THE VARIABILITY OF POMPE'S DISEASE

A clinical, biochemical and genetic study of glycogen storage disease type 2, or acid maltase deficiency.

PROEFSCHRIFT

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Aan mijn ouders
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J.C. POMPE 1901-1945

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ABBREVIATIONS

AcP	: acid phosphatase
CPK	: creatine phosphokinase
ECG	: electrocardiogram
EM	: electronmicroscopy
EMG	: electromyography (or: electromyogram)
Gn	: glycogen
LCM	: lower costal margin
MCV	: motor conduction velocity
MU-glucoside	: 4-methylumbelliferyl-α-glucoside
PAS	: periodic acid Schiff
pNP-glucoside	: para-nitrophenyl-α-glucoside
TLC	: thin-layer chromatography (or: chromatogram)
UDPG	: uridine diphosphate glucose

GENERAL INTRODUCTION

Pompe's disease is an autosomal recessive metabolic disorder, characterized by storage of glycogen in organs and tissues, and associated with deficiency of the lysosomal enzyme acid maltase (acid α-1, 4-glucosidase, E.C. 3.2.1.20). Synonyms for Pompe's disease are "glycogen storage disease type 2" and "acid maltase deficiency". Several clinical variants have become known. In this thesis a *generalized form* and a *muscular form* are distinguished.

The *generalized form* is a progressive disease of infancy. It is characterized by a failure to thrive, cardiorespiratory difficulties and weakness with hypotonia, and leads to death within the first two years of life. The *muscular form* may occur in childhood or even in adults. It causes a slowly progressive myopathy which is ultimately fatal, usually because of respiratory insufficiency. Despite the wide variability in clinical manifestations, one and the same enzyme, lysosomal acid maltase, is deficient in both forms.

The present study was prompted by two circumstances. The first was an exceptional combination of both clinical forms in one family: an infant suffered from the generalized form and her grandfather form the muscular form. The second reason was that data of 20 patients with this rare disorder (seven with the generalized form, and 13 with the muscular form) were available for study. This series consisted of patients known to the pediatrician Fernandes and the biochemist Koster in Rotterdam, both of whom have a special interest in the field of the glycogenoses, and of patients diagnosed by the neurologists Bethlem (Amsterdam), Busch (Rotterdam) and Jennekens Utrecht.

The first aim of the study was to collect the clinical, morphological and biochemical findings in these patients and to supplement these where possible. Analysis of a series of this size might give little more insight in some unsolved problems, such as a possible explanation of the muscular form by the presence of a residual enzyme activity, and the relation between glycogen accumulation and weakness. Moreover, pedigree studies

might provide more information on the genetics of the disease. The second objective was to test the feasibility of heterozygote identification. For this purpose, 21 parents and 10 siblings of patients could be investigated. The emphasis was on the biochemical study of the urine, a sample with obvious practical advantages. To this end, Schram and Tager (Laboratory of Biochemistry, B.C.P. Jansen Institute, Amsterdam) developed an immunological assay of acid maltase, and Blom (Laboratory of Metabolic Diseases, Sophia Children's Hospital, Rotterdam) performed thin-layer chromatograms of oligosaccharides. Moreover, in some cases activity of acid maltase was assayed in skeletal muscle, fibroblasts and total leucocytes. An additional question in the study of the relatives was wheter the same combination of the muscular form and the generalized form might be present as in the family mentioned above. For this purpose, 13 grandparents of five infants with the generalized form were examined.

The final aim of this study was to obtain more insight into the clinical variability of acid maltase deficiency and into the genetic relationship between the two forms of the disease by studying the family in which both clinical forms occurred as an "experiment of nature".

1 A SHORT HISTORY OF GLYCOGEN STORAGE DISEASE TYPE 2 NOW KNOWN AS ACID MALTASE DEFICIENCY

J.C. Pompe's discovery

J.C. Pompe, pathologist in Amsterdam, deserves the honour of having postulated in 1932 the existence of a new form of glycogen storage disease. He proposed the following terms: "Cardiomegalia glycogenica", in German: "Glycogenspeicherkrankheit des Herzens" or, because of the generalized character of the glycogen storage, "Glycogenspeicherkrankheit".

On December 27th, 1930, he performed a post-mortem examination on a seven-month old girl who had died after an illness of four days' duration. The child had been reported to be normal before the onset of the illness and was presented with fever and respiratory difficulties. On examination she looked seriously ill, had a greyish-pale colour and was inactive. Percussion of the heart was normal, but auscultation revealed only soft sounds. Pulmonary examination showed a dull percussion and soft breathing sounds on the left side. The liver was slightly enlarged. She died on December 26th. The diagnosis was pneumonia.

The post-mortem examination showed an enormously enlarged heart, weighing 190 grams (normal 36 grams). There were no congenital defects of the heart, of the coronary system or of other vessels. The lungs showed an atelectasis of the left lower lobe and purulent bronchitis, bronchiolitis and pneumonia in other parts of the left lung. The abdominal organs were normal. The brain was not examined. The diagnosis was: idiopathic cardiac hypertrophy with bronchitis, bronchiolitis and bronchopneumonia. The heart was kept in formalin and after two days the first frozen sections were made. To Pompe's surprise he found no recognizable cardiac tissue. Instead, he observed a network of round or oval meshes with scattered nuclei, but on higher magnification the walls of the meshes proved to be cross-striated. He concluded that he was dealing with a vacuolar alteration of the cardiac muscle fibres. A negative reaction with Sudan III ruled out that the vacuoles were filled with fat. Remembering that the vacuoles resembled cardiac Purkinje fibres, he examined the vacuolar content for glycogen. Best's

stain demonstrated a strongly positive reaction, which was the proof of glycogen storage. Large quantities of glycogen were also found in the liver, the spleen, the kidneys, the adrenals, the thyroid gland and the skeletal muscles. Pompe decided to make a descriptive diagnosis of: " ...glycogen infiltration in virtually all organs, accompanied by excessive enlargement of the heart. He interpreted his findings in terms of a disturbance of glycogen metabolism.

One year before Pompe's discovery, von Gierke (1929) had described an eight-year-old patient with glycogen storage in the liver and kidneys ("Hepatonephromegalia glycogenica" or "Glycogenspeicherkrankheit der Leber und Nieren"). An important difference between von Gierke's and Pompe's patient was that the heart of von Gierke's patient was not enlarged (80 grams) and that the glycogen storage was confined to the liver and the kidneys.

It should be mentioned that a few months after Pompe's first publication in 1932, W. Putschar, a German pathologist, had also described a patient with glycogen storage of the heart. Pompe claimed priority in his thesis "Cardiomegalia Glycogenica" (1936). Strictly speaking, Pompe was entitled to the precedence, though his first publication in 1932 was in Dutch and in small print, being a report of a meeting of the "Genootschap ter bevordering van de Natuur-, Genees- en Heelkunde". The next year his discovery became more accessible by his paper in the French Journal "Annales d' Anatomie Pathologique" (Pompe, 1933).

A metabolic disease?

The pathogenesis of "idiopathic cardiac hypertrophy" had for a long time been a matter of discussion (Pompe, 1936). Virchow (1864) supposed that idiopathic cardiac hypertrophy was the result of a diffuse benign tumour growth (rhabdomyomatosis) and this view gained general acceptance. On the third meeting of the German Society of Pathology in 1900 Seifert demonstrated a case of idiopathic cardiac hypertrophy and wondered what the content of the vacuoles could be: fat, fluid? In the discussion Marchand suggested that it might be glycogen and Askenazy claimed to have seen glycogen in the form of large spheroids in an identical case. One should realize that it was not before 1903 that Best introduced a reliable method of staining glycogen.

The Russian pathologist Abrikosov found glycogen with Best's stain in a case of cardiac rhabdomyoma in 1919. He did not recognize the significance of his discovery, perhaps because the time had not yet come to leave

Virchow's tumoral explanation in favour of the metabolic concept of Garrod (1909 and 1923).

Von Gierke and Pompe, in co-operation with experts in biochemistry, discovered glycogen storage as the cause of the disease. Schönheimer (1929) demonstrated in von Gierke's patient that the glycogen content of the liver did not decrease after six days of incubation in a solution of NaCl and chloroform at 37° C. Under the same conditions, normal liver tissue could be shown te degrade four fifths of its glycogen within three days. Therefore, Schönheimer and von Gierke concluded that a glycogen degrading ferment might be absent in the liver of their patient. Snapper and van Creveld (1928) had arrived at the same conclusion by in vivo studies of carbohydrate metabolism in a patient with hepatic enlargement: glycogen had accumulated in the liver, but could be mobilized only with great difficulty.

Pompe's case had been under the clinical care of Professor Snapper. Snapper and particularly his co-worker, the pediatrician van Creveld, were pioneers in the field of the glycogenoses. Reading Pompe's thesis one might presume that especially van Creveld stimulated him to postulate a metabolic cause for the glycogen storage in the patient. And perhaps van Creveld should be considered as the chief promoter of the eponym "Pompe's disease".

Thus, around 1930, the concept emerged of glycogen storage being an inborn error of glycogen metabolism. Two different forms were distinguished: one, described by von Gierke, with glycogen storage in the liver and kidneys, and another, described by Pompe as well as by Putschar, with a generalized accumulation of glycogen and excessive enlargement of the heart.

Other types of glycogen storage disease

According to Garrod's concept of Inborn Errors of Metabolism (1909 and 1923) the accumulation of glycogen might be explained in terms of a block of its breakdown due to the absence of one or more enzymes. The first evidence in this direction had already been given by Schönheimer (1929) in his elegant experiments.

In 1952 G.T. Cori and C.F. Cori demonstrated an extremely low activity of the enzyme glucose-6-phosphatase in the liver of two patients with von Gierke's disease. In 1957 G.T. Cori was able to list four distinct types of glycogen storage disease (table 1). In three of these the associated en-

Table 1. Types of glycogen storage disease + the underlying enzyme deficiencies (G.T. Cori, 1957).

Type	Organ involvement	Enzyme deficiency
1	liver and kidney	glucose-6-phosphatase
2	generalized	?
3	liver, heart, muscle	debrancher enzyme
4	liver, probably other organs	brancher enzyme

zyme deficiency had been discovered, but the enzyme deficiency in Pompe's disease (glycogen storage disease type 2) remained as yet unknown. In this connection she speculated: " ...It is still possible that this type of storage is due to the genetic loss of an enzyme, an enzyme which so far has evaded detection".

The enzyme deficiency in Pompe's disease

In 1955 de Duve and his co-workers introduced the concept of "lysosomes", being intracellular granules, rich in hydrolytic enzymes. They suggested that lysosomes might be involved in intracellular digestion at acid pH and that they might have little to do with major metabolic processes.

In 1963 Lejeune et al. demonstrated by tissue fractionation studies that lysosomes in the liver of the rat contain an acid maltase. They also discovered the presence of a neutral maltase in the soluble and microsomal tissue fraction. In the same year Hers described the occurrence of the lysosomal enzyme acid maltase (acid α-glucosidase) in normal human tissues as well as the absence of this enzyme in the liver, the heart and the skeletal muscles of patients with Pompe's disease. His was the first description of an inborn error resulting from a deficiency of a lysosomal enzyme.

Ultrastructural studies in liver tissue of patients with Pompe's disease by Baudhuin et al. (1964) demonstrated that the storage of glycogen was present mainly within the lysosomes.

It seemed that with these findings the pathogenesis of Pompe's disease had been largely resolved:" ...the pathological manifestations are not due to the enzyme defect itself but rather to the progressive glycogen deposition, which causes the disruption of the muscular fibres" (Hers, 1963).

Clinical variants

Patients with weakness caused by glycogen storage within the skeletal muscles but without enlargement of the heart had been described by Gunther (1939), Zellweger et al. (1955), and Gutmann (1960). The existence of an enzyme defect in those "atypical" cases was still unknown. In 1963 a similar case was reported by van Creveld: an 18-month-old girl had progressive muscle weakness and hepatomegaly but no cardiomegaly. A muscle biopsy showed marked degeneration and vacuolization of the muscle fibres. A diagnosis of glycogen storage disease of the muscle was made. Van Creveld carefully excluded all known types of glycogen storage disease by performing tolerance tests. Additional enzyme studies by Hers on a liver biopsy were normal, except for acid maltase activity, which was absent (this patient was presented as case 5 in Hers' publication of 1963). This was the first description of a patient suffering from the muscular form of acid maltase deficiency in whom the diagnosis was confirmed by enzyme studies.

Two years later acid maltase deficiency was described in juvenile patients by Courtecuisse et al. (1965) and by Zellweger et al. (1965). The patient reported by Courtecuisse et al. was examined at the age of 14 years because of a slowly progressive weakness from the age of four. Zellweger et al. described two brothers, one 15 years old with mild and slowly progressive weakness from infancy, the other four and a half years old with mild weakness of the trunk and pelvic muscles. The muscle biopsy of these three patients showed a vacuolar myopathy. On electronmicroscopy the vacuoles proved to contain membrane-bound accumulations of glycogen. Acid maltase was decreased in muscle tissue.

That acid maltase deficiency may be found even in adulthood was demonstrated by Engel and Dale (1968) and by Hudgson et al. (1968). Their patients also presented a slowly progressive weakness, which was originally diagnosed as limb girdle dystrophy, Biopsies from skeletal muscles showed vacuoles filled with glycogen. Biochemical studies demonstrated a distinct decrease in activity of acid maltase in muscle tissue.

Thus, glycogen storage associated with acid maltase deficiency appears to be a disorder that may be present at any age. In infants the disease has a generalized character; in older patients the disease seems to be limited to the skeletal muscles. All aspects of the disorder at different ages are summarized in two excellent reviews: "Glycogen storage disease of the heart" (Di Sant' Agnese et al., 1950) and "The spectrum and diagnosis of acid maltase deficiency" (Engel et al., 1973) It should be mentioned that Di Sant'Agnese

et al. (1950) based the diagnosis only upon clinical criteria and the demonstration of glycogen because the deficiency of the enzyme acid maltase had not yet been demonstrated.

More than ten years after the first description of the clinical and laboratory features of patients with the muscular form (van Creveld, 1963; Courtecuisse et al., 1965; Zellweger et al., 1965; Engel and Dale, 1968; Hudgson et al., 1968) the results of the post-mortem examination in two patients were reported (Martin et al., 1976a; DiMauro et al., 1978). Glycogen storage could be demonstrated only within the skeletal muscles by light-microscopial examination, and within several cell types of the skin by electronmicroscopical examination. It was not found in the heart, the liver, the kidneys, or in the central nervous system. On the contrary, biochemical analysis demonstrated a severely reduced activity of acid maltase not only in muscle tissue and in cultured fibroblasts, but also in the heart, in the liver, in the bladder, and in several parts of the central nervous system. These two post-mortem studies indicate the generalized nature of the enzyme deficiency in patients with the muscular form. They do not explain the curious fact that the glycogen storage is nevertheless largely restricted to the skeletal muscles.

Genetics

The delineation of the enzyme defect in various types of glycogen storage disease is the basis for classification in types 1 - 8. Table 2 summarizes clinical and biochemical data and data on genetics. An autosomal recessive mode of inheritance has been suggested for most types. In types 2 and 5 an as yet unexplained preponderance of male patients has been reported (Sidbury, 1967; Huying, 1975), in type 3 an unexplained excess of female patients (Spencer-Peet et al., 1971; Huying, 1975). Autosomal dominant inheritance was demonstrated in one family for type 5 by Chui and Munsat (1976), others (Dawson et al., 1968) reported an autosomal recessive inheritance. An X-linked inheritance for type 8 has been described in a large family by Huying and Fernandes (1969).

Facts and missing links at present

Facts

1. There are two main clinical forms of glycogen storage associated with acid maltase deficiency:
   a. *The infantile form* with a uniform clinical picture (see chapter 2). The patients die in their first or second year from cardiorespiratory failure.

Table 2. Types of glycogen storage disease at present.*

Type and eponym	Clinical and laboratory features	Enzyme deficiency	Estimate of incidence among newborns	Inheritance
1 (von Gierke)	hepatomegaly, severe hypoglycaemia, convulsions, growth retardation, tendency to lactic acidosis progressive course, in 50% early mortality	glucose-6-phosphatase	1/200,000	autosomal recessive
2 generalized form (Pompe)	cardiomegaly, hepatomegaly, enlarged tongue, hypotonia, weakness, cardiorespiratory failure progressive course, death in first or second year of life	acid maltase	see chapter 5	autosomal recessive; male preponderance
muscular form	weakness of limb girdle and of trunk muscles slowly progressive course	acid maltase	?	autosomal recessive
3 (Cori-Forbes) subtypes A-F	hepatomegaly, hypoglycaemia, weakness, growth retardation (moderate) fairly mild course	debranching enzyme system	1/200,000	autosomal recessive; female preponderance
4 (Andersen)	liver cirrhosis, weakness of skeletal and respiratory muscles, failure to thrive death in second year of life	branching enzyme	extremely rare	autosomal recessive
5 (McArdle)	cramps after exercise, weakness, lack of increase of venous lactate level after ischaemic exercise onset in second or third decade; mild course †	muscle phosphorylase	rare	autosomal recessive, or dominant; male preponderance
6 (Hers)	hepatomegaly (resembles mild form of type 3) mild course	hepatic phosphorylase	?	autosomal recessive
7 (Tarui)	like type 5	muscle phosphofructokinase	extremely rare	autosomal recessive
8	hepatomegaly mild course	hepatic phosphorylasekinase	1/100,000	X-linked recessive

* Sidbury (1967), Huying (1975), Howell (1978), McKusick (1978).

† A fatal infantile form of myophosphorylase deficiency was recently reported by DiMauro and Hartlage (1978).

Most organs show a massive accumulation of glycogen, especially the heart and the skeletal muscles. Acid maltase is absent or extremely low in all tissues.

b. The form with *later onset* in which the symptoms and signs are confined to the skeletal muscles. The clinical picture is variable with regard to the age at onset, the severity of the weakness, and the amount of glycogen stored. The patients die many years after the first symptoms, most frequently from respiratory failure. Vacuoles filled with glycogen may be found in a muscle biopsy and acid maltase activity is abnormally low in skeletal muscle tissue.However, it appears from two post-mortem studies that acid maltase activity is also decreased in the heart, but without glycogen accumulation (Martin et al., 1976a; DiMauro et al., 1978).

2. The activity of the enzymes involved in the extra-lysosomal degradation of glycogen is normal in patients with either form of acid maltase deficiency (Hers, 1963; Hudgson et al., 1968; Engel, 1970).

Missing links

The problems concerning the clinical and biochemical heterogeneity of glycogen storage diseases of muscles have been lucidly discussed by Rowland (1971). Some interesting points about acid maltase deficiency are the following:

1. The infantile and late-onset patients show such a different clinical picture that it is hard to assume that they suffer from the same disease.
2. There is no well-defined biochemical basis for the clinical heterogeneity. It has been suggested that a *residual* acid maltase activity might be the reason for the later onset and the milder course in some cases or that *neutral* maltase plays a compensatory role (Angelini and Engel, 1970). DiMauro et al. (1977) suggested that neutral maltase might be a precursor of acid maltase and that the two different clinical forms are due to different defects in the conversion of the neutral to the acid enzyme. One might even ask whether the lack of acid maltase is really the primary abnormality in the disease or merely the consequence of an as yet unknown, more basic defect.
3. The pathogenesis of acid maltase deficiency is ill-understood. Weakness is a predominant symptom in all patients. Muscular glycogen accumulation is abundant and distorts the normal architecture of the muscles in classical Pompe's disease. Glycogen excess is also found in the anterior horn cells of the spinal cord. Both findings provide an acceptable explanation for the weakness in the infants. On the other hand, in the late-onset

patients the cause of the weakness is more difficult to understand. Since the increase of glycogen in the muscles of these patients is moderate or non-existent, the explanation cannot be merely mechanical. Furthermore, glycogen has not been found in the grey matter of the spinal cord (Martin et al., 1976a; DiMauro et al., 1978).
It is also difficult to understand why deficiency of this lysosomal enzyme may lead to such a disabling myopathy, because normal skeletal muscle tissue contains few lysosomes (Fischman et al., 1973) and because acid maltase has catalytic activity in an ill-understood pathway of glycogen degradation (Hers and van Hoof, 1973).

A large proportion of the accumulated glycogen is not within lysosomes, but free in the cytoplasm (Baudhuin et al., 1964; Hug et al., 1966; Garancis, 1968; Martin et al., 1973). Why do the normal phosphorolytic enzymes not degrade this cytoplasmic glycogen?

We can only conclude that acid maltase deficiency, a disorder that at first seemed to be fully explained as a storage disease due to deficiency of a particular lysosomal enzyme, has now turned into a disease that still presents many puzzling features.

2 REVIEW OF BIOCHEMICAL, CLINICAL AND GENETIC FEATURES OF ACID MALTASE DEFICIENCY

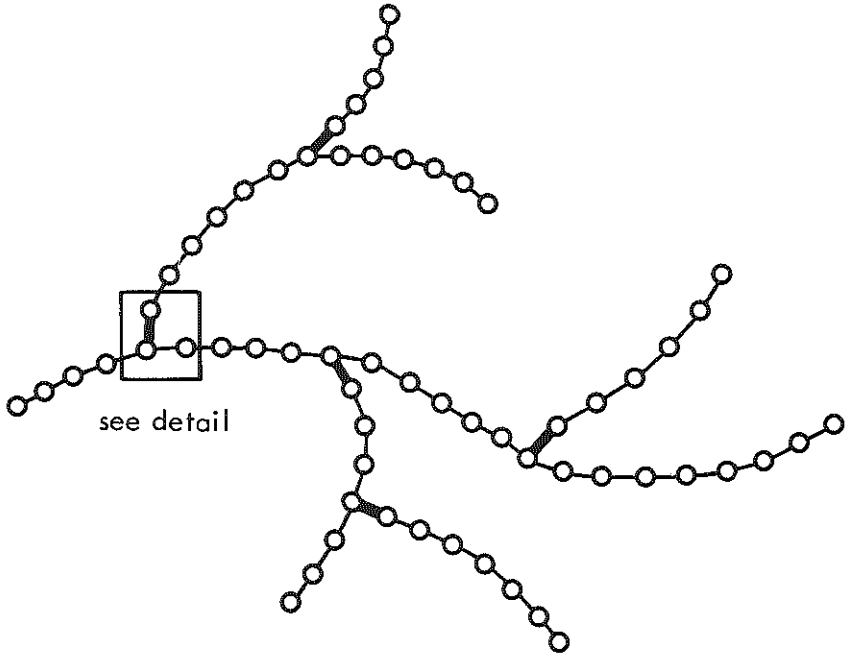
Glycogen metabolism

Glycogen is a polysaccharide with a molecular weight varying from six to several hundreds of millions, depending upon the type of tissue and the method of extraction. Its molecules have a spherical form. They consist of D-glucose molecules, which are attached to each other by 1,4 or 1,6 linkages (figure 1).

In glycogen synthesis, units of four to ten glucose molecules are linked by 1,4 bonds. This is catalyzed by the enzyme glycogen synthetase. The newly formed chains are then sideways attached to each other by 1,6 bonds by the branching enzyme which results in the multibranched glycogen molecule.

The degradation of glycogen takes place along two different pathways: a major pathway by *phosphorolysis*, which occurs in the cytoplasm, and a minor pathway by *hydrolysis*, which takes place in the lysosomes.
In the process of *phosphorolysis* the non-reducing terminal glucose molecules are split off by phosphorylase in the presence of inorganic phosphate (Pi), to produce glucose-1-phosphate. At the branching points glucose molecules are released under the action of the debranching enzyme system. Glucose-1-phosphate is either oxidized to pyruvic acid and lactic acid via the glycolytic pathway, or it is transformed to uridine diphosphate glucose (UDPG) to be used for the synthesis of new glycogen molecules (figure 2). Usually, glycogen molecules are incompletely degraded. A nucleus of glycogen remains present to act as a primer for the rebuilding of glycogen molecules. The synthesis and breakdown of glycogen in the liver are under strict control of activating and inhibiting enzymes and hormones (Hers et al., 1970). When glycogen breakdown prevails, glycogen synthesis is suppressed by a feedback mechanism. In skeletal muscles the metabolism of glycogen is much more under the control of local influences.
Glycogen breakdown by *hydrolysis* takes place by the action of the lysosomal enzyme acid maltase. The enzyme shows affinity to both 1,4 and 1,6

bonds of the glycogen molecule and it is therefore capable of degrading glycogen that has entered into the lysosomes by the process of autophagy. The functional importance of this pathway in skeletal muscle is not clear, especially in man. In newborn rats, Schiaffino and Hanzlíková (1972) demonstrated that glycogen in skeletal muscles was digested in autophagic vacuoles. The rate of digestion appeared to be related to the degree of maturity of the muscles and was not affected by denervation.



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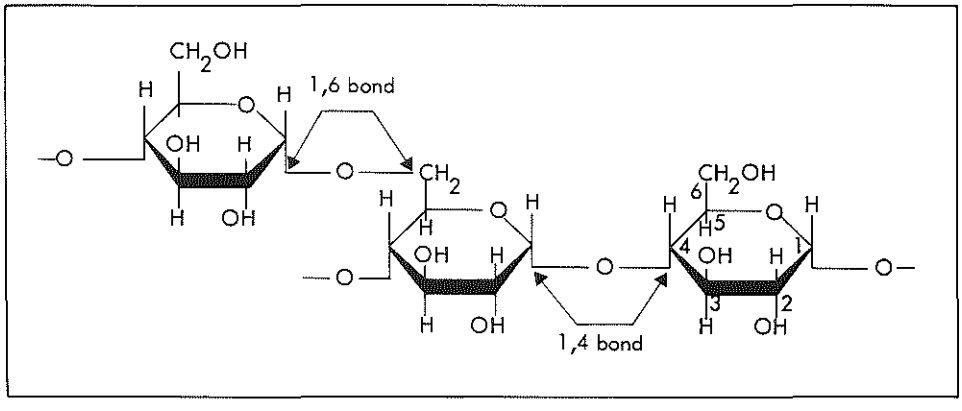


Figure 1. 1,4 (o-o) and 1,6 (o=o) bonds of glucose molecules in glycogen.

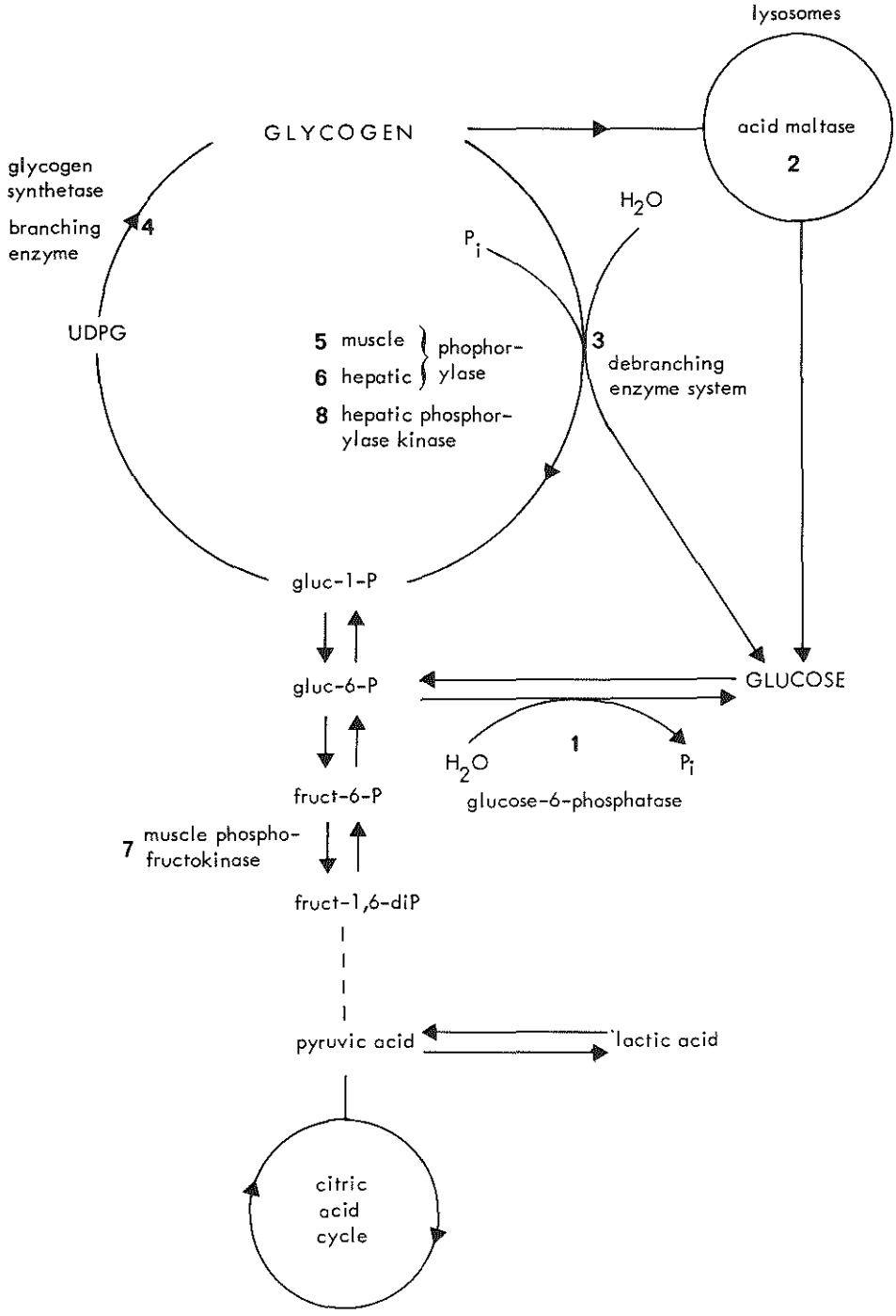


Figure 2. Simplified scheme of glycogen metabolism. The enzyme deficiencies in the different types of glycogen storage diseases (1-8).

Glycogen storage disease type 2 is the only type in which the deficient enzyme is of lysosomal origin. In all other types of glycogen storage disease the deficiency affects one of the extralysosomal enzymes of the phosphorolytic pathway.

Acid and neutral maltase

Acid maltase activity has been demonstrated in most human tissues and body fluids, including the liver, the heart and skeletal muscles (Hers, 1963), leucocytes (Huying et al., 1963), urine (Franzini and Bonini, 1967), cultivated fibroblasts (Nitowsky and Grunfeld, 1967), amniotic fluid cells (Nadler and Messina, 1969) and the placenta (de Barsy et al., 1972). Acid maltase has a pH optimum between 4.0 and 4.5. Its molecular weight is about 10^5 (Jeffrey et al., 1970).

The activity of acid maltase can be measured with glycogen or maltose as a substrate, or with artificial substrates like 4-methylumbelliferyl-α-glucoside (MU-glucoside) or para-nitrophenyl-α-glucoside (pNP-glucoside).

The interpretation of the results is complicated by the presence of a neutral maltase in most human tissues and body fluids (Illingworth Brown and Brown, 1965). Neutral maltase has also considerable activity at acid pH. It is located in the microsomal fraction of tissue cells and its physiological role is unknown. In vitro it hydrolyses maltose and artificial substrates but it is reported to have a low activity towards glycogen (Ushakova and Lukomskaya, 1976).

Clinical manifestations

As indicated in chapter 1, the clinical features of acid maltase deficiency are quite heterogeneous.

In the *classical generalized form*, first described by Pompe and comprehensively reviewed by Di Sant'Agnese et al. (1950), the symptoms start in the first months of life. The infants may present feeding difficulties, failure to thrive, attacks of dyspnoea, perioral cyanosis particularly during feeding, or weakness with hypotonia. On examination they often look severely ill. They have a greyish-pale or pale colour and are dyspnoeic. The tongue may be enlarged. Crying is weak and the patients are hypo-active. On percussion the cardiac dullness may be found markedly enlarged and sometimes there is even a left-sided prominence of the thoracic wall. In some cases a systolic murmer is heard over the base of the heart. The liver may also be enlarged. If profound weakness and hypotonia are present, the

legs assume a "frog posture". On palpation the muscles may feel firm. Most patients die in their first year of cardiorespiratory failure.

In the *muscular form* no cardiomegaly is found and the presenting complaints are skeletal or respiratory muscle weakness. The symptoms appear in late infancy, childhood or adulthood. Weakness is most marked in the trunk and the proximal limb muscles. The respiratory muscles are frequently involved and eventually most patients die of respiratory failure, many years after the onset of the first symptoms (Engel et al., 1973, Gullotta et al., 1976).

In the literature a variety of terms are used for these two different clinical forms, according to the age at onset, the course of the disease, or the site of the main pathological findings. Table 3 summarizes these synonyms. Provisionally, we prefer to use the terms *generalized* and *muscular* forms of acid maltase deficiency, because these cover more accurately the clinical and morphological (but not the biochemical!) characteristics of the two variants.

Ancillary studies

Since the symptoms and signs are by no means specific, the following investigations will usually be performed before the measurement of acid maltase activity in tissues or body fluids is requested.

Roentgenogram of the chest

In patients with the generalized form, the roentgenogram of the chest shows that the heart is enlarged in all directions ("globular heart"). Cardiac enlargement may be so extreme that the heart occupies most of the left half of the thorax. The cardiac shadow may be obscured by pulmonary infections, which frequently occur.

In the cases with the muscular form no evidence of cardiac enlargement has been found by Engel et al. (1973) and Gullotta et al. (1976).

Electrocardiogram (ECG) and echocardiogram

The ECG of infants with acid maltase deficiency shows the following helpful characteristics (Caddell and Whittemore, 1962; Ehlers et al., 1962):

- a short PR interval, 0.05 – 0.09 seconds, (the normal value depends upon the age and the cardiac rate, but is > 0.12 seconds according to Rendle-Short et al., 1978) indicating an acceleration in the atrio-ventricular conductive system.
- high voltage QRS complexes, suggesting hypertrophy of the ventricular walls, the septum, or both.

– supraventricular tachycardia and other atrial arrhythmias also occur. The short PR interval appears to be the most specific ECG abnormality in the generalized form of acid maltase deficiency, although it may also be found in patients with glycogen storage disease type 3 (Dubowitz, 1978) and in patients with muscular dystrophy with cardiac involvement (Harris and Nghiem, 1972). The cause for the short PR interval in glycogen storage disease of the heart is not clear. It has been assumed that the increased glycogen content in the myocardium and in the atrio-ventricular bundle may accelerate the conduction (Caddell and Whittemore, 1962).

Echocardiography in patients with the generalized form has been reported in a few studies (Bloom et al., 1974; Rees et al., 1976). Thickening of the interventricular septum and the posterior left ventricular wall could reliably be demonstrated.

In the muscular form no characteristic abnormalities have been found on the ECG (Gullotta et al., 1976), while echocardiography has – to my knowledge – not been reported.

Electromyography (EMG)

In both the generalized and the muscular form electromyographic abnormalities can be found (Lenard et al., 1974). These indicate a myopathy in general. During voluntary contraction, the motor unit potentials are of low amplitude with normal or short duration and often with a complex form. On inserting or on moving the needle, abnormal irritability is seen. At rest, fibrillation potentials and positive denervation potentials may be recorded. Moreover, bizarre high frequency potentials are occasionally a striking phenomenon. These discharges lack the typical crescendo-decrescendo character of myotonic volleys and have therefore been called "pseudo-myotonic". Clinically, there is no myotonia in these patients. According to Engel et al. (1973) these bursts are recorded less often in patients with the muscular form, and preferentially in the paraspinal muscles.

Creatinephosphokinase (CPK)

Serum CPK has been found to be moderately elevated in all cases with either form of acid maltase deficiency (Engel et al., 1973; Gullotta et al., 1976).

