were supplied through the root system, for rapid growth.

Our results seem to indicate that the principal source of Pb^{210}, and thus of Po^{210}, in tobacco is the soil and that its nuclides are absorbed by the plant roots. This finding differs from that reported by Berger *et al.* (*7*), but is in agreement with Marsden's conclusion (*8*) that the contribution of Po from fallout to the total activity of the plant is minor compared to the Po absorbed from the soil by the roots. However, other factors may also contribute to the final concentration of Po^{210} in tobacco.

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Pancreatic Carboxypeptidases: Activities in Zinc-Deficient Rats

Abstract. *Zinc deficiency in rats caused decrease in the activity of pancreatic carboxypeptidase A, but it had no effect on pancreatic carboxypeptidase B and liver alcohol dehydrogenase. The observed reduction of enzymic activity may be related to the poor utilization of feed that occurs in zinc-deficient rats.*

Zinc is essential in animal nutrition primarily because of its participation in the structure of several enzymes. The effect of zinc deficiency upon zinc metalloenzymes, however, has not been established. Earlier observations by Hove *et al.* (*1*) indicated that zinc deficiency in rats decreased activity of intestinal phosphatase but had no effect on bone phosphatase. Day and McCollum (*2*) reported that the activity of carbonic anhydrase per unit of erythrocytes in zinc-deficient rats was unchanged from that of normal animals. This report presents the results of a study on the activity of pancreatic carboxypeptidase in zinc-deficient rats; the effect of zinc on the activity of liver alcohol dehydrogenase is also recorded.

Table 1. Activities of pancreatic carboxypeptidases and hepatic alcohol dehydrogenase in zinc-deficient and zinc-supplemented rats. Results are expressed as means ± standard error of the mean. Numbers in parentheses are the numbers of rats used. Activities of the pancreatic carboxypeptidases are expressed as the change in optical density per minute per milligram of protein; the activity of hepatic alcohol dehydrogenase as micromoles of NAD reduced per minute per milligram of protein.

Enzyme	Zinc-deficient rats	Zinc-supplemented rats
Pancreatic carboxypeptidase A		
Assay 1	0.057 ± 0.004 (3)	0.097 ± 0.004* (3)
Assay 2	.042 ± .004 (4)	.057 ± .003† (4)
Pancreatic carboxypeptidase B	.277 ± .10 (5)	.248 ± .031 (5)
Hepatic alcohol dehydrogenase	.11 ± .01 (3)	.10 ± .00 (3)

* $P < 0.01$. † $P < 0.05$.

Three- to four-week-old male rats (52 to 70 g) from our own colony were randomly divided into two groups. The first group received a diet low in zinc, essentially as employed by Miller and associates (*3*), except that the percentages of egg albumin and casein hydrolyzate were reversed. The second group received the same zinc-low diet with a daily supplementation of 150 μg of zinc as zinc sulfate. All rats were housed in individual plastic cages, with pyrex feed cup and polyethylene drinking bottle; feed and deionized water were freely available. After feeding for 103 days, the rats were anesthetized with sodium pentobarbital and exsanguinated through the abdominal aorta. The pancreatic glands were excised quickly, cleaned, weighed, and stored at −20°C until they were assayed.

For preparation of acetone powders of the pancreas, frozen glands were broken into several pieces and blended in a Waring blendor with ice-cold acetone (50 ml/g). Homogenization was continued for 1 minute at full speed. The resulting suspension was filtered on a Buchner funnel with Whatman filter paper No. 2. Residue removed from the filter paper was reextracted successively, by hand, in a Potter-Elvehjem glass homogenizer, first with 20 ml of acetone, next with 20 ml of a mixture of acetone and ether (1:1), and finally with 20 ml of ether. The defatted tissue was dried to a constant weight at room temperature and stored in a desiccator at 4°C overnight.

Dried acetone powders were extracted with cold distilled water (50 ml per gram of powder) for 15 minutes with gentle stirring at 4°C. The suspension was centrifuged in a refrigerated centrifuge for 10 minutes at 3000 rev/min. A portion of supernatant fluid was added to trypsin-tris buffer solution (*p*H 7.65) for activation which was completed in 30 minutes at room temperature. The trypsin-treated extract was subsequently used for enzyme assay. Activities of carboxypeptidase A and carboxypeptidase B were determined according to the outlined method of Folk and Schirmer (*4*) and Folk *et al.* (*5*), respectively. The rates of hydrolysis of hippuryl-L-phenylalanine and hippuryl-L-arginine were measured by the increase in absorbancy at 254 mμ at 25°C in a thermostated Beckman DU spectrophotometer. Activity is expressed as change in absorbance per minute per milligram of protein.

For the assay of liver alcohol dehydrogenase the method of Racker (*6*) as modified by Vallee and Hock (*7*) was used. One unit of activity is defined as one micromole of nicotinamide-adenine dinucleotide (NAD) reduced per minute per milligram of protein. Protein was estimated according to the method of Lowry, Rosebrough, Farr, and Randall (*8*).

The mean gain in body weight over 103 days was 57 g for the rats receiving a diet poor in zinc and 265 g for those on a diet with adequate zinc. In addition to the depression of growth, external symptoms of zinc deficiency were noted as early as the 3rd week of experimental feeding. These symptoms included graying of black hair, alopecia, and scabby skin lesions. The

results of enzyme studies summarized in Table 1 indicate that activity of pancreatic carboxypeptidase A of zinc-deficient rats was consistently lower than that of rats that had been fed zinc. However, zinc deficiency did not alter the activities of pancreatic carboxypeptidase B and liver alcohol dehydrogenase.

