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Citrullinemia

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Summary. The pathological findings in two cases of neonatal citrullinemia are reported. One patient survived for eight months with treatment using alpha keto-analogues of essential amino acids. The untreated patient expired at eight days of age. Necropsy findings in these two cases are compared. The major histopathological changes were present in the brain and liver. They were much less prominent in the treated patient. Changes in a section of rib from the untreated patient were consistent with growth arrest and suggest than damage may occur in utero in neonatal citrullinemia.

Key words: Citrullinemia – Urea cycle – Argininosuccinic acid synthetase.

Introduction

Citrullinemia is a rare inherited disorder of metabolism, characterized by very high levels of citrulline, ammonia, and glutamine in the plasma. The defect is a deficiency of the urea cycle enzyme argininosuccinic acid synthetase (E.C. 6.3.4.5.).

Thirteen patients have been reported in the literature (McMurray et al., 1963; Van der Zee et al., 1971; Ghisolfi et al., 1972; Wick et al., 1973; Roedink et al., 1973; Danks et al., 1974; Buist et al., 1974; Scott-Emaukpor et al., 1972; Vidailhet et al., 1971; Morrow et al., 1967; McMurray et al., 1964; Thoene et al., 1977). These patients can be divided into two groups; those presenting during the neonatal period (nine patients) and those presenting after five months of age (four patients). In those presenting in the neonatal period, the disorder is usually lethal very early in life. It is the purpose of this paper to report

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on the pathological findings in two patients with the neonatal form of citrullinemia, one not previously reported, and one who survived for eight months with treatment using alpha ketoanalogues of essential amino acids (Thoene et al., 1977). Necropsy findings in these patients have been compared in an effort to determine the extent to which clinical success with keto-acid treatment is reflected morphologically. Changes in the bones of the infant who died at eight days of age suggest that damage may occur in the developing fetus in utero. Histopathological changes were found in brain and liver. They were much less prominent in the patient who had been treated. The contrast in the clinical courses of these two patients re-emphasizes the need for a low threshold of suspicion of metabolic disease in patients who present with overwhelming illness in the newborn.

Materials and Methods

Tissue obtained at autopsy was fixed in buffered formalin, dehydrated, and embedded in paraffin, and 4 μ sections were cut and stained with hematoxylin and eosin. In addition, some sections were stained with Luxol Fast Blue. Frozen sections were cut from some tissues and stained with Oil Red O.

Concentrations of amino acids in blood and urine were measured using a Beckman 120 Amino Acid Analyzer. Samples were kept at 3° C until deproteinized with 3% sulfosalicylic acid and then either analyzed immediately, or kept frozen until analysis. Concentrations of ammonia in the blood were determined by the Hyland modification of the Berthelot method. Argininosuccinate synthetase activity in fibroblasts was determined by the method of Spector et al. (1975).

Case Reports

Case 1. J.S., a female infant, was born at term to Caucasian parents in whom the paternal grandfather and maternal grandmother were from the same sibship. The family history was also remarkable for three unexplained infant deaths among first degree relatives. Gestation, labor and delivery were uneventful and the infant was discharged on the third day of life. She returned in shock the same day. She was thought to have sepsis and was treated with Gentamicin, penicillin, and oxacillin. She was transferred to University Hospital on day 3. She was ashen gray, cold, and on a respirator. There was no response to painful stimuli. She had a cardiac arrest 39 h after admission. Renal failure ensued and peritoneal dialysis was begun. She remained comtose and displayed intermittent seizure activity. Electrolyte imbalances present on admission were corrected during dialysis; however, her clinical status did not improve and she expired on the eighth day of life (Table 1). Evaluation of her metabolic status was begun on the eighth day, but the result was not available until the day following death, that the concentration of citrulline in the plasma was 3.85 mm (normal less than 0.032). Cultured fibroblasts derived from a skin biopsy demonstrated an absence of activity of argininosuccinate synthetase in the patient (Table 2). The values obtained in both parents were consistent with heterozygocity.

