

## Plasma amino-acids in hereditary retinal disease Ornithine, lysine, and taurine

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In one previous report plasma free amino-acids were found to be normal in seven patients with hereditary retinitis pigmentosa (Campbell and Tonks, 1962); however, the genetic types studied and the amino-acids measured were not specified. In more recent investigations, a reduction of plasma taurine and an increase in plasma ornithine have been demonstrated in association with retinal degenerations. A severe and selective plasma taurine deficiency was accompanied by the development of photoreceptor cell degeneration in cats which were given a taurine-free casein diet (Berson, Hayes, Rabin, Schmidt, and Watson, 1976; Hayes, Carey, and Schmidt, 1975; Hayes, Rabin, and Berson, 1975; Rabin, Hayes, and Berson, 1973; Schmidt, Berson, and Hayes, 1976). Delays in the cone electroretinogram (ERG) similar to those seen in patients with early stages of hereditary retinitis pigmentosa (Berson, Gouras, and Hoff, 1969; Berson and Howard, 1971; Berson, 1974) were detected in these taurine-depleted cats. Nondetectable ERGs with plasma ornithine levels 10 to 20 times higher than normal have been reported in patients with recessively inherited gyrate atrophy of the choroid (Simell and Takki, 1973; Takki, 1974a, b; Takki and Simell, 1974).

The present study was undertaken in order to determine plasma ornithine and taurine concentrations in patients who had different genetic types of retinitis pigmentosa and allied chorio-retinal degenerations. Precursors and metabolites of ornithine and taurine as well as levels of lysine (an analogue of ornithine) were also measured.

### Methods

Plasma free amino-acids were measured in 41 patients with representative types of hereditary retinal degenerations, six relatives of patients with gyrate atrophy of

the choroid, and 13 normal subjects. Patients included those with retinitis pigmentosa (dominant, four; sex-linked, three; autosomal recessive, 15; Usher's syndrome, two; and Laurence-Moon-Biedl (LMB) syndrome, two); juvenile macular degeneration, four; choroideremia, three; generalized choroidal sclerosis, three; and gyrate atrophy of the choroid, five. Among the patients with autosomal recessive retinitis pigmentosa, six had signs of early macular degeneration with visual acuity of less than 20/50. Two patients with juvenile macular degeneration had Stargardt's disease, and two had fundus flavimaculatus.

Patients with gyrate atrophy (Patients 1-5) and their relatives (Patients 6-11) were from four families. Patients 3 (parents 8 and 9) and 4 (sibling 10) were of Portuguese extraction; Patients 1 and 2 (parents 6 and 7) were of Swedish extraction; and Patient 5 (parent 11) was of English extraction. Parents of Patient 3 were first cousins. All patients had myopia. Refractive errors ranged from -1.00 dioptre (Patient 1) to -16.00 dioptres (Patient 2). Ophthalmoscopic examination was performed on all patients and their relatives. Full-field ERG testing was done on the patients using techniques described previously (Berson and others, 1969). For comparison, fundus examination and ERG were carried out on one child (Patient 12), aged 11 years, previously described (Shih, Efron, and Moser, 1969) and known to have hyperammonaemia, hyperornithinaemia, and homocitrullinuria. In addition to plasma amino-acids, levels of blood ammonia (NH<sub>3</sub>) and blood urea nitrogen (BUN) were measured in four of the five patients with gyrate atrophy and in five of the six relatives.

The amino-acid analyses were performed as described previously (Stein and Moore, 1954) using a Durrum 500 automatic amino-acid analyzer. Fasting blood samples were collected and centrifuged, and the plasma was immediately deproteinized with 30 mg/ml sulphosalicylic acid (Perry and Hansen, 1969). The deproteinized supernatants were stored at a temperature of -70°C.

Before amino-acid analysis, the pH of each extract was adjusted to pH 2.2 with lithium hydroxide crystals, and an aliquot of 40 µl was applied to the ion exchange chromatography column (Durrum DC-4A resin). The samples were eluted from the column by a continuous lithium buffer gradient (from 0.24 M, pH 2.89 to 1.19 M, pH 6.5) at a rate of 6.3 ml/h and at three temperatures (41°C, 45°C, and 65°C). The results obtained using this method were reproducible within a ±8 per cent range.