Excretion pattern of oligosaccharides in urine

An abnormal amount of oligosaccharides can be found in the urine of patients with diseases that affect the metabolism of glycoconjugates (glyco-

Table 3. Synonyms for the clinical forms of acid maltase deficiency

according to:	generalized form	muscular form
1. age of onset	infantile form	late infantile, childhood, adult form
		late onset form
2. site of pathological findings	heart: cardiomegalia glycogenica (diffusa)	muscle: muscular form
	glycogen storage disease of the heart	pseudomyopathic form
	cardiac type of glycogen storage disease	
	Pompe's disease of the heart	
	generalized: generalized form	
	generalized glycogen storage disease	
	generalized glycogenosis	
3. course	severe form	mild form
1, 2 and 3	classical form	atypical form

proteins, glycolipids, polysaccharides), such as glycogen storage diseases, gangliosidoses, fucosidosis, mannosidosis, or I-cell disease (Sjöblad, 1977). To detect these oligosaccharides, thin-layer chromatography (TLC) of a urinary sample can be used as a simple screening procedure (Humbel and Collart, 1975). A more distinct separation of the oligosaccharides can be obtained after desalting of the urine (Friedman et al., 1978).

Sjöblad demonstrated the excretion of an increased amount of a glu-

cose-containing tetrasaccharide in the urine of patients with glycogenosis type 2, 3 or 6, and also in the urine of patients with classified (Duchenne muscular dystrophy) or unclassified myopathies. This tetrasaccharide was composed of three glucose molecules linked by 1, 4 bonds and a fourth attached to this chain by a 1,6 bond. This strongly indicates a glycogen origin. Sjöblad further pointed out the feasibility of the quantitative determination of this compound by means of gas-chromatography and mass-spectrometry.

Muscle biopsy

Morphological and histochemical studies in biochemically verified cases of acid maltase deficiency have shown characteristic changes in skeletal muscle (Engel and Dale, 1968; Hudgson et al., 1968; Engel, 1973; and Martin et al., 1976a). They consist of vacuolization of the muscle fibres due to abnormal accumulation of glycogen lying either free in the sarcoplasm or, more typically, sequestered in small, membrane-bound sacs. In addition, there is an increased number of autophagic vacuoles.

In the generalized form gross vacuolar changes are present in most muscle fibres, giving the appearance of a lace-work. In the muscular form similar changes are present, but to a lesser degree, and the biopsy may show only a small number of fibres with a few inconspicuous vacuoles. Acid phosphatase activity is increased in the abnormal regions, but also in the fibres showing no light-microscopic alterations.

Both fibre types are involved in most cases. In the muscular form type I fibres may be almost exclusively involved (Engel and Dale, 1968; Gullotta et al., 1976; Schlenska et al., 1976; Heene and Ernst, 1977; Karpati et al., 1977; and DiMauro et al., 1978). In view of the preferential glycolytic metabolism of the type II fibres it is surprising that a selective involvement of type II fibres has been reported only once (Horoupian et al., 1978).

A basophilic substance showing metachromasia has been noted in biopsies of patients with the generalized form (Engel et al., 1973). According to Martin et al. (1973) the composition of this material is closely related to that of glycogen.

"Globular structures" in type I fibres of a patient with the muscular form were reported by Horoupian et al. (1978). They proved to consist of large, complex aggregates of lysosomal profiles.

Ultrastructural examination of a skin biopsy

Hug et al. (1970) reported that the important ultrastructural clue to

the diagnosis in muscle tissue: membrane-bound vacuoles filled with glycogen particles, may also be found in many different cell types of a skin biopsy. Martin and Ceuterick (1978) described such findings in nine patients with different forms of acid maltase deficiency. The vacuoles were more numerous and easy to discover in young patients.

Enzyme studies

The investigations discussed so far may be suggestive, but confirmation of the diagnosis is, of course, based upon enzyme assays. Acid maltase activity can now routinely be measured in easily accessible samples such as leucocytes (Koster et al., 1974), urine (Mehler and DiMauro, 1976; Schram et al., 1979), muscle (Angelini and Engel, 1972; Mehler and DiMauro, 1977), and cultured fibroblasts (Reuser et al., 1978). Some studies seem to indicate that acid maltase activity is not detectable in patients with the generalized form, and decreased to 5-15 percent of the control values in patients with the muscular form (in muscle by Mehler and DiMauro, 1977; in fibroblasts by Reuser et al., 1978).

Differential diagnosis

In *infants* with an enlarged heart other conditions should be considered such as endocardial fibroelastosis, myocarditis, tumours of the heart, anomalous left coronary artery, and "idiopathic cardiac hypertrophy". It should be stressed that glycogen storage is a relatively rare cause of cardiac enlargement in infants (Di Sant'Agnese et al., 1950). However, a short PR interval on the ECG, combined with giant QRS complexes, is highly suggestive for glycogen storage disease type 2.

Hypotonia in infants, "the floppy infant syndrome", has many causes but the combination of hypotonia and weakness limits the differential diagnosis to a number of neuromuscular diseases as (neonatal) myasthenia gravis, infantile spinal muscular atrophy (Werdnig-Hoffmann disease) and congenital myopathies (Dubowitz, 1978). Various types of glycogen storage disease should also be considered (see chapter 1, table 2). Glycogen storage disease type 2 is highly probable if cardiomegaly is present.

Enlargement of the tongue may lead to a mistaken diagnosis of Down's syndrome or hypothyroidism.

The differential diagnosis of the *muscular form* of acid maltase deficiency includes other conditions with proximal muscle weakness such as various forms of muscular dystrophy, polymyositis, spinal muscular atrophy and myopathies caused by endocrine disorders or drugs (Gullotta et al., 1976).

Treatment

Various therapeutic strategies have been attempted in patients with acid maltase deficiency:

- stimulating glycogenolysis by epinephrine administration combined with carbohydrate restriction (Hug et al., 1966; Swaiman et al., 1968; Engel et al., 1973; Horoupian et al., 1978).
- labilization of lysosomes by vitamin A (Swaiman et al., 1968; Nevsimal and Kocura, 1973, (cited by Gullotta et al., 1976)).
- acid maltase replacement by intravenous administration (Baudhuin et al., 1964; Hug and Schubert, 1967; Lauer et al., 1968; de Barsy, 1976; Tyrell et al., 1976).

In patients with the *generalized form* none of these treatments has been successful.

In some patients with the *muscular form* some improvement in the strength of less severely affected muscles has been noted after carbohydrate restriction and epinephrine injections (Engel et al., 1973; Horoupian et al., 1978). Although Swaiman and co-workers (1968) observed no improvement by administration of vitamin A, Nevsimal and Kocura reported a good result of this therapy in one patient.

Genetics

An autosomal recessive mode of inheritance for acid maltase deficiency is now generally accepted.

In an analysis of the genetics of the *generalized form*, Sidbury (1967) studied personally 21 families and collected 59 families from the literature, with a total number of 116 affected infants. Calculating the percentage of affected infants, after subtracting the probands he arrived at 21.4 per cent. This proportion of affected infants is in keeping with the expected 25 per cent for autosomal recessive inheritance. There was a preponderance of male patients (57 males versus 37 females). Parental consanguinity (10 per cent) was higher than in the general population. In parents of patients with the generalized form, intermediate levels of acid maltase activity have been found in leucocytes (Williams, 1966; Nitowsky and Grunfeld, 1967), in lymphocytes (Taniguchi et al., 1978), in fibroblasts (Nitowsky and Grunfeld, 1967) and in muscle (Hug et al., 1966; Engel and Gomez, 1970). However the number of investigated heterozygotes was small.

In the *muscular form*, various sibships with several affected siblings have been reported (Zellweger et al., 1965; Ketelsen et al., 1973; Carrier et

al., 1975; de Barsy, 1976; Martin et al., 1976a; Tanaka et al., 1979). Of the total number of 13 affected sibs in six sibships nine patients were male. Data on parental consanguinity were seldom given: once its presence was reported (Tanaka et al., 1979) and once its absence (Ketelsen et al., 1973). In parents of patients with the muscular form intermediate levels of acid maltase activity have been found in skeletal muscle tissue (Engel and Gomez, 1970; Tanaka et al., 1979) and in urine (Mehler and DiMauro, 1976; Tanaka et al., 1979).

The incidence of the *generalized form* in the state of North Carolina (U.S.A.) was estimated as 1 per 400,000 live births, which corresponds to a heterozygote frequency of about 1 in 300 (Sidbury, 1967). Incidence estimates for several European countries (see table 16, chapter 5) show large variations in different countries. The cause for these variations is unknown and they might be due to genetic differences between populations, or to different standards and facilities for diagnosis.

Estimations on the frequency of the *muscular form* could not be traced in the literature.

Recently, Solomon and co-workers (1979) proved evidence that in man the gene for acid maltase may be located on chromosome 17.

Post-mortem studies

In all cases with the *generalized* form the outstanding feature is a massive cardiac enlargement. Di Sant'Agnese et al. (1950) found that the weight of the heart was 2 to 5.6 times the normal value for age. The hypertrophy and dilatation affected only the ventricles, and the size of the auricles was not increased. The weight of the liver was 11 to 60 per cent above normal for age, that of the kidneys varied from 16 per cent below normal to 20 per cent above normal for age. On light-microscopic examination the authors observed glycogen storage in all tissues and organs.

Martin et al. (1973) studied the nervous system of one patient with the generalized form, and they reviewed previous reports. They stressed the selective topography of the neuronal storage: the anterior horns and the motor nuclei of the brain stem showed a considerable amount of glycogen storage. It was variable, or even absent in other areas of the grey matter within the central nervous system. Large deposits of glycogen were also found in the glial cells. Myelination was normal. In the peripheral nervous system they found large amounts of glycogen in the spinal ganglia and in the neurons of the parasympathetic myenteric plexus.

Acid maltase activity has been found to be absent in the heart and in the brain, and very low, or absent in the liver (Hers, 1963; Angelini and Engel, 1972; Mehler and DiMauro, 1977)

Autopsy studies in the *muscular* form are rare. Martin et al. (1976a) reported the findings in a 24-year-old male, and DiMauro et al. (1978) those in a 31-year-old male. As already mentioned in chapter 1, only in the skeletal muscles and in several cell types of the skin accumulations of glycogen were found on morphological examination. Moreover, Martin and co-workers stressed the extremely variable involvement of different muscles.

A surprising finding from the biochemical studies was a severely reduced acid maltase activity in the morphologically normal heart.

3 METHODS

The investigations performed in the patients and relatives presented in the next chapter consisted of history taking and physical examination, and one or several of the following studies: cardiological investigation, serum creatine phosphate kinase (CPK) assay, electromyography (EMG), histological, histochemical and ultrastructural examination of a muscle biopsy, and ultrastructural examination of a skin biopsy. Studies of enzymatic activities of maltase were done in one or several of the following samples: total leucocytes, lymphocytes, skeletal muscle tissue, cultured fibroblasts, urine, the liver, or the heart.

Serum CPK

The activity was measured according to the "optimized standard method", recommended by the "Deutsche Gesellschaft für Klinische Chemie" (Anon., 1972), by G.J.M. Boerma and Miss J.J.H. Veelbehr, Department of Clinical Chemistry, Erasmus University, Rotterdam. The upper limit for normal in adults in this laboratory is 50 U/L.

Muscle biopsy

Muscle biopsies were obtained under local anaesthesia by the conventional open surgical procedure. Part of the tissue was fixed in susa solution for histological examination. Another part was frozen in liquid nitrogen-cooled isopentane (-160° C) for cryostat sections and for biochemical assays. For electronmicrscopy a small strip of muscle was fixed at resting length in glutaraldehyde 3 per cent with Sörensen's phosphate buffer at pH 7.26.

Cryostat sections were prepared histochemically according to accepted methods (Dubowitz et al., 1973). Acid phosphatase (AcP) activity was demonstrated by the method of Burstone (1958), or by the technique with semipermeable membranes reported by Meijer (1972). Apart from the routine periodic acid Schiff (PAS) technique described by Dubowitz et al., the PAS dimedon procedure (Barka and Anderson, 1963) was sometimes used.

Histological and histochemical studies were performed by J. Bethlem (Department of Neurology, University of Amsterdam, Amsterdam), H.F.M. Busch (Departments of Neurology and of Pathology, Erasmus University, Rotterdam) and F.G.I. Jennekens (Department of Neurology, State University, Utrecht).

The glutaraldehyde-fixed tissue was postfixed in osmiumtetroxide, dehydrated in graded ethanols, and embedded in Epon. One-μm (semithin) sections were stained with uranyl acetate and lead citrate.

Electronmicroscopic studies were performed by J.J. Martin (Born-Bunge Stichting en Universitaire Instelling Antwerpen, Belgium) and by A. Stadhouders (Department of Submicrsocopic Morphology, University of Nijmegen).

The amount of glycogen in muscle tissue was determined according to Huijing (1970). These assays were done by J.F. Koster and Miss R.G. Slee (Department of Biochemistry I, Erasmus University, Rotterdam).

Skin biopsy

Skin biopsies were obtained after local anaesthesia with an ethyl-chloride spray. One piece was put in Ham's F 10 cell culture medium and prepared for the culture of skin fibroblasts (M.F. Niermeijer, Miss M. Mekes and W.J. Kleijer, Department of Clinical Genetics, Erasmus University, Rotterdam).
Occasionally, a second piece was fixed in glutaraldehyde 3 per cent with Sörensen's phosphate buffer at pH 7.26. This was further prepared for electronmicroscopy and studied by J.J. Martin (Born-Bunge Stichting en Universitaire Instelling Antwerpen, Belgium).

Assays of acid and neutral maltase activities

Samples

Total leucocytes were isolated from heparinized blood with dextran (Wyss et al., 1971). Samples for control values were obtained from 14 clinically healthy adults.

Lymphocytes were prepared by the Ficoll procedure ("Lymphoprep", Nyegaard & Co, Oslo) according to the method of Böyum (1968). Lymphocytes for control values were obtained from five clinically healthy adults.

Muscle biopsies, and cardiac and hepatic tissues were homogenized in 0.05 M NaF. The homogenate was centrifuged for 10 minutes at 12,000 g. Biopsies taken from non-weak muscles of 11 subjects with different neuromuscular diseases were used as controls. Cardiac and hepatic tissues for the establishment of control values were obtained from young children who had died of other causes than a metabolic disorder.

Fibroblasts were cultured under standardized cell cultivation procedures in Ham's F 10 with 15 per cent foetal calf serum as the medium (Reuser et al., 1978). Acid maltase activity was measured in fibroblast cultures from earlier subculture numbers two weeks after the last subculture. Fibroblasts of six healthy adults not at-risk of being heterozygous for acid maltase deficiency were used as controls. In the investigation of family B (chapter 7) the fibroblasts of four spouses from this family and of three adults not at-risk of being heterozygous for acid maltase deficiency were taken for control values.

Fresh morning urine was collected, and a urinary protein preparation by dialysis was made within six hours after voiding. The enzyme assays were performed within 48 hours (Schram et al., 1979). Control values were determined in 39 healthy subjects between 8 months and 68 years of age.

Maltase assays

Acid and neutral maltase activities were measured in total leucocytes, skeletal muscle tissue, the liver and the heart according to a modified method of Nitowsky and Grunfeld (1967) at pH 4.0 and at pH 6.5 using maltose (13.8 mM), glycogen (10, 20 or 50

mg/ml), 4-methylumbelliferyl-α-glucoside (MU-glucoside, 1.0 mM) as a substrate (Koster et al., 1972). The assays were performed by J.F. Koster and Miss R.G. Slee (Department of Biochemistry I, Erasmus University, Rotterdam), or by A.E.F.H. Meijer (Laboratory of Pathological Anatomy, University of Amsterdam, Amsterdam). Acid maltase activity in the fibroblasts was measured using glycogen (50 mg/ml) or MU-glucoside (2.2 mM) as a substrate by Miss M. Mekes (Department of Clinical Genetics, Erasmus University, Rotterdam).

Urinary acid maltase was specifically assayed after separation from the urine by antibodies against acid maltase immobilized on Sepharose 4B (A.W. Schram, Mrs. B. Brouwer-Kelder and J.M. Tager, Laboratory of Biochemistry, University of Amsterdam, B.C.P. Jansen Institute, Amsterdam). By this method (Schram et al., 1979) acid maltase is quantitatively removed from the urine by incubation with an excess of immobilized antibodies (antibodies raised against lysosomal acid maltase purified from human liver and coupled to Sepharose 4B). This method allows a separate assay of acid α-1,4-glucosidase (acid maltase) without interference from other enzymes in the urine that hydrolyse α -1,4-glucosidic linkages as amylase, neutral maltase, or renal maltase. After adsorption to immobilized antibodies, the acid maltase activity is measured with paranitrophenyl-glucoside (pNP) as a substrate and this activity accounts for 91 $\pm$ 3% of the total maltase activity at pH 4.0 in normal urine. The influence of diuresis is corrected by expressing the activity as a ratio to the activity of another lysosomal enzyme, β-hexosaminidase, assuming that the excretion of both enzymes is similarly affected by diuresis (Paigen and Peterson, 1978).

Statistics

In co-operation with H.J.A. Schouten (Department of Biostatistics, Erasmus University, Rotterdam), a probability of heterozygosity for acid maltase deficiency was calculated using the ratio of acid maltase/ β-hexosaminidase in the urine from several persons (see chapters 6 and 7) according to Bayes' theorem (Duda and Hart, 1973:

$$P(H \mid x) = \frac{P(H)\, f_H(x)}{P(H)\, f_H(x) + P(N)\, f_N(x)},$$

where

1. $P(H \mid x)$ is the posterior probability of being a heterozygote given the urinary ratio x of acid maltase/ β-hexosaminidase.
2. $P(H)$ is the prior probability of heterozygosity for acid maltase deficiency.
3. $P(N)$ is the prior probability of having a normal genotype for acid maltase deficiency.
4. $f_H(x)$ may be calculated as

$$f_H(x) = \frac{1}{s_H \sqrt{2\pi}} \exp \left\{ - \tfrac{1}{2} \left(\frac{x - \bar{x}_H}{s_H} \right)^2 \right\},$$

where $\bar{x}_H$ and s_H are the mean and the standard deviation of the urinary enzyme ratios measured in the obligate heterozygotes for acid maltase deficiency (see chapter 6, table 21B).

5. $f_N(x)$ may be calculated as

$$f_N(x) = \frac{1}{s_N \sqrt{2\pi}} \exp \left\{ - \tfrac{1}{2} \left(\frac{x - \bar{x}_N}{s_N} \right)^2 \right\},$$

where $\bar{x}_N$ and s_N are the mean and the standard deviation of the urinary enzyme ratios measured in the controls (see chapter 6, table 21B). The last two expressions are normal densities, so it is assumed that the ratios approximately follow a normal distribution.

Excretion pattern of oligosaccharides in urine

Oligosaccharides were estimated in fresh morning-urine portions by thin-layer chromatography (TLC) after desalting of the urine by W. Blom, Mrs. H.H. Kelholt-Dijkman and Mrs J.C. Luteijn (Laboratory of metabolic diseases, Sophia Children's Hospital, Erasmus University, Rotterdam).
The urine portions were kept deep-frozen until analysis. After centrifugation of 20 ml urine, the supernatant was desalted by elution with 400 ml of aqua bidest over successively a Bio-Rad A6 50W-X8 (200 - 400 mesh) and a Bio-Rad A6 3-X4A (100 - 200 mesh) column (comparable with the method reported by Friedman et al., 1978). The eluate was evaporated to the original volume of 20 ml with a Rota-Vapor. TLC was done according to the method described by Humbel and Collart (1975).

The amount of sample to be spotted on the thin-layer plate was calculated depending on the age of the person and the creatine concentration in the urine:

$$\text{ml to be spotted} = 2\frac{F}{C} *$$

Plate 1 gives an example of the TLC of oligosaccharides in pure and in desalted urines of three patients with the muscular form of acid maltase deficiency and in pure urine of one control subject. A dark band is seen in the tetrasaccharide region of the oligosaccharide pattern of the patients' desalted urine.

For the present study the urinary TLC of 86 persons was done according to this procedure. To avoid possible bias in the interpretation of the thin-layer chromatograms, each urine portion had been provided with a code-number by someone who was not involved in the study (J.H.M. van Eijndhoven, Department of Neurology, Erasmus University, Rotterdam). Three persons (W. Blom, H.F.M. Busch, M.C.B. Loonen) independently attempted to identify "patients" (dark band in the tetrasaccharide region), "heterozygotes" (faint band), and "normals" (absence of band).

Treatment

In co-operation with J. Fernandes and Miss A.E.H. Hart (Sophia Childrens' Hospital, Erasmus University, Rotterdam), two patients with the muscular form (H III 2 and K III 2) were given a low-carbohydrate and high-fat diet for a period of three months. Physical examination, pulmonary function tests, dynamic exercise on bicycle, and biochemical studies in blood were done at the onset, after six weeks, and at the end of the three-months period.

* C = creatinine of the urine in nmol/l
F = age-dependent factor:

age	F
0 - 1 year	0.075
1 - 2 years	0.100
2 - 8 years	0.150
> 8 years	0.200

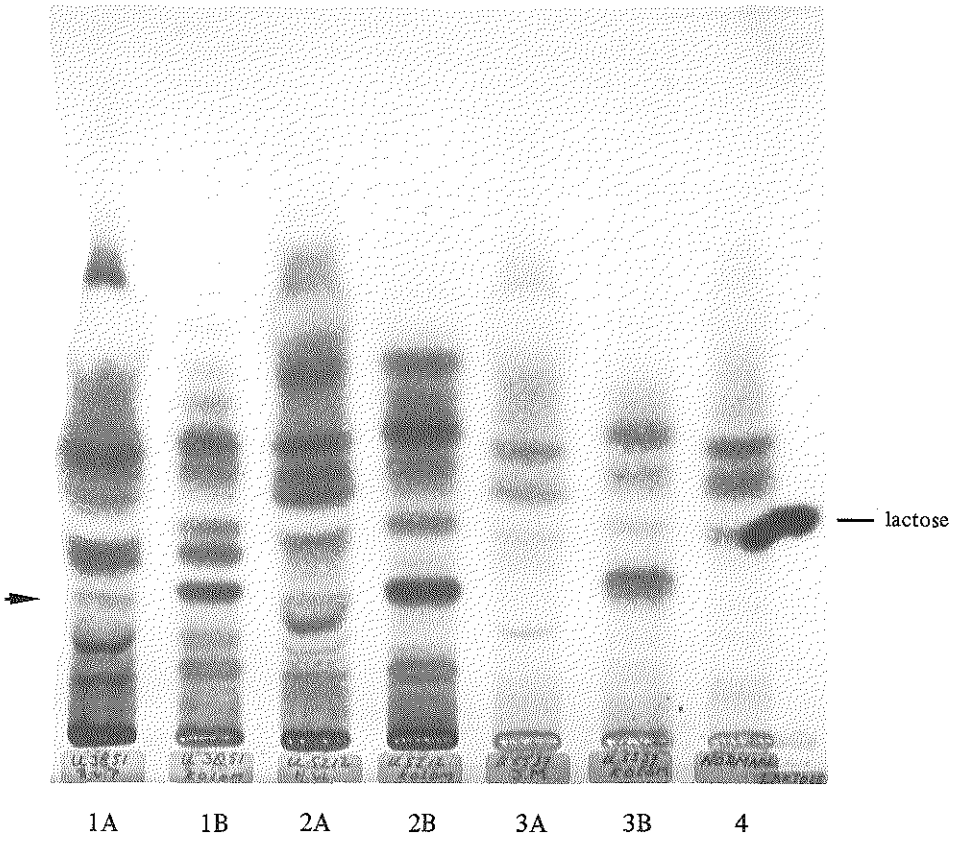


Plate 1.
Thin-layer chromatograms of oligosaccharides in urine of three patients (1, 2, and 3) with the muscular form of acid maltase deficiency, and of a control urine (4). "A" stands for pure urine and "B" for the same, but desalted urine. The arrow indicates the tetrasaccharide band which is evident in the desalted urine of the patients.
Overlapping with the control urine (4), the disaccharide lactose was spotted as a reference.

Genetics

To investigate the presence of consanguinity, the ancestors of ten index cases, six with the generalized form and four with the muscular form, as well as the ancestors of family B in which both forms occurred were traced as far as possible over five generations by gathering data from municipal population registries and from provincial archives, with the help of Mrs A.C.M. den Brok-Loonen, Mrs. F. de Kruik-Postma, Mrs A.Th. Loonen-Walen, and J.C. Loonen.

The ancestors of the patient in Pompe's original description (1932) were also traced.

In addition, it was attempted to estimate the frequency of acid maltase deficiency in The Netherlands. Through an inquiry at the "Hoofdafdeling Gezondheidsstatistieken

van het Centraal Bureau voor de Statistiek"* and at the pediatric departments of the seven university hospitals in this country, information has been obtained about the number of infants with the generalized form, diagnosed from 1967 until 1977. Furthermore, the members of the Dutch Study Group on Neuromuscular Diseases gave information about their cases with the muscular form. Practically all known Dutch cases suffering from this variant have been diagnosed by them, between 1969 and 1979.

The proportion of affected sibs in the sibships was calculated according to Fisher's method of segregation analysis by the single incomplete ascertainment method (Emery, 1976).

All patients and all selective abortions after prenatal diagnosis of acid maltase deficiency in the present series have been arranged according to their sex for purposes of comparison with the report of Sidbury (1967) about the preponderance of male patients in the generalized form.

* "Section for Health Statistics of the Netherlands Central Bureau of Statistics".

CASE HISTORIES AND FAMILY DATA

Introduction

The present study was initiated in 1977. Data of 20 patients were available for analysis: seven patients with the generalized form and 13 with the muscular form of acid maltase deficiency. The diagnoses had been made between 1969 and 1977. Nine patients could be examined personally. Most of the remaining patients have been investigated by J. Bethlem (Department of Neurology, University of Amsterdam, Amsterdam), J. Fernandes (Sophia Children's Hospital, Erasmus University, Rotterdam), or H.F.M. Busch, (Department of Neurology, Erasmus University, Rotterdam).

The pedigrees of 12 families are shown in figure 3. The generalized form occurs in the families A and C-G. Only two of the seven patients have been investigated personally. In addition, all parents, all sibs, and 13 of the 18 living grandparents have been investigated.
The muscular form occurs in the families H-M. Seven patients, their parents when still alive, and five of the 11 sibs have been examined personally. Further, it can be seen that in family B both the generalized and the muscular form occur. This family has been the subject of a separate study, which is presented in chapter 7.

No relatives have been examined of five patients with the muscular form (N-S) and virtually nothing is known about their families.

The patients and their relatives

Prior to the description of the individual patients and their relatives, the following general remarks are appropriate:

- In alle cases with the muscular form the examination showed no abnormality of the cranial nerves, no sensory loss or cerebellar disturbances, and the plantar responses were flexor. The grading of the strength was according to the MRC scale (Medical Research Council, 1943).
- In all cases with the generalized form an enlarged heart has been found;

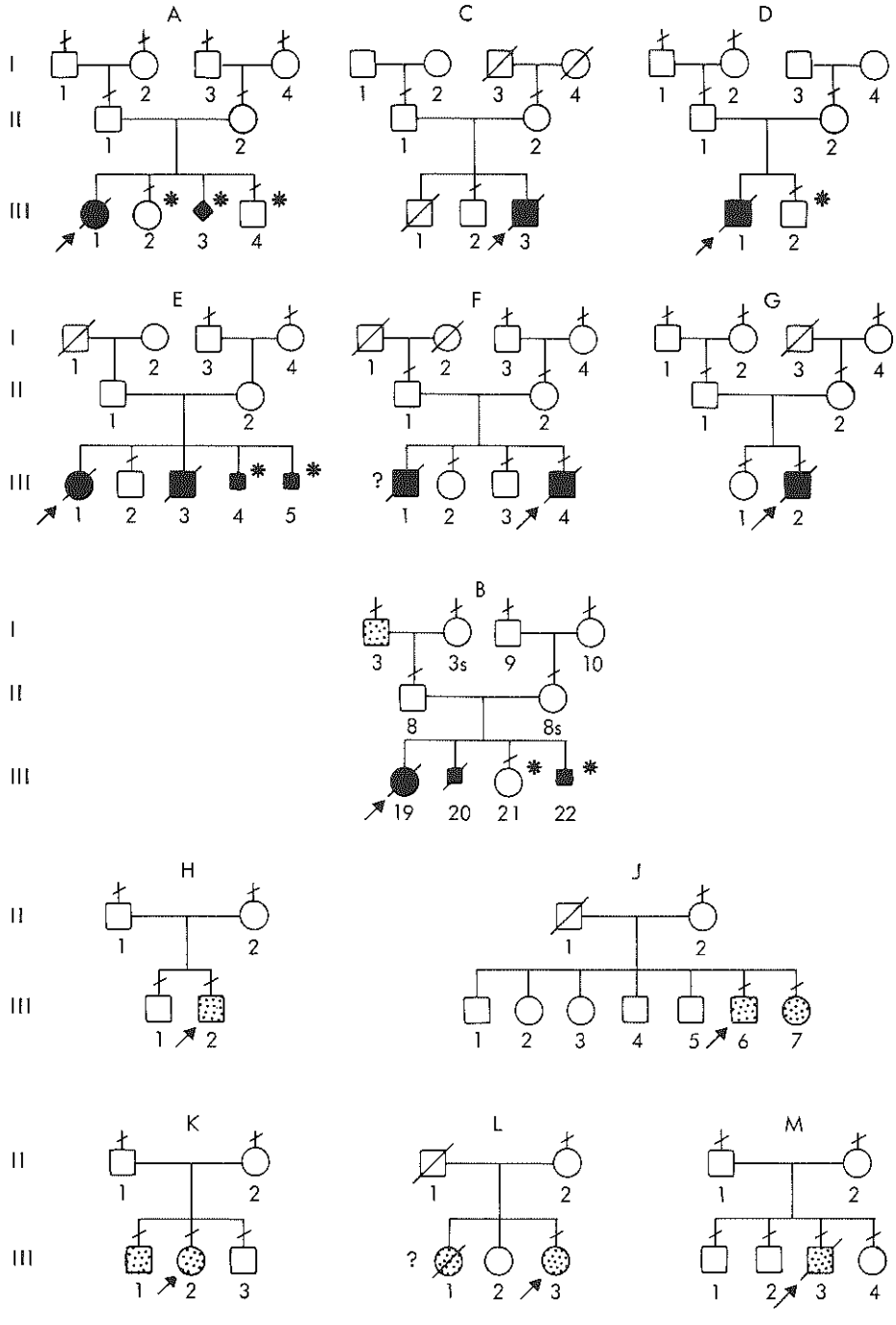


Figure 3. Pedigrees of the seven index cases with the generalized form, and of five index cases with the muscular form.

Legends to figure 3.

 male, generalized form

 female, muscular form

 immature childbirth, affected, proven by enzymatic assay

 male, selective abortion after prenatal diagnosis of generalized form

 selective abortion after prenatal diagnosis of generalized form, sex unknown

 index case

 deceased

 personally investigated

* prenatal enzyme studies

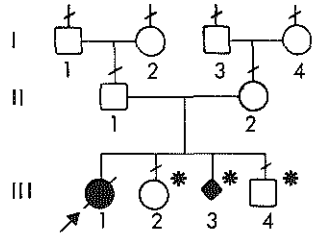
? diagnosis not confirmed by biochemical studies

the ECG (electrocardiogram) showed a short PR interval and suggested biventricular hypertrophy.

- EMG (electromyography) included the sampling of three or more limb muscles. Motor unit potentials of low amplitude with normal or short duration, or polyphasic potentials were taken to indicate a "myopathic" pattern. MCV (motor conduction velocity) has been studied in one or two peripheral nerves.
- The serum CPK (creatine phosphokinase) was considered to be elevated if above 50 U/L (Anon., 1972). CPK values indicated only as "normal" or as "elevated" have been assessed in laboratories of other hospitals.
- The activities of acid maltase in the samples of the patients and their relatives are given in chapters 5, 6 and 7.
- The prenatal diagnoses have been carried out by M.Jahoda (Department of Obstetrics and Gynecology, Erasmus University, Rotterdam), M.F. Niermeyer and W.J. Kleijer (Department of Clinical Genetics, Erasmus University, Rotterdam), and by J.F. Koster and R.G. Slee (Department of Biochemistry I, Erasmus University, Rotterdam). Data on the prenatal investigations in families A and E have been reported by Niermeyer et al. (1975).
- Reports on family B have been published by Koster et al. (1978), and by Busch et al. (1979).

For legends to the pedigrees see "Legends to figure 3" page 43.

FAMILY A



III 1 *Index case:* girl, born in 1969
This girl was born in breech position after 37 weeks' gestation. Her birth-weight was 2440 grams. The immediate post-natal period was uneventful, but "soon" after birth the parents observed increasing dyspnoea and failure to thrive. At two months she was admitted with the provisional diagnosis of congenital cardiac disease. She was seriously ill, with a greyish-pale colour and severe respiratory distress. A soft, mid-systolic murmur was heard to the right of the sternum at the fourth intercostal space. On pulmonary auscultation numerous moist bubbling rales were heard. The liver was palpable two cm below the lower costal margin (LCM).

The roentgenogram of the chest showed a massive cardiac enlargement and pulmonary vascular congestion.

Despite treatment of the cardiac failure and artificial respiratory support, the infant died five days after admission.

At post-mortem examination the heart proved enormously enlarged. Histochemical examination showed numerous PAS positive vacuoles. Glycogen accumulation was also found in the brain, the liver, the tongue and the skeletal muscles. Acid maltase activity was absent in the heart and very low in the skeletal muscles and the liver.

III 2: girl, born in 1970
Prenatal diagnosis at 16 weeks' gestation: normal acid maltase activity in the amniotic fluid supernatant.
Physical examination at the age of seven years: normal girl.

III 3: 1972
Amniocentesis in the 15th week of gestation yielded an insufficient number of cells for acid maltase assay. A spontaneous abortion followed four weeks later. An activity suggestive for acid maltase deficiency was found in the foetal muscle tissue.

III 4: boy, born in 1973
Following amniocentesis in the 16th week of pregnancy a normal acid maltase activity was found in the cultured amniotic fluid cells.
Physical examination at the age of five years: normal boy.

II 1: father, born in 1943
Physical examination at the age of 34: healthy male.

II 2: mother, born in 1942
Physical examination at the age of 35: healthy female.

I 1: paternal grandfather, born in 1915
Normal on physical examination at the age of 62.

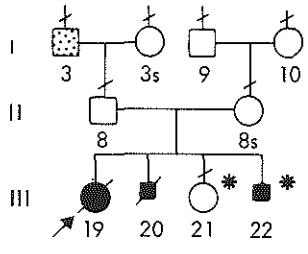
I 2: paternal grandmother, born in 1918
Complained of tension headache and low back pain. No abnormalities on physical examination at the age of 59.

I 3: maternal grandfather, born in 1898
Suffered from diabetes. Otherwise normal on physical examination at the age of 79.

I 4: maternal grandmother, born in 1899
Complained of myalgia and showed some proximal weakness of the arms and the legs on physical examination at the age of 78.

The first child in this sibship suffered from Pompe's disease. In two subsequent pregnancies antenatal examination for acid maltase deficiency gave normal results and two healthy children were born. In one pregnancy antenatal examination was unsuccessful. This pregnancy ended at 19 weeks by a spontaneous abortion.

