

Further studies are in progress to determine if 'Gibrel', a water-soluble potassium salt of gibberellic acid, has a significant effect on nitrogen fixation by *Azotobacter*.

The gibberellic acid was kindly supplied by Dr. L. T. Blaney of the Horticulture Department. It was originally obtained from Dr. P. W. Brian of Imperial Chemical Industries, Ltd., England. The 'Gibrel' was supplied by Merck and Co., Inc., New Jersey.

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Enzymes in the Ileal Juice of the Horse

So far as can be ascertained there is no information about the enzymes secreted by the horse's small intestine. Since a pony with a Thiry-Vella loop in the middle of the ileum was available¹ the opportunity was taken to determine whether the enzymes present in man and dogs were present in the horse. The juice was obtained by gently rubbing the mucosa with a rubber catheter. Juice thus obtained was, on occasion, slightly contaminated with blood. However, the enzymic activity of such samples did not differ from the uncontaminated.

Ileal juice is a watery fluid with a yellow tinge and sometimes contained mucus. The pH was 7.8-8.5. Enzymic activity was measured on the supernatant fluid from juice centrifuged at 3,000 r.p.m. for 5 min.

Amylase, lactase, maltase, and sucrase activity was detected by Lagerlof's² method, lipase activity by Cherry and Crandall's³ method, peptidase by Cajori's⁴ technique using a peptone substrate and protease by Lagerlof's⁵ method with casein as substrate. Enterokinase activity was detected by using the protease method after adding 2 ml. of 0.01 per cent trypsinogen (Worthington Biochemical Corporation) to 6 ml. incubating mixture and using controls without trypsinogen and also with boiled juice. This method showed marked enterokinase activity in the ileal juice of freshly killed rats and guinea pigs. The experimental findings are shown in Table 1; the dilution of juice was the highest at which activity could be detected by the methods used.

Table 1. ENZYMES IN HORSE ILEAL JUICE

Enzyme	No. of observations	Activity present	Dilution of juice
Amylase	18	14	1:1,000
Sucrase	14	12	1:20
Lactase	19	17	1:1,000
Maltase	19	16	1:1,000
Lipase	4	0	Undiluted
Lipase	6	0	1:5
Protease	16	15	1:10
Peptidase	14	14	1:10
Enterokinase	7	1	Undiluted

It was interesting to compare the results of these experiments with those of Doller and Porter⁶, who studied the enzymes of the calf's digestive tract. They found no sucrase activity in the calf's ileum and that the lactase activity decreased with age.

The absence from the horse juice of lipase activity was noteworthy, although Reale⁷ has stated that lipase was absent from fresh ileal juice in man but increased with cellular autolysis, and Roger and Binet⁸ suggested that bile increased lipolytic activity by destroying cells. However, in our experiments both centrifuged juice, macerated mucus and cellular debris were free from lipolytic activity. In control experiments the method employed showed marked lipolytic activity in ileal juice of rats and guinea pigs.

The absence of enterokinase activity could be explained by the Thiry-Vella loop being six years old, as Foa⁹ showed the enzyme to disappear from fistulae of dogs within six months of operation, and Sawitsch¹⁰ found the enzyme to disappear from fistulae deprived of pancreatic juice.

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Effect of Electrolytes on the Uptake by Bacteria of Labelled Glutamate

In a recent paper, Krobs, Whittam and Hems¹ reported an accelerating effect of potassium salts on the rate of oxidation of various substrates by *Alcaligenes faecalis*. All the potassium salts tested were equally active. The ionic composition of the medium was found to affect respiration also in *Escherichia coli* (strain B), and *Pasteurella tularensis* (strain S + D)². The effect of the anion was also apparent with these two micro-organisms. For the maximal rate of oxidation of glutamate and glucose by *P. tularensis* the halogen salts of potassium and rubidium were found to be required; in the case of *E. coli* potassium chloride or sulphate was needed. In *E. coli* addition of minute amounts (10^{-4} M) of sodium salts rendered the accelerating effect of potassium independent of the nature of the anion. Conditions known to diminish the rate of penetration of metabolites into the bacterial cell (rising pH, low substrate concentration) rendered the accelerating effect of potassium more pronounced. The latter observation suggested regulation of permeability as a possible mode of action of the electrolytes. In order to test the validity of this assumption, the effect of electrolytes on the rate of uptake of labelled glutamate was examined.

The composition of the reaction mixture was as follows: 15 mgm./ml. (wet weight) of the bacterial cells, washed thoroughly with 0.25 M sucrose, 10^{-2} M of the electrolytes and 0.25 M sucrose.