werden, dass hier zu diesem Zeitpunkt keine vergleichbare organologische Differenzierung stattfindet.

Messungen an Explantaten von nicht-induziertem Ektoderm aus einer frühen Gastrula stimmen vollkommen überein mit den Werten für die Epidermis in situ: Auftreten der ersten, Fehlen der zweiten DNS-Zunahme. Das stützt die Annahme, dass die erste Zunahme mit der Cytodifferenzierung (Ektodermzelle → atypische Epidermiszelle), die zweite mit der organologischen Differenzierung in Verbindung steht. (Das explantierte, nicht-induzierte Ektoderm ist bekanntlich zu keinerlei organologischer Differenzierung befähigt.) Unter Dauereinwirkung von Actinomycin D (1 µg/ml) werden die DNS-Werte vollständig nivelliert, das heisst auch die erste DNS-Vermehrung unterbleibt. Dieser Befund ist sehr überraschend, zumal, wie aus den Karyogrammen ersichtlich ist, die interphasische DNS-Replikation sowie die Kern- und Zellteilung erwartungsgemäss ungestört ablaufen. Es ist daher anzunehmen, dass die Synthese der kurzfristig zusätzlich erscheinenden DNS unabhängig ist von der normalen interphasischen DNS-Verdoppelung. Eine eingehendere Analyse der Karyogramme, durch die weitere Aufschlüsse über das Synthesegeschehen während der Interphase zu erwarten sind, ist im Gange. Ähnliche Untersuchungen an Explantaten von induziertem Ektoderm einer späten Gastrula (ohne und mit Actinomycin) sowie an Explantaten von Chordamesoderm und Dotterentoderm wurden durchgeführt, bedürfen aber noch der Auswertung.

Summary. The DNA content of isolated cells of *Triturus vulgaris* embryos (morula through early tail-bud) was measured cytophotometrically. In various regions (entoderm, chordamesoderm, neuroectoderm, presumptive epidermis) considerable differences occur. In particular, some phase-specific DNA increases (approximately 11%) of short duration were detected, which are correlated with the onset of cyto- and organodifferentiation, respectively, and which are caused independent of the interphase DNA reduplication.

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Glycogenosis Type II: Glycogen Storage in Cell Cultures from Muscle

Glycogenosis type II or Pompe's disease is characterized by extensive deposition of normal glycogen with massive involvement of striated muscle. The absence of the α-1-4-glucosidase, a lysosomal enzyme, has been demonstrated in this condition by HERS[1]. The α-1-4-glucosidase deficiency is reported in almost all tissues of affected subjects[2]; nevertheless, there is disagreement as to whether this enzymatic defect alone can cause glycogen storage[3–5].

Recently NITOWSKY and GRUNFELD[6] have studied fibroblast cell cultures derived from the skin of a 5-month-old patient. The cultured fibroblasts showed no glycogen deposits even though α-1-4-glucosidase was absent.

We had the opportunity to study with histochemical reactions, cell cultures from the skin and muscle of a 1-year-old female with glycogenosis type II. The diagnosis was based on clinical features, histological patterns of muscle biopsy and α-1-4-glucosidase deficiency biochemically determined in the fresh muscle and skin.

Tiny fragments obtained from a surgical biopsy of the skin and the gastrocnemius were plated on to coverslips placed in T-flasks with Eagle's synthetic medium plus 15% calf foetal serum and antibiotics. The muscle cultures were allowed to grow for 13 days and the skin cultures for 32 days. Then the coverslips were removed and stained for phosphorylase[7], ATPase[8] and glycogen, with periodic acid-Schiff reagent before and after digestion with diastase. Fibroblast cultures from normal skin and muscle cultures from 4 children aged 6–13 months, without muscular or metabolic diseases, were used as controls.

The fibroblast cultures from the patient's skin and those of the control subjects demonstrated some difference in staining with PAS reagent. A more evident PAS positivity was noticed in the patient's fibroblasts. However it was not reduced by diastase incubation in a significant way.

The muscle cultures of our patient and those of the controls after 13 days of growth were mainly composed of fibroblast-like cells. They were rather dumpy, often connected by cytoplasmic bridges or aggregated in bi- or multinucleated cells. In the muscle cultures of the control subjects PAS positive material was present in 2 different patterns, one diffused throughout the cytoplasm and the other confined to a few cytoplasmic granules. Only the latter were absent in the slides incubated with diastase.

In the muscle cultures of the patient with glycogenosis type II, PAS positive material was plentifully present in the majority of the cells (Figure 1). This material appeared in intensely stained granules which almost completely filled the cytoplasm in some cells (Figure 2). After diastase digestion the PAS positive granules were absent and a faint PAS positivity could be seen.

Phosphorylase and ATPase reactions were strongly positive in the cell cultures from the muscle of both patients and control subjects, while they were weakly positive in the fibroblast skin cultures.