FAMILY B (see also chapter 7)



III 19 *Index case:* girl, born in 1972
She was born after an uneventful gestation and delivery. Her birth-weight was 4220 grams. A few days after birth crying was noticed to be somewhat weak and at times the colour was greyish. In the first months she took the bottle slowly. At the age of three months she showed progressive dyspnoea and failure to thrive.
On examination at the age of three months she was severely ill, looked greyish-pale and had an enlarged tongue. The heart sounds were soft. The liver was palpable 2-3 cm below LCM and the spleen 1 cm. Generalized hypotonia and areflexia were noted. Pompe's disease was considered and a reduced acid maltase activity in the leucocytes confirmed this diagnosis.

She died at the age of 16 weeks from progressive dyspnoea and respiratory infection.

At post-mortem examination the heart weighed 200 grams (normal for this age: 27 grams), the liver 260 grams (normal: 160 grams), and the spleen 20 grams (normal: 16 grams). Histological examination confirmed a massive glycogen accumulation in the

heart (figure 4a), the liver, the tongue and the skeletal muscles. Acid maltase activity proved not detectable in the heart and a reduced activity was found in skeletal muscles and in the liver.

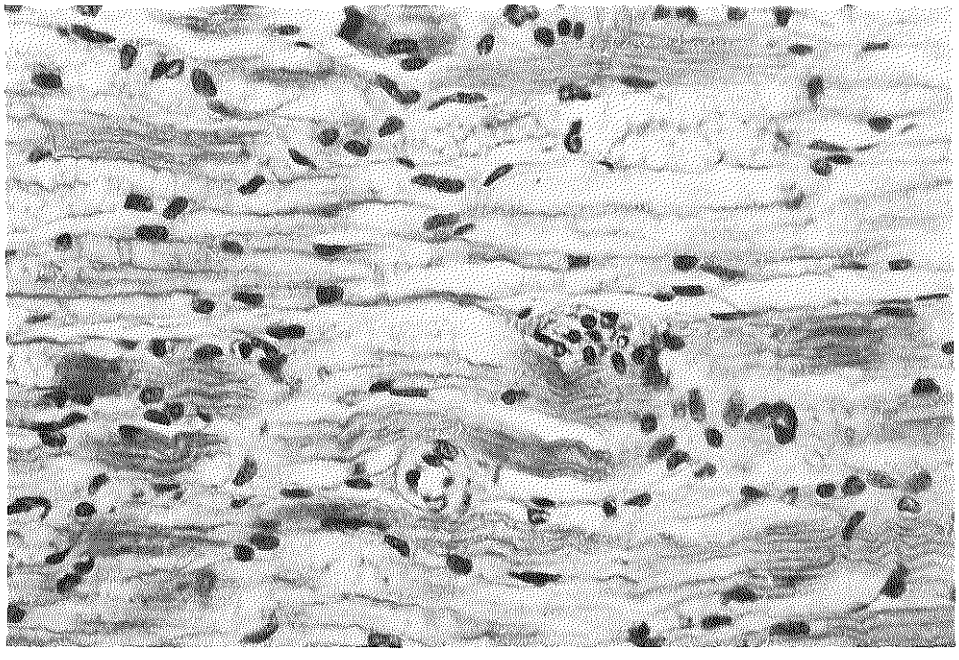


Figure 4a. Case B III 19. Extensive vacuolization of the myocardium giving the appearance of a lace-work pattern (paraffin section, haematoxylin and eosin, x 280).

III 20: 1973
Amniocentesis at 15 weeks was unsuccessful. At 25 weeks an immature male baby was born, length 34 cm. He died after half an hour. Fibroblasts were cultured and the acid maltase activity proved nine per cent of the lowest control value, suggesting acid maltase deficiency, but routine microscopical examination of paraffin sections failed to detect glycogen storage in the heart, the liver of the tongue.

III 21: girl, born in 1976
Following amniocentesis in the 16th week of pregnancy a normal acid maltase activity was measured in cultured amniotic fluid cells.
Physical examination at the age of one year: normal girl.

III 22: 1979
A very low activity of acid maltase was measured in the cultured amniotic fluid cells. The pregnancy was interrupted. In foetal samples (liver, brain and cultured fibroblasts) acid maltase proved virtually absent, confirming acid maltase deficiency.

II 8: father, born in 1945
Was normal on physical examination at the age of 32.

II 8s: mother, born in 1947
Showed no abnormalities on physical examination at the age of 30.

I 3: paternal grandfather, born in 1916
This printer was in good health until he experienced difficulty in climbing the stairs of the printing machine at the age of 53 years. His gait became waddling and he was unable to do his work. A diagnosis of chronic polymyositis was made. He was treated with corticosteroids without success. At the age of 59 he was admitted to an intensive care unit because of acute respiratory failure. At that time the family history had become known and the diagnosis was reconsidered.
He had an athletic stature. Some muscles (deltoid, biceps and calf muscles) looked hypertrophic but others (trapezius and pectoral muscles) showed wasting. There was profound winging of the left scapula (figure 4b). He was dyspnoeic and his gait was waddling. A severe weakness (strength grade 1-3) was found in the shoulder girdle muscles. The anterior neck muscles had a normal strength. The pelvic muscles showed a severe weakness (strength grade 1-3), the gluteal muscles being most severely involved. The strength of the quadriceps and hamstring muscles was grade 4. The tendon reflexes of the arms were normal, but the knee jerks and ankle jerks were decreased.

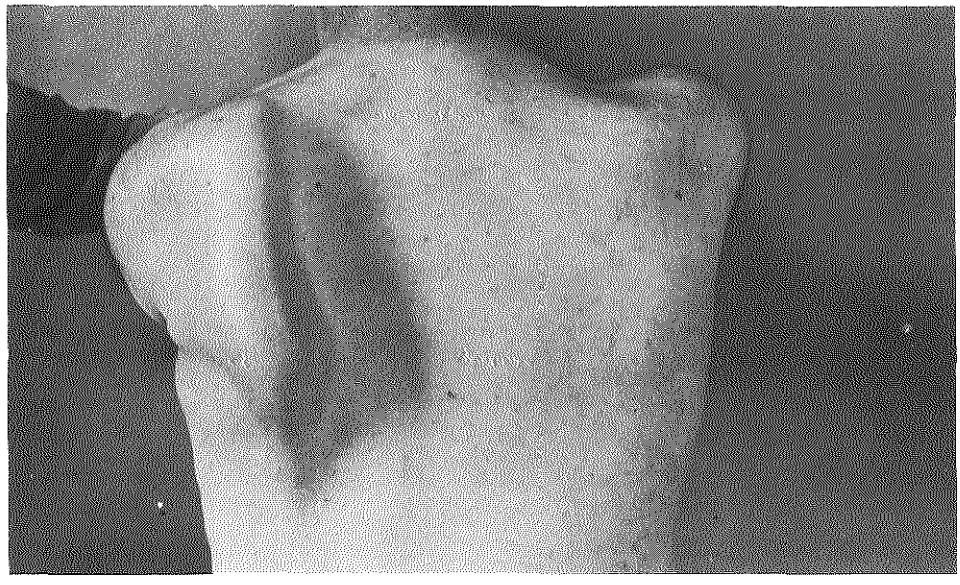


Figure 4b. Case B I 3. Winging of the left scapula.

The serum CPK activity was initially normal, later on slightly elevated: 91 U/L. On fluoroscopy the movements of the diaphragm proved decreased. ECG: normal. Echocardiogram: normal. EMG: myopathic pattern. MCV: normal. Pectoralis muscle

biopsy at the age of 53: increase of fatty tissue, muscle fibre atrophy and some aggregates of nuclei. Quadriceps biopsy at the age of 59: very few fibres contained multiple small vacuoles, which coloured intensely with AcP and were PAS positive. Ultrastructural examination of the muscle biopsy demonstrated glycogen enclosed within membranes, but free glycogen was also observed in the perinuclear region and between the myofibrils. EM of a skin biopsy disclosed the presence of a few membrane-bound accumulations of glycogen, and of autophagic vacuoles. Both muscle tissue and cultured skin fibroblasts showed a reduced acid maltase activity. The glycogen content of the skeletal muscle tissue was only slightly increased.

For the last three years the patient was in a clinically stable condition. He needed artificial respiration only at night and he was moderately disabled by his weakness.

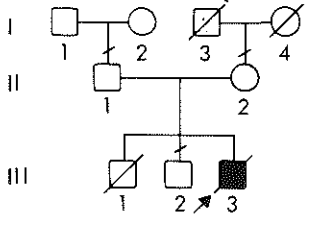
I 3s: paternal grandmother, born in 1917
Physical examination at the age of 60: healthy female.

I 9: maternal grandfather, born in 1916
Had hypertension. On physical examination at the age of 61 the blood pressure was 160/105; otherwise normal findings.

I 10: maternal grandmother, born in 1918
Had had a myocardial infarction in 1974, suffered from diabetes mellitus and complained of pain in the right arm. Physical examination at the age of 60 showed a frozen shoulder on the right. Moreover, she showed a slight inco-ordination of movements.

In this family the generalized form and the muscular form of acid maltase deficiency proved to occur. A detailed study of this family is presented in chapter 7.

FAMILY C



III 3 *Index case:* boy, born in 1971
Gestation and delivery were normal. The birth-weight was 4000 grams. During the first weeks of life perioral cyanosis was observed intermittently. He took his feedings slowly. At six weeks the baby was hospitalized because of bronchiolitis and cardiac failure. At

four months a second admission followed because of failure to thrive and suspicion of congenital heart disease. At that time he proved a slender boy, with a pale colour, perioral cyanosis, and dyspnoea. He weighed 3600 grams (400 grams below his birth-weight). The cardiac sounds were soft. On pulmonary auscultation many moist bubbling rales were heard. The liver was enlarged (5 cm below LCM). There was a generalized hypotonia, and the muscles felt firm on palpation. The possibility of Pompe's disease was rejected because of a normal acid maltase activity in the leucocytes (substrate: maltose).

He died six days after admission from frequent paroxysms of supraventricular tachycardia (300 per min.).

At post-mortem examination the heart was enormously enlarged (the recorded weight was illegible). The liver weighed 370 grams (normal: 160 grams), the spleen 34 grams (normal: 16 grams), the kidneys 65 grams (normal: 41 grams) and the brain 716 grams (normal: 770 grams). Glycogen storage was found in the sections of all these tissues. Acid maltase activity proved absent in the heart, the liver and skeletal muscles.

III 1: boy, born in 1966
This boy died at the age of two and a half years of a "congenital heart disease". No clinical records were available.

III 2: boy, born in 1967
Physical examination at the age of ten years: normal boy.

II 1: father, born in 1939
Normal findings on physical examination at the age of 38.

II 2: mother, born in 1945
Physical examination at the age of 32: healthy female.

I 1: paternal grandfather, born in 1907
Was said to have "narrowing of the blood vessels".

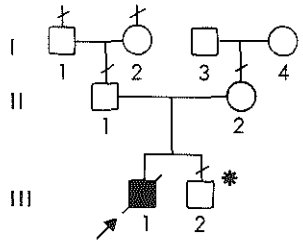
I 2: paternal grandmother, born in 1910
Was said to suffer from "balance disturbances".

I 3: maternal grandfather, born in 1897, died in 1976
Was said to have died of "chronic bronchitis".

I 4: maternal grandmother, born in 1899, died in 1974
Was said to have died of "intracerebral bleeding".

The eldest sib died of a "congenital heart disorder". The data were insufficient to substantiate a diagnosis of Pompe's disease. The second sib was a healthy boy. The third was the index case with Pompe's disease. During life the diagnosis was rejected because of the finding of a normal acid maltase activity in leucocytes (substrate: maltose). Later studies demonstrated maltose not to be a suitable substrate for the assay of acid maltase in leucocytes. The autopsy findings were characteristic for acid maltase deficiency.

FAMILY D



III 1 *Index case:* boy, born in 1976
The pregnancy and delivery were normal. The birth-weight was 3420 grams. Immediately after birth crying was weak. In the first week of life rapid breathing, bradycardia and soft heart sounds were noted. Based on the clinical manifestations and the ECG, the provisional diagnoses were endocardial fibroelastosis, or an anomalous origin of the coronary artery. He did fairly well until the age of six weeks, when he began to take the feedings slowly. After three months hypotonia and subsequently dyspnoea were observed, while perioral cyanosis and tachycardia were present intermittently.
During the physical examination at four months he was whining and he showed a greyish colour and perioral cyanosis. A soft apical protosystolic murmur was heard. The liver was enlarged (4 cm below LCM). The hands and feet were oedematous.

He died at four and a half months. The clinical diagnoses were: cardiac failure and respiratory infection.

At autopsy the heart was grossly enlarged with thickened ventricular walls. It weighed 180 grams (normal for this age: 27 grams). The weight of the liver was 210 grams (normal: 160 grams). The pathologist suggested Pompe's disease and his diagnosis was subsequently confirmed by the presence of glycogen storage in the cardiac, hepatic and muscular tissues, and by the absence of acid maltase activity in the heart.

The parents were informed about antenatal diagnosis in case of a subsequent pregnancy.

III 2: boy, born in 1978
A normal acid maltase activity was found in cultured amniotic fluid cells.Physical examination at nine months: healthy boy.

II 1: father, born in 1946
Physical examination at the age of 31: normal male.

II 2: mother, born in 1947
No abnormalities were found on physical examination at the age of 30.

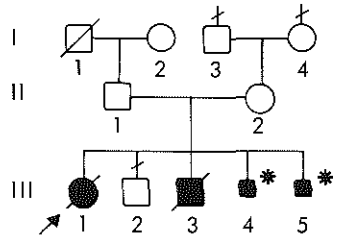
I 1: paternal grandfather, born in 1919
On physical examination at the age of 58 normal findings, except for obesity.

I 2: paternal grandmother, born in 1915
Suffered from osteoporosis. A small tumour in the left breast and a slightly enlarged liver were found on physical examination at the age of 62.

I 3 and I 4: maternal grandparents
Were said to be healthy.

In the index case the diagnosis of Pompe's disease was made by the pathologist at post-mortem examination. In retrospect, suspicion of this diagnosis might have been raised by the ECG abnormalities, especially by the short PR interval (0.08 sec.).
Following amniocentesis and the finding of a normal acid maltase activity in cultured cells a second, healthy, child was born.

FAMILY E



III 1 *Index case:* girl, born in 1965
This girl died at six months of age from progressive cardiorespiratory failure. The heart was enlarged and the ECG showed a pattern that was suggestive of Pompe's disease. The diagnosis was confirmed by the finding of glycogen storage and a severely reduced acid maltase activity in muscle tissue (K.K. Bossina, Department of Pediatrics, Academic Hospital, Groningen).

The parents were informed of the risk of recurrence in subsequent children.

III 2: boy, born in 1967
Physical examination at the age of ten years: normal boy.

III 3: boy, born in 1970
This boy was delivered after a full-term and uncomplicated pregnancy. His birth-weight was 3650 grams. After two months he took his feedings slowly and hypotonia was noticed. At four months he proved an inactive boy with a somewhat enlarged tongue and generalized muscular hypotonia. The chest was bulging and an apical systolic murmur was heard. The liver was enlarged (3 cm below LCM). The tendon reflexes were brisk.
Because of the diagnosis of Pompe's disease in the eldest sib, it was also suspected in this patient. Confirmation was obtained by a low ratio of acid over neutral maltase activity in leucocytes and a decreased acid maltase activity in a muscle biopsy.

He died at the age of five months from progressive dyspnoea and cardiac failure. Post-mortem examination was not done.

At that time the possibility of prenatal diagnosis could be offered to the parents.

III 4: 1972
A very low acid maltase activity was found in cultured amniotic fluid cells. The pregnancy was interrupted. In the foetal tissues the diagnosis was confirmed by the absence of acid maltase activity in the heart, and a very low acid maltase activity in the liver and in the skeletal muscles.

III 5: 1975
Enzyme assays in cultured amniotic cells again established acid maltase deficiency in this pregnancy. In the aborted foetus acid maltase activity was absent in cardiac, skeletal muscle, hepatic and nervous tissues.

II 1: father, born in 1935
Physical examination at the age of 42: normal male.

II 2: mother, born in 1939
No abnormalities were found on physical examination at the age of 38.

I 1: paternal grandfather, born in 1901
Had died. The cause of death is unknown.

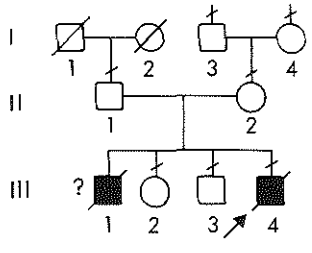
I 2: paternal grandmother, born in 1901
Was not examined because of a recent myocardial infarction (in 1977).

I 3: maternal grandfather, born in 1908
Had complaints of pain in both arms, restless legs and arthrosis of the knees. On examination at the age of 69 atrophy of the skin was found on the distal part of the legs as a result of earlier infections (erysipelas).

I 4: maternal grandmother, born in 1911
Had a somewhat enlarged thyroid gland on examination at the age of 66.

In this sibship two babies had died of Pompe's disease. In two subsequent pregnancies acid maltase deficiency was prenatally diagnosed, and verified by assays in the foetal tissues. There is one healthy son.

FAMILY F



III 4 *Index case:* boy, born in 1973
This boy was delivered at term after a normal gestation. The birth-weight was 4000 grams. Poor feeding, moderate weight gain and listlessness were noted from birth. At

five months he had an upper respiratory tract infection with cardiac failure. At six months he was a poorly nourished boy with generalized hypotonia. Weight 6420 grams. He had a pale colour. No dyspnoea was present. The liver was enlarged (3 cm below LCM). The tendon reflexes were normal, only the ankle jerks were brisk.

The serum CPK was 320 U/L. The tentative diagnosis of Pompe's disease was confirmed by a low ratio of acid over neutral maltase activity in leucocytes.

He died at the age of eight months from respiratory insufficiency.

At post-mortem examination the heart was moderately enlarged: 73 grams (normal for this age: 37 grams). On histological examination a typical lace work pattern and glycogen storage were found in the heart and in skeletal muscles. Acid maltase proved absent in the heart, the liver and the skeletal muscles.

III 1: boy, born in 1966
This boy died at the age of fifteen months. He had always been floppy and was unable to sit unsupported at the age of one year. A respiratory tract infection was followed by a regression in the development and he died three months later. On post-mortem examination the heart and the liver were enlarged: 120 grams (normal for this age: 48 grams) and 470 grams (normal: 331 grams) respectively. On microscopical examination glycogen accumulation was found in the heart, in the skeletal muscles as well as in the liver. Enzyme studies were not done.

III 2: girl, born in 1967
Physical examination at the age of ten years: normal girl.

III 3: boy, born in 1970
Physical examination at the age of seven years: normal boy.

II 1: father, born in 1939
Physical examination at the age of 38: normale male.

II 2: mother, born in 1940
Showed no abnormalities on physical examination at the age of 37.

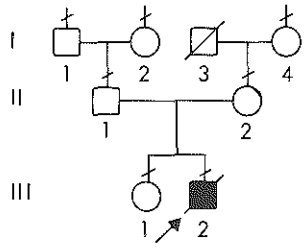
I 1 and I 2: paternal grandparents
Had died. The cause of death is unknown.

I 3: maternal grandfather, born in 1905
Had hypercholesterolaemia. No abnormalities found on physical examination at the age of 72.

I 4: maternal grandmother, born in 1913
No abnormalities on physical examination at the age of 64.

The first child in this sibship presumably suffered from Pompe's disease. The second and third sibs were normal. The fourth sib, the index case, suffered from classical Pompe's disease.

FAMILY G



III 2 *Index case:* boy, born in 1973
This boy had a normal birth after an uneventful pregnancy. The birth-weight was 3380 grams. The initial development seemed normal but at the age of six months he could not lift his head and his limbs became hypotonic and weak. At eleven months he proved a reasonably nourished boy. The tongue was enlarged and the liver was palpable 4-5 cm below LCM. The head control was extremely poor (figure 4c). The skeletal and trunk muscles were hypotonic and weak and the tendon reflexes were decreased. During his stay in hospital chewing and swallowing proved difficult.

Apart from an enlarged heart, the roentgenogram of the chest showed a consolidation in the left apical region. The serum CPK was 120 U/L. EMG: myopathic pattern and pseudomyotonic discharges. MCV: normal. The presumed diagnosis of Pompe's disease was confirmed by acid maltase assays in leucocytes, fibroblasts and skeletal muscles. Microscopical examination of a muscle biopsy showed a massive vacuolization by glycogen storage. The glycogen content of the muscle tissue was increased to ten times the upper normal value.

There was no progress in motor and mental development. He frequently whined with weak sounds. Swallowing proved difficult. Suddenly he died after choking at the age of two years.

Figure 4c. Case G III 2. Poor head control at the age of 11 months.

III 1: girl, born in 1969
Physical examination at the age of eight years: normal girl.

II 1: father, born in 1939
He had been examined at the age of eight years because of "cardiac enlargement". A definite diagnosis could not be made.

History and physical examination at the age of 38: no cardiovascular complaints. Normal on physical examination, in particular no cardiac abnormalities. The blood pressure was 130/80.

II 2: mother, born in 1944
Physical examination at the age of 33: normal female.

I 1: paternal grandfather, born in 1911
No abnormalities on physical examination at the age of 66.

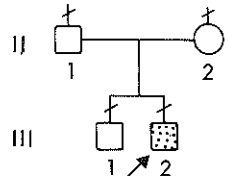
I 2: paternal grandmother, born in 1912
Suffered from diabetes mellitus and hypertension. On physical examination at the age of 65 the blood pressure was 190/90, otherwise no abnormalities.

I 3: maternal grandfather, born in 1913
Died at the age of 41 from a "heart attack".

I 4: maternal grandmother, born in 1922
Had arthrosis of the right hip on physical examination at the age of 55.

The index case suffered from Pompe's disease with a somewhat protracted course. He died at the age of two years. The other sib was normal. The father had been examined at the age of eight years because of "cardiac enlargement", but thirty years later his subsequent history proved uneventful and no abnormalities of the cardiovascular system were detected on physical examination.

FAMILY H



III 2 *Index case:* boy, born in 1966
This boy was examined at the age of ten because of problems during judo lessons: he could not let himself fall properly and was unable to lift his head when lying on his

back. Furthermore, he walked awkwardly and could hardly run. The developmental milestones had been normal, although his parents remembered in retrospect that he had never been able to lift his head, and had to roll on to his side before he could rise from the supine position.
At physical examination no wasting of the muscles was seen. He walked awkwardly and was unable to run. The anterior neck muscles were severely weak. Muscular strength was grade 4 in the shoulder muscles and grade 3-4 in the pelvic muscles. In the distal limb muscles only a slight weakness could be detected. The tendon reflexes were absent.

The serum CPK was elevated: 539 U/L. Serum lactic acid increased normally during exercise under ischaemic conditions. Roentgenogram of the chest: normal cardiac configuration; cardiothoracic ratio: 40 per cent. ECG: normal. Echocardiogram: thickening of the posterior wall of the left ventricle and of the interventricular septum. EMG: myopathic pattern and pseudomyotonic discharges. MCV: normal. Pulmonary function tests: vital capacity reduced to 70 per cent of normal. Quadriceps biopsy: severe vacuolar changes in both fibre types with glycogen storage within the vacuoles. Electronmicroscopy (EM): glycogen in membrane-bound sacs. Ultrastructural study of a skin biopsy: membrane-bound vacuoles filled with glycogen in several cell types. Biochemical studies: no acid maltase activity in skeletal muscles and considerable increase in muscular glycogen content.

A low-carbohydrate and high-fat diet for three months did not result in improvement of strength.

There had been no progression in the past three years. Once he had had severe muscle cramps after an hour's walk.

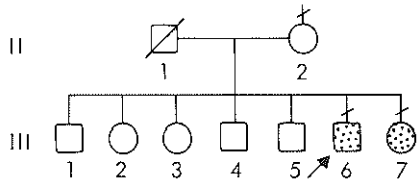
III 1: male, born in 1962
Physical examination at the age of 15 years: healthy young man.

II 1: father, born in 1933
No abnormalities were found on physical examination at the age of 44.

II 2: mother, born in 1932
On physical examination at the age of 45: normal female.

The index case was a boy of ten years with the muscular form of acid maltase deficiency. He had always had difficulty in lifting his head when lying on his back. The respiratory function appeared to be mildly reduced. The echocardiogram indicated thickening of the left posterior ventricular wall and of the interventricular septum. Therapy with a low-carbohydrate and high-fat diet for three months was unsuccessful. The second sib was normal.

FAMILY J



III 6 *Index case:* male, born in 1952
In this 25-year-old male the serum CPK was determined as a routine preoperative screening test for the risk of malignant hyperthermia. Laparotomy had been planned because of abdominal pain of long duration. The serum CPK was raised and the patient was referred for a neurological examination. He had a history of clumsiness in gymnastics at school. He had played soccer but could not run properly. Since a few months he had noticed some difficulty in climbing stairs.
At physical examination he was a strongly built young man. No muscular wasting was seen, but his calves were somewhat hypertrophic and felt firm on palpation. His gait was slightly waddling. The strength of the muscles of the shoulders and arms was normal with the exception of a grade 4 strength in the infraspinatus and the supinator muscles. The pelvic girdle muscles had a grade 3-4 strength, the gluteal and iliopsoas muscles being the weakest. Rolling over from a supine position was carried out with hand support. The tendon reflexes were absent.

Serum CPK: 850 U/L. ECG: normal. Echocardiography: normal. Pulmonary function tests: no abnormalities. Quadriceps biopsy: some fibres with one or several small vacuoles which stained positive for PAS. There was type I preponderance. The vacuoles were observed in fibres of both types. Acid maltase activity in muscle tissue proved very low. The glycogen content was slightly increased.

III 1-5
These sibs were said to be normal.

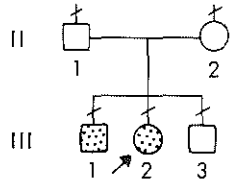
III 7: female, born in 1955
After the examination of her brother this 22-year-young woman surmised that she probably had the same disorder. She had never been very agile and had never performed well in gymnastics at school. As long as she could remember she had had problems with opening the screw cap tops of jars and wringing out wet clothes. Recently she had noticed difficulty in rising from a chair and from a supine position. During the night she often woke up with cramps in the calf muscles. After a strenuous walk she once had had a severe cramp in her calves which took several days to disappear.
At physical examination she was a strongly built young woman. Her gait was slightly waddling and her calves seemed to be somewhat hypertrophic. Weakness was observed in both the shoulder girdle muscles and in the lower arm and hand muscles. Further, the pelvic girdle muscles, and especially the gluteal muscles were weak. The tendon reflexes of the arms were normal, but they were absent in the legs.
Serum CPK: 110 U/L. ECG: a right bundle branch block was found; repolarization was normal. The echocardiogram and the pulmonary function tests were normal. The quadriceps biopsy showed occasionally fibres with one or several centrally located vacuoles. Acid maltase activity in skeletal muscle tissue was severely reduced. The glycogen content was normal.

II 1: father, born in 1911
Had never shown symptoms of weakness when still alive. Died in 1979 at the age of 68 of an acute myocardial infarction.

II 2: mother, born in 1915
No abnormalities were found on physical examination at the age of 64.

The muscular form of acid maltase deficiency was discovered by chance in the index case. Both he and his affected sister were not aware of the muscular weakness, probably because of the very slow progression of their disability. Weakness of the hands, as was found in III 7, was not observed in the other patients with the muscular form. The other sibs were not examined.

FAMILY K



III 2 *Index case:* female, born in 1951
Since childhood this young woman had had an equinus foot on the right side. For several years she had complaints of low back pain, and the orthopedic surgeon attributed this to the foot deformity. Since she was not able to do her work as a teacher in a nursery school, a right Achilles tenotomy was performed in 1976 followed by bed rest for two months. Since then she noted difficulty in climbing stairs. Moreover, she discovered that the right calf had become thinner.
On physical examination at the age of 26 she showed a somewhat waddling gait, and could not walk on the heels. The right calf was three cm thinner than the left one. A mild equinus foot was also found on the left (figure 4d). The anterior neck muscles and the shoulder girdles showed a mild weakness (strength grade 4). The pelvic girdle was more severely involved: in most muscles the strength was assessed as grade 2. The left quadriceps was weaker than the right. Turning over when lying on her back was possible only with the help of her hands. The tendon reflexes were decreased in the arms and absent in the legs.

Serum CPK: 150 U/L. The ECG indicated a left ventricular hypertrophy. The echocardiogram was normal. Initially the pulmonary function tests were normal, but six months later the vital capacity was slightly reduced (75 per cent of the normal value). The EMG showed myopathic changes and pseudomyotonic discharges. MCV: normal. Quadriceps biopsy: vacuolar myopathy, the vacuoles being located exclusively in type I fibres. Most vacuoles did not react with PAS, but the AcP reaction was strongly positive. Acid maltase was grossly deficient in the skeletal muscles.

A low-carbohydrate and high-fat diet for three months did not result in improvement of strength.

The equinus foot on the left side became more manifest and was treated with a corrective plaster. Some wasting of the rhomboid and supraspinatus muscles had developed two years after the initial physical examination. The weakness was slightly progressive. She emphatically declared that she felt at her best with regular but not too strenuous activity and she resumed her work at the nursery school.

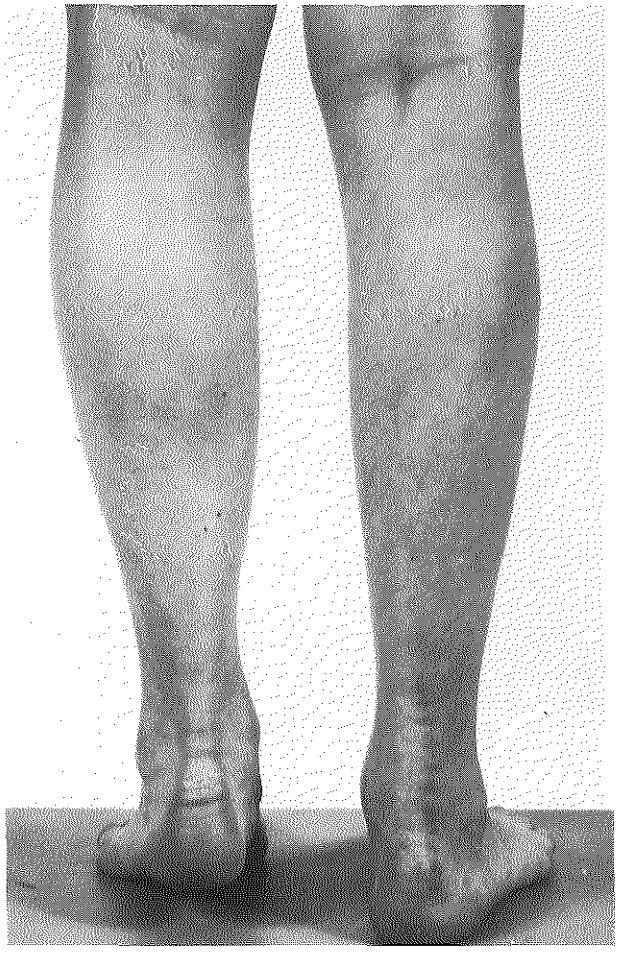


Figure 4d. Case K III 2. Atrophy of the right calf after Achilles tenotomy.Mild equinus foot on the left side.

III 1: male, born in 1948
Although this young male probably knew that he suffered from the same disorder as his sister, he did not consult a physician. He agreed, however, to participate in the family investigation and then told that he had noticed problems in climbing stairs for several

months. Putting on his socks was also difficult. After a minor effort he became short of breath. At school he had been a clumsy athlete, he could never run properly and failed in obtaining a swimming diploma. Moreover, he had been suffering from low back pain for many years..
On physical examination at the age of 29 he showed an asthenic stature with severe wasting of the pectoral, rhomboid and supraspinatus muscles and with winging of the scapulae. The right quadriceps was more wasted than the left. He had a waddling gait with the shoulders thrown backwards and a lumbar lordosis with protrusion of the abdomen ("aldermanic posture", Brooke, 1977, page 20). There was a severe weakness of the trunk muscles, but the strength in the anterior neck muscles was normal. Strength was decreased in the shoulder muscles but normal in the arms. A moderate weakness was found in the pelvic muscles (strength grade 3-4). The gluteal muscles were more severely involved (strength grade 2). The tendon reflexes in the arms and the knee jerks were decreased, the ankle jerks were absent.

Serum CPK: 402 U/L. The ECG indicated a right ventricular hypertrophy. The echocardiogram was normal. A reduced vital capacity (65% of the normal value) was found at pulmonary function testing. Quadriceps biopsy showed a vacuolar myopathy, the vacuoles being present predominantly in type I fibres. The vacuolized fibres showed a diffuse PAS reaction and sometimes the vacuoles were PAS positive. The AcP reaction was strongly positive. Acid maltase activity in the muscle biopsy was severely reduced.

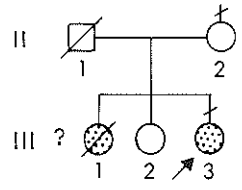
III 3: male, born in 1956
Apart from complaints of low back pain this young man had a normal history and he showed no abnormalities on physical examination.

II 1: father, born in 1920
Had suffered from a nephrotic syndrome at the age of 55. Corticosteroid treatment was successful. Physical examination at the age of 57: normal male.

II 2: mother, born in 1920
No abnormalities were found on physical examination at the age of 57.

The index case had highly unspecific complaints: low back pain, probably due to an equinus foot. Following a long period of bed rest after tenotomy, muscle weakness and an atrophic right calf were noticed. The muscle weakness was moderately severe but slowly progressive. Treatment with a ketogenic diet had no favourable influence on the course of the disease. Her elder brother proved more severely affected with weakness of the limb girdles and of the respiratory muscles. The youngest sib complained of low back pain but he proved otherwise healthy. The father had suffered from a nephrotic syndrome.

FAMILY L



III 3 *Index case:* female, born in 1949
The early motor development of this young female had been slow. She had never been able to lift her head from the supine position She walked without aid at 20 months, but the gait had never been normal and she had a tendency for falling. At the age of six years a muscle biopsy was performed, and a vacuolar myopathy was found. This was thought to be due to glycogen storage.
At the age of twelve no muscular wasting was seen on physical examination, but there was a severe weakness of the trunk muscles. Acid maltase activity in the leucocytes proved decreased and the activities of the phosphorolytic enzymes were normal. On the basis of these findings and considering the clinical picture J. Fernandes concluded in 1963 that the patient was suffering from a clinical variant of acid maltase deficiency.
At the age of twenty she suddenly had a respiratory arrest. Artificial respiration was required continuously for several months, subsequently only at night. Since that time she was unable to walk. A muscle biopsy was repeated and showed a severe vacuolar myopathy. Acid maltase activity was severely decreased.
On physical examination at the age of 29 she was a lean young woman who managed to sit up only with the utmost effort. There was an enormous lumbar kyphoscoliosis. She could stand only when bent forward, holding herself on to the bed. The trunk and shoulder muscles, as well as the sternomastoid and trapezius muscles seemed absent and were accordingly very weak. The neck extensors had normal strength. The arm muscles had normal strength save for a mild weakness (strength grade 3-4) in the atrophic biceps muscles. The pelvic muscles and the proximal muscles of the legs showed very severe weakness. The distal muscles of the legs had normal strength. No contractures were present. The tendon reflexes were absent with the exception of weak ankle jerks. There was some doubt about fasciculations in the tongue being present or not. Co-ordination could not be tested because of the severe weakness. Despite her severe disability she was able to work as a telephone operator.

In addition to the studies already mentioned the following investigations were performed: serum CPK 131 U/L. Electronmicroscopic examination of a skin biopsy: membrane-bound vacuoles filled with glycogen in several cell types.

