Electron-microscopic study of measles virus in lymphocytes of affected children

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SUMMARY

Two different types of inclusion bodies have been found by electron-microscopy in the cytoplasm of sectioned lymphocytes from children infected with measles.

The first is tubular, 18 nm. internal diameter, embedded in osmiophilic material, and is found during the incubation period. It is not thought to be specific for measles, and is probably identical with structures recently reported in HEp-2 cell lines and tumour tissue.

The second type is less obviously tubular and morphologically resembles measles nucleocapsid. This was found only after the rash had appeared.

INTRODUCTION

Although measles virus can be recovered with greater ease from the white cell *fraction than from other elements of blood (Todd, 1928; Daubney, 1928; Smith,* 1929; Douglas & Smith, 1930; Fenner & Woodroofe, 1953; Gresser & Chany, 1963) there appear to have been few electron-microscope studies of measles lymphocytes themselves. In the present work these cells have been examined early in the disease and the morphology of intracytoplasmic inclusions described.

MATERIALS AND METHODS

Blood from ten children was examined: 2 were incubating the disease (7 days before the rash appeared), 2 were in the first day, 4 were 2 days after the appearance of the rash, and 1 each at 14 and 17 days. For comparison, blood from ten normal children who had had measles within the last 2–5 years was also studied.

Lymphocytes were separated from about 5 ml of venous blood by the carbonyl iron and methyl cellulose method (Coulson & Chalmers, 1967; modified by Hughes & Caspary, 1970) and the cells in 199 solution were fixed for 1 hr. by addition of the same volume of double-strength (i.e. 8 %) chilled buffered glutaraldehyde in 0·4 M sodium cacodylate solution at pH 7·2. The cells thus fixed in suspension were lightly centrifuged (250 g for 15 min.), the pellet post-fixed in 2 % osmic acid and embedded in Epon. Sections were cut on an LKB 'Ultrotome', stained with lead citrate and uranyl acetate and examined in a Philips 200 or 300 electron microscope.

In one experiment lymphocytes which had been rapidly frozen in liquid nitrogen were thawed, and negative contrast staining carried out with 2 % sodium phospho-

tungstate at pH 7·0. In another, fresh unfixed lymphocytes were disintegrated in a Griffiths tube and examined immediately by techniques as described by Narang, Bell & Gibson (1971).

RESULTS

In the incubation stage specimens many of the lymphocytes showed pseudo-podial outgrowths and the cytoplasmic volume was increased and there were many ribosomes free from endoplasmic reticulum. Here and there were regularly arranged tubular elements sometimes cut in cross-section (Pl. 1, figs. 1, 2) and sometimes longitudinally (Pl. 2, figs. 3, 4). They were deeply stained and apparently embedded in osmiophilic material so that at first sight under low power they resembled ordinary dense bodies. The clear internal diameter of the individual tubular structures was 18 nm and their outer surface was often rather blurred where they were embedded in dense background material. Despite the superficial resemblance to dense bodies no relation either to these or to mitochondria was apparent.

On the second day of rash a second type of cytoplasmic inclusion characteristic of measles nucleocapsids was found in addition to those described above. Lightly staining rounded islands of cytoplasm, usually close to the nucleus, were seen. They were devoid of bounding membrane (Pl. 3, fig. 5). Sometimes more than one inclusion body was present in a single lymphocyte (Pl. 3, fig. 6, arrows). These islands comprised a network of tubular structures often with rather ill-defined boundaries, though here and there was evidence of a fine cross-striation (Pl. 4, figs. 7, 8). Sometimes most of the structures constituting an inclusion were cut transversely (Fig. 7) and their tubular nature was then clear. The diameter of the internal translucent area was 16 nm. The mass of tubules appeared embedded in an electron-dense 'fuzzy' matrix. Sometimes the inclusions were related to endoplasmic reticulum (Fig. 8) but no structural connexion was apparent.

Despite the large number of villous protrusions, no budding of virus particles from affected lymphocytes was seen. As a rule lymphocytes with inclusion bodies showed evidence of stimulation (e.g. increased endoplasmic reticulum and mitochondria) and often the large numbers of pseudopodial outgrowths associated with beginning degeneration of the cell.

