Congenital ophthalmoplegia, floppy baby syndrome, myopathy, and aminoaciduria

Report of a family

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The simultaneous occurrence of apparently disparate disorders poses the question of a common pathogenesis or the coincidental association of unrelated processes. This report presents a family in which the two children were found to have marked limitation of ocular movement and limb girdle weakness: both children and the parents have an unusual pattern of urinary amino acid excretion. Hurwitz, Maguire, and Fannin (1968) have described the ocular features in the children and the electromyographic and histopathological evidence of myopathic involvement of the eye muscles. Evidence of a more generalized myopathy and its possible relationship to the familial aminoaciduria is now detailed and discussed.

CLINICAL FINDINGS

CASE 1 J. F. (III), the propositus, a boy aged 15 years, has been examined several times over a period of two years. The mother was healthy during the pregnancy; delivery was with forceps, after a labour lasting 36 hours. He weighed 7 lb 14 oz (3.6 kg) at birth and measured 21 in. (53.3 cm) in length. Hospital notes (Professor F. M. B. Allen) record that he was slow to cry and had a weak cry for some days. The limbs were very floppy and he was also considered to have bilateral wrist drop. Neonatal had no clinical effect and a diagnosis of amyotonia congenita was made. He was unable to suck and had to be tube fed for at least six weeks. There was a convergent squint at birth. Physical development was delayed, but intellectual development appeared normal. He was unable to support his head for several months and at the age of 14 months could not stand. In the first two years he had three episodes of pneumonia, and it was during treatment of the third episode that impaired ocular movement was noted. Although the full degree of ocular movement was not documented until the age of 4 years, the impairment was considered to be present from birth. From the age of 4 years there has been no change in the ocular signs. He started to walk between the age of 2 and 3 years but weakness in limb girdle muscles was noted and a muscular dystrophy was suspected. However improvement in walking continued and by the age of 6 years Professor Allen considered the diagnosis of muscular dystrophy no longer tenable. The cerebrospinal fluid (CSF), examined shortly after birth and again at 7 years, had a normal cell count and protein content with a negative Wassermann reaction.

The patient was able to attend school, to use stairs, and travel by public transport, although weakness was noted at times. The presenting complaint at the age of 13 years was that over the previous four years he had been more liable to stumble and fall and that he had increasing difficulty in climbing stairs and getting up from a lying position. At the age of 8 years he developed psoriasis over the trunk, and two years later he had an episode of acute guttate psoriasis with a good response on both occasions to local treatment. On examination he was of normal intelligence. His height was 66 in. (1.7 m), arm 64 in. (1.6 m), and crown to pubis measurement 32 in. (0.81 m). He had an expressionless face, but eyelid closure and movements of the face during smiling and whistling were good. There was no ptosis or abnormality of the pupils. There was a convergent squint. There was no abduction of the eye beyond the midline on looking to either side and virtually no upward or downward movement. The range of adduction of each eye was normal. The sternomastoids were normal but there was slight weakness of the shoulder girdle muscles without winging of the scapula. There was some slight weakness of the quadriceps and hamstring muscles. The other muscles of the body were of normal strength. The tendon reflexes were not elicited and the plantar reflexes were flexor. Sensation was intact. He could not attain a sitting from a lying position without using his upper limbs and when asked to rise to a standing position he was severely handiaapped and had to use Gower's manoeuvre. General medical examination revealed no abnormality. He had moderately high arched feet.

The following investigations were carried out, with normal results except where indicated. Haemoglobin 13 g%, with a white blood cell count of 5,500/c.mm. The serum sodium was 138 m-equiv/l., potassium 4-6 m-equiv/l., CO₂CP. 25-0 m-equiv, serum urea 25 mg%.}

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calcium 9.9 mg%, phosphorus 4.6 mg%. The calcium and phosphorus estimations were repeated on two other occasions with similar results. The alkaline phosphatase level on four occasions at intervals of a few months was 19, 22, 43, and 22 u. (normal range 3 to 13 King Armstrong u.). The blood pH was 7.36, and PCO2 34 mm Hg, base excess -5.2 m-equiv/l., buffer base = 42 m-equiv/l., standard bicarbonate 20.0 m-equiv/l. Plasma total bilirubin was 0.4 mg/100 ml., zinc sulphate turbidity 3 u., pseudocholinesterase 144 u., SGOT 35 u., and the SGPT 20 u. The serum folie acid was 8.0 m l./ml. Serum carotene level was 70 u. and the d-xylene excretion was 33% of the ingested dose. Xanthurenic acid excretion was normal after oral tryptophan. Faecal fat was 8 g/three-day specimen. Glucose tolerance test: normal with a maximum rise in blood glucose of 36 mg after 50 g glucose given orally. The urine did not contain glucose or albumin. On three occasions the serum aldolase was 20, 16, and 50 u. (normal range 7 to 21 u.). The serum creatine phosphokinase on two occasions was 0.55 and 2.1 u. The 24 hour urinary excretion of creatine was 0.94 g, which is abnormally high, and of creatinine 0.24 g giving a ratio of 4:1. Blood Wassermann reaction was negative; Kahn test +1. Fluorescent treponemal antibody reaction was negative. Radiographs of the skull and chest were normal and the bone age, judged from radiographs of the wrist and elbow, was appropriate with normal bone density.

Otolological examination showed mild perceptive hearing loss in the left ear. On caloric testing by the method of Fitzgerald and Hallpike there was no response with water at 44°C in either ear. With water instilled in the left ear at 10°C there was a wide amplitude slow beating nystagmus away from the stimulated ear which lasted for 110 seconds. There was no response with water at 10°C in the right ear.

