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16. L. D. Stamp, in *Africa: A Study in Tropical Development* (Wiley, New York, 1964), p. 144. In fertile southern Nigeria, an average family of 3.6 persons required the produce from $8 \times 10^3$ m$^2$ of cultivated land annually. In less fertile northern Nigeria, an average of 3.3 persons per family would be supported by $12 \times 10^3$ m$^2$. Thus, the average land requirement amounts to $3 \times 10^3$ m$^2$ per capita for shifting cultivation. The average size of family is 3.5 persons per shifting cultivator.
17. *Yearbook of Forest Products, 1963-1974* (FAO, Rome, 1976). In 1974, the world production of fuel wood and charcoal was estimated to be $1.17 \times 10^9$ m$^3$. Conversion factors of 725 kg m$^{-3}$ for density, 0.5 for the dry weight of woody material, and 0.5 for the carbon content are used. Also, a total of $1.52 \times 10^8$ metric tons of paper products were produced.
18. It is assumed that half of the paper products produced (*17*) are burned as waste. The net input is calculated on the basis of a 1 percent increase in paper demand per year.
19. Loss of soil carbon in agricultural burning and forest fires is based on the following data: a total area of $350 \times 10^{10}$ m$^2$ year$^{-1}$ (Table 1), a fractional change of 0.08 in soil carbon before and after an experimental slash burning [R. F. Watters, *Shifting Cultivation in Latin America* (FAO, Rome, 1971), p. 43], and a soil carbon content in the top 10 cm of soil of $1.5 \times 10^3$ g m$^{-2}$ estimated from *Soil Map of the World, North America* [(UNESCO, Paris, 1975), vol. 2]. An average area for new tropical forest clearing of $16 \times 10^{10}$ m$^2$ year$^{-1}$ is used for the calculation of the net input.
20. Desertification of the savanna and farmland is estimated to be $5.7 \times 10^{10}$ m$^2$ year$^{-1}$ (U.N. Conference on Desertification, Nairobi, Kenya, 1977), with a change in phytomass from about $1.1 \times 10^3$ to 0.3 g m$^{-2}$ (*7*). Thus, the decrease in $CO_2$ uptake from the atmosphere amounts to $0.05 \times 10^{15}$ g year$^{-1}$.
21. In California each city dweller requires an urban area of $10^3$ m$^2$. In Great Britian the area is $6 \times 10^2$ m$^2$ [see also N. W. Pire, *Food Resources* (Penguin, New York, 1976), p. 39], with an average of $0.8 \times 10^3$ m$^2$ per urban dweller. The world's annual urban population increase, estimated from the degree of urbanization (*15*) and population increase (*14*, pp. 331-340), is about $24 \times 10^6$ persons. The urban encroachment on farmland is occurring at a rate of $1.9 \times 10^{10}$ m$^2$ year$^{-1}$ and, on the basis of a decrease in the phytomass $CO_2$ uptake from $0.5 \times 10^3$ g m$^{-2}$ for farmland to zero for concrete paved area, a net $CO_2$ input into the atmosphere of $0.01 \times 10^{15}$ g year$^{-1}$ is estimated.
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30. I thank Dr. R. W. Stewart for helpful comments on the first draft.

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## Gyrate Atrophy of the Retina: Inborn Error of L-Ornithine:2-Oxoacid Aminotransferase


Abstract. *Cultured fibroblasts from a patient with gyrate atrophy of the retina do not convert* L-*ornithine, uniformly labeled with carbon-14, to proline. This metabolic block is caused by deficient* L-*ornithine:2-oxoacid aminotransferase activity in the patient. Her heterozygote father has intermediate activity of this enzyme.*


Gyrate atrophy of the retina is a blinding human disease characterized by progressive retinal degeneration, autosomal recessive inheritance, and an increase of ornithine in the blood and urine (*1*). We now report a specific inborn error of ornithine metabolism in this disease. A recent in vivo metabolic study (*2*) has sug-

