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Carbamylphosphate synthetase I
homocitrullinuria
hyperammonemia
hyperornithinemia
leukocytes
liver

Hyperornithinemia, Hyperammonemia, and Homocitrullinuria Associated with Decreased Carbamyl Phosphate Synthetase I Activity

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Extract

Six related subjects with severe feeding problems in infancy, hyperammonemia, hyperornithinenia, and homocitrullinuria are reported. The clinical picture includes episodic motor and mental impairment, seizures, intellectual deficits that vary from severe to very mild and, in two cases, a bleeding tendency early in life. The pedigree indicates autosomal recessive inheritance.

In a liver biopsy in one patient decreased activity of carbamyl phosphate synthetase I (CPS I, EC. 2.7.2.5) was found. Enzymatic assays on peripheral leukocytes in all six subjects confirmed the liver results. Loading studies with ornithine and citrulline were consistent with a defect early in the urea cycle. Homocitrullinuria appeared to arise from excessive synthesis from lysine, but there was no impairment of the main lysine catabolic pathway.

Large and bizarrely shaped hepatic mitochondria with curious periodic (350–400 Å) membranes were observed ultrastructurally.

Speculation

Clinical entities in which liver CPS I deficiency has been reported represent a heterogeneous group. This heterogeneity may arise because the assays currently in use do not differentiate between individual steps in the complex synthetic reaction catalyzed by this enzyme. Associated abnormalities in ornithine transport into the hepatic mitochondria in some may also contribute to the varying expression of this group of disorders. It is conceivable that some unusual periodic structures observed just inside the inner mitochondrial membranes are related to altered ornithine mitochondrial entry.

This disorder adds to the evidence from other inborn errors that there are one or more ways in which lysine metabolism and the urea cycle are specifically interrelated, but the details remain obscure.

In 1969 Shih *et al.* (23) described a patient with hyperammonemia who could be differentiated from the growing group of specific inherited hyperammonemias by the presence of markedly elevated blood ornithine and urinary homocitrulline in addition to the high levels of blood ammonia. Wright and Pollitt (28) reported briefly a second patient with these features.

During the past several years we have had the opportunity to study six related subjects with this disorder, and carry out assays of the urea cycle enzymes on peripheral leukocytes of all, and on liver biopsy in one of these. In addition, ultrastructural examination was carried out on the biopsied hepatic tissue.

It is the purpose of this communication to report on the results of the above study with special reference to clinical manifestations, localization of the metabolic block, possible mechanism of production of homocitrullinuria, and correlation of the biochemical and morphologic data.

CLINICAL SUMMARIES

CASE 1

This severely mentally retarded boy, Figure 1 IV-8, first came to our attention at the age of 7 years when increased excretion of ornithine and lysine was noted on a screening urine chromatogram. He was then found to have hyperornithinemia (350–550 μM) and postprandial hyperammonemia (168–270 μg N/100 ml).

Birth weight had been 3.6 kg and the neonatal period was normal. The infant was breast fed initially and was described as a very good baby. At the age of 6 months he weighed 7.3 kg. Weaning at that time was followed by refusal to eat and vomiting. At the age of 10 months he was hospitalized for vomiting which progressed to episodes of lethargy; these culminated in a period of semicoma lasting several days. At that time blood sugar, BUN, calcium, phosphorous, sodium, potassium, chloride, glucose tolerance, and vitamin A absorption tests were all normal. Blood pH was 7.42 and CO_2 combining power 23.5 mEq/liter. SGOT was persistently elevated around 220 units. During his stay in hospital sporadic, infrequent generalized seizures occurred for the first time and a falling hemoglobin was noted. Occult blood was present in the stool and a prolonged partial thromboplastin time and prothrobin time were found; "some response" to vitamin K was reported.

The patient had previously sat alone and was beginning to creep at 8 months, but there was a delay in development after the above episode and he did not stand until 23 months of age. An inguinal hernia repair at the age of 2 years was followed by several days of marked drowsiness and confusion.

At the present time the boy is severely retarded, not toilet trained, and has a speech impairment. His height and weight are below the 3rd percentile. His gait is abnormally wide-based and shuffling, and tendon reflexes are increased. The liver is slightly enlarged and firm; bone age is markedly delayed.

Dietary protein was restricted to 1.25 g/kg/24 hr at the age of 7 years. Blood ornithine and postprandial ammonia remained elevated although blood ammonia fell to normal after overnight fasting. In the following months the patient seemed more alert but failed to gain weight. Protein intake was, therefore, increased to 2 g/kg/24 hr. With this mild restriction, growth occurred and there was slight development of speech. To test further the usefulness of protein restriction at this late stage of development, EEG's were done before and after ingestion of 1.3 g/kg protein. There was a marked increase in irregular slow background and spike-wave

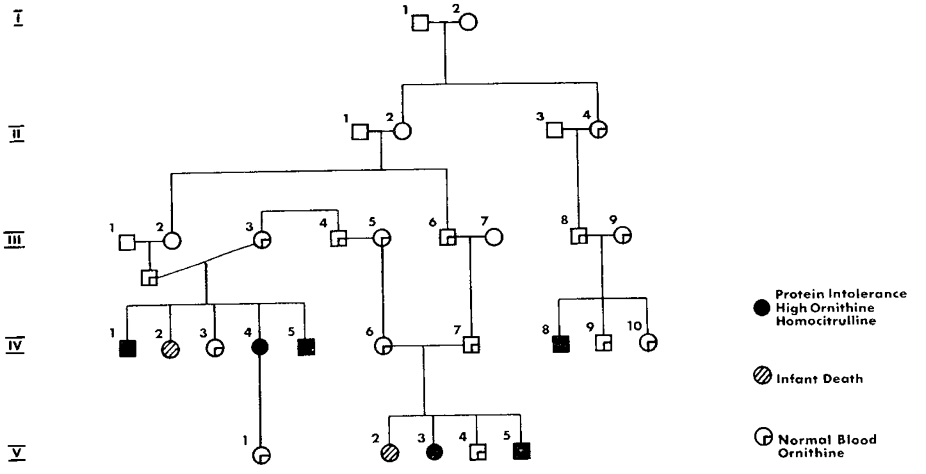

Fig. 1. Abbreviated pedigree. The infant deaths (*IV-2, V-2*) were both consistent with hyperammonemia. *Infant IV-2* was breast fed for three months and healthy until the age of 1 year. She developed vomiting and coma and died suddenly after an illness of about 6 weeks. Death was attributed to meningitis but no necropsy was performed. *Infant V-2* appeared healthy and well developed at birth. At a few days of age she had convulsive twitching and died at the age of 5 days. There was no vomiting. At autopsy the lobular structure and cell outlines of liver were noted to be obliterated but no satisfactory cause of death was found.

activity. Blood ammonia rose to 268 μg/100 ml; the patient vomited and was drowsy.

