Spongy degeneration of the white matter of the central nervous system associated with hyperglycinurina

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SYNOPSIS Five infants from two families with the clinical features of hyperglycinaemia and hyperglycinurina are described. In four of these cases spongy degeneration of the central nervous system is associated with lipid-filled glial cells and retarded myelination. The origin of these changes is discussed and the relationship of the lesions to the metabolic defect is reviewed. The importance of such cases in the understanding of the normal metabolism of the nervous system is stressed.

The clinical syndrome of weak cry, poor sucking reflex, vomiting, irregular, laboured breathing, drowsiness, hypotonia and flaccidity associated with a metabolic acidosis, ketonuria, and hyperglycinaemia and hyperglycinurina was first described by Childs, Nyhan, Borden, Bard, and Cooke (1961). Since this report further cases have been reported from France (Freycon, 1961), Japan (Tada, Yoshida, Morikawa, Minakawa, Wada, Ando, and Shimura, 1963), the United States (Nyhan, Chisholm, and Edwards 1963; Cochrane, Scriver, and Krause, 1963), the Netherlands (Visser, Veenstra, and Pik, 1964), and Germany (Schreier and Müller, 1964).

The majority of cases are fatal within three months of birth though the first case reported by Childs et al. (1961) was discovered when the patient was 3 years old. Necropsy of fatal cases has not revealed any characteristic lesion though Donohue (1967) has described severe spongy degeneration of the central nervous system in this condition.

Five infants from two families are described with the clinical features indicated above. Three of these showed raised cerebrospinal fluid or urinary glycine levels. In each of the four cases where necropsy material was available, severe spongy degeneration of the central nervous system was demonstrated. Frozen sections from three of these cases showed numerous lipid-filled glial cells which were not related to demyelination.

CASE REPORTS

The clinical features are summarized in Table I.

Table I

<table>
<thead>
<tr>
<th>Pregnancy Complications</th>
<th>Parity</th>
<th>Gestation (weeks by dates)</th>
<th>Baby (at birth)</th>
<th>Length (cm)</th>
<th>Head circumference (cm)</th>
<th>Sex</th>
<th>Clinical features</th>
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FAMILY W The mother was 22 years of age at her first pregnancy, which was uneventful, and was delivered following a normal labour (case 1). The second pregnancy was also uneventful and delivery was normal (case 2). Subsequently she has produced a third unaffected infant. She has had no miscarriages.

Case 1 (C.W.) At birth he cried well but failed to feed adequately from the breast. He became progressively more drowsy with vomiting and slight jaundice. On the fourth day he was admitted to this hospital unconscious and moribund with shallow gruntng respiration. He was...
limp, jaundiced, peripherally cyanosed, and hypothermic (temperature 92°F). Lumbar puncture produced a yellow fluid containing 432 red cells per c mm and 8 white cells per cmm. Culture was negative after 48 hours incubation. His progress was unsatisfactory and respiratory failure led to death when aged 109 hours.

No investigations were made into the amino acid content of the urine or blood.

The body was that of a slightly jaundiced male with no external abnormalities.

The left lung weighed 22 g (normal 18 g), the right 19 g (normal 21 g). Both were poorly aerated, pinkish-brown and the lower lobes were firm and collapsed. Slicing showed marked congestion. The treachea and bronchi contained viscid mucus and the mucosa was congested.

Histological examination confirmed the poor aeration. Masses of intrabronchial and intraalveolar squames were present in all lobes, and focal areas of intrapulmonary haemorrhage were associated with vascular congestion. A scanty cellular exudate, mainly macrophages but with occasional polymorphs, affected all lobes.

The heart weighed 23 g (normal 17 g) and was unremarkable. Histology of the myocardium showed striking vacuolation of the muscle fibres. The liver, 106 g (normal 78 g), was slightly enlarged. Microscopy showed marked fatty change and excess iron present, mainly in the Kupfer cells but also within the liver cells. The spleen, 18 g (normal 9 g), was enlarged and on histology contained excess iron. The adrenals were of normal size and weight. Histological examination showed a medullary neuroblastoma extending through the remnants of the foetal cortex to involve the adult cortex in one gland.