An electroencephalogram (EEG) showed a slight generalized abnormality.

After nine months of observation it was decided to try the effect of treatment with a sulphur-containing amino acid combined with vitamin E. For the past 14 months the patient has been receiving orally methionine 250 mg four times daily and a tocopherol 50 mg twice daily. The patient and his parents have reported improvement in muscle strength. The outcome of this treatment will be described in a later communication.

CASE 2 K.F. (III1) was a girl aged 11 years. The mother was well during the pregnancy and the delivery was normal. The birth weight was 7 lb. (3.2 kg) and the baby was found to be limp after birth. She had a convergent squint from birth. The hospital notes (Professor F. M. B. Allen) stated that at the age of 1 year 7 months she could not stand unaided and made only a poor attempt at sitting up alone and that she could not roll over from a prone or supine position. She was thought to be mentally normal. It was noted that she had little facial expression and considerable ocular weakness. The child was considered to be 'very like her brother' and to have generalized hypotonia. By the age of 3 years she was walking well and there were no complaints.

On examination at 11 years of age she was 58 in. tall the arm span was 59 in. (1.49 m), crown to pubis 29+ in. (0.74 m), pubis to ground measurement 28½ in. (0.72 m). There was considerable poverty of facial expression, although there was only a little objective weakness. There was no ptosis and no pupillary abnormalities. She had a right convergent squint. As in her brother, there was considerable though less severe weakness in ocular gaze, except for addition of either eye which was normal. She showed very slight limb girdle weakness in that she tended to use Gower's manoeuvre in rising from a lying to a standing position. The tendon reflexes in the arms were impaired and were not elicited in the legs. The plantar responses were flexor. There were no sensory changes. General medical examination was normal. The results of investigations were as follows: blood Wassermann negative, blood Kahn reaction +1, fluorescent treponemal antibody negative; serum sodium 133 m-equiv/l., CO2CP. 23.8 m-equiv/l., serum urea 23 mg/100 ml.; serum calcium 9.4 mg/100 ml.; serum phosphorus 4.6 mg/100 ml., alkaline phosphatase 24 K.A. units; blood pH 7.37; blood PCO2 33 mm Hg; base excess -5.2, buffer base 42 m-equiv/l.; standard bicarbonate 20.0 m-equiv/l. The urine did not contain glucose or albumin. Serum aldolase was 23 u. and serum creatine phosphokinase 0.5 u. The urinary 24 hour excretion of creatine was 0.1 g, which is considered normal in this laboratory, and of creatinine was 0.5 g, giving a creatine/creatinine ratio of 1.5:1.

An EEG showed a slight generalized abnormality.

An otological examination by Mr. G. D. L. Smyth showed a mild left perceptive deafness. Caloric testing on two occasions with water at 10°C produced no response in either ear.

PARENTS (II4 and II11) The mother, aged 38, and the father, age 47 years, were of average stature and intelligence and showed no abnormal signs. The Wassermann and Kahn reactions in both parents were negative. The serum aldolase was 10 u. and the serum creatine phosphokinase was 0.55 u. in the mother and the serum aldolase was 5 u. in the father. The creatine/creatinine ratio in the mother was 1:14 and in the father 1:5.

ELECTROMYOGRAPHY Electromyography (EMG) was carried out in cases 1 and 2 and their parents. The deltoid muscle was examined by concentric needle electrodes 0.65 mm in diameter and using a three-channelled DISA machine. In each case, nine different muscle points at three depths and thus 27 different motor unit territories were sampled as formulated by Buchthal (1957). The duration of the motor unit action potential was measured for each position and a mean value determined. A value deviating more than 20% from the normal for the muscle in relevant age groups was considered definitely abnormal (Buchthal, 1957). The incidence of polyphasia was determined and the upper limit was taken as 12% (Fitzgerald and Hallpike, 1965).

EMG RESULTS

The results are summarized in Table I. There was no spontaneous activity in the form of fibrillation
TABLE I

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Mean duration of all motor action potentials (^1) (msec)</th>
<th>Incidence of polyphasic potentials (%)</th>
<th>Amplitude of interference patterns (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.F.</td>
<td>M</td>
<td>15</td>
<td>9.0 (24.3)</td>
<td>18.5</td>
<td>4.4</td>
</tr>
<tr>
<td>K.F.</td>
<td>F</td>
<td>11</td>
<td>8.6 (21.7)</td>
<td>13.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Mother</td>
<td>F</td>
<td>38</td>
<td>9.2 (30.8)</td>
<td>16.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Father</td>
<td>M</td>
<td>47</td>
<td>12.1 (12.3)</td>
<td>4.1</td>
<td>4.6</td>
</tr>
</tbody>
</table>

\(^1\)In parentheses is the percentage decrease from the normal value in the mean duration of the motor action potentials for deltoid muscle for the appropriate age group (Buchthal, 1957).

or fasciculation potentials. The maximum effort in all patients revealed interference pattern of normal voltage. Reduced duration of motor unit potential greater than 20% was found in cases 1 (J.F.) and 2 (K.F.) and their mother. These three also had an abnormally high incidence of polyphasic potentials (Fig. 1). The significant reduction in duration of the motor unit potential and the polyphasia suggest a myopathic lesion in the muscle.