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**Table I** Plasma amino-acids (nmol/ml) in normal subjects, patients with retinal degenerations, and relatives of patients with gyrate atrophy

	Retinitis pigmentosa						Juvenile macular degeneration	Choroid-eremia	General choroidal sclerosis	Gyrate atrophy	Gyrate atrophy relatives
	Normal	Dominant	Sex-linked	Autosomal recessive	Usher's syndrome	LMB syndrome					
No. of subjects	13	4	3	15	2	2	4	3	3	5	6
Age range (years)	9-58	10-28	14-28	11-52	11-31	6-17	11-25	30-33	17-39	7-18	13-44
Taurine	57 ±28	40 ±8	40 ±0	44 ±8	60 —	50 —	45 ±13	50 ±17	57 ±12	46 ±9	45 ±5
Aspartic acid	12 ±4	10 ±0	13 ±6	12 ±4	—	—	—	10 ±0	10 ±0	10 ±0	8 ±4
Glutamine	863 ±232	733 ±99	723 ±331	983 ±170	950 —	—	640 ±113	955 ±245	965 ±295	706 ±308	717 ±191
Serine	119 ±17	118 ±10	117 ±6	123 ±30	100 —	70 —	110 ±18	103 ±21	113 ±46	126 ±15	93 ±21
Glutamic acid	32 ±13	28 ±5	27 ±6	39 ±18	35 —	30 —	—	43 ±23	33 ±15	24 ±9	32 ±10
Proline	181 ±66	138 ±69	207 ±74	201 ±90	130 —	110 —	128 ±17	193 ±58	170 ±92	224 ±77	270 ±117
Citrulline	32 ±8	33 ±6	30 ±10	29 ±12	15 —	10 —	48 ±13	30 ±10	27 ±6	26 ±5	270 ±5
Cysteine	120 ±22	116 ±10	126 ±12	124 ±22	90 —	90 —	80 ±34	120 ±20	100 ±20	100 ±20	90 ±10
Methionine	28 ±9	28 ±5	33 ±12	29 ±5	30 —	20 —	28 ±5	20 ±0	23 ±6	22 ±4	25 ±8
Ornithine	61 ±16	65 ±10	63 ±31	65 ±12	80 —	60 —	45 ±10	73 ±15	57 ±15	*1,026 ±277	†102 ±29
Lysine	167 ±19	158 ±5	160 ±40	175 ±47	200 —	130 —	155 ±13	193 ±40	167 ±21	†102 ±37	158 ±19
Arginine	103 ±24	95 ±24	103 ±15	101 ±28	75 —	70 —	85 ±24	107 ±46	77 ±12	88 ±8	80 ±13

The values represent the mean ± SD  
Significantly different from normal \*P < 0.0005, †P < 0.0025, ‡P < 0.005

**Results**

Table I shows that in five patients with gyrate atrophy of the choroid the mean plasma ornithine is 17 times higher than in normal subjects (Student's two-tailed *t* test, P < 0.0005). The mean plasma ornithine value for relatives of patients with gyrate atrophy is 1.7 times higher than normal (P < 0.005). Mean plasma lysine of the patients with gyrate atrophy is lower than normal (P < 0.0025) while that of the relatives of patients with gyrate atrophy is normal. All other plasma amino-acids measured in patients with gyrate atrophy, relatives of patients with gyrate atrophy, and patients with other representative types of hereditary chorio-retinal degenerations are within normal limits.

Table II shows plasma ornithine and lysine values for each patient with gyrate atrophy. The minimum increase is 10 times above normal, and the maximum is 22 times. Four out of five patients

have lower-than-normal plasma lysine. Four out of six relatives have plasma ornithine levels outside the normal range, and all have normal lysine values. Blood NH<sub>3</sub> and blood urea nitrogen levels are normal in all patients.

Ophthalmoscopic examination showed typical changes in gyrate atrophy in Patients 1-5. Atrophy appeared most extensive in the midperipheral fundus of Patients 2 and 3 and could also be seen in the posterior pole. Atrophy was visible only in the midperiphery in Patients 1 and 4. ERGs were nondetectable in Patients 2-5 and were 75 per cent below normal in amplitude in Patient 1. Relatives had no visible fundus abnormalities.

