gle band of intermediate  $R_{*}$  and zonal length (Fig. 1, samples 9 and 15). The greater the proportional amount of the normal hemolyzate in the mixture, the higher the rate of migration of the catalase band. Measurements reveal that the length of a catalase zone-normal, abnormal, or mixed-is a linear function of the hemolyzate concentration between 2 and 1/2 percent. Below 1/2 percent the zones appear clouded and measurement becomes inaccurate. As the concentration of a hemolyzate decreases, the rate of migration of catalase increases; however, under standard conditions the relative rates of migration, as well as the relative lengths of the zones, can be satisfactorily reproduced.

One way to explain the combination of the phenomena of elongation of the abnormal zone, of its lower catalase concentration in front and rear regions, and of the intermediate zonal length and  $R_u$  of mixtures of normal and abnormal catalase, is to assume that more than one fraction in the zymogram of the heterozygote is present. The following experimental arrangement may support the hypothesis of heterogeneity: it is possible to produce a facsimile of the catalase zymogram of a 2 percent abnormal hemolyzate by placing .67 percent normal hemolyzate samples on three consecutive starting lines (Fig. 1, samples 7 and 8). Such an array would correspond to an assumption of three catalase fractions of approximately equal concentrations in the abnormal hemolyzate. A similar but not identical zymogram results from a 1 percent normal hemolyzate on two sequential starting lines (Fig. 1, samples 12 and 13). Analogously, in the arrangement in Fig. 1, 10 duplicates the zymogram 9 of the mixed hemolyzate (normal to abnormal, 1:1 volume) better than 14 duplicates 15. Although these results indicate neither a specific number of catalase fractions nor their ratios in the abnormal hemolyzate, they do illustrate the possibility of heterogeneity of the abnormal band. The demonstration of separate but closely adjoining catalase zones with the currently used specific H2O2-iodine stain is impeded by diffusion of the reactants. A protein stain, such as amido black, would perhaps be more suitable for making the zonal fractionation visible, but it requires purification and high concentration of catalase.

The hypothesis that there are more than two fractions in the abnormal catalase is in accordance with findings by Boyer (11) on placental alkaline phosphatase and Schwartz (12) on maize esterase. Both reported the simultaneous occurrence of parental and hybrid enzymes in the heterozygote.

The reaction velocity constants  $K_{o}$ for the atypical erythrocyte catalase of the six subjects were 18, 23, 25, 27, 29, and 30. The  $K_{\circ}$  values obtained from determinations on hemolyzates of 150 normal subjects ranged from 15 to 37 (mean 24.4, standard deviation 5.4).

The hemolyzates of 350 subjects were investigated by electrophoresis. Thirty Negroes, eleven Orientals, one American Indian, and ten newborns were included. Because of the joint occurrence of abnormal catalase and multiple sclerosis in one member of family S, 23 cases with multiple sclerosis were included in our investigations. These 23 subjects possessed a normal catalase. The other five carriers of the abnormal catalase appear to be healthy. No abnormal catalase was found outside family S. The ancestors of the grandfather, who is the carrier of the abnormal trait in the P generation, came from Norway and Great Britain. Family S also includes two known carriers in the F1 and three in the  $F_2$  generation. Whereas sex linkage of the abnormal character can be ruled out by the occurrence of the mutant gene in males and females of the F1 and F2 generations, the results of an examination for autosomal linkage with either the haptoglobin or the ABO blood group locus were inconclusive. Abnormal hemoglobins do not occur in family S.

The structural modification in the catalase variant, presumably only a small alteration in the primary structure of the protein, results in a slight decrease of net charge, but the modified protein retains the enzymic activity. The mutation thus differs significantly from the cases of acatalasemia reported in Japan and Switzerland.

By designating the normal catalase gene as A and the mutant allele of family S as B, the genotype of the carrier can be symbolized by  $Ct^A/Ct^B$ . The heterozygous abnormality may be described as allocatalasia AB and the theoretical homozygosity for the variant as allocatalasia B (13).