Protein intake was subsequently kept at 1.5 g/kg/24 hr. The patient showed slow improvement in development and gains in height and weight. In recent months there has been sporadic vomiting, an increase in momentary clinical seizures, and a worsening of the EEG in spite of good control of protein intake at that level. Seizures consist of momentary eye blinking, staring, and loss of awareness, and on some occasions are followed immediately by vomiting. These have responded to reduction of protein to 1.2 g/kg/24 hr and anticonvulsant medication. Attempts at increasing protein intake have resulted repeatedly in vomiting.

At the age of 9 years hepatic tissue was obtained by a needle biopsy for enzymatic assays and morphologic studies.

CASE 2

This boy, Figure 1, V-5, now 5 years of age, was normal at birth and showed good growth on low protein milk. After switching to cow's milk there were recurrent feeding difficulties and at 23 months there was a sudden development of lethargy, ataxia, spastic hemiplegia, and papilledema that prompted surgery for suspected brain tumor. This episode was followed by a period of marked obtundation with choreiform movements and focal seizures. After the recognition of hyperammonemia and institution of protein restriction there was clinical improvement but the patient is now severely mentally retarded. His clinical condition has stabilized on a protein intake of 1.5 g/kg/24 hr, but excess protein ingestion still results in irritability and ataxia. The clinical details on this patient will be published separately.

When it was discovered that *case 1* and *2* were distantly related (see below), attempts were made to examine other relatives. This resulted in the identification of four more affected individuals.

CASE 3

This 17-year-old student, Figure 1, V-3, is the sister of the subject of *case 2*. She also has shown a pronounced life-long intolerance of cow's milk. Ingestion of ordinary amounts of protein is usually followed by nausea. If vomiting occurs there is prompt recovery. She has always voluntarily avoided meat, milk, eggs, and cheese. Growth and physical development are normal. She has repeated two grades in school and has been described by her parents as slow, but recently her school performance is good and on superficial examination she appears to be near normal mentally. The parents maintain that her mental abilities have improved in the last few years. There is no history of episodic motor or mental impairment or seizures.

CASE 4

This subject is a 31-year-old man, Figure 1,IV-1, who is presently unemployed and living at his parents' home. He appears to be moderately mentally retarded and was unable to maintain his position at a sheltered workshop because of behavior problems. For the first 2 months of life he was partially breast fed and there is a history of only a moderate amount of feeding difficulty. He first walked at the age of 3 years, and other developmental milestones were similarly delayed. Seizures began at the age of 18 years. There has been only one observed that was typically grand mal in type; most are atypical and it has not been clear whether they are psychomotor seizures or manifestations of emotional problems.

CASE 5

This 22-year-old woman, Figure 1, IV-4, is a sister to the subject in *case 4*. She had a severe feeding problem in infancy and was twice hospitalized for vomiting and once for "suspected meningitis." She has repeated several grades in school but was, until recently, successfully employed in clerical work. Nausea and vomiting still recur after intake of milk or large amounts of meat, and she spontaneously has avoided milk, cheese, and other high protein foods throughout life. There is no history of episodes of motor or mental impairment or of seizures. During a recent pregnancy (her first) a protein intake of 1.0 g/kg/24 hr was maintained. The child was born at 35 weeks of gestational age with a birth weight of 2,155 g. Length, head circumference, and clinical condition were appropriate for gestational age. Blood ornithine was normal at 2 weeks of age. The child progressed well on a commercial milk formula containing 3.0 g protein/kg/24 hr and shows normal development at the age of 6 months.

CASE 6

This 19-year-old boy, Figure 1, IV-5, is a brother to the subjects in *cases 4* and *5*. He developed well while breast fed until age 8 months. Switching to cow's milk was followed by persistent vomiting and refusal to feed. Milestones were not noted to be delayed. He walked at 16 months. At the age of 2 years there was severe bleeding subsequent to a minor mouth cut sustained in a fall. Thorough hematologic investigation at another hospital resulted in a diagnosis of factor VII deficiency; he was successfully treated with transfusion. There has since been no recurrence of severe bleeding and no easy bruising. He has repeated one grade in school, but on superficial examination appears to be intellectually near normal and well developed physically. He now eats all foods except milk and notes no intolerance to protein.

Of the six subjects studied, three (*cases 1*, *2*, and *4*) are definitely mentally retarded, whereas minor degrees of intellectual loss are probably present in subjects of *cases 3* and *5*, and possibly also in *case 6*. The detailed testing necessary to substantiate that clinical impression has been declined by the families involved. Main clinical features are summarized in Table 1.

All six subjects may be traced to common ancestors three and four generations back (Fig. 1). In addition, there are two infant deaths in sibs of the six reported subjects. On the basis of the pedigree the mode of inheritance appears to be autosomal recessive.

ANALYTICAL METHODS

Amino acids were measured with the Beckman 120 C analyzer under standard conditions. Blood ammonia was determined according to the method of Seligson and Hirahara (22). Urea cycle enzymes in a needle biopsy of liver from *case 1* were assayed by the method of Brown and Cohen (2) but mercuric acetate was used for the blanks in the ornithine transcarbamylase (OTC, EC 2.1.3.3) assay (26). The same methods were adapted for CPS and OTC in leukocytes (27). Because the leukocyte assays require large blood samples (30 ml), the remaining cycle enzymes and ornithine-keto-acid transaminase (OKT, EC. 2.6.1.13) were assayed in serum (27). Serum and liver OKT were measured by the method of Shih and Schulman (24) using *O*-aminobenzaldehyde (OAB) from Sigma Chemical Co., St. Louis, and a millimolar extinction coefficient of 2.71. Frozen or heparinized plasma gave lower values; therefore, fresh samples of serum were used. Urine glutamic semialdehyde (GSA) was measured in a similar fashion by the method of Efron (4). The urine gave a troublesome mauve color leading to high blank values with trichloroacetic acid. This was reduced by substituting 5% metaphosphoric acid in the deproteinizing step. Heating with OAB was also reduced to 5 min at 60° for the same purpose. Blanks for the urine alone (no OAB) and for OAB alone (no urine) were determined separately and both were subtracted from the sample values. Blood creatinine was measured by the method of Owen *et al.* (18). Urinary orotic acid was measured by the method of Rogers and Porter (20).

RESULTS

AMINO ACIDS

Serum amino acids of the six patients under various dietary conditions are shown in Table 2. Blood ornithine is elevated three- to fivefold and is not significantly influenced by dietary manipulation. There is no correlation of ornithine concentration with degree of clinical impariment in the different patients. Blood glutamine + glutamate is increased. Blood lysine is normal when protein intake is high (*patient 2*) but is abnormally low when protein intake is reduced in all subjects.

