ately after the treatment with light, the samples were stored in complete darkness for 40 minutes. The percentage of cells in which the chloroplast had turned to face position was scored. From induction until scoring, all manipulations were conducted in green safelight (*6*).

With increasing concentrations of cytochalasin B, the response dropped to nearly zero (Fig. 1); the result is the same if the inhibitor was applied only 5 minutes before induction rather than 15 minutes before. The effective concentrations of cytochalasin and the rapid effect agree with results in other systems (*9*).

We then tested the reversibility of the inhibition. After the response was scored, the samples were rinsed with inhibitor-free medium (all manipulations in green safelight). The response induced by a second treatment with red light compares well with that of the controls for cytochalasin concentrations of 12.5 μg/ml or less. It is only after inhibition with a concentration of 50 μg/ml that the response is not restored completely (Fig. 2).

Another experiment was designed to ascertain whether photoperception or the mechanism of movement is affected by cytochalasin. Immediately after the induction, the inhibitor was replaced by control medium, and the normal response was obtained during the following dark period of 40 minutes in spite of the fact that the inhibitor was acting during the induction. This result shows that the mechanism of movement rather than the photochemical process is blocked by cytochalasin B, and confirms the reversibility of this inhibition.

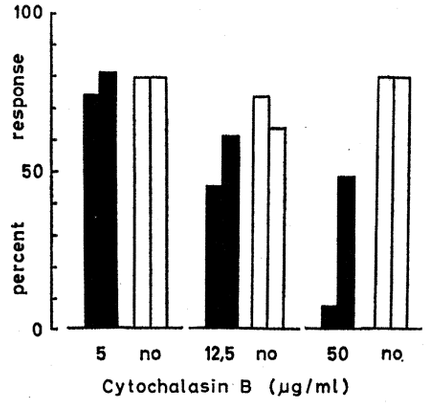

Fig. 2. Light-induced chloroplast movement with and without cytochalasin B. In each pair of black columns, the left column shows the response after the first induction under the influence of different concentrations of cytochalasin; the right column shows the response of the same sample after the inhibitor is replaced by control medium and treated again by light. White columns, controls without cytochalasin B. Ordinates are as in Fig. 1.

From these experiments, which confirm similar results of Schönbohm [in preparation, as suggested by (*3*)], we conclude that contractile protein fibrils are essential for the mechanism of chloroplast movement in *Mougeotia*.

GOTTFRIED WAGNER
WOLFGANG HAUPT, ANNELIESE LAUX
Botanisches Institut der Universität Erlangen–Nürnberg, 852 Erlangen, Germany

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10. Supported by the Deutsche Forschungsgemeinschaft.

27 December 1971

Hyperprolinemia and Prolinuria in a New Inbred Strain of Mice, PRO/Re

Abstract. *A hyperprolinemia was discovered, in a new inbred strain of mice, which was equivalent to about a sevenfold elevation above the concentration of proline in the blood of either of the original parental lines, or of 12 other inbred strains with diverse genetic constitution. In addition, mice of this PRO/Re strain exhibited a marked prolinuria, whereas the other 14 inbred strains had no proline detectable in their urine.*

Genetic aberrations that affect amino acid metabolism are documented for various organisms. Studies on mutations in *Neurospora* (*1*), for example, which block the biosynthesis of tryptophan (*2*) helped to establish the biochemical link between gene action and control of enzyme activity. Approximately 40 hereditary disorders of amino acid metabolism in humans are known, many of which are due to defective regulation of liver enzyme function (*3*, *4*); two such disorders involve the heterocyclic amino acids proline and hydroxyproline. Hyperprolinemia and hydroxyprolinemia are rare "inborn errors of metabolism" characterized by an elevation of the concentration of proline or hydroxyproline in plasma. These amino acids also accumulate in the urine. At least two variations of hyperprolinemia are known in man (*5*). Type I hyperprolinemia is characterized by a hereditary renal disease, congenital anomalies of the genitourinary tract, and mild mental retardation. Type II hyperprolinemia has been accompanied by mild mental retardation, but neither the patient nor any of his relatives had renal disease.

Recently we investigated various inbred strains and mutant stocks of mice for inherited diseases of enzyme control that affect amino acid metabolism. During these studies, one particular line (*6*), since designated PRO/Re, was observed to excrete in the urine a substance that produced a yellow spot on paper chromatograms treated with ninhydrin.