HISTOPATHOLOGICAL INVESTIGATION IN CASE 1

Further sections were cut from blocks of the right deltoid muscle which had been biopsied in 1952 and were stained with haematoxylin and eosin. A biopsy from the right deltoid muscle taken in 1967 was examined by light microscopy. A biopsy of the right quadriceps muscle in 1967 was examined by light and electron microscopy and by standard histochemical techniques. The materials and methods used in the histopathological studies were the same as those described by Hurwitz, Carson, Allen, Fannin, Lyttle, and Neill (1967). The following enzymes were studied histochemically: phosphorylase, NADH and NADPH diaphorases, dehydrogenases (lactic, succinic), acid phosphatase, non-specific esterase, and acetylcholinesterase.

HISTOPATHOLOGICAL RESULTS

RIGHT DELTOID MUSCLE (1952) The biopsy was obtained under general anaesthesia at the age of 6 weeks and showed muscle fibres relatively uniform in size with no features which could lead to a diagnosis of the cause of the floppiness. Some of the fibres contained central nuclei but the histological appearance was considered normal for the age.

RIGHT DELTOID MUSCLE (1967) The biopsy was obtained under local anaesthesia.

Light microscopy Many of the fibres were healthy but some showed marked rounding of their outline and hyaline degeneration. Some of the nuclei showed clumping and were centrally placed. Small atrophic fibres measuring less than 10 \( \mu \) in diameter were scattered at random, while occasional large hyaline fibres measuring up to 84 \( \mu \) were also seen. The average muscle fibre diameter was 29 \( \mu \). The muscle spindles were normal. There was slight fibrous tissue proliferation but no increase in fat.

RIGHT QUADRICEPS MUSCLE (1967) The biopsy was obtained under general anaesthesia.

Light microscopy (Fig. 2) Most of the muscle fibres were healthy but there were occasional fibres in which the nuclei were grouped in short chains

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FIG. 1. Electromyography of the deltoid muscle showing motor unit action potentials of short duration in cases 1 and 2 and in the mother. The potentials in the father are normal.
FIG. 2. Some small atrophic fibres are seen close to some rounded hyalinized fibres. H and E × 220.

beneath the sarcolemma. A few hyaline rounded fibres were present and in addition a number of small fibres (10 to 15 μ in diameter) were seen, but these did not show any other abnormality. The average diameter was 54 μ (range 10 to 110 μ). There was a little fatty infiltration but no fibrous tissue proliferation.

**Electronmicroscopy** In general the fibre architecture was well-preserved but there was some variation in fibre diameter and a proportion of fibres showed mild degenerative changes (Fig. 3). The sarcolemma was not thickened but there were a few areas in which there was slight ballooning of the membrane. The subsarcolemmal nuclei were mostly normally placed, though a few central nuclei were present. The myofibrils showed focal degenerative changes with loss of sarcomere alignment and an accompanying increase in sarcoplasm granularity and vacuole formation. No mitochondrial abnormality was detected. The histological features with variation in individual fibre size and hyaline degeneration of some fibres combined with the electron microscopic finding of disruption of sarcomere pattern is considered characteristic of a myopathy.

**Histochemistry** The results did not show any abnormality.

**BIOCHEMICAL INVESTIGATION**

Paper chromatographic techniques for amino acids in urine were those used by Carson and Neill (1962). The Standard Technicon Automatic Amino Acid Analyzer was used for quantitative studies of amino acids in blood and urine. Five separate 24 hour specimens of urine were examined in case 1 and three in case 2, at least two 24 hour samples being collected in hospital after two days on a standard hospital diet. The 24 hour specimens from the mother and father were collected at home by the subjects while they were taking their normal diet.

In order to assess the influence of intestinal flora on the urinary pattern of amino acid excretion, oral neomycin (5 g daily for three days) was given in an attempt to produce gut sterilization. The possible influence of ingested food was investigated by the use of various diets: a meatless diet and a diet low in all protein was given to cases 1 and 2 for three days while in hospital.

FIG. 3. There is focal fibre degeneration extending along several sarcomeres. × 7,500.
and the urinary amino acids were estimated on the third day of each diet. Further, a diet consisting mainly of chicken (which is rich in anserine, a precursor of 1-methyl histidine) was given for one day and of beef steak on another day and the urinary amino acids estimated during the 24 hours of the special diets. The mother and father were also given a chicken diet and the urine collected for the 24 hours. In the appendix the estimated amino acid content of the diets is given (McCance and Widdowson, 1960).

Oral loading tests of L-cystine and L-lysine were given to both patients and the parents. Histidine, methionine, leucine, and glycine loads were also performed on case 1, and histidine on case 2.

Renal clearance studies and per oral amino acid loading tests were performed. The techniques used for these are as described by Hurwitz et al. (1967).

**BIOCHEMICAL RESULTS**

Table II gives the range of values of the daily excretion of the basic amino acids, cystine and glycine, in cases 1 and 2 and the parents, and the values are compared with those found in normal individuals and in the homozygous and heterozygous patient with cystinuria. Lysine, histidine, and 1-methyl histidine were excreted in excess in case 1 and lysine was in excess in case 2 and in the mother and father. The excretion of histidine in the father was at the upper limits of normal but the diet taken by the father may have been high in protein (appendix). The urinary excretion of cystine was slightly higher than normal on occasions in case 1, although at other times cystine was not detected in the urine. Glycine was excreted at the higher level of normal values in case 1 and the mother. Ornithine was not estimated in the urine because of interference by tryptophan which is eluted in the same position on the chromatogram.