Case 2. J.P.N. was the term product of an uncomplicated gestation and delivery. He had unrelated European parents. He experienced the onset of lethargy progressive to coma on the second day of life. This was accompanied by culture proven staphylococcal sepsis. He developed signs of disseminated intravascular coagulation and was treated with repeated exchange transfusion. Observations that his coma appeared to lighten some with exchange transfusion, as well as the depth and persistence of coma prompted investigation for possible metabolic causes of his clinical condition. Enormous elevations in the concentrations of citrulline and ammonia in the blood provided the diagnosis of neonatal citrullinemia. The infant was maintained alive, though comatose, by the use of multiple exchange transfusions, until treatment with the mixture of alpha keto-analogues

Table 1. Clinical course of neonatal citrullinemia in Case 1

Day of life	3	4	5	6	7	8
Clinical status	Shock	Seizures	Edematous	Seizures	Unresponsive	Death
Plasma citrulline	–	–	–	–	–	3.85
Therapy	Whole blood Dopamine oxacillin penicillin Gentamicin	Phenobarbital Solu-Cortef	Plasma whole blood		Peritoneal dialysis →	
Oral intake	NPO →					

Table 2. Argininosuccinate synthetase activity in fibroblast (nmol/mg/h)

Patient J.S.	< 1.0	Patient J.P.N.	< 1.0
Mother of patient J.S.	15.1 ± 3.2	Mother of patient J.P.N.	3.4 ± 1.9
Father of patient J.S.	16.2 ± 5.3	Father of patient J.P.N.	9.6 ± 4.7
Control	21.2 ± 6.2	Control	36.0 ± 9.0

Table 3. Clinical course of neonatal citrullinemia in Case 2 treated with α-keto acids

Day of life	18	22	24	26	30	36
Blood NH_3 (μM)	302	513	240	132	85	64
Plasma citrulline (mM)	3.88	2.68	2.34	1.05	1.26	0.83
Clinical status	Opisthotonic coma seizures	Same	Same	Responsive feeding	Awake	Alert
Keta acid therapy	–	–	–	→		
Oral intake	NPO	NPO	Lytren	Lytren	0.25 g protein/ day	0.50 g protein/ day

of essential amino acids could be instituted on the 26th day of life. The infant's response to this therapy was dramatic (Table 3). Details of his course have been reported (Thoene et al., 1977).

He died at eight months of age, following a presumed viral infection. The activity of argininosuccinate synthetase in cultured fibroblasts was demonstrated to be essentially absent in this patient. Intermediate levels were found in his parents (Table 2).

Autopsy Findings

Case 1. Postmortem examination of J.S. was performed $2^1/_2$ h after death. The most striking pathologic findings were in the brain and liver.

The brain weighed 524 g (normal 429.6 g) and appeared soft and edematous. On microscopic examination of H & E and Luxol Fast Blue stained slides normal