Abnormal excretion of proline in the urine of PRO mice was first detected by the resolution of amino acids in urine with paper chromatography. Fresh, untreated urine, in amounts varying from 10 to 30 μl, was spotted on Whatman No. 1 paper and developed with a water-saturated phenol solvent for about 24 hours in a descending chromatography system. The amino acids were detected by means of the ninhydrin reaction; proline produced its characteristic yellow spot. In other experiments, an isatin reagent, 0.2 percent solution of isatin in *n*-butanol acidified to 5 percent glacial acetic acid, was utilized to detect proline on either paper chromatograms or paper electrophoretigrams.

Figure 1 is a paper chromatogram on which proline, after being resolved from the urine of two PRO/Re mice, was treated with the isatin reagent to give the characteristic blue-colored product. A proline standard was present in the same chromatographic position as the substance detected as urine proline.

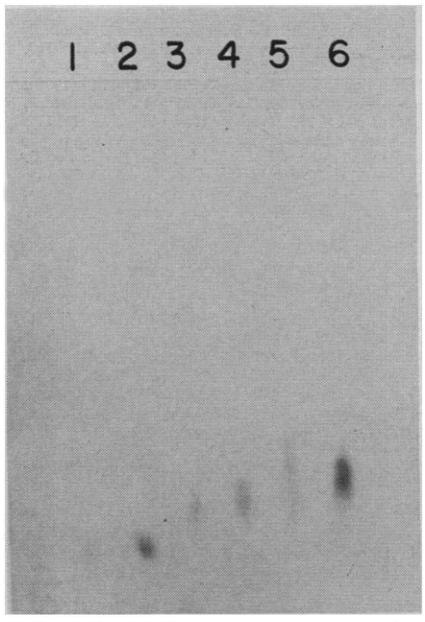

Fig. 1. Detection of urinary proline in PRO/Re mice by isatin reaction on paper chromatograms. A proline standard was run in positions 1 and 2. Untreated urine of PRO/Re mice was run in positions 3 and 5. A combination of the urine and the proline standard was added in positions 4 and 6 to determine whether the standard would reinforce the spot originally identified as proline, or whether the two would chromatograph differently, indicating that the mice were excreting some other isatin-reacting substance.

In order to further characterize this substance present in the urine of the PRO/Re mice, the amino acids were resolved by a two-dimensional system with high-voltage paper electrophoresis. Figure 2 is the electrophoretigram after reaction with ninhydrin. When a proline standard was added to the urine sample it moved to the same position as did the urinary proline. The evidence on the nature of this substance—that is, its distinctive qualitative reaction to both ninhydrin and isatin, and its identical mobility to proline in two different resolving systems—strongly suggests that the compound is indeed proline, although this is not definitive chemical proof.

We tested 21 females and 9 males for proline in urine in ages varying from 9 to 16 months. All urine samples produced a positive proline reaction to the isatin reagent. The concentration of proline in the urine of 30 mice was determined by a method similar to that described by Blackburn (*7*), and was found to be an average of 105 ± 20 μl in freshly voided urine. This corresponds approximately to 9.1 μmole per milliliter of urine.

Neither of the parental inbred strains used to develop PRO/Re (C57BL/6J and 129/ReJ mice) excrete any proline in the urine, as detected by the isatin method. In addition, no urinary proline was detected in the following 12 inbred strains: AKR/J; AU/SsJ; DBA/2J; LP/J; P/J; PL/J; RIII/2J; RF/J; SM/J; SJL/J; SWR/J; and ST/bJ. These 12 strains were selected on the basis of previous studies by Roderick *et al.* (*8*), which indicated that they may exhibit the greatest genetic variations among the many inbred strains of mice now available at the Jackson Laboratory.

The accumulation of proline in the urine of PRO/Re mice might result from either a defect in the renal reabsorption mechanism, or from an "over-flow" due to a metabolic error that possibly involved a liver enzyme. Separation of the blood amino acids by phenol solvent on paper chromatograms demonstrated that the proline concentration was elevated significantly, as compared to that in C57BL/6J and 129/ReJ mice, an indication of an "over-flow" type disorder. A 50-μl portion of blood from the orbital sinus was mixed with two volumes of a solution of 3.1 percent picric acid in 50 percent ethanol. The mixture was centrifuged, and 20-μl aliquots of the supernatant were spotted on Whatman No. 1 paper. The papers were developed, and the concentration of proline in blood was determined by the same procedure used to analyze proline in urine. Analyses of 30 PRO/Re mice demonstrated an average concentration of proline in blood of 35.3 ± 2.1 μg per 100 μl of blood, corresponding approximately to 3.1 μmole of proline per milliliter of blood. By comparison, the proline concentration in blood in the C57BL/6J and 129/ReJ mice, as well as in the 12 additional srains, was an average of about 5 μg per 100 μl of blood.

