

First, in order to determine whether species-specific G-6-PD of these interbreeding hares can be distinguished from each other by electrophoresis, two males and three females of *Lepus europaeus* were compared with two males and one female of *L. timidus*. Regardless of sex, each animal of both species demonstrated a single sharp band of G-6-PD at both pH's, and that of *L. europaeus* consistently migrated further toward the anode than that of *L. timidus*. The difference in rate of migration was comparable to the difference that exists between A and B variants in man.

Next, all available F_1 hybrids were examined. A cross between a male *L. timidus* and a female *L. europaeus* was represented by two males and two females of the same litter. The sire of this litter was no longer available, but hemolyzate of the dam was used for comparison. The reciprocal cross to the above was represented by one male. Neither his sire nor his dam was alive.

As shown in Fig. 1, the hemolyzates of each of the two hybrid males with the *L. europaeus* mother revealed a single fast-moving band of G-6-PD identical with the maternal band. One male hybrid of the reciprocal cross had a single slow-moving band of G-6-PD inherited from the *L. timidus* mother. Each of the two female hybrids showed two bands of G-6-PD, the fast-moving band corresponding to that of their mother *L. europaeus*, the slow-moving band corresponding to that of their father *L. timidus*. Thus, sex-linkage of this enzyme was suggested.

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References and Notes

1. S. Ohno, W. Beçak, M. L. Beçak, *Chromosoma* **15**, 14 (1964); S. Ohno, *Ann. Genet.* **8**, 3 (1965).
2. V. A. McKusick, *Quart. Rev. Biol.* **37**, 69 (1962).
3. C. K. Mathai, J. M. Trujillo, S. Ohno, E. Beutler, *Fed. Proc.* **24**, 440 (1965); J. M. Trujillo, B. Walden, P. O'Neil, H. B. Anstall, *Science* **148**, 1603 (1965); C. K. Mathai, S. Ohno, E. Beutler, *Nature*, in press.
4. I. Gustavsson, in preparation.
5. We thank E. Beutler for advice.
6. V. F. Fairbanks and E. Beutler, *Blood* **20**, 591 (1962).
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Polonium-210 Analyses of Vegetables, Cured and Uncured Tobacco, and Associated Soils

Abstract. Analysis of the edible portion of vegetables and samples of green leaf tobacco failed to show polonium-210. The cured samples of leaf tobacco and the soils that were analyzed all contained small quantities of the element. Muck soils contained three times as much Po^{210} as did mineral soils. Solutions used commonly to extract "available" forms of many mineral elements failed to extract a detectable amount of Po^{210} . Indications are that Po^{210} or its radioactive precursors are not taken up from the soil directly by plant roots but rather by sorption in dead, moist plant materials at the atmosphere-plant interface.

Mayneord *et al.* (1) suggest that Po^{210} in plants is derived from the soil, the air or from both. Po^{210} may originate from the radioactive decay of in precursors such as Pb^{210} , Rn^{222} , or Ra^{226} . It may also originate from radioactive decay of the daughters of Rn^{222} deposited on the leaves (1). Radford and Hunt (2) suggest that Po^{210} in tobacco may initiate neoplasms in the bronchial epithelium of cigarette smokers. Po^{210} , an alpha emitter, is volatile above $500^\circ C$ —well below the temperature of a burning cigarette (2). It binds rapidly and strongly to surfaces and thus may be firmly attached to smoke particles (2). In cats, significant amounts of added Po^{210} were absorbed from the stomachs (3), and, since the amounts of Po^{210} in vegetables passing through the body could be vastly greater than that which enters by way of cigarette smoke into the lungs, it seemed imperative, that the amounts of Po^{210} in vegetables be determined.

Air-cured leaf tobacco, grown in the summer of 1964 in Wisconsin, was dried at 50° to $60^\circ C$ and ground to pass through an eight-mesh screen. Samples of three different tobacco varieties and several soil types were analyzed for Po^{210} .

Edible portions of fresh vegetables were collected in the summer of 1964 and stored under dry ice for 6 months. Samples of these were then dried and analyzed for Po^{210} . Duplicate samples were stored for 5 more months and then analyzed.

For analyses, 2-g samples of plant tissue (dry weight) were digested with 20 ml of 12N HCl for 40 minutes at

60° to $70^\circ C$; 2-g samples of soils were digested with 10 ml of 12N HCl for the same period. After digestion, the solution was quantitatively transferred to a plating flask by washing with 0.5N HCl until the final plating volume was 75 ml. To eliminate the interference of the ferric ion, 0.1 g of ascorbic acid, a mild reducing agent, was added to the flask. The contents were then heated (90° to $100^\circ C$), continuously stirred for 4 hours, and plated on a silver planchet. The planchet was then removed, washed with distilled water, and air dried, and the Po^{210} was counted in a solid-state alpha-particle counter for 4 hours (4). The background was 0.02 ± 0.01 count/min and it was similar for the blank. The efficiency of the counter and the plating procedure for a known amount of Po^{210} was 10.5 ± 1.5 percent. Although digestion of plant tissue or soil was incomplete with hot, concentrated HCl, when known amounts of Po^{210} were added recovery was quantitative.