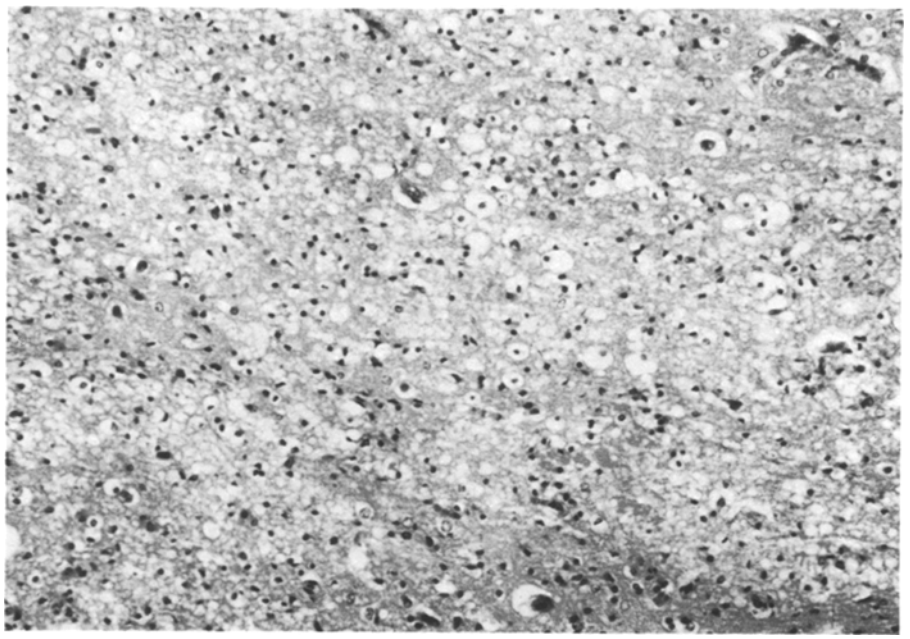

Fig. 1. Basal ganglia of Case 1 demonstrating severe edema and a spongiform appearance (H & E, ×100)

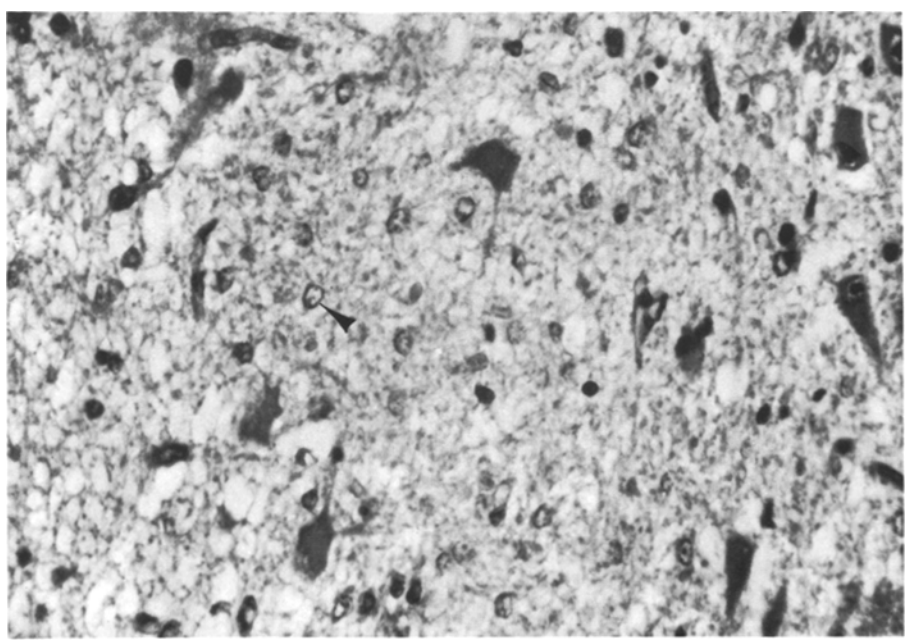

Fig. 2. Dentate nucleus of Case 1 showing a group of necrotic neurons. Also note the presence of an Alzheimer Type II glial cell (*arrow*) with a water clear nucleus (H & E, ×250)

