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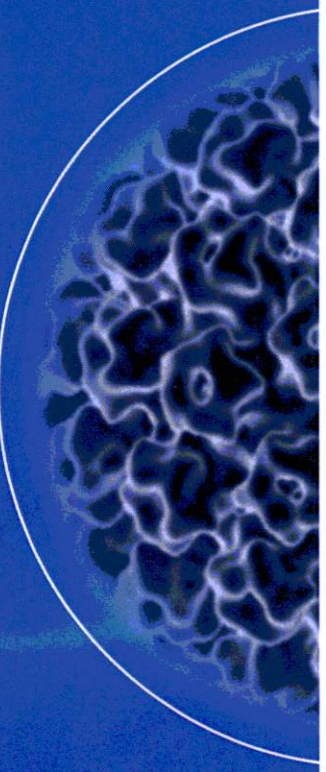

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FOCUS ON

Morphogenesis of Yellow Fever Virus: an ultrastructural study

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RIASSUNTO

La morfogenesi del virus della febbre gialla è stata studiata mediante infezione di colture di cellule Vero. La penetrazione e la perdita dell'envelope del virus della febbre gialla si verificano per endocitosi con la con la formazione di vescicole, simili a quelle descritte per gli altri virus provvisti o non provvisti di envelope. I corpi inclusi associati con nucleocapsidi di recente formazione durante il ciclo replicativo si rinvengono solitamente presso la regione perinucleare. Una eccessiva proliferazione di membrane che coinvolge vacuoli e reticolo endoplasmatico è caratteristica degli stadi tardivi dell'infezione. Non sono stati osservate alterazioni morfologiche del nucleo o dei mitocondri. Il rilascio dei virus si verifica per movimento dei virioni neoformati attraverso il reticolo endoplasmico, concludendosi con la fusione delle vescicole contenenti i virus con il plasmalemma.

The members of the Flaviviridae family, are small enveloped viruses and include the genera Flavivirus Hepacivirus, and Pestivirus.

The genus Pestivirus includes the bovine viral diarrhea virus (BVDV) and the classical suine fever virus (CSFV), two animal viruses responsible for economic losses in the livestock industry.

Hepatitis C virus (HCV) is the best studied member of the genus Hepacivirus, as HCV infection is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma in humans affecting 170 million people worldwide.

The genus flavivirus comprises more than 70 viruses, many of which are arthropodborne human viruses causing a range of important diseases including fevers, encephalitis, and hemorrhagic fever. Flaviviruses including dengue virus (DENV), Yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus (TBEV).

Yellow fever in man varies from an inapparent infection to a fulminating disease which is invariably fatal. Despite the effectiveness of mosquito eradication in reducing the incidence of infection and the availability of an effective vaccine epidemics continue to occur, particcularly in Africa and there is some evidence of foci of infection mantaining reservoir between epidemics. Yellow fever is is an infection principally of primates and is transmitted from man to man by Aedes mosquitoes. Clinically, infection involves the spleen, kidneys and heart as well as the liver. Infected hepatocytes show a number of characteristic changes, the most prominent being the formation of Counicilman bodies resulting from acidophilic degeneration. The appearence of these inclusion bodies correlates well with the virus replication and precedes biological and histological signs of liver cell damage.

Yellow fever virus is enveloped, of average diameter 38 nm, and is indistinguishable from other flaviviruses on morphological criteria alone. The YFV replication has been associated with a proliferation of cytoplasmatic membranes with the accumulation of nascent virus particles within the cisternae of RER or inside large vacuoles.

In the present study, the morphogenesis of a vaccine strain (17D) of a YFV has been examined in acutely infected cell culture. Thin sections of infected Vero cell cultures were examined within the first 30 min of infection in order to elucidate

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events. An initial thickening of the plasmalemma at the site of virus adsorption was followed by the appearence of pedicular attachment between the virus and the membrane. Contact between the virus and the cell membrane appeared to trigger the uptake of the virus exclusively by a process of endocytosis. The formation of a membrane invagination with the appearance of a coated pit was seen to precede the encirclement of the virus particles. Virus particles were carried further into cytoplasm within coated vesicles after the thickening of the plasma membrane in the area of virus-containing vesicles. Enveloped viruses are thought to release their nucleocapsid into the cytosol by fusion of the envelope with the inner lysomal membrane. The finding of vesicles containing YFV in the process of disintegration suggests the release of infectious RNA occurs within the endosomes.

After 24 of infection mature virus particles were seen, both within cytoplasmic vacuoles and as extracellular virus aggregates. Infectious virus was released continously until cytolysis. Vacuolization was the most prominent feature on the first day post infection. The second and the third day post infection were characterized by the appearance of virus particles, mostly inside cytoplasmic vacuoles and the RER. Vacuoles of various size were distributed through the cytoplasm, particularly around the nucleus. These virus-containing vacuoles tend to fuse with larger vacuoles prior to transport of the virions into the extracellular environment. During these phases of virus growth, the Golgi complex became sparse and diffucult to detect compared to unifected control cells. In contrast the mitochondria and the nucleus remain unchanged.