Table III gives the values obtained in cases 1 and 2 of 1-methyl histidine, 3-methyl histidine, histidine, and lysine after the various diets with the exception of the chicken diet. The urinary amino acids were considerably reduced after the low protein diet and after neomycin. Because of the consistently high urinary concentration of 1-methyl histidine in case 1, it was decided to investigate the urinary excretion of imidazol compounds in the family and in four healthy controls after a diet rich in chicken. Table IV gives these results. Anserine, carnosine, and β-alanine appeared in the urine in various concentrations in the controls and in the family, with the exception of the mother. The increase after chicken diet of histidine, 1-methyl histidine, and 3-methyl histidine is similar for the subjects and for the controls. The differences in the quantity of β-alanine, anserine, and carnosine excreted by the subjects and the controls suggested a normal variation in the ability of individuals to hydrolyse these amino acids.

The renal clearance (Table V) for lysine, histidine, and glycine was abnormal in case 1 and glycine clearance alone was slightly abnormal in case 2. The mother showed an increase in the renal clearance of glycine and lysine with histidine at the upper limits of normal and the father had an increase in histidine clearance. The serum amino acid values were within the normal range in each subject.

The results of the loading tests suggest that case 1 has an intestinal absorption defect for lysine, histidine, and glycine, while methionine, cystine, and leucine loading gave normal results. The lysine and cystine loads on the parents and sibling and the histidine load in case 2 were all normal. The results can be seen in graphic form in Figures 4 to 9. Although intravenous loads were not given to test the utilization and excretion of lysine, histidine, and glycine in the propositus, it is considered most
### TABLE III

**DAILY EXCRETION OF AMINO ACIDS IN MICROMOLES AFTER VARIOUS DIETS**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Mixed diet</th>
<th>Mixed diet and Neomycin (3 days)</th>
<th>Low protein</th>
<th>Muscle free</th>
<th>Steak</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Daily total protein (approx. 60 g)</td>
<td>Daily total protein (Average 60 g)</td>
<td>Daily total protein (23 g)</td>
<td>Daily total protein (80 g)</td>
<td>Daily total protein (107 g)</td>
</tr>
<tr>
<td>Case 1</td>
<td>J.F.</td>
<td>K.F.</td>
<td>J.F.</td>
<td>K.F.</td>
<td>J.F.</td>
</tr>
<tr>
<td>Case 2</td>
<td>J.F.</td>
<td>K.F.</td>
<td>J.F.</td>
<td>K.F.</td>
<td>J.F.</td>
</tr>
<tr>
<td>1 me Hist</td>
<td>848 to 2,084</td>
<td>N.E. to 4,192</td>
<td>35 to 1,265</td>
<td>35 to 1,265</td>
<td>0 to 985</td>
</tr>
<tr>
<td>3 me Hist</td>
<td>1,907 to 2,084</td>
<td>386 to 4,192</td>
<td>35 to 1,265</td>
<td>35 to 1,265</td>
<td>0 to 985</td>
</tr>
<tr>
<td>Histidine</td>
<td>1,171 to 2,084</td>
<td>141 to 2,025</td>
<td>122 to 793</td>
<td>122 to 793</td>
<td>80 to 159</td>
</tr>
<tr>
<td>Lysine</td>
<td>884 to 2,084</td>
<td>593 to 2,025</td>
<td>302 to 1,595</td>
<td>302 to 1,595</td>
<td>180 to 159</td>
</tr>
<tr>
<td>β-alanine</td>
<td>0 to 2,084</td>
<td>0 to 2,025</td>
<td>0 to 1,595</td>
<td>0 to 1,595</td>
<td>0 to 159</td>
</tr>
<tr>
<td>Anserine</td>
<td>0 to 2,084</td>
<td>0 to 2,025</td>
<td>0 to 1,595</td>
<td>0 to 1,595</td>
<td>0 to 159</td>
</tr>
<tr>
<td>Carnosine</td>
<td>0 to 2,084</td>
<td>0 to 2,025</td>
<td>0 to 1,595</td>
<td>0 to 1,595</td>
<td>0 to 159</td>
</tr>
</tbody>
</table>

N.E. = Not estimated because of technical fault, but small quantity present.

### TABLE IV

**IMIDAZOLE COMPOUNDS IN URINE BEFORE AND AFTER CHICKEN DIET**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Amount of chicken (oz)</th>
<th>Histidine Before chicken</th>
<th>Histidine After chicken</th>
<th>1-Methyl histidine Before chicken</th>
<th>1-Methyl histidine After chicken</th>
<th>β-Alanine Before chicken</th>
<th>β-Alanine After chicken</th>
<th>Anserine Before chicken</th>
<th>Anserine After chicken</th>
<th>Carnosine Before chicken</th>
<th>Carnosine After chicken</th>
<th>3-Methyl histidine Before chicken</th>
<th>3-Methyl histidine After chicken</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>8 1/2</td>
<td>1,199</td>
<td>1,357</td>
<td>426</td>
<td>976</td>
<td>Trace</td>
<td>52</td>
<td>0</td>
<td>Trace</td>
<td>0</td>
<td>106</td>
<td>264</td>
<td>299</td>
</tr>
<tr>
<td>Mother</td>
<td>16</td>
<td>903</td>
<td>1,080</td>
<td>395</td>
<td>6,002</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>217</td>
<td>469</td>
</tr>
<tr>
<td>K.F.</td>
<td>14</td>
<td>423</td>
<td>1,041</td>
<td>386</td>
<td>2,993</td>
<td>0</td>
<td>N.E.</td>
<td>813</td>
<td>0</td>
<td>101</td>
<td>213</td>
<td>469</td>
<td>477</td>
</tr>
<tr>
<td>J.F.</td>
<td>16</td>
<td>2,084</td>
<td>3,039</td>
<td>401</td>
<td>5,877</td>
<td>0</td>
<td>189</td>
<td>0</td>
<td>2,073</td>
<td>704</td>
<td>54</td>
<td>296</td>
<td>207</td>
</tr>
<tr>
<td>Four</td>
<td>155</td>
<td>752</td>
<td>176</td>
<td>2,288</td>
<td>0</td>
<td>43</td>
<td>Trace</td>
<td>0</td>
<td>Trace</td>
<td>119</td>
<td>207</td>
<td>Trace</td>
<td>Trace</td>
</tr>
<tr>
<td>Healthy</td>
<td>16</td>
<td>1,292</td>
<td>2,919</td>
<td>372</td>
<td>7,583</td>
<td>72</td>
<td>444</td>
<td>1,568</td>
<td>154</td>
<td>665</td>
<td>333</td>
<td>978</td>
<td>978</td>
</tr>
</tbody>
</table>