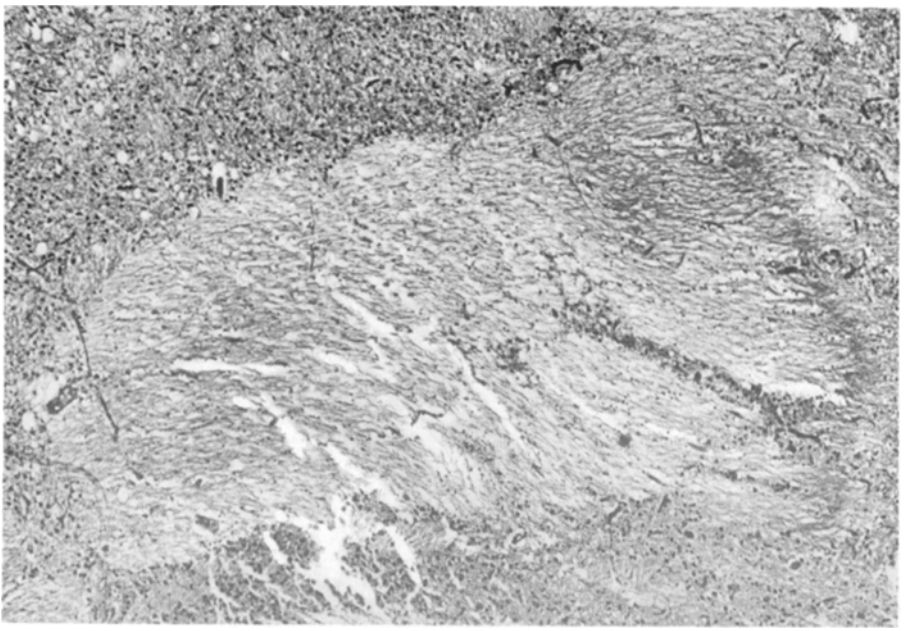

Fig. 3. Pons of Case 1 demonstrating focal necrosis (H & E, ×40)

myelination was noted. There was severe cerebral edema giving a spongiform appearance to the cerebral parenchyma (Fig. 1). Widespread focal necrosis was present, most prominently in the dentate nucleus of the cerebellum (Fig. 2) and the pons (Fig. 3). These areas of necrosis contained neurons with pyknotic nuclei and eosinophilic cytoplasm. These changes were also present focally in the cerebral cortex and basal ganglia. Alzheimer Type II glia were present.

The *liver* was normal in weight (151.5 g). The cut surface had a mottled yellow appearance, and microscopic examination revealed severe diffuse fatty change (confirmed by Oil Red O stain of frozen sections) with focal hepatocellular necrosis and hemorrhage (Fig. 4 and 5). Bile stasis was present as evidenced by bile thrombi in small bile ducts.

The *lungs* contained areas of patchy atelectasis alternating with hyperinflated alveoli. Focal hemorrhage and intraalveolar collections of fat-filled histiocytes were also present.

Disorganization of *ossifying cartilage* was present at the line of growth in a rib, characterized by some disorganization and shortening of the usual columns of hypertrophic cartilage in a line of growth (Fig. 6). This change is consistent with growth arrest and is considered to be of some weeks' duration.

Other pathologic findings included *acute renal tubular necrosis,* hydronephrosis of the right kidney with a stricture at the ureteropelvic junction, a splenic infarct, and vacuolar myopathy.

Case 2. Postmortem examination of J.P.N. was performed at 27 h after death. The most striking pathologic findings were in the brain.

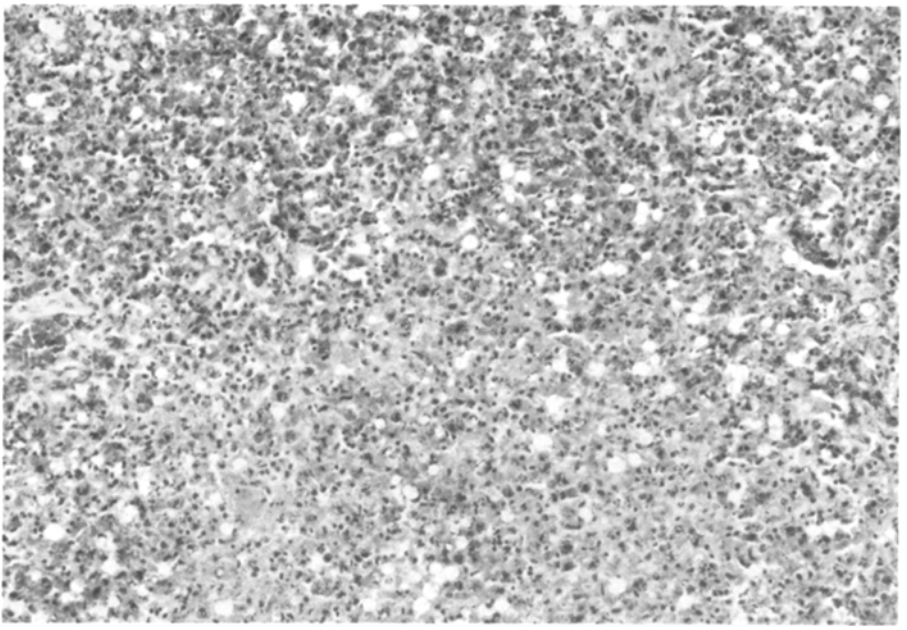

Fig. 4. Liver of Case 1 demonstrating necrosis of hepatocytes. (H & E, ×100)

