

served for mitotic interphases. Lane (16), however, in work which came to our attention after our interpretation of our own results had been arrived at, has recently concluded, on the basis of evidence obtained by him, that in *Tradescantia* microspores the union of broken ends is, contrary to the previously accepted interpretation for such material, long delayed. His conclusion is at present under dispute (17, 18).

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## Effect of a Cationic Detergent on the Digestion of Raw Cornstarch *in Vitro*<sup>1</sup>

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Recent reports have indicated that certain detergents increase the growth rate of chickens (1), rats (2), and pigs (3). The mechanism of such growth stimulation is not understood, but it has been suggested that it is related to surface-active properties (4).

During a study of the digestion of raw cereal starches by  $\alpha$ -amylases from various sources, it was noted that the presence of "Roccal,"<sup>2</sup> a mixture of high molecular weight alkyl-dimethyl-benzyl-ammonium chlorides, was effective in increasing the rate of action of the  $\alpha$ -amylases from fungal, bacterial, and animal sources. This detergent was effective in increasing the activity of the amylases on the raw starches but not on cooked starches.

The exceedingly slow rate of digestion of raw starches is well known. It is possible that some of the energy value of a feed is not made available to the animal because of incomplete digestion during the relatively short period in the intestinal tract—an in-

<sup>1</sup> Preliminary report; published with the approval of the director as Paper No. 562, Journal Series, Nebraska Agricultural Experiment Station.

<sup>2</sup> Roccal is the brand name of a product sold by Sterwin Chemicals, Inc., New York 18.

crease in the rate or extent of digestion should increase the efficiency of gain.

The data presented in Table 1 indicate the effect

TABLE 1

EFFECT OF 0.1% ROCCAL ON THE DIGESTION OF 500 MG RAW CORNSTARCH BY 4 MG PANCREATIN\*

pH	Raw starch digested		
	Without detergent (%)	With detergent (%)	Increase of activity (%)
5.4	25.7	27.6	7
6.2	37.2	54.1	45
7.0	37.1	58.3	57
7.8	31.8	51.2	57

\* Twenty-hr digestions carried out at 30° C on 20 ml 2.5% raw cornstarch suspension, buffered with phosphate and constantly agitated.

tiveness of Roccal in increasing the rate of action of pancreatic amylase on raw starch. These data show that the increased rate of action was evident over a pH range from 5.4 to 7.8, but was most effective near the pH of maximum activity. Table 2 compares the

TABLE 2

ELECTROLYTE STIMULATION OF THE DIGESTION OF 500 MG RAW CORNSTARCH BY 1.5 MG PANCREATIN, PH 6.5\*

Digestion condition	Raw starch digested	
	Amount (%)	Increase of activity (%)
<i>Without detergent</i>		
Control	28	—
0.05% NaCl	37	32
0.10% NaCl	38	35
0.10% CaCl <sub>2</sub>	37	32
<i>With 0.1% detergent</i>		
Control	43	54
0.05% NaCl	45	61
0.10% NaCl	46	64
0.10% CaCl <sub>2</sub>	56	100

\* Twenty-hr digestions carried out at 30° C on 20 ml 2.5% raw cornstarch suspension, buffered with phosphate and constantly agitated.

effect of the detergent, an organic electrolyte, with two inorganic electrolytes that are known to stimulate the action of pancreatic amylase on cooked starch.

The demonstrated stimulation of pancreatic  $\alpha$ -amylase by calcium or sodium chlorides, and the stimulation of raw starch digestion by Roccal, suggest that these two effects may be the same or similar in mechanism. To obtain information concerning the action of these two types of enzyme stimulant a series of determinations was made in which the stimulants were studied individually and in combination on raw starch digestion. Digestions were carried out with 0.10% Roccal alone, with .05% and .10% sodium chloride, and 0.10% calcium chloride, both individually and in combination with the 0.10% Roccal, each digest being

adjusted to pH 6.5 with *M*/15 phosphate. A control digestion at the same pH was carried out with no activator present.