Single virus particles could be seen in the process of transport and release either through a vacuole or the RER. Extracellular, isolated particles appeared to form large aggregates. Frequently, virions were also seen within dilated regions of the perinuclear space, but virus particles were rarely organized into symmetrical cristalline arrays. Membraneous structures formed into an amorphous mesh of converging RER membranes within which necleocapsid-like structures and virions frequently protruded into cytoplasmic cisternae. The area for nucleocapsid assembly could not be clearly established. Although virus particles were present in most areas in the infected cell, a concentration in and around the membraneous amorphous material which contained nucleocapsid-like structures. In addition these inclusion-like areas were usually located in close proximity to the cell nucleus, an area which rapidly degenerated as the infection progressed.

Nucleocapsids were not found associated with the plasmalemma and complete virions were not in direct contact with the cytosol. It appeared that both the richly developed vacuolated area and the canalicules formed by the RER contributed to centrifugal movement of the virus from the centre of cell to the external medium. The cell at the fourth and fifth day post infection showed a similar profile of cytological changes, although by this stage of infection cells had progressively degenerated or lysed.

Few virus particles were seen in the process of acquiring an outer envelope. It appeared that during this process the nucleocapsid became flattened as protrusion began through the membrane, with the virus outer envelope encircling the nucleocapsid. Appendages and links between the envelope and the inner side of the organelle membrane were frequently present.

Internalization of YFV follows a mechanism similar to that reported for other enveloped and unenveloped viruses, with virus particles entering predominantly by endocitosis. Enveloped viruses are thought to release their nucleocapsid into the cytosol by fusion of the envelope with the inner lysosomal membrane. The finding of vesicles containing YFV in the process of disintegration suggests the release of the infectious RNA occurs within the endosome.

Extensive proliferation of membraneous organelles appears to be a unique feature of flavi-virus infected cells, with large number of vesicles already apparent by the end of the eclipse period. This excessive production of membranes may be either induced or controlled by the virus, or both. The proliferation of membrane-bound organelles, in particular RER and vacuoles, would favour both virus protein production and glycosilation, together with an enhancement of virus release involving the exocitic pathway.

The inclusion bodies appeared to be closely associated with nucleocapsids. The major nucleocapsid protein aggregates are detected around newly synthesized RNA. This is an area rich in membranes

and resembles the ultrastructural changes observed previously for YFV and other flavivirus. Such inclusion consist of protein material only. Release of newly formed YFV is achieved by the peripheral movement of virions through the extensively proliferated RER followed by the exocitic fusion fusion of small and larger vacuoles containing virions with the plasmalemma. The Golgi apparatus is generally involved in the cellular exocitic pathway, although the direct involvement appeared not to be the case with YFV. It has been shown that flavivirus glycoproteins also contain complex oligosaccharide side-chains, suggesting that the production and initial glycosilation of the envelope glycoproteins in the RER is followed by subsequent to the Golgi apparatus for further processing, and then insertion into the membrane of Golgi apparatus derived vesicles. These vesicles would acquire the completed glycoprotein either by fusing with vacuoles or with the RER resulting in the encirclement of the nucleocapsid with the envelope glycoprotein The extensive vacuolization within the infected cells could thus be explained by the intracellular transport between two organelles.

SUMMARY

The morphogenesis of yellow fever virus (YFV) replication was examined in infected Vero cell cultures. Penetration abd uncoating occured by endocytosis with the formation of coated vesicles, similar to that demonstrated for other enveloped and unenveloped viruses. Inclusion bodies associated with newly formed nucleocapsids were evident in the cytoplasm during the growth cycle. An excessive proliferation of membrane bound organelles inv olving both vacuoles and endoplasmic reticula was the most striking feature of virus infected cells late in infection. No morphological changes in the appearence of noclei or mitochondria were detected. Virus release appeared to occur by movement of nascent virions through the prolifetated endoplasmic reticula followed by exocytic fusion of virus-containig vesicles with the plasmalemma.