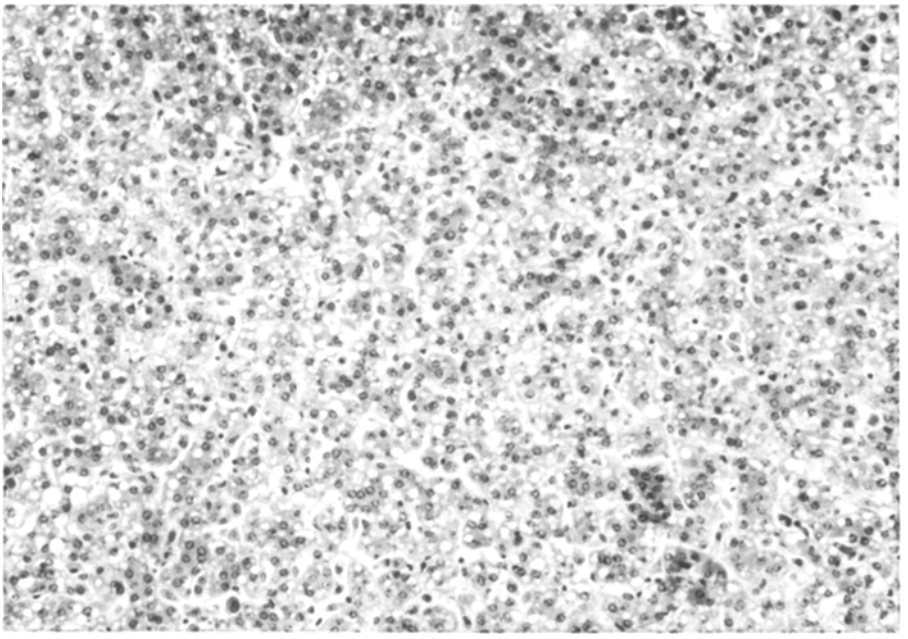

Fig. 5. Severe fatty change of the liver of Case 1 (H & E, ×100)

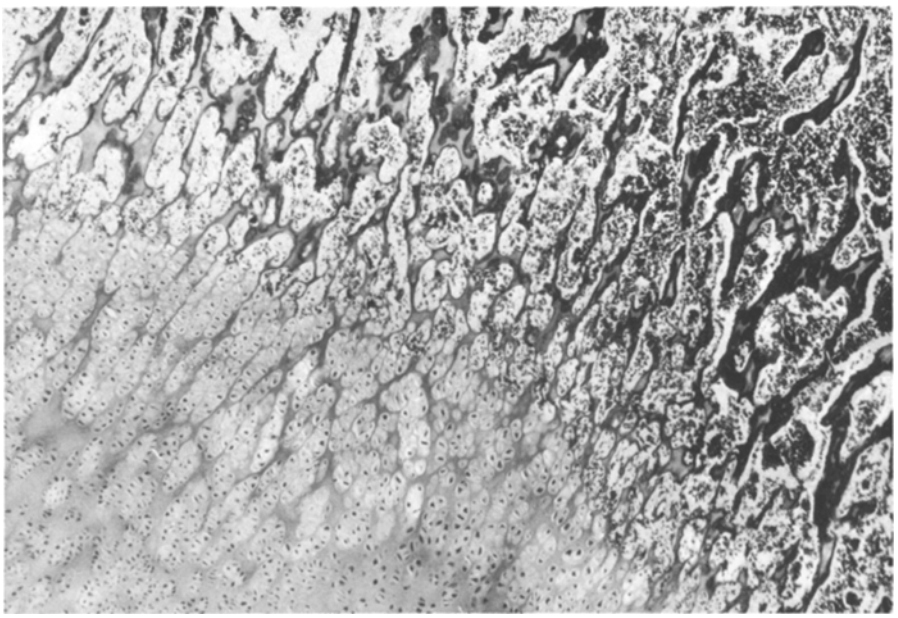

Fig. 6. Disorganization at the line of growth of a rib in Case 1 (H & E, ×40)

