cite and taenite in the residue reflects mechanical dislodgment by the vibrator during electrolysis. The absence of identifiable troilite from the residue is somewhat surprising, but the samples were fairly small and only a few millimeters of kamacite was dissolved; complete dissolution would no doubt leave troilite nodules.

At currents of 0.5 amp or less, only the iron appeared to stay in solution on dissolution of kamacite, while nickel ended up as a metallic sludge at the bottom of the anode compartment. More nickel dissolved when higher currents were applied, even though the standard potential of the nickelousnickel couple (-0.24 volt) is 0.2 volt more positive than the standard potential of the ferrous-iron couple (-0.44)volt). At currents exceeding 1 amp, some ferric iron was formed. The standard potential of the ferric-ferrous couple is +0.773 volt-considerably more positive than the nickelous-nickel couple. At low currents, however, the extent of nickel dissolution was slight and the taenite structure was maintained while kamacite dissolved entirely.

A Canyon Diablo specimen measuring about 2 by 2 by 0.8 cm was electrolyzed until about 35 g of metal was taken into solution; finally it appeared identical with the Canyon Diablo specimen in Fig. 4; analysis of the electrolyte solution showed that nickel comprised only 1.5 percent of the total dissolved metal. Since kamacite contains approximately 5.5 percent nickel, the remaining nickel must have either plated-out on the taenite lamellae or separated from the solution as metallic nickel. X-ray fluorescence analysis of the residue showed a high nickel content. Analysis of a sample of taenite that was carefully removed from the specimen gave 40.1 percent iron and 59.9 percent nickel, a composition within the normal range of taenite but somewhat richer than the 31 to 34 percent nickel reported for taenite in the Canyon Diablo meteorite (7). The nickel content found here for taenite was still too low to account for the remainder of nickel from the kamacite phase; thus it was concluded that the metallic nickel had indeed collected at the bottom of the anode compartment.

If metallic iron is brought into contact with a solution of a more noble metal, the other metal is reduced by the iron; it plates out onto the iron and an equivalent amount of iron dissolves.

Dissolution of the iron dislodges the noble metal which settles to the bottom of the container as a metallic sludge. This principle is used in copper mining; "tin" cans are added to vats of acidic copper solutions, and metallic copper is recovered. Nickel from the dissolved kamacite may have been deposited analogously.

Electrolytic oxidation of iron meteorites in a suitable electrolyte solution results in preferential dissolution of metallic iron. Nonmetallic inclusions and, for the most part, metallic nickel remain undissolved. The high nickel content of taenite, and especially the nickelricher borders in the taenite phase, result in preservation of its structure, while the high iron content of kamacite results in its dissolution. The remaining specimen shows a network of taenite plates in octahedral arrangement, with the kamacite removed. Nonmetallic inclusions and any oxide coating were untouched by the dissolution process (Figs. 1-4).

The electrolytic process is an excellent and previously unavailable technique for chemically separating metallic and nonmetallic phases of meteorites and for preferentially dissolving only the kamacite in octahedrites. Since iron dissolves first because of its favorable potential, a three-dimensional Widmanstätten pattern can be easily revealed as desired. The dissolution of iron is followed by dissolution of nickel, but all nonmetallic phases remain completely untouched.

STANFORD L. TACKETT\* WALLACE M. MEYER, JR. FRANK G. PANY CARLETON B. MOORE Department of Chemistry, Arizona State University,

Tempe

#### **References and Notes**

- J. I. Goldstein and R. E. Ogilvie, Geochim. Cosmochim. Acta 29, 893 (1965).
   B. Mason, Meteorites (Wiley, New York, 1962), pp. 53-69.
   R. W. Gurry, J. Christakos, C. D. Stricker, Trans. Amer. Soc. Metals 50, 105 (1958).
   J. D. Buddhue, The Oxidation and Weathering of Meteorites (Univ. of New Mexico Press, Albuquerque, 1957), pp. 30-43.
   J. M. Short and C. A. Andersen, J. Geophys. Res. 70, 3745 (1965).
   Analysis by C. F. Lewis, Arizona State University.