The brain weighed 411 g (normal 335 g). The meninges were congested. Slicing showed no macroscopic abnormality. The histological appearances are described below.

Case 2 (C.W.) Though in good condition at birth the baby cried little and soon became drowsy. Breast feeding failed because of inability to suck and she was admitted to this hospital on the first day of life. Examination showed a drowsy, hypotonic, mildly jaundiced infant with hypothermia (temperature 95°F falling to 93°F necessitating the use of an incubator). Progressive respiratory failure with apnoeic attacks required artificial respiration. These attacks increased in frequency and duration and death ensued at 112 hours of age.

Haemoglobin was 21·9 g per 100 ml, white cells 9,900 per c mm (non-segmented polymorphs 8%, segmented polymorphs 58%, eosinophils 2%, lymphocytes 26%, monocytes 6%). Serum bilirubin 2·1 mg per 100 ml, blood sugar 58% mg per 100 ml. Serum electrolytes: sodium 154 m-equiv per litre, potassium 7·2 m-equiv per litre, chloride 115 m-equiv per litre, calcium 9·8 mg per 100 ml, phosphate 10·0 mg per 100 ml. Blood pH 7·155, pCO2 68 mmHg, standard bicarbonate 8·5 m-equiv per litre, base excess-7·5 m-equiv per litre.

The total excretion of amino acids in urine assessed visually was greater than normal, due mainly to a very large amount of glycine. Ethanolamine and cystine were both fairly prominent; alanine and glutamine were present in normal amounts.

The amino acids detected in serum in groups according to their relative concentrations assessed visually are set out below.

<table>
<thead>
<tr>
<th>Serum Amino Acid</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Alanine, glutamine, glycine, serine</td>
<td>++ +</td>
</tr>
<tr>
<td>Glutamic acid, leucine, valine, lysine, histidine</td>
<td>+ +</td>
</tr>
<tr>
<td>Threonine, cystine, asparagine, proline</td>
<td>+</td>
</tr>
<tr>
<td>Tyrosine, methionine and possibly tryptophan</td>
<td>trace</td>
</tr>
</tbody>
</table>

It is difficult to assess the significance of the serum amino acid pattern, glycine is probably present in a normal concentration in this specimen and the pattern as a whole seems qualitatively normal.

The cerebrospinal fluid was xanthochromic but there were no red cells present. Normally in cerebrospinal fluid glutamine dominates the amino acid pattern and although it was very prominent in this specimen, other amino acids were much more prominent than usual and the presence of proline, cystine, histidine, methionine, and lysine confirms the impression of a serum rather than a cerebrospinal fluid picture. The relative concentrations assessed visually were:

<table>
<thead>
<tr>
<th>Amino Acid in Spinal Fluid</th>
<th>Concentration</th>
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<tbody>
<tr>
<td>Glutamine</td>
<td>+++ +</td>
</tr>
<tr>
<td>Glutamic acid, alanine, glycine, serine, threonine</td>
<td>+++ +</td>
</tr>
<tr>
<td>Leucine, valine, histidine</td>
<td>+ +</td>
</tr>
<tr>
<td>Methionine, tyrosine, cystine, proline, asparagine, tryptophan, lysine</td>
<td>+</td>
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</table>

The body was that of a thin female with marked head moulding. The eyes were unusual in that the palpebral fissure sloped upwards and outwards. The ears were slightly low set. There was slight jaundice.

The trachea and bronchi contained large amounts of rust-coloured tenacious mucus. Many areas of the lungs were collapsed and anoxic haemorrhages were present in the pleura. Both lungs weighed 22 g (normal). Histology showed good expansion. Scanty clumps of eosinophilic hyaline material, occasional intraalveolar macrophages and focal intrapulmonary haemorrhages were evident in all lobes. A Meckel's diverticulum 2 cm in length was present 15 cm from the ileocaecal junction. The liver, 100 g (normal 80 g), was enlarged, tawny brown and mottled. Fatty vacuolation, mainly in the centriflobular zones, foci of erythrophoetic cells and excess iron are evident histologically. The spleen weighed 12 g (normal 9 g) and was slightly enlarged. Microscopy shows excess iron in the splenic pulp. The remaining thoracic and abdominal organs were normal macroscopically and histologically.