No inclusions of the measles type shown in Figs. 5–8 were found in convalescent or uninfected subjects. However, the dark inclusion bodies (Figs. 1–4) seen in the incubation phase (continuing on into the early clinical stage) have also been seen in lymphocytes from four patients with leukaemia and one with carcinoma of ovary (Pl. 5, fig. 9) and cannot be regarded as specific for measles.

Negative contrast staining of frozen and thawed lymphocytes from a child on the second day of rash revealed nucleocapsids resembling those of measles virus (Pl. 5, fig. 10) though clear herring-bone formations were not discernible, but this may be because frozen-thawed material was used. The width of the structure was about 15 nm.

DISCUSSION

Although there is so much evidence that measles virus may grow in leucocytes, especially lymphocytes, virus has not been seen within these cells during clinical infection.

The patients from whom the lymphocytes were studied developed well-marked measles, with rash at the time of study or (when this was in the incubation stage) subsequently. Collateral study *in vitro* of the lymphocyte sensitization to measles antigens on the same patients showed this to be present in all cases (Field, Caspary, Shenton & Madgwick, 1973). Thus in one case macrophage electrophoretic slowing (Field & Caspary, 1971) rose from 2·4 % and 4·1 % for encephalitogenic factor and measles respectively, 7 days before onset of the rash, to 9·0 % and 12·2 % on the second day of the rash. This cellular sensitization preceded the appearance of humoral antibody demonstrated by complement fixation and haemagglutination inhibition tests on the same children (B. McLaughlin, H. Madgwick & E. J. Field, unpublished). In some cases blood was taken during the incubation stage and retaken during the rash or 17, 18 and 47 days after measles rash. Complement fixation went up from a titre of 1/2 on the 2nd day of rash to 1/512 on the 18th day after rash. On the other hand, haemagglutination inhibition was 1/32 on the 2nd day and went up to a titre of 1/256 on the 18th day in the same patient, thus showing the appearance of measles antibody. From these findings together with the absence of the structures seen in Figs. 5–8 in lymphocytes from other conditions it is probable that the structures described are limited to natural measles infection. This is further supported by the morphological resemblance of the inclusions to known measles nucleoprotein in the brain of mice infected by intracerebral inoculation with the Edmonston strain of measles virus (Figs. 11, 12) (E. J. Field, H. K. Narang and T. M. Bell, unpublished) and *in vitro* (Bell *et al.* 1972). Most lymphocytes which contained virus also showed evidence of stimulation and many were furnished with numerous villous projections – a feature commonly seen in degenerating cells. Dunn & Kernahan (1957) have postulated that the lymphopenia present in the early stages of certain virus infections may be due to destruction of affected cells and perhaps also their bone-marrow precursors.

The depression of delayed hypersensitivity reactions immediately after measles first described by von Pirquet in 1908 has since been several times confirmed. It may be that it is associated with viral colonization of lymphocytes.

Clarke, Attridge & Gay (1969) described tubular structures similar in morphology to the 1st type (Figs. 1–4) in HEp-2 cells infected with type 1 foamy agent. More recently, Berthiaume & Joncas (1973) in a study of the morphogenesis of respiratory syncytical virus have observed similar tubular structures, both in infected and control HEp-2 cells. The specificity of these structures is uncertain and their presence in lymphocytes from cancer and measles patients and HEp-2 cell line may suggest that they are occasional normal structures of unknown function, or abnormal structures resulting from disease or some other agent.