- Analysis by C. I. Levin, versity.
   T. B. Massalski and F. R. Park, J. Geophys. Res. 67, 2925 (1962).
   Aided by NSF undergraduate research participation program (W.M.M. and F.G.P.).
   \* Present address: Department of Chemistry, Indiana University of Pennsylvania, Indiana, Departmentation

16 May 1966

## Source of Lead-210 and Polonium-210 in Tobacco

Abstract. Test plants were grown within a chamber enriched with radon-222 in the atmosphere, in tobacco fields with different sources of phosphate-containing fertilizer, and in culture containing lead-210 in the nutrient solution. Harvested leaves were subjected to three curing conditions. The major portion of the lead-210 in the plant was probably absorbed through the roots. Airborne radon-222 and its daughters contributed much less to the plant's content of lead-210 and of polonium-210. The stage of leaf development and the methods used to cure the leaf affected the final amount of polonium-210 in tobacco leaf.

Current interest in the presence of Po<sup>210</sup> in tobacco and tobacco smoke (1, 2) and recent reports on the variation of the amount of Po<sup>210</sup> in leaf tobacco produced in different parts of the world (3) led us to investigate the source of Pb<sup>210</sup> and Po<sup>210</sup> in tobacco. Of the nuclides in the uranium series with longer half-lives, U<sup>238</sup>, Ra<sup>226</sup>, Pb<sup>210</sup>, and Po<sup>210</sup> are principally present in soil and fertilizer, while Rn<sup>222</sup>, a chemically inert gas, and some of its short-lived daughter products, are present in the air. To determine whether any one of these nuclides is a possible source of Po<sup>210</sup> in tobacco, the following experiments were conducted: (i) tobacco plants were grown in an atmosphere enriched with Rn<sup>222</sup>, (ii) tobacco plants grown in the field and supplied

with regular commercial fertilizer containing superphosphate were compared with those supplied with specially mixed fertilizer containing chemically pure, secondary calcium phosphate; and (iii) tobacco was grown in a nutrient solution containing Pb<sup>210</sup> as lead nitrate in equilibrium with Po<sup>210</sup>. Methods used for the measurement of the activities of Ra<sup>226</sup> and Po<sup>210</sup> were essentially the same as those previously described (1), with the exception that Po<sup>208</sup> was added as a tracer to correct for the radiochemical yield of Po<sup>210</sup>.

Eight well-developed plants (Nicotiana tabacum L. cv. Catterton, about 45.72 cm tall) were transplanted from the field to buckets 35.56 cm in diameter and 40.61 cm deep and were placed inside a small, closed greenhouse

(28.32 m<sup>3</sup>). A jar of radium-bearing sludge was placed in the rear center between benches and under an air conditioner. The temperature was maintained at about 27.8 °C. Plants grew in this greenhouse for a total of 6 weeks; the plants were decapitated 2 weeks before the end of this period. Radon in the greenhouse was maintained at a concentration of approximately 50 pc/liter, which is about 500 times greater than that in the normal atmosphere (4).

After they were harvested, tobacco leaves were divided into two groups for 2 weeks of air-curing; one group was hung inside the greenhouse containing  $Rn^{222}$ , the other group in the regular greenhouse. Control plants grew in similar soil-filled buckets outside the greenhouse and were harvested and cured in the same way as the test plants. Samples of leaves and soil were analyzed for  $Ra^{226}$  and  $Po^{210}$  after sufficient time had elapsed to allow  $Po^{210}$ to equilibrate with  $Pb^{210}$  in the sample (Table 1).

Tobacco leaf produced inside the greenhouse containing Rn<sup>222</sup> had approximately twice the amount of Po<sup>210</sup> as the control did. This result indicates that Rn<sup>222</sup> (and therefore an increased concentration of Pb<sup>210</sup> in the air) is not a major source of Po<sup>210</sup> in tobacco. The air contained dust particles; there were some from the soil and a small number from the Ra<sup>226</sup>. These were carried around inside the greenhouse by air constantly circulated by the air conditioner. The hairy, waxy surface of tobacco leaves could have easily attracted such particles. There was no rainfall, but the two sprayings inside the greenhouse within the 6 weeks would have simulated some of the effects of rainfall.