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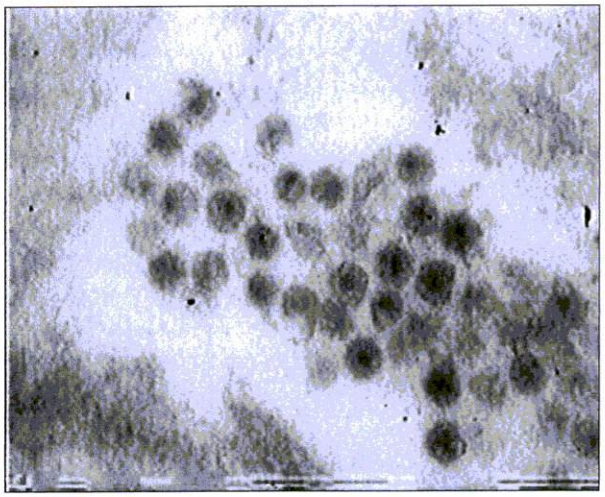

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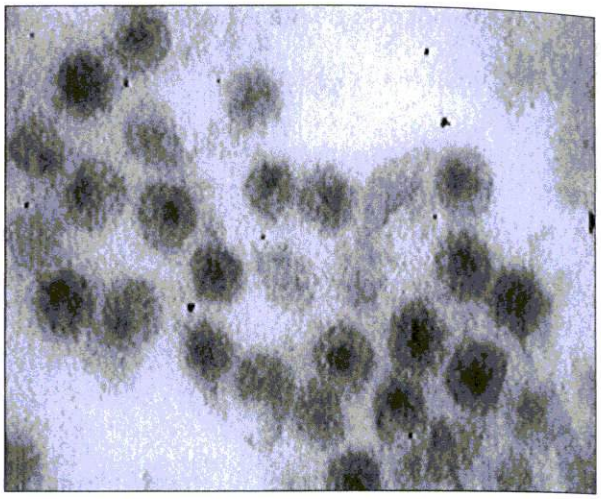

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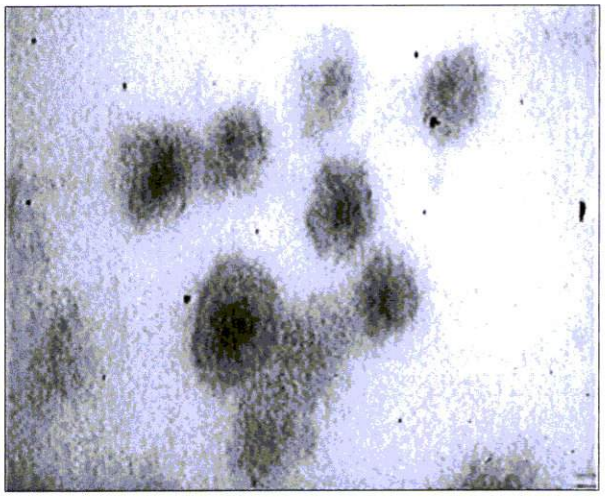

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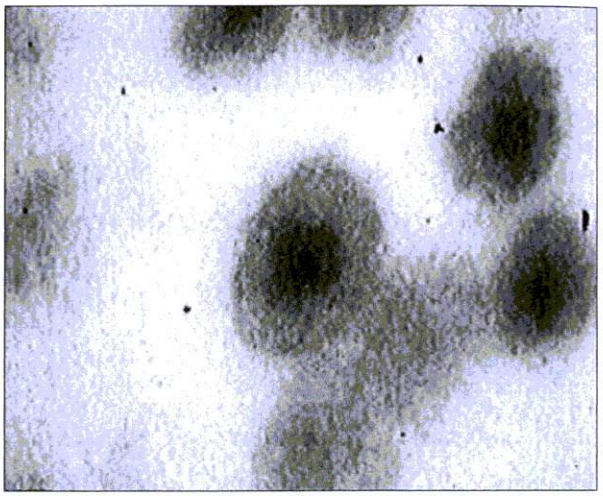

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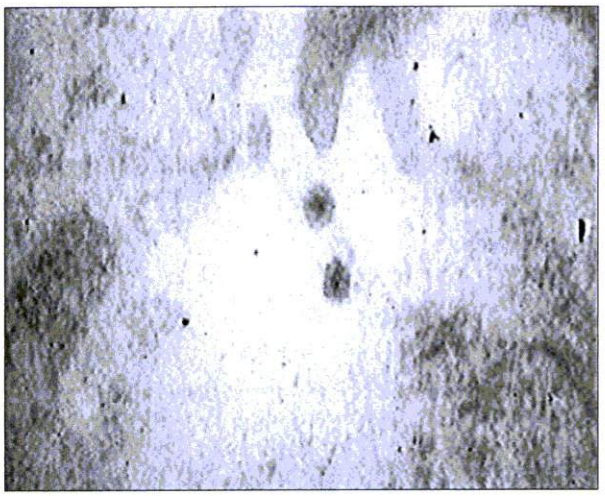