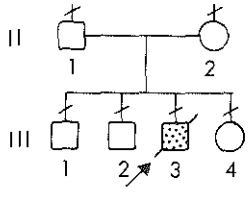
III 1: girl, born in 1942
Died at the age of 12 years, presumably from the same disorder. Her mother reported that she had shown muscular weakness especially of the legs. Breathing had been "peculiar". She died after an illness of less than 24 hours, possibly a respiratory infection. No investigations were performed before or after death.

II 1: father, born in 1902
Died, 64 years old, of a "heart attack".

II 2: mother, born in 1912
On physical examination at the age of 66 no abnormalities were found.

The index case showed a long-lasting progressive weakness and wasting of the trunk, shoulder and pelvic girdle muscles. She needed artificial respiratory support at night and proved severely disabled at the age of 29 years. As early as in 1963, the same year that Hers discovered the existence of acid maltase in human tissues and the deficiency of its activity in Pompe's disease, J. Fernandes, on the basis of the clinical findings and of the biochemical studies by F. Huijing, made the diagnosis of a clinical variant of Pompe's disease. The eldest sib died at the age of 12 years and presumably she had suffered from the same disorder.

FAMILY M



III 3 *Index case:* male, born in 1945

This farmer's son seemed to have had a normal early motor development. However, he had always shown problems with lifting weights and he could not obtain a swimming diploma. Neither could he participate normally in the activities on the farm. From the age of 23 a waddling gait was observed.

At 26 years he was examined at the Department of Psychiatry, Erasmus University, Rotterdam, where he had been admitted because of endogenous depression. His gait was waddling and he showed wasting of the paraspinal and shoulder girdle muscles. On bending forward, the spine seemed to be "stiff" (figure 4e). The shoulder muscles showed more weakness than the proximal arm muscles. A more pronounced weakness was found in the pelvic girdle and in the proximal leg muscles. The hamstrings were the least involved. The tendon reflexes in the arms were depressed. The knee jerks were absent, the ankle jerks diminished.

Serum CPK: 185 U/L. The ECG indicated a partial right bundle branch block. On electromyography no abnormalities were found initially. At a second examination pseudomyotonia was detected in the paraspinal muscles. The MCV was normal. Pulmonary function tests disclosed a severely reduced vital capacity (35% of the normal value), a decrease of the arterial PO_2 (55-60 mm Hg) and an elevated arterial PCO_2 (61 mm Hg). A quadriceps biopsy showed vacuolization in a few fibres which were mainly of type I. The PAS reaction was negative, whereas the AcP reaction was strongly positive in several fibres. Acid maltase in leucocytes and in skeletal muscles proved decreased.

At the age of 27 a severe respiratory insufficiency developed which necessitated continuous artificial respiratory treatment for three days. Subsequently he required respiratory support only at night. Three years later he refused artificial respiration. He died at the age of 30.

At post-mortem examination the cardiac weight was increased (500 grams, normal: 325 grams). Both the right and the left ventricular wall showed hypertrophy. There

was pulmonary oedema. About 500 ml serous fluid was found free within the abdominal cavity. The liver and the spleen were enlarged, weighing 1800 and 250 grams respectively. The shoulder and pelvic muscles as well as the respiratory muscles showed a severe atrophy. Unfortunately, no tissues were frozen for biochemical and histochemical studies. On histological examination no vacuolization of the cardiac muscle was found, but the skeletal muscles as well as the smooth muscle layers in the bladder, in the pylorus, and in some of the arteries showed vacuolar changes. In the liver a severe central congestion was seen, associated with damage of the hepatic cells. The cause of death was thought to be heart failure.

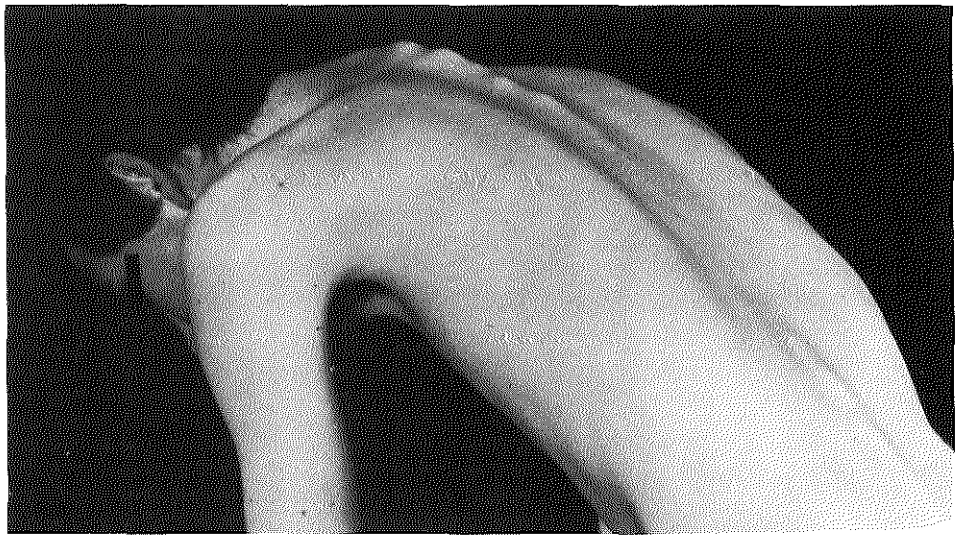


Figure 4e. Case M III 3. Wasting of paraspinal muscles and restricted flexibility of the spine.

III 1, 2 and 4: sibs, born in 1942, 1943 and 1949 respectively
Were all normal on physical examination.

II 1: father, born in 1910
Was normal on physical examination at the age of 62.

II 2: mother, born in 1912
No abnormalities were found on physical examination at the age of 60.

This patient with long-standing weakness of the trunk and of the limb girdles showed considerable respiratory problems. He died at the age of 30 after refusing further artificial respiratory support. The cause of death was presumed to be heart failure. The sibs were normal on physical examination.

CASE N

Case N: male, born in 1927
This farmer had participated normally in gymnastics as a child. At the age of 20 he entered the Army. A vaccination for smallpox was followed nine days later by high fever and headache. One month later he noticed difficulty with running. Walking became increasingly difficult and he began to complain of low back pain. Although he completed the military service, he was from that time unable to perform strenuous labour. At the age of 37 he was admitted to a hospital with an acute respiratory distress and he received artificial respiration for four months continuously and subsequently only at night.
On physical examination at the age of 45 a slight dyspnoea was noted and he used his auxiliary respiratory muscles. The shoulder girdle muscles were severely atrophic and mildly weak. The anterior neck muscles and the proximal muscles of the arms showed a mild weakness as well. The trunk muscles and especially the paraspinal muscles were severely atrophic and weak. In the pelvic muscles, notably in the iliopsoas muscles, strength was severely decreased, whereas the quadriceps muscles showed only minimal weakness. The left knee jerk was absent, the other tendon reflexes were normal.

The serum CPK activity was normal. EMG: myopathic pattern. A quadriceps biopsy showed vacuolization of some fibres. The vacuoles reacted positively for PAS and AcP. There was type I preponderance. Acid maltase activity in muscle tissue was severely reduced.

In this patient symptoms became apparent at the age of 20 years following a period of bed rest for a severe reaction to smallpox vaccination. Because of respiratory insufficiency he needed artificial support at night from the age of 37.

CASE O

Case O: female, born in 1924
From the age of approximately 35 years this woman walked with protrusion of the abdomen. Several years later she experienced low back pain. Rising from a chair became difficult and she was unable to climb stairs without holding on to the bannister. At the age of 43 she noticed a shuffling gait with the right leg.
Physical examination at that time showed atrophy and weakness of the paraspinal muscles. She had a waddling gait. A mild weakness was noted in the shoulder and pelvic muscles, with a symmetric distribution except for the right iliopsoas, which was more involved than the left. The tendon reflexes were normal.

The serum CPK activity was normal. EMG: myopathic changes. MCV: normal. Quadriceps biopsy: occasionally a fibre with one or more small vacuoles. The initial diagnosis was "myopathy". Two years later the tendon reflexes were noted to be brisk. A muscle biopsy was repeated, but again minimal changes were found: some vacuoles which did not react for routine PAS, but which were positive with AcP. Some of the vacuoles contained globular structures which stained intensely red in Gomori's

trichrome. Acid maltase deficiency was considered and this was confirmed subsequently by enzyme studies in leucocytes, fibroblasts and muscle tissue.

The patient died suddenly at the age of 54.

The post-mortem examination showed an acute pancreatitis as the probable cause of death. No abnormalities were found on macroscopical and microscopical examination of the heart and the brain. Unfortunately, no tissues were frozen for biochemical and histochemical studies.

The cause of the long-lasting atrophy and weakness in the paraspinal, shoulder and pelvic muscles in this patient remained obscure for several years. The diagnosis of acid maltase deficiency was made after a second muscle biopsy, which was done at a time when the adult-onset variant of this disorder had been reported in the literature. The patient died at the age of 54, presumably of an acute pancreatitis.

CASE P

Case P: female, born in 1923

This woman complained of muscle weakness since the birth of her youngest child in 1962, but she remembered that already as a child she had had difficulty in walking and running. At the time of examination her main problems were rising from a chair and climbing stairs.

At the age of 52, physical examination showed a waddling gait with a lumbar lordosis. The anterior neck muscles and the muscles of the shoulders and arms had a normal strength. The pelvic girdle muscles were mildly weak. The quadriceps muscles showed atrophy and weakness, the right one being the most involved. In addition, the dorsiflexors of the feet were weak. The strength in the paraspinal muscles was also diminished. The tendon reflexes in the arms and the ankle jerks were normal, but the knee jerks were absent.

The serum CPK was elevated to twice the normal value. EMG and MCV were normal. The quadriceps biopsy showed occasional fibres with multiple vacuoles. The vacuoles reacted positively for PAS and AcP. The diagnosis of acid maltase deficiency was confirmed by enzyme studies in muscle and leucocytes.

CASE R

Case R: male, born in 1918

This man had been a fencer in the Dutch national team and he had participated in the pentathlon during military service. Nevertheless, he remembered very well that he never had been able to pull himself up to the rings or to a horizontal bar. From the age of 32

he walked with a protruded abdomen, and climbing stairs had become difficult from the age of 55.
On physical examination at the age of 57, he walked with an "aldermanic posture". The trunk muscles were wasted and weak. The strength was diminished in the anterior neck muscles, the shoulder and pelvic girdle muscles, and the dorsiflexors of the feet. The tendon reflexes were normal.

Normal CPK values were found on three occasions. Pulmonary function tests: vital capacity slightly reduced to 85 per cent of the normal value. EMG: myopathic pattern. MCV: normal. Deltoid biopsy: only non-specific changes. No vacuoles were found. The AcP reaction, however, proved strongly positive in several fibres. Acid maltase activity turned out to be absent.

A younger sister had died at the age of 52, presumably of the same disorder. A muscle biopsy had shown a myopathy with PAS positive vacuoles. Enzyme assays were not carried out.

This case illustrates that performing sports at a high level is not incompatible with a slowly progressive metabolic disorder of the skeletal muscular system. There were only minimal changes in the muscle biopsy. His sister had presumably suffered from the same disorder.

CASE S

Case S: female, born in 1918
This woman had had a gait disturbance for at least five years and she had become short of breath on exertion.
On physical examination at the age of 54, she had a waddling gait and a thoracolumbar scoliosis. Besides weakness of the pelvic girdle muscles, there was a more conspicuous weakness of the quadriceps muscles, especially on the left. Shoulder and arm muscles were normal. The knee and the ankle jerks were decreased.

The serum CPK activity was normal. EMG: myopathic features and pseudomyotonic discharges. Pulmonary function tests: reduced vital capacity (50 per cent of the normal value). Quadriceps biopsy: fibres with multiple small vacuoles regularly dispersed through the tissue. The vacuoles did not react for routine PAS but were strongly positive for AcP. In the muscle biopsy acid maltase activity proved absent.

5 RESULTS OF THE INVESTIGATIONS IN THE PATIENTS

Clinical manifestations

The generalized form (summarized in table 4)

Five boys and two girls were included as part of this study.

The first symptoms were apparent before the age of three months in six infants and they had all died before the age of nine months. In one boy (G III 2) the disease had a more protracted course: the first symptoms were observed at six months and he suddenly died of aspiration at the age of two years. Dyspnoea, failure to thrive, feeding difficulties, weak crying, or hypotonia were the presenting symptoms. With one exception the infants had been born after a full-term pregnancy and with a normal birth weight. One baby (A III 1) was delivered by the breech at 37 weeks' gestation and her birth weight was 2440 grams. The neonatal period had been uneventful in all infants.

Four children were first-borns. In two families an elder sib had died young: one of a disorder similar to that of the patient and one of a "congenital cardiac disease".

Six infants were examined by their physicians shortly before death. They all looked pale or greyish pale. Four were noted to be dyspnoeic and in three an apical systolic murmur was heard over the heart. The liver was enlarged in all patients, and a slightly enlarged spleen was found in two. The tongue was large in two, doubtfully enlarged in one, and normal in three of the patients. A reduced muscle tone was recorded in five patients. Absent or diminished tendon reflexes were found in two patients, but it was remarkable that in two other patients the tendon reflexes were brisk. Data on the lengths and the weights of five patients were available. The rate of growth in length was normal, whereas the increase in weight lagged behind (figure 5).

Table 4. History and physical examination of the seven patients with the *generalized* form

family, patient number, and sex	age (in months) at			physical examination								
	onset	death	examination	weight (g)	colour	dyspnoea at rest	enlarged tongue	auscultation	enlarged liver (cm below costal margin)	enlarged spleen (cm below costal margin)	muscle tone*	tendon reflexes†
A III 1 ♀	soon after birth	2½	2	3090	greyish-pale	yes	?	soft syst. murmur	6	1–2	?	?
B III 19 ♀	1	3½	3	5300	greyish-pale	yes	+	no murmur	2–3	1	↓	absent
C III 3 ♂	1½	4	4	3600	pale, peri-oral cyanosis	yes	–	no murmur	5	–	?	?
D III 1 ♂	1½	4½	4	4600	greyish-peri-oral cyanosis	yes	–	soft syst. murmur	4	–	↓	?
E III 3 ♂	2½	5	4	5690	pale	no	±	syst. murmur	3	–	↓	brisk
F III 4 ♂	soon after birth	8	6½	6420	pale	no	–	no murmur	3	–	↓	KJ normal AJ brisk
G III 2 ♂	6	24	11	8400	normal	no	+	no murmur	4–5	–	↓	↓

Legends: * ↓ : reduced
† KJ : knee jerk; AJ: ankle jerk; ↓ : decreased
? : : no data available on tongue, muscle tone, or tendon reflexes

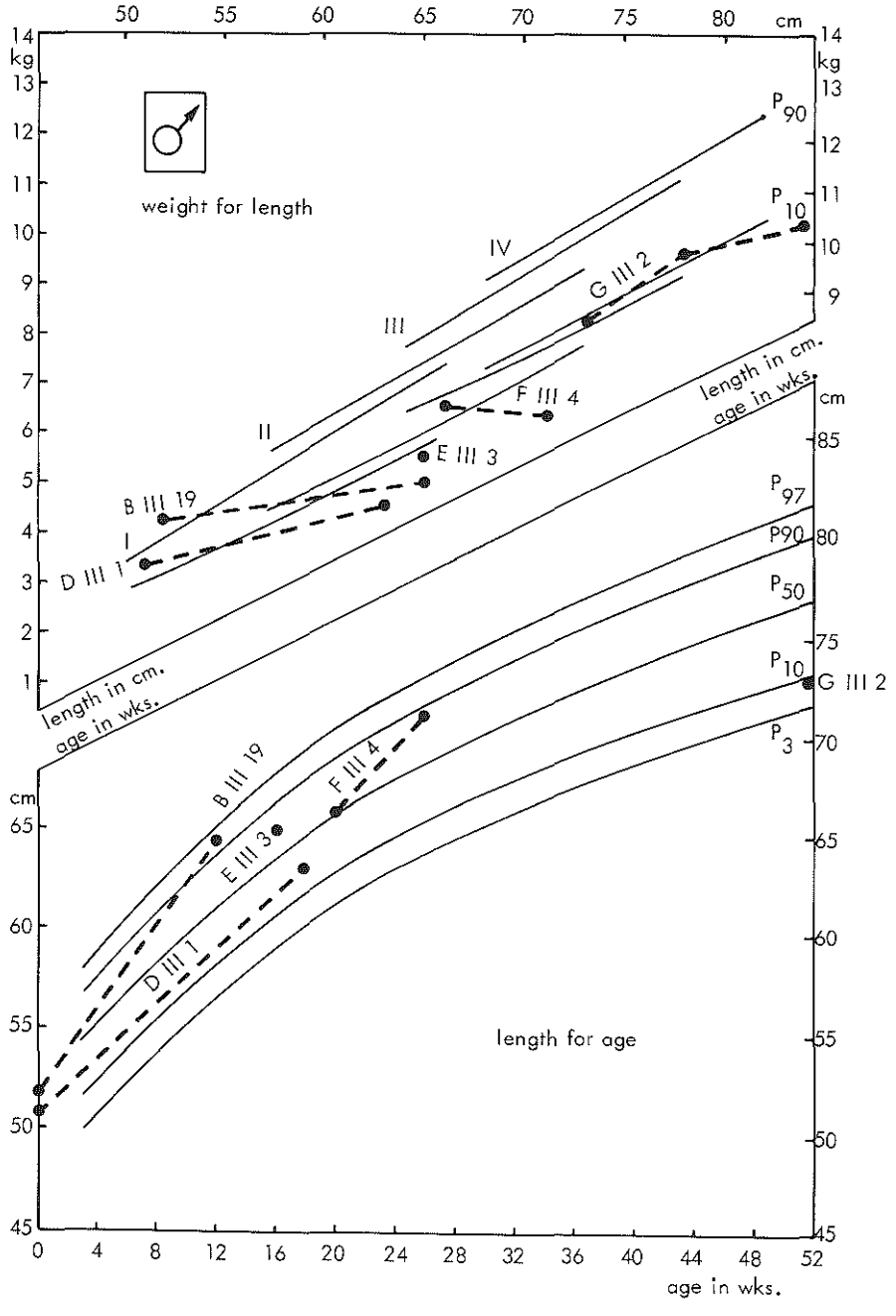


Figure 5. Lengths and weights of five patients with the generalized form.
"Growth diagram 1965" in male infants (van Wieringen, 1973). For the sake of simplicity, the data of the only female patient of these five (B III 19) have been depicted in the same diagram.

The muscular form (summarized in figure 6 and table 5).

Seven males and six females were included as part of this study.

The age at onset of the symptoms proved difficult to establish in the patients with the muscular form, as is illustrated in figure 6. There had often been difficulties in the motor performance many years before the onset of the weakness. In two patients the weakness in retrospect dated from infancy: their parents indicated that they could not lift their heads when trying to sit up. Most patients were clumsy in childhood, they could not run well, had difficulties in gymnastics, or did not succeed in obtaining a swimming diploma. They were often simply considered "stiff". In two patients (N and B I 3) the onset seemed to have been sub-acute.

It was striking that the patients often had no spontaneous complaints notwithstanding the presence of advanced weakness.

In two families two sibs were affected and in another family an elder sib had died, presumably of the same disorder.

In all patients but one (J III 7) the proximal muscles were more severely involved. The pelvic girdle was more affected than the shoulder girdle in eight, in four the pelvic and shoulder girdle muscles were involved more or less equally, and in one patient the weakness was restricted to the pelvic muscles. A slight asymmetry of the weakness was found in seven patients. In three patients a severe respiratory insufficiency was present, and in six a subclinical respiratory insufficiency was found by pulmonary function tests. In all patients except one the tendon reflexes were normal, diminished, or absent. In patient O the tendon reflexes were brisk. The plantar responses were flexor in all patients.

In six cases acid maltase deficiency was the first diagnosis. In the remaining cases the initial diagnosis had been: "myopathy" (2), muscular dystrophy (2), polymyositis (1), "muscular glycogenosis" (1), and "equinus foot" (1).

Two patients had died at the ages of 54 (0) and 30 (M III 3) of acute pancreatitis and of heart failure respectively. The latter cause of death may be considered secondary to chronic alveolar hypoventilation (Turino et al., 1965).

Ancillary studies

Roentgenogram of the chest

An enlarged heart was found in all cases with the generalized form.

In the patients with the muscular form the X-rays showed no characteristic abnormalities.

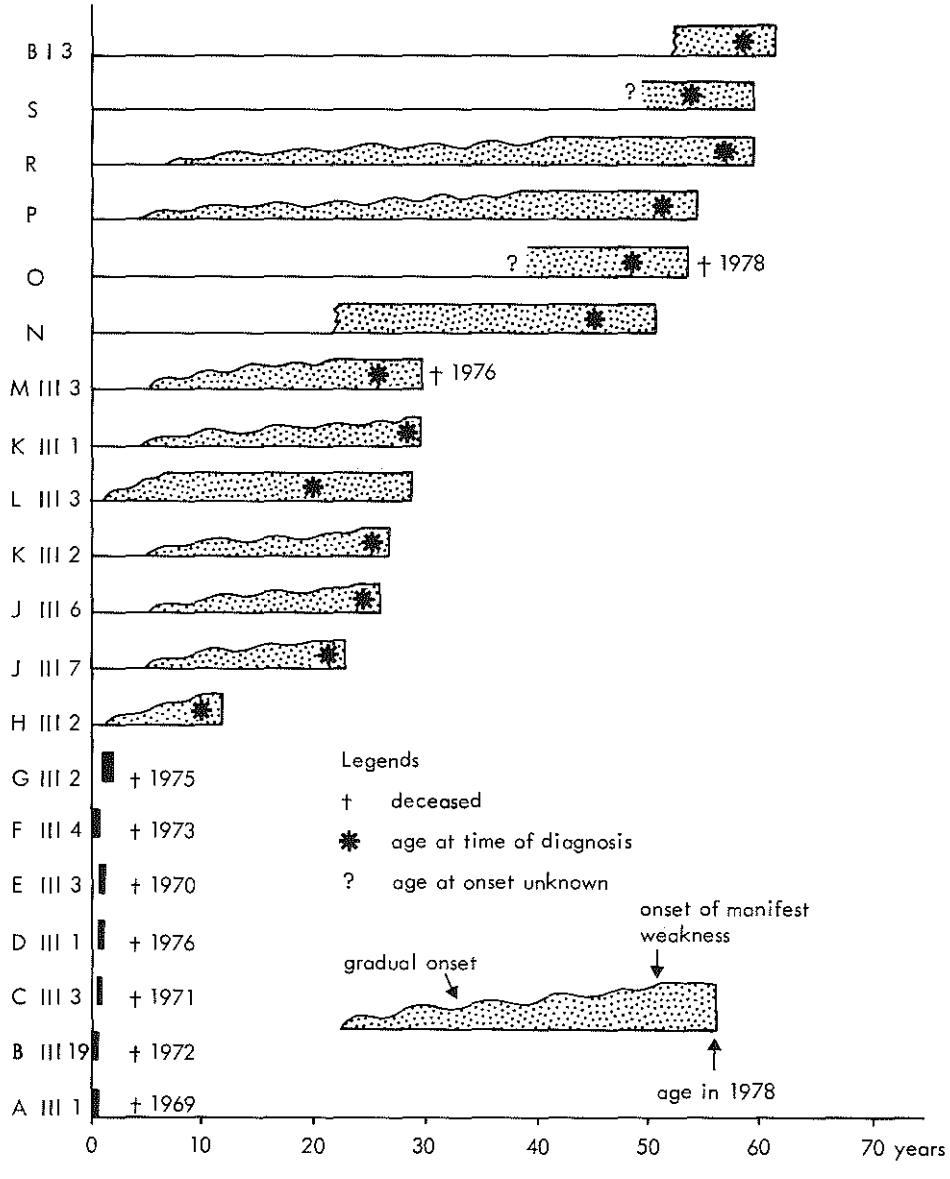


Figure 6. Onset and course of the disease in the patients with acid maltase deficiency.

Table 5. History and physical examination of the 13 patients with the *muscular* form

family, patient number, and sex	onset of motor disturbances	age (in years) at onset of weakness	age (in years) at diagnosis	age (in years) at death	muscle weakness: proximal vs. distal*	muscle weakness: pelvic vs. shoulder-girdle†	muscle weakness: respiratory ‡	tendon reflexes ϕ: arms	tendon reflexes ϕ: legs
H III 2 ♂	infancy	10	10		>	>	± (10)	absent	absent
J III 7 ♀	childhood	gradually	21		<	=	– (22)	normal	absent
J III 6 ♂	childhood	gradually	24		>	>	– (25)	absent	absent
K III 2 ♀	childhood	25	25		>	>	± (26)	↓	absent
L III 3 ♀	infancy	7	20		>	=	+ (20)	absent	KJ absent AJ ↓
K III 1 ♂	childhood	29	29		>	>	± (29)	↓	KJ ↓ AJ normal
M III 3 ♂	childhood	23	26	30	>	>	± (26)	↓	KJ absent AJ ↓
N ♂	21 y	21	45		>	>	+ (37)	normal	left KJ absent
O ♀	?	35	48	54	>	=	?	brisk	brisk
P ♀	childhood	39	52		>	only pelvic girdle	?	normal	KJ absent AJ normal
R ♂	childhood	32	57		>	=	± (55)	normal	normal
S ♀	?	49?	54		>	>	± (54)	?	↓
B I 3 ♂	53 y	53	59		>	>	+ (59)	normal	↓

Legends: * > weakness proximally more pronounced than distally

† > pelvic mucles weaker than shoulder muscles

= pelvic and shoulder muscles equally affected

ϕ KJ: knee jerk; AJ: ankle jerk; ↓ : decreased

‡ ± subclinical respiratory insufficiency, assessed by pulmonary function tests

\+ respiratory insufficiency requiring artificial respiration

– no respiratory problems, pulmonary function tests normal

? no data available on onset, respiratory function, or reflexes

ECG and echocardiogram

The analysis of the ECG data in the patients with the generalized form is shown in table 6. An acceleration in the atrio-ventricular conductive system, as indicated by a short PR interval, was seen in all cases, as well as signs indicating biventricular hypertrophy (including hypertrophy of the interventricular septum). Indications for hypertrophy of the right atrium were found in two cases. Four patients showed the characteristics of a left ventricular overload, and one patient those of a right ventricular overload. One patient (C III 3) showed a supraventricular tachycardia with a cardiac rate of 300 per minute.
No characteristic signs of cardiac involvement were seen on the ECG of eight patients with the muscular form.

Echocardiography was not performed in the patients with the generalized form.
In five cases with the muscular form no abnormalities were detected by this investigation. However, in one patient (H III 2) thickening of the posterior wall of the left ventricle and of the septum was present (10 mm, normal value: 7-8 mm, plate 2). Repeat study after one year showed no increase in thickening of the cardiac walls or of the septum, and the ECG was normal.

EMG

Data of ten patients (one with the generalized form and nine with the muscular form) were available. In seven cases a myopathic pattern was found. Fibrillations and complex action potentials during relaxation were observed infrequently. Bizarre high-frequency discharges ("pseudomyotonic discharges") were found in five patients. In one patient these discharges were the sole abnormality and they were detected only in the trunk muscles. Motor conduction velocities, measured in nine patients, were normal.

CPK

The routine serum CPK values of 14 cases are summarized in figure 7. The activity was moderately raised in most patients, with values between 91 and 850 U/L (normal value below 50 U/L). In four, all adults above 45 years of age, the CPK activity proved normal. In patient B I 3 a normal value was found at the onset of the disease, and a slightly raised value six years later.

Table 6. ECG data of the patients with the generalized form

patients	PR interval (sec) *	L ventricular hypertrophy †	R ventricular hypertrophy ‡	biventricular hypertrophy ϕ	R atrial hypertrophy ‖	septal hypertrophy q	L ventricular overload **	R ventricular overload ††
A III 1	0.06	+	+	+	+	+	+	—
B III 19	0.08	+	+	+	—	+	—	—
C III 3	0.06	+	+	+	—	+	+	—
D III 1	0.08	+	+	+	—	+	+	—
E III 3	0.06	+	+	+	—	+	+	—
F III 4	0.08	+	+	+	+	+	—	+
G III 2	0.10	+	+	+	—	+	—	—

Legends: * normal value $>$ 0.12 sec.

† + if the sum of the amplitudes of the R wave in V_6 and the S wave in V_3R (or V_1) is $\geqslant$5 millivolt (mv) (Sokolov index)

‡ + if in infants $>$1 month and $<$1 year the amplitude of the R wave in V_3R (or in V_1) is $\geqslant$ 1.5 mv

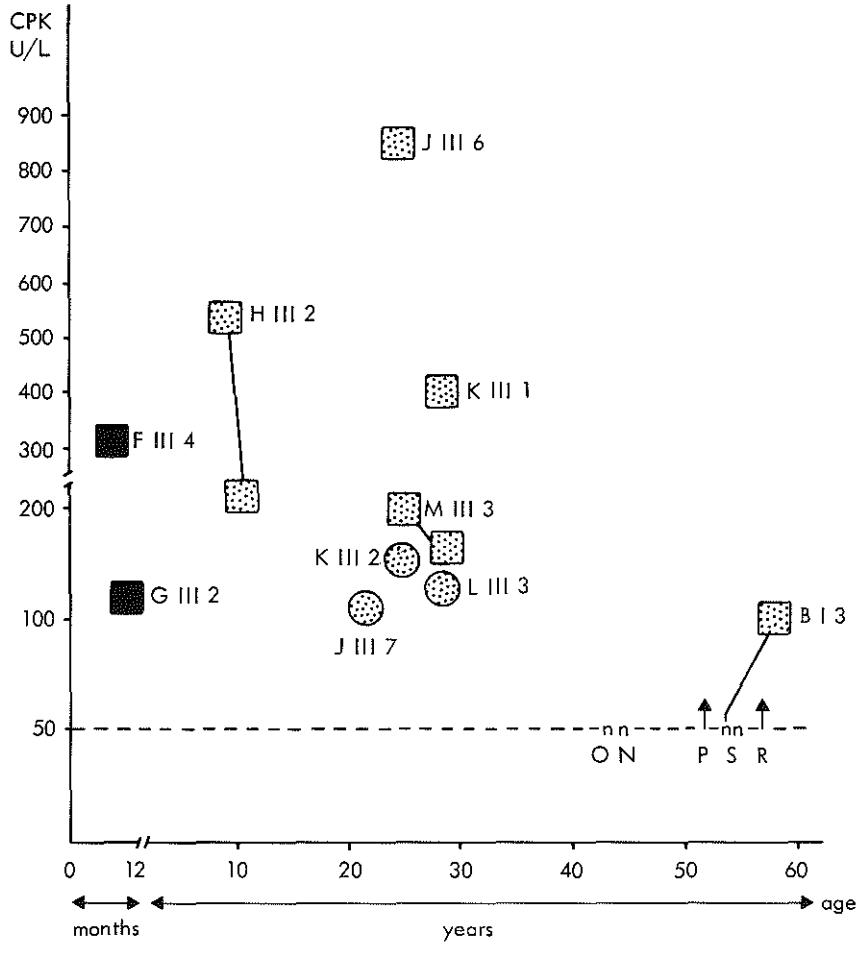
ϕ + if the sum of the amplitudes of the R and S waves in the mid-precordial lead is $>$ 6 mv (Katz index)

‖ + if the P wave is spiked and has an amplitude of $\geqslant$ 0.25 mv

q + if the amplitude of the Q wave in V_6 is $\geqslant$ 0.4 mv

** + if depressed T wave in the left precordial leads is seen

†† + if depressed T wave in V_3R is seen



* according to Anon. (1970). Normal value less than 50 U/L

male, generalized form

female, muscular form

n normal value measured in other hospital

↑ elevated value measured in other hospital

O, N, P, S, R = patients without pedigree data

F III 4, G III 2 etc. = patients with pedigree data

Figure 7. Serum CPK values* in 15 patients with acid maltase deficiency.

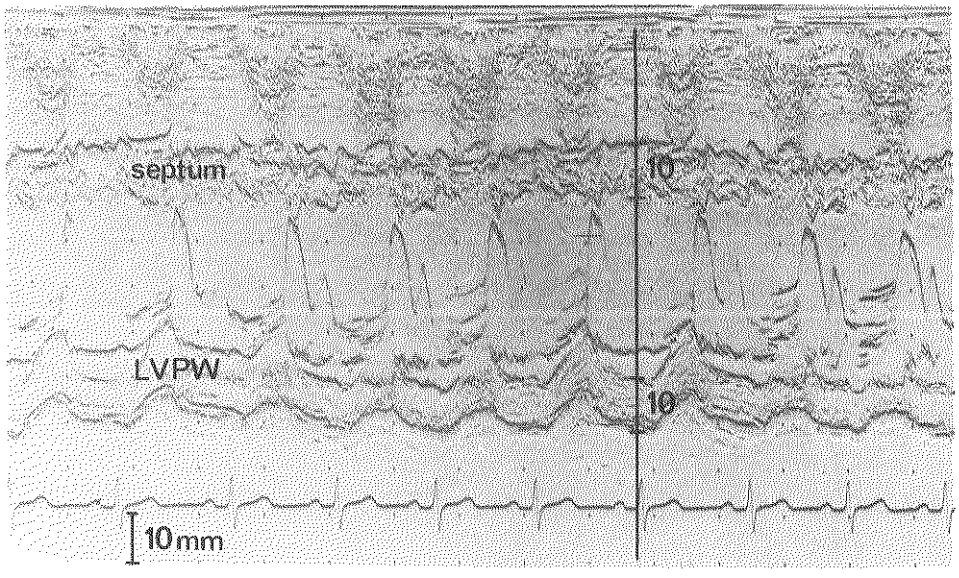


Plate 2. Echocardiogram of case H III 2.
Thickening of the posterior wall of the left ventricle (LVPW) and of the septum (10 mm, normal value: 7-8 mm).

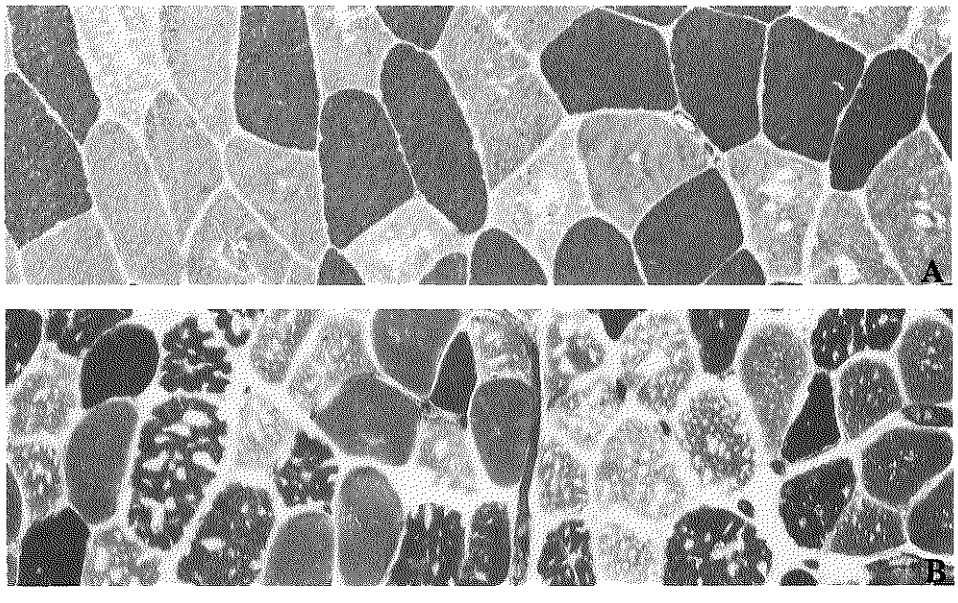


Plate 3.
A. Quadriceps biopsy from patient K III 2. Vacuoles almost exclusively in type I fibres. ATPase reaction pH 9.4; x 150.
B. Quadriceps biopsy from patient H III 2. Vacuoles in both fibre types. ATPase reaction pH 9 4; x 150.

