Metachromasia in Cultured Fibroblasts of Subjects with Glycogenosis Type II

In some genetic diseases, whether in the affected individuals or in the clinically normal carriers, metachromatic material has been found in skin-fibroblast cultures stained with toluidine blue 0[1-9].

Recently we have observed the presence of metachromasia in cultured fibroblasts of 2 subjects with glycogenosis type II (L.U. and L.M. from different families) and from several of the subject's relatives. L.U., a 12 month old girl, was affected by the cardiac variant of the disease, and L.M., a 6 month old male, by the muscular variant. The diagnosis of glycogenosis type II was made according to clinical features, histological patterns of the muscle and biochemical determination of the acid α-1,4-glucosidase in the muscle.

The skin biopsies, taken without anesthesia from the extensor surface of the upper arm, were cut in tiny fragments and then plated on coverslips placed in T-flasks. Eagle's medium with 15% foetal calf serum was used. When the halos of the cellular growth from the explants were dense (generally between the 4th and the 6th week) the subculture was carried out by trypsinization. The staining was made with 0.1% toluidine blue 0 in 30% ethanol on both primary cell cultures and subcultures which had been fixed in methanol. The α-1,4-glucosidase was measured by the method of HUDGSON et al.[10] in all cases on leukocyte extracts and in some cases also on cultured cell extracts. Leukocytes were isolated from the blood by sedimentation in 3% polyvinylpyrrolidone in 0.85% saline followed by a 1 min hypotonic shock[11] to lyse contaminating erythrocytes. The enzyme activity is expressed as millimicromoles maltose hydrolyzed per min per mg protein, measured by the method of LOWRY et al.[12].

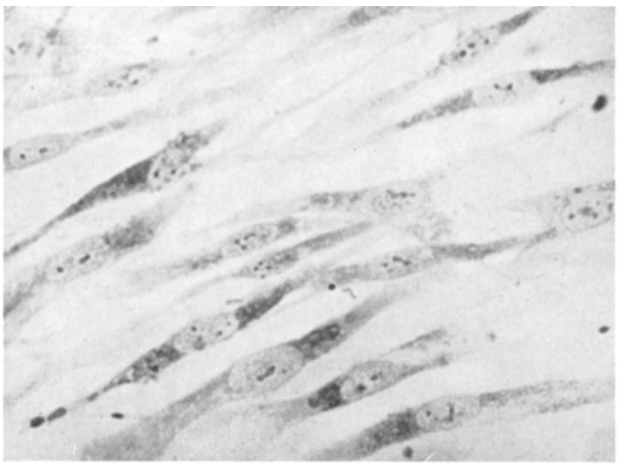
No difference in cellular morphology was noted between normal cultures and those of the patients. Metachromatic material was present in primary skin cultures of the two patients and in their relatives after the fourth week (see Figure). This material demonstrated the same features even after several subcultures. The morphological distribution of metachromatic material was variable. The fibroblast cultures derived from the family of the patient L.U. showed a metachromasia diffusely distributed throughout the cytoplasm, while it was vesicular in the affected child. The metachromatic cultures derived from L.M. and his parents and siblings showed a prevalent vesicular pattern, but several cells with granular cytoplasmic metachromatic material were also present.

The α-1,4-glucosidase activity of the leukocytes was not detectable in the patients and significantly reduced in their parents and siblings. A reduction of the glucosidase activity was also observed in the fibroblast cultures of L.U. and her family (see Table).

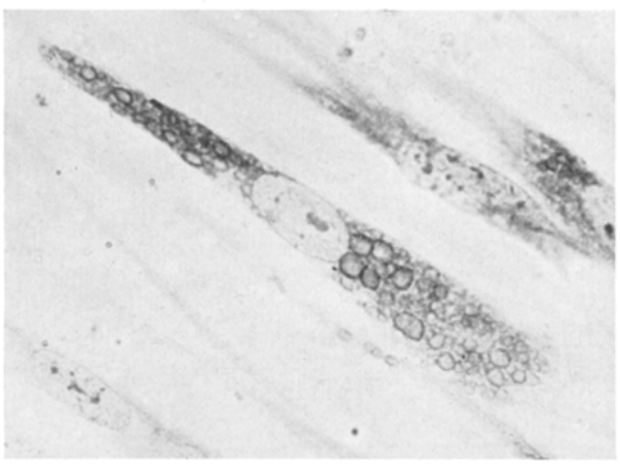
Glycogenosis type II is inherited as an autosomal recessive trait[13]. The deficiency of α-1,4-glucosidase discovered by HERS[14] is almost complete in the affected homozygotes and probably intermediate in the apparently healthy heterozygotes.

