various combinations of the B-vitamins were also studied. All the B-vitamins used were crystalline products. All treatments were triplicated.

Results and Discussion

In a basal medium containing no known B-vitamins but 0.5 percent of pure cellulose, approximately one-half of the cellulose was usually digested during an incubation period of 24-hr. For the convenience of comparison, the amount of cellulose digested for the basal group in an experiment was assigned a value of 100 and the amounts of cellulose digested for the test groups were computed proportionally to this basal value.

The results of the effects of vitamin B_{12}, pseudo-vitamin B_{12}, biotin, folic acid, para-aminobenzoic acid, pyridoxine and riboflavin on cellulose digestion by rumen microorganisms are shown in Table 2. All these vitamins appeared to increase the amounts of cellulose digested when added at levels ranging from 0.1 to 100

Table 2

The Stimulatory effect of Certain B-Vitamins upon Cellulose Digestion by Rumen Microorganisms

B-Vitamin	Amount added μg/20 ml. medium	Cellulose digested (relative value)
	None	100
B_{12}	1	109
	5	124
	10	116
	None	100
	1	107
	5	119
Pseudo B_{12}	10	113
	25	126
	50	132
	100	127
	None	100
Biotin	0.1	123
	1	122
	10	124
	None	100
Folic Acid	1	121
	10	136
	100	142
	None	100
para-Aminobenzoic acid	1	109
	10	115
	100	122
	None	100
Pyridoxine	1	119
	10	119
	100	145
	None	100
Riboflavin	10	107
	100	126

micrograms per 20 ml. of washed suspension. Apparently these vitamins were required either by the cellulolytic microorganisms or other microorganisms in symbiosis with them.

The additions of either choline chloride, inositol, niacin, pantothenic acid or thiamine at levels ranging from 1 to 100 micro-

grams did not result in a significant increase in cellulose digestion. It appears that either these vitamins were not required by rumen microorganisms or that they were synthesized in sufficient quantities under the conditions of these experiments.

Further studies involved the addition of more than one individual vitamin into the medium. Various combinations of B-vitamins were tried but only the combination of vitamin B_{12} and biotin was found to stimulate microbial cellulose digestion more than did either of these vitamins alone. This synergistic effect of vitamin B_{12} and biotin is shown in Table 3. It is further shown in Table 3 that yeast

Table 3

Effect of Biotin, Vitamin B_{12}, and Yeast Extract Upon Cellulose Digestion by Rumen Microorganisms

Addition to basal medium	cellulose digested (relative value)
None	100
5 μg vitamin B_{12}	115
1 μg biotin	112
5 μg vitamin B_{12} + 1 μg biotin	124
50 mg. yeast extract	148
50 mg. yeast extract + 5 μg. vitamin B_{12} + 1μg. biotin	148

extract was more stimulatory to cellulose digestion than the combination of biotin and vitamin B_{12}. However, the addition of these two vitamins did not enhance the stimulatory effect of the yeast extract. Apparently the yeast extract contains additional cellulolytic factors other than biotin and vitamin B_{12}.

The influences of the additions of other B-vitamins to the combination of vitamin B_{12} and biotin into the basal medium on cellulose digestion are shown in Table 4. It is seen that folic acid, p-

Table 4

Influence of Various B-Vitamins in Addition to B_{12} and Biotin on Cellulose Digestion by Rumen Microorganisms

Addition to basal medium	Cellulose digested (relative value)
5 μg B_{12} + 1 μg biotin	100
5 μg B_{12} + 1 μg biotin + 10 μg folic acid	96
5 μg B_{12} + 1 μg biotin + 1 μg inositol	103
5 μg B_{12} + 1 μg biotin + 10 μg p-aminobenzoic acid	95
5 μg B_{12} + 1 μg biotin + 100 μg pyridoxine	92
5 μg B_{12} + 1 μg biotin + 10 μg riboflavin	108
5 μg B_{12} + 1 μg biotin + 10 μg thiamine	97

aminobenzoic acid, pyridoxine and thiamine did not stimulate any increase in cellulose digestion. The slightly stimulatory effect

of inositol and riboflavin was probably not significant. It is possible that in the presence of B_{12} and biotin, the microorganisms were able to synthesize other B-vitamins in sufficient quantities to supply their needs.

These data confirm our earlier report (7) and that of Bentley et al. (1) which demonstrated that biotin, vitamin B_{12}, and para-aminobenzoic acid stimulated cellulose digestion by rumen microorganisms in vitro. The stimulatory action of the B-vitamins was not equal to that obtained from such sources as yeast extract and autoclaved rumen liquid. Thus, while these B-vitamins are not the complete answer to the unidentified cellulolytic factors, it would appear that they are at least partially responsible for the cellulolytic properties of such substances as yeast extract, rumen liquid, and possibly other sources of these factors.

It is not yet known whether the addition of these B-vitamins which stimulated cellulose digestion in vitro to ruminant rations would increase rumen microbial activity in vivo. Normally, rumen microorganisms are thought to synthesize sufficient quantities of the B-vitamins to meet their needs as well as that of their host. It is conceivable that these B-vitamins required by cellulolytic microorganims may be supplied by the microbial synthesis of other types of rumen bacteria in as much as symbiotic relationships undoubtedly exist in the normal rumen. It should also be mentioned that throughout these studies carbohydrates which furnish readily available energy for microbial activity were not added into the medium. The effects of such materials upon in vitro cellulose digestion will be reported later.

Summary

Certain B-vitamins were found to stimulate cellulose digestion by washed suspensions of rumen microorganisms. These were: Vitamin B_{12}, pseudo-vitamin B_{12}, biotin, para-aminobenzoic acid, pyridoxine, riboflavin, and folic acid. Of the combinations of vitamins studied, vitamin B_{12} and biotin were the only vitamins in which the effect of one was synergistic to that of the other. Despite the stimulatory effect of these B-vitamins, no combination of them was found which stimulated cellulose digestion as much as that obtained from yeast extract. This substance apparently contains unidentified factors stimulatory to cellulose digestion in addition to the B-vitamins.

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