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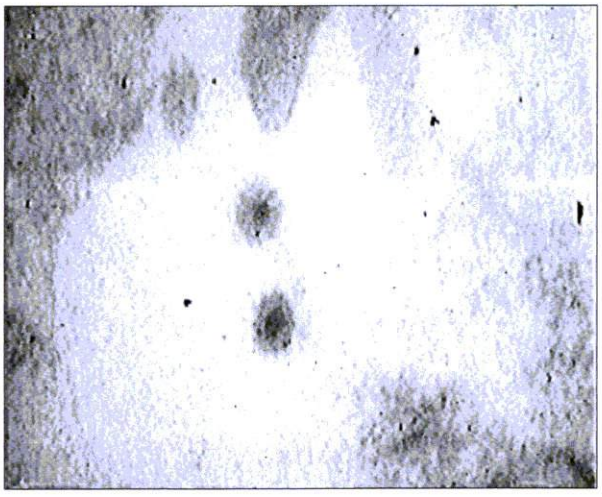

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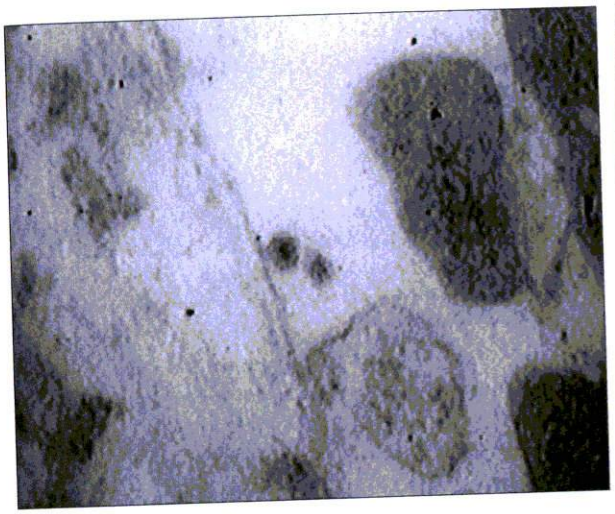

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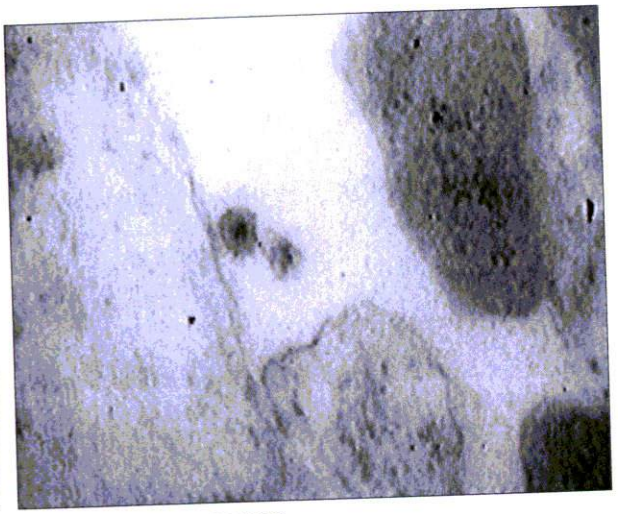

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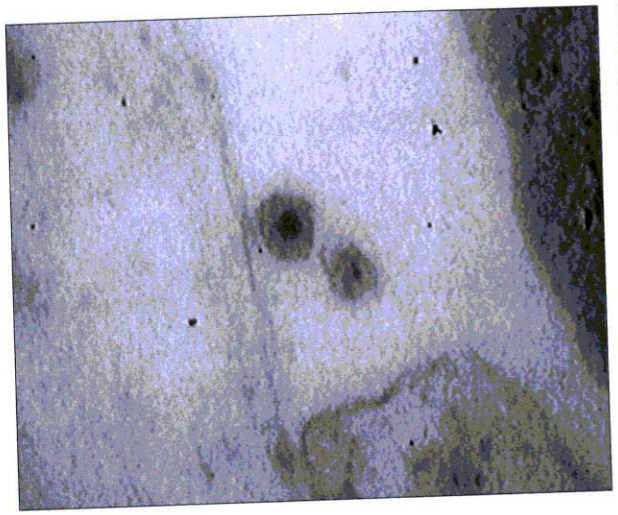

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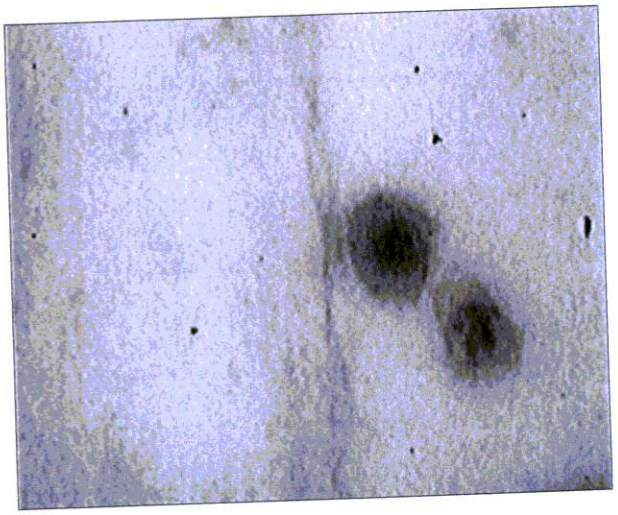