The outstanding features of the urine amino acid pattern (Table 3) appear to vary with the stage of the illness. In the acute phase (*case 2*, Table 3, column 1) there is a marked increase in ornithine, cystine, lysine, and glutamine. The pattern, especially on paper chromatography, could be mistaken for a severe form of cystinuria unless careful attention is paid to glutamine. There is also increased homocitrulline but it is not prominent in the acute phase. In quiescent periods ornithine excretion is only slightly increased, and the pattern could be mistaken for very mild insignificant cystinuria and overlooked unless close attention is paid to the homocitrulline. Excretion of the latter is increased 3 20-fold and it serves as a good marker for the disease because it runs to vacant areas of the chromatogram in both ion exchange column and common paper partition chromatographic systems.

Table 1. *Clinical summary*

Case	Protein intolerance, vomiting	Episodic motor and mental impairment	Seizures	Intellectual deficit
1[1]	++++	++++	++	++++
2	++++	++++	++	++++
3	+++	–	–	+
4	++	+	+	++
5	+++	–	–	+
6[1]	++	–	–	+?

[1] *Case 1* also had gastrointestinal blood loss that responded to vitamin K at the age of 1 year. *Case 6* had recurrent bleeding after trivial trauma requiring transfusion at the age of 2 years.

AMMONIA

Blood ammonia also varies widely depending on the stage of the illness and on recent dietary intake. In the acute semicomatose stage (*case 2*), values observed were 155, 181, and 252 μg N/100 ml, and similar transient values in that range were observed immediately after protein intake in *cases 1* and *3*. The high postprandial values in *case 1*, *e.g.*, 270, 168, 266, repeatedly fall to normal after an overnight fast. Only semifasting (*i.e.*, 2–3 hr after a small breakfast) samples were available in *cases 4–6*. They contained 119, 111, and 80 μg ammonia N/100 ml, respectively. Two of these values exceeded the commonly regarded upper limit of 100; however, they were within the range of our observed control values, *i.e.*, mean 70, SD 27, when age and dietary conditions were similar.

ENZYMES

Observed enzyme values in liver biopsy in *case 1* (Table 4) indicate decreased CPS activity in comparison with autopsy controls. Unfortunately, in the initial assay performed 2 hr after biopsy CPS I and II (EC. 2.7.2.9), activities were not differentiated. When the same homogenate stored overnight at −15° was assayed 24 hr later, the CPS II activity was 0.35 μmol/mg protein/hr. We subsequently found that CPS II in autopsy controls was stable under these conditions. If we assume no change in the patient's CPS II his CPS I activity was 19% of the mean of autopsy controls. The fraction of total activity due to CPS II is much lower in controls (consistently about 2%) than in *case 1*. This is attributable to a sevenfold increase in CPS II activity as well as the decrease in CPS I in the patient.

The leukocyte-serum enzyme activities in all six patients are shown in Table 5. *Cases 1* and *2* has no detectable activity of total CPS on two occasions. On a third assay in *case 2* the low synthetase activity was all found to be attributable to CPS II. *Cases 3–6* showed low normal values in the combined assay. When this was repeated in *cases 4–6* with separate determination of each synthetase, the CPS II was above the control mean and CPS I was 27%, 0, and 25% of the control mean, respectively. No separate CPS I values are available in *case 3*.

ORNITHINE AND CITRULLINE

In order to confirm the location of the metabolic block L-ornithine was given to *patient 1* and three control subjects of similar age. At 6 hr the patient's ornithine level was still high, whereas it had returned to normal in control subjects (Fig. 2). Arginine and proline showed no change. The significance of the early small fall and subsequent rise in glutamate + glutamine is difficult to judge. When L-citrulline, 0.2 g/kg, was given the blood citrulline response in the patient was similar to that in control subjects (Fig. 3). Ornithine increased more than in control subjects and was still high in the patient at 6 hr. Serum proline, arginine, and glutamate + glutamine responses were similar in patient and control subjects. In this patient it appears that the urea cycle was intact from citrulline to ornithine but defective from ornithine to citrulline.

Table 2. *Serum amino acids (micromolar concentrations*

Dietary protein[1] (g/kg/24 hr)	*Ad lib.*	*Case 1*			*Case 2*			*Case 3, ad lib.*,	*Case 4, ad lib.*,	*Case 5, ad lib.*,	*Case 6, ad lib.*,	Controls (mean ± SD)
		2.0[2]	1.25	1.5	3.0[2]	1.0	1.5[2]	1.0[3]	1.2[2]	1.0[2]	1.5[2]	
Taurine	160	73	85	129	220	177	146	147	160	146	171	164 ± 38
Aspartic acid		45			96	75	111		37	52	35	44 ± 20
Threonine	85	94	140	49	125	66	136	129	104	109	91	202 ± 44
Serine	160	146		102	245	152	307	180	151	158	151	162 ± 47
Glutamine[4]	1,130	850	1,100	786	1,032	723	878	962	844	803	931	635 ± 88
Proline	121	156	203	148	397	276	348	262	158	131	186	286 ± 72
Glutamic acid[4]	84	66	112	228	226	169	285	216	147	74	97	72 ± 21
Citrulline	22	34	27	28	42	23	31	38	33	18	34	29 ± 9
Glycine	267	230	330	286	232	353	457	254	238	165	251	249 ± 49
Alanine	195	226	405	318	393	648	798	446	428	533	485	460 ± 88
α-Amino-1-butyric acid	42	38	25	11	65		12	18	21	16	24	
Valine	217	145	199	116	318	127	200	196	184	178	253	248 ± 43
Methionine	47	37	44	37	42	18	31	31	20	26	40	35 ± 6
Isoleucine	68	41	51	36	77	35	75	86	45	54	64	73 ± 21
Leucine	103	68	102	66	119	42	102	91	101	91	110	135 ± 34
Tyrosine	66	42	66	47	112	36	69	76	56	43	68	61 ± 20
Phenylalanine	77	44	73	44	73	45	85	69	46	45	55	63 ± 12
Ornithine	372	540	448	386	330	209	255	380	519	430	483	94 ± 29
Lysine	169	138	118	84	195	47	81	81	56	88	61	187 ± 36
Histidine	139	127	158	105	140	61	95	163	104	114	109	110 ± 16
Arginine	124	84	112	99	97	106	100	126	76	65	61	101 ± 22

[1] The protein intake for *patients 1* and *2* was well controlled. The values for adults, *cases 3–6*, are estimates made from their descriptions of the diets they had found suitable from their own experience.

[2] Mean of two samples.

[3] Mean of three samples.

[4] Glutamine is converted to glutamate after venipuncture to an extent that varies with the length of time until deproteinization and time of storage until analysis. Since these factors were not well controlled, the sum of glutamate and glutamine is considered to be a very good reflection of the *in vivo* sum, whereas individual values may vary. In addition the glutamine value includes a small (usually less than 10%) amount of asparagine in the Beckman analytical system used.