The defective activity of the acid glucosidase in leukocytes of the parents and siblings of our patients would indicate that they are all carriers of this genetic defect. Therefore it is probable that there is a relation between the appearance of metachromatic material in the cultures and the α-1,4-glucosidase deficiency.

It is known that a great number of metabolic substances can be stained metachromatically. In Hurler's syndrome[15,16], in Marfan's syndrome[6] and also in cystic



a



b

a) Skin cultured cells stained with toluidine blue 0. Positive cells appear with dark cytoplasm.
b) Typical appearance of cell with cytoplasm filled with vesicular metachromatic material.

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fibrosis[17,18], the metachromatic material is made of mucopolysaccharides.

In the other diseases with metachromasia positive fibroblast cultures, the nature of this material has not yet been established. In vivo, in glycogenosis type II, metachromasia located in the glycogen rich areas of the multivacuolated muscle cells has been observed, after staining with toluidine blue 0[19,20]. Histochemical analysis results indicated that these metachromatic areas contain a glycogen complex only partially digestible with diastase, resembling acid mucopolysaccharides[19,20]. This compound probably needs acid glucosidase for degradation[21]. We are not able to say whether the metachromatic material found in the skin fibroblast cultures of our patients has the same features as that found in vivo. Further investigation clarifying this point could contribute to the knowledge of the nature of metachromasia in glycogenosis type II.

Skin fibroblast metachromasia, leukocyte and fibroblast α-glucosidase activity in patients with glycogenosis type II and in their next of kin

Subjects	Metachromasia (% positive cells)	α-1,4-Glucosidase activity[a]	
		Leukocytes	Fibroblasts
Patient L.U.	40–80	0	4.5
Family of L.U.:			
Father	10–50	7.6	–
Mother	30–70	4.8	16.5
Brother A	40–80	5.9	23.1
Brother B	40–80	6.1	16.0
Patient L.M.	40–90	0	–
Family of L.M.:			
Father	40–90	6.0	–
Mother	40–80	5.8	–
Brother	40–90	5.2	–
Controls 10	1–5	–	–
Controls 9	–	11.9 ± 3.6	–
Controls 1	–	–	36.2
Controls 1	–	–	45.7

[a] Millimicromoles maltose hydrolyzed per min/mg protein.

Riassunto. Frammenti di cute di due soggetti affetti da glicogenosi tipo II e dei loro genitori e fratelli sono stati coltivati in vitro. Sia nelle culture dei pazienti che dei loro familiari si è osservata la presenza di materiale metacromatico dopo colorazione con blu di toluidina 0. Nei leucociti di tutti i familiari esaminati l'attività della α-1,4-glucosidasi è risultata ridotta.

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Fetal Encephalopathy Following Ingestion of Tellurium

Non-obstructive hydrocephalus may be present in newborn rats if their mother ingests elemental tellurium during gestation. There are pathological changes present in the ependymal cells of the lateral ventricles of these rats during fetal life at the site of the CSF-ependymal barrier.

There are 2 types of hydrocephalus, obstructive and non-obstructive. In obstructive hydrocephalus there is an impediment to the flow of the cerebrospinal fluid (CSF) from the choroid plexus where it is secreted, to the venous sinuses, where it is absorbed. Non-obstructive hydrocephalus may result from atrophy of the neural tissue in the brain, or from overproduction of CSF by the choroid plexus. There is an experimental method to produce non-obstructive hydrocephalus, which consists of adding elemental tellurium to the diet of a gestating rat. This can result in the birth of a litter of hydrocephalic animals without apparent ill-effects on the mother[1,2]. The present communication is the report of a study of the pathological changes present in the brain of fetal rats, whose mothers had ingested tellurium in their diet and eventually gave birth to hydrocephalic animals.

The amount of tellurium needed in the diet of the mother to produce hydrocephalus varied from 500 to 3500 ppm of the diet[1,2]. 50 to 100% of the gestating rats who ingested tellurium gave birth to hydrocephalic animals and 10 to 100% of the animals in the litter were hydrocephalic. The incidence of hydrocephalus was proportional to the amount of tellurium present[1,2].

Elemental tellurium is much less toxic than tellurites, tellurates and tellurous acid. Its minimal lethal dose is unknown[3,4]. Tellurium is easily absorbed into the body[5] and it remains there a long time. The classic sign of tellurium intoxication in man and rat is the garlic odor of the breath caused by dimethyl telluride, a breakdown product of tellurium[3,4]. Patients receiving i.m. injections of elemental tellurium in suspension, for the treatment of syphilis, complained of a persistant odor of garlic in the breath, 2 years after the end of the treatment[6]. Repeated injections of a suspension containing elemental tellurium to animals resulted in the presence of granular

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