The results of this study, presented in Table 2, show the extent of stimulation realized by each of the treatments. With the pancreatic enzyme the inorganic salts increased the activity, but the increase seemed to be independent of concentration or kind of salt. A considerably greater increase in activity was observed when Roccal was used—about double the amount of activation afforded by the inorganic salts. Even with Roccal present, however, additional stimulation resulted from the addition of the sodium chloride. When calcium chloride and Roccal were both present, the activity was increased to 200% of the unstimulated digestion.

These data suggest a possible explanation of the growth stimulation obtained with chickens, rats, or pigs when they are fed detergents. It seems probable that the presence of the detergent permits a more ready penetration of the starch granule by the enzyme. This would make available a greater portion of the substrate for enzyme attack during the limited time in the digestive tract, and accordingly would increase the rate of starch digestion by the enzyme.

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### Putrescine as a Growth Requirement for *Neisseria*<sup>1</sup>

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Recently Herbst and Snell (1) have shown that the bacterium *Hemophilus parainfluenzae* requires the diamine putrescine for growth in a chemically defined medium. This was the first instance in which one of the putrefactive diamine compounds was shown to be essential for bacterial growth. The compounds spermine, spermidine, and agmatine could replace putrescine, and it is conceivable that their activity is via putrescine, which would be formed as a result of hydrolytic cleavage.

Previous work in our laboratory, by Nemes *et al.* (2), on the nutritional requirements of nonpathogenic *Neisseria* has shown that many of these organisms require biotin in a vitamin-free casein hydrolysate medium. Attempts to cultivate *N. perflava* in media where the casein hydrolysate was replaced with com-

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TABLE 1  
COMPOSITION OF BASAL MEDIUM SUPPORTING  
GROWTH OF *N. perflava*

Amino acids	Amount/liter
DL-aspartic acid	1.0 (g)
L-glutamic acid	1.0
DL-alanine	1.0
L-arginine	0.20
DL-methionine	0.20
DL-threonine	0.20
DL-serine	0.20
DL-tryptophane	0.20
DL-valine	0.20
DL-phenylalanine	0.20
DL-isoleucine	0.20
L-lysine	0.20
L-leucine	0.10
L-proline	0.10
L-histidine	0.10
L-cystine	0.10
L-tyrosine	0.10
Glycine	0.10
Biotin	1.0 µg
Putrescine	1.0 mg
Salt solution A	5.0 ml
K <sub>2</sub> HPO <sub>4</sub> 25 (g)	
KH <sub>2</sub> PO <sub>4</sub> 25	
H <sub>2</sub> O 250 ml	
Salt solution B	5.0 ml
NaCl 0.5 (g)	
FeSO <sub>4</sub> · 7H <sub>2</sub> O 0.5	
MnSO <sub>4</sub> · 4H <sub>2</sub> O 0.5	
MgSO <sub>4</sub> · 7H <sub>2</sub> O 10.0	
H <sub>2</sub> O 250 ml	

plex amino acid mixtures were unsuccessful. Numerous vitamins and growth-factor compounds were then tested, and during the course of this work it was found that putrescine, in addition to biotin, was essential for growth of some strains of *N. perflava*.

Employing the usual technical procedures for studies of bacterial growth in chemically defined media, it was found that the medium illustrated in Table 1 was capable of supporting growth of this organism on continued subculture.

The growth response of *N. perflava* (876) to graded amounts of putrescine is illustrated in Fig. 1. For this determination the medium was dispensed in 10 ml

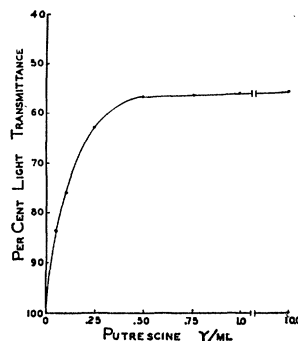


FIG. 1. Growth response of *N. perflava* to graded amounts of putrescine in a chemically defined medium.