Table 3. *Urine amino acids (micromoles per gram of creatinine)*

	Case 1		*Case 2*			*Case 3*		*Case 4*	*Case 5*	*Case 6*	Controls ± SD
Taurine		366	[1]	280	[2]	64		112	62	60	927 ± 879
Threonine	94	123	320	320	200	122	276	106	262	125	391 ± 174
Serine	207	339	780	970	660	408	576	241	620	313	803 ± 421
Glutamine	1,510	1,099	3,740	1,340	720	1,440	1,390	590	1,420	538	1,020 ± 573
Glutamic acid	106	36	282	110		56	26			20	80 ± 39
Citrulline		<15	190		60	25	27	<15	<15	<15	<15
Glycine	2,990	2,265	1,560	4,860	1,960	1,860	2,440	618	2,580	659	1,617 ± 975
Alanine	446	644	1,300	1,200	1,120	500	756	124	412	142	450 ± 294
Valine		40	90					25	48	33	68 ± 26
Half-cystine	142	100	2,400	3,000	1,270	470	506	68	102	54	71 ± 45
Tyrosine	69	138			140	100	114	56	107	63	121 ± 196
Phenylalanine	47	79			100	54	58	32	56	29	107 ± 47
Homocitrulline	284	205	732	300	230	214	148	273	1,190	492	51 ± 38
Ornithine	82	167	8,160	2,500	620	370	387	90	97	73	14 ± 10
Lysine	117	186	5,550	1,000	580	219	438				124 ± 68
Histidine	1,280	3,115	3,250	1,650	1,320	1,450	1,350	670	2,650	1,365	1,669 ± 1,184
Arginine	4	28	380	150	16	10	22	17	10	11	37 ± 24

[1] Acutely ill, 3.0 g protein/kg/24 hr.

[2] During recovery, 1.0 g protein/kg/24 hr.

In addition to conversion to citrulline a second established path of ornithine metabolism is that to proline or glutamate via GSA. The flat serum proline response to ornithine and citrulline in both the patient and control subjects is not informative regarding the status of flux through this pathway. The excretion of GSA itself is equivocal. Its elimination in the urine in the 6-hr period after ornithine administration, 0.2 g/kg, was compared with that from a control urine sample collected from the same individual while fasting the previous day. The patient showed a decrease from 73 to 65 μmol. Three control subjects showed increases from 37 to 57, 15 to 24, and 66 to 100 μmol. It seems unlikely that the failure to show a rise in the patient could be due to OKT deficiency when his basal GSA excretion is as high as that of control subjects. Such levels might result because the patient's pathway is at capacity even under fasting conditions and cannot increase further with a load. In the five subjects studied, serum OKT assays yielded

Table 4. *Liver urea cycle enzymes (micromoles of product per milligram of liver protein per hour)*[1]

	CPS I	CPS II	OTC	ASS + L	ARG	OKT
Patient 1	0.43	0.35	44.3	0.59	115	0.123
Controls (7)	2.24 ± 0.88[2]	0.05 ± 0.03	20.4 ± 5.2	0.34 ± 0.16	76.8 ± 80.8	0.089 ± 0.040

[1] The patients values were obtained from biopsy, the controls were from necropsy on adults who had expired from a variety of diseases that did not involve the liver. CPS I: carbamyl phosphate synthetase (acetyl glutamate dependent) for urea synthesis; CPS II: carbamyl phosphate synthetase for pyrimidine synthesis (CPS I is the difference between activity in the presence of acetyl glutamate (CPS I + II) and its absence (CPS II)); OTC: ornithine transcarbamylase; ASS + L: argininosuccinate synthetase + lyase combined as a single assay; ARG: arginase; OKT: ornthine-keto acid transaminase.
[2] SD.

Table 5. *Leukocyte and serum enzymes*[1]

Case	CPS I + II[2]	CPS I[2]	CPS II[2]	OTC[2]	ASS + L[3]	ARG[3]	OKT[3]
1	0, 0			144	0.92	0.83	1.01
2	0, 0, 1.3	0	1.3	242	1.80, 3.30	3.16, 2.66	0.56
3	3.0, 4.1			228	1.60, 1.66	1.20, 1.33	
4	3.8, 3.0	1.2	1.8	86, 128	1.50, 0.75	1.33, 0.55	0.61
5	3.0, 1.8	0	1.8	53, 331	0.67, 0.42	0.33, 1.16	0.63
6	3.1, 3.2	1.1	2.1	40, 44	0.75, 1.66	2.00, 0.76	0.63
Controls	5.9 ± 1.4[4]	4.4 ± 1.4	1.5 ± 0.8	128 ± 67	1.94 ± 1.76	2.15 ± 1.66	0.88 ± 0.23
Range	3.2-9.3	1.8-7.1	0-3.3	50-275	0.42-6.60	0.60-8.30	0.62-1.38
n	14	14	14	21	29	30	10

[1] For explanation of abbreviations, see Table 4.
[2] Micromoles/gm leukocyte protein/hr.
[3] Micromoles per milliliter of serum per hour.
[4] SD.

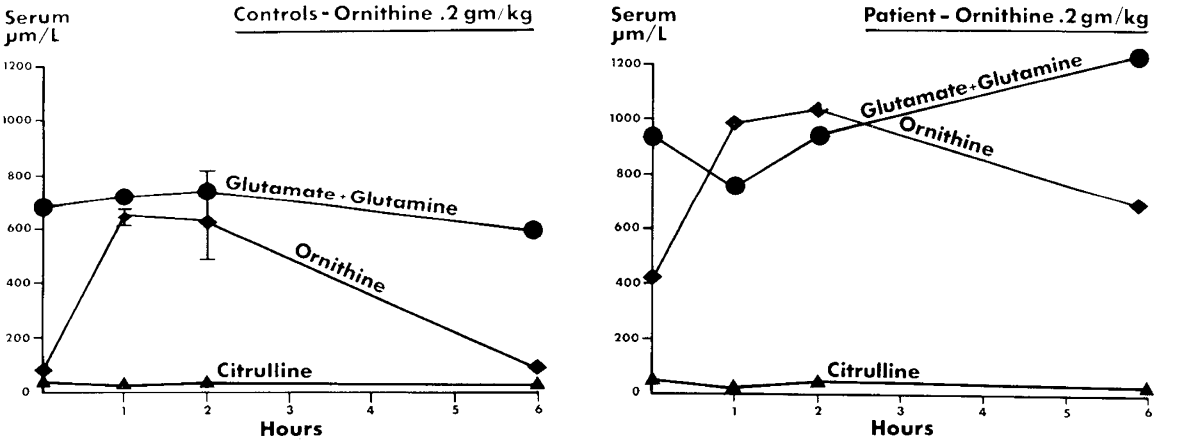

Fig. 2. On day 1, after an overnight fast, the subject voided and urine was then collected for a 6-hr period. Water only was given *ad libitum* except for 10 g glucose at 3 hr. On *day 2*, under similar conditions, L-ornithine, 0.2 g/kg, was given by stomach tube. Blood was collected at 0, 1, 2, and 6 hr and urine from 0-6 hr.

normal values (Table 5). It should be noted that the urinary GSA yield is well under 1% of the approximately 30 mmol ornithine administered and the differences between the patient and control subjects are very small. The only permissible conclusion from these results is that very little GSA accumulates if flux through the pathway is from ornithine to glutamic acid and proline. In summary, there is no evidence from these substrate studies to implicate defective ornithine metabolism outside the urea cycle.