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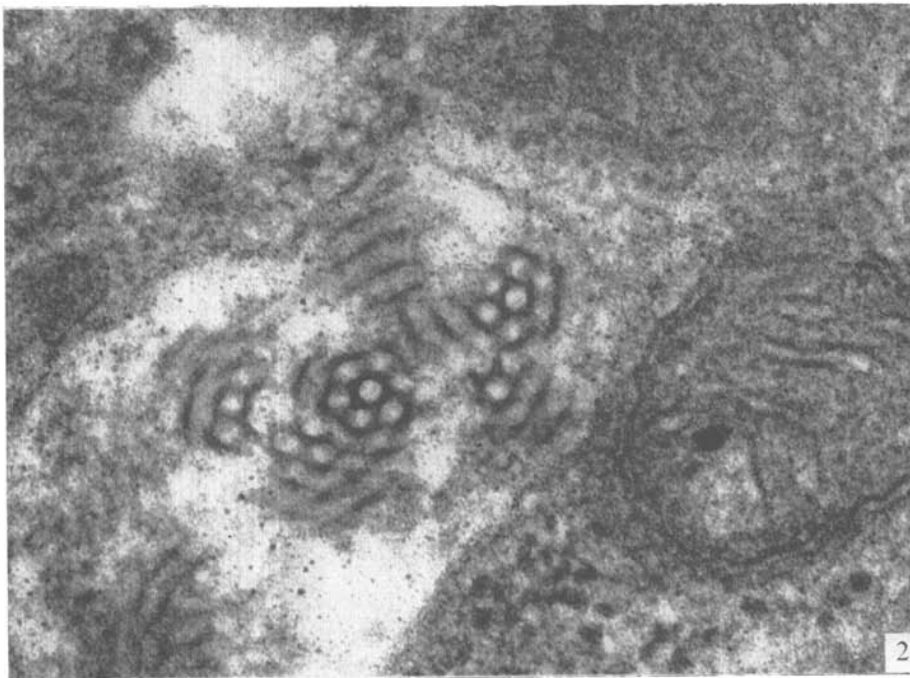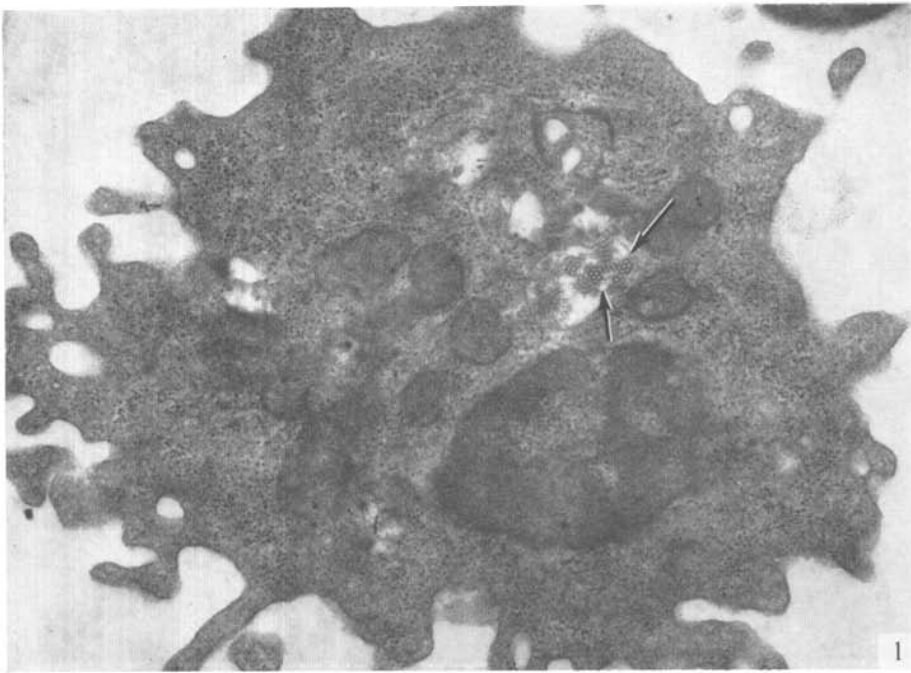## EXPLANATION OF PLATES

PLATE 1

Fig. 1. Lymphocytes from child on second day of rash. Note multiple pseudopodial villi and the density of ribosomes within the cytoplasm. The number of mitochondria is increased. The arrows show an accumulation of tubular structures mostly circular but some in longitudinal section (detail in Fig. 2). × 25,000.