N.E. = Not estimated because of technical fault.

### TABLE V

**RENAI CLEARANCES OF NINE AMINO ACIDS (ML./MIN./1.73 M²)**

<table>
<thead>
<tr>
<th>Glutamic acid</th>
<th>Glycine</th>
<th>Alanine</th>
<th>Tyrosine</th>
<th>Phenylalanine</th>
<th>Ornithine</th>
<th>Lysine</th>
<th>Histidine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2 (K.F.)</td>
<td>1-5</td>
<td>9-5</td>
<td>1-7</td>
<td>1-5</td>
<td>1-3</td>
<td>0-7</td>
<td>1-2</td>
<td>9-3</td>
</tr>
<tr>
<td>Age 11 yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal range1</td>
<td>1-2</td>
<td>9-1</td>
<td>1-1</td>
<td>1-2</td>
<td>1-9</td>
<td>0-9</td>
<td>5-3</td>
<td>18-0</td>
</tr>
<tr>
<td>Case 1 (J.F.)</td>
<td>1-2</td>
<td>9-1</td>
<td>1-1</td>
<td>1-2</td>
<td>1-9</td>
<td>0-9</td>
<td>5-3</td>
<td>18-0</td>
</tr>
<tr>
<td>Age 15 yr</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal adult range</td>
<td>0-1-0.8</td>
<td>2-5-6.6</td>
<td>0-2-0.9</td>
<td>0-8-2.4</td>
<td>0-2-1.4</td>
<td>0</td>
<td>0-2-0.9</td>
<td>3-3-9.7</td>
</tr>
<tr>
<td>(Values from literature)²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Scriver and Davies (1965).
2. Doolan, Harper, Hutchin, and Shreeve (1955); Christiansen, Date, Schanheyder, and Volquartz (1957); Cusworth and Dent (1960); Scriver, Efron, and Schafer (1964).
Congenital ophthalmoplegia, floppy baby syndrome, myopathy, and aminoaciduria

**FIG. 4.** 1-lysine load in cases 1 and 2 (J.F. and K.F.), parents, and in three healthy controls.

**FIG. 5.** 1-cystine load in cases 1 and 2 (J.F. and K.F.) and in one healthy control.

**FIG. 6.** 1-histidine load in cases 1 and 2 (J.F. and K.F.) and in eight normal siblings and their parents in a family with histidinaemia and one healthy control.

**FIG. 7.** Glycine load in case 1 (J.F.) and in one healthy control.

**FIG. 8.** 1-leucine load in case 1 (J.F.) and in two healthy controls.

**FIG. 9.** 1-methionine load in case 1 (J.F.) and one healthy control.
likely that the low blood levels were due to defective absorption across the gut.

FAMILY STUDIES The family pedigree is shown in Figure 10. There was no evidence of ocular or limb girdle weakness among 14 relatives who were examined. There was no consanguinity. The father, II_4, and the paternal grandmother, I_2, had high arched feet. A history of limb paralysis was reported in a paternal cousin, II_5, but a recent clinical examination revealed no abnormality. Hospital records of that patient suggested a demyelinating episode some years previously. The daughter of II_5, a girl aged 14 years (III_7), was mentally defective. On examination she had microcephaly and her intelligence quotient was about 40 but there was no muscle disease. The amino acid chromatograms of random urine samples in members of the family other than the parents and the two children showed a normal pattern of excretion.

DISCUSSION

CLINICAL CLASSIFICATION OF PATIENTS In case 1 electromyography and histopathology of the eye muscles were compatible with a myopathic lesion. As the EMG findings in the deltoid muscle and the histopathology of the deltoid and quadriceps muscles suggested a myopathy one can reasonably infer that the ophthalmoplegia and limb girdle weakness in case 1 have a common myopathic aetiology. One can assume that in his sister (case 2) the clinically similar ophthalmoplegia was also of myopathic origin. In addition in this patient the diminished tendon reflexes, the slight limb girdle weakness, and the myopathic EMG pattern in the deltoid muscle suggested a more general myopathy. A muscle biopsy has not been done.

Ophthalmoplegia is rare in muscular dystrophy. It is not a feature of most cases of Duchenne type muscular dystrophy, nor is it present in the scapulo-humeral and limb girdle dystrophies. It is occasionally found in dystrophia myotonica, though not as severe as in the present cases. In the acquired adult ocular myopathies a descent of the myopathic processes to involve other skeletal muscles has been well described (Lees and Liversedge, 1962).

