

Technical methods

tional force of 2100 g. The plastic tubes were lifted out of the glass tubes, and grids were picked out with fine forceps after tilting the tubes on the side. The grids were dried on filter paper for a few minutes and then floated on a drop of phosphotungstate (PTA) pH 6.6 for half to one minute. Excess stain was blotted off with a filter paper. All grids were left under ultraviolet light for at least 15 minutes to inactivate viruses before examination in the AEI electron microscope at a standard magnification of $\times 25\,000$.

Results

Of the 29 samples examined, 27 were positive for virus particles by electron microscopy (EM): herpes simplex was found in 9, chicken pox/zoster in 7, vaccinia in 6, orf in 4, and molluscum contagiosum in 1. Both negative EM samples were negative by culture and serology for virus infection. One of these was subsequently diagnosed as Sweets' syndrome and the other as an allergic rash. In the majority of cases examination of the grids from both tubes revealed many virus particles. In some instances where there was a lot of cell debris in the vesicle fluid some grid holes in the first grid were thick and black, but many other squares could be examined as virus particles were found in almost all holes. Many of these particles were found individually, but in some cases they were also seen in small clumps of 2-6 particles.

Comments

A frequent difficulty when examining specimens from skin lesions is the paucity of material. Very

often it is not possible to extract more than a few microlitres (2-10 μ l) of vesicle fluid. In these circumstances the specimen may easily be lost in transport on the side of the container. Since it is necessary to have at least one drop of fluid, 20-25 μ l, to process a grid conventionally, eluting the fluid by adding a drop of distilled water to the specimen may impose an unacceptable dilution factor. Further, even in an adequate specimen processed by dipping, the grid usually results in a very uneven distribution, that is, more particles are seen in certain areas than in others. Both these difficulties are overcome by the present centrifugation method as in scanty specimens all the vesicle fluid can be transferred into the water in the tube overlying the grid by rinsing. On centrifugation many of the particles were uniformly distributed on the grid. It took only a few minutes to find virus particles by EM and their absence from the first few grid squares examined implied a negative sample. This contrasts with a conventionally prepared grid where it is frequently found that particles are absent from the first five or six grid squares but subsequently appear to be plentiful in other areas examined.

Reference

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Letters to the Editor

Fetal adrenal cytomegaly

We report a case of fetal adrenal cytomegaly associated with maternal Metrolen ingestion.

Case history

A 30-year-old woman, who had two normal, healthy children, was first seen in outpatients when she was 16 weeks' pregnant by size. Because of prolonged vaginal bleeding starting at four weeks in an undiagnosed pregnancy she had been given a two-month course of

Metrolen from six to 14 weeks. At the end of this time she remained amenorrhoeic, and pregnancy had become obvious. In view of the possibility of the hormone administration causing fetal abnormality, such as a major cardiac anomaly or masculinisation of a female, a prostaglandin termination was performed at 17 weeks.

Macroscopic findings

An autolysed male fetus weighed 115 g, with a crown-rump length of 13.5 cm, a crown-heel length of 19.5 cm, and a head circumference of 15 cm. The combined kidney weight was 0.85 g. These

measurements are consistent with a 15-week gestational age. Both adrenal glands were enlarged but of a normal morphology. They had a combined weight of 0.88 g, which is over the second standard deviation above the normal for this body weight (Tanimura *et al.*, 1971). There were no other unusual features.

Microscopic findings

Cytomegalic cells were present in the inner third of the cortex of one adrenal gland. These cells were large with large nuclei, which showed a prominent chromatin network (Figure). There were no other unusual features.

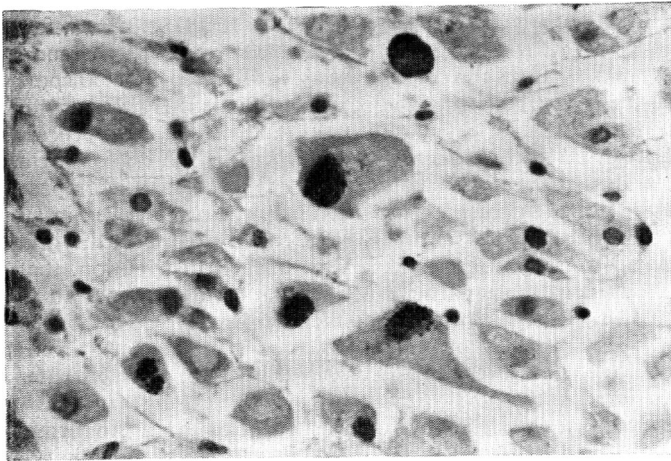


Figure Inner one-third of adrenal cortex showing marked cytomegaly (Haematoxylin and eosin $\times 600$).