ORIGIN OF HOMOCITRULLINE

Urinary homocitrulline is derived in part from lysine in the heating of infants' prepared formulas (8) and, to a minor extent, as a normal metabolite of lysine. In an attempt to determine the origin of the increased urinary homocitrulline in our subjects the blood and urine response to oral homocitrulline was determined in *patient 1* and three control subjects of similar age. The blood homocitrulline response at 0, 1, 3, and 6 hr was similar in the patient and control subjects after both 0.01 and 0.1 g L-homocitrulline/kg. The amount and the fractions of the homocitrulline loads excreted in the urine over 6 hr was also similar in the patient and control subjects. It appears that the homocitrullinuria does not arise from defective catabolism of homocitrulline.

LYSINE

The patient and four control subjects were also given oral L-lysine in a manner similar to the homocitrulline loads. The high 6-hr lysine value found initially was not present when the procedure was repeated (Fig. 4). It was concluded that there was no block in lysine catabolism and consequently the homocitrullinuria could not be attributed to diversion of lysine through homocitrulline because of a block in the main lysine pathway. Homocitrulline excretion in the 6 hr after lysine in *patient 1* rose to 87.4 μmol from 66.0 μmol in the comparable fasting period in the same patient. In four control patients the mean values were 15.0 μmol after lysine, 2.9 before.

Homocitrulline is normally not detectable in serum with com-

mon methods of automatic column analysis. In our subjects there is a detectable but not quantifiable peak in the homocitrulline area. It is possible that the homocitrullinuria arises from defective renal tubular reabsorption of this relatively low plasma homocitrulline. This might occur if the transport mechanism for homocitrulline is defective or if it shares a common path with ornithine or some other amino acid present in excess.

To examine the possibility of shared renal transport, the percentage reabsorption of the filtered load of several amino acids was calculated from the 3-hr blood values and the 0–3-hr urine collections in the presence and absence of the homocitrulline load. There was no effect of the homocitrulline on any other urinary amino acid measured. When ornithine and citrulline loads were given to control subjects there was excessive ornithine and citrulline excretion but no increase in urinary homocitrulline as should occur if they shared a common path.

Deficiency of a reabsorption mechanism exclusive for homocitrulline is also possible; however, the amounts of administered homocitrulline excreted in both patient and control subjects were similar in the presence of similar blood concentrations. This suggests that the renal clearance of homocitrulline is normal in our patient.

Oral lysine on three occasions produced an increase in urinary homocitrulline (Table 6). This may have arisen from bacterial flora in the gastrointestinal tract. Oyanagi *et al.* (19) described increased urinary homocitrulline excretion in two patients with a defect in intestinal lysine absorption. In our patient oral Neomycin did not decrease homocitrulline excretion, nor did it modify the increase seen with the administration of extra oral lysine (Table 6). The gastrointestinal bacteria do not appear to be implicated.

In summary, the homocitrullinuria does not arise from defective catabolism of homocitrulline, from a block in catabolism of lysine resulting in shunting through homocitrulline, or from defective renal transport of homocitrulline. By exclusion, it arises from increased homocitrulline synthesis and this is related to lysine intake.

Hepatic urea cycle enzyme activities have been found to vary to some extent directly with dietary protein intake (16, 21). All of our subjects, at the time enzyme assays were done, were restricting their protein intake in varying degrees. To determine whether protein restriction alone might cause the leukocytic CPS activities observed, four normal control subjects adopted a diet containing 0.5 g protein/kg/24 hr for 2 weeks. No effect on CPS activity was found.

MORPHOLOGIC STUDIES

On light microscopic examination there was no apparent distortion of the hepatic architecture. Often, the parenchymal cells showed fine reticulation with an increased positive reaction to the periodic acid-Schiff stain.

Ultrastructurally the most striking features were observed in mitochondria. These were very long (Fig. 5), and/or had bizarre

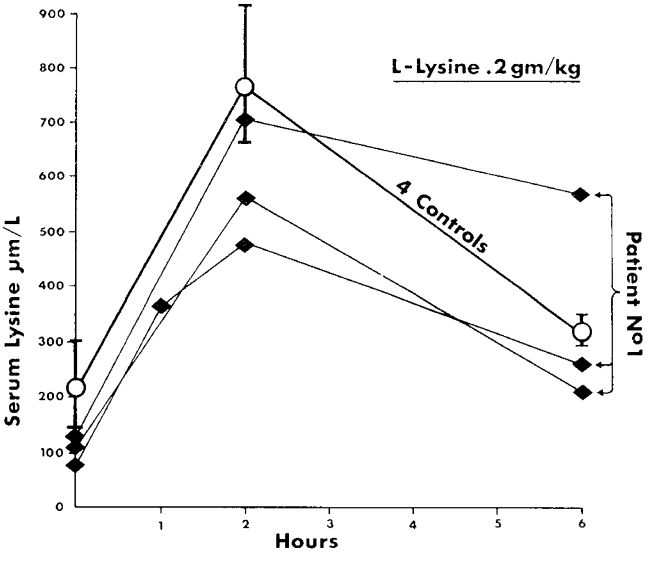

Fig. 4. Oral lysine was given as for ornithine in Figure 2.