Fig. 2. High-power view of inclusions in Fig. 1. Note groups of circular tubular structures of internal diameter 18 nm. × 154,000.
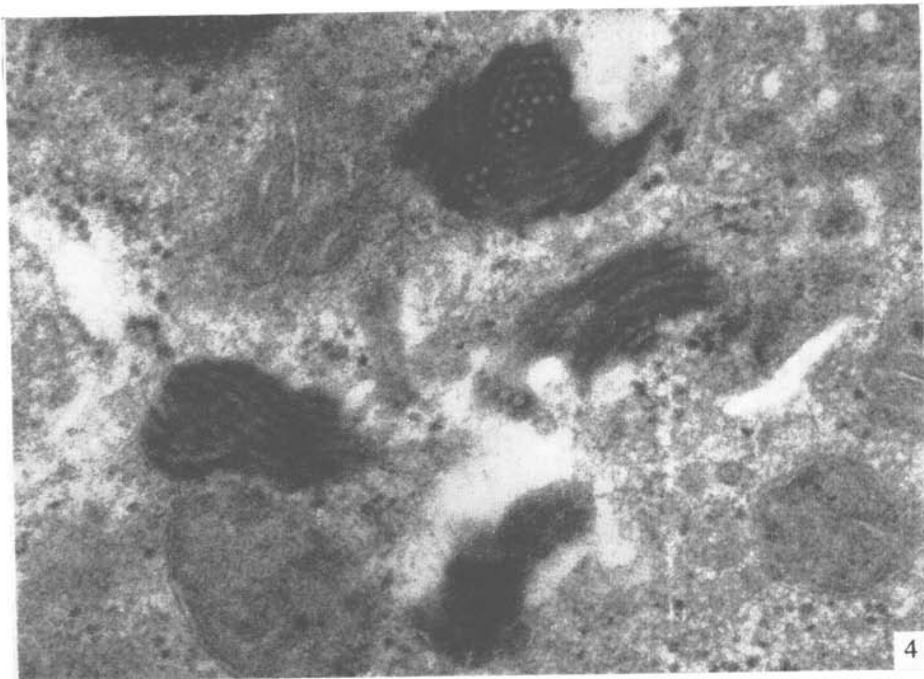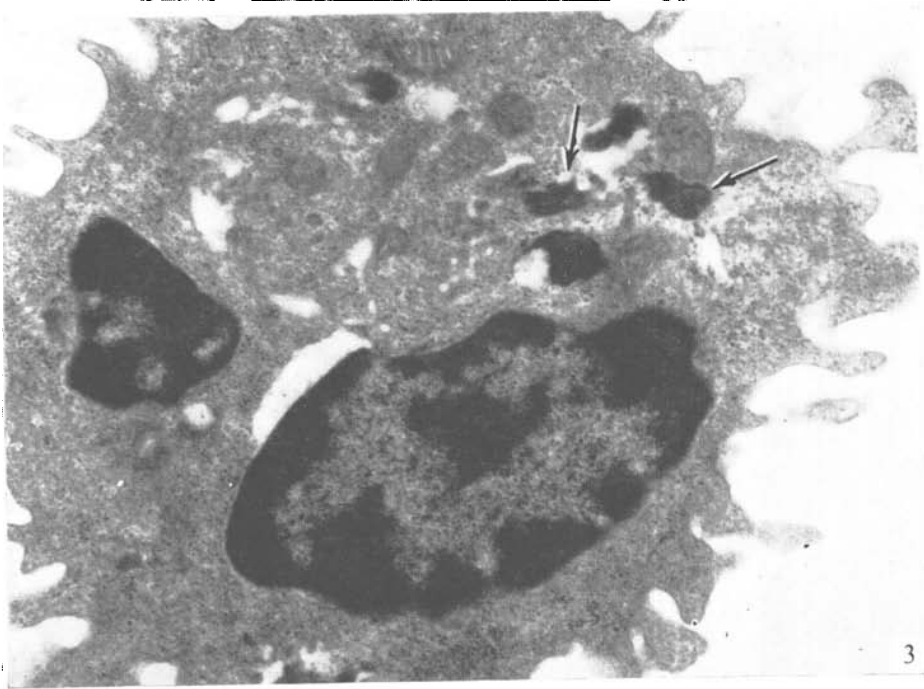
PLATE 2

