

more, the time changes in the spectrum also repeat, with virtually identical slopes in the time frequency domain. Jupiter's dynamic spectrum represents a kind of permanent map of the surface of the planet, where radio frequency and time act as latitude and longitude, respectively. Possible physical implications of the existence of this permanent dynamic spectrum are discussed in the references below.

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#### References and Notes

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### Catalase Abnormality in a Caucasian Family in the United States

**Abstract.** *An erythrocyte catalase with atypical electromigration velocity was discovered in three generations of a family of Scandinavian-British extraction. Six members are heterozygous for the hereditary autosomal character; no abnormal homozygotes were found. The condition is associated with normal erythrocyte catalase activity and with no clinical or subclinical disease.*

Acatalsmia is the only known catalase disorder. This rare hereditary abnormality represents the homozygous phenotype for a mutant catalase gene. Acatalsmia and its heterozygous analogue, hypocatalasemia, were both discovered in Japan and described by Takahara and the Okayama University group (1, 2). Later, two familial cases were reported from Switzerland (3). Recent efforts to obtain evidence of other catalase disorders or to reveal multiple molecular forms of catalase have failed (4). Nevertheless, heterogeneity of this enzyme occurs, though rarely.

An atypical erythrocyte catalase, demonstrated by electrophoresis, was found in six members of a Caucasian

family (hereafter designated as S), all six of whom are heterozygous for this inherited character. In contrast to the abnormality in hypocatalasemia, their erythrocyte catalase activity is normal. Electrophoresis with thin-layer starch gel and the staining and plasticizing methods for catalase, peroxidase, and proteins were used as described previously (5). The catalase stain is a modification of the staining method of Thorup *et al.* (6). Catalase activity was determined with potassium permanganate by the titrimetric method of von Euler and Bonnichsen (7) at 60, 120, and 180 seconds. The reaction velocity constants ( $K_t$ ) were calculated according to the equation (7)

$$K_t = \frac{2.3 \times 10^3}{t} \log \frac{X_0}{X_t}$$

where  $X_0$  is the initial amount of potassium permanganate and  $X_t$  the amount at  $t$  seconds. Values for  $K_t$  were extrapolated to time zero ( $K_0$ ). Hemolyzates were prepared at 4°C from red blood cells that had been washed four times in normal saline, lysed by the toluene method (8), and used within 24 hours for titration and electrophoresis (9). Although electrophoretic artifacts of catalase are rarely observed with fresh hemolyzates, the possibility of such incidence caused us to examine at least three blood samples of each member of family S. Consistent results were obtained with all samples.

When a buffer system of tris-citrate and borate at pH 8.6 is used, the location of the common human erythrocyte catalase after electrophoresis is indicated by a single white zone on the iodine-stained blue background at the anodic side of the gel. Catalase moves slightly more slowly than hemoglobin  $A_2$  (Fig. 1). The atypical catalase also exhibits a single white zone but has a relative rate of electromigration ( $R_u$ ) of .89 (10). Its zonal length exceeds the normal by an average of one-tenth. The fastest and the slowest moving components of the abnormal catalase band show signs of low enzyme concentration, as indicated by the clouded appearance and by unsharp borders. Clouding of the entire zone of normal catalase occurs when hemolyzate concentrations of below 1/2 percent are used.

Efforts were made to fractionate the atypical catalase band through electrophoresis with different buffer systems (phosphate, borate, acetate, tris-borate-ethylenediamine-tetraacetate) at pH 8.9 to 5.5. Although a mobility difference between normal and variant catalase

remained, no separation into more than one zone could be obtained, even when electrophoresis was prolonged to 64 hours; moreover, catalase did not subfractionate with the highest practical dilutions (1/8 percent) of normal, abnormal, and mixed hemolyzates.

In a mixture of normal and abnormal hemolyzates, catalase migrates as a sin-

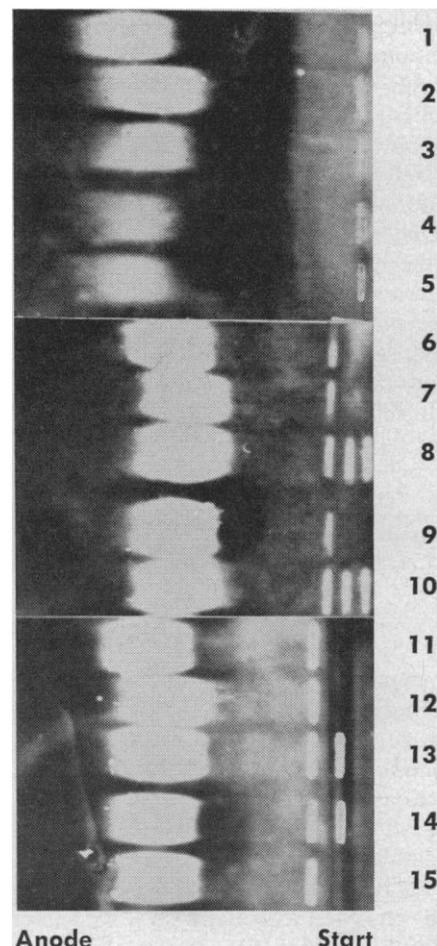


Fig. 1. Electrophoresis of hemolyzates with thin-layer starch gel; catalase stain. Samples 1-5 developed in inverted-J type, 6-10 and 11-15 in inverted-V type electrophoresis cells. For each application strip 10  $\mu$ l of hemolyzate were applied. Multiple application strips are listed from anode toward cathode. Hemolyzate concentrations in percentages follow (where A is from a normal homozygote; AB, from a heterozygote of family S).

|    | %       |     | %      |
|----|---------|-----|--------|
| 1. | A 2     | 9.  | A 2    |
| 2. | AB 2    | 10. | AB 2*  |
| 3. | AB 1    | 11. | A 1.33 |
| 4. | AB 0.5  | 12. | A 0.33 |
| 5. | A 0.5   | 13. | A 0.33 |
|    | AB 0.5* | 14. | A 2    |
| 6. | A 2     | 15. | A 2    |
| 7. | AB 2    |     | AB 2*  |
| 8. | A 0.67  |     |        |
|    | A 0.67  |     |        |
|    | A 0.67  |     |        |

\* 1:1 volume

gle band of intermediate  $R_u$  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 ½ percent. Below ½ 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  $H_2O_2$ -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  $K_0$  for the atypical erythrocyte catalase of the six subjects were 18, 23, 25, 27, 29, and 30. The  $K_0$  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  $F_1$  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  $F_1$  and  $F_2$  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 struc-

ture 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).

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#### References and Notes

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9. Hemolyzate concentrations mentioned refer to cyanmethemoglobin contents. Unless otherwise stated 2 percent solutions were used. For electrophoresis 10  $\mu$ l were applied per sample.
10. The rates of migration  $R_u$  refer to the electrophoretic mobility of the zonal center of normal catalase ( $R_u$ , 1.0) in a 2 percent hemolyzate in tris-citrate buffer pH 8.6 and represent mean values obtained from a number of gels.
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13. I thank Dr. A. G. Motulsky for his advice and Mrs. J. Morriss for her technical assistance.

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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 previ-

ously 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