The brain weighs 342 g (normal 335 g). The frontal lobes were small relative to the remainder of the brain. The gyri of all lobes were flattened indicating oedema. Slicing revealed no macroscopic abnormality.

The histological appearances of the central nervous system are described below.

Family C In 1955 the mother had a full-term normal delivery, and the baby developed normally.

In 1960 a second infant was born (case 3), and in 1962 she had a full-term normal delivery, the baby again developing normally. In 1962 she had a miscarriage. In
1964 she was delivered of case 4, and in 1965 of case 5. There was no relevant family history, both parents being healthy.

Case 3 (A.C.) Full-term normal delivery. The baby was admitted to hospital aged 5 days, because of an inability to suck and a weak cry. The baby showed marked generalized flaccidity and poor respiratory movement with inadequate air entry at both bases. A diagnosis of cerebral damage was made and therapeutic measures were without success, the baby dying on the seventh day. Post-mortem examination showed a left tentorial tear with posterior fossa haemorrhage and poorly aerated lungs.

Case 4 (B.C.) Pregnancy and delivery were normal though the liquor was stained by meconium. The infant cried well at birth (but not subsequently) and fed well for two days. During this period she became increasingly drowsy and the mother became agitated since she felt the infant was behaving as the sib who died in 1960. By the third day the drowsiness prevented adequate feeding. Hypotonia, shallow irregular respiration, irregular heart rate, falling temperature (93·4°F) and severe generalized convulsions on the fourth day of life necessitated admission to this hospital. Examination revealed a severely ill, hypotonic baby with intermittent flexor spasms affecting the legs in particular, absent Moro, grasp, sucking, rooting, and gag reflexes, gross hypoventilation and oedema around the eyes and feet. Respiratory failure increased, necessitating artificial respiration, and spontaneous respiration ceased. Death was confirmed at 112 hours of age.

Serum electrolytes: sodium 146 m-equiv/litre, potassium 4·4 m-equiv/litre, chloride 108 m-equiv/litre; blood urea 12 mg/100 ml, calcium 10 mg/100 ml; bilirubin 4·6 mg/100 ml (direct bilirubin 0·1 mg/100 ml), blood sugar 67 mg/100 ml. Blood pH 7·22, pCO2 69 mmHg, standard bicarbonate 23·5 m-equiv/litre, base excess 0·5 m-equiv/litre. Cerebrospinal fluid, faintly xanthochromic, red cells 45/cmm, white cells 3/cmm, protein 140 mg/100 ml. Culture was sterile.

The amino acid pattern in urine was neither quantitatively nor qualitatively normal but on the other hand a generalized gross amino aciduria was not observed.

Glycine was the dominating amino acid, and cystine, lysine, and glutamine were also prominent. Threonine, alanine, ethanolamine, glutamic acid, proline, hydroxyproline, methionine, and ornithine were also detected. The presence of the last four amino acids might only have been a reflection of the very young age of the patient.

The urine also gave a positive Clinistix test (specific for glucose) and Benedict's reagent gave a green colour. The mellituria consisted of glucose (30 mg%); a trace of lactose and some fructose were detected by chromatography.

The body was that of a slightly cyanosed oedematous infant (see Table 1).

The lungs showed multiple focal areas of collapse and intrapulmonary haemorrhages. The tracheal and bronchial mucosa was congested and there was much bloodstained mucus. Microscopy showed irregular expansion. There was moderate congestion and groups of alveoli contained haemorrhages. There were scanty foci of polymorphs. A few alveoli contained hyaline membrane-like material lying free within the lumina associated with masses of red cells. There was acute tracheitis and bronchitis with epithelial necrosis. The lungs weighed 28·5 g each (normal 24 and 22 g). The pericardial cavity contained 2·3 ml of bloodstained fluid and petechial haemorrhages were present in the coronary sulcus. The liver 109 g (normal 96 g) was yellow and mottled with a normal consistency. No excess iron was seen microscopically but there was marked fatty vacuolation. The remaining thoracic and abdominal organs were unremarkable.