ERNST W. BAUR

Biochemistry Department, Mental Health Research Institute, Fort Steilacoom, Washington

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### **Carbon: A New Crystalline Phase**

Abstract. The electrical resistance of single crystal graphite shows a very sharp increase at above 150 kilobars, accompanied by a drifting upward with time. The behavior is typical of a first-order phase transition, and is irreversible. X-rays on the material after removal from the cell show lines of a new material with a structure which can be indexed as a cubic lattice with a unit cell edge of 5.545 angstroms. The density of the new phase is estimated at 2.80 grams per cubic centimeter.

Electrical resistance measurements on single crystal graphite at high pressure have revealed a new crystalline form of carbon metastable at atmospheric pressure. The single crystal graphite was natural material obtained from L. Zumwalt and R. B. Duffield of General Atomics. The high-pressure resistance techniques have been previously discussed (1). By placing a small flake between crossed electrodes, resistance could be measured along the c-axis of the crystal. By using electrodes with a gap between them, resistance perpendicular to the *c*-axis could be measured. Isotherms were obtained at room temperature and 77°K. Isobars (77° to 296°K) at both low

Table 1. X-ray diffraction lines: new carbon phase. a = 5.545 Å;  $\rho = 2.803$  (estimated); 24 atoms per unit cell.

| hkl         | d     | Intensity |  |
|-------------|-------|-----------|--|
| (111)       | 3.208 | Weak      |  |
| (200)       | 2.770 | Medium    |  |
| (210)       | 2.467 | Medium    |  |
| (220)       | 1.961 | Weak      |  |
| (221) (300) | 1.844 | Weak*     |  |
| (222)       | 1.600 | Weak*     |  |
| (321)       | 1.485 | Weak      |  |

\* These lines appear also in untransformed graphite, but their relative intensity is markedly higher in the transformed material.

and high pressures were also obtained. Previous measurements on pyrolytic graphite (2) at  $296^{\circ}$ C were confirmed and extended by isotherms at  $77^{\circ}$ K and several isobars.

Figure 1 shows a 296°K isotherm measured perpendicular to the c-axis. The resistance drops slowly with increasing pressure to about 150 kb. There is a sharp rise with pressure at this point, accompanied by drifting upward with time, behavior typical of a sluggish first-order phase transition. As the pressure increased, the rise of resistance with pressure and the drift gradually decreased, but never ceased completely, which indicates that the transition was never complete. (See the discussion of our x-ray results.) Upon release of pressure the resistance increased, and the transition did not run backwards. The dashed line shows an isotherm on pyrolytic graphite for comparison. The rise of resistance (and the drift) is much smaller and there is an actual decrease in resistance with



Fig. 1. Resistance of graphite versus pressure measured perpendicular to *c*-axis of single crystal (below transition). Solid line, single crystal; dashed line, pyrolytic.

increasing pressure at high pressures. The same irreversibility is noted as for single crystal graphite.

Figure 2 shows a room-temperature isotherm on single crystal graphite measured along the c-axis. There is a rapid drop in resistance at low pressure. The resistance then levels, and then rises sharply at 150 kb. The total rise is only a factor of 7 to 8. Again, the rise is irreversible. The dashed line shows comparative data for pyrolytic graphite. No significant rise in resistance could be noted at high pressure.

The transition did not run at any available pressure at  $77^{\circ}$ K. On heating a sample at 410 kb, the transition initiated at 180° to 190°K.

At the lowest pressures for which measurements could be made (10 to 30 kb), the single crystal exhibited an increase in resistance with increasing temperature in both directions in the crystal. In contrast, the pyrolytic graphite was semiconducting in both directions at low pressure.

At high pressures (that is, above 300 kb) both single crystal graphite and pyrolytic graphite show a positive temperature coefficient of resistance (metallic behavior) along the *c*-axis. Perpendicular to the *c*-axis both materials showed a small but definite negative temperature coefficient of resistance. Figure 3 illustrates the behavior for single crystal graphite.

It should be noted that at these high pressures the material is partially converted to the new phase, so that neither the resistance nor the temperature coefficient of resistance is necessarily characteristic of either phase, nor, a fortiori, of a particular direction in graphite. Nevertheless, it is of interest that some directional characteristics are measurable.