Previous reports (1) indicate that fertilizers containing uranium and its daughters may contribute to the amount of  $Ra^{226}$  in the soil and of  $Po^{210}$  in tobacco. Two kinds of fertilizers, a commercial fertilizer containing superphosphate and a specially mixed fertilizer containing chemically pure, secondary calcium phosphate, were compared. These two fertilizers were applied in bands along rows of plants. The activities of  $Ra^{226}$  and  $Po^{210}$  in the tobacco plants are given in Table 2.

The activity of  $Ra^{226}$  in the commercial fertilizer we used was 13 times greater than that of the specially mixed material; the activity of  $Po^{210}$  in the commercial fertilizer was 6.5 times greater than that of the specially mixed

19 AUGUST 1966

Table 1. Activity of radium-226 and polonium-210 in tobacco grown in, and in samples of soil from, a greenhouse containing radon-222.

Conditions for samples	Ra-226 (pc/g)	Po-210 (pc/g)
Tobacco samples		
Grown inside greenhouse, cured under regular conditions Grown inside greenhouse, cured inside greenhouse Grown under regular conditions, cured inside greenhouse Grown and cured under regular conditions	$\begin{array}{c} 1.78 \pm .17* \\ 2.10 \pm .03 \\ 0.07 \pm .005 \\ .06 \pm .006 \end{array}$	$0.67 \pm .01^{+}$ .78 ± .01 .34 ± .01 .29 ± .01
Soil samples after harvest		
From inside greenhouse From control plants	$\begin{array}{c} 0.75 \pm .06 \\ .91 \pm .10 \end{array}$	$.44 \pm .01$ $.58 \pm .01$
* One stondard deviation (Counsian) of dualizated and an emeration	+ One standar	a dentadan dere

\* One standard deviation (Gaussian) of duplicated radon emanation. † One standard deviation due to counting error.

material (Table 2). When the fertilizer was applied at 1560 kg/hectare in a formula of 4 percent nitrogen, 8 percent  $P_2O_5$ , and 12 percent  $K_2O$ , there was an apparent increase in the activity of  $Po^{210}$  in soil as well as in the tobacco. In the large volume of soil treated, the amount of fertilizer used in such a short period did not appear to have a significant effect on the amounts of radioelements which could be absorbed by plants.

Lead-210 nitrate (3.4  $\mu$ c) in equilibrium with Po<sup>210</sup> was added to the nutrient solution in which tobacco plants were grown. The plants were harvested 6 weeks later; they absorbed and translocated Pb<sup>210</sup> and Po<sup>210</sup> from solution cultures. The concentration of Po<sup>210</sup> rose to  $150 \pm 1$  pc/g in the leaves whereas the concentration in the controls was  $0.16 \pm .01$  pc/g. The presence of lead in soil and in tobacco has been the subject of many studies (5), and there have been various reports of absorption of Pb<sup>210</sup> from the soil (6).

The effects of quick-oven drying, aircuring, and flue-curing on the radioactivity of harvested tobacco leaves was investigated. The activity of Po<sup>210</sup> in tobacco leaves quickly oven-dried immediately after harvest was similar to that of air-cured leaves  $(0.24 \pm .01)$ pc/g). However, when these samples were flue-cured a small increase in the Po<sup>210</sup> content was apparent  $(0.31 \pm 0.1)$ pc/g). The combustion products from the fuel used for flue-curing may contribute to this increase. Polonium-210 from the fuel is volatile at the temperature of combustion and readily attaches to the leaf surface.

During the course of the experiments on plants grown in atmospheres supplemented with  $Rn^{222}$ , secondary leaf growth from axillary buds was stimulated by decapitation. These tobacco "suckers" were allowed to develop for 4 weeks and then were harvested and immediately freeze-dried in liquid nitrogen. A comparison was made of the amounts of  $Ra^{226}$  and  $Po^{210}$  in old tobacco leaves grown in air enriched with  $Rn^{222}$ , in normal tobacco "suckers" from plants grown outside, and in these young suckers (Table 3). The activity of  $Ra^{226}$  in young, secondary leaves was 12 times that in old leaves. The activity of  $Po^{210}$  in the young leaves was three times that in the old. It appears that, after the plant was decapitated, the axillary buds were the only actively developing tissues and that they used the precursors of  $Po^{210}$ , which

Table	2.	Radium-22	6 an	d p	olonium-210	in
sample	es c	of fertilizer,	soil,	and	tobacco.	

