broadening of neural tuning curves reflects, not an alteration in basilar membrane vibration, but a disruption of the functioning of a second filter of unknown nature, whose existence is considered necessary by some authors (*2, 3, 10*). The disruption could involve a structural change, perhaps a reversible displacement of the tectorial membrane (*16*), or an alteration in the flow of current in some important extracellular pathway. The lack of effect of fluid removal on spontaneous activities, however, implies that standing current levels through the hair cells are not altered, since it is known that polarization of the cochlear partition strongly affects rates of spontaneous activity in primary fibers (*17*). The results reported here do not make it possible to choose between these various hypotheses. However, by revealing a major source of error in some previous mechanical measurements, they again pose the question whether a second filter is necessary in addition to the basilar membrane to account for the sharpness of primary auditory frequency selectivity.

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Leukocyte Peroxidase Deficiency in a Family with a Dominant Form of Kuf's Disease

Abstract. *Use of a spectrophotometric assay of peroxidase with* p-*phenylenediamine as cosubstrate demonstrated deficient enzyme activity in leukocytes from two patients with a dominantly inherited form of ceroid lipofuscinosis (Kuf's disease) and a clinically healthy unaffected sibling. When the reaction was performed in the absence of added hydrogen peroxide, oxidation of the* p-*phenylenediamine cosubstrate (indicating the presence of endogenous peroxide) occurred only with enzyme samples from the three siblings but not with those from a large number of unrelated, unaffected controls. This demonstrates that the deficiency of peroxidase found previously in the recessively inherited infantile and juvenile forms of ceroid lipofuscinosis (Batten-Spielmeyer-Vogt disease) is also present in an adult form with dominant inheritance.*

In a previous report (*1*) we described a deficiency of leukocyte peroxidase in patients afflicted with the late infantile and juvenile forms of ceroid lipofuscinosis (Batten-Spielmeyer-Vogt disease). These disorders represent forms of neuronal storage disease characterized by progressive cerebral degeneration, accumulation of lipopigments in nerve cells, and recessive inheritance (*2*). Another form of ceroid lipofuscinosis, Kuf's disease, is characterized by onset during adulthood (*3*). Although most reports of cases of Kuf's disease suggest recessive inheritance, one pedigree showing inheritance of the disease as a dominant trait has been described (*4*). It was important, therefore, to determine whether the peroxidase deficiency found in the late infantile and juvenile forms of ceroid lipofuscinosis could also be demonstrated in the adult form and particularly in a pedigree in which the trait was dominant. Accordingly, we examined a peroxidase that acts on hydrogen peroxide with *p*-phenylenediamine as cosubstrate in leukocyte preparations from members of a pedigree in which ceroid lipofuscinosis is expressed as a dominant trait after the age of 30.

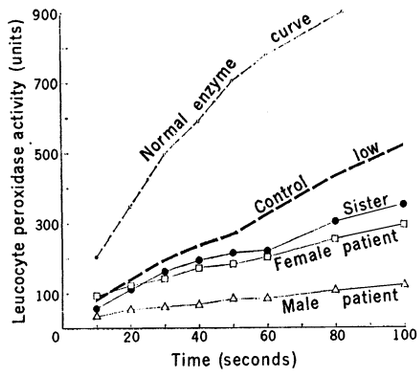

Fig. 1. Progress curves of peroxidase obtained from equivalent leukocyte samples. Enzyme was prepared from white blood cells as reported (*1*). The incubation was performed at 25°C and consisted of 0.15*M* phosphate buffer, *p*H 7.3, 0.28*M* *p*-phenylenediamine, and 1 m*M* hydrogen peroxide in a volume of 3.2 ml. Following addition of enzyme preparation derived from 5×10^4 cells, progress of the reaction was determined by recording, at 10-second intervals, the absorbance at 485 nm, which indicated appearance of the oxidized form of the hydrogen donor. Each curve is derived from a single incubation. The absorbance measurements were converted to units of enzyme activity by comparison with a standard curve prepared with horseradish peroxidase; these values were divided by the number of milligrams of total protein in each sample and plotted; patient IV/11, ●; patient IV/13, △; patient IV/15, □.

In Fig. 1 we have plotted the progress curves for equivalent samples of leukocyte enzyme from three siblings. The sibling designations have been used previously (*4*). Two of the siblings, a male of age 38 (IV/13) and a female of age 35 (IV/15), are patients with clinical manifestations of the disease, whereas the other, a female of age 41 (IV/11), is unaffected clinically. Also plotted are control data comprising the mean progress curve for 46 individuals who were neither affected with ceroid lipofuscinosis nor related to the affected kindred; the lowest values for a progress curve obtained from a control individual are also shown. The curves from the two siblings with clinical manifestations of the disease are well below that of the lowest control except at the earliest reading. The curve for the unaffected female sibling (IV/11) is below the lower control but above the curves for her two clinically affected siblings.