In humans with hyperprolinemia, hydroxyproline and glycine are also excreted in the urine in excessive amounts. Apparently these three amino acids share a common renal absorption mechanism in man. However, tests for hydroxyproline in the urine of the PRO/Re mice by the isatin-Ehrlich aldehyde method (*9*) were negative. In contrast

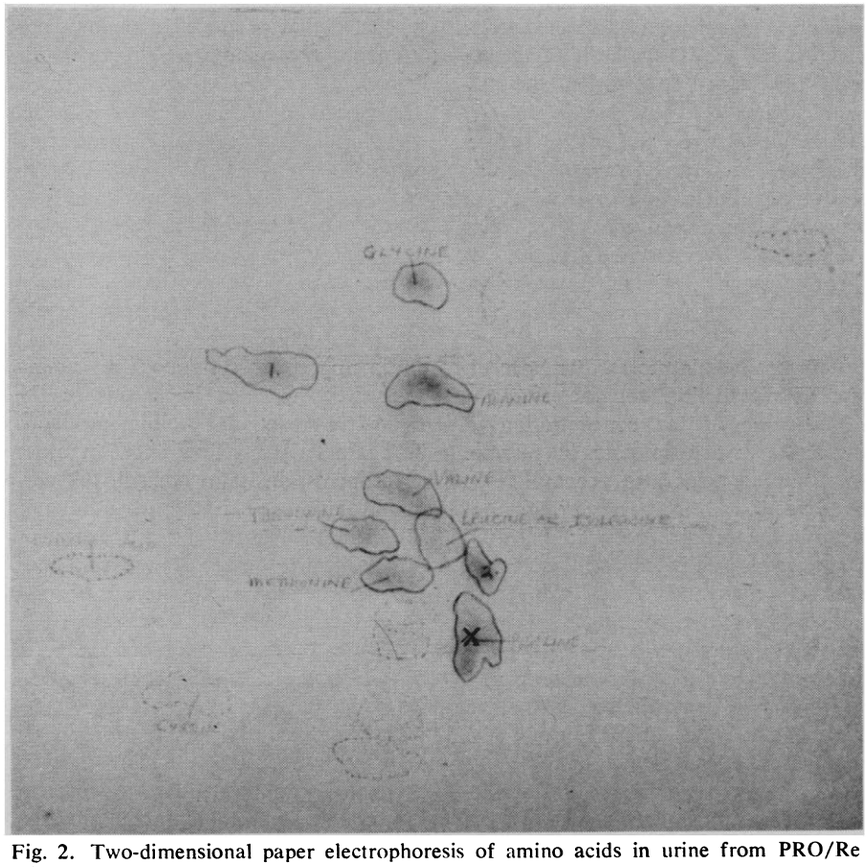

Fig. 2. Two-dimensional paper electrophoresis of amino acids in urine from PRO/Re mice.

to proline and hydroxyproline, glycine is normally excreted in mouse urine; we did not observe abnormally high amounts of glycine in the urine of PRO/Re mice. The mice do excrete a substance(s) that causes the pine shavings to exhibit a bright yellow color (*10*). This might be indicative of some type of abnormal renal transport mechanism (*11*), although the plasma appeared clear on visual examination. Proline did not stain pine shavings. When the shavings were treated with aqueous proline, proline mixed with urine from the parental lines, or proline mixed with urine from PRO/Re mice, no unusual stain was observed when compared to control shavings treated with only water or urine.

In summary, this report describes the discovery of an unusual biochemical characteristic of proline metabolism, hyperprolinemia, occurring in a new inbred strain of mice now designated the PRO/Re strain. An elevation of the blood concentration several times that in the normal animal is indicative of an "over-flow" type disorder of amino acid metabolism, perhaps similar to one of the types of hyperprolinemias known to occur in human beings. We believe that the PRO/Re strain may serve as an animal model for similar types of biochemical disorders in man and may also be useful in studies on (i) the comparative biochemistry and physiology of mammalian proline metabolism, (ii) the genetic transmission of biochemical traits, and (iii) the structural and functional organization of the genome in *Mus musculus* (*12*).