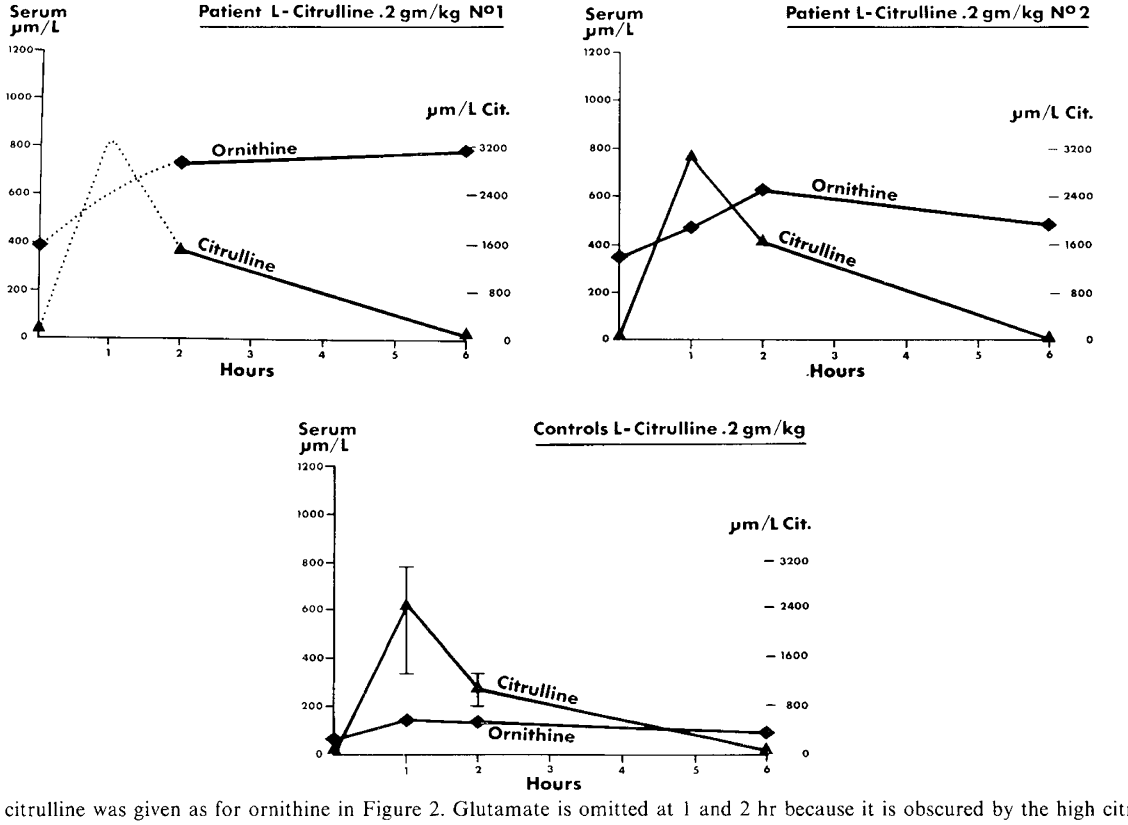

Fig. 3. Oral citrulline was given as for ornithine in Figure 2. Glutamate is omitted at 1 and 2 hr because it is obscured by the high citrulline in the column assays.

Table 6. *Dietary effect on homocitrulline excretion, patient 1*

Dietary condition	Homocitrulline, 24-hr excretion, mmol
1.5 g protein/kg/24 hr	0.266, 0.284, 0.423
1.5 g protein/kg/24 hr + 0.2 g lysine/kg	0.880, 0.673
1.5 g protein/kg/24 hr + Neomycin for 48 hr	0.650
1.5 g protein/kg/24 hr + Neomycin for 72 hr + 0.2 g lysine/kg	0.778
1.2 g protein/kg/24 hr	0.143
1.2 g protein/kg/24 hr + 0.2 g lysine/kg	0.458

shapes (Figs. 6 and 7). The extremely long forms contained what superficially resembled "crystalloid" structures, but may have represented, in essence, elongated systems of cristae or tubules. The width of the outermost of these on either side was approximately 1.5 times larger than was that of the other cristae (Fig. 5). Occasionally, mitochondrial matrix was interposed in a 'bulge'-like fashion between the mitochondrial limiting membranes and the "crystalloids" (Figs. 5 and 6). Many mitochondria showed a peculiar 350–400 Å periodicity below the level, but not at the site, of the inner limiting membrane (Fig. 7). Occasionally, small mitochondria appeared to originate within the larger forms (Fig. 8).

DISCUSSION

Previously reported patients with CPS I deficiency differ from those described here, often in different ways, although none have shown increased ornithine and homocitrulline. The patient of Gelehrter and Snodgrass (7) had hyperammonemia and severe hepatic CPS I deficiency and died at the age of 3 days, and that of Odivre *et al.* (17) showed hyperammonemia, CPS I deficiency, and signs of severe renal disease. The patient of Arashima and Matsuda (1) had severe vomiting and cerebral atrophy, but mild hyperammonemia. In patients described by Freeman *et al.* (5) and Kirkman and Kiesel (13), acidosis was prominent. In the latter, a primary defect of methylmalonate metabolism was subsequently found. The patient of Hommes *et al.* (10 had only a small reduction of CPS I activity, to 42% of a single control, and a minimal increase in blood ammonia, yet showed severe brain damage at autopsy.

This heterogeneity may, in part, be attributable to a lack of specificity in the methods of assay. Carbamyl phosphate synthesis is a complex multistep reaction involving four substrate molecules (NH_3, 2ATP, CO_2) in addition to *N*-acetylglutamate and magnesium. Several distinct heritable defects might be expected to result from derangement of different parts of this complex system. Commonly used methods that assay the overall reaction would not differentiate them.

Significantly reduced CPS I activity has been demonstrated in leukocytes in five of the six subjects tested. The combined assay in the sixth, *case 3*, shows only a slight decrease. Regretfully, no assays to separate CPS I and II were carried out and the extent of CPS I contribution to the total value is unknown. Leukocytic CPS I activity appears to reflect and parallel liver activity. In patients with two other demonstrated liver defects of urea synthesis, OTC and arginosuccinate synthetase (ASS) deficiencies, this also has been shown to be the case (27).

Failure to demonstrate any CPS II activity in *cases 1* and *2* is unexpected, especially in view of the high liver biopsy activity of CPS II observed in *case 1*. Pyrimidine synthesis, as reflected by urinary orotic acid excretion, is normal or increased in several instances including *case 1* (Table 7). We suspect that the inability to demonstrate CPS II activity does not indicate an absence of the enzyme. It may, in part, arise from the fact that the assay conditions are intended to be optimal for CPS I and do not accurately reflect CPS II activity.

The absence of either synthetase activity suggests that an inhibitor might be active. When leukocytes from *case 1* and *case 2* were mixed with those from normal control subjects the activities were additive. No evidence of the presence of an inhibitor was found.

The high ornithine concentrations found in our subjects could be due to the absence of carbamyl phosphate, ornithine's cosubstrate in citrulline synthesis. If that were the only cause, however, ornithine accumulation should also occur in OTC and in other CPS deficiencies. Such is not the case. An alternative explanation may lie in the observations of Gamble and Lehninger (6) on ornithine transport into mitochondria. They point out that the first two enzymes of the urea cycle are in the mitochondrial matrix, whereas the last three are cytoplasmic. Operation of the full cycle requires entrance of ornithine into the mitochondrion and exit of citrulline. Their studies indicate that the ornithine transport is unidirectional, respiration and permeant anion dependent, and stereospecific, and they postulate a specific electrogenic uniport carrier for ornithine. If an abnormality in ornithine transport were associated with the CPS I defect it would account for the elevated ornithine concentrations in our patients in contrast to the normal blood ornithine seen in other CPS I and OTC deficiencies. In the latter, ornithine might enter the mitochondrion where it could be diverted to glutamic semialdehyde by mitochondrial OKT.

In this respect it is of interest to speculate on the significance of the ultrastructural observations on the hepatic mitochondria. Morphologic alterations may be summarized as changes concerning the growth (shape and length) of these organelles and thus involving the matrix, and changes at the level of the limiting mitochondrial membrane (appearance of a "periodic" structure just below the inner limiting membrane). It is possible to ascribe to the presence of the periodic structure the inability of ornithine to traverse from the cytoplasmic into the intramitochondrial compartment. Thus, the interposition of this morphologically identifiable "structure" may represent a barrier to the unidirectional transport into mitochondria. Obviously, such an interpretation may be too simplistic; what is observed morphologically may represent a consequence of the disturbed ornithine transport rather than its cause.