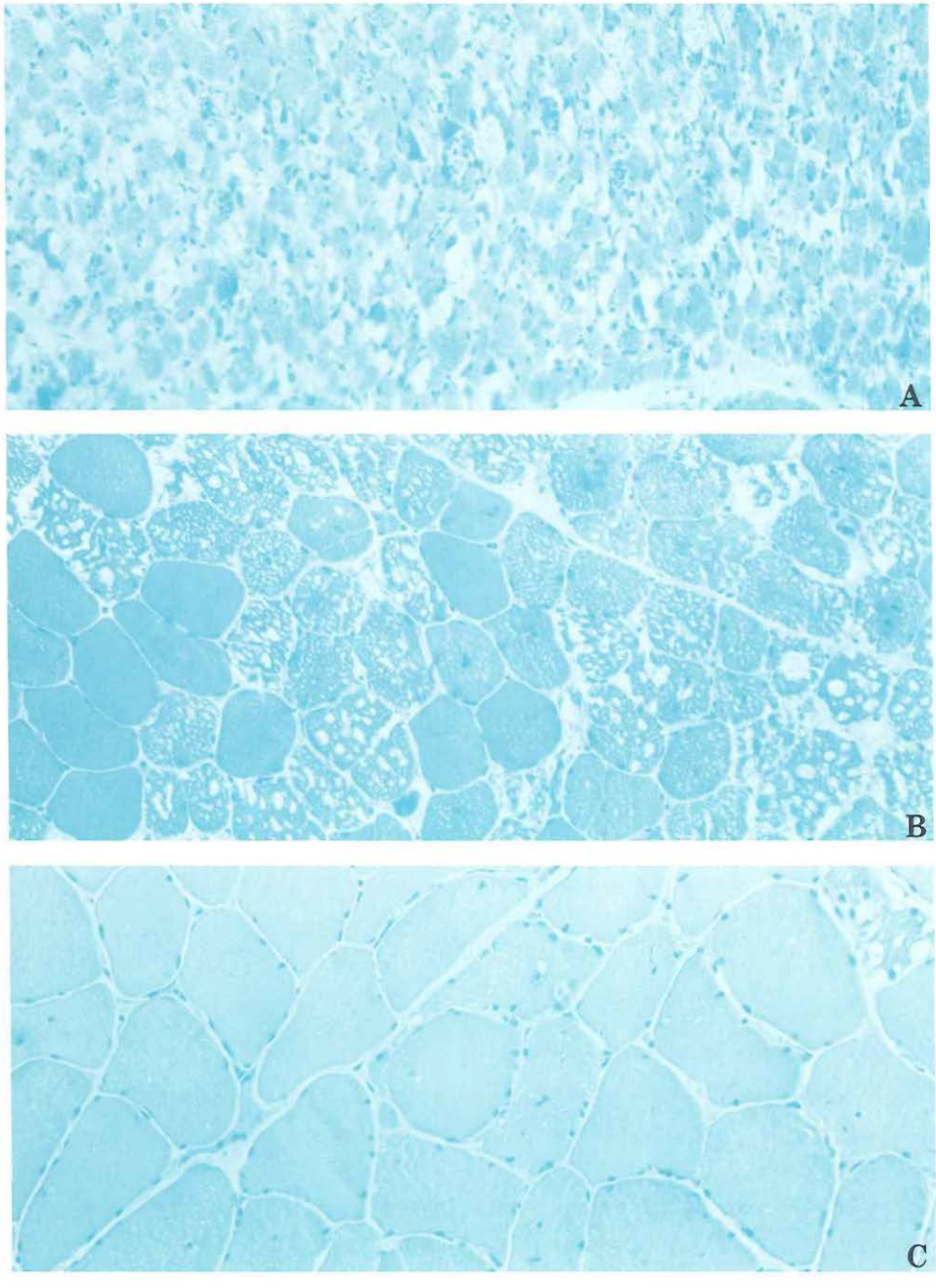


Plate 4. Varying degrees of vacuolization in biopsies of different patients.
A. Gastrocnemius biopsy from patient G III 2 (generalized form), at the age of 11 months.
B. Quadriceps biopsy from patient H III 2 (muscular form), at the age of 10 years.
C Quadriceps biopsy from patient B I 3 (muscular form), at the age of 59 years.

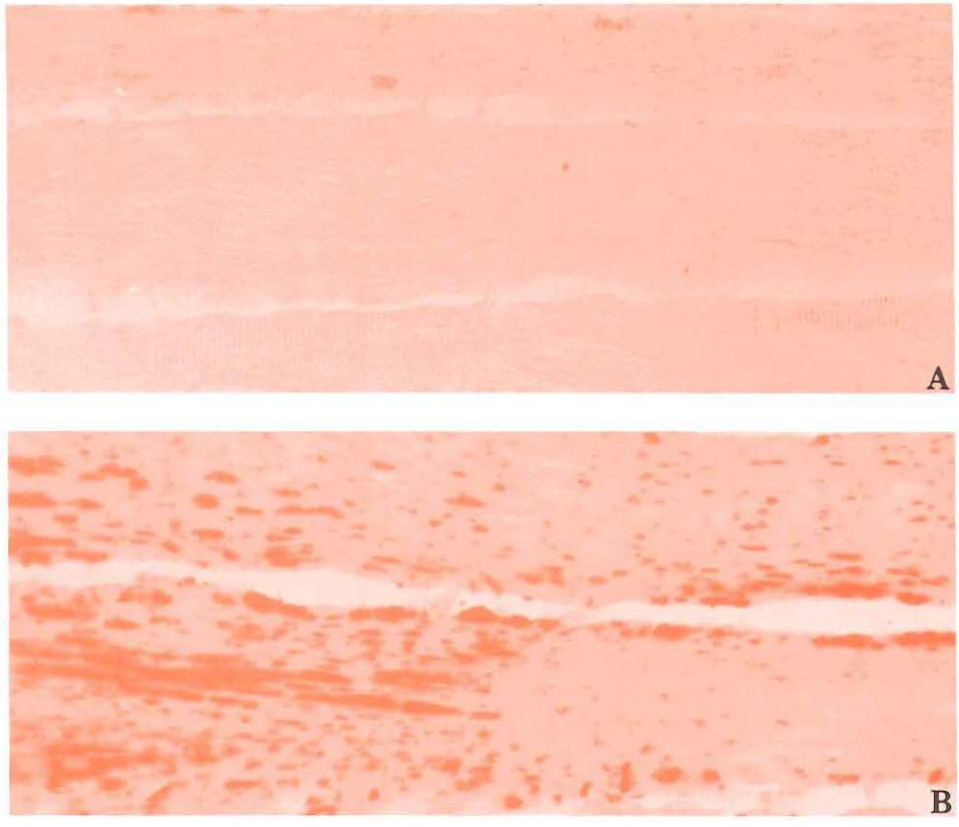


Plate 5. Acid phosphatase (AcP) reaction. Quadriceps biopsy from patient B I 3; x 280.
A. According to Burstone (1958).
B. Same reaction with the use of a semipermeable membrane (Meijer 1972). Strongly AcP reactive sites are evident

Oligosaccharide excretion pattern in the urine (table 7)

After decoding, the group of 86 persons consisted of: seven patients with the muscular form, including B I 3; 17 obligate heterozygotes and 18 possible heterozygotes for the generalized form or for the muscular form; 34 members of family B (possible heterozygotes), and ten spouses of members from family B as controls.

Of the 86 thin-layer chromatograms, rated in a "blind" fashion, five showed such a dark tetrasaccharide band that they were identified by each of the three observers as excretion patterns of patients. This proved correct after the code was broken.

Eighteen persons had, according to all three observers, a faint tetrasaccharide band which was thought to correspond with heterozygosity. It appeared that two patients (B I 3 and J III 7) were included in this series of "faint-band excretors". Futher, only one of the obligate heterozygotes showed a faint band. On the other hand, five of the ten spouse-controls belonged to the group of "faint-band excretors".

Thus, it can be concluded that TLC of oligosaccharides may be used for the identification of patients with acid maltase deficiency, but that this method cannot be used for the identification of heterozygotes. Moreover, a dark tetrasaccharide band is by no means diagnostic for acid maltase deficiency since this band may also be found in patients with other types of glycogen storage disease or with other neuromuscular diseases.

Table 7. Results after decoding following "blind" rating acoording to the colour intensity of the tetrasaccharide band in 86 urinary thin-layer chromatograms

		heterozygotes			
	patients	obligate	possible	controls	total
colour intensity	n=7	n=17	n=52	n=10	n=86
dark	5				5
faint	2	1	10	5	18
absent		16	42	5	63

Morphological examination of the muscle biopsies

Muscle biopsies from three patients with the generalized form and from all patients with the muscular form of acid maltase deficiency were available for reviewing.

Extensive vacuolization by glycogen storage was found in the psoas muscle and the tongue, obtained at post-mortem in a patient with the generalized form. Similar abnormalities were present in a biopsy from the gastrocnemius muscle of another infant and in a biopsy from an unspecified muscle of the third case. A basophilic substance was found by the Alcian blue method in the biopsy of the second patient.

Of the patients with the muscular form a quadriceps biopsy had been performed in 12 and a deltoid biopsy in one. On light-microscopic examination, non-specific features (variation in fibre size and displacement of subsarcolemmal nuclei towards the centre of the fibre) were present in all but one of the biopsies. In the deltoid biopsy these were even the only abnormalities. Vacuoles were present in widely varying amounts (plate 4). Sometimes they had to be carefully looked for. At the histochemical examination, it was often difficult to decide whether the vacuoles showed a positive reaction for PAS when the routine PAS procedure had been applied. The PAS dimedon procedure (Barka and Anderson, 1963) proved more sensitive. The AcP reaction appeared to be a valuable screening procedure, especially if the technique with semipermeable membranes (Meijer, 1972) was used (plate 5). In sections with barely detectable vacuoles, the reaction for AcP was often prominent. It has to be emphasized, however, that this procedure is by no means diagnostic. It was not always possible to determine whether the vacuoles were located in the fibres of type I, or type II, or both. In three patients (H III 2, J III 6, and S) they were present in both fibre types and in three cases (K III 1, K III 2, and M III 3) they were mainly found in type I fibres (plate 3). Type I preponderance was seen in two patients (J III 6 and N). "Globular structures" as described by Horoupian et al. (1978) were observed in three cases (H III 2, L III 3, and 0).

Ultrastructural examination was performed in three patients suffering from the muscular form. Vacuoles limited by a membrane were seen in the subsarcolemmal space as well as between the myofibrils (figure 8). They looked empty or were filled with beta glycogen particles. Autophagic vacuoles filled with glycogen particles and with lamellar structures were found as well. Occasionally, lipofuscine inclusions were observed. Free glycogen was seen in one patient (K III 1) in whom glycogen was also present in numerous mitochondria.

It is interesting to investigate the possibility of a correlation between the strength of the muscle from which the biopsy was taken, the severity of the morphological abnormalities, and the level of acid maltase activity in this muscle. Sufficient data of ten patients were available for this purpose. These are shown in figure 9. If a strength grade 4 or 5, barely detectable vacuoles in the biopsy, and a decreased acid maltase activity were taken as the "least-severe" combination, and if a strength grade 3, a considerable or a moderate amount of vacuoles, and a no-detectable acid maltase activity were considered as the "most severe" combination, two

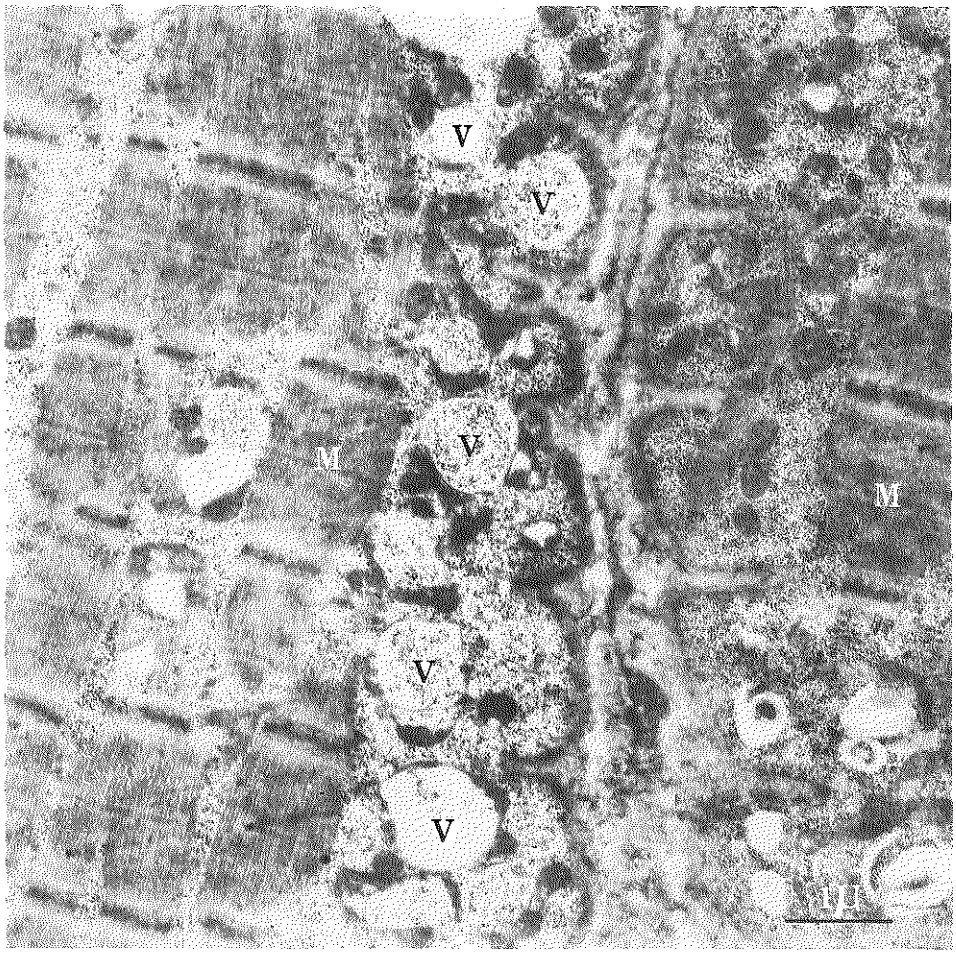


Figure 8. Electronmicrograph showing vacuoles (V) filled with varying amounts of glycogen. They are localized mainly in the subsarcolemmal space. M: myofibrils.
Quadriceps biopsy from patient K III 2, original magnification x 22,275.

patients (J III 7 and B I 3) showed the least severe combination, and two (K III 2 and S) showed the most severe combination. The remaining patients showed random combinations of the three criteria. Therefore, there seems to exist no strict correlation between these three parameters.

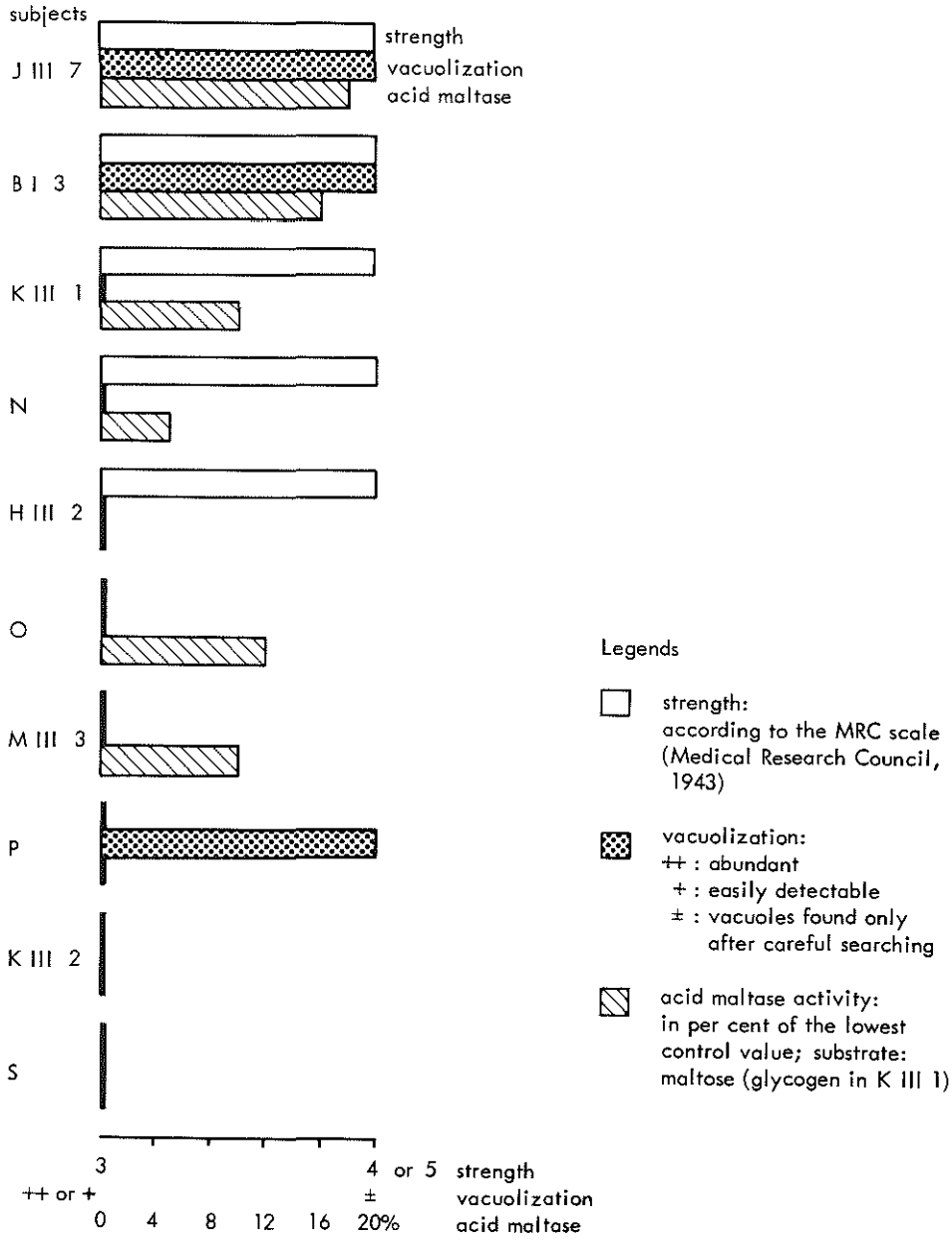


Figure 9. Strength, vacuolization, and acid maltase activity in the quadriceps of ten patients with the muscular form.

Maltase assays

Acid and neutral maltase measurements were done in several samples of all patients. They were carried out with different substrates (maltose, glycogen, MU-glucoside, or pNP-glucoside).

Urine (see table 8)

Seven patients with the muscular form were investigated. The ratio of acid maltase / β-hexosaminidase was reduced in all to 9 to 16 per cent of the lowest control value. Although the ratio of acid to neutral maltase was also reduced, a better distinction between patients and controls was obtained with the ratio versus β- hexosaminidase.

Table 8. Acid maltase assays, expressed as ratio of this enzyme to β-hexosaminidase and to neutral maltase in the *urine* of seven patients with the muscular form

patients	acid maltase/ β-hexosaminidase	acid maltase/ neutral maltase
J III 6	0.051	0.8
L III 3	0.053	1.0
H III 2	0.061	1.1
O	0.065	1.6
B I 3	0.071	1.5
K III 2	0.085	0.9
J III 7	0.09	0.6
controls	n=39	n=38
range	0.56-2.30	1.8-15.3

Total leucocytes (see table 9)

Maltose was used in total leucocytes of four patients with the generalized form and of ten patients with the muscular form. The value was normal or near the lowest control value in all patients but three. The ratio of acid over neutral maltase was diminished in ten.

With glycogen, however, the activity at acid pH as well as the ratio of acid

over neutral maltase was severely reduced in all seven patients (three with the generalized form and four with the muscular form) in whom the assays were carried out.

Table 9. Maltase activities* in *total leucocytes* of 14 patients with acid maltase deficiency

	[maltose]		[glycogen]			
	13.8 mM		10 mg/ml		20 mg/ml	
patients	pH 4.0	4.0/6.5	pH 4.0	4.0/6.5	pH 4.0	4.0/6.5
generalized form						
G III 2	3.00	0.69	—	—		
E III 3	4.25	0.60	0.09	0.27		
F III 4	5.06	0.62	—	—		
C III 3	7.77	0.80				
muscular form						
P	3.95†	0.64				
H III 2	4.78	0.77			0.05	0.16
O	5.34†	0.67				
R	5.52	0.80				
S	6.31	0.66				
J III 7	6.58	0.74			0.06	0.18
J III 6	6.94	0.65			0.10	0.38
M III 3	7.15†	0.61	0.07	0.19		
L III 3	8.36†	0.62				
B I 3	9.50†	0.67				
controls	n=14	n=14	n=13	n=13	n=4	n=4
range	5.8-14.5	0.7-1.1	0.44-0.94	1.05-1.83	0.66-1.15	0.70-2.0

* expressed in nmol glucose /min per mg protein

— not detectable

† reported by Koster et al. (1972)

Muscle (see table 10)

Using maltose, acid maltase activity proved not detectable in two patients with the generalized form and in six patients with the muscular form. In

the remaining four patients with the generalized form and six with the muscular form a reduced activity of 4 to 28 percent of the lowest normal value was detected. Neutral maltase appeared to be essentially normal in four infants, and in nine patients with the muscular form. It proved severely reduced or not detectable in two infants and in three adults.

In six patients with the muscular form, acid maltase was measured with glycogen. In one patient no activity was detectable and in the other

Table 10. Maltase activities* in *skeletal muscles* of 19 patients with acid maltase deficiency

	[maltose] 13.8mM		[glycogen] 20 mg/ml	
patients	pH 4.0	pH 6.5	pH 4.0	pH 6.5
generalized form				
C III 3	–	–		
F III 4	–	0.13		
A III 1	0.17	0.65		
B III 19	0.44	2.89		
G III 2	1.09	0.89		
E III 3	1.16	2.54		
muscular form				
P	–	–	–	1.49
R	–	–		
S	–	–		
J III 6	–	0.76	0.11	1.76
K III 2	–	1.74	0.19	1.87
H III 2	–	2.69		
N	0.20	0.86		
O	0.49	1.02		
B I 3	0.69	1.03	0.09	2.06
J III 7	0.77	0.86	0.20	1.53
K III 1			0.06	
controls	n=11	n=11	n=11	n=11
range	4.20-21.39	0.72-4.11	0.58-1.83	1.36-2.70

* expressed in nmol glucose / min per mg protein

— not detectable

five the activities ranged between 10 to 34 per cent of the lowest control value. The neutral maltase activities were normal.

Fibroblasts (see table 11)

The fibroblasts were cultured under standardized conditions and the assays were carried out with MU-glucoside or with glycogen. Using MU-glucoside, acidic activity proved severely reduced in the three patients with the generalized form and it showed an activity of 7 to 19 percent of the lowest control value in the six patients with the muscular form.

With glycogen, the activities were not detectable in the patients with the generalized form and were between 8 and 25 per cent of the lowest control value in the six patients with the muscular form.

Table 11. Acid maltase activity* in *fibroblasts* of nine patients with acid maltase deficiency

patients	[MU-glucoside] 2.2 mM	[glycogen] 50 mg/ml
generalized form		
E III 3	0.03	–
F III 4	0.03	–
G III 2	0.03	–
muscular form		
P	0.23	3
N	0.28	1
L III 3	0.38	2
H III 2	0.40	2
K III 1	0.62	3
O	0.62	3
controls	n=6	n=6
range	3.25-5.10	12-22

* expressed in nmol glucose / min per mg protein

– not detectable

Heart (see table 12)

Acid maltase activity was determined in the heart of five infants with the generalized form. It was not detectable in all, as was neutral maltase in three. Neutral activities amounted to 38 and 47 per cent of the lowest control value in the two other cases.

Table 12. Maltase activities* in the *heart* of five patients with the generalized form

	[maltose] 13.8 mM	
patients	pH 4.0	pH 6.5
B III 19	–	–
C III 3	–	–
D III 1	–	–
F III 4	–	0.38
A III 1	–	0.47
controls	n=3	n=3
range	4.70-27.69	1.00-3.60

* expressed in nmol glucose / min per mg protein

– not detectable

Liver (see table 13)

Maltase activity proved not detectable or severely reduced in the liver of all four patients with the generalized form in whom this was determined.

Glycogen content

In the heart of one patient with the generalized form the glycogen content was 560 μg per mg protein.

Table 14 shows the glycogen content in the skeletal muscles of ten

Table 13. Maltase activities* in the *liver* of four patients with the generalized form

patients	[maltose] 13.8 mM pH 4.0	pH 6.5
C III 3	–	–
F III 4	–	1.65
A III 1	0.15	0.47
B III 19	0.54	2.52
controls	n=3	n=3
range	21.74-38.85	5.66

* expressed in nmol glucose / min per mg protein
– not detectable

patients. In three patients with the generalized form and in two with the muscular form it was grossly increased; in five patients with the muscular form it was only slightly increased or normal. There seemed to exist a fair correlation between the degree of vacuolization and the glycogen content.

Treatment

The treatment with a low-carbohydrate and high-fat diet in cases H III 2 and K III 2 did not result in an increase of their strength. The only change in the laboratory investigations was a rise in serum CPK levels during treatment, but this was probably caused by the exercise on bicycle which was done on the day before the laboratory investigations. Acid maltase was not studied during the course of the treatment, and biopsies of skin or muscle were not repeated. The TLC of oligosaccharides showed no alteration of the patterns during the period of treatment.

Genetics

Pedigree analysis

Due to the geographical position of Rotterdam, most of the patients were referred from the south-western or from the middle-western area of the

Table 14. Glycogen content and vacuolization in the muscles of ten patients with acid maltase deficiency

patients	age	glycogen content*	vacuolization†
generalized form			
F III 4	6 months	1832	++
B III 19	3 months	1480	++
G III 2	11 months	903	++
muscular form			
H III 2	10 years	647	++
L III 3	20 years	622	++
J III 6	24 years	128	+
K III 2	25 years	95	++
B I 3	59 years	86	±
J III 7	21 years	61	±
P	52 years	58	±
controls (13)		32-80	

* in μg per mg protein

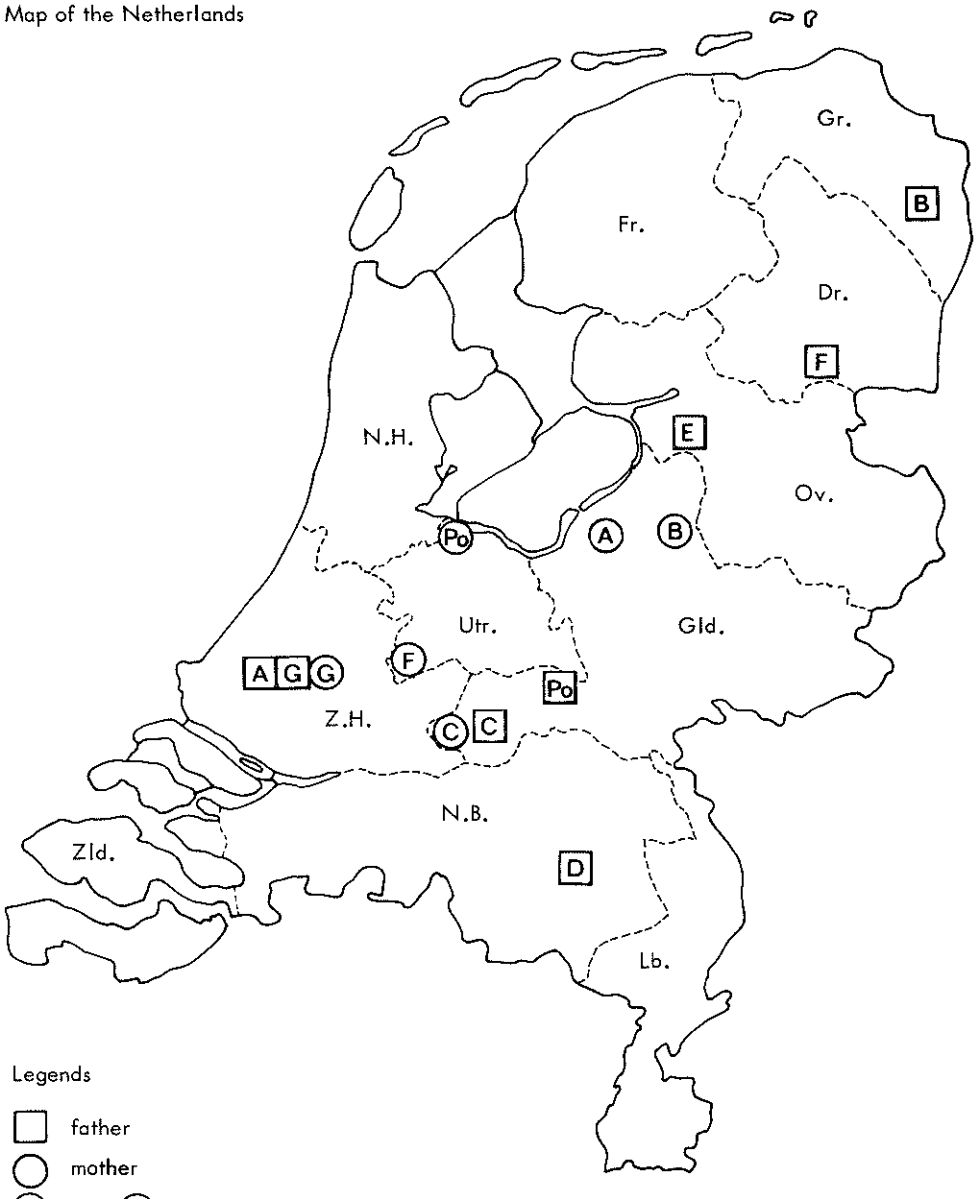
† ++: abundant; +: easily detectable; ± vacuoles found only after careful searching

country. An exception is formed by the patients of family B who lived in the north-eastern region of The Netherlands.

The birth-places of the parents of the patients with the generalized form of acid maltase deficiency (figure 10) show no specific geographical distribution with the exception of the parents of case C III 3 and those of G III 2 who were not born far apart. Charting the ancestry of these patients for five generations (in the case of family D for three generations) failed to reveal any relationship (not even geographical) between the different ancestors.

The parents Po (figure 10) of the patient in Pompe's original description were born in different provinces and there was no close consanguinity (second cousin marriage or closer).

Map of the Netherlands



Legends

□ father

○ mother

(D) and (E) were born in Indonesia

Po and [Po] : parents of the patient originally described by Pompe

Capitals indicate family

N.H., Gr., etc.: abbrevations of names of the Dutch provinces

Figure 10. Birth-place of parents of the patients with the generalized form.

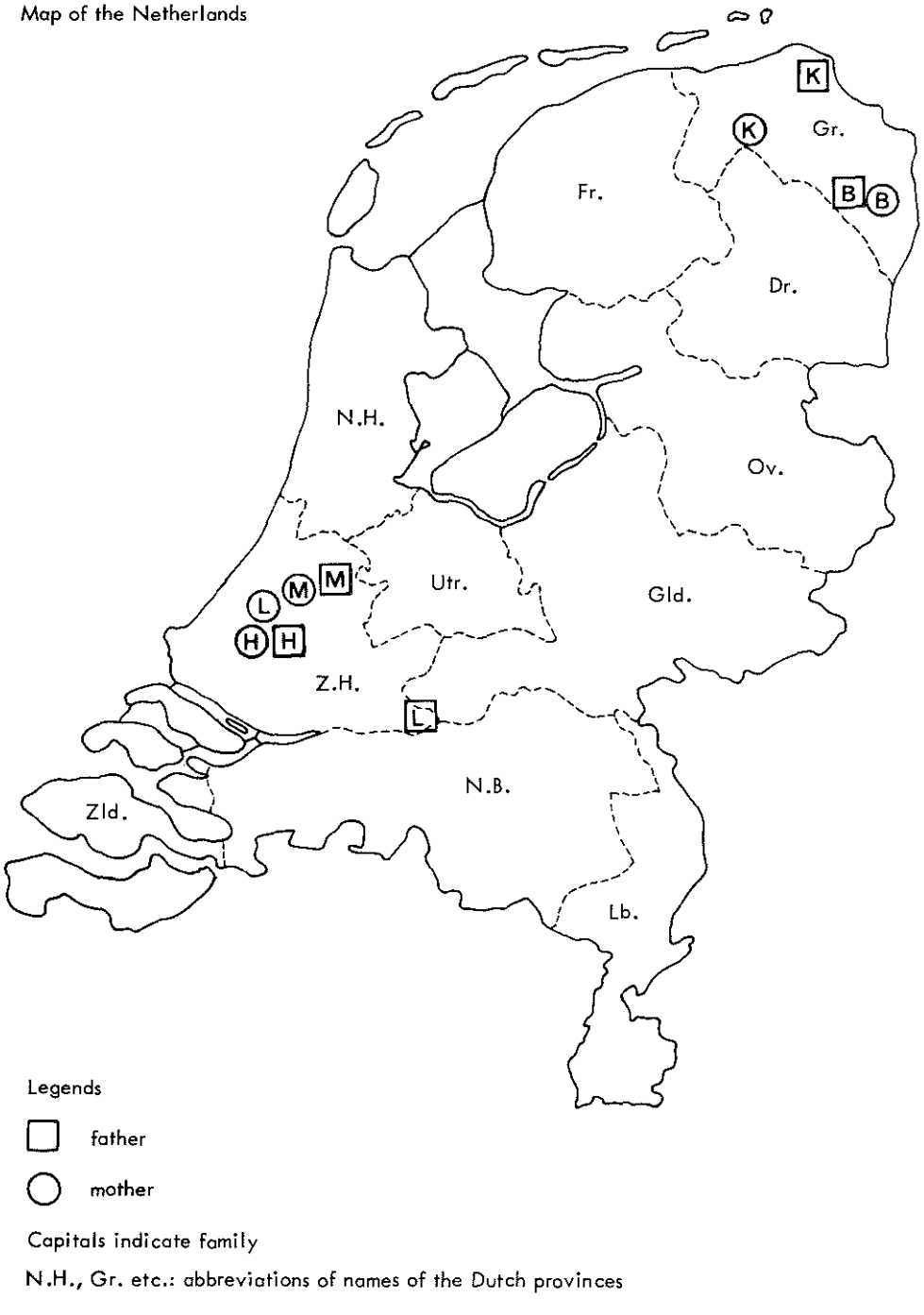


Figure 11. Birth-place of parents of five patients with the muscular form.

Figure 11 shows that the parents of four of the five index cases with the muscular form were born in localities close to each other, but tracing the ancestry over five generations did not reveal any blood-relationship.

Segregation analysis

It is evident that the ascertainment of the affected individuals in the present study was incomplete and non-random. In view of the small and selected family material that has been studied, it has been impossible to derive a better correction for the bias of ascertainment. The figures obtained in figure 12 are not in disagreement with the assumption of autosomal recessive inheritance with an expected proportion of 0.25 of affected subjects in sibships.