In case 2 there is certainly some facial weakness and in case 1 facial weakness was commented on at birth. If the eye signs in the present cases can be deemed congenital, for which there is good evidence, then the ophthalmoplegia and the slight facial weakness could be broadly classified as being related to the Möbius syndrome of congenital facial diplegia. Ophthalmoplegia is present (Henderson, 1939) in a high proportion of patients with the typical Möbius syndrome of facial diplegia, while
Congenital ophthalmoplegia, floppy baby syndrome, myopathy, and aminoaciduria

in cases of congenital ophthalmoplegia facial weakness is common (Danis, 1945). A myopathic aetiology in some cases of Möbius syndrome has been discussed by Pitner, Edwards, and McCor- mick (1965). Also the occurrence of limb girdle weakness after some years in patients with congenital ophthalmoplegia has been reported by other authors. Among these, Ross (1963) has described three members of a sibship of five who were affected by ptosis and ophthalmoplegia, probably congenital, with limb girdle weakness coming on at the age of 9 years. These patients showed excessive sensitivity to curare but muscle biopsy showed myopathic features. Two other patients reported by this author developed limb girdle weakness some years after the eye signs were noted. In our patients there was no improvement in the ocular signs with edrophonium hydrochloride but curare sensitivity was not tested. Verzella, Fiume, and Luppi (1963) studied two families with congenital external ophthalmoplegia. Two sisters were affected in one family and a father and son in the other. Histopathological examination of the ocular and skeletal muscles suggested myopathy in one member of each family, but clinically limb girdle weakness was not notable. Floppy baby syndrome is not mentioned as being present in the patients of Ross (1963) or Verzella et al. (1963).

The floppy baby syndrome in both our patients is again likely to be due to a myopathic process. Walton (1956) gives congenital dystrophy as one of the causes of the floppy baby syndrome and Sandifer (1967) described 'flaccid' weakness being replaced by limb girdle weakness as the child grows older. Dystrophia myotonica may be responsible for flaccipness at birth and for the presence of congenital facial diplegia (Vanier, 1960; Watters and Williams, 1967). The six cases described by Vanier with dystrophia myotonica were floppy babies and this author emphasized that without the family history of dystrophia myotonica in case 5 the diagnosis would have been Möbius syndrome. Vanier also recorded four patients who had congenital facial diplegia with weakness of certain other muscles and three patients with congenital facial diplegia and benign congenital hypotonia, in whom there was no evidence of dystrophia myotonica. Vanier was thus able to suggest that, although dystrophia myotonica may occasionally be responsible for the floppy baby syndrome, the coexistence of Möbius syndrome and flaccipness at birth could be caused by some other muscular disease. In the family with dystrophia myotonica described by Parker (1963), one member (D16) was a floppy baby and presented with congenital facial diplegia, which appeared to be the earliest expression of the disease. Seven of the 10 cases considered by Graham (1964) as having congenital flaccid bulbar palsy of probable myopathic aetiology showed retarded motor development and two were floppy babies. Case 3 of Graham had eye signs similar to our two patients.

The limb girdle weakness in case 1 of the present family became the main complaint about six years ago. However it would appear that some limb girdle weakness could have been present much earlier and one could not state with certainty that as the infantile 'hypotonia' improved average muscle strength was established. The evolution of the floppy baby syndrome and the limb weakness in our two cases resemble most closely patients described by Batten (1915). In Batten's family six of 13 siblings, all in one generation, were affected and Turner (1940, 1949) and Turner and Lees (1962) in a long term follow-up have demonstrated that the patients were suffering from a non-progressive myopathy. The members of the family had features of amyotonia congenita in infancy changing to that of myopathy as the children grew older. The tendon reflexes were diminished in the affected cases. Follow-up over 45 to 50 years in three affected members (Turner and Lees, 1962) showed that the disease was not progressive. Our patients are too young to classify with certainty as belonging to the Batten and Turner group. Case 2 seems likely to be non-progressive but case 1 has shown evidence in recent years of more progression than the cases of Batten. Ophthalmoplegia, floppy baby syndrome, and limb girdle weakness has been described in myotubular myopathy (Spiro, Shy, and Gonatas, 1966). The histopathological features in our case do not resemble the changes described in myotubular myopathy.

The blood Kahn reaction was weakly positive in the presence of a negative Wassermann reaction in both siblings but this was not considered to be indicative of a luetic infection because the blood Wassermann and Kahn reactions were negative in the parents and the fluorescent treponemal antibody test was negative in the children. Further, the CSF in case 1 was normal when examined at birth and at age 7 years. The reason for the weakly positive Kahn reaction is unknown.

The clinical features in the two siblings in the present study thus represent a generalized myopathy and combine in a particular way characteristics of Möbius syndrome and congenital myopathy.