Long and bizarre mitochondria containing "crystalloids" have been reported in certain metabolic and other disorders in man, and in some apparently normal animals (for review see Reference 9), but these reported herein do have distinctive features. This problem must await further studies on other patients with this disease and other related disorders.

These patients add to the intriguing frequency with which abnormalities of the urea cycle and lysine metabolism occur together. In addition to homocitrullinuria, the low plasma lysine, even when protein restriction is very mild, is striking in all of our subjects. The patient of Shih *et al.* also had low lysine on a protein intake of 1.5 g/kg/24 hr. No other amino acid is low, therefore nonspecific protein deficiency seems unlikely. Levine *et al.* (15) have pointed out that the homocitrullinuria seen in citrullinemia is not likely to be simply a reflection of an alternate urea cycle since it is not seen in several other specific inherited urea cycle defects. It is limited to ASS deficiency and the disease considered herein. Levin has proposed that inhibition of the main lysine pathway through saccharopine by citrulline is the mechanism of homocitrulline production (15). This seems unlikely in our patients since serum lysine response to a load is normal and blood lysine is low, not high. There appears to be no single set of circumstances that would explain these observations, either the striking citrullinemia in saccharopinuria (3), and the lysinuria of familial protein intolerance (12). There is no common cofactor requirement for these reactions. Nor can intracellular location and common transport mechanisms by themselves be involved. ASS is cytoplasmic, whereas OKT, CPS I, and the enzymes catalyzing

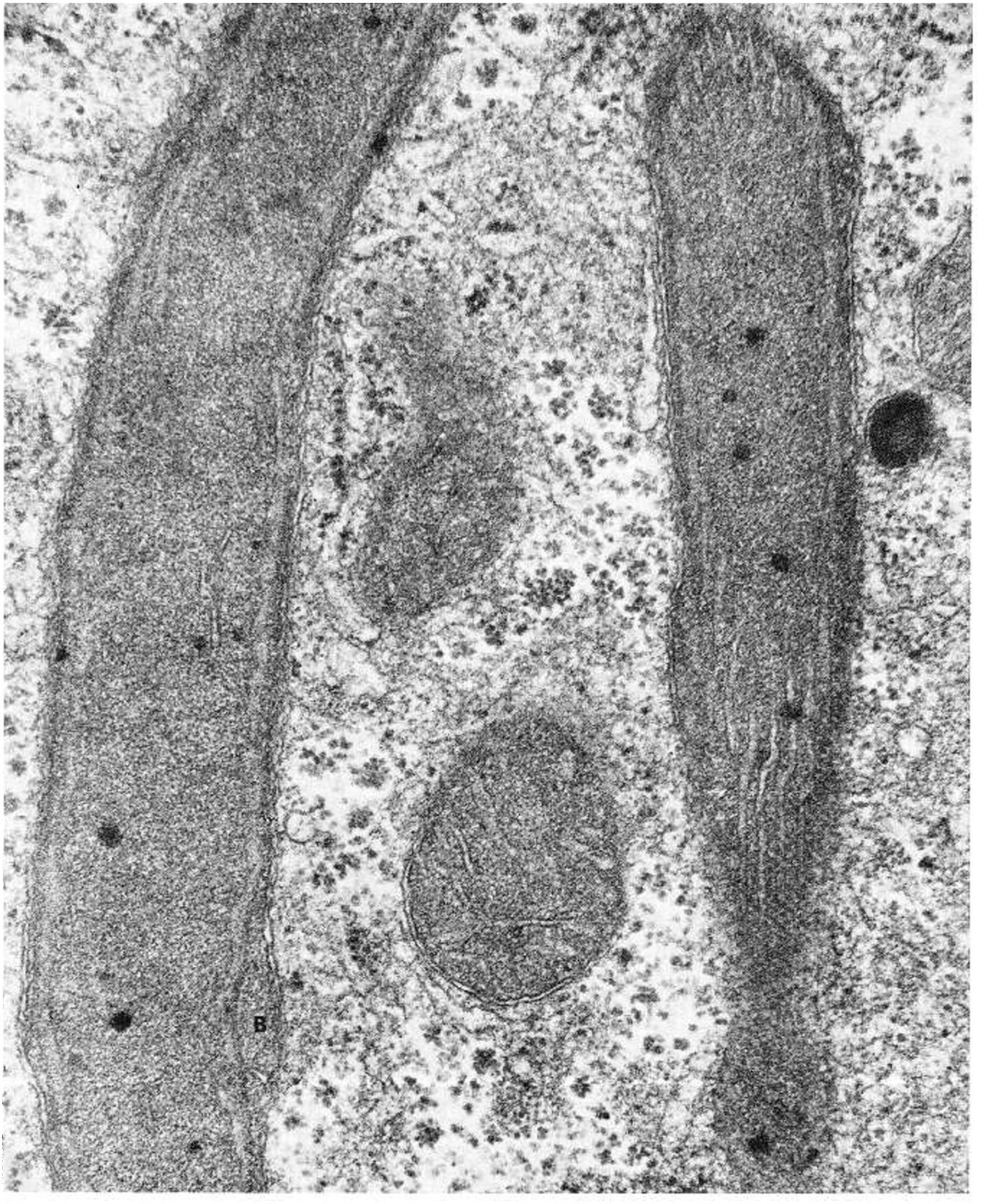

Fig. 5. Long hepatic mitochondria; only approximately one-half of a long mitochondrion is seen on the left of the photograph. Parallel tubules with diameters of cristae extend along the longitudinal axis into some distance from both ends of the organelle. The outermost tubules are wider and appear to run along the entire length of the mitochondrion. They are separated from the inner limiting mitochondrial membrane by a dark "fuzzy" line that on tangential cuts shows "periodicity" from 350-400Å. A mitochondrial "bulge" (*B*) is seen in the left lower corner of the photograph. All electronmicrographs were taken of sections cut from Epon 812-embedded hepatic tissue, prefixed in glutaraldehyde, postfixed in osmium tetroxide, and stained with uranyl acetate and lead citrate. Magnification, × 51,300.