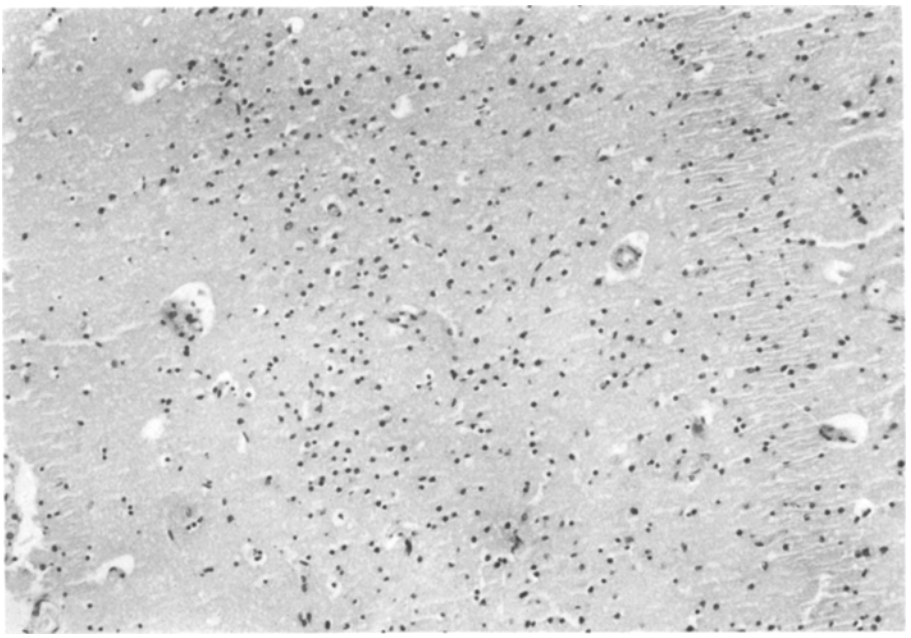

Fig. 7. Laminar neuronal drop-out and gliosis of the cortex of Case 2 (H & E, ×100)

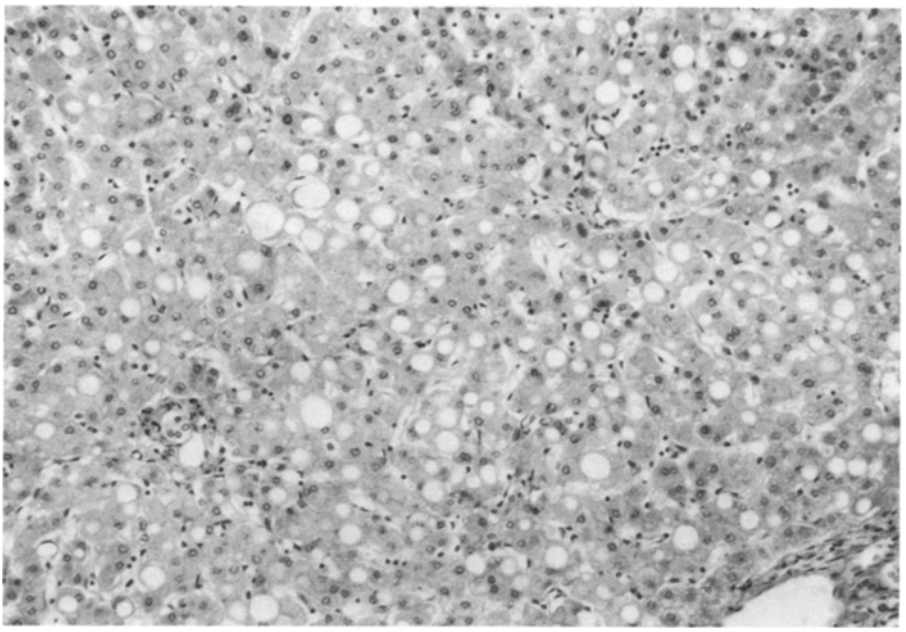

Fig. 8. Moderate fatty change of the liver of Case 2 (H & E, × 100)