Sex of the affected subjects

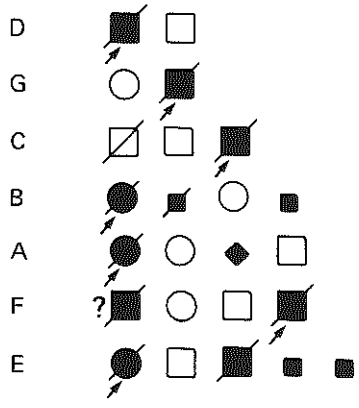
As is shown in figure 12, the number of males (affected + unaffected) in the seven sibships with the generalized form is much larger than that of females (16 males versus 7 females). Of the total number of affected subjects ten were males, three were females, and the sex of one was unknown (abortion after prenatal diagnosis of acid maltase deficiency). According to the exact test of Fisher, the larger number of males affected with the generalized form in the present series was not significant. Neither could sex preponderance be demonstrated in the subjects affected with the muscular form.

Frequency

Patients who died of a glycogen storage disease, except of von Gierke's disease, are coded in our country by the Section of Health Statistics of the Netherlands Central Bureau of Statistics under number 271.1 of the International Statistical Classification of Diseases, Injuries, and Causes of Death (W.H.O., 1965). From this Section a list was obtained covering data such as type of glycogen storage disease, sex, age at death, and year of death of the patients who, during the period of 1967 to 1977, were coded under number 271.1 on their death certificate, giving a total of 25 subjects. Eleven of these 25 patients were specified as having died from the generalized form of acid maltase deficiency. Unfortunately, it has not been possible to trace in whom of these cases the diagnosis was verified by enzyme studies.

GENERALIZED FORM

family

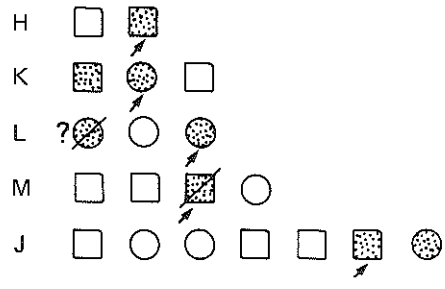


$$p = \frac{14 - 7}{24 - 7} = 0.41$$

s.e. = 0.12

MUSCULAR FORM

family



$$p = \frac{8 - 5}{19 - 5} = 0.21$$

s.e. = 0.11

general formula: $p = \frac{R-N}{T-N}$

variance: $\frac{pq}{T-N}$

standard error: $\sqrt{\frac{pq}{T-N}}$

where R is the number of affected individuals
T is the total number of individuals
N is the number of sibships
p is the estimated proportion
q = 1-p

For legends see "Legends to figure 3" page 43.

Figure 12. Segregation analysis for the generalized and the muscular forms by the single incomplete ascertainment method according to Fisher (Emery, 1976).

Table 15A shows the results of the inquiry into the number of infants with Pompe's disease diagnosed from 1967 to 1977 at the pediatric departments of the seven university hospitals in our country, giving a total of 13 infants.

By comparing the year of death, the age at death, and the sex of the patients diagnosed in the pediatric departments with the data recorded on the list of the Section for Health Statistics, seven of the 11 children on the list could be indentified. The remaining four cases had apparently been diagnosed in other hospitals. It is striking that six of the 13 infants who had been diagnosed in the pediatric departments had been coded incorrectly on their death certificate. An explanation for this might be that the correct diagnosis had not been made before autopsy was done as could be verified in two of our own seven cases.

From the above-mentioned figures it may be concluded that the total number of infants diagnosed with Pompe's disease in our country in the period of 1967 to 1977 was 17 (11 on the list of the Section for Health Statistics, and six diagnosed in the pediatric departments of the university hospitals, but coded incorrectly on their death certificates). Since 2,139,544 children were born from 1967 to 1977 (Netherlands Central Bureau of Statistics, personal communication), the minimal incidence among newborns for the generalized form of acid maltase deficiency is 17/2,139,544 which is about 1/125,000. Calculated according to the Hardy-Weinberg principle (Emery, 1975), this leads to a frequency of 1/175 heterozygotes for the generalized form of acid maltase deficiency. In table 16 these figures are compared with the reports of other authors.

Between 1969 and 1979 the muscular form of acid maltase deficiency was diagnosed in 20 individuals by the members of the Dutch Study Group for Neuromuscular Diseases (table 15 B). The age of these patients at the time of diagnosis varied from 10 to 59 years. In 1975 all of them were alive, and in that year the Dutch population between 5 and 65 years amounted to 11,242,911 individuals (Statistisch Zakboek, 1977). Therefore, the minimal prevalence for the muscular form of acid maltase deficiency in the Dutch population between 5 and 65 years is 20/11,242,911 or about 1/550,000. However, it seems reasonable to assume that over the period of analysis (1969 to 1979) a considerable number of patients has not been diagnosed as such. Therefore, it seems incorrect to calculate an approximate frequency of heterozygotes for the muscular form of acid maltase deficiency.

Table 15. Results of the inquiry of the Dutch pediatric departments and of the Dutch Study Group for Neuromuscular Diseases

Town	A. pediatric departments generalized form*		B. study group muscular form*	
	number of patients	year of diagnosis	number of patients	year of diagnosis
Amsterdam (1)	1	1972	0	
Amsterdam (2)	0		7	1971 (1), 1972 (4), 1975 (2)
Groningen	2	1972, 1973	2	1975, 1978
Leiden	2	1967, 1973	0	
Nijmegen	1	1968	5	1977 (2), 1978 (3)
Rotterdam	7	1969, 1970, 1971, 1972†, 1973, 1974, ‡ 1976	4	1969, 1971, 1976 (2)
Utrecht	0		2	1976 (2)
total	13		20	

* period of analysis for the generalized form: 1967 tot 1977, and for the muscular form: 1969 to 1979

(1) "Free" University

(2) Municipal University

† this case was initially diagnosed in the Liduina Hospital, Apeldoorn

‡ this case was initially diagnosed in the St. Franciscus Hospital, Rotterdam

Post-mortem studies

On five patients (A III 1, B III 19, C III 3, D III 1, and F III 4) with the generalized form and on two patients (M III 3 and 0) with the muscular form of acid maltase deficiency a post-mortem examination was performed. Unfortunately, the data that have been obtained were so scanty that the findings have been summarized in the case studies reported in chapter 4.

Table 16. Frequency of patients with the generalized form and of the heterozygotes

author(s)	period of analysis	number of patients	country or state	incidence among newborns	frequency of heterozygotes*
Sidbury (1967)	1950-1963	4	North Carolina	1/400,000	1/300
Moe et al. (1972)	27 years	1	Norway	1/1,500,000	1/600
Öckerman (1972)	1961-1970	2	Sweden	1/517,000†	1/350
Schaub and Bayerl (1975)	1965-1972	12	Germ.Fed. Republ.	1/548,062	1/370
de Barsy (1976)	?	?	Belgium	1/75,000	1/140
present study	1967-1977	17	The Netherlands	1/125,000	1/175

* Calculated from incidence among newborns according to the Hardy-Weinberg principle

† Derived from other data in Öckerman's paper

Summary of the results

Clinical features

Our findings in the patients confirm the previous reports of different clinical forms of acid maltase deficiency (Courtecuisse et al., 1965; Zellweger et al., 1965; Hudgson et al., 1968; Engel et al., 1973; and Gullotta et al., 1976).

In seven patients with the generalized form the manifestations were uniform as to the clinical features and the course. A normal growth in length, but a lag in increase of weight despite a massive storage of glycogen in the visceral organs were an additional finding in five infants.

Thirteen patients with the muscular form showed a wide variability with regard to the age of onset, the severity of the clinical manifestations, and the distribution of weakness. In view of the relatively mild complaints, the pronounced weakness in several patients was a surprising finding. All patients showed a slowly progressive weakness of the proximal limb girdles and of the trunk muscles, and a lack of clinical involvement of the visceral organs. It cannot be excluded that there may be several variants of the muscular form, but it seems premature now to define criteria for a subdivision.

Genetics

More than one affected sib (the abortions after prenatal diagnosis are excluded) were found in two families with the generalized form and in three families with the muscular form. Both forms did not occur within the same sibship. However, in one family (B) an infant suffered from the generalized form and her paternal grandfather from the muscular form (see chapter 7).

No close consanguinity (second cousin marriage or closer) was established in seven families in which the generalized form occurred, nor in five families with the muscular form. The number of families with acid maltase deficiency was too small to demonstrate an increase in inbreeding compared with the inbreeding in the general Dutch population.

A minimal incidence amongst newborns of 1/125,000 was calculated for the generalized form of acid maltase deficiency in The Netherlands, and in 1975 at least 1/550,000 individuals in the Dutch population from 5 to 65 years suffered from the muscular form of acid maltase deficiency.

No definite sex preponderance could be established for either form.

Ancillary studies

Cardiac enlargement and diagnostic features on the ECG were present in all infants with the generalized form, whereas no enlargement of the heart and no specific findings on the ECG were found in the patients with the muscular form.

An echocardiogram, performed in five patients with the muscular form, showed normal dimensions of the cardiac walls in four. In one case (H III 2) it indicated thickening of the left ventricular wall and of the interventricular septum. The significance of this finding remains unexplained as yet.

By thin-layer chromatography of the urine of seven patients with the

muscular form the excretion of an abnormal amount of a tetrasaccharide was demonstrated in five. According to Sjöblad (1977), this tetrasaccharide originates from glycogen, but the reason why patients with acid maltase deficiency and patients with other types of glycogen storage or with other neuromuscular diseases excrete small bits of glycogen in the urine is unclear. Sjöblad suggested that undegraded glycogen is released into the circulation and that the oligosaccharide in the urine represents the end-product of serum amylase activity.

The variability of the morphological picture in the muscle biopsies of the patients with the muscular form was striking. In some muscles many fibres showed severe vacuolization, whereas in others vacuoles were barely detectable or absent. However, acid maltase deficiency may be suspected if in a biopsy with mild non-specific changes the acid phosphatase activity is clearly increased. The method with semipermeable membranes (Meijer, 1972) proved useful in this respect.

No relationship between the strength, the degree of vacuolization, and the level of acid maltase activity was found in the quadriceps muscle of 10 patients with the muscular form. But a positive correlation seemed to exist between the glycogen content and the degree of vacuolization in the muscles of three patients with the generalized form and of seven patients with the muscular form. This confirms the findings of Martin et al. (1976 a and b).

Since type II fibres derive their energy from glycogen breakdown, one would expect these fibres to be predominantly involved. However, a number of authors reported a preferential affection of type I fibres (Engel and Dale, 1968; Gullotta et al., 1976; Schlenska et al., 1976; Heene and Ernst, 1977; Karpati et al., 1977; DiMauro et al., 1978). We also observed this curious phenomenon in patients K III 1, K III 2, and M III 3. This is another unexplained finding in acid maltase deficiency.

Enzyme studies

After physical examination and ancillary studies as CPK, EMG, and muscle biopsy, the diagnosis acid maltase deficiency was confirmed by the assay of acid maltase. In all patients acid maltase, and often neutral maltase, were measured in one or several of the following samples: the urine, total leucocytes, skeletal muscles, or fibroblasts. Heart and liver obtained post-mortem were studied as well. A deficiency of acid maltase was found in all these samples. The extent of the decrease of activities proved quite variable.

In the *urine* (muscular form) the activity was 9-16 per cent of the lowest value in controls.

In *total leucocytes* the reduction depended considerably upon the substrate used. With maltose, the acid maltase activity was always normal or nearly normal. The ratio of the acidic versus the neutral activity was similarly unrewarding. This confirms the conclusions of Koster et al. (1972), which were based on some of the measurements presented in table 9. However, with glycogen the enzyme deficiency could reliably be established.

In *skeletal muscle tissue* (both the generalized form and muscular form) acid maltase activity towards maltose proved not detectable in some patients while in others it was reduced to 4-28 percent of the lowest control value. No consistent differences in the degree of deficiency were noted between the muscles of patients with the generalized form and those with the muscular form. In fact, in two infants the acidic activity towards maltose proved well above that in the muscles of all patients with the muscular form. Thus, the different clinical forms of acid maltase deficiency cannot be distinguished on the basis of biochemical findings in the muscle which agrees with Angelini and Engel (1972).

In the *fibroblasts* of the infantile patients, acidic activity was not measurable or extremely decreased, while in the patients with the muscular form the activity amounted to 7-25 per cent of the values in controls. This confirms the findings of Reuser et al. (1978).

Acid maltase in the *heart* of five infants was not detectable.

In the *liver* of four infants the acidic activity proved virtually absent.

For **neutral maltase** there were also no consistent differences between the two clinical forms. In skeletal muscle tissue its activity proved normal, severely decreased, or absent in both forms. In the heart of two of the five infantile patients neutral maltase showed an activity of 38 and 47 per cent of the lowest control value. The role of neutral maltase in the pathogenesis of the disease is even more obscure than that of acid maltase.

Guidelines for the diagnosis of acid maltase deficiency

For the diagnosis of acid maltase deficiency, the following guidelines emerge:

The *generalized form* will soon be suspected in a severely ill infant with an enlarged heart on the roentgenogram of the chest, and with a short PR interval and high voltage QRS complexes on the ECG. In such a patient the

diagnosis has to be confirmed by enzymatic and morphological studies. Measurement of the activity of acid maltase is possible in samples that are easily obtained, i.e. urine (immunological assay!) or isolated leucocytes (with glycogen as a substrate – maltose is unsuitable). Glycogen storage can be demonstrated in a biopsy of muscle or skin. A skin biopsy in the less invasive procedure of the two, and electromicroscopic (EM) examination shows the membrane-bound vacuoles filled with glycogen in nearly all cell types. Moreover, a piece of skin for a culture of fibroblasts may be taken at the same time, in view of genetic counseling of the relatives. In this respect it is also advisable to perform a skin biopsy in the patients for the cultivation of fibroblasts.

Children or adults with the *muscular form* present with less specific features. Therefore, the diagnosis may be overlooked. The main signs are a slowly progressive weakness of the trunk and of the limb girdle muscles which may be associated with respiratory insufficiency. Serum CPK activity may be slightly elevated or normal. The EMG may be suggestive for the diagnosis when "pseudo-myotonic" discharges are found, especially if the paraspinal muscles are examined. As a rule, a muscle biopsy will be performed in these patients. The suspicion of a glycogen storage disease will arise if this shows fibres with multiple small vacuoles, which are filled with glycogen. However, sometimes vacuolization is almost absent. In such cases the finding of a prominent acid phosphatase reaction may indicate a lysosomal abnormality and this should lead to ultrastructural study and to the assay of acid maltase activity in muscle tissue or in other specimens.

Patients with acid maltase deficiency may excrete a large amount of a tetrasaccharide in the urine. This is demonstrable by thin-layer chromatography of the oligosaccharides. This finding, however, is not specific for acid maltase deficiency, but it may be a reason to suspect the diagnosis.

6 RESULTS OF THE INVESTIGATIONS IN THE RELATIVES; AN ATTEMPT AT IDENTIFICATION OF HETEROZYGOTES

Introduction

An autosomal recessive mode of inheritance for acid maltase deficiency has been established by genetic studies (Sidbury, 1967) and by biochemical investigations (Hug et al., 1966; Williams, 1966; Nitowsky and Grunfeld, 1967; Engel and Gomez, 1970; Mehler and DiMauro, 1976; Taniguchi et al., 1978; Tanaka et al., 1979). The biochemical studies demonstrated in parents of patients enzyme activities intermediate between the values measured in patients and in controls. The number of subjects was often small, and in most cases acid maltase activity was measured in not more than one specimen; only Nitowsky and Grunfeld performed assays in leucocytes and in cultivated fibroblasts.

The purpose of this part of the study was to test the feasibility of a reliable identification of heterozygotes.

I had the opportunity to investigate 21 parents (obligate heterozygotes) of known patients with the generalized form or with the muscular form, and ten siblings (who could be patient, heterozygote, or normal). In addition, I investigated 13 grandparents of five infants with the generalized form, to see if a similar situation might be present as was found in family B, where the paternal grandfather of an infant with the generalized form suffered from the muscular form.

Subjects

Twelve parents and seven sibs of patients with the generalized form were investigated, and nine parents and three sibs of patients with the muscular form. Thirteen of the 19 living grandparents of patients with the generalized form were examined. None of the remaining six and none of the deceased grandparents were reported by relatives to have suffered from weakness or respiratory insufficiency.

The subjects were investigated according to the methods described in chapter 3.

Table 17 shows the number of persons who were investigated by each of these various methods.

Table 17. Investigations carried out in the relatives

	generalized form			muscular form	
	grandparents n=13	parents n=12	sibs n=7	parents n=9	sibs n=3*
physical examination	13	12	7	8	3*
serum CPK	13	12	7	7	3*
light micr. muscle				4	3*
EM muscle				2	2*
EM skin				2	2*
oligosaccharides urine	11	12	6	4	3*
acid maltase assay:					
– leucocytes		6		6	1
– muscle				4	3*
– fibroblasts		6	1	4	3*
– urine	13	12	7	9	2

* including patient K III 1 who proved to be affected on physical examination

Results

Physical examination, and studies other than acid maltase assays

Clinical manifestations of acid maltase deficiency were found in one of the sibs (K III 1). The results of the studies in this patient have been presented in the preceding chapters and, after designation as a patient, he is not considered in the heterozygote study.

The serum CPK activity was normal in all grandparents, and it was slightly raised in four parents of patients with the generalized form as

well as in two parents and in one sib of patients with the muscular form.

Histological and histochemical investigations of the muscle biopsies of four parents of patients with the muscular form gave normal results. The ultrastructural examination of the muscle biopsies of two parents of patients with the muscular form and of the skin biopsies of two parents of patients with the muscular form was also negative.

The oligosaccharide excretion pattern in the urine, which was judged by a "blind" procedure, was inconsistent in the obligate heterozygotes as well as in the possible heterozygotes (see chapter 5, oligosaccharide excretion pattern in the urine).

Acid maltase assays in the obligate heterozygotes

The results of the acid maltase assays in the parents are shown in figure 13 and in tables 18 - 21, together with the values measured in the patients.

The main findings are that separation between obligate heterozygotes and controls seems good in the assays of muscle (with maltose or with glycogen as a substrate) and in total leucocytes (glycogen), but few heterozygotes were investigated by these methods. Some overlap between the enzyme activity of heterozygotes and controls was found in fibroblasts (MU-glucoside) and in the urine, and the overlap was wide in fibroblasts (glycogen) and in total leucocytes (maltose).

Separation between heterozygotes and patients was reasonable in fibroblasts (MU-glucoside or glycogen), in total leucocytes (glycogen), and in the urine; the overlap was wide within muscle (maltose or glycogen) and in total leucocytes (maltose).

Acid maltase assays in the possible heterozygotes

The results of the acid maltase assays in the *siblings* are presented in table 22. Table 22 A shows the ratio of acid maltase/ β -hexosaminidase in the urine of nine sibs. In three the ratio was below the lowest control value, in three the ratio was in the range of the lowest value of controls, and a ratio in the normal range was found also in three. The results of the enzyme assays in the urine could be compared with those in other samples in three of the sibs (table 22 B). Besides a low ratio in the urine of H III 1 low activities of acid maltase were found in his leucocytes, fibroblasts, and skeletal muscle tissue. In K III 3 a normal activity was found not only in the urine, but also in his fibroblasts and in the muscle biopsy. And finally in G III 1 the normal enzyme activity in the urine corresponded with a normal activity in her fibroblasts. Thus, the sibs K III 3 and G III 1

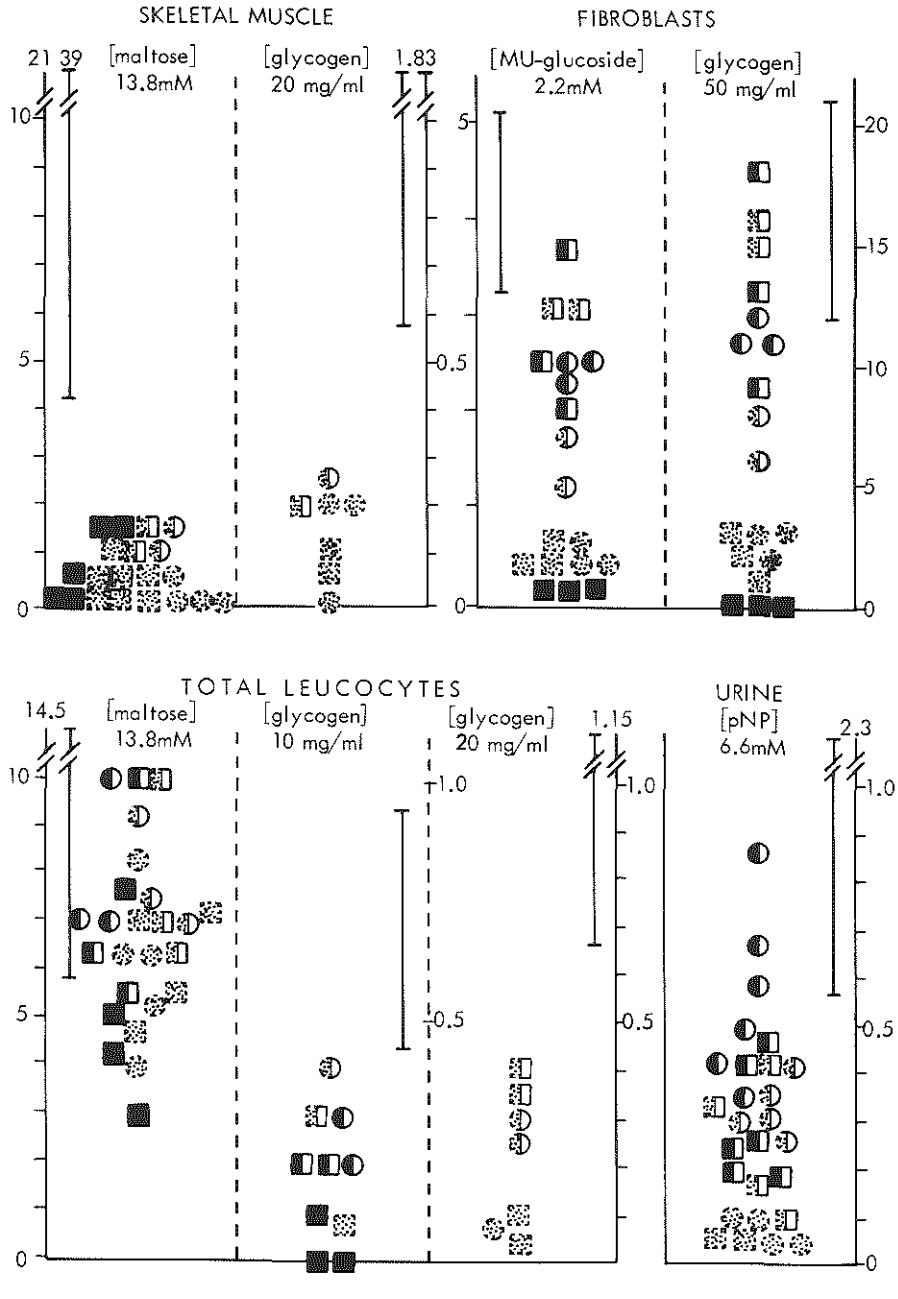


Figure 13. Acid maltase activities* in samples of obligate heterozygotes and of patients (for numerical values see tables 18-21).

Legends to figure 13: Acid maltase activities in samples of obligate heterozygotes and of patients.


range of control values


male patient, generalized form


female patient, muscular form


obligate heterozygote, generalized form


obligate heterozygote, muscular form

In SKELETAL MUSCLE acid maltase activity, assayed with maltose or with glycogen shows very low activities in the patients as well as in the obligate heterozygotes. In both assays there is an overlap between the values of the patients and the heterozygotes.

In FIBROBLASTS the assays of acid maltase with MU-glucoside or with glycogen show a separation between the patients and the heterozygotes, but there is an overlap between the heterozygotes and the controls. The activities measured in the patients with the generalized form are lower than those found in the patients with the muscular form. There is no separation between the heterozygotes for the generalized and those for the muscular form.

In TOTAL LEUCOCYTES acid maltase activity measured with maltose shows an evident overlap between values found in patients, heterozygotes and controls, although patients tend to have lower values. With glycogen as a substrate, used in only a few cases, there is a obvious separation between the patients and the heterozygotes, and between the heterozygotes and the controls.

The ratio of acid maltase/β-hexosaminidase in the URINE showed that one of the heterozygotes had a value in the range of the patients. There was also an overlap with controls, but most of the 21 obligate heterozygotes had a ratio below the lowest control value.

Table 18. Acid maltase assays* in *skeletal muscles* of obligate heterozygotes and of patients (for diagrammatic representation see figure 13). The patients and their enzyme values are printed in heavy type

generalized form		muscular form		
	[maltose]		[maltose]	[glycogen]
subjects	13.8 mM	subjects	13.8 mM	20 mg/ml
C III 3	–	**H III 2**	–	
F III 4	–	**J III 6**	–	**0.11**
A III 1	**0.17**	**K III 2**	–	**0.19**
G III 2	**1.09**	**P**	–	–
E III 3	**1.16**	**R**	–	
		S	–	
		N	**0.20**	
		L III 3	**0.32**	
		M III 3	**0.41**	
		O	**0.49**	
		K III 1		**0.06**
		K II 2	0.71	
		K II 1	0.72	
		J III 7	**0.77**	**0.20**
		H II 1	1.03	0.21
		H II 2	1.23	0.25
			[maltose] 13.8 mM	[glycogen] 20 mg/ml
	controls		n=11	n=11
	range		4.20-21.39	0.58-1.83

* expressed in nmol glucose / min per mg protein

– not detectable

Table 19. Acid maltase assays* in *fibroblasts* of obligate heterozygotes and of patients (for diagrammatic representation see figure 13). The patients and their enzyme values are printed in heavy type

generalized form			muscular form		
subjects	[MU-glucoside] 2.2 mM	[glycogen] 50 mg/ml	subjects	[MU-glucoside] 2.2 mM	[glycogen] 50 mg/ml
E III 3	**0.03**	–	**P**	**0.23**	**3**
F III 4	**0.03**	–	**N**	**0.28**	**1**
G III 2	**0.03**	–	**L III 3**	**0.38**	**2**
F II 1	2.07	9	**H III 2**	**0.40**	**2**
F II 2	2.30	11	**K III 1**	**0.62**	**3**
E II 1	2.53	13	**O**	**0.62**	**3**
G II 2	2.55	11	K II 2	1.47	6
E II 2	2.67	12	H II 2	1.75	8
G II 1	3.70	18	K II 1	3.12	15
			H II 1	3.15	16

	[MU-glucoside] 2.2 mM	[glycogen] 50mg/ml
controls	n=6	n=6
range	3.25-5.10	12-22

* expressed in nmol glucose / min per mg protein

— not detectable

Table 20. Acid maltase assays* in total *leucocytes* of obligate heterozygotes and of patients (for diagrammatic representation see figure 13). The patients and their enzyme values are printed in heavy type.

generalized form			muscular form			
	[maltose]	[glycogen]		[maltose]	[glycogen]	[glycogen]
subjects	13.8 mM	10 mg/ml	subjects	13.8mM	10 mg/ml	20 mg/ml
G III 2	**3.00**	–	**P**	**3.95**		
E III 3	**4.25**	**0.09**	**H III 2**	**4.78**		**0.046**
F III 4	**5.06**	–	**O**	**5.34**		
E II 1	5.58	0.17	**R**	**5.52**		
F II 2		0.15	M II 1	6.28	0.28	
F II 1		0.20	**S**	**6.31**		
C II 1	6.34		**J III 7**	**6.58**		**0.063**
C II 2	6.63		H II 1	6.78		0.35
E II 2	7.00	0.25	H II 2	6.80		0.25
C III 3	**7.77**		**J III 6**	**6.94**		**0.099**
A II 1	10		**M III 3**	**7.15**	**0.07**	
A II 2	10		M II 2	7.58	0.37	
			L III 3	**8.36**		
			J II 2	9.21		0.30
			J II 1	10.14		0.39
controls	n=14	n=13		n=14	n=13	n=4
range	5.8-14.5	0.44-0.94		5.8-14.5	0.44-0.94	0.66-1.15

* expressed in nmol glucose / min per mg protein

– not detectable

Table 21. Ratio of acid maltase/ β-hexosaminidase in *urine* of the obligate heterozygotes and of six patients with the muscular form. (for diagrammatic representation see figure 13). The patients and their enzyme values are printed in heavy type.

A. Ratio of obligate heterozygotes and of patients

generalized form		muscular form	
subjects	ratio	subjects	ratio
A II 1	0.19	**J III 6**	**0.05**
F II 1	0.20	**L III 3**	**0.05**
C II 1	0.24	**H III 2**	**0.06**
E II 1	0.26	**O**	**0.07**
F II 2	0.36	**J III 7**	**0.09**
G II 2	0.42	**K III 2**	**0.09**
D II 1	0.43	J II 1	0.10
G II 1	0.46	M II 1	0.17
E II 2	0.48	L II 2	0.26
D II 2	0.58	M II 2	0.29
C II 2	0.67	K II 2	0.30
A II 2	0.86	K II 1	0.33
		H II 2	0.35
		H II 1	0.42
		J II 2	0.43

B. Range, mean and standard deviation in controls and in obligate heterozygotes

		obligate heterozygotes		
	controls	generalized form + muscular form	generalized form	muscular form
	n=39	n=21	n=12	n=9
range	0.56–2.30	0.10–0.86	0.19–0.86	0.10–0.43
mean	1.19	0.37	0.43	0.29
s.d.	0.49	0.18	0.20	0.11

may be considered as non-carriers, and sib H III 1 is presumably a heterozygote. Of the six sibs in whom only the urinary enzyme ratio was measured, one (A III 4) may be considered as a non-carrier, and five (A III 2, C III 2, E III 2, F III 2, and F III 3) as heterozygotes.

In the *grandparents* the acid maltase assay was done only in the urine. The results are shown in figure 14 and in table 23. In figure 14 they are compared with matched controls of approximately the same age. Figure 14A depicts the values of the controls and the grandparents (50 - 91 years). In four of the 13 controls, who turned out to be older than 70 years, the ratio of acid maltase / β -hexosaminidase was below 0.56 (which is the lowest value in a series of 39 controls with ages varying between 8 months and 68 years). Surprisingly, the ratios of all grandparents were near or below 0.56. Figure 14B shows the values of six couples of grandparents. Only two couples (A I 1-2 and D I 1-2) demonstrated a fairly large difference between their ratios, but only the first couple (A I 1-2) fulfils the theoretical expectation that in a given, unrelated couple of grandparents one should have a normal enzyme activity and the other an activity below the lowest control value.

Discussion

This study of 21 obligate heterozygotes for acid maltase deficiency (12 for the generalized form and nine for the muscular form) demonstrates the limitations in the identification of heterozygotes. Physical examination was not helpful in this respect, neither were serum CPK assays, light-microscopical examination of a muscle biopsy, ultrastructural examination of a muscle biopsy or of a skin biopsy, nor the oligosaccharide excretion pattern in the urine.

In some autosomal recessive disorders the mutant gene product is identifiable in a heterozygote, e.g. in sickle cell anaemia on electrophoresis of hemoglobins. Such a test, however, is not available for lysosomal storage diseases such as acid maltase deficiency. Like in other autosomal recessive disorders with an underlying enzyme deficiency the aberration is based upon a gene-dosage effect: the heterozygote will have in theory half of the normal enzyme activity, but in practice enzyme measurements for the identification of heterozygotes may have limitations (Nitowsky, 1975):
- biological variations in the control-population which will introduce overlap with the group of obligate heterozygotes.

Table 22. Acid maltase assays* in nine possible heterozygotes for acid maltase deficiency

A. Urinary assays

subjects	ratio	p (H|x)†
H III 1	0.27	0.96
A III 2	0.31	0.96
C III 2	0.42	0.95
F III 2	0.52	0.91
E III 2	0.56	0.88
F III 3	0.59	0.84
K III 3	0.92	0.06
A III 4	1.29	<0.001
G III 1	1.32	<0.001
controls	n=39	
range	0.56-2.30	

B. Other assays

	TOTAL LEUCOCYTES		FIBROBLASTS		SKELETAL MUSCLE	
	[maltose]	[glycogen]	[MU-glucoside]	[glycogen]	[maltose]	[glycogen]
subjects	13.8 mM	20 mg/ml	2.2 mM	50 mg/ml	13.8 mM	20 mg/ml
H III 1	4.13	0.25	2.72	12	2.32	0.49
K III 3			4.13	20		1.04
G III 1			4.30	25		
controls	n=14	n=4	n=6	n=6	n=11	n=11
range	5.8-14.5	0.66-1.15	3.25-5.10	12-22	4.20-21.39	0.58-1.83

* expressed in urine as ratio of acid maltase / β-hexosamindase; in other samples in nmol glucose / min per mg protein

† P (H| x): posterior probability being a heterozygote for acid maltase deficiency calculated according to Bayes' theorem (see chapter 3). The prior probability in each sib is assumed to be 0.66

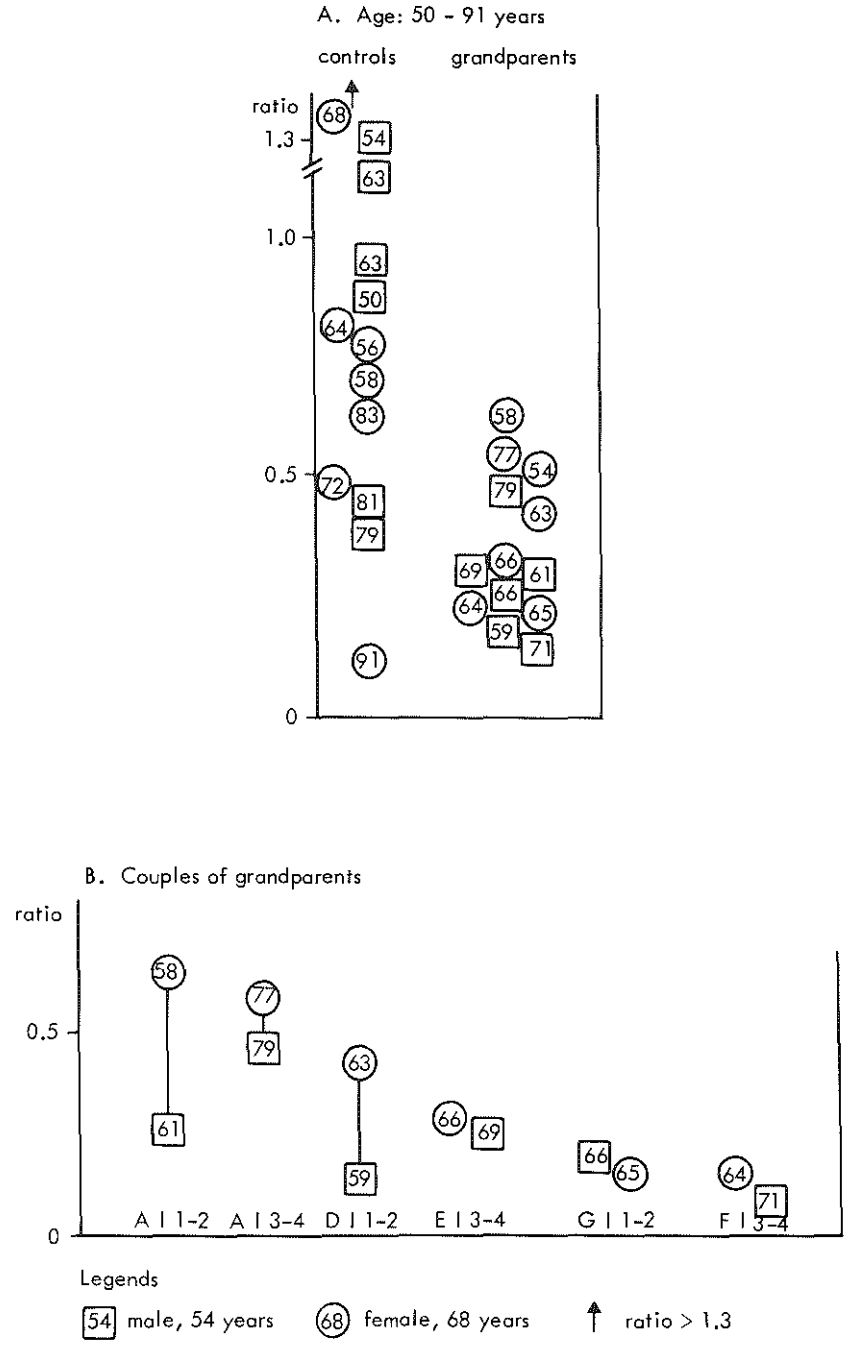


Figure 14. Ratio of acid maltase/ β-hexosaminidase in urine of 13 grandparents of five patients with the generalized form, compared with controls of approximately the same age.