Patient 12 who was known to have hyperornithinaemia, hyperammonaemia, and homocitrullinuria (Shih and others, 1969) had a tenfold increase in plasma ornithine (650 nmol/ml) at a time when the fundus examination and ERG were normal.

**Table II** *Biochemical findings in patients with gyrate atrophy and their relatives*

		Age	Sex	Plasma ornithine nmol/ml	Plasma lysine nmol/ml	Blood NH <sub>3</sub> µg per cent	BUN mg per cent
Patients	1	7	Female	1170	110	86	17
	2	8	Female	880	60	110	16
	3	18	Female	1140	90	57	16
	4	11	Male	620	160	82	20
	5	17	Male	1320	90	—	—
Relatives	6	31	Male	150	170	77	13
	7	30	Female	70	130	84	16
	8	44	Male	110	180	60	10
	9	40	Female	90	140	60	11
	10	13	Female	110	170	55	17
	11	50	Male	80	160	—	—
Normal range				40-80	130-210	< 110	10-20

### Discussion

This investigation has confirmed previous reports (Simell and Takki, 1973; Takki, 1974a, b; Takki and Simell, 1974) that the level of plasma ornithine was much higher than normal in patients with gyrate atrophy of the choroid. Other hereditary degenerations involving the choroid and retina, in particular choroideremia and generalized choroidal sclerosis, were not associated with abnormal levels of plasma ornithine. In addition, the level of lysine, an analogue of ornithine, was lower than normal in four of five patients with gyrate atrophy of the choroid, and the mean value was significantly different from normal ( $P < 0.0025$ ). Takki (Takki, 1974a) also showed that the range of lysine values was low in comparison with the normal range in the plasma and aqueous humour of her patients, but no statistical analysis was given.

High plasma ornithine levels alone may not necessarily lead to the development of gyrate atrophy of the choroid. A threefold increase in plasma ornithine was reported in two siblings with mental retardation and normal fundi (Bickel, Feist, Müller, and Quadbeck, 1969). Patient 12 in this study with hyperornithinaemia, hyperammonaemia, and homocitrullinuria (Shih and others, 1969), had had a tenfold increase of plasma ornithine for at least nine years but was found to have a normal fundus appearance and a normal ERG. The plasma ornithine of Patient 12 was comparable with that observed in Patient 4 who was of the same age with fundus changes of gyrate atrophy and a nondetectable ERG.

It is unlikely that the high plasma ornithine in gyrate atrophy has resulted from an enzyme deficiency in the Krebs-Henseleit urea cycle (see

Fig. 1). Patients with a deficiency of ornithine transcarbamylase (OTC) have shown high levels of blood ammonia NH<sub>3</sub> and normal plasma ornithine (Efron, 1967; Levin, Abraham, Oberholzer, and Burgess, 1969). Other known enzyme defects in the cycle have usually been associated with high levels of blood ammonia (NH<sub>3</sub>) and an increase of one or more amino-acids in the cycle (Efron, 1967; Shih and Efron, 1972). In contrast, patients with gyrate atrophy have the unusual combination of normal levels of NH<sub>3</sub> and high levels of ornithine as well as normal citrulline and arginine. Therefore, the high level of ornithine is probably due to some abnormality not involving the urea cycle. Possible explanations include decreased activity of ornithine ketoacid transaminase (OKT) or ornithine decarboxylase.

A reduction of 70 to 80 per cent in the activity of OKT has been observed in two siblings with normal levels of blood NH<sub>3</sub> and a threefold increase in plasma ornithine (Bickel and others, 1969). This raises the question of whether an OKT deficiency alone could account for an increase of 10 to 20 times of ornithine in patients with gyrate atrophy. In one patient with gyrate atrophy, liver biopsy showed an OKT deficiency, but this finding awaits confirmation (Takki, personal communication). Shih found normal OKT activity (Shih and Schulman, 1970) and a 70 per cent reduction in ornithine decarboxylase activity (Shih and Mandel, 1974) in her patient (Patient 12) with a tenfold increase of plasma ornithine. Decarboxylase activity has not been measured in gyrate atrophy. If a decarboxylase had higher affinity for lysine than for ornithine, this could explain the high mean plasma ornithine and the low mean plasma lysine in patients with

