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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black deposits in the cytoplasm of renal and neuronal cells, without clinical manifestations. These animals survived these experiments for years[7-9].

The mechanism by which tellurium produces hydrocephalus is unknown. It obviously acts during fetal life, because the hydrocephalus has been observed during fetal life and at birth[1,2]. AGNEW et al.[2] demonstrated that radioactive tellurium, Te 127m, as tellurous acid, injected into a pregnant rat fed the tellurium diet, crossed the placental barrier and was present in fetal blood, CSF and brain, 4 h later.

The purpose of the present study was to examine the brains of rat fetuses, during early fetal life on days 13 and 15[10]. The instruments employed in this study were light and electron microscopes. Twenty female Wistar rats were fed a diet consisting of 3000 ppm elemental tellurium added to Purina Lab Chow Meal, every day of pregnancy. In my experience, this amount of tellurium in the diet results in 50% of the rats, giving birth to litters whose every member is hydrocephalic. The first day of pregnancy was determined by the presence of sperm in the vaginal swabs. The animals weighed an average of 250 g at the beginning of pregnancy, ate about 15 g of food per day, containing 45 mg of elemental tellurium. It has been estimated that 63 to 84% of ingested elemental tellurium is excreted by rats[3] so that an average of 14 mg of tellurium was retained per day. A control group of 20 pregnant female rats of the same stock were fed Purina Lab Chow Meal. Fetuses were removed from the tellurium fed and normal gestating animals through an opening in the abdominal wall, on days 13 or 15 of pregnancy. The abdominal wall was then closed and the animals terminated their pregnancy and gave birth. Only the fetuses of tellurium-fed animals (henceforth referred to as 'tellurium' fetuses) who eventually gave birth to hydrocephalic animals, and fetuses of

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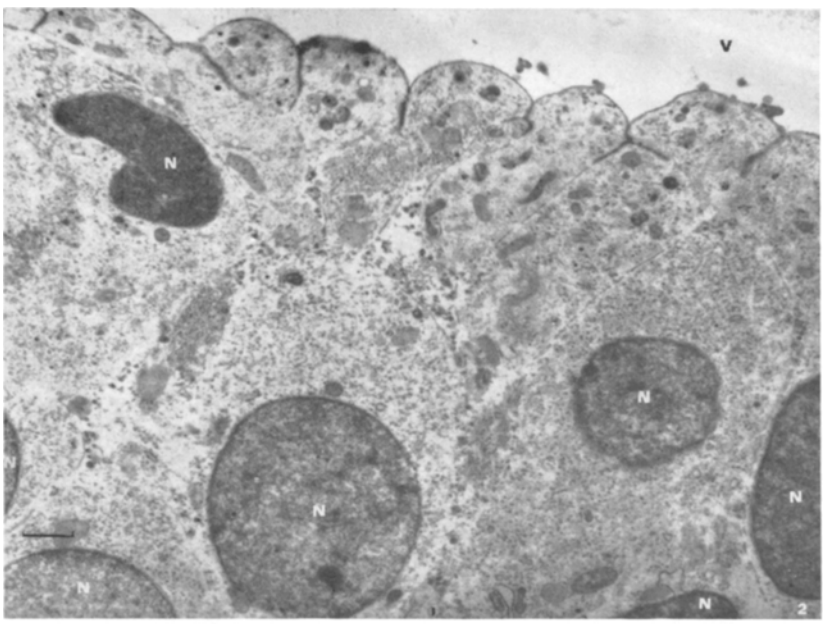
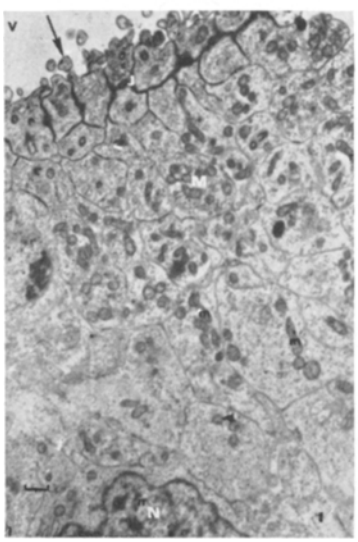


Fig. 2. Electron micrograph (× 7000) of the ependyma bordering the lateral ventricle of a 'tellurium' fetal rat, age 15 intrauterine days. Note the absence of microvilli and the smaller number of mitochondria (M) compared to that in the ependymal cells in Figure 1. The line = 1 μ.



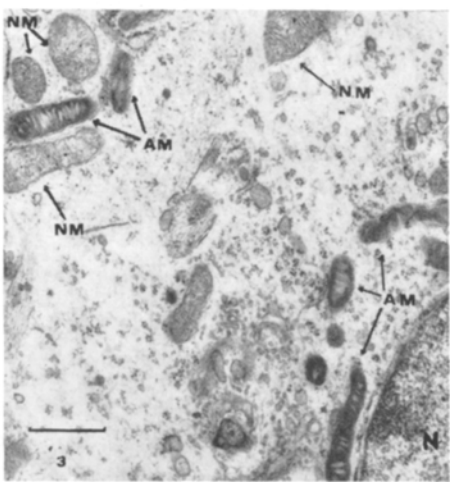


Fig. 1. Electron micrograph (× 3300) of the ventricular portion of the ependyma of a normal fetal rat, age 15 intrauterine days. Note the microvilli (arrow) projecting into the lateral ventricle, the mitochondria (M), the junctional complexes (J), and the nucleus (N). The line = 1 μ.