Comment

Adrenal cytomegaly has been described as a feature of Beckwith's syndrome as well as in isolation, generally as a totally unexpected finding, unrelated to race, maturity, or gross abnormality but possibly related to erythroblastosis fetalis or prenatal viral infection (Birdwell and Dimmette, 1967; Morison, 1970; Aterman *et al.*, 1972; Potter and Craig, 1975). A theory that adrenal cytomegaly may be an indication of polyploidy occurring when the adrenal cortical cells are intensely stimulated was proposed by Aterman *et al.* in 1972, who thought that that stimulus could be chronic stress. However, using their criteria, the majority of cases coming to necropsy must have been exposed to chronic stress, and the incidence of cytomegaly should be higher.

In this case, as well as a possible stressful episode of vaginal bleeding there was exposure to exogenous steroids (that is, Metrulen composed of 2 mg ethinodiolacetate and 0.1 mg Mestranol). The unsubstantiated suggestion by Craig and Landing (1951) that adrenal cytomegaly may be pretumorous becomes worrying in the light of the anxiety expressed by Gal (1976) over the use of oestrogens in early pregnancy and the evidence of Herbst and colleagues (1975) on the administration of diethylstilboestrol in pregnancy. Our patient's ingestion of 4.8 mg oestrogens and 96 mg progestogens overall may have been contributory to the fetal adrenal cytomegaly. Possibly hormone therapy is the historical aspect of Aterman's cases that has not been

noted, yet it may be the stimulus causing adrenal polyploidy. Regardless of that, this case should be used to highlight the possible hazards to the fetus of maternal hormone therapy in early pregnancy.

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Rapid diagnosis of primary meningoencephalitis due to Naegleria: Detection of organisms with bacterial stains

Amoebic meningoencephalitis due to *Naegleria* is a relatively rare, extremely serious, fulminating infection usually following exposure to contaminated bodies of fresh water, especially warm water (Lancet, 1977). The probability of successful treatment is minimal unless early diagnosis is effected (Duma *et al.*, 1971).

We recently encountered a case of amoebic meningoencephalitis detected by fortuitous observation of amoebic trophozoites while performing a spinal fluid cell count. After prompt initiation of therapy the affected 9-year-old child recovered completely.

While the organisms could be seen in wet mounts (Fig. 1), survived in refrigerat-

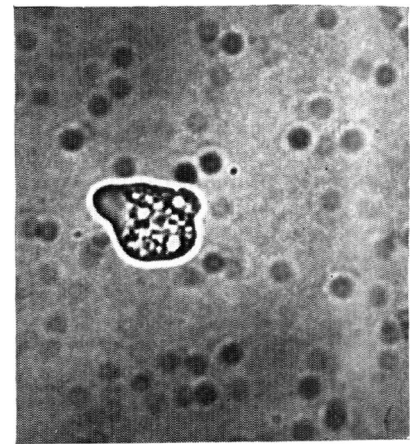


Fig. 1 Unstained wet mount of *Naegleria* trophozoite. ($\times 505$).

ed spinal fluid for several days, and were successfully cultured (Culbertson, 1974), we could also visualise the amoebic trophozoites microscopically in routine smears of spinal fluid concentrate stained to demonstrate bacteria by Wayson's and Gram's stains (Paik and Suggs, 1974). The organisms appeared in those preparations as irregular, ovoid structures with vacuolated cytoplasm and non-stainable nuclei, purple-pink with the superior Wayson's stain (Figs 2 and 3) and pink with Gram's stain. We did not observe similar structures in identically stained smears from 10 cases of bacterial meningitis. In particular, white blood