R. L. BLAKE
E. S. RUSSELL

Jackson Laboratory,
Bar Harbor, Maine 04609

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6. The PRO/Re strain was developed from crosses between 129/ReJ (A^w/A^w, c^{ch}/c^{ch}, p/p) and C57BL/6J (a/a) inbred strains. After the genotype of particular mice was determined to be a/a, c^{ch}/c^{ch}, p/p, they reproduced by brother-sister inbreeding for over 25 generations without interruption to produce a highly inbred strain now designated the PRO/Re strain. Gene symbols referred to here are as follows: a, nonagouti; A^w, white-bellied agouti; c^{ch}, chinchilla; p, pink-eyed dilution.
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9. The amino acids in urine were first resolved by paper chromatography using the water-saturated phenol solvent that effectively separates proline from hydroxyproline. The developed chromatograms were dipped in the isatin reagent, dried, and then dipped in freshly prepared Ehrlich aldehyde reagent. A red-purple spot of increasing intensity appears if hydroxyproline is present.
10. We thank Ethel Anthony who helped maintain the PRO/Re colony of mice and first noticed the staining of the pine shavings, and Margaret Singleton and Marilyn Dolliver for their technical assistance.
11. In type I hyperprolinemia in man, there is a renal defect which occurs simultaneously with a deficiency of liver proline oxidase. In type II hyperprolinemia, there is no evidence of a renal defect, and the enzyme studies suggest a deficiency of Δ^1-pyrroline-5-carboxylate dehydrogenase. The existence of renal abnormalities or of enzyme lesions in the liver of the PRO/Re mice is yet to be determined. The mode of inheritance of the hyperprolinemia is also undetermined at this time. In type I hyperprolinemia, the renal disease appears to be transmitted from generation to generation as a single unit factor. However, the hyperprolinemia is not present in either the parents or the children of affected patients, a suggestion of a more complex genetic mechanism for the expression of the metabolic abnormality.
12. During the preparation of this manuscript, we observed that both the hyperprolinemia and prolinuria can be detected in PRO/Re mice at 4 weeks of age.
13. We thank NIH for financial support (grants AM 14769-01 and CA 01074), and the Southwaite Foundation for partial support. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

14 February 1972; revised 20 March 1972

Unit Responses to Moving Visual Stimuli in the Motor Cortex of the Cat

Abstract. *Neurons in the pericruciate cortex of the cat were tested with moving visual stimuli for responses to specific properties of the visual receptive field. Specific response patterns were shown by cells of origin of the pyramidal tract as well as by other cells.*

Processing of visual information at the cortical level has been investigated, and complex receptive field properties (that is, specificity to movement, orientation, and shape) have been demonstrated for unit responses in areas 17 to 19 of the cat visual cortex (*1, 2*). Similar studies of unit responses to stationary and moving visual stimuli in the anterior middle suprasylvian association area (AMSA) of the cat have revealed movement and orientation specificity for a number of "association" neurons (*3*).

The work of Buser and Imbert (*4*) and others (*5, 6*) revealed that unit responses from the cat motor cortex can be elicited by visual, auditory, and somatosensory stimuli and hence are polysensory. The study reported here indicates that some neurons in the pericruciate "association" cortex (PCA) of the cat are also responsive to oriented and moving stimuli. Both cells of origin of the pyramidal motor system (PT units) and nonpyramidal tract units (NPT units) in the PCA display such specific visual properties.

Fourteen cats were anesthetized with chloralose (70 mg/kg), and standard surgical techniques were used to expose the PCA. The area investigated was limited to the PCA, and the region of the frontal eye fields was not invaded (*7*). The nictitating membranes were removed, and the eyes were dilated with topical methyl atropine. A concentric bipolar electrode was placed in the ipsilateral medullary pyramidal tract for identification of PT cells by antidromic stimulation (*6, 8*). After surgery the animal was placed in a special atraumatic head holder that did not interfere with the presentation of visual or auditory stimuli (*9*). Standard procedures were used in recording individual unit responses from the tungsten microelectrodes.

Units were tested for polysensory properties by presenting an auditory free-field click, a visual flash from a photostimulator, and a tactile single pulse shock to the ipsilateral forepaw. Moving white stimuli were projected on a darkened tangent screen (1 by 1 m), centered 1 m from the eyes, by