THE DECOMPOSITION OF ALLANTOIN BY INTESTINAL BACTERIA

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Despite the fact that allantoin is widely distributed in both plant and animal tissues, no specific study, to our knowledge, has been made of its degradation by bacteria. When administered orally to man, dog and rabbit a percentage varying from 0 to 90 is recoverable in the urine whereas if given subcutaneously or intravenously the recovery is essentially complete (Young, Wentworth and Hawkins, 1943). Hunter and Givens (1914) had previously shown this to be true in the monkey. Furthermore we have been unable to demonstrate any allantoin in rabbit feces after administering half a gram in solution by stomach tube and no excess allantoin was recoverable from the urine. An explanation was sought in a decomposition by the intestinal flora. Givens (1914) has shown that when allantoin was incubated for 24 hours in a filtered suspension of monkey feces it was destroyed to the extent of 90 per cent, half of which he attributed to bacteria. Mendel and Dakin (1909) found that a solution of allantoin was partially decomposed when inoculated with bacteria from urine undergoing ammoniacal fermentation.

Since uricolysis to ammonia and carbon dioxide is an enzymic property of *Proteus vulgaris*, *Bacillus mesentericus* and other organisms (Truszowski, 1930) a similar reaction for allantoin was to be expected and has been found by us for several intestinal organisms.

EXPERIMENTAL

Most of the cultures used came from the American Type Culture Collection in Washington. Those common to the human intestine have been tried in various media containing allantoin. The organisms used were *Aerobacter aerogenes*, *Bacillus mesentericus*, *Escherichia coli*, *Lactobacillus bulgaricus*, *Proteus vulgaris*, *Streptococcus fecalis* and *S. hemolyticus*, *Staphylococcus aureus* and *Clostridium putrificum* and *Bacteroides bifidus* as strict anaerobes. The cultures were verified and checked for possible contamination.

Bacto nutrient broth was prepared and in this was dissolved pure solid allantoin (Eastman Kodak Co.) at 50°–60°C. This solution was sterilized by passage through a Mandler filter candle and tubed in 10 ml. quantities. Inoculated and control tubes were incubated at 40° and removed for analysis as required. *Lactobacillus bulgaricus* was cultured in litmus milk. Allantoin was estimated by the method of Young and Conway (1942) using the preliminary purification of the solution with phospho-24 tungstic acid, basic lead acetate and sulfuric acid as described by Larson (1931–32).

The results are summarized in table 1. We have also noted a loss of 22% of allantoin in a culture of *Staphylococcus aureus* in 9 days. *Streptococcus hemo-*

lyticus did not decompose the allantoin in a period of 14 days. One experiment with *Bacillus mesentericus* showed no loss of allantoin in 18 days. No growth could be obtained on an allantoin medium with the strict anaerobes.

TABLE 1

Decomposition of allantoin

ORGANISM	PERIOD OF INCUBATION	CONCENTRATION OF ALLANTOIN		Loss
		Control	Culture	
	days	*mgm. per 100 ml.*	*mgm. per 100 ml.*	*per cent*
E. coli	0	20.0	20.0	55
	5	16.0	8.6	
	9	15.5	7.0	
E. coli	0	250	250	100
	2		197	
	6		Trace	
E. coli	0	25.0	25.0	100
	3	20.0	0.0	
A. aerogenes	0	20.0	20.0	100
	5	16.0	9.8	
	9	15.5	Trace	
P. vulgaris	0	65.6	65.6	78
	5	59.0	62.2	
	8	65.6	42.0	
	11	65.6	29.0	
	14		14.8	
P. vulgaris	0	16.0	16.0	100
	4	15.5	14.7	
	9	16.0	16.4	
	18	19.7	Trace	
B. mesentericus	0	20.0	20.0	10
	5	16.0	17.3	
	9	15.5	14.0	
L. bulgaricus	0	49.2	49.2	15
	5	51.3	43.8	
	8	59.0	59.0	
	11	75.7	64.7	
Strep. fecalis	0	20.0	20.0	0
	5	16.0	14.4	
	9	15.5	16.5	
	14	19.7	19.7	

Certain hydrogen ion concentration changes were noted during the experimental period in several cultures. The initial pH of the allantoin-nutrient broth was about 6.6. With *E. coli* this rose to 8.0 in 6 days. Changes in the concen-

tration of allantoin in the control tubes were probably due to two factors, a slow decomposition of allantoin in aqueous solution (Givens, 1914) and evaporation. By the uneven and retarded rate of disappearance of allantoin it would appear as though the utilization of allantoin as a source of nitrogen was secondary to that of peptone in most cultures.

The loss of allantoin as compared to the controls is not considered by us convincing in the experiments with *L. bulgarius* and *B. mesentericus*. The results however establish the power of destroying allantoin for *E. coli*, *A. aerogenes* and *P. proteus*.

The probable chain of degradation would be anticipated as allantoin → allantoic acid → urea + glyoxylic acid → ammonia, carbon dioxide and oxalic acid. In a culture of *E. coli* freshly isolated from human feces determinations of ammonia were carried out after all allantoin had disappeared in the course of 3 days. An aliquot of fermented and of control liquids was aerated into sulfuric acid and nesslerized as in the determination of urea in blood by the Folin-Wu technique. A tube of inoculated nutrient broth without allantoin was also done. Results obtained showed concentrations of ammonia of 11.4 mgm. per 100 ml. for inoculated allantoin culture, 10.8 mgm. per 100 ml. for inoculated control culture, and 0.0 mgm. per 100 ml. for uninoculated control. There should have been a difference of 8.8 mgm. to account for the allantoin decomposition at an original concentration of 25 mgm. The treatment of allantoin was therefore not as simple as anticipated.

SUMMARY

Allantoin in nutrient broth was decomposed by *Escherichia coli*, *Aerobacter aerogenes* and *Proteus vulgaris*. This reaction was shown to a very slight extent or not at all by cultures of *Bacillus mesentericus*, *Lactobacillus bulgaricus*, *Streptococcus fecalis* and *S. hemolyticus* and *Staphylococcus aureus*.

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