Further evidence of reduced capacity for removal of peroxide in the presence of a *p*-phenylenediamine cosubstrate was obtained as follows. When the enzyme reaction as described in Fig. 1 was performed in the absence of added hydrogen peroxide and the incubation

was extended to 5 minutes, a purple color indicating oxidation of the *p*-phenylenediamine hydrogen donor was noted in the three siblings with the low progress curves (Table 1). In the absence of an inflammatory response (a count of less than 9×10^3 white cells per cubic millimeter of venous blood), enzyme samples from controls failed to give any measurable color after long incubation in the absence of added hydrogen peroxide. This finding is interpreted to indicate the presence in the sample from the three siblings of increased amounts of endogenous peroxide, probably hydrogen peroxide, which is gradually reduced by the residual enzyme. It is proposed that demonstrable intracellular peroxide levels are a result of the peroxidase enzyme deficiency.

Acid phosphatase (*5*), catalase (*6*), and superoxide dismutase (*7*) were assayed in the leukocyte preparations; the activities of the three siblings did not differ from controls. Thus, the abnormality of peroxidase in the leukocyte preparations did not extend to an enzyme representative of the lysosomal hydrolases, to another hydrogen peroxide–consuming enzyme, or to an enzyme known to produce hydrogen peroxide. In azurophilic hypergranulation (*4*) in which the Giemsa stain at *p*H 6.5 was used, mild hypergranulation was found in both patients affected with the disease. No hypergranulation was observed in the unaffected sister. The deficiency in leukocyte peroxidase *p*-phenylenediamine thus could be used to correctly diagnose both patients with Kuf's disease independently of previous hypergranulation studies. However, the superiority of the peroxidase assay is shown by the detection of an abnormal enzyme in the apparent heterozygous unaffected sister (IV/11).

The peroxidase reaction studied is extremely rapid. In the normal progress curve (Fig. 1), the rate decreases after 30 seconds of incubation. If one takes the difference in readings between 10 and 30 seconds of incubation as an approximation of the true initial rate, then the samples from the two clinically affected siblings have initial rates of activity much below that of the lowest control and the sample from sibling IV/11, for which the progress curve falls below that of the lowest control, has, in fact, a normal initial rate. It is important, therefore, to examine the entire curve (10 to 100 seconds) as well as the initial rate. Thus, enzyme activity for patient IV/13 falls in the low normal range at 10 seconds, but formation of reduced substrate then neither continues at the normal rate nor reaches the full normal value. Therefore, by extending the assay time, the full extent of the peroxidase deficiency is revealed. The significance of the abnormal progress curve in sibling IV/11 is not apparent. Although she is beyond the age when other members of her family first developed symptoms of the disease, she might carry the trait. If she does, the progress curve determined from her leukocytes suggest that the enzyme abnormality may be in the stability of the protein rather than in initial catalytic activity.

Table 1. Oxidation of *p*-phenylenediamine in the absence of added hydrogen peroxide by white cell preparations. The reaction described in Fig. 1 was performed in the absence of added hydrogen peroxide and the absorbance at 485 nm was measured after 5 minutes of incubation.

Enzyme source	White cell count (cell/mm^3)	Absorbance at 485 nm
Controls	<9000	0.000
IV/11	6000	.006
IV/13	4900	.024
IV/15	6400	.054

The use of a specific hydrogen donor (*p*-phenylenediamine) is stressed because when other donors are employed, the enzyme deficiency may not be demonstrated. Others have confirmed this observation (*8*). Porphyria (*9*) is the only dominantly inherited disease in which an enzyme abnormality has been detected. Our findings suggest an enzyme abnormality in another dominantly inherited disorder with chronic progressive degeneration of the nervous system. Further studies of this pedigree and of the nature of the enzyme abnormality should shed light on an additional mechanism for expression of the biochemical phenotype of dominantly inherited diseases and should enable precise biochemical detection of individuals bearing the trait.

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Responses in Pavlovian Conditioning Studies

Wasserman (*1*) reported that in a cooled chamber, young chicks came to approach and peck a small disk whose illumination was repeatedly followed by heat lamp activation. One central conclusion he drew from these data was: "Approach and contact of conditioned stimuli does not depend on similar or compatible conditioned stimulus– and unconditioned stimulus–controlled responses. The chicks approached and pecked or snuggled with the lighted key even though the heat stimulus evoked none of these behaviors." This conclusion is important because it appears to be an exception to a generally accepted interpretation of previous studies using similar procedures (*2*): Behavior toward the conditioned stimulus has always been similar either to behavior elicited by the unconditioned stimulus or to behavior related to the motivational state associated with the unconditioned stimulus.