Conditions for samples	Ra-226 (pc/g)	Po-210 (pc/g)
Ferti	lizer samples	
Commercial fertilizer	$13.4 \pm .6*$	$6.72 \pm .14^{+}$
Special mix	$0.95 \pm .06$	1.05 ± .09
Soil sample	s (after harvest	ing)
From commercial fertilizer plot	$0.72 \pm .04$	0.48 ± .02
From special mix plot	$.63 \pm .04$	.28 ± .02
Tobacco s	amples (air-cur	ed)
From commercial fertilizer plot	$0.12 \pm .01$	$0.26 \pm .01$
From special	$.06 \pm .005$	$.22 \pm .01$

\* One standard deviation (Gaussian) of duplicated radon emanation. † One standard deviation due to counting error.

Table 3. Comparison of activities of radium-226 and polonium-210 in secondary leaf growth and in old leaves from plants grown in air enriched with radon-222. All tissues were freeze-dried while still green.

Sample	Ra-226 (pc/g)	Po-210 (pc/g)
Rn-2	22 greenhouse	
Old leaves	$0.12 \pm .01*$	$0.45 \pm .01$ †
Secondary leaf growth (suckers	$1.41 \pm .38$	$1.32 \pm .01$
	Control	
Secondary leaf	$0.38 \pm .02$	.22 + .01

growth (suckers)

mix plot

\* One standard deviation (Gaussian) of duplicated radon emanation. † One standard deviation due to counting error. were supplied through the root system, for rapid growth.

Our results seem to indicate that the principal source of Pb<sup>210</sup>, and thus of Po<sup>210</sup>, 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<sup>210</sup> in tobacco.

T. C. Tso

Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

## NAOMI HARLEY

Health and Safety Laboratory, U.S. Atomic Energy Commission, New York L. T. ALEXANDER

Soil Conservation Service,

U.S. Department of Agriculture,

Beltsville, Maryland

### **References and Notes**

- T. C. Tso, N. A. Hallden, L. T. Alexander, Science 146, 1043 (1964).
   E. P. Radford and V. R. Hunt, *ibid.* 143, 247 (1964); T. F. Kelley, *ibid.* 149, 537 (1965); E. Marsden, New Zealand Med. J. 64, 367 (1965); J. B. Little, E. P. Radford, H. L. McCombs, V. R. Hunt, New Eng. J. Med. 273, 1343 (1965).
   L. P. Gregory, Science 150, 74 (1965).
   W. Jacobi, Biophysik 1, 175 (1963).
   T. C. Tso, Bot. Bull. Acad. Sin., Taipei 7, 1 (1966).
   United Nations Scientific Committee on the

- (1966).
  United Nations Scientific Committee on the Effects of Atomic Radiation, *Report General Assembly* (United Nations, New York, 1962).
  K. C. Berger, W. H. Erhardt, C. W. Francis, *Science* 150, 1738 (1966).
  E. Marsden, *Nature* 203, 230 (1964).
  We thank J. Harley for his interest and for divergence of the processing of the interest of the Science Science
- discussions of this manuscript, G. L. Steffens for the studies of the fertilizers, and J. Allen, J. M. Carr, E. Hardy, and M. Meyer for their assistance.
- 5 May 1966

# **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 zincdeficient 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

Table 1. Activities of pancreatic carboxypeptidases and hepatic alcohol dehydrogenase in zincdeficient and zinc-supplemented rats. Results are expressed as means  $\pm$  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	$0.057 \pm 0.004$ (3)	$0.097 \pm 0.004^{*}$ (3)	
Assay 2	$.042 \pm .004$ (4)	$.057 \pm .003$ (4)	
Pancreatic carboxypeptidase B	$.277 \pm .10$ (5)	$.248 \pm .031$ (5)	
Hepatic alcohol dehydrogenase	.11 ± .01 (3)	.10 ± .00 (3)	

\* P < 0.01.† P < 0.05.

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 Mc-Collum (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.

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  $\mu$ g 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  $-20^{\circ}$ 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 (pH 7.65) for activation which was completed in 30 minutes at room temperature. The trypsintreated 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-Lphenylalanine and hippuryl-L-arginine were measured by the increase in absorbancy at 254 m<sub> $\mu$ </sub> 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 graving of black hair, alopecia, and scabby skin lesions. The

SCIENCE, VOL. 153