THE NATURE OF THE AMINOACIDURIA In case 1 the excess urinary excretion of lysine was similar quantitatively to that found in the incomplete recessive heterozygote cystinuric. The excess histi-
cystinuria or ridge histidine urinary transport histidine, lysine, acids in the kidney. In healthy controls, except in one child under 10 years of age reported by Sriver and Davies (1965). An abnormal renal clearance of histidine and glycine (also increased in case 1) has not hitherto been described in association with abnormal renal clearance of the dibasic amino acids. It is also notable that histidine has not been described as excreted in excess in patients with cystinuria, but one cystinuric has been reported in whom there was an abnormal clearance of glycine (Frimpter, Horwith, Furth, Fellows, and Thompson, 1962). Oral loading tests in the propositus suggested impaired absorption of lysine, histidine, and glycine across the gut wall. Impaired absorption from the gut rather than an abnormal plasma clearance of these amino acids is suggested in our patient because of the associated cell transport defect in the kidney. In conditions like cystinuria with a renal tubular cell transport disturbance, abnormal cell transport across the gut wall has been demonstrated (Milne, Asatoor, Edwards, and Loughridge 1961; Milne, 1967). A gut absorption defect to histidine and glycine is not a feature either of cystinuria or of histidinaemia.

The response in the boy to neomycin therapy, low protein and to low muscle fibre (low histidine) diets demonstrated that a peculiarity in dietary habits as a cause of the aminoaciduria was unlikely. The urinary excretion of histidine in case 1 was generally in excess of the upper limit of normal by other authors. Even on a low protein diet histidine was excreted in substantial quantities in cases 1 and 2. 1-Methyl histidine, although excreted in high concentration in the urine on an average protein intake, disappeared to trace quantities on a muscle free diet and was markedly reduced on a low protein diet and also when neomycin was given. This suggests the 1-methyl histidine in the urine was of exogenous origin. The ratio of 1-methyl histidine to histidine in the urine was less than unity and so was not like the abnormal amino acid findings in the urine of patients with psoriasis (Hubbard, Steele, Spear, and Block, 1962; Curtis and Block, 1964), a disease of which our patient had two episodes in the past. It was shown by per oral loading tests in the propositus that there was no absorption defect of glucose, D-xylose, cystine, methionine, leucine, glycine, vitamin B₁₂, and folic acid. However, the abnormal oral loading tests to lysine, histidine, and glycine in case 1 (the amino acids for which there was increased renal clearance) cannot be taken as proof of a cell transport defect across the gut. The family reported by Perheentupa and Visakorpi (1965) is particularly relevant in this respect. These authors originally described defective absorption of lysine and arginine in familial protein intolerance. Later Kekomäki (1968) demonstrated in the same patients that there was no impairment in vitro of lysine or arginine uptake using biopsy specimens obtained from the jejunum. Therefore factors other than cell transport may operate.

There was no evidence of a general impairment of intestinal absorption. The serum alkaline phosphatase was somewhat elevated in the children but the significance of this finding at puberty is uncertain (McGeown, personal communication). There was no clinical or radiological evidence of bone or renal disease. Therefore any cell transport defect present in the gut and in the kidney must be an highly selective one.

A POSSIBLE RELATIONSHIP BETWEEN THE AMINOACIDURIA AND THE MYOPATHY Excess aminoaciduria has been noted previously in muscular dystrophy but it has usually been regarded as either an inconstant finding or a non-specific generalized increase in urinary amino acids because of muscle wasting. However, Hurley and Williams (1955) and Blahd, Bloom, and Drell (1955) among others have described significantly increased urinary amino acids in such patients. In muscle disease increased creatinuria blocking amino acid reabsorption (Pitts, 1943) could explain aminoaciduria in some instances. In one of our patients (case 1) there was a moderate creatinuria with a renal clearance of creatine of 182 ml./min. However such a creatinuria is unlikely to lead to the type of aminoaciduria found in case 1 with a greatly increased excretion of only two amino acids while the other amino acids were excreted in normal quantities. There was no creatinuria in the mother of our cases, yet she had an excess excretion of lysine in the urine. Berger (1962) has found a partial or universal hyper-aminoaciduria at some time in the course of pseudohypertrophic muscular dystrophy.

In recent years aminoacidurias with a predominant excretion of lysine have been described. Hurwitz, Lyttle, and Neill (1965) and Hurwitz et al. (1967) described a family with facio-scapulo-humeral dystrophy, where an aminoaciduria essentially involving lysine (quantitatively greater than in the present cases) and to a lesser extent cystine, ornithine, and arginine was found. In one of these patients with dystrophy and aminoaciduria (case Π₁), an oral loading test with L-lysine suggested an intestinal absorption defect, while a lysine loading test in a sister, Π₅, with muscular dystrophy...
but no aminoaciduria showed normal absorption. Renal clearance for lysine and ornithine was also abnormal in two patients with aminoaciduria and normal in two patients without aminoaciduria. The authors, while considering that a relationship of the aminoaciduria to the dystrophy was unlikely, postulated that a possible inter-relationship lay in a disturbance of cell-transport mechanisms affecting some essential muscle metabolite. Although the aminoaciduria and the muscular dystrophy segregated independently, the inheritance of both traits was compatible with an autosomal dominance. The inheritance and the pattern of amino acid excretion and the results of oral loading tests to L-lysine in the family of Hurwitz et al. (1967) accord with the inherited disorder of amino acid transport described later by Whelan and Scriver (1968). In a family of 33 members reported by Whelan and Scriver, 13 had the aminoaciduria and the proband was said to be of small stature and to have symptoms compatible with a mild malabsorption syndrome. Gross, Comfort, and Ulrich (1958) described hyper-lysinuria in association with the hereditary form of chronic pancreatitis and Fleming, Avery, Morgan, and Cone (1963) described gastrointestinal malabsorption associated with a basic aminoaciduria. A defect in the intestinal absorption of lysine was shown by lysine oral loading. In a syndrome described by Clara and Lowenthal (1965) four of five children in the family were affected and showed hypotonia at birth, delayed motor development, dwarfism, failure to gain weight normally, and decreased circumference of the skull. Only the hypotonia had improved clinically. The four affected children had a similar pattern of aminoaciduria as the family described by Hurwitz et al. (1967), while the parents and unaffected eldest brother had no excess aminoaciduria. Another pattern of aminoaciduria has been described in a family by Rowley, Mueller, Watkin, and Rosenberg (1961), with increased urinary excretion of threonine, serine, lysine, arginine, and tyrosine. This aminoaciduria was found in the parents and other relatives as well as in the affected family members, who had muscle weakness, growth retardation, minimal osteoporosis, and severe cor pulmonale.