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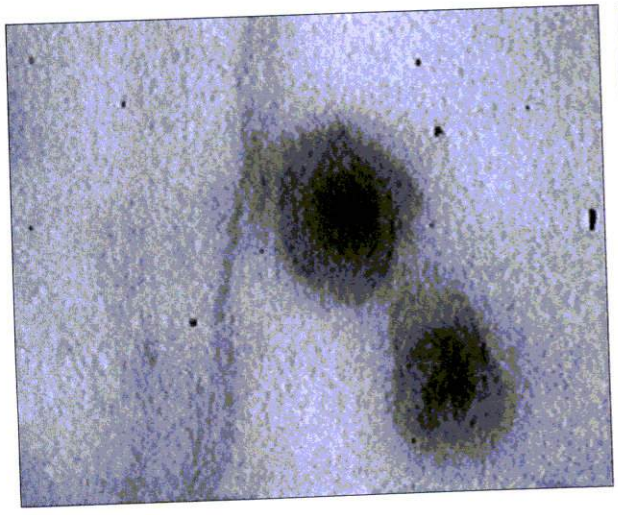

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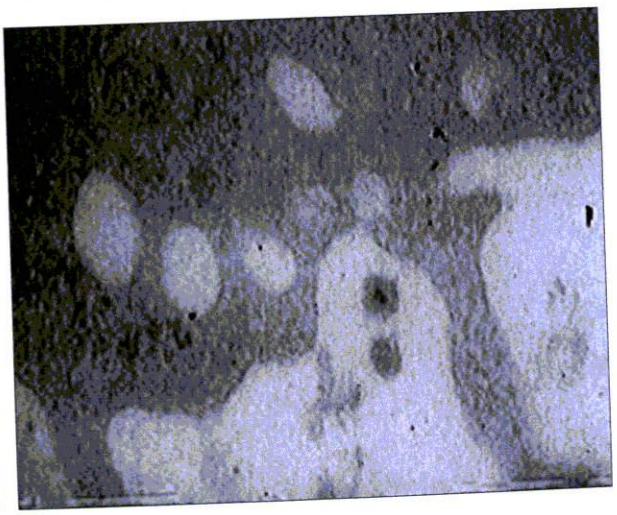

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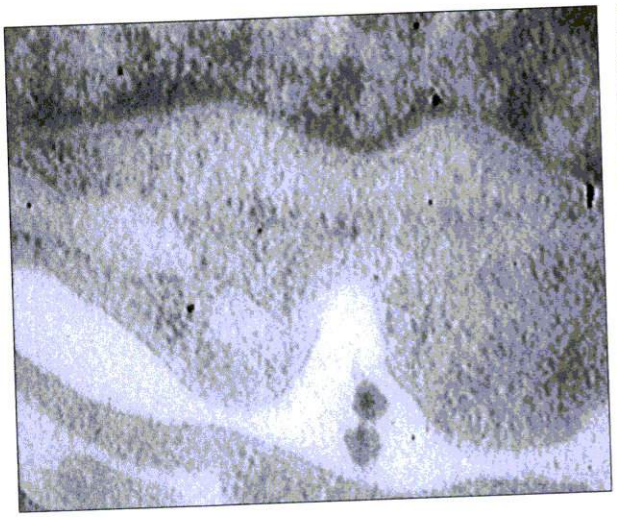

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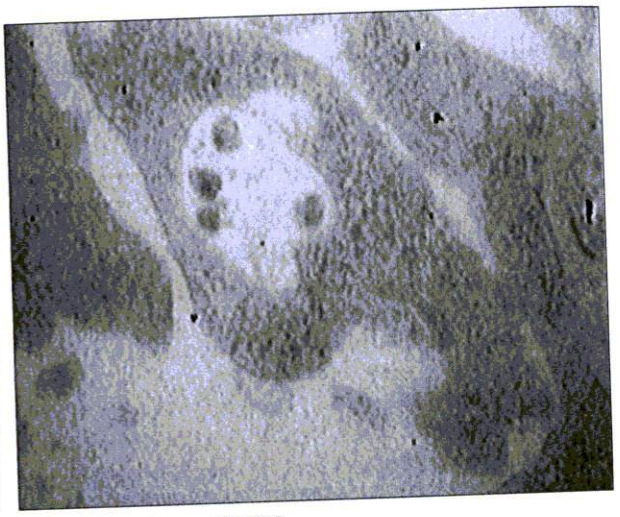