Analyses were made on 30 samples of the edible portion of each of the following plants: sweet corn, field corn (grain and leaves), cabbage, carrots, red beets, cucumbers, radishes, snap beans, and potatoes; there was no detectable Po^{210} . We then assumed that the Po^{210} present in the plant tissue was not being released upon chemical treatment with hot 12N HCl. When a wet-ashing method (HNO_3 , H_2SO_4 , and $HClO_4$) (5) was used to completely digest 1 g of dry sweet corn grain, no Po^{210} was detected. Using this method on 1 g of dry cigarette tobacco, we found a significant amount of Po^{210} . The wet-ashing method gave results similar to those obtained by digestion with 12N HCl. We then assumed that the size of the sample (1 g dry weight) was too small for the detection of Po^{210} in vegetables. However, when samples of 2-, 5-, 10-, and 100-g dry weight were used, the count was still not detectable. Known amounts of Po^{210} were added to these samples of various sizes and quantitatively recovered.

Because the difference between the number of counts in the sample and the number in the background obtained from any sample of the vegetables, either in the moist or in the dried state, was less than or equal to the standard deviation of the number of counts, we conclude that the amounts of Po^{210} in these samples are below the limits of detection (Table 1). As we mentioned, radioactivity was significant in one 1-g sample of dry cigarette tobacco and

Table 1. Amounts of Po^{210} in 20 vegetable samples and their associated soils. One tissue and one soil sample were taken from each site. The standard deviation is the ratio of the square root of the number of counts per minute to the time. The relative error is the ratio of the standard deviation to the observed result. The background was subtracted from the values.

Sites (No.)	Soil type	Mean activity of $Po^{210} \pm S.D.$ (pc/g)	
		Soil	Tissue
2	Loamy sand	1.92 ± 0.13	$-0.04 \pm 0.09^*$
13	Silt loam	2.05 ± 0.12	$-0.04 \pm 0.09^\dagger$
5	Muck	5.92 ± 0.27	$0.00 \pm 0.09^\ddagger$

* Carrots, snap beans, red beets, sweet corn.
 $\dagger$ Cabbage, cucumbers, Carrots, onions, potatoes, radishes, red beets.

not in samples of as much as 100 g (dry weight) of sweet-corn tissue. There were no detectable amounts of Po^{210} in the vegetable samples, whether storage was for 6 or for 11 months.

Table 1 shows the content of Po^{210} in soils in which various vegetable crops were grown. More Po^{210} was found in muck soils than in mineral soils. The average value of Po^{210} in five muck soils was 5.92 pc/g, while the 15 mineral soils contained an average of 2.03 pc/g. The higher concentration of Po^{210} in muck soils may have been caused by an accumulation of Po^{210} or its radioactive precursors in the organic matter. A likely precursor would be Rn^{222} , which is known to be in the soil atmosphere in relatively large concentrations (10^5 to 10^6 pc/m³) (6), as well as in the atmosphere in concentrations of 50 to 200 pc/m³ (7).

Table 2. Amounts of Po^{210} in cured tobacco leaves, in green leaf tobacco, and in their associated soils. With the cured leaf tobacco one tissue and one soil sample from each site were tested; for the uncured tobacco eight tissue and three soil samples were tested.

Site	Mean activity of $Po^{210} \pm S.D.$ (pc/g)		
	Soil	Leaf after 6 months' storage	Leaf after 11 months' storage
<i>Cured leaf tobacco</i>			
Coon Valley	3.56 ± 0.17	1.20 ± 0.08	1.03 ± 0.08
Westby	$2.92 \pm .17$	$0.77 \pm .06$	$0.69 \pm .06$
Viroqua	$2.83 \pm .17$	$1.29 \pm .10$	$1.54 \pm .08$
Soldiers Grove	$2.53 \pm .16$	$0.34 \pm .04$	$0.34 \pm .04$
Viroqua	$2.44 \pm .17$	$1.29 \pm .10$	$1.50 \pm .10$
Edgerton	$2.28 \pm .13$	$0.98 \pm .08$	$0.64 \pm .08$
Edgerton	$2.28 \pm .13$	$0.77 \pm .04$	$0.87 \pm .06$
Coon Valley	$2.27 \pm .13$	$0.60 \pm .06$	$0.17 \pm .01$
Ferryville	$2.14 \pm .17$	$1.33 \pm .08$	$1.50 \pm .08$
Coon Valley	$1.76 \pm .13$	$1.03 \pm .06$	$1.13 \pm .08$
<i>Uncured green leaf tobacco</i>			
	$3.46 \pm .17$	$0.00 \pm .09$	$0.00 \pm .09$

Po^{210} activities in samples of cured leaf tobacco and in the soils associated with these samples are shown in Table 2. The average amounts of Po^{210} in cured leaf tobacco after 6 months of storage and in soil samples were 0.96 and 2.50 pc/g dry weight, respectively. All cured tobacco samples were analyzed again for Po^{210} after 11 months of storage, and the results were similar.