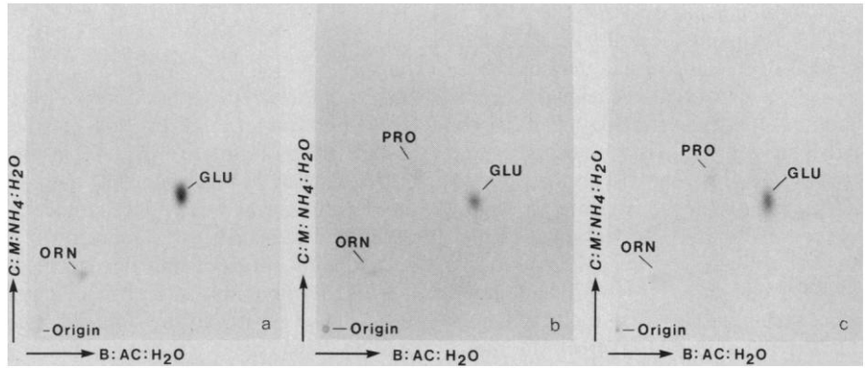


Fig. 1. Autoradiography of $^{14}$C-labeled amino acids in acid soluble cell fraction separated by thin-layer chromatography. (a) Normal; (b) heterozygote father; and (c) patient. The mother was not available. Skin fibroblasts were cultured in Dulbecco's medium with 10 percent fetal calf serum in an incubator (5 percent $CO_2$) at 37°C. All cultures were free of mycoplasma contamination (*6*). Confluent cultures were incubated for 24 hours in media containing 0.133 $\mu$Ci of L-[U-$^{14}$C]ornithine per milliliter (200 $\mu$Ci/nmole; New England Nuclear). Cells were harvested and protein was precipitated with perchloric acid. The acid-soluble cell fraction was lyophilized and redissolved in distilled water so that 1 $\mu$l contained an acid-soluble cell fraction proportional to 11 $\mu$g of cell protein (*7*). A portion (10 $\mu$l) was applied to silica gel thin-layer chromatography plates (Analtech). The first dimension was run in a chloroform, methanol, ammonium hydroxide, water system (60:60:18:9) for 1 hour and the second dimension in a butanol, acetic acid, water system (9:3:3) for 2.5 hours. The migrations of ornithine, glutamate, and proline standards were used as controls. Autoradiography was accomplished by apposing the plates to film (Kodak NoScreen) in darkness for 1 month. The ornithine, proline, and glutamate spots identified from this autoradiography and from ninhydrin or isatin stain were scraped into vials containing 10 ml of liquid scintillation fluid (Aquasol; New England Nuclear) and 0.5 ml of water. A spot equal in size to the glutamate spot adjacent to the glutamate spot was used as a control spot. Spots removed and counted produced (counts per minute): (a) Normal; ornithine, 74; proline, 91; glutamate, 382; control, 11. (b) Heterozygote father; ornithine, 65; proline, 83; glutamate, 366; control spot, 14. (c) Patient; ornithine, 179; proline, 19; glutamate, 438; control, 13. Abbreviations: *ORN*, ornithine; *PRO*, proline; *GLU*, glutamate, *C:M:NH$_4$:H$_2$O*, chloroform, methanol, ammonium hydroxide, water; and *B:AC:H$_2$O*, butanol, acetic acid, water.



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gested a deficiency of L-ornithine:2-oxoacid aminotransferase (E.C. 2.6.1.13) in gyrate atrophy.

We studied cultured fibroblasts from the 14-year-old daughter of clinically normal, nonconsanguineous parents of Finnish ancestry. The patient complained of decreased vision at age 7. She has been otherwise well, and specifically has not had seizures, as have some patients with this disease. On examination, she had central vision of 20/25 in each eye with myopic correction, impaired dark adaptation, an extinguished scotopic electroretinogram, constricted peripheral field of vision, and retinal atrophy with a scalloped margin except at the posterior pole of both eyes. Ornithine in her blood plasma was ten times that of normal individuals. Blood ammonia was normal.

Cultured skin fibroblasts were incubated with [$^{14}$C]ornithine. $^{14}$C-Labeled amino acids from the nonprotein cell fraction were detected by autoradiography of thin-layer chromatography plates (Fig. 1). The intracellular pool of ornithine became radioactive, and the label was found in proline and glutamate in cells from a normal control and from the obligate heterozygote father. In the patient's cells, the label was increased in ornithine and was decreased in proline. The glutamate label was slightly increased in the patient's cells. Since the conversion of labeled ornithine to proline is blocked, we assayed sonically disrupted cultured cells for L-ornithine:2-oxoacid aminotransferase (*3*), one of the enzymes that converts ornithine to proline through the intermediates glutamic-$\gamma$-semialdehyde and $\Delta^1$-pyrroline 5-carboxylate. L-Ornithine:2-oxoacid aminotransferase activity was absent in the patient's cells and was approximately half of normal in the heterozygote father (Fig. 2). Mixing patient cell extract with normal cell extract did not inhibit the enzyme activity, an indication that the deficient activity was not caused by a diffusible inhibitor in the patient's cells.