X-rays have been obtained on both the partially transformed and starting materials, with a 114.6-mm Philips powder camera. Table 1 shows lines which we associate with the new phase, in most cases because they do not appear in the old phase, but in two cases because the lines in the transformed material seemed relatively more intense than in the original graphite. In all cases, even the strongest lines in the new phase were markedly weaker than the stronger graphite lines, which probably indicates that only a minority of the material was transformed. In pyrolytic graphite only two or three of the stronger lines from the new phase appeared, which indicates very low con-



Fig. 2. Resistance of graphite versus pressure measured parallel to *c*-axis of single crystal (below transition). Solid line, single crystal; dashed line, pyrolytic.

version; this is consistent with the resistance results.

Because of the very small samples obtained in our apparatus and the relatively low conversion, the precise identification of the structure is a very difficult matter. However, the new phase could be indexed as cubic with a unit cell edge of 5.545 Å. The lines present would be consistent with the space groups Pm3m, P432, P43m, Pm3, P4232, Pa3, or possibly P23 or P213.

By a series of float-sink tests, the partially transformed mixture was found to have a density of 2.35 to 2.40. If the new phase has 24 atoms per unit cell it would have a density of 2.80. The mixture density would



Fig. 3. Resistance versus pressure at high pressure (296°K and 77°K) for both initial orientations of crystal.

then correspond to 17- to 26-percent conversion, which is certainly consistent with the relative intensity of the lines of the new phase compared with the untransformed graphite.

The new phase is relatively stable at atmospheric pressure, as heating in a vacuum at 450°C for 6 hours gave no appreciable diminution in the intensity of the x-ray lines characteristic of this phase.

As we have shown, the single crystal graphite is only partially transformed. Pyrolytic graphite is only slightly transformed, and no indications of transformation have been found from powdered graphite. Evidently, the transformation can occur at a measurable rate only where there are relatively large areas of rather perfect crystal. It would seem possible that with unusually fine single crystals of graphite a very substantial conversion could be obtained. This would permit the identification of more lines, and reasonably accurate intensity determinations, so that the details of the structure could be determined. It would seem at present that this form of carbon may be analogous, in a general way, to the denser forms of germanium and silicon which have recently been reported as metastable at 1 atm (3), although it is formed from graphite, not from the diamond structure. In view of the marked increase in resistance at the transition, this new structure is not similar to the metallic forms of Si and Ge previously found at high pressure (4), which are reported by Jamieson (5) to have the white tin structure (6). R. B. AUST

# H. G. DRICKAMER

Department of Chemistry and Chemical Engineering and Materials Research Laboratory, University of Illinois, Urbana

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## Cadmium: Uptake by Vegetables from Superphosphate in Soil

Abstract. Phosphates in fertilizers contain cadmium. When vegetables usually devoid of cadmium were grown in soil heavily fertilized with 20 percent superphosphate, they absorbed it. Vegetables normally containing cadmium absorbed larger quantities in the presence of superphosphate and little or none in its absence. The superphosphate showed 7.25 parts of cadmium per million. Five grains usually containing cadmium were grown in unfertilized soil poor in this element; four did not absorb it. Phosphate fertilizers may be a source of the cadmium in some vegetable foods.

Cadmium accumulates with age in the human kidney and liver (1, 2), although variations in concentration in these organs from different areas of the world are large (3). The principal sources of cadmium for man are mollusks, crustaceans, and grains (2). Although cadmium is not always present in food, certain vegetables and grains produced with heavy applications of commercial fertilizer (4) have shown relatively high concentrations (2). We found 9 to 36 parts of cadmium per million in the phosphate fractions of five fertilizers. Others have reported even larger amounts in phosphorites from Jamaica (5) and Oceania (6), although a sample from Tennessee contained only 0.41 ppm. The source of cadmium in phosphates is undoubtedly sea water, for natural phosphate rock is partly composed of the hard parts of marine animals of Plio-Pleistocene or Tertiary time (5). Marine phosphorite deposits in Florida provide most of the phosphates in fertilizers used in the United States. In 1961 almost 3 million metric tons of P<sub>2</sub>O<sub>5</sub> for domestic agricultural uses and 1 million tons for export were produced in this state (7). Therefore, two questions required answers: Could phosphates supply cadmium to growing vegetables? Could grain be grown free of cadmium?