Twenty-one freshly harvested tobacco leaf samples, 11 greenhouse-cultured and 10 field-grown samples, failed to show any detectable activity of Po^{210} , even after some samples were stored 6 months in a dry, ground state. A significant amount of Po^{210} was found, however, in the associated soils. Also fresh bluegrass leaf samples, harvested in May 1965, did not contain detectable Po^{210} , nor did the same samples 3 months later. Since freshly harvested tobacco samples showed no detectable Po^{210} even after considerable time for ingrowth, whereas cured tobacco contained significant quantities of Po^{210} , it appears that Po^{210} accumulates in the tobacco leaf during curing.

Analysis of dead tree leaves (7.2 pc/g) and of dead bluegrass (6.6 pc/g) showed that they had 7 to 8 times more Po^{210} than did cured tobacco (0.96 pc/g). These moist samples were taken near the soil surface in the spring of 1965. Heads of dead cabbage left in the field over winter had in their outer leaves 5 to 6 times more Po^{210} than did cured tobacco. A definite decrease in Po^{210} content was found with increasing distance from the outermost leaves. The first and second leaves contained 5.4 pc of Po^{210} per gram of dry tissue, whereas the fourth, fifth, and sixth leaves contained 1.03 pc/g. The inner portion, from the seventh leaf to the core of the cabbage heads, showed no detectable Po^{210} . Most of the Po^{210} in the cabbage heads was found in the outermost two leaves. The dead inner portion of the cabbage heads should have also contained Po^{210} if this element or one of its radioactive precursors was taken from the soil. Therefore, the sorption of Po^{210} or of its radioactive precursors may occur at the atmosphere-plant interface.

There are then three kinds of evidence suggesting that Po^{210} is not taken up from the soil directly by plant roots and translocated to other plant organs. First, no detectable quantity of Po^{210} was found in the center of the cabbage heads. Second, common extracting solutions used to evaluate "available" forms of many mineral elements failed

to extract from various vegetables a detectable amount of Po^{210} . Third, even after sufficient time for ingrowth, there was no detectable quantity of Po^{210} in green leaf tobacco grown in a soil medium containing significant amounts of Po^{210} .

A common extracting solution (0.1N HCl and 0.03N NH_4F) for available soil phosphorus and potassium was used to determine how much available Po^{210} was in the above soils (5); none was detected. Extracting solutions of 1N ammonium acetate and 0.2N HCl gave similar results, suggesting that Po^{210} does not exist in an available form in the soil complex.

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References and Notes

- W. V. Mayneord, R. C. Turner, J. M. Radley, *Nature* **187**, 208 (1960).
- E. P. Radford, Jr., and V. R. Hunt, *Science* **143**, 247 (1964).
- P. E. Morrow, F. A. Smith, R. J. Della Rosa, L. J. Casarett, J. N. Stannard, *Radiation Res. Suppl.* **5**, 60 (1964).
- E. P. Radford, Jr., V. R. Hunt, D. Sherry, *Radiation Res.* **19**, 298 (1963).
- M. L. Jackson, *Soil Chemical Analysis* (Prentice-Hall, Englewood Cliffs, N.J., 1958), pp. 161, 331.
- K. G. Vohra, M. C. Subboramu, A. M. Mohan Rao, *Nature* **201**, 37 (1964).
- W. Jacobi, *Biophys. Zentralbl.* **1**, 175 (1963).
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Centrifugal Homogenizer

Abstract. *Tissue can be homogenized in conventional ground-glass tissue grinders in conjunction with centrifugation. The method may be of special value for separating soluble from insoluble material during homogenization of tissue. As yet only yeast cells and spores of slime mold have been homogenized, but the method may be useful for many tissues.*

Tissue is homogenized by various mechanical devices including ground-glass tube and pestle grinders, Waring blenders, VirTis grinders, French hydraulic presses, and ultrasonic vibrators (1). I have developed a new technique of homogenization in conventional ground-glass tissue grinders in conjunction with centrifugation (Fig. 1). In the pilot model a tissue grinder was adapted for use with an Inter-