Fig. 3. (× 10,000) Normal (M) and abnormal (AM) mitochondria in the cytoplasm of an ependymal cell of a 'tellurium' fetal rat, age 15 intrauterine days. N, nucleus. The line = 1 μ.

similar age from the control rats, were examined and are reported in this study.

The size and appearance of the 'tellurium' and control fetuses were similar. No anomalies were noted in sections of the brains of the tellurium fetuses, stained with hematoxylin-eosin. The tissues destined for electron-microscopic studies, were fixed in 2% gluteraldehyde, buffered with 0.1 *M* sodium cacodylate, to pH 7.2. The tissues were fixed for 1 h, postfixed for 30 min in 1% osmic acid, buffered with 0.1 *M* sodium phosphate to pH 7.2, washed dehydrated, embedded in Epon Resin 812 (Fisher Scientific Co.).

There were morphological anomalies in the cells in the ependymal layer of the tellurium fetuses, 13 and 15 intrauterine days old. The ependymal layer of the normal fetal rat resembled that described in the human fetus[11], fetal rabbit[12] and chick[13]. In the normal fetal rat (Figure 1) microvilli were abundant on the ventricular surface of the ependymal cells. The mitochondria were grouped in the apical portion of the cytoplasm surrounded by small, presumably pinocytotic, vesicles. The nucleus was in the basal portion of the cytoplasm. In the ependymal cells of all 'tellurium' fetuses (Figure 2) the ventricular plasmalemma was without microvilli and the number of mitochondria was greatly diminished. The mitochondria were often abnormal, smaller and darker than normal and showed distortion of cristae (Figure 3). The cells in the rest of the telencephalon appeared to be normal.

There is evidence that tellurium crosses the placenta and reaches the cerebrospinal fluid (CSF) and the fetal brain after it is injected into the mother[2]. Since the telencephalic wall is so poorly vascularized during fetal life[14] and the choroid plexuses are well vascularized, it appears that the tellurium reaches the ependyma of the lateral ventricles by way of the CSF, where the lesions were found[15].

Résumé. L'ingestion de tellure, associé à la diète normale, par une rate en gestation, peut donner des ratons hydrocéphaliques. Des anomalies cellulaires s'observent dans le cerveau du foetus de 13 à 15 jours.

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The Pathogenic Role of the Inflammatory Reaction in Poliomyelitis. Immunofluorescence, Electronmicroscopic and Virological Studies with Type 3 Poliovirus

In most histological studies of poliomyelitis, the inflammatory reaction has been regarded as a defense reaction. New information about the relationship of the virus to the inflammatory elements was obtained with immunofluorescence techniques, which have demonstrated the presence of virus antigen within the mesenchymal cells of the central nervous tissues, including the inflammatory elements[1-4]. These findings raise the question whether or not some of the inflammatory cells play a pathogenic role in the course of the infectious process. Our study deals with this problem and a preliminary report is given here. 98 rhesus and cynomolgus monkeys were inoculated intraspinally (0.1 ml) or s.c. (0.5 ml) with undiluted virus suspension of virulent and attenuated type 3 poliovirus of titre from 5.6 to 6.8 $\log_{10}$ TCD_{50}/0.1 ml. 2 control animals were given 0.1 ml of bovine-globulin conjugated with fluorescein isothiocyanate (FITC). The animals were sacrified at time intervals between 12 h and 21 days after inoculation. Light, fluorescence and electron microscopic studies, and virus assays, were carried out on the central nervous system of the inoculated animals.

The virulent strain produced the expected histopathological picture of severe, rapidly progressive neuronal damage with extensive inflammatory reaction. After intraspinal inoculation this inflammatory response comprised the early appearance of polymorphonuclear leukocytes and macrophages (within 12 h) and represented the 'secondary' non-specific reaction to neuronal destruction. Lymphoid cells and macrophages predominated later (between 48 and 72 h); in our opinion, this is the 'primary' specific reaction and represents the local immune response to viral antigen. A similar sequence of changes was observed 4 days after s.c. injection.

With the attenuated virus, lesions in the central nervous system only occurred after intraspinal inoculation. They were of the 'primary' specific character and were seen between 48 and 72 h. The majority of nerve cells were intact, the remainder exhibited degenerative changes, mostly of a reversible nature[5-8]. No changes were seen in the brain or cord after introduction of attenuated poliovirus by the subcutaneous route. In the animals inoculated with the virulent strain, 30–60% of the motor neurons showed some degree of fluorescence. This could be seen already after 12 h. With the attenuated strain of poliovirus, an exceptional neuron could be observed to fluoresce after an interval ranging from 48 to 72 h. However, fluorescence was seen after inoculation with either strain of virus in the non-neuronal elements, i.e. in the inflammatory and glial cells as well as in the vascular walls. After intraspinal inoculation, this finding was observed soonest, i.e. after 12–24 h, in the inflammatory cells of

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