Fig. 3. Lymphocytes from child about 4 days before appearance of rash. Note more densely staining inclusions which may be taken at first sight for dense bodies (arrows) (detail in Fig. 4). × 20,000.
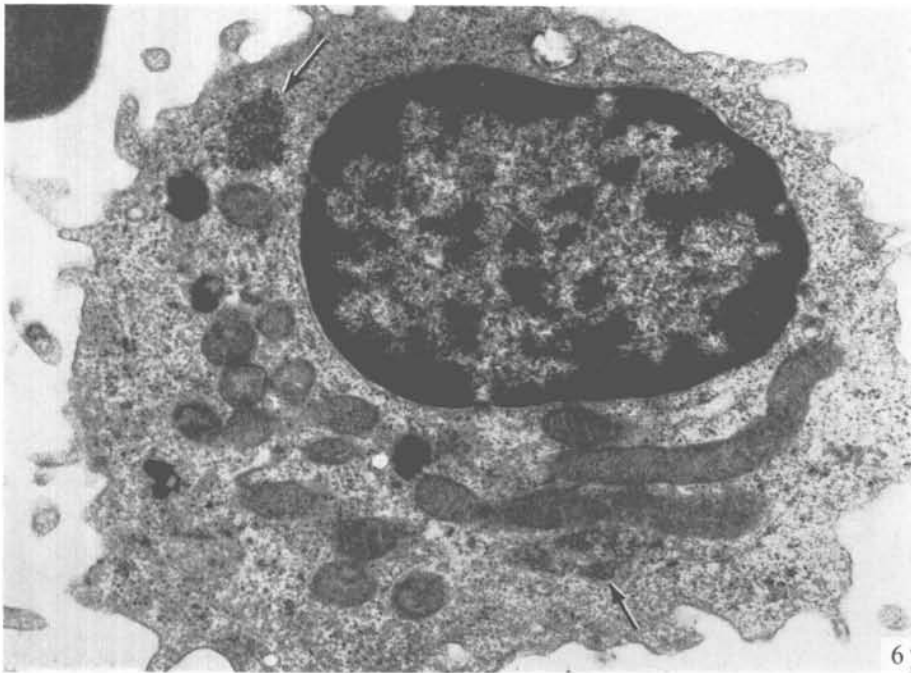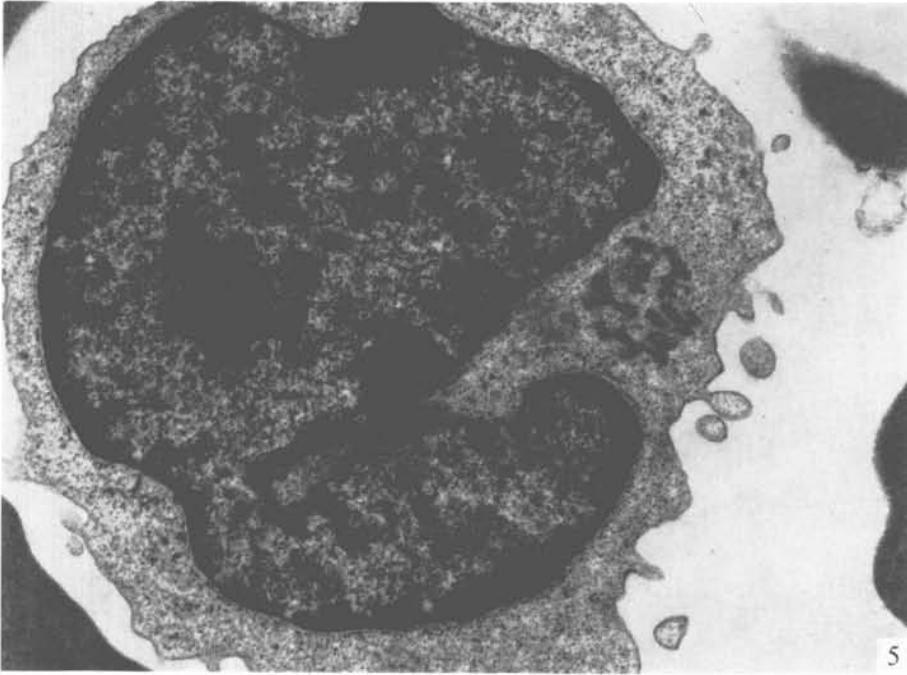
Fig. 4. High-power view of inclusions in Fig. 3. × 82,000.