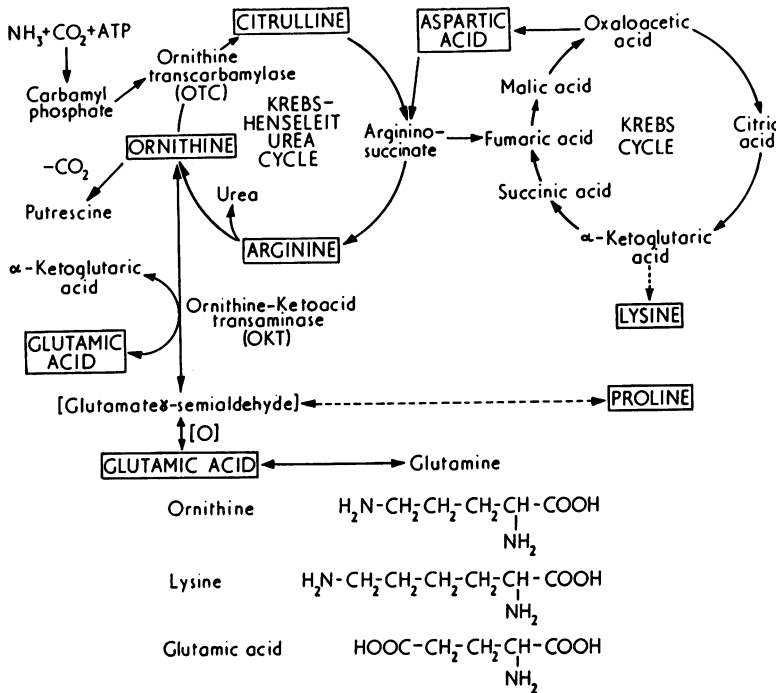


FIG. 1 Ornithone metabolism: known pathways of ornithine metabolism are illustrated. Amino-acids (capitalized) were measured in plasma of patients with hereditary chorio-retinal degenerations, relatives of patients with gyrate atrophy and normal subjects (Efron, 1967; Schepartz, 1973; Shih and Efron, 1972; White and others, 1968)

gyrate atrophy, and this could also result in altered ratios of polyamine metabolites of ornithine and lysine (putrescine and cadaverine, respectively) in the plasma and in the retina. The role, if any, of ornithine and lysine as well as their polyamine metabolites in retinal function is not known.

Several free amino-acids have been found in the normal retinae of many species. These include not only ornithine and lysine but also taurine, glutamine, glutamic acid, glycine, alanine,  $\gamma$ -amino-butyric acid, aspartic acid, and histidine (Pasantes-Morales, Klethi, Ledig, and Mandel, 1972). Evidence suggests that glycine (Ehinger and Lindberg, 1974) and  $\gamma$ -aminobutyric acid (Graham, 1974) are neurotransmitters in the retina, but the role of the other free amino-acids is not known. Taurine and ornithine have not been shown to be incorporated into proteins (White, Handler, and Smith, 1968). Taurine, present in the normal human retina (Kubiček and Dolének, 1958), exceeds by threefold the level of any other free amino-acid in the retinae of many species (Berson and others, 1976; Cohen, McDaniel, and Orr, 1973; Hayes and others, 1975a; Kennedy and Voaden, 1974; Macaione, Ruggeri, De Luca, and Tucci, 1974; Pasantes-Morales and others, 1972; Schmidt and others, 1976).