We are not able to assess the nature of the cells deriving from the muscle. However, the peculiar morphology and the strong phosphorylase and ATPase activity in these cells allow us to distinguish them from common fibroblast ones. A lack of glycogen accumulation, at least histochemically detectable, in the skin cultures in contrast

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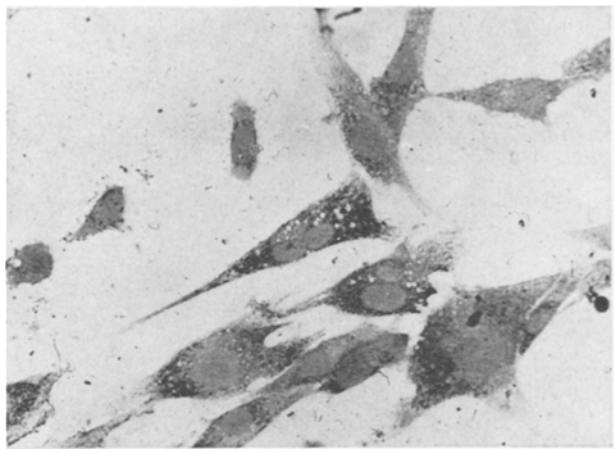

Fig. 1. Fibroblast-like cell cultures derived from α-1-4-glucosidase deficient muscle.

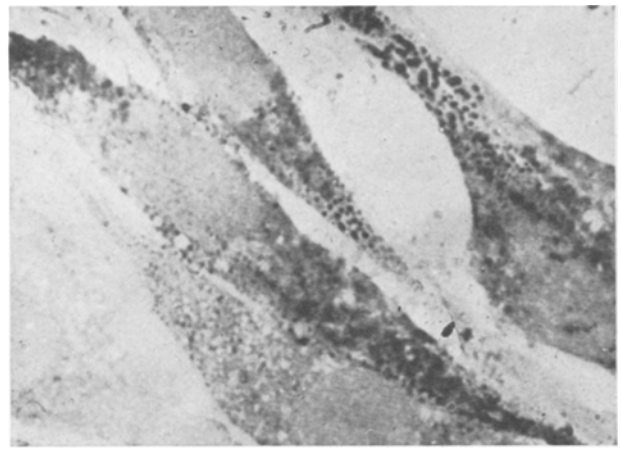

Fig. 2. High magnification: the cytoplasm is filled with PAS positive diastase digestible granules.

to muscle cultures, could be related to the different features of glycogen metabolism of these cells.

It is possible, because the glycogen synthesis occurs in the cell cultures[9], that it comes about slowly in skin cultures and very rapidly in muscle cultures. In the former, but not in the latter, glycogenolysis could take place completely by an alternate pathway to the α-1-4-glucosidase degradation. However, our observation could also mean that the α-1-4-glucosidase defect is not the only cause of glycogen deposition. This would be in agreement with the presumption that there is another defect apart from the α-1-4-glucosidase one. On the whole, in vitro culture results also show the difficulty of simplifying the pathogenesis of glycogenosis type II to a lysosomal enzymatic defect.

These results demonstrate that the disease's features can be reproduced in vitro. Further studies of tissue cultures might produce a better understanding of glycogenosis type II.

Riassunto. È stata eseguita la coltura in vitro di frammenti di muscolo e di cute di un soggetto affetto da glicogenosi di tipo II°. È stato osservato un accumulo di materiale PAS positivo, digeribile con la diastasi, nelle cellule derivate dal muscolo ma non in quelle derivate dalla cute.

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Partial Nervous Control of the Avian Ultimobranchial Body

There is now strong evidence that the mammalian thyroid parafollicular, or C, cells are the cells of origin of the hypocalcaemic hormone calcitonin[1,2]. Embryological[3] and cytochemical[4] studies have shown that the C cells are derived from the ultimobranchial bodies.

In birds there are no thyroid C cells but the ultimobranchial bodies persist as distinct structures into adult life. Recently Copp et al.[5] have shown that they contain high levels of calcitonin activity.

Unlike the mammalian thyroid C cells the ultimobranchial body of the fowl is well innervated. The vagus nerve lies adjacent to its dorso-lateral border and the recurrent nerve runs close to its medial boundary. Nerve fibres supplying the ultimobranchial come mainly from the vagus and to a lesser extent from the recurrent nerve and the sympathetic system[6]. Nonidez[7] described a single branch, often initially in association with the accessory depressor nerve, which leaves the vagal ganglion nodosum to supply the ultimobranchial body. The situation is, however, more complex than this. An interanastomosing group of several fine nerve bundles leaves the ganglion nodosum to supply both the carotid body and the ultimobranchial body, and the 2 bodies may also be interconnected by nerves. Similarly, there may be more than one nerve bundle passing from the recurrent nerve into the ultimobranchial[6,8].

Within the ultimobranchial body the nerves branch and pass out into the tissues but, before doing so, some of the fasciculi, particularly from the vagus, may become associated with small groups of ganglion cells located superficially within the gland[6,9].

The structure and innervation of the ultimobranchial gland of the laying hen has been studied with light microscope, silver impregnation techniques and by

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