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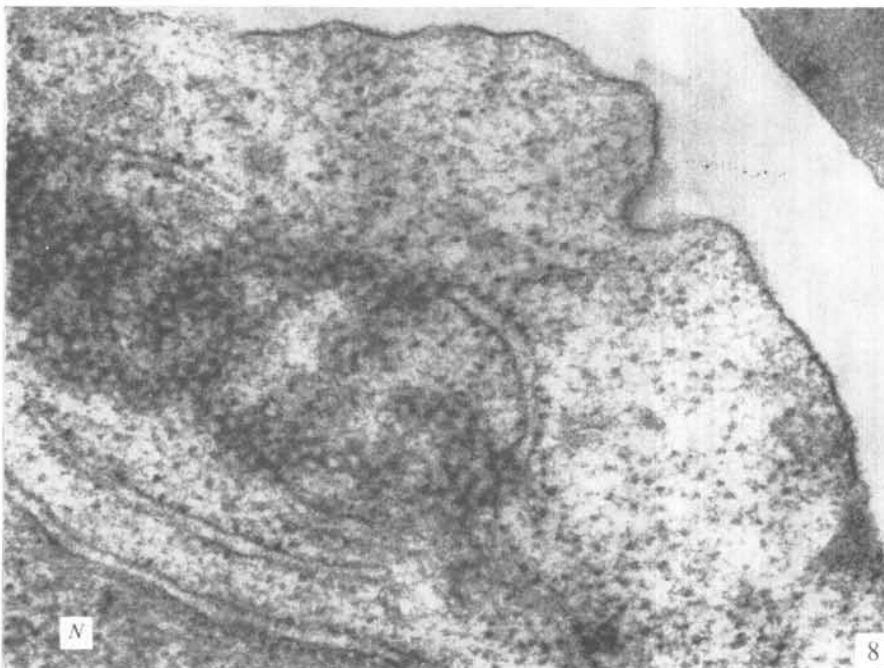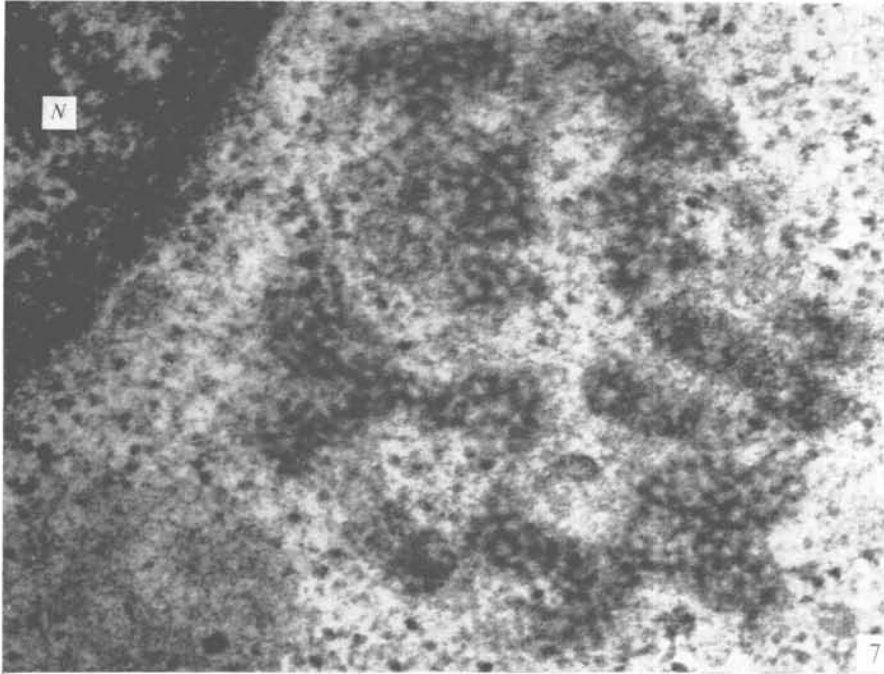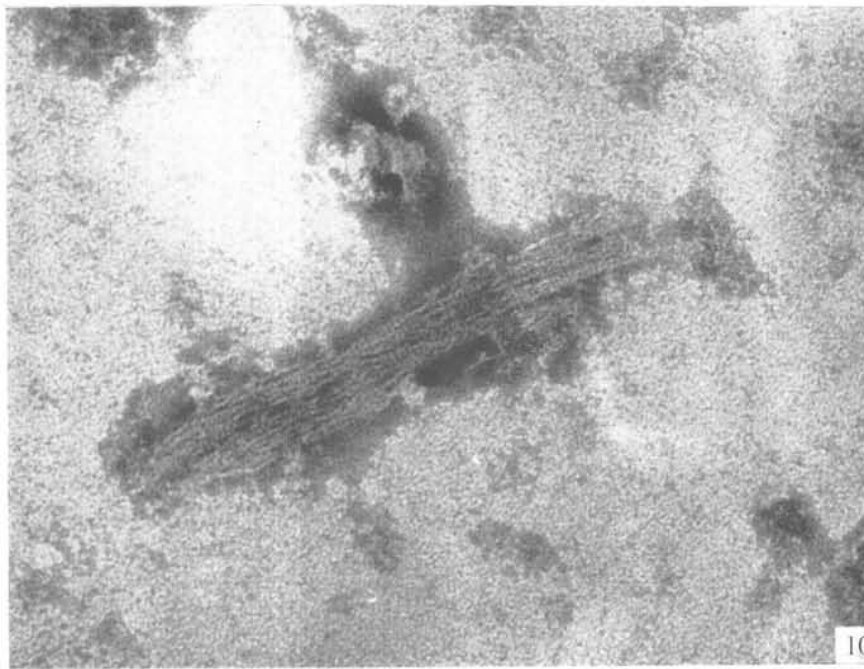