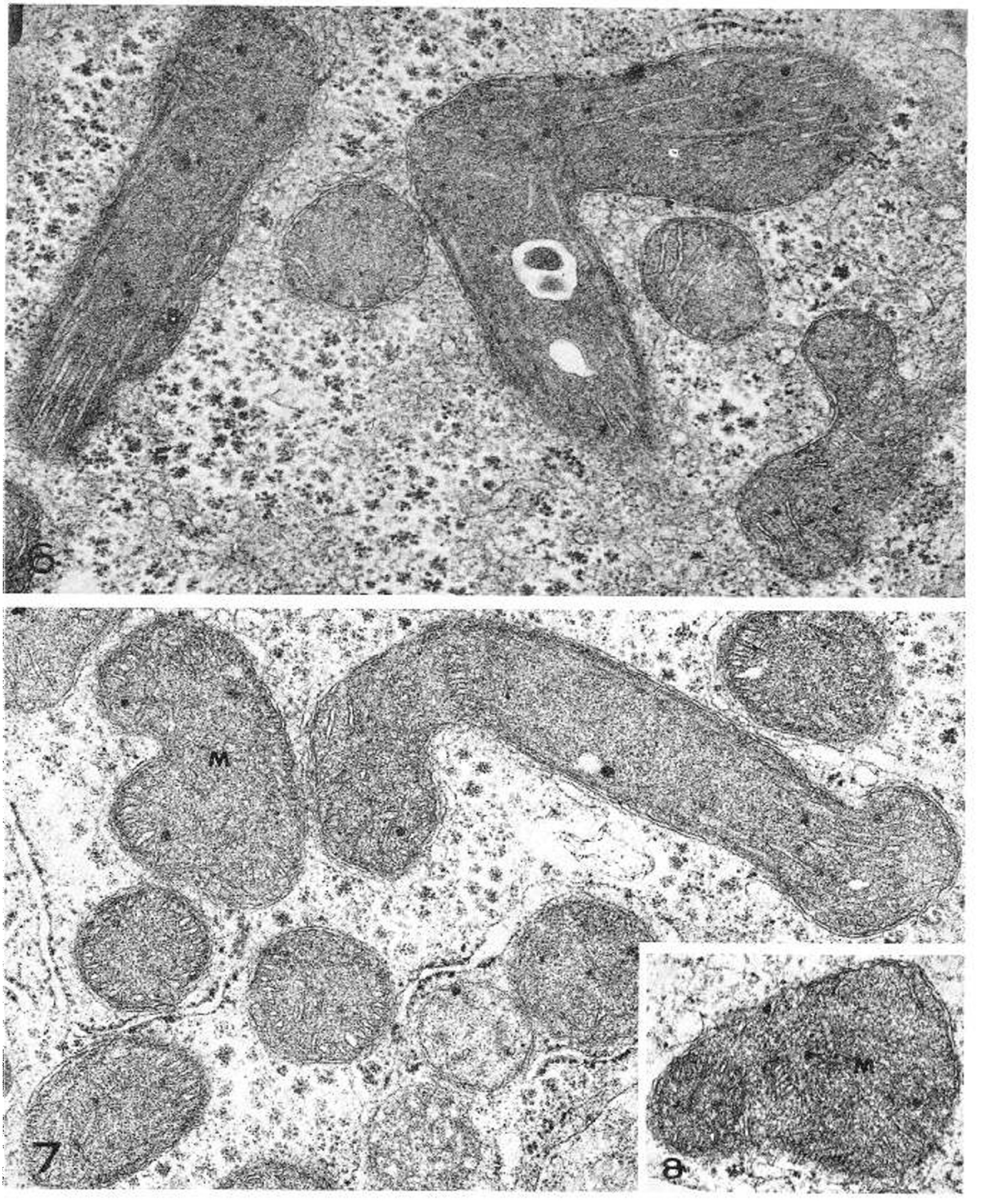

Fig. 6. Long and angulated hepatic mitochondria with degenerative changes in the latter. Features as described for Fig. 3. Note the mitochondrial bulge (*B*). Magnification, × 40,000.

Fig. 7. Abnormally sized and shaped hepatic mitochondria. Note the "periodicity" beyond the inner limiting mitochondrial membrane on the right side of the mitochondrion (*M*) on the left. Magnification, × 40,000.

Fig. 8. A small hepatic mitochondrion is seen "inside" a larger misshapen mitochondrion (*M*). Mangification, × 31,000.

Table 7. *Urinary orotic acid*[1]

Case	mg/g creatinine	mg/24 hr
1	109	34.5, 26.0, 39.2
2		2.1, 1.3, 2.0
3	23.8, 8.2	
4	9.2	
5	27.5	
6	11.7	

[1] Control subjects: adults, 8.3 ± 5.7, n = 20; 2.6 ± 1.4, n = 10; children, 10.1 ± 6.2, n = 26.

synthesis and splitting of saccharopine are mitochondrial. Mitochondrial transport of ornithine, lysine, and citrulline all appear to depend on separate mechanisms (6).

Hyperammonemia has been associated with various primary abnormalities in short chain fatty acid metabolism (11, 14). Examination of urine for organic acids by gas-liquid chromatography did not reveal any abnormal peaks for *patient 1*. Similarly, the optic fundi in our patients fail to show the retinal and choroidal atrophy described by Simell and Takki (25) in association with hyperornithinemia.

SUMMARY

Six subjects from three sibships with hyperornithinemia, homocitrullinuria, and hyperammonemia are described. Assays of liver biopsy in one showed decreased CPS I and leukocyte assays indicate a similar defect in all six. Loading studies with ornithine and citrulline are consistent with a block early in the urea cycle between ornithine and citrulline. They thus support the results of the enzymatic assays. Similar studies with lysine and homocitrulline indicate there is excessive homocitrulline biosynthesis that is related to lysine intake, but there is no evidence of a block in the main lysine catabolic pathway.

The younger more severely affected patients require protein restriction to 1.2 and 1.5 g/kg/24 hr to control hyperammonemia; hyperornithinemia remains unaffected. Adult subjects avoid large protein meals but tolerate a diet that is almost normal. The mode of inheritance of this disorder appears to be autosomal recessive.

The fine structure of liver shows the presence of large and abnormally configurated mitochondria. There is a peculiar periodic structure situated closely to the inner mitochondrial membrane, and it is possible that the presence of this may be related to the impairment of transport of ornithine into the mitochondria; this in turn may give rise to hyperornithinemia.

This disorder adds to the metabolic errors that suggest that there are close links of lysine metabolism to the urea cycle but the details are yet to be defined.

ADDENDUM

After submission of this manuscript the report of Fell *et al.* (Amer. J. Dis. Child., *127:* 753 (1974)) concerning the patient initially recorded by Wright and Pollitt (28) came to our attention. Those authors describe the beneficial effects of oral ornithine in lowering blood ammonia and attribute it to the ability of very high ornithine concentrations to overcome a block in mitochondrial ornithine transport. We subsequently observed a similar response in *case 2*. L-Ornithine was given 1 hr postprandially. The blood ammonia nitrogen was 192 μg/100 ml pre-ornithine (1 mmol/kg) and 157 at 1 hr. On a second occasion it was 197 pre-ornithine (2 mmol/kg), 137 at 1 hr, and 101 at 3 hr. On a control day the values were 109, 134, and 129 at 1, 2, and 3 hr postprandially, respectively. This surprising observation provides support for the presence of a transport defect. It suggests that the decreased CPS activity observed in our patients does not present a significant hindrance to urea synthesis from ammonia, at least when ornithine is sufficiently increased.

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