The *brain* weighed 725 g (normal for age 714 g). The external surface and coronal sections were unremarkable. Microscopic examination revealed marked laminar neuronal loss with accompanying gliosis in the cerebral cortex (Fig. 7). In addition, there was focal acute necrosis, as evidenced by neurons with pyknotic nuclei, and eosinophilic cytoplasm. Luxol Fast Blue staining revealed normal myelination. No Alzheimer Type II glial cells were present. Sections from the cerebellum demonstrated evidence of recent necrosis in the dentate nucleus.

The *liver* weighed 350 g (normal for age 254), and was grossly unremarkable. Histologic examination revealed only moderate diffuse fatty metamorphosis (Fig. 8). There was no evidence of hepatocellular necrosis.

The *lungs* were heavy (combined weight 136 g, normal for age 97 g), and congested. Microscopic examination demonstrated severe congestion and edema of the intralobular and alveolar septa with some desquamation of pneumocytes.

Other pathologic findings were confined to bone and kidney. Microscopic examination of a rib demonstrated the bony cortex to be somewhat dysplastic and to contain very young bone. Acute *renal tubular necrosis* and multiple minute calcifications in the renal tubules were present.

Discussion

These two cases provided an opportunity to observe the pathologic findings in untreated neonatal citrullinemia and to contrast them with those seen in

a child with citrullinemia who dies following eight months of treatment with alpha ketoacids. In the untreated infant, the liver contained focal areas of necrosis in addition to severe fatty infiltration while the liver of the treated infant showed rather less severe fatty change without evidence of hepatocellular necrosis. Hepatocellular necrosis has been reported to be present in only two of five babies who died of neonatal citrullinemia (Van der Zee et al., 1971; Ghisolfi et al., 1972; Wick et al., 1973; Roerdink et al., 1973); hence the absence of necrosis in our second patient cannot with assurance be attributed to treatment. It is nevertheless provocative that the histology of liver of the treated patient looked much better.

The neuropathologic findings in the untreated patient were similar to those previously reported (Van der Zee et al., 1971; Wick et al., 1973; Roerdink et al., 1973). There was widespread focal necrosis and severe cerebral edema, with a spongy appearance. In Case 2, there was, in addition to recent focal necrosis, evidence of previous damage, consisting of laminar neuronal loss and gliosis. This picture was consistent with the patient's clinical course. He had an acute insult prior to treatment with keto-acids that could have led to the laminar neuronal necrosis and a terminal catabolic state resulting in acute necrosis. The acute neuropathologic findings have been previously described in other cases of neonatal citrullinemia (Wick et al., 1973). These findings included focal necrosis and severe cerebral edema with severe focal involvement of the dentate nucleus and pons. Similar changes have been reported in other infants with hyperammonemia due to defects in the urea cycle (Hopkins et al., 1975; Ebels, 1972; Baumgartner et al., 1968). These pathologic changes in the brain have been attributed to the toxic effects of ammonia. Alzheimer Type II glial cells have been associated with elevated levels of ammonia (Cavanagh and Kyu, 1971), and were present in our first patient. However, other than for the presence of these cells, it is impossible to differentiate the lesions observed from those caused by anoxia.

The disorganization of the line of growth found in a section of bone is of interest in a child who died at eight days of age. The changes are consistent with growth arrest. It is possible that they occurred during the child's eight days of extra-uterine life, but it also raises the possibility that some of the effects of citrullinemia may be present prior to birth. Intra-uterine effects of a urea cycle defect have previously been suggested in argininosuccinic aciduria on the basis of decreased myelination (Baumgartner et al., 1968). Since in utero diagnosis of citrullinemia is possible, treatment of the mother with alpha keto-acids might be considered in patients in whom abortion is not desired.

Survival of a patient with the neonatal type of citrullinemia to eight months of age is unprecedented. It effectively demonstrates the utility of the alpha keto-acid treatment in this disorder. The availability of a treatment and comparison with outcome of Case 1 strongly emphasize the need for a high index of suspicion in the newborn period to permit a timely diagnosis. The data obtained in both families indicate that heterozygosity can be established by the assay of the activity of argininosuccinic acid synthetase in cultured fibroblasts.