A plot, situated on a slope on an isolated hilltop in Vermont, measuring 20 by 18 m was divided into four subplots each 5 by 18 m. There was little drainage between them, as their slopes were similar. Fertilizer had not been applied to our knowledge, and the chance of industrial contamination in this location is remote. The soil contained no detectable cadmium. Two subplots served as controls. A third

received a heavy application of composted wood chips and manure, approximately 100 kg being spread thickly in seed rows. One horse provided the manure; he had fed on oats, containing 0.02 ppm Cd, and on pasture grass, hay, and water which had no detectable Cd. The wood chips showed 0.05 ppm Cd. The fourth subplot received an application of 20 percent superphosphate containing 7.25 ppm Cd; 25 kg were broadcast and 20 kg were laid in drills in close contact with seed. A garden was planted in rows passing through all four sections at right angles to the slope; it was watered only by rainfall. The mature vegetables and others purchased from local chain stores were carefully washed in deionized water, ashed at 400°C, and analyzed by the Saltzman method (8) with minor modifications (2). Fertilizers and soil samples were extracted with concentrated HCl or aqua regia (Table 1).

In order to expose growing plants to high concentrations of phosphate, three wooden window boxes were filled with forest top soil containing 0.34 ppm cadmium. One window box served as control, the soil in the second was mixed with about 25 percent by volume

Table 1. Cadmium in garden vegetables and soil (in micrograms per 100 g wet weight). N.D., not detected. There was no uptake of cadmium from superphosphate by corn, turnip leaves, lima beans, or radish leaves. Commercial lima beans and locally grown corn contained no detectable cadmium; frozen corn and corn meal showed 7.5 and 6.5  $\mu$ g/100 g, respectively. Turnip and radish leaves were not available commercially.

| Vegetable              | Con-<br>trol | Com-<br>post-<br>manure | Super-<br>phos-<br>phate | Chain-<br>store<br>vege-<br>tables |
|------------------------|--------------|-------------------------|--------------------------|------------------------------------|
|                        | Gard         | den                     |                          |                                    |
| Lettuce, iceberg       | N.D.         | N.D.                    | 4.0                      | 1.0                                |
| Lettuce, early curled  | N.D.         | 0.3                     | 0.5                      | 1.7                                |
| Turnips, roots         | N.D.         | N.D.                    | 3.0                      | 2.0                                |
| Radishes, roots        | N.D.         | N.D.                    | 2.0                      | 1.5                                |
| Parsnips               | 3.0          | 3.0                     | 14.0                     | 2.8                                |
| Potatoes               | N.D.         | N.D.                    | 0.3                      | N.D.                               |
| String beans           | N.D.         | N.D.                    | 0.3                      | N.D.                               |
| Beets                  | N.D.         | N.D.                    | 0.9                      | N.D.                               |
| Onions                 | N.D.         | N.D.                    | 0.3                      | N.D.                               |
| Peas, green            | N.D.         | N.D.                    | 1.0                      | N.D.                               |
| Carrots                | N.D.         | 0.7                     | 0.8                      | N.D.                               |
| Garden soil            |              |                         |                          |                                    |
| (dry wt.)*             | N.D.         | 18.8                    | 31.1                     |                                    |
|                        | Window       | v box                   |                          |                                    |
| Peas, green, leaves†   | 0.3          | 1.0                     | 13.0                     |                                    |
| Peas, green, shelled†  | N.D.         | N.D.                    | 3.3                      | N.D.                               |
| Swiss Chard            | ‡            | ‡                       | 1.7                      | 2.9                                |
| Beets                  | ‡            | ‡                       | 4.1                      | N.D.                               |
| Turnips, roots         | 0.3          | 0.3                     | 1.0                      | 2.0                                |
| Forest soil (dry wt.)§ | 34.0         | 67.1                    | 130.5                    |                                    |

\* The pH of all sections of the garden was 7.0. The soil had been limed the previous year. The sample from the phosphate-treated plot was taken from between rows.  $\dagger$  Pea seeds contained 1.0  $\mu$ g of Cd per 100 g.  $\ddagger$  Poor growth, insufficient size for analysis. § Forest topsoil was used in the window boxes. Foliage from nearby trees contained Cd (2). The pH was 7.0 in the boxes treated with compost and manure; in the other boxes it was 6.0.