The different responses in the observed enzymic activities by zinc-deficient animals are of particular interest. It is known that zinc is a functional component of carboxypeptidase. The nature of the binding of zinc to carboxypeptidase A appears to be different from that of binding to carboxypeptidase B (*9*, *10*). According to Vallee and Coombs (*11*), zinc is bound strongly in alcohol dehydrogenase. Thus it is possible that in dietary deprivation of zinc the activities of each of the zinc metalloenzymes may be affected differently.

Nevertheless, the reduction of the specific enzyme, carboxypeptidase A, in zinc-deficient rats suggests that zinc deficiency may decrease proteolysis within the intestinal tract and result in poor utilization of feed. This possibility is supported by the observation of Stirn *et al.* (*12*) which indicates that the zinc-deficient animals require 52 percent more ration to gain 1 g of body weight.

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Cylindrical Inclusions in the Cytoplasm of Leaf Cells Infected with Tobacco Etch Virus

Abstract. *Combined tracings from electron micrographs of serial sections of leaf tissue infected with tobacco etch virus show that one type of cytoplasmic inclusion, when sectioned in different planes, can produce configurations which have been interpreted as being two distinct types of inclusion bodies.*

Electron micrographs of sections of plant tissue infected with certain flexuous rod viruses characteristically exhibit cytoplasmic inclusions of the pinwheel and bundle types, as shown in a portion of a parenchyma cell of tobacco leaf infected with tobacco etch virus (Fig. 1). Inclusions very similar to these have been described in various host tissues infected with tulip mosaic (*1*), turnip mosaic (*2*), tobacco etch (*3*, *4*, *5*), wheat streak mosaic (*6*), bean yellow mosaic (*5*, *7*), watermelon mosaic, lettuce mosaic, bean common mosaic, potato Y, or sugarcane mosaic viruses (*5*). These inclusions have been variously interpreted as distinct types of inclusion bodies (*2*, *3*, *6*) or the same structure viewed from different angles (*1*, *4*, *8*).

Recently (*5*) these pinwheels and bundles have been considered as different aspects of a single type of inclusion, which was assumed to be cylindrical in shape and composed of curved plates with their inner edges converging around the central axis of the cylinder. Outer edges of the plates were assumed to diverge to form the boundary of the cylinder. Pinwheels would arise from cross sections of the hypothetical cylindrical inclusion, and longitudinal sections of the inclusion would show bundles.

Electron micrographs used in this study were obtained from portions of tobacco leaf that had been fixed in 6.5 percent phosphate-buffered glutaraldehyde for 17 hours, fixed again in 1 percent OsO_4 for 3 hours, and embedded

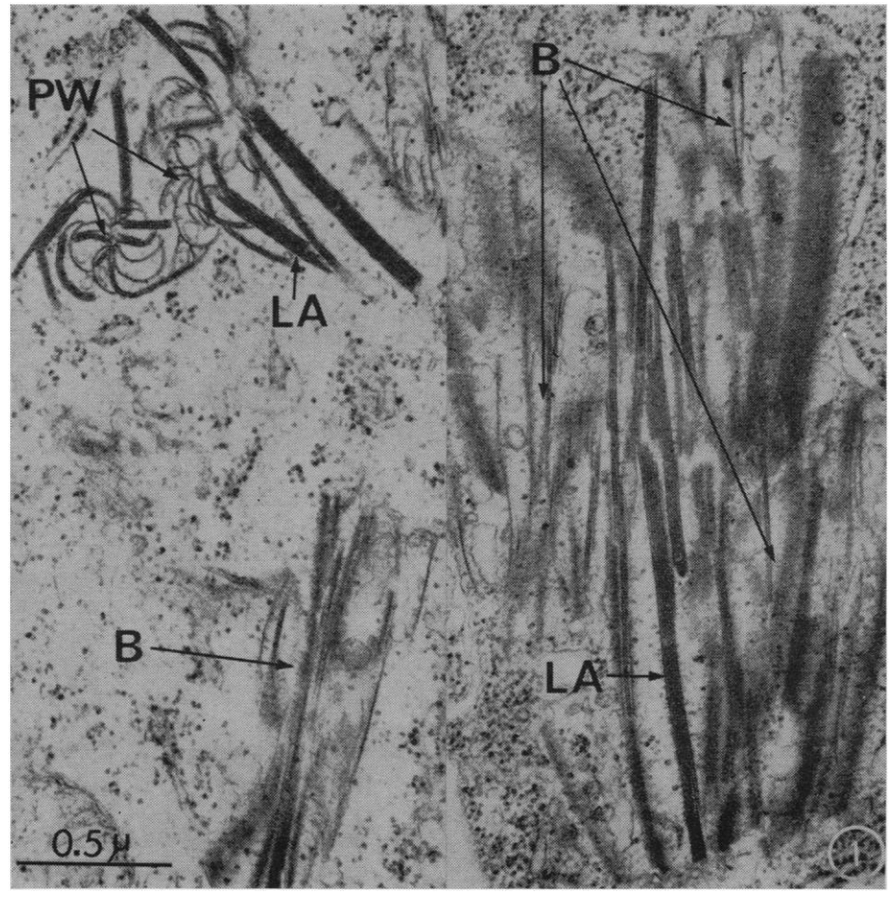

Fig. 1. Portions of leaf cells from tobacco infected with tobacco etch virus, which contain pinwheel (*PW*), bundle (*B*) inclusions, and laminated aggregates (*LA*).