The brain weighed 374·5 g (normal 358 g) and was macroscopically normal. Slicing revealed no abnormality. The histological appearances are described below.

Case 5 (S.C.) The sixth pregnancy of the mother of case 4. The baby was very vigorous in utero. Pregnancy was terminated at 41 weeks by artificial rupture of membranes and pitocin infusion. The infant was of good colour but did not cry till one hour after birth. She did not cry again. At 48 hours she became flopppy, quiet, and refused to suck and was admitted to this hospital. Examination revealed a motionless infant lying in full extension giving sudden convulsive jerks of the lower limbs if handled. There was moderate jaundice, absent Moro and sucking reflexes, slight hypothermia (97·2°F). Hypotonia was more severe in the upper limbs. The liver was palpable, one fingerbreadth below the costal margin and the spleen could be tipped. In view of the previous family history hyperglycaemia was suspected. The baby became oedematous and slightly jaundiced and respiratory movement deteriorated. She was given bicarbonate and amino acids by gastric tube and a nasotracheal tube was inserted because of respiratory difficulties. The diet consisted of a mixture of amino acids from which glycine, threonine, valine, and methionine were excluded. Calories were provided in the form of corn oil and 10% dextrose. After beginning the diet the patient first developed a marked metabolic alkalosis (pH 7·57, standard bicarbonate 38 m-equiv/litre, base excess +15 m-equiv/litre) which was restored to normal over a few days. She showed some improvement with an increase in general activity and spontaneous respiratory effort. This improvement was not maintained and further deterioration began on the tenth day when spontaneous respiration ceased and there was slight oral bleeding. Bleeding continued from the mouth and rectum and death ensued when the infant was 2 weeks old.

On admission haemoglobin was 21·61 g/100 ml, white cells 15,000/cmm, platelets 130,000/cmm (unsegmented polymorphs 3%, segmented polymorphs 60%, eosinophils 7%, lymphocytes 23%, monocytes 7%). Serum electrolytes were: sodium 137 m-equiv/litre; potassium 7·2 m-equiv/litre, chloride 113 m-equiv/litre; urea 23 mg/100 ml, total bilirubin 1·7 mg/100 ml. Blood pH 7·21, pCO2 74 mmHg, standard bicarbonate 21 m-equiv/litre, base excess 4 m-equiv/litre. Calcium level was 9·0 mg/100 ml, phosphorus 6·4 mg/100 ml, alkaline phosphatase 13 K.A. units, bilirubin 3·4 mg/100 ml, blood sugar 97 mg/100 ml, SGOT 18 SF units, SGPT 15 SF units.

The total amino aciduria was within normal limits found in older children. Glycine was the most prominent
of the amino acids but was nothing like as prominent as the spot found in the urine of her sibling (case 4). Taurine was also fairly prominent but is known to be excreted in fairly large amounts during the first days of life.

The body was that of a pale female infant with slight icterus. The medial upper thighs and groins were extensively excoriated. Blood was leaking from the nose.

The nares, nasopharynx, mouth, and larynx contained bloodstained fluid which is also evident in the trachea and bronchi. The bronchi on the left contained purulent fluid. The left lung weighed 70·5 g (normal 26 g) and was firm, solid and slightly nodular with numerous subpleural abscesses in the upper lobe. The right lung weighed 71 g (normal 29 g) and was of similar consistency. Slicing of both lungs showed intrapulmonary haemorrhage and extensive pneumonic consolidation. Post-mortem culture of lung grew *Ps. pyocyanea* which was also isolated during life from the suction catheter. Microscopy showed extensive intra-alveolar haemorrhage associated with congestion. There were masses of pale eosinophilic material similar to that in hyaline membrane disease. Section from the left upper lobe showed extensive necrosis with masses of basophilic material in which numerous Gram-negative rods and mononuclear cells were dispersed. There was severe tracheitis and bronchitis. The heart weighed 22 g (normal 19 g) and the myocardium was pale. There was marked vacuolation of the myocardial fibres similar to that seen in case 1. The liver, 142·5 g (normal 123 g), was pale and mottled, yellow, with a greasy cut surface. Histology showed dilated sinusoids and occasional minute foci of erythropoietic cells. Fatty vacuolation was prominent.