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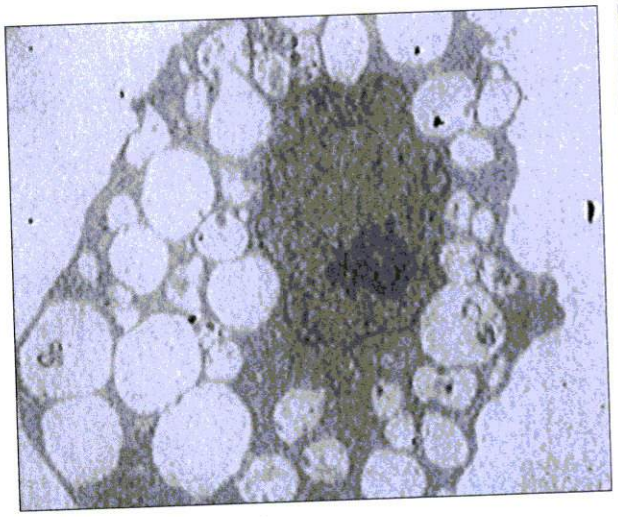

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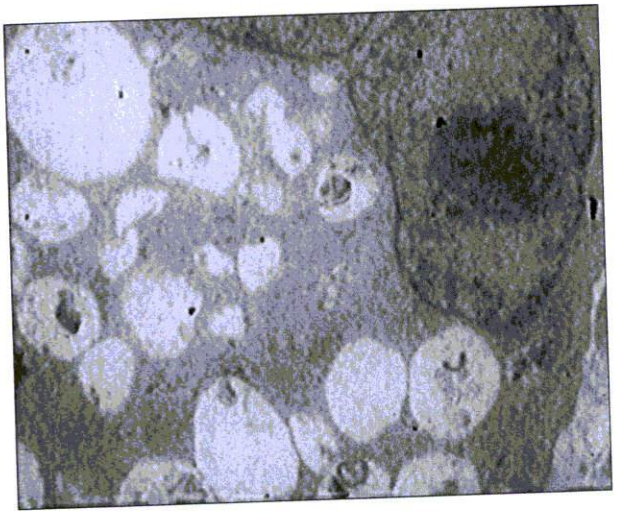

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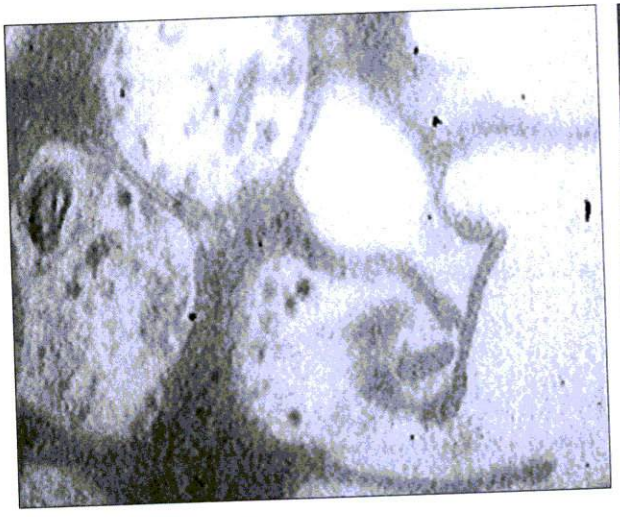

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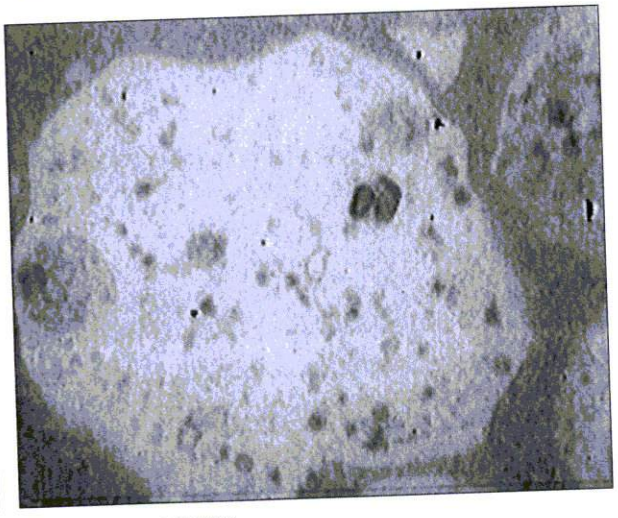

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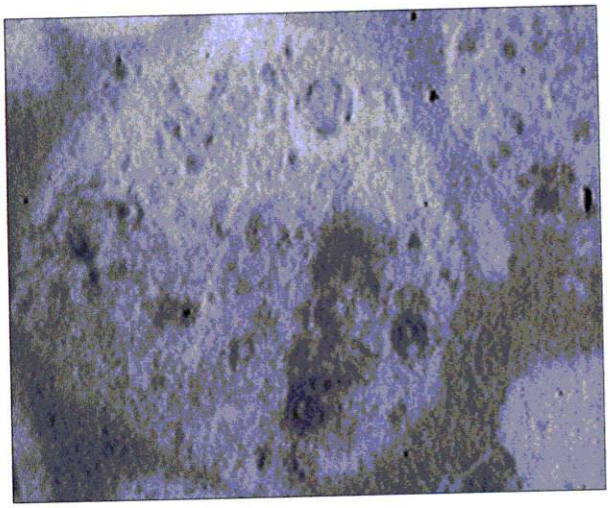