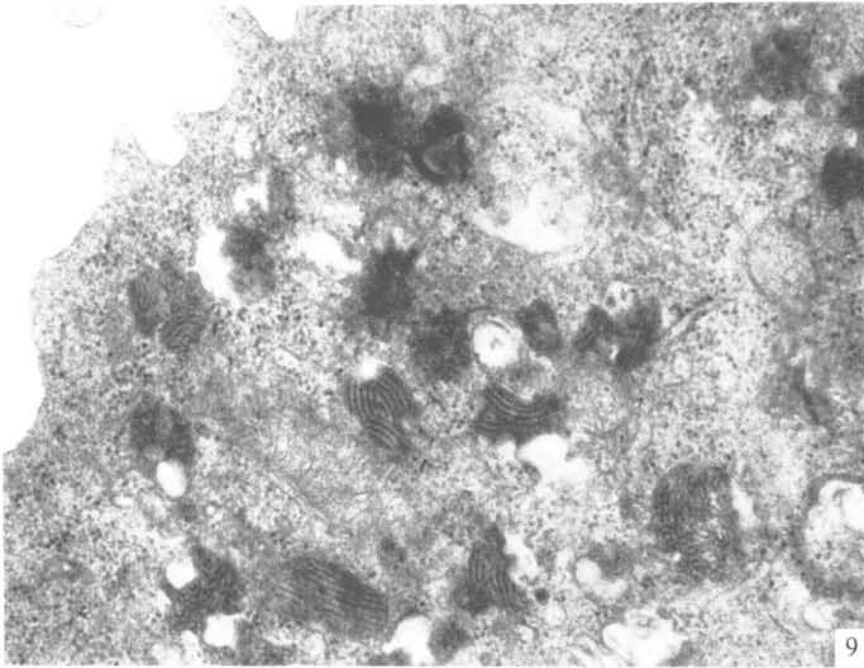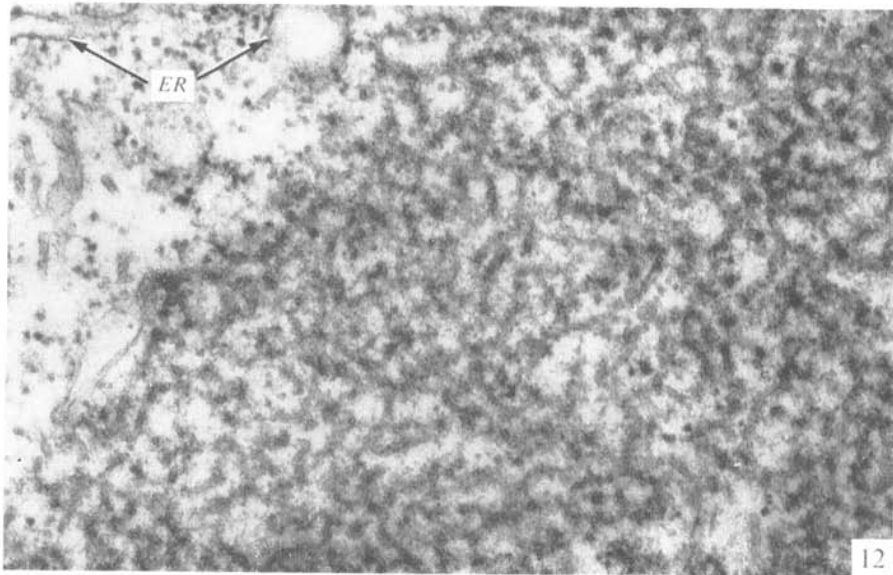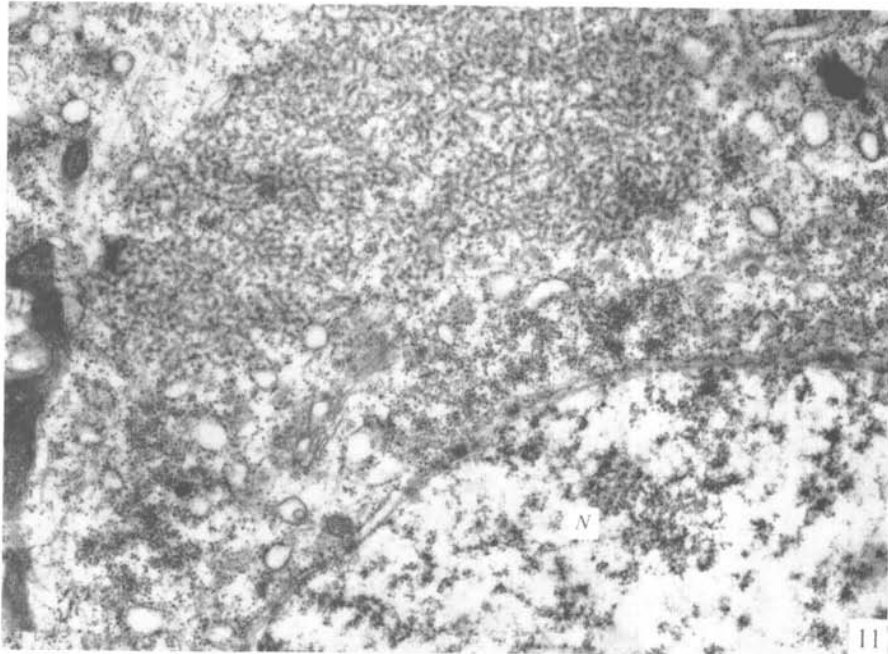
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PLATE 3