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References

Baumgartner, R., Scheidegger, S., Stalder, G., Hottinger, A.: Argininbernsteinsaure-Krankheit des Neugeborenen mit letalem Verlauf. Helvet. Paediatr. Acta **1**, 77–106 (1968)

Buist, N.R.M., Kennaway, N.G., Hepburn, C.A., Strandholm, J.J., Ramberg, D.A.: Citrullinemia: Investigation and treatment over a four year period. J. Pediatr. **85**, 208–214 (1974)

Cavanagh, J.B., Kyu, M.H.: Type II Alzheimer change experimentally produced in astrocytes in the rat. J. Neurol. Sci. **12**, 63–75 (1971)

Danks, D.M., Tippett, P., Zenter, G.: Severe neonatal citrullinemia. Arch. Dis. Child **49**, 579–581 (1974)

Ebels, E.J.: Neuropathological observations in a patient with carbomylphosphate-synthetase deficiency and in two sibs. Arch. Dis. Child **47**, 47–51 (1972)

Ghisolfi, J., Augier, D., Martinez, J., Barthe, P.H., Andrien, P., Besse, P., Regnier, C.: Forme néo-natale de citrullinémie à évolution mortelle rapide. Pediatrie **27**, 55–59 (1972)

Hopkins, I.J., Connelly, J.F., Dawson, A.G., Hird, F.J.R., Maddison, T.G.: Hyperammonaemia due to ornithine transcarbamylase deficiency. Arch. Dis. Child **44**, 143–148 (1969)

McMurray, W.C., Rathbun, J.C., Mohyuddin, F., Koegler, S.J.: Citrullinuria. Pediatr. **32**, 347–357 (1963)

McMurray, W.C., Mohyuddin, F., Bayer, S.M., Rathbun, J.C.: Citrullinuria, a disorder of amino acid metabolism associated with mental retardation. Proceedings of the International Copenhagen Congress on the Study of Mental Retardation. Copenhagen, Denmark, p. 117, August 7–14 (1964)

Morrow, G., Barness, L.A., Efron, M.L.: Citrullinemia with defective urea production. Pediatr. **40**, 565–574 (1967)

Roerdink, F.H., Gouw, W.L.M., Okken, A., Van der Blij, J.F., Luit-de Haan, G., Hommes, F.A.: Citrullinemia, report of a case, with studies of antenatal diagnosis. Pediat. Res. **7**, 863–869 (1973)

Scott-Emaukpor, A., Higgins, J.V., Kohrman, A.F.: Citrullinemia: A new case, with implications concerning adaptation to defective urea synthesis. Pediatr. Res. **6**, 626–633 (1972)

Spector, E.B., Lockridge, O., Bloom, A.D.: Citrulline metabolism in normal and citrullinemic human lymphocyte lines. Biochem. Genet. **13**, 471–485 (1975)

Thoene, J.G., Batshaw, M., Spector, E., Kulovich, S., Brusilow, S., Walser, M., Nyhan, W.: Neonatal citrullinemia: Treatment with keto-analogues of essential amino acids. J. Pediatr. **90**, 218–224 (1977)

Van der Zee, S.P.M., Trisbells, J.M.F., Monnens, L.A.H., Hommes, F.A., Schretlen, E.D.A.M.: Citrullinemia with rapidly fatal course. Arch. Dis. Child **46**, 847–851 (1971)

Vidailhet, M., Levin, B., Dautrevaux, M., Paysant, P., Gelot, S., Badonnel, Y., Pierson, M., Neimann, N.: Citrullinémie. Arch. Franc Ped. **28**, 521–532 (1971)

Wick, H., Bachmann, C., Baumgartner, R., Brechbuhler, T., Colombo, J., Wiesmann, P., Mihatsch, M.J., Ohnacker, H.: Varients of citrullinemia. Arch. Dis. Child **48**, 636–641 (1973)

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