The remaining thoracic and abdominal organs were unremarkable. There was extensive retroperitoneal haemorrhage extending from the pelvic brim to the pelvic floor.

The brain, 303 g (normal 382 g), was considerably underweight but otherwise macroscopically normal. Slicing showed no abnormality. The histology is described below.

Limited histochemical studies were carried out on the liver and kidney. Acid phosphatase, alkaline phosphatase, and non-specific esterase were demonstrated in control material using the methods described by Pearse (1960). In liver and kidney from the necropsy material of case 5 acid phosphatase and non-specific esterase were present in normal quantities. Alkaline phosphatase could not be demonstrated by either the Gomori or azo techniques though it was present in similar control material. The significance of this finding is unknown.

HISTOLOGY OF THE CENTRAL NERVOUS SYSTEM

Paraffin sections of the central nervous system from cases 1, 2, 4, and 5 were stained with haematoxylin and eosin, PTAH, Holzer, and Lillie's myelin stains. Frozen sections were stained for fat from cases 2, 4, and 5.

The following description is based mainly on case 5, but similar lesions were demonstrable in cases 1, 2, and 4. These cases were studied retrospectively and only limited material is available.

FIG. 1. *Spongy change in cerebral white matter. PTAH × 128.*

CEREBRUM The cerebral cortex is well preserved in all cases and no vacuolation or fat-containing cells are demonstrable. All cases show marked spongy degeneration of the white matter (Fig. 1). The distribution of this change is diffuse and shows no predilection for the immediate subcortical zones. The spongy areas appear to originate from swollen glial cells and many contain pyknotic nuclei. Associated with the spongy changes numerous fat-filled glial cells are evident but show no relationship to blood vessels. In the areas of spongy change there are occasional hypertrophied glial cells.

Myelination is retarded as compared with that in a brain from an infant of similar age and weight dying from congenital heart disease. There are short segments of myelinated fibres but none of the areas investigated revealed a degree of myelination as seen in the control.

Areas particularly severely affected in the cerebrum include the internal capsule and midline tracts. The basal ganglia are well preserved but occasional fat-filled cells are seen in the globus pallidus.

CEREBELLM The cerebellum was examined in cases 2, 4, and 5. The outer molecular layer is present in all three
cases. The Purkinje cells are reduced in number and occasional cells are displaced towards the molecular layer. There is extensive vacuolation of the white matter with early changes in the lamina dissecans (Fig. 2). In case 5 these changes are associated with numerous lipid-filled cells in the white matter, most prominent in the cerebellar peduncles. Lesser changes of a similar nature were seen in cases 2 and 4. The white matter in the region of the dentate nucleus shows very severe changes though the neurones are well preserved (Fig. 3). Myelination is retarded.

BRAIN STEM Spongy lesions are widespread (Fig. 4) particularly in the cerebrospinal, spinocerebellar, and corticobulbar tracts. The cranial nerve nuclei and other brain stem nuclei are normal but are surrounded by severe spongy lesions. These are particularly severe in the floor of the fourth ventricle. Myelination is retarded (Figs. 5 and 6).

SPINAL CORD Sections of spinal cord were examined from cases 1 and 5. Spongy change is present in the white matter but becomes progressively less marked with the descent from cervical to lumbar regions. Fat-containing cells are very infrequent.

FIG. 2. Cerebellum: severe spongy change. PTAH × 128.

FIG. 3. Dentate nucleus: spongy lesions. PTAH × 128.

DISCUSSION

Donohue (1967) in a review of the lesions of the central nervous system associated with inborn errors of amino acid metabolism notes that spongy degeneration of the white matter is, in his experience, most severe in hyperglycinemia. In the previously reported cases of hyperglycinemia this change had not been described. Donohue noted that similar lesions had also been reported in oasthouse urine disease, maple syrup urine disease, homocystinuria, alanaemia, phenylketonuria, and tryrosinaemia. He concludes that damage to the central nervous system is not uncommon in errors of amino acid metabolism and that it may be the result of an abnormality in the synthesis or homeostasis of myelin. This concept is supported by the present cases.