Recent studies in the cat have shown that taurine has a biological role in maintaining photoreceptor cell viability. All cats fed a taurine-free

casein diet developed a selective decrease in plasma and retinal taurine concentrations within 10 weeks and evidence of retinal degeneration was visible with the ophthalmoscope by 23 weeks in the area centralis. Initially the area centralis (comparable to the macula in man) appeared to be most affected, but taurine was similarly reduced in the central and peripheral retina, and by 1 year, cats had degeneration of most photoreceptor cells. The cat, as well as man, has a low level of liver decarboxylase activity (Jacobsen and Smith, 1968) such that conversion of cysteic acid or cysteine sulphinic acid (see Fig. 2) to taurine proceeds at a slow rate. In the cat, supplementation of the casein diet with taurine precursors (methionine and cysteine) did not prevent the development of either taurine deficiency or the retinal degeneration (Berson and others, 1976). Delayed cone b-waves in the ERGs recorded from the taurine-deficient cat were similar to those recorded from patients with early stages of retinitis pigmentosa. The present investigation shows that taurine and its precursors (Fig. 2) were normal in the plasma of patients with different genetic types of retinitis pigmentosa and patients with hereditary juvenile macular degeneration. However, a local defect in taurine uptake or metabolism in the photoreceptor cells of these patients has not been excluded.

The associations of raised levels of plasma ornithine and low plasma lysine with gyrate atrophy of

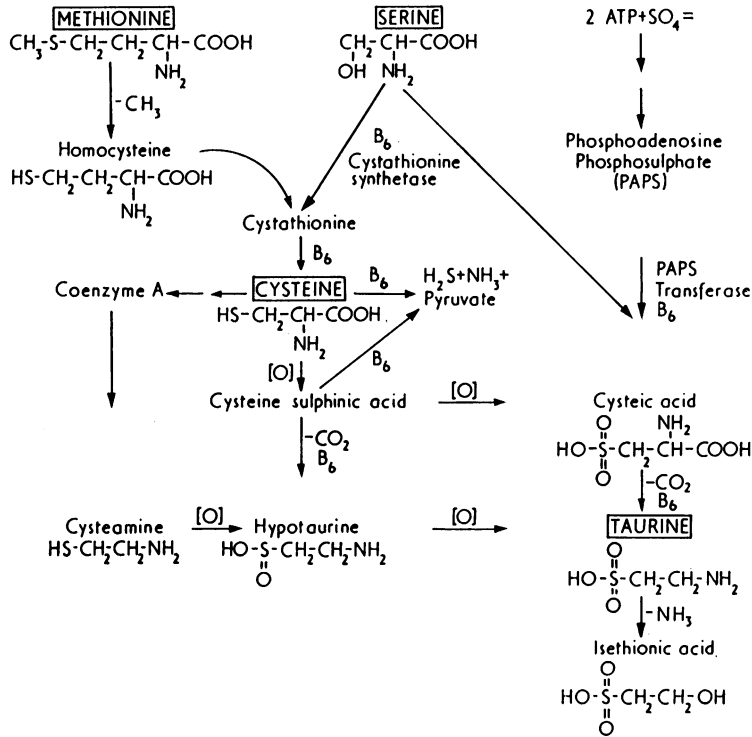


FIG. 2 *Taurine metabolism: known pathways of taurine metabolism are illustrated. Amino-acids (capitalized) were measured in plasma of patients with hereditary chorio-retinal degenerations, relatives of patients with gyrate atrophy and normal subjects (Efron, 1967; Jacobsen and Smith, 1968; Martin, Truex, Tarka, Hill, and Gorby, 1974; Roy and Trudinger, 1970)*

the choroid in man, and low plasma and retinal taurine with retinal degeneration in the cat, have suggested new areas for research into the pathogenesis of retinal degenerative diseases. Further studies are needed to quantitate the free amino-acids in the human retina and to study the metabolism of these amino-acids in normal animals and in animals with retinal degenerations.

### Summary

Plasma free amino-acids were measured in 41 patients with hereditary chorio-retinal degenerations including 26 with retinitis pigmentosa and five with gyrate atrophy of the choroid, six relatives of patients with gyrate atrophy, and 13 normal sub-

jects. Patients with gyrate atrophy had very increased levels of ornithine and slightly decreased mean lysine values. Most relatives had slightly increased ornithine. Taurine, known to be deficient in the plasma of casein-fed cats with photoreceptor degeneration, was normal in all patients. Amino-acid precursors and metabolites of ornithine and taurine were also normal in the plasma. Although the association of high ornithine and gyrate atrophy appears constant, high levels of ornithine alone do not necessarily lead to this degeneration; one patient with known hyperammonaemia, homocitrullinuria and a tenfold increase in plasma ornithine was found to have a normal fundus appearance and normal electroretinogram.

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