Fig. 5. Lymphocyte from child on second day of rash. Note inclusion body made up of islands of circular tubular structures. × 15,000.

Fig. 6. Lymphocyte from another child on second day of rash. Note two similar inclusions (arrows). × 19,000.

PLATE 4

Fig. 7. High-power view of inclusion shown in Fig. 5. Note circular profiles about 16 nm. diameter embedded in electron dense 'fuzzy' material. N = nucleus. × 82,000.

Fig. 8. Lymphocyte from a different child on second day of rash showing similar rather diffuse inclusion in proximity to endoplasmic reticulum though no functional association is suggested. N = nucleus. × 67,000.

PLATE 5

Fig. 9. Lymphocyte from patient with cancer of ovary. Note same type of cytoplasmic inclusions as shown in Figs. 1–4. × 37,000.

Fig. 10. Negative contrast preparation of disintegrated lymphocyte from another child on second day of rash. Cells had been frozen before preparation was made in P.T.A. Note structures resembling nucleocapsids of measles. × 154,000.

PLATE 6

Fig. 11. Electron micrograph through Ammon's horn of a baby mouse inoculated with Edmonston strain of measles virus. Note nucleoprotein tubules within the neuron and accumulations of ribosomes. N = nucleus. × 22,000.

Fig. 12. High magnification of Fig. 11. Note typical nucleoprotein tubules. ER = endoplasmic reticulum. × 52,000.