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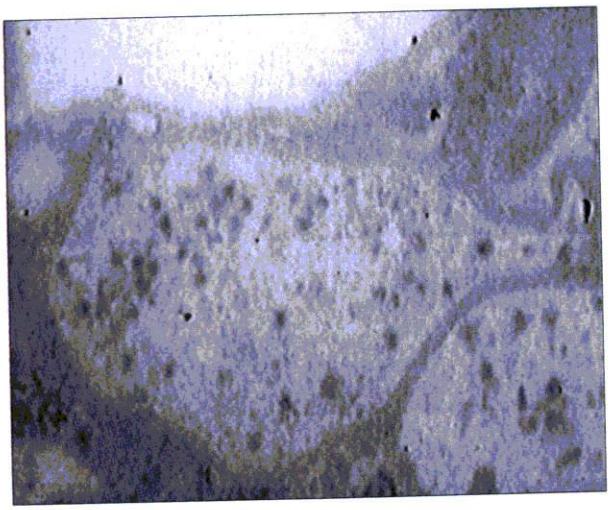

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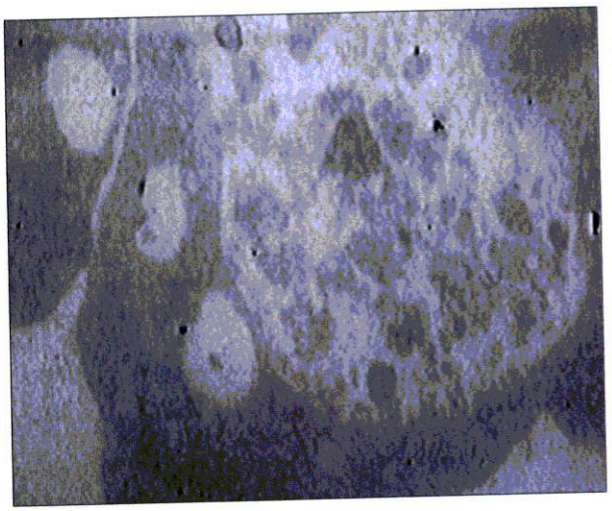

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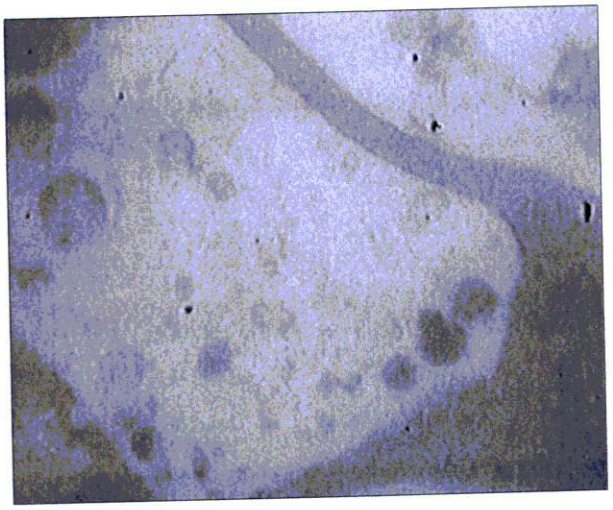

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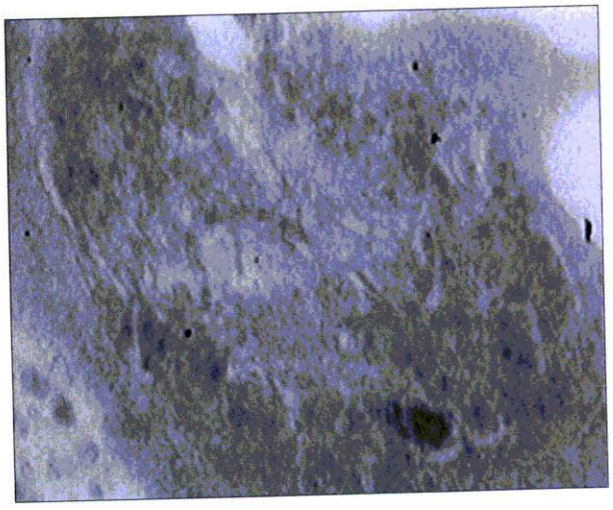