Table 23. Ratio of acid maltase / β- hexosaminidase in the *urine* of 13 grandparents of five patients with the generalized form compared with controls of approximately the same age

grandparents				controls of approximately the same age		
subjects	sex	age	ratio	sex	age	ratio
F I 3	m	71	0.18	f	91	0.12
D I 1	m	59	0.21	m	79	0.39
F I 4	f	64	0.23	m	81	0.45
G I 2	f	65	0.23	f	72	0.48
G I 1	m	66	0.27	f	83	0.63
A I 1	m	61	0.31	f	58	0.70
E I 3	m	69	0.31	f	56	0.78
E I 4	f	66	0.33	f	64	0.81
D I 2	f	63	0.44	m	50	0.88
A I 3	m	79	0.49	m	63	0.94
G I 4	f	55	0.54	m	63	1.14
A I 4	f	77	0.57	m	54	1.30
A I 2	f	58	0.62	f	68	1.78

controls (8 months - 68 years of age) n=39
range 0.56-2.30

- biological variations in heterozygotes which might lead to either higher or lower values than the expected 50 per cent activity.
- presence of various "wild type" alleles and various types of mutant alleles in the population.

For practical purposes, statistical methods may be used to increase the discriminative power of the data (see for instance table 22A).

In the present study an overlap between the enzyme values of obligate heterozygotes and controls was seen for the assays in the fibroblasts, the leucocytes and the urine. Moreover, there was an overlap between the enzyme values measured in the heterozygotes and in the patients in the skeletal muscles, in the leucocytes (substrate: maltose) and in the urine. In view of this last finding, the obvious fact must be stressed that physical examination should precede the interpretation of enzyme activities. But

when there are no physical signs, it is still possible that a person with low activities will develop clinical manifestations in the future.

With the methods that were used, we have not been able to distinguish obligate heterozygotes for the generalized form from those for the muscular form. This confirms the findings of Engel and Gomez (1970).

Surprisingly, all 13 grandparents (including six couples) of five patients with the generalized form showed a low ratio of acid maltase / β-hexosaminidase in the urine. Williams (1966) studied acid maltase by the transglucosylation technique (Hers, 1963) in the leucocytes of three grandparents of a patient with the generalized form. The paternal grandfather showed a normal activity and the paternal grandmother had an activity of approximately one-half of the mean of the control values. An activity near the lowest control value was measured in the maternal grandmother. These results might be in line with the expectation. To my knowledge no other studies exist of grandparents of patients with acid maltase deficiency. I have no reasonable explanation for the unforeseen finding of a low urinary enzyme ratio of acid maltase / β-hexosaminidase in all 13 grandparents. Age-matched controls showed an obviously higher ratio of acid maltase/β-hexosaminidase. Pedigree analysis demonstrated no consanguinity in the six couples. The frequency of heterozygotes for the generalized form of acid maltase deficiency in the Dutch population, calculated in chapter 5 to be about 1/175, is too low to assume six chance marriages between two heterozygotes. Further studies on grandparents of patients with acid maltase deficiency are necessary to explain this curious finding.

As far as the identification of heterozygotes is concerned, for practical purposes the examination of the urine by means of the immunological assay (Schram et al., 1979), or of total leucocytes with glycogen (Koster et al., 1974) appears to be suitable, although to a limited extent because of the overlap with control values. The finding that three female obligate heterozygotes for the generalized form showed a urinary ratio of acid maltase / β-hexosaminidase in the normal range needs further clarification. It is hard to believe that this was due to chance and it might be possible that there is an influence for instance of the ovulatory cycle, or of blood cells in the urine upon the enzyme excretion. Nevertheless, the assays in the urine and in total leucocytes (substrate: glycogen) should be preferred above the time-consuming culturing of fibroblasts and the more invasive method of a muscle biopsy.

In practice, the identification of the heterozygous state may be desirable for the prospective spouses of sibs of patients. In this respect there are several problems. For instance, the consequences of a marriage between an obligate heterozygote for the generalized form and an obligate heterozygote for the muscular form are unknown: a child with the generalized form, or one with the muscular form? Further, it has already been mentioned that it has not been possible to distinguish between heterozygotes for the generalized form and those for the muscular form. In chapter 5 the frequency of heterozygotes for the generalized form in the Dutch population was calculated to be about 1/175. If it is assumed that the generalized form and the muscular form occur in approximately the same frequency, the combined frequency of heterozygotes for either form may be estimated upon 2/175, which is about 1/100.

The prior probability to a child with acid maltase deficiency from the marriage of a clinically healthy sibling of an index case with a non-related partner may be calculated as: 2/3 x 1/100 x 1/4 = 1/600. This is a low risk and additional biochemical tests will usually not be indicated. If there is a possibility of consanguinity, or if families from both partners originate from the same (isolated) region, enzyme tests may be used. If both partners show values in the heterozygous range in the leucocytes (using glycogen as a substrate), or in the urine, or both, their pregnancy may be at risk and it may be indicated to offer prenatal diagnosis. If they both score in the "grey zone" where values of obligate heterozygotes and non-carriers overlap, the risk is less easier to define and it might be prudent to offer prenatal diagnosis in a pregnancy from such a mating.

7 OCCURRENCE OF THE MUSCULAR FORM AND THE GENERALIZED FORM OF ACID MALTASE DEFICIENCY IN ONE FAMILY

Introduction

The occurrence of both the muscular form and the generalized form of acid maltase deficiency as was found in family B is an exceptional finding which has not been reported in other families with acid maltase deficiency.

The muscular form was diagnosed in the grandfather I 3 (figure 15), a 59-year-old printer, with a history of a progressive weakness for six years. The diagnosis acid maltase deficiency was suspected by the neurologist T.W. van Weerden (Department of Neurology, University Hospital, Groningen), who learned from the patient that one of his grandchildren (III 19) had died from Pompe's disease. Biochemical studies in I 3 confirmed the diagnosis.

This family provided the opportunity to study the modes of inheritance and the genetic relationship between the two forms of the disease.

The pedigree (figure 15, page 163)

First generation (1,1-8)

All seven living siblings were investigated as well as five of the spouses.

1 3 was the third in a sibship of eight. No consanguinity was established between his parents for the four previous generations. He was in good health until the age of 53 when he experienced difficulty in climbing stairs. Chronic polymyositis was diagnosed. Treatment with corticosteroids was unsuccessful. At the age of 59 he was admitted to an intensive-care-unit with an acute respiratory insufficiency. As mentioned above, at that time the diagnosis of polymyositis was rejected because of the family history. Acid maltase deficiency was confirmed by the results of the microscopic and ultra-structural examination of a muscle biopsy, and by a reduced enzyme activity in muscle tissue and in cultured fibroblasts.

The patient remained in a clinically stable condition. He was moderately disabled and artificial respiration at night was necessary.

I 2 had died from a traffic accident at the age of 20 years.

I 1 and I 7 had emigrated to Canada. They were personally examined there (in co-operation with Professor J.L. Hamerton, Department of Paediatrics, The University of Manitoba, Winnipeg), and the enzyme assays were performed in The Netherlands.

Second generation (II, 1-11)

All ten living siblings and seven of the nine spouses were examined.

II 1 had died of jaundice at the age of ten years.

II 4 and II 11 had emigrated to Canada and they were personally examined there (in co-operation with Dr. D.A. Applegarth, Department of Paediatrics, University of British Columbia, Vancouver). II 9 had emigrated to Australia and she was examined by Professor B.A.Kakulas, Royal Perth Hospital, Western Australia. The enzyme assays of these three persons were carried out in The Netherlands.

Third generation (III, 1-24)

Twenty of the 29 living children were examined personally. Clinical records were available of III 19 and of III 20 (Dr. J.M.J.P. Gadiot and Dr. A.J.W. Leenders, pediatricians in Apeldoorn; Dr. J.H.van der Vegt, pathologist in Deventer).

III 19, the index case, was the first child of healthy parents who were unrelated according to the pedigree analysis of five previous generations. She was examined at the age of three months for progressive dyspnoea and failure to thrive. She looked severely ill, had a greyish-pale colour, and showed hypotonia with weakness and areflexia. The tongue was definitely enlarged, the liver and the spleen were slightly enlarged.
The roentgenogram of the chest showed cardiac enlargement. The ECG indicated a biventricular and septal hypertrophy. The PR interval was shortened, 0.08 seconds (normal > 0.12 seconds).
She died at 16 weeks. At post-mortem examination the heart weight was 200 grams (normal for this age: 27 grams). Microscopical examination

confirmed a massive glycogen accumulation in the heart, the liver, the tongue, and skeletal muscles. Acid maltase was not detectable in the myocardium, and a severely reduced activity was measured in muscle tissue, in the liver, and in cultured fibroblasts.

III 20 was born immaturely after 25 weeks of gestation. He died after half an hour. In the 15th week of gestation amniocentesis had been unsuccessful. In fibroblasts, cultured from a skin biopsy obtained post-mortem, acid maltase activity proved to be virtually absent.

III 22 was a selective abortion after prenatal diagnosis of acid maltase deficiency.

The patients and the relatives were examined according to the methods described in chapter 3.

Results

History and physical examination

No further patients with weakness were found, although I 5, a sister of the patient I 3, showed some weakness in the limb girdles. Acid maltase deficiency was excluded as a cause for her weakness, however.
In II 2 sarcoidosis with a benign course was diagnosed in 1976.

Muscle biopsies

A myopathy with PAS reactive vacuoles was found in the grandfather I 3 and in his grandchild III 19. In the biopsy of the grandfather only a few fibres with multiple small vacuoles were found, but vacuolization was abundant in the skeletal muscle of the grandchild. In agreement with these findings, the glycogen content in the muscle of the grandfather was slightly above normal (86 μg per mg protein; normal values: 32 - 80 μg per mg protein) and it was considerably increased in the infant's muscle (1480 μg per mg protein).

The muscle biopsies of I 3s, of four siblings in the first generation (I 4,5,6, and 8), of six sibs in the second generation (II 2,3,5,7,8, and 10), and of II 8s showed no abnormalities on routine microscopical and on histochemical examination.

In the biopsy of I 3 electronmicroscopy demonstrated the presence of membrane-bound accumulations of glycogen, but also of free glycogen in the perinuclear region and between the myofibrils (figure 16).

The electronmicroscopic examination of the muscle biopsy from I 3s, I 5, and II 8 was unremarkable.

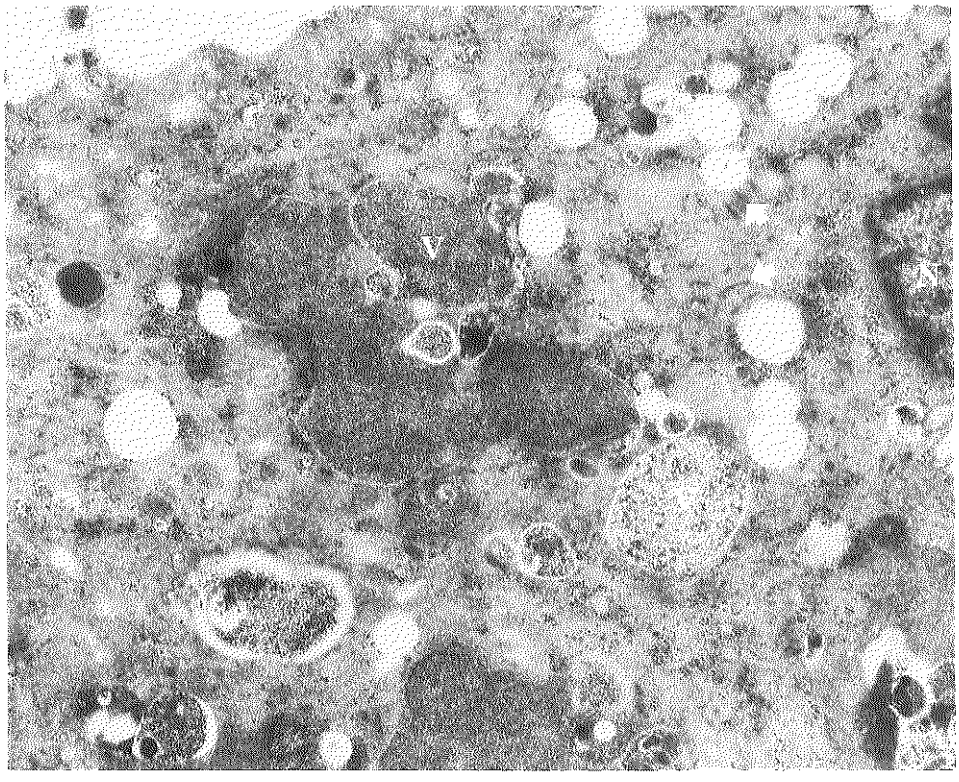


Figure 16. Electronmicrograph showing vacuoles filled with glycogen (V). A few small leptomere fibrils are also present (arrows). N: nucleus.
Quadriceps biopsy from patient B I 3, original magnification x 17,5000.

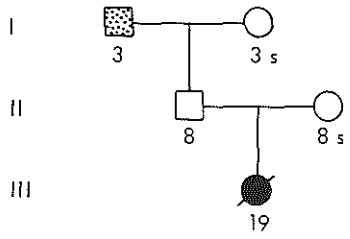
Skin biopsies

The ultrastructural examination of the skin biopsy of I 3 showed a few, small, membrane-bound accumulations of glycogen in some fibroblasts and in smooth muscle fibres.

No abnormalities were found in the biopsies of I 3s, I 5, and II 8.

Enzyme assays

The key-persons.
Detail of figure 15.



With respect to the inheritance of acid maltase deficiency in this family, the *key-persons* are of course the diseased grandfather and his wife (I 3 and I 3s), and the parents (II 8 and II 8s) of the infant with Pompe's disease (III 19). The enzyme levels in these persons are represented in figure 17 and in table 24. The enzyme levels in the grandfather were very low in all assays. On the other hand, those in his wife (I 3s) were quite normal (except for a low value in the lymphocytes using MU-glucoside), suggesting that she is not a carrier of a mutation affecting acid maltase activity.

In their son (II 8) as well as in his wife (II 8s) the enzyme levels were all between the lowest control value and those of the affected father (I 3). This suggests that they are heterozygotes which is in accordance with the expectation in parents of an infant affected with Pompe's disease. But the interesting point is that II 8 presumably is a heterozygote for the MUSCULAR FORM of acid maltase deficiency. This will be considered further in the discussion.

In the *second generation* (figure 18 and table 24), low activities were found in all siblings, with the exception of sibs 2 and 9. The activity in the muscle of II 2 (using glycogen as a substrate) was near the mean control value, but in the other tests the activities were near, or clearly below the lowest control value. In II 9 only lymphocytes and urine were studied, and the activities in the lymphocytes were at, or slightly above the lowest control values, but in the urine the ratio was very low. With one obvious exception (II 9, lymphocytes, substrate MU-glucoside) the values in all ten sibs of this generation were between those of their parents. It appears reasonable to conclude that the results in the siblings of generation II suggest heterozygosity in all and accordingly that their father, the patient I 3,

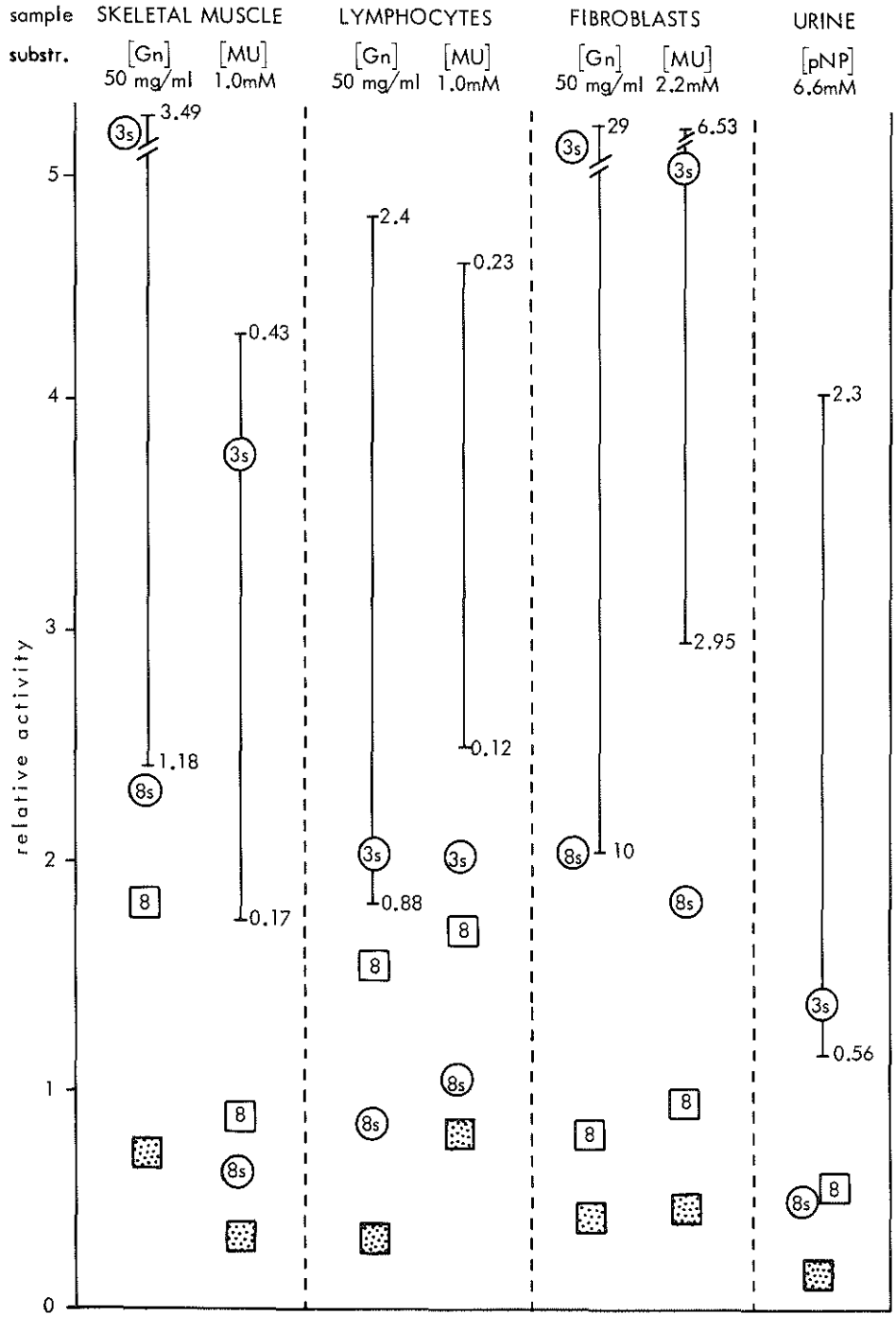


Figure 17. Enzyme studies in the key-persons I 3, I 3s, II 8, and II 8s.

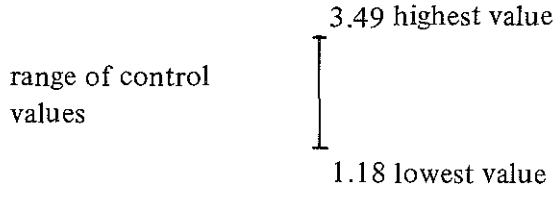
Legends to figures 17, 18 and 20.

These figures give a diagrammatic representation of the acid maltase activities measured with various substrates in skeletal muscles, lymphocytes, fibroblasts and urine. They are compared with the range of values in controls. The absolute values of the highest and the lowest activities in the controls are included in the figures, those of the subjects are summarized in table 24. They are expressed in nmol glucose/min per mg protein for skeletal muscles, lymphocytes and fibroblasts, and as the ratio of acid maltase/ β -hexosaminidase in urine. To facilitate comparison between different samples, the activity on the vertical axis has been designated as "relative activity" which means that the magnification scale by which the range of each sample is depicted is different. The numbers within the squares and the circles correspond with the numbers of the individuals in the pedigree (figure 15).


I 3, patient with the muscular form


male


female



Gn glycogen
MU MU-glucoside
pNP pNP-glucoside

Table 24. Acid maltase assays* in generations I and II, and in spouses of family B. The key-persons and their enzyme values are printed in heavy type

	skeletal muscle		lymphocytes		fibroblasts		urine
	[Gn]	[MU]	[Gn]	[MU]	[Gn]	[MU]	[pNP]
subjects	50 mg/ml	1.0 mM	50 mg/ml	1.0 mM	50 mg/ml	2.2 mM	6.6 mM
GENERATION I							
1			0.38	0.07			0.48
3	**0.33**	**0.03**	**0.15**	**0.04**	**2**	**0.43**	**0.07**
4	1.53	0.15	1.87		10	2.15	0.28
5	2.28	0.29	0.96	0.10	10	2.80	1.09
6	1.77	0.23	1.44	0.16	9	2.23	0.49
7			0.44	0.09			0.32
8	2.39	0.26	1.53	0.15	19	5.17	0.82
GENERATION II							
2	1.85	0.18	0.47		11	1.87	0.70
3	1.13	0.11	0.69		10	2.33	0.24
4							0.39
5	1.05	0.11	0.68	0.07	9	1.62	0.30
6			0.37	0.05	7	1.20	0.36
7	1.25	0.12	0.43	0.04	7	2.17	0.31
8	**0.90**	**0.09**	**0.77**	**0.08**	**4**	**0.93**	**0.26**
9			1.04	0.12			0.18
10	1.07	0.12	0.53		8	1.83	0.29
11							0.65
SPOUSES							
I 3s	**3.02**	**0.38**	**0.96**	**0.10**	**27**	**5.02**	**0.68**
II 8s	**1.15**	**0.06**	**0.41**	**0.05**	**10**	**1.80**	**0.21**
CONTROLS	n=12	n=7	n=5	n=5	n=7	n=7	n=39
range	1.18-3.49	0.17-0.43	0.88-2.40	0.12-0.23	10-29	2.95-6.53	0.56-2.30
mean	2.14	0.29	1.58	0.18	18	4.20	1.19
s.d.	0.63	0.09	0.49	0.04	5.59	1.11	0.49

* expressed in nmol glucose / min per mg protein for skeletal muscles, lymphocytes, and fibroblasts, and as the ratio of acid maltase / β-hexosaminidase in the urine

Gn = glycogen, MU = MU-glucoside, pNP = pNP-glucoside.

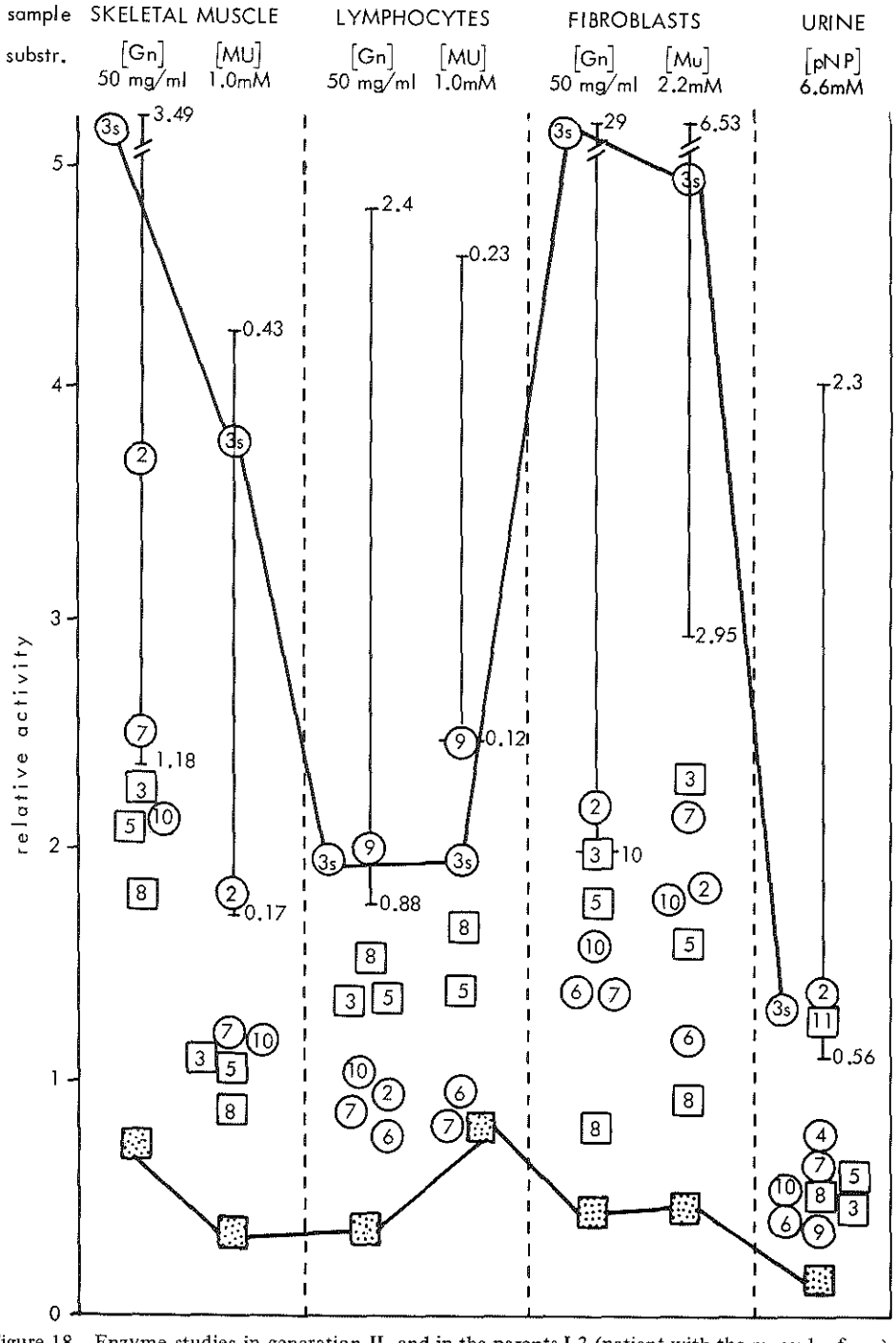


Figure 18. Enzyme studies in generation II, and in the parents I 3 (patient with the muscular form) and I 3s. For legends see page 125.

is a homozygote for acid maltase deficiency. Therefore, it seems that in this family acid maltase deficiency follows the usual pattern of autosomal recessive inheritance.

From the urinary enzyme studies in *six spouses of generation II* (as specified in table 25), it appears that they all have a very low probability of being a heterozygote for acid maltase deficiency. Since each of these spouses is married to a sib of generation II, their children may be assumed to have one parent with the normal genotype and one who is presumably a heterozygote for acid maltase deficiency. Accordingly, each of these children has a prior probability of 0.50 of being a heterozygote for acid maltase deficiency. Figure 19 and table 26 show the ratio of acid maltase/ β-hexosaminidase in the urine of these 20 children of *generation III.* In six a ratio below or near the lowest control value was found, which corresponds with a posterior probability for heterozygosity of 0.66 and higher. The clinically healthy sister (III 21) of the infant with Pompe's disease has a prior probability of 0.66 of being a heterozygote for acid maltase deficiency. The enzyme levels in her urine further increased this probability to 0.96.

Table 25. Ratio of acid maltase / β-hexosaminidase in the urine of six spouses in generation II

subjects	ratio (x)	P(H|x)*
II 2s	0.53	0.04
II 3s	1.09	< 0.001
II 5s	1.08	< 0.001
II 6s	1.10	< 0.001
II 7s	0.72	0.006
II 10s	0.98	< 0.001
controls	n=39	
range	0.56 - 2.30	
mean ± s.d.	1.19 ± 0.49	

* P(H | x): probability of being a heterozygote for acid maltase deficiency calculated according to Bayes' theorem (see chapter 3). It is assumed that the prior probability of heterozygosity for acid maltase deficiency in the general population, and thus in each of the above-mentioned persons, is 0.01.

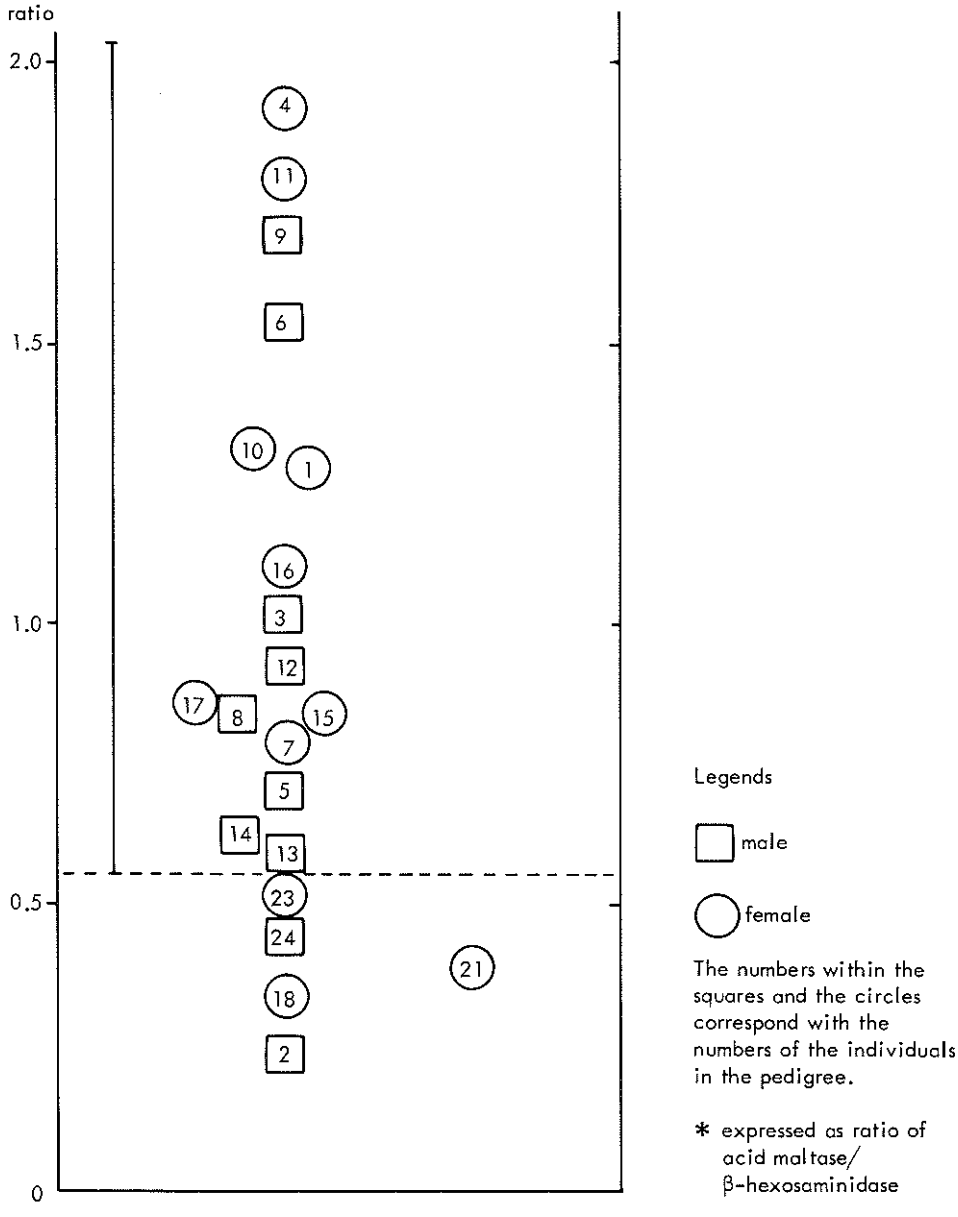


Figure 19. Urinary enzyme studies* in generation III. On the left the ratios of 20 sibs having one parent who is presumed to be a heterozygote for acid maltase deficiency; on the right the ratio of sib 21, whose parents are both heterozygotes. For numerical values see table 26.

Table 26. Ratio of acid maltase / β-hexosaminidase in the urine of 21 sibs in generation III. For diagramatic representation see figure 19.

subjects	sex	age (y)	ratio (x)	P(H|x)*
III 1	f	16	1.28	< 0.001
III 2	m	14	0.23	0.93
III 3	m	7	1.02	< 0.001
III 4	f	11	1.91	< 0.001
III 5	m	9	0.71	0.39
III 6	m	7	1.54	< 0.001
III 7	f	6	0.78	0.20
III 8	m	8	0.84	0.07
III 9	m	6	1.70	< 0.001
III 10	f	4	1.31	< 0.001
III 11	f	13	1.79	< 0.001
III 12	m	12	0.92	0.01
III 13	m	9	0.59	0.73
III 14	m	8	0.62	0.66
III 15	f	9	0.84	0.07
III 16	f	8	1.10	< 0.001
III 17	f	4	0.86	0.06
III 18	f	1	0.34	0.93
III 23	f	5	0.52	0.84
III 24	m	3	0.46	0.89
III 21	**f**	**2**	**0.39**	**0.96**

controls n=39
range 0.56-2.30
mean 1.19
s.d. 0.49

* P(H | x): probability being a heterozygote for acid maltase deficiency calculated according to Bayes' theorem (see chapter 3). The prior probability in each sib, except **III 21** , is assumed to be 0.5. The prior probability in **III 21** is assumed to be 0.66.

In *generation I* (figure 20 and table 24) the results in I 8 suggest that she may not be a carrier of the mutation affecting acid maltase activity, and those in I 1 and I 7 (in whom only lymphocytes and urine were studied) may be compatible with heterozygosity. Those in I 4, I 5, and I 6 are difficult to interpret. Assuming autosomal recessive inheritance, two thirds of the clinically healthy sibs in this generation may be expected to be heterozygotes, but a satisfactory identification of all genotypes on the basis of the enzyme assays was not possible.

Discussion

An autosomal recessive mode of inheritance for acid maltase deficiency in this family is strongly suggested by the results of the enzyme assays, especially those in the key-persons (I 3 and I 3s, II 8 and II 8s) and in the members of the second generation. The low activities in all samples of I 3 suggested homozygosity for the muscular form of acid maltase deficiency. For his wife I 3s there were no indications that she was a carrier of the mutation that affects acid maltase activity. The results in II 8 and II 8s suggested that both were heterozygotes, in keeping with the fact that their first child (III 19) suffered from the generalized form of acid maltase deficiency. None of the ten children of I 3 and I 3s scored with their enzyme activities unequivocally in the range of the controls and with one obvious exception all showed enzyme levels that were intermediate between those of their parents. This suggests heterozygosity in all individuals of generation II.