The fact that the heterozygote's intermediate enzyme activity appears to be sufficient to convert ornithine to proline in the cultured cells may explain why the heterozygote does not manifest clinical disease. The conversion of ornithine to glutamate in the patient's cells is unexplained by known mammalian metabolic pathways. Perhaps this experiment of nature has unmasked the presence in humans of pathways known in microorganisms. Since vitamin $B_6$ is a cofactor for L-ornithine:2-oxoacid aminotransferase, it may be possible to treat some patients having gyrate atrophy with massive doses of $B_6$ where the altered enzyme coded by the mutant gene has a lowered affinity for this cofactor, as has been done in some cases of homocystinuria (*4*). Deficient L-ornithine:2-oxoacid aminotransferase activity by itself does not explain the pathophysiology of the retinal degeneration. A deficit in the enzyme's product, proline, could impair synthesis of an essential protein such as the collagen basement membrane (Bruch's membrane) of the retinal pigment epithelium. Alternatively, precursor accumulation of ornithine could cause retinal toxicity. The absence of gyrate atrophy in other hyperornithinemia conditions argues against this latter possibility of ornithine toxicity (*1*).

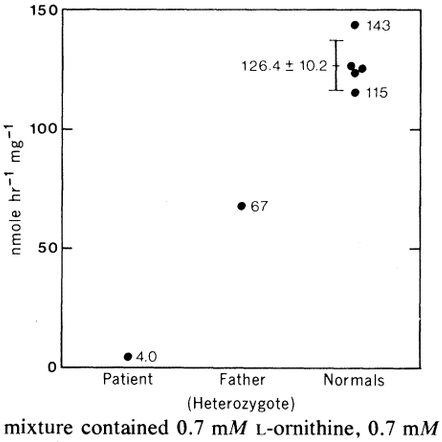


Fig. 2. Activity of L-ornithine:2-oxoacid aminotransferase in cultured fibroblasts from patient with gyrate atrophy, father (heterozygote) of patient, and five separate normal lines. Cells were cultured as described in Fig. 1 and harvested at midlog growth as observed by inverted phase-contrast microscopy. The cells were suspended in 0.1*M* phosphate (*p*H 8) buffer containing pyridoxal phosphate (4 $\mu$g/ml) and sonicated (Sonifier W185; Ultrasonics) at 75 W for three 15-second intervals in an ice bath. L-Ornithine:2-oxoacid aminotransferase was assayed as described (*8*); this assay measures the radioactivity incorporated into the dihydroquinozolinium compound formed by $\Delta^1$-pyrroline 5-carboxylate, which is the reaction product, and by *O*-aminobenzaldehyde (Sigma). The reaction mixture contained 0.7 m*M* L-ornithine, 0.7 m*M* $\alpha$-ketoglutarate, 4 $\mu$g of pyridoxal phosphate per milliliter in a 0.1*M* phosphate buffer (*p*H 8), and 0.8 $\mu$Ci of L-[U-$^{14}$C]ornithine per milliliter. Blank reaction controls were run without the sonicated cellular material. Radioactivity in the assay was at least four times that of the blank. Under the conditions used, the reaction was linear with time and with added enzyme in the sonicated material. The activity is expressed as nanomoles of $\Delta^1$-pyrroline 5-carboxylate formed per hour per milligram of protein. Each normal point represents the mean of two determinations. The heterozygote activity is the mean of four determinations (range 57 to 72). For normals, the mean ± standard deviation is noted. The activity in the heterozygote is significantly ($P < .01$) lower than the normals. The activity in the patient is ≦ 4.0 within the sensitivity limit of this assay.

Gyrate (Latin, *gyratus* turned round) was originally used to describe this disease because the margin of the retinal atrophy in the early disease stages curves as circular segments. As such, this disease is one of the few retinal degenerations that can be morphologically identified in the group of diseases known collectively as retinitis pigmentosa. This study demonstrates a specific inborn error of metabolism in one of the human retinal degenerations. Other hereditary human retinal degenerations may be caused by as yet undiscovered inborn errors of metabolism.

Intermediate activity of L-ornithine:2-oxoacid aminotransferase may identify carriers of gyrate atrophy. Detection of ornithine aminotransferase deficiency in cultured amniotic fluid cells (*5*) may permit prenatal diagnosis of this disease in the fetus. Applied carrier screening programs and prenatal diagnosis, however, should await further data on the statistical distribution of enzyme activity and development of a technically simple assay.

JAMES J. O'DONNELL
ROBERT P. SANDMAN
SUSAN R. MARTIN
*Department of Ophthalmology, U-490, University of California, San Francisco 94143*

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