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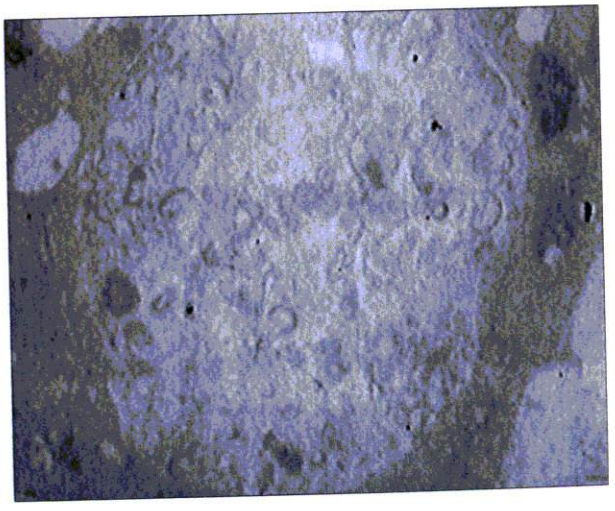

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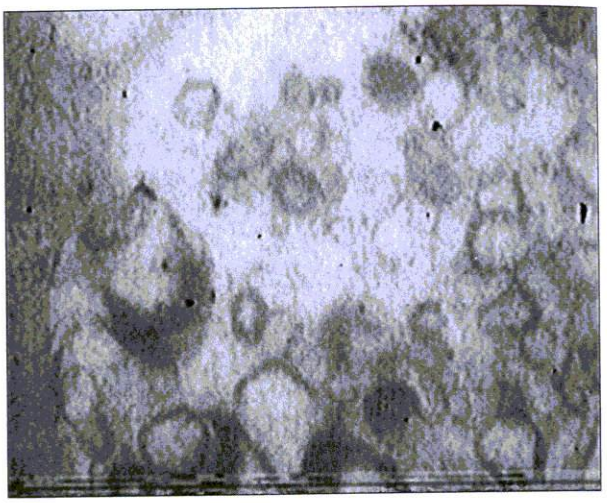

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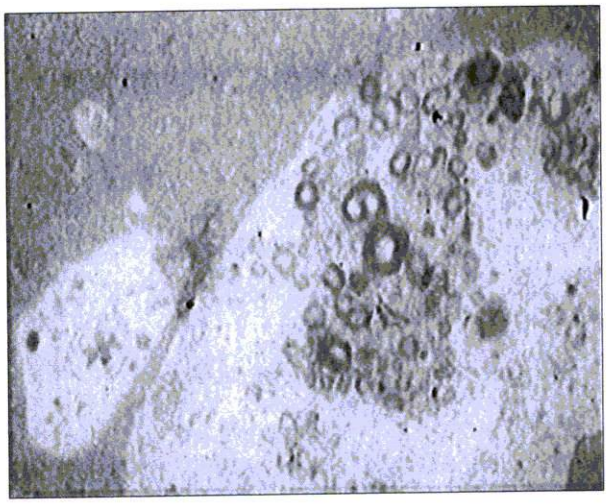

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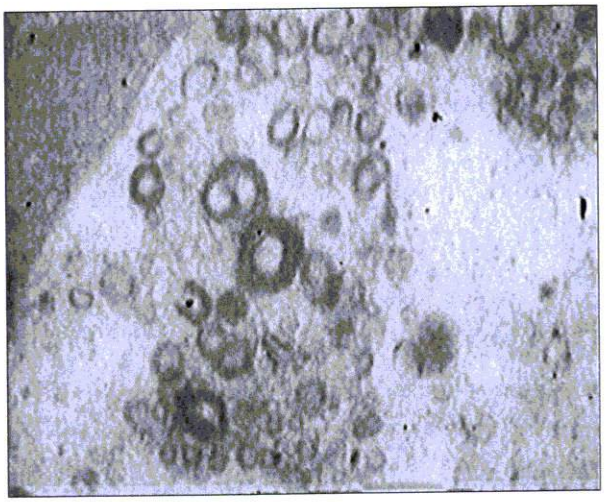

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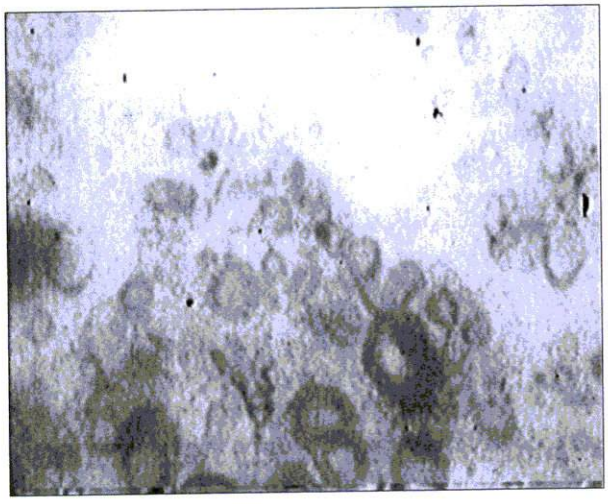

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