Few studies are available on the genetics of the muscular form. Hudgson et al. (1968) suggested that the clinical picture of acid maltase deficiency in adults represents a manifestation of the heterozygous state. This is contradicted by the finding of intermediate levels of acid maltase activity in muscle biopsies of both parents of an adult patient (Engel and Gomez, 1970). These authors classified the parents as heterozygotes and the patient with the muscular form as a homozygote. In the present study this question was studied the other way round. From the findings in the ten children of the patient I 3 with the muscular form, the conclusion seems inevitable that the clinical manifestation of acid maltase deficiency in this adult patient corresponds to a homozygous genotype.

Finally, the occurrence of the two clinically different forms of acid maltase deficiency in this family remains to be explained. Presumably this

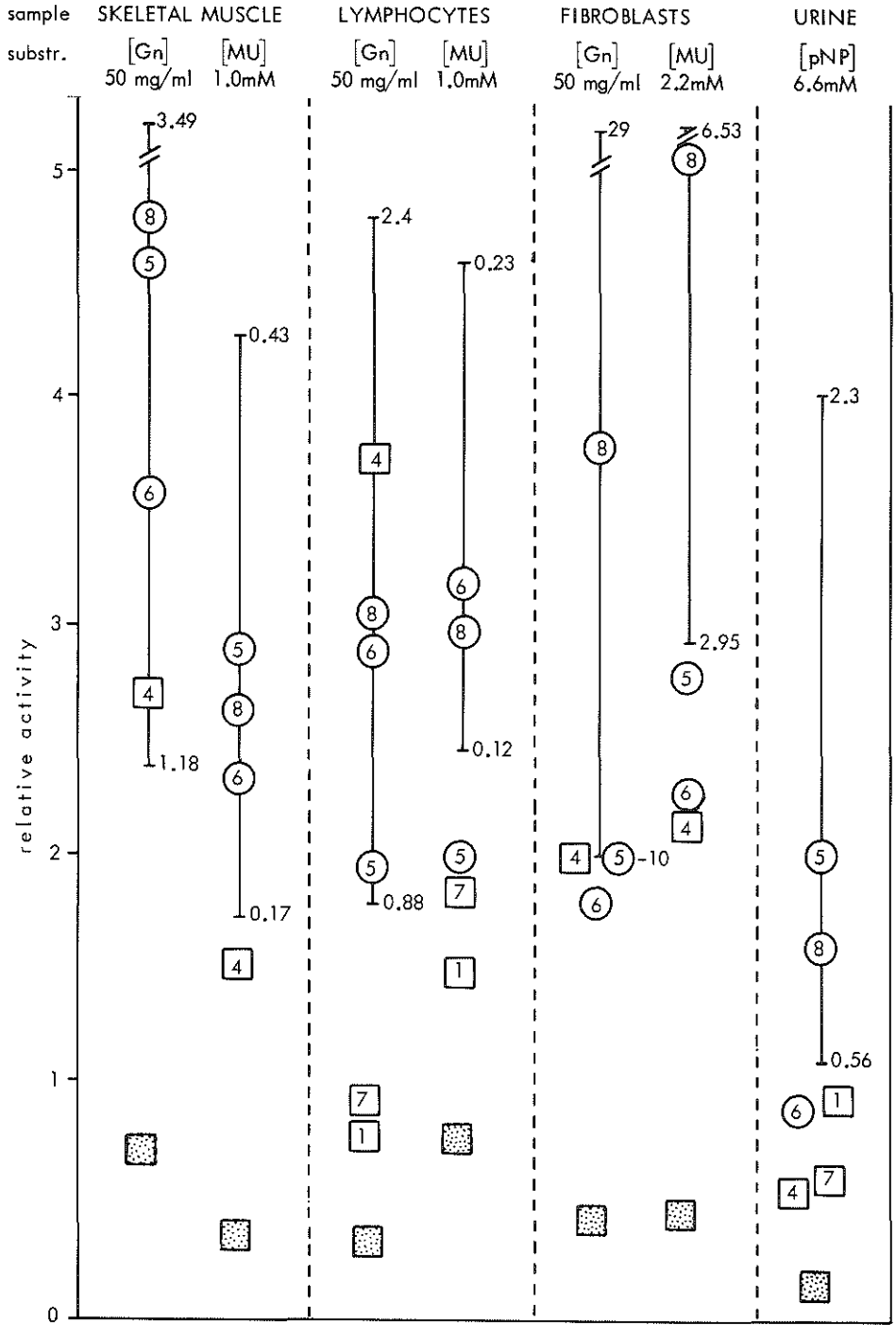


Figure 20. Enzyme studies in generation I. For legends see page 125.

was a matter of chance: one of the adult patient's sons (II 8), assumed to be a heterozygote for the muscular form of acid maltase deficiency, married an unrelated woman (II 8s) who was probably also a heterozygote for acid maltase deficiency. Their first child (III 19) suffered from the generalized form. Accordingly, it is likely that II 8s is not a heterozygote for the muscular form, but for the generalized form or for some other as yet unknown mutation affecting acid maltase activity. However, as noted in the previous chapter, it is impossible with present techniques to discriminate between heterozygotes for the muscular form and those for the generalized form.

Two different hypotheses might explain the occurrence of the muscular form and the generalized form in this family. The first is that the two forms are caused by gene mutations at different loci. In this case the father (II 8) of the affected infant would have to be a heterozygote for both diseases. But this possibility is largely ruled out, although not definitely excluded, by the experiments of Reuser et al. (1978). They demonstrated by complementation experiments that mutual correction of the enzyme deficiency (which might be expected in case of non-allelic mutations) did not occur when fibroblasts of patients with the muscular form were grown together with those of patients with the generalized form. The other explanation is that either the grandfather or the grandchild is a genetic compound, i.e. having different mutant alleles on the same locus, analogous to genetic compounds described for the mucopolysaccharidoses by McKusick et al. (1972). Assuming allelic mutations, patients with the muscular form would be homozygotes for the "muscular" allele ("mm") and patients with the generalized form homozygotes for the "generalized" allele ("gg"), while a genetic compound should carry the "muscular" allele on one autosome and the "generalized" allele on the same locus of the homologous chromosome ("mg"). Genetic compounds sometimes have phenotypical features that are intermediate between those of the "pure" homozygotes (Winters et al., 1976). However, the clinical picture of the adult patient I 3 and that of the infantile patient III 19 was similar to that found in other patients with the muscular form and with the generalized form respectively. In other words, the grandfather did not show any clinical feature that is typical for the generalized form, such as cardiac enlargement or a rapid progression of the disease, and mutatis mutandis the same is true for the infant. Biochemical studies also failed to give evidence for the compound hypothesis: the enzyme activities in I 3 and in III 19 fell within the range of values obtained for other patients with either the mus-

cular form or the generalized form (table 27). However, it cannot be excluded that some of the other patients might be genetic compounds too. The biochemical techniques used at present (measurement of the enzyme activity or immunological assay of the enzyme) are not sufficiently sensitive to detect subtle differences in mutant enzymes and no further data can be obtained where no enzyme protein is produced (as in the generalized form in which no cross-reacting material was demonstrated by de Barsy et al., 1972; Brown et al., 1975; Koster and Slee, 1977; and Reuser et al., 1978; see however General Discussion, Beratis et al., 1978).

Table 27. Comparison of acid maltase activity* in I 3 and in III 19 with other patients with the muscular form or with the generalized form, and with controls

	SKELETAL MUSCLE		FIBROBLASTS		URINE	HEART	LIVER
	[maltose]	[glycogen]	[MU]	[glycogen]	[pNP]	[maltose]	[maltose]
subjects	13.8 mM	20 mg/ml	2.2 mM	50 mg/ml	6.6 mM	13.8 mM	13.8 mM
I 3	0.69	0.09	0.43	2	0.07		
other patients (musc.form)	n=11 0.20- 0.77	n=5 0.06- 0.20	n=6 0.23- 0.62	n=6 1-3	n=6 0.05- 0.09		
III 19	0.44		0.02	–		–	0.54
other patients (gen.form)	n=5 0.17- 1.09		n=3 0.03	n=3 –		n=4 –	n=3 – to 0.15
controls	n=11 4.29- 21.39	n=11 0.58- 1.83	n=7 2.95- 6.53	n=7 10- 29	n=39 0.56- 2.30	n=3 4.70- 27.69	n=3 21.74- 38.85

* expressed in urine as ratio of acid maltase / β-hexosaminidase, in other samples in nmol glucose/min per mg protein

– not detectable

To summarize the findings in this family: an autosomal recessive mode of inheritance for the muscular form is strongly suggested by the results of the enzyme assays. Although it is an attractive hypothesis to consider either the grandfather (I 3) or the grandchild (III 19) as a genetic compound for acid maltase deficiency, no definite proof for this hypothesis has been obtained from the clinical investigations nor from the biochemical studies. However, this in itself is no proof against the possibility of a compound genotype in either the grandfather or the grandchild.

GENERAL DISCUSSION

Reconsidering the "Facts and missing links at present" as mentioned in chapter 1, it is evident that the following points were considered as the main problems in acid maltase deficiency:
1. the variability in clinical expression
2. the pathogenesis

In the following discussion these two problems will be further elaborated by comparing the present findings with previous reports.

Ad 1. The clinical variability

The variability of clinical manifestations of patients with acid maltase deficiency is most intriguing, but this is also found in several other lysosomal storage diseases.

Once suspected, there will be no difficulty with the diagnosis of the *generalized form* of acid maltase deficiency in a hypotonic and weak infant with cardiac enlargement and characteristic findings on the ECG. The clinical picture of this form is uniform and its course predictable.
If, on the other hand, the clinician is confronted with children or with adults suffering from the *muscular form*, he may have diagnostic difficulties since the clinical picture shows few striking features and is quite variable in the degree of muscle involvement and in the rate of progression. The patients present themselves with weakness of the limb girdles and of the trunk muscles, and sometimes with an acute respiratory insufficiency. Occasionally, the presenting symptoms are quite unspecific, such as chronic low back pain or an equinus foot. In some patients the progression may be insidious for many years while others develop severe weakness within few years. The weakness may not even manifest itself until at an advanced age. In the muscle biopsy the characteristic finding of a vacuolar myopathy due to storage of glycogen may be obvious, but also inconspicuous. Although the diversity of clinical expressions in this form is striking, there is, in my opinion, no firm basis for subdivisions at the present state of knowledge.

Patients with a different phenotype from apparently the same enzyme deficiency have been found in several other lysosomal storage diseases such as metachromatic leucodystrophy, GM_1-gangliosidosis and mucopolysaccharidosis type 1. In some of these disorders the variability of clinical expressions may be due to a different structural defect of the enzyme as has been demonstrated for metachromatic leucodystrophy by Luijten (1979). Another possibility is that the enzyme deficiency is due to secondary inhibition of the enzyme by storage products originating from another lysosomal enzyme deficiency. This was demonstrated by O' Brien (1978) who measured a primary deficiency of neuraminidase in an adult patient originally reported as a patient with β-galactosidase deficiency by Loonen et al. (1974), Galjaard et al. (1975), and Koster et al. (1976). Different allelic mutations at the same locus (genetic compounds) have been suggested for the different clinical manifestations in mucopolysaccharidosis type 1 by McKusick et al. (1972).
Regarding acid maltase deficiency several explanations for the clinical variability have been put forward:

a. A difference in distribution of the enzyme deficiency

According to Hudgson et al. (1968), the deficiency would be present in most tissues in patients with the generalized form, whereas in patients with the muscular form it might be restricted to skeletal muscle tissue. But in patients with the muscular form we and also others found a decreased activity of acid maltase in fibroblasts (Angelini et al., 1972; Reuser et al., 1978), in leucocytes (Koster et al., 1972), and in urine (Mehler and DiMauro, 1976; Schram et al., 1979). Moreover, Martin et al. (1976a) and DiMauro et al. (1978) measured low activities in the heart and the central nervous system in autopsy material from patients with the muscular form. Thus, these observations do not support the supposition that the enzyme deficiency has a different distribution in the two forms of acid maltase deficiency.

b. A difference in the degree of deficiency

In patients with the generalized form the enzyme activity in skeletal muscles might be undetectable or severely reduced, whereas a residual enzyme activity might be present in patients with the muscular form (Zellweger et al., 1965; Hudgson et al., 1968).
In fibroblasts such a difference in acid maltase activity has been demon-

strated by Reuser et al. (1978) as well as in the present study. Schram et al. (1979) were not able to demonstrate acid maltase activity in the urine of one patient with the generalized form and they found a residual activity of about 5 per cent of the control values in urine of six patients with the muscular form. Less conclusive evidence is available on acid maltase activity in muscle tissue: a difference in activity was reported by Mehler and DiMauro (1977), but Engel et al. (1973) stated that they had been unable to distinguish between the generalized form and the muscular form on the basis of acid maltase levels in muscle tissue. Our findings are in agreement with those of Engel and his co-workers. Thus, it appears that residual activity of acid maltase in muscle may occur both in the generalized form and in the muscular form. On the other hand, both clinical forms can be distinguished by the enzyme levels in fibroblasts or in urine: the presence of a residual activity in the muscular form and absence of enzyme activity in the generalized form.

c. An additional role of neutral maltase

Angelini and Engel (1972) suggested that in the muscular form of the disease neutral maltase might partially compensate for the lack of acid maltase. In patients with the generalized form they demonstrated only traces of neutral maltase in the heart and a significantly decreased activity of this enzyme in skeletal muscles and in the liver, and no decrease was found in patients with the muscular form. In contrast, we found a neutral maltase activity within the control range in skeletal muscles of some patients with the generalized form, and a very low activity in some patients with the muscular form.

DiMauro et al. (1977) speculated that the enzyme defect affects the carbohydrate component rather than the polypetide constituent of the acid maltase molecule. The sugars become attached to the polypeptide chain in the endoplasmic reticulum and in the Golgi apparatus before the enzyme enters into the lysosomes. According to these authors neutral maltase, known to be localized in the microsomal fraction of the tissue cells, might be a precursor of acid maltase, and the difference between the generalized form and the muscular form of acid maltase deficiency might be due to different defects in the conversion of the neutral to the acid enzyme. This attractive hypothesis needs verification. It might explain the absence of neutral maltase in skeletal muscle tissue which was found in some of our patients with the muscular form (see chapter 5, table 10).

d. Different non-allelic mutations

Reuser et al. (1978) performed complementation studies in which cultured fibroblasts of patients with the generalized form were fused with those of patients with the muscular form. If two non-allelic gene mutations should be present, an increase in acid maltase activity should occur after fusion of the cells. However, these investigators found no restoration of the enzyme defect and therefore their experiments provided no evidence for different non-allelic gene mutations to explain the variability of the phenotype in acid maltase deficiency. Non-allelic gene mutations seem also unlikely in view of our findings in family B (see chapter 7). The presence of both the generalized and the muscular form in this family suggests that these forms are in some way genetically related. It is attractive to invoke different allelic mutations at the same locus (genetic compound) in this family, but this possibility was not borne out by clinical and biochemical findings.

Ad 2. Pathogenesis

The pathogenesis of acid maltase deficiency remains ill-understood.

Schiaffino and Hanzlíková (1972) demonstrated the participation of the lysosomal system in glycogen mobilization in the liver and skeletal muscles in rats during the first post-natal days. Assuming a similar role for the lysosomes during the post-natal period in man, they suggested that in Pompe's disease the normal uptake of large amounts of glycogen within lysosomes during the first months of life is not followed by its degradation. The clinical manifestations of the disease in later months would be explained by progressive lysosomal storage of glycogen. This assumption corresponds with the "mechanical" concept put forward by Hers (1963), who further presumed the progressive deposition of glycogen to cause a disruption of muscle fibres. Although this "mechanical" concept seems to offer an attractive explanation for the dysfunction in patients with the generalized form, it cannot be applied to the weakness found in patients with the muscular form. The importance of lysosomal glycogen metabolism in human muscle in relation to muscular function is incompletely understood. On microscopic examination the muscle biopsies of the patients with the muscular form show a large variation in the degree of involvement. This might be due to sampling error, but no studies have been performed in which scrutiny of entire muscles demonstrated only

focal vacuolization. A variability in the degree of involvement between different muscles of the same patient has been reported by Martin et al. (1976a). We have not been able to demonstrate a correlation between muscle power, the degree of vacuolization, and the enzyme activity in the quadriceps muscle in ten patients with the muscular form (see chapter 5, figure 9). This is in agreement with the findings of Martin et al. (1976a). On the other hand, like Martin et al. (1976b) we found a correlation between the glycogen content and the degree of vacuolization in skeletal muscles of patients with either form.

The enzyme deficiency in patients with the muscular form can be demonstrated not only in skeletal muscles, but also in other samples such as leucocytes, cultured fibroblasts, urine, the heart and the central nervous system. Apparently all these tissues tolerate the deficiency of acid maltase much better than skeletal muscle since it does not lead to storage of glycogen nor to clinically evident dysfunction of these tissues.

Refined techniques for enzyme investigation might unravel the problem of the clinical variability of acid maltase deficiency. Pena and co-workers (1978) used a double labeling technique with radioisotopes to detect molecular abnormalities in acid maltase deficiency. In their experiments one radioisotope (^{3}H-leucine) was labeled to control fibroblasts and a different one (^{14}C-leucine) to fibroblasts of a patient with the generalized form. This was followed by extensive fractionation and subsequent analysis. The same procedure was used in fibroblasts of a patient with the muscular form. They established the deficiency of a protein in the fibroblasts of both patients. This protein had a minimum molecular weight of 29,000 and it was structurally unrelated to acid maltase. They speculated this finding to be a "new" molecular defect in acid maltase deficiency.

Beratis et al. (1978) studied acid maltase by immunological techniques in fibroblast lysates of controls and of patients with acid maltase deficiency. They identified cross-reacting material in controls, in one patient with the generalized form and in one patient with the muscular form. In the patient with the muscular form the amount of cross-reacting material was significantly reduced. The immune complexes showed enzyme activity in the controls and in the patient with the muscular form, but enzyme activity proved absent in the patient with the generalized form. They concluded that in the muscular form there is a reduction of the amount of functionally normal enzyme protein, and that a catalytically inactive enzyme protein was present in their patient with the generalized

form. These findings are unexpected as far as the generalized form is concerned, since other investigators have not found cross-reacting material in patients with this form (de Barsy et al., 1972; Brown et al., 1975; Koster and Slee, 1977; Reuser et al., 1978). Beratis et al. considered genetic heterogeneity as an explanation for this discrepancy. Their results in the muscular form are in agreement with those of Reuser et al. (1978).

Further studies are necessary in view of the small number of patients investigated with these refined enzyme techniques.

As long as the pathogenesis of acid maltase deficiency remains obscure, there is no firm basis for therapy. Up to the present, enzyme replacement therapy has been without success. One of the reasons for this failure may be that the administered enzyme does not penetrate into the lysosomes (Tager et al., 1979). Further studies on the pathogenesis of acid maltase deficiency are important, and perhaps they will open the gate to an effective therapy.

SUMMARY

This thesis presents a clinical, biochemical and genetic study of 20 patients with acid maltase deficiency, seven with the generalized form and 13 with the muscular form. In addition, the parents of six patients with the generalized form and of five patients with the muscular form have been investigated, as well as the siblings of four patients with the generalized form and of two patients with the muscular form. Further, a large family has been extensively studied because a male patient suffered from the muscular form whereas one of his grandchildren died from the generalized form. Finally, thirteen grandparents of five infants with the generalized form have been investigated to see if such a combination might occur more often.

Chapter 1 reviews the history of acid maltase deficiency in relation to other glycogen storage diseases. It is outlined how in the thirties ideas about the etiology of idiopathic cardiac hypertrophy changed from "tumor growth" to "glycogen storage". The enzyme deficiency was identified in the fifties. The description of other clinical forms soon followed, and this clinical heterogeneity prompted new questions about the pathogenesis.

Chapter 2 reviews recent reports on acid maltase deficiency, with emphasis on ancillary studies, differential diagnosis, treatment and genetics.

Chapter 3 describes the methods of clinical examination, and of biochemical and genetic studies in the patients and their relatives. A calculation of the a posteriori probability of heterozygosity was developed according to Bayes' theorem, on the basis of the ratio of acid maltase / β-hexosaminidase.

Chapter 4 contains the 20 case histories and the clinical findings in the relatives.

Chapter 5 summarizes the clinical, biochemical and genetic findings in the patients, and these are compared with previous reports. A uniform clinical pattern and course in the patients with the generalized form could be confirmed, but patients with the muscular form showed a wide clinical variation. Although this variability might warrant a subdivision into genetic subtypes, no strict morphological or biochemical criteria could be defined that would allow such a subdivision. The enzyme studies in the patients only partially confirm the results of previous authors who have found absent or extremely low activities of acid maltase in the generalized form, and residual activities (10-20 per cent of control values) in the muscular form. This could only be confirmed in fibroblasts, but not in muscle tissue or in leucocytes.

No relationship between the strength, the degree of vacuolization and the level of acid maltase acitvity was found in the quadriceps muscle of 10 patients with the muscular form. On the other hand, a positive correlation did seem to exist between the glycogen content and the degree of vacuolization in three patients with the generalized form and in seven patients with the muscular form.

Treatment with a low-carbohydrate and high-fat diet during three months in two patients with the muscular form did not result in an increase in muscle power. Since no effective therapy in available at present, prenatal diagnosis remains a possibility to prevent further cases in the family. No consanguinity was found in eight families with the generalized form including the parents of Pompe's original patient. A blood-relationship was also absent in five families with the muscular form.

The incidence of the generalized form in The Netherlands has been calculated to be about 1/125,000 which corresponds to a heterozygote frequency of 1/175. For the muscular form an approximate frequency of 1/550,000 was estimated in the Dutch population of 5-65 years. This represents a minimum since it is probable that there is a number of unidentified patients.

No sex preponderance was found in either form of the disease.

The chapter concludes with guidelines for the diagnosis of acid maltase deficiency.

Chapter 6 is devoted to the investigations in the relatives, with emphasis on heterozygote indentification. Heterozygotes could not be detected by physical examination, serum CPK assay, morphological examination of muscle or skin biopsies, or by TLC of oligosaccharides in the urine. Most

obligate heterozygotes could usually be indentified by the assay of acid maltase activity in muscle tissue, leucocytes, or urine, but in some the enzyme values overlapped with those of the controls (figure 13).

Heterozygotes for the generalized form could not be distinguished from those for the muscular form.

The exceptional combination of the muscular form in a patient and the generalized form in one of his grandchildren was not found again by the examination of 13 grandparents of five infants with the generalized form. Surprisingly, all grandparents (including six couples) showed a low ratio of acid maltase/β-hexosaminidase in urine, while it was expected that only one subject of each couple should have a low ratio. An explanation could not be given.

Chapter 7 describes the family in which the generalized form and the muscular form of acid maltase deficiency occurred together. No further patients were found by physical examination of the six siblings of the patient with the muscular form, his children, and 21 of his grandchildren. In this family an autosomal recessive mode of inheritance seemed very likely in view of the enzyme studies in muscle tissue, lymphocytes, cultured fibroblasts, and urine. An attractive explanation for the occurrence of both clinical forms in this family seemed the postulation that either the grandfather or the grandchild is a genetic compound for acid maltase deficiency. However, no definite proof could be obtained for this hypothesis.

The General Discussion deals with the clinical variability and the pathogenesis of acid maltase deficiency. Clinical variability is well known in other lysosomal storage diseases, and a reasonable explanation is available in some. With the present state of knowledge of the morphological, the biochemical and the genetic features of acid maltase deficiency, it remains hard to understand the considerable clinical variability of Pompe's disease.

SAMENVATTING

Dit proefschrift bevat een klinische, biochemische en genetische studie over 20 patiënten met zure maltase deficiëntie, waarvan 7 met de gegeneraliseerde vorm en 13 met de musculaire vorm. De ouders van 6 patiënten met de gegeneraliseerde vorm en die van 5 patiënten met de musculaire vorm werden onderzocht, evenals de broers en zusters van 4 patiënten met de gegeneraliseerde vorm en van 2 patiënten met de musculaire vorm. Bovendien worden de resultaten beschreven van het onderzoek van een familie, waarin een patient leed aan de musculaire vorm, terwijl één van zijn kleinkinderen was overleden aan de gegeneraliseerde vorm. Om na te gaan of een dergelijke combinatie vaker voorkwam werden tenslotte 13 grootouders onderzocht van 5 patiënten met de gegeneraliseerde vorm.

Hoofdstuk 1 behandelt de historie van de ziekte in relatie tot andere glycogeenstapelingsziekten. Allereerst is geschetst hoe in de dertiger jaren een omschakeling plaatsvond in het etiologische denken over de zogenaamde idiopatische hypertrofie van het hart, en wel van "gezwelgroei" naar "glycogeenstapeling". In de vijftiger jaren werd het enzymedefect bekend en spoedig daarna werden klinische varianten van de ziekte beschreven. Het bekend worden van deze varianten leidde al snel tot nieuwe vragen over de pathogenese, die wat betreft de gegeneraliseerde vorm eerst zo goed begrepen leek.

Hoofdstuk 2 bevat de recente literatuur over zure maltase deficëntie, waarbij de nadruk is gelegd op de diagnostiek, differentiële diagnostiek, therapie en erfelijkheid.

In hoofdstuk 3 worden de methoden vermeld, waarmee de patiënten en hun familieleden klinisch en biochemisch onderzocht zijn. Een formule volgens het principe van Bayes werd gebruikt voor het berekenen van de a posteriori kans op dragerschap voor zure maltase deficiëntie op basis van de

gemeten enzymwaarden in de urine. Tenslotte is in dit hoofdstuk aangegeven op welke wijze de gegevens voor genetische analyse verkregen zijn.

Hoofdstuk 4 bevat de ziektegeschiedenisssen van 20 patiënten, tesamen met de bevindingen van anamnese en onderzoek bij een aantal familieleden.

In hoofdstuk 5 zijn de klinische, biochemische en genetische gegevens van de patiënten bijeengezet en getoetst aan gegevens uit de literatuur. Een uniform beeld van de klinische verschijnselen en het ziektebeloop bij de patiënten met de gegeneraliseerde vorm kon worden vastgesteld, maar bij de patiënten met de musculaire vorm was er echter een grote klinische variabiliteit. Alhoewel deze variabiliteit bij de musculaire vorm het bestaan van subtypen doet vermoeden, konden geen stricte morphologische of biochemische criteria aangegeven worden op grond waarvan een onderverdeling zou kunnen worden gemaakt.

De enzymstudies bij de patiënten bevestigden slechts ten dele de conclusie van sommige auteurs, dat bij de patiënten met de gegeneraliseerde vorm géén of slechts een zeer geringe enzymactiviteit wordt gemeten, terwijl bij de patiënten met de musculaire vorm een rastactiviteit (van 10-20% van de controlewaarden) kan worden vastgesteld. Dit kon alleen voor fibroblasten aangetoond worden, maar niet voor spierweefsel of voor leucocyten.

Er werd geen relatie gevonden tussen spierkracht, mate van vacuolisatie en percentage van de gemeten acitiviteit van zure maltase in de m. quadriceps van 10 patiënten met de musculaire vorm. Wel leek er een positieve correlatie te bestaan tussen de mate van vacuolisatie en de hoeveelheid glycogeen bij 3 patiënten met de gegeneraliseerde vorm en 7 patiënten met de musculaire vorm.

Een koolhydraat-arm, vetrijk dieet, gedurende 3 maanden gebruikt door 2 patiënten met de musculaire vorm, leidde niet tot toename van de spierkracht. Tot op heden is er geen oorzakelijke therapie en kan alleen antenatale diagnostiek worden aangeboden ter preventie van nieuwe patiënten in het gezin.

Er werd geen consanguiniteit vastgesteld in 7 families met de gegeneraliseerde vorm en ook niet in 5 families met de musculaire vorm. Evenmin werd gevonden dat de ouders van het patientje, oorspronkelijk beschreven Pompe, aan elkaar verwant waren.

De frequentie van de gegeneraliseerde vorm in Nederland werd berekend op omstreeks 1:125.000 pasgeborenen, hetgeen overeenkomt met een

heterozygotenfrequentie van 1:175. De frequentie van de musculaire vorm werd geschat op 1:550.000 in de Nederlandse bevolking van 5-65 jaar. Dit laatste echter een minimum omdat waarschijnlijk bij een aantal patiënten de diagnose niet gesteld is.

Tenslotte werd nog aangetoond, dat in de hierbeschreven groep van patiënten, geen voorkeur bestond voor een vaker aangedaan zijn van één der geslachten, noch wat betreft de gegeneraliseerde vorm, noch wat betreft de musculaire vorm.

Hoofdstuk 6 is gewijd aan het onderzoek van familieleden, waarbij de voornaamste vraag was of dragerschap voor de ziekte zou kunnen worden vastgesteld. Dit bleek niet mogelijk te zijn door lichamelijk onderzoek, CPK bepaling, onderzoek van een spier- of een huidbiopsie, of door dunne laag chromatografie van de oligosacchariden in de urine. Met de bepaling van de zure maltase activiteit in spierweefsel, gekweekte fibroblasten, leucocyten of urine leek vaststellen van dragerschap mogelijk, zij het niet bij alle individuen, vanwege het overlappen van de enzymwaarden van de dragers met die van controles (figuur 13).

Heterozygoten voor de gegeneraliseerde vorm konden niet onderscheiden worden van die voor de musculaire vorm.

Het onderzoek van 13 grootouders van 5 patiëntjes, overleden aan de gegeneraliseerde vorm, heeft niet geleid tot het ontdekken van eenzelfde combinatie als in de familie, die in hoofdstuk 7 beschreven wordt. Een merkwaardige vondst was de lage enzymactiviteit in de urine bij alle 13 onderzochte grootouders (waarvan 6 paren), terwijl verwacht werd dat slechts de helft van hen een lage waarde zou hebben. Hiervoor was geen verklaring te vinden.

Hoofdstuk 7 beschrijft het onderzoek van een familie, waarin zowel de musculaire als de gegeneraliseerde vorm van zure maltase deficiëntie waren gediagnosticeerd. Lichamelijk onderzoek van de 3 broers en de 3 zusters van de patiënt met de musculaire vorm, zijn 10 kinderen, en 21 van de 29 kleinkinderen heeft niet geleid tot het vinden van nieuwe patiënten in deze familie. Een autosomaal recessieve overervingsmodus leek het meest waarschijnlijk te zijn op basis van de enzymstudies in spierweefsel, lymphocyten, gekweekte fibroblasten en urine. Het voorkomen van de beide varianten binnen deze familie leek het beste verklaard te kunnen worden door aan te nemen dat óf de patiënt met de musculaire vorm, óf de patiënt met de gegeneraliseerde vorm, een "genetic compound" voor zure maltase

deficiëntie is. Hiervoor kon echter geen afdoende bewijs worden geleverd.

In een Algemene Discussie wordt aandacht besteed aan de klinische variabiliteit en aan de pathogenese van zure maltase deficiëntie. Ook bij andere lysosomale stapelingsziekten wordt variabiliteit van de klinische verschijnselen gevonden en bij een aantal van deze ziekten is hier een aannemelijke verklaring voor. Wat betreft zure maltase deficiëntie was het echter niet goed mogelijk om vanuit de huidige biochemische en genetische kennis de aanzienlijke klinische variabiliteit te verklaren, die gevonden wordt bij de ziekte van Pompe.

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In datzelfde jaar begon zij de studie in de geneeskunde aan de Gemeenteuniversiteit te Amsterdam. Deze studie werd in 1959 vervolgd aan de Stichting Klinisch Hoger Onderwijs te Rotterdam, alwaar in 1960 het artsexamen behaald werd.

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Sinds 1971 is zij als kinderneuroloog verbonden aan de afdeling neurologie van het Academisch Ziekenhuis Rotterdam.

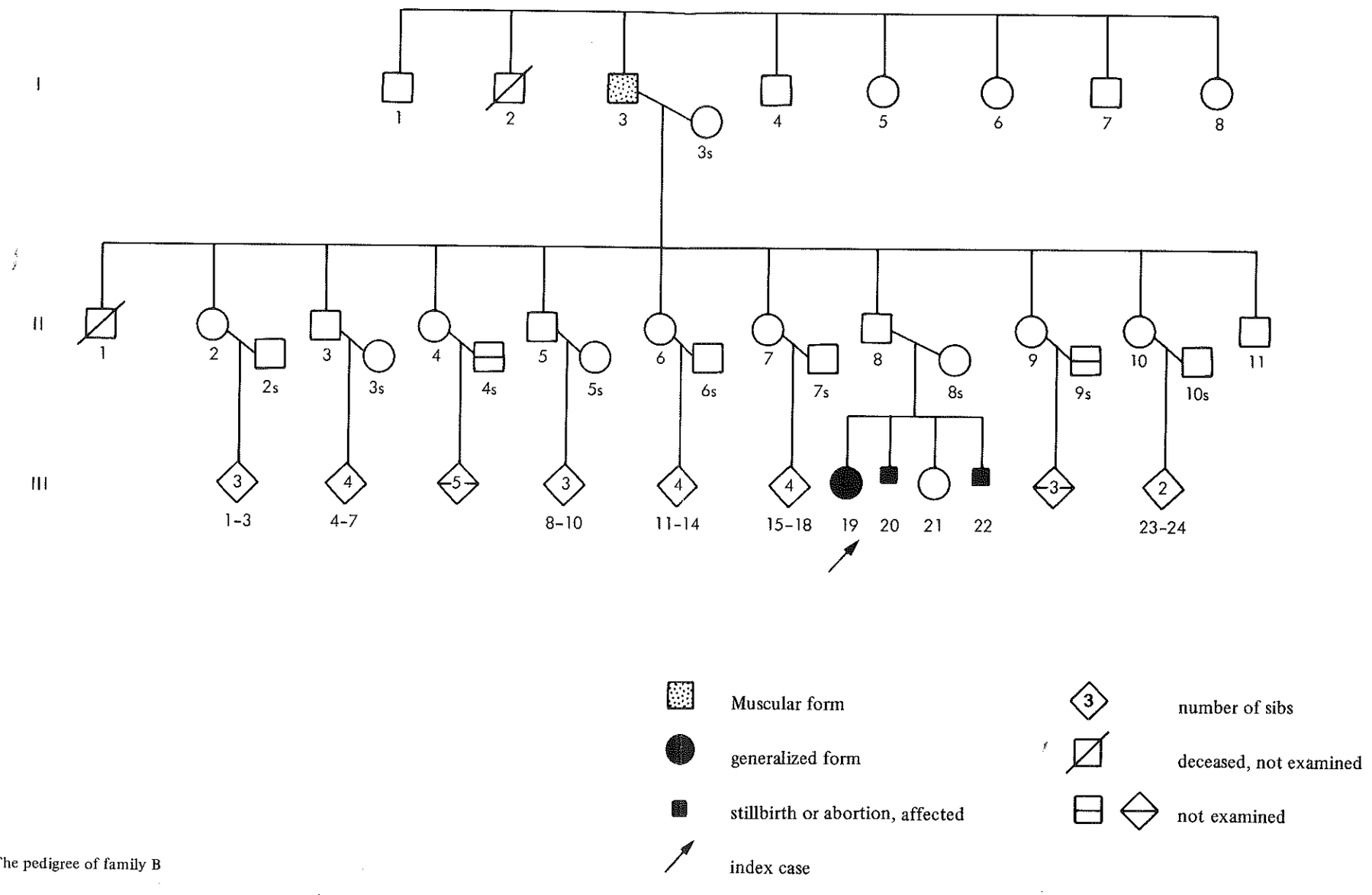

Figure 15. The pedigree of family B