In considering the relationship between aminoaciduria and myopathy it seems unlikely that the loss to the body of the excreted amino acids is playing a major part. Thus in cystinuria, where there is much greater loss than in the present cases of the essential amino acid lysine and of cystine, a muscular lesion has not been described in the reviews by Stein (1951), Knox (1960), Harris (1963), and Milne (1967).

In rabbits with vitamin E deficiency, Fink, Williams, and Fink (1959) have described 1-methyl-histidinuria. These authors argued that a possible cause of myopathy in this deficiency was the loss to the body of its reserve of histidine which would be protected by giving vitamin E supplements. In our case 1, 1-methyl histidine was often noted in excess.

One should consider also a structural lesion in the gut from involvement of smooth muscle in muscular dystrophy and Harvey, Sherbourne, and Siegel (1965) have described such lesions in dystrophia myotonica.

A urinary amino acid pattern similar to that in the present patients has therefore been reported not only in patients with myopathy alone or in association with many inherited defects including muscular weakness but also in patients with differing types of intestinal absorption without clinical myopathy. In the present patients we consider that either the myopathy and the aminoaciduria are coincidental traits or that the relationship is a genetically determined defect in a cell transport mechanism either in the gut, kidney or muscle, varying in expression in different members of the family.

GENETIC ASPECTS Both children (III, and III) in this two sibship family, had congenital ophthalmoplegia and limb girdle muscle weakness. The pedigree is consistent with an autosomal recessive mode of inheritance. In the mother, the electromyography of the deltoid muscle was suggestive of a myopathy, which would also be consistent with recessive inheritance. However, electromyography is not usually a satisfactory way of recognizing the carrier state (Caruso and Buchthal, 1965; Gardner-Medwin, 1968).

With reference to the aminoaciduria, the lysine pattern of excretion was present in the parents and the children but not in 14 other relatives in whom urine was examined by paper chromatography. In the father, the amino acid excretion was within normal limits but the excretion pattern was similar to that of his wife and children. Thus it would appear that in both parents, who are probably heterozygous for the gene for myopathy, and in the affected children, there is an unusual amino acid excretion pattern for which the parents might be considered heterozygous and the children homozygous or heterozygous with full penetrance. The genes for myopathy and aminoaciduria are segregating together but there is not enough evidence from the family studies to state whether one or two genes are involved.
SUMMARY

In a two sibship family the propositus (case 1) a boy aged 15 years and his sister (case 2) aged 11 years have severe limitation of ocular movement which probably is congenital and of myopathic aetiology. Both were floppy babies with delayed motor development. Case 1 has developed moderate to severe pelvic girdle weakness in the past six years, and case 2 has slight pelvic girdle weakness. Electromyographic and histopathological evidence is presented which suggests a myopathic aetiology for the limb girdle weakness and it is considered that the floppy baby syndrome was a manifestation of congenital myopathy. The parents showed no clinical abnormality, although the mother had some EMG changes of a myopathic type.

The patients and the parents had a similar pattern of urinary amino acid excretion. There was an excess urinary excretion of lysine and histidine in case 1 and on occasions of lysine in case 2 and the mother. There was an abnormal renal clearance of lysine, histidine, and glycine in case 1, of glycine in case 2, of glycine and lysine in the mother, and histidine in the father. Oral loading tests with L-lysine, L-histidine, and glycine in the propositus suggested impaired intestinal absorption of these amino acids. Estimation of urinary amino acids in the patients after neomycin therapy and with various diets did not suggest any dietary cause for the aminoaciduria. The ophthalmoplegia and limb girdle myopathy and aminoaciduria are each compatible with an autosomal recessive inheritance. Although it is considered unlikely that there is a direct aetiological relationship between the myopathic features and the aminoaciduria, a possible inter-relationship is considered.

We are grateful to Mr. Charles Maguire and to Mr. Gordon Smyth for ophthalmological and otological opinions, and to Dr. N. Nevin for advice on the genetics. The biochemical aspects of this investigation formed part of the work being carried out under a grant from the Medical Research Council to the Department of Child Health of the Queen's University of Belfast, for the study of inborn errors of metabolism. We wish to thank the Muscular Dystrophy Group of Great Britain for secretarial help.

REFERENCES


— (1949). On amyotonia congenita. Ibid., 78, 25-34.


L. J. Hurwitz, Nina A. J. Carson, Ingrid V. Allen, and J. S. Chopra

APPENDIX

FOOD INTAKE OF CASE 1 (J.F.)

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<tr>
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Home diet

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AMINO ACID COMPOSITION OF FOOD (g/DAY) OF CASE 1 (J.F.)

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Father's Home diet

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Taken from the Dietetic Department, Royal Victoria Hospital, Belfast—F. Kathleen Acheson, S.R.N., S.R.D. (chief dietitian).
L J Hurwitz, N A Carson, I V Allen and J S Chopra

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