The evidence indicates that myelination is retarded and there is no evidence of demyelination. The degree of spongy change appears to be related to the degree of failure of myelination and the presence of lipid-filled cells.
Spongy degeneration of white matter of central nervous system associated with hyperglycinuria

FIG. 4. Medulla: spongy change. PTAH × 128.


FIG. 6. Olivary nucleus (Fig. 5). Control case with more advanced myelination. Lillie's myelin × 52.
Lipid-filled glial cells are usually associated with areas of demyelination or nervous tissue destruction. These cells eventually migrate into the vicinity of the blood vessels. In the current cases the lipid-filled cells are not associated with histological evidence of demyelination nor are they to be found related to blood vessels. These cells may be found in normal infant brains but the numbers present in these cells are in excess of normal. Zu Rhein, Eichman, and Puletti (1960) suggested that similar lipid-containing glial cells in the von Bogaert type of spongy change may be the result of a metabolic disturbance within these cells. They based this hypothesis on the evidence of Duncan and Nall (1959) that degeneration of the mitochondria of cultured human amnion cells leads to the formation of lipid inclusions.

The nature of the underlying defect to spongy change is disputed. The change is in itself non-specific though it is associated with familial idiocy, spasticity, blindness, and occasionally deafness (Canavan, 1931). Von Bogaert and Bertrand (1949) considered the change as primarily oedematous but Blackwood and Cumings (1954) suggested that the familial type was due to defective myelination which begins in utero. Adachi, Wallace, Schneck, and Volk (1966), in a study of the fine structure of spongy degeneration of the von Bogaert type, have demonstrated that the vacuoles are located in the myelin sheaths and to a lesser degree in the astrocytes. The vacuoles may rupture into the intercellular spaces. These authors also noted a marked reduction in the number of mitochondria in the astrocytes, an interesting feature in light of the hypothesis suggested by Zu Rhein et al. (1960). Davison and Dobbing (1966) have indicated that the vulnerable period in human myelination extends from the seventh month of intrauterine life into the first few months of postnatal life and that small restrictions imposed during this period may cause permanent damage to the developing central nervous system. It would appear that inborn areas of amino acid metabolism may be a very important cause of disturbed myelination during this period.

The finding of spongy degeneration of the white matter in hyperglycinuria allows some interpretation of the clinical presentation. The presence of retarded myelination supports the hypothesis of Blackwood and Cumings (1954) though the underlying disease process is different. The exact nature of the vacuolation is not apparent but there is evidence of astrocytic involvement. Light microscopy does not allow of the identification of involved myelin sheaths. It is likely that the spongy change occurs after birth but the retardation of myelination probably begins in utero. The poor obstetric history of some of the mothers of hyperglycinuric infants (Visser et al., 1964) may be an indication of a severe intrauterine form of the disorder leading to intrauterine death and abortion. It is impossible to predict at present whether the lesions can be corrected when a rational therapy based on knowledge of the metabolic defect is available.

The association of spongy degeneration of the central nervous system with inborn errors of amino acid metabolism is important in the understanding of neuropathology and biochemistry. It is also important because the finding of these lesions in some infants dying of undetermined causes may be a pointer to a metabolic disorder. Hyperglycinuria and hyperglycinemia is a rare metabolic disturbance though it is possible that cases are missed among neonatal deaths of unknown aetiology. Awareness of its existence may explain some hitherto inexplicable deaths.

I wish to thank Dr Margaret Barton, Dr B. D. Bower, and Dr W. H. P. Cant for permission to publish the clinical details of these cases; Dr D. N. Raine for his assistance with the biochemistry, and Dr A. H. Cameron for his help in preparing the manuscript. I am grateful to Mr R. H. Bailey for preparation of the histological material and Miss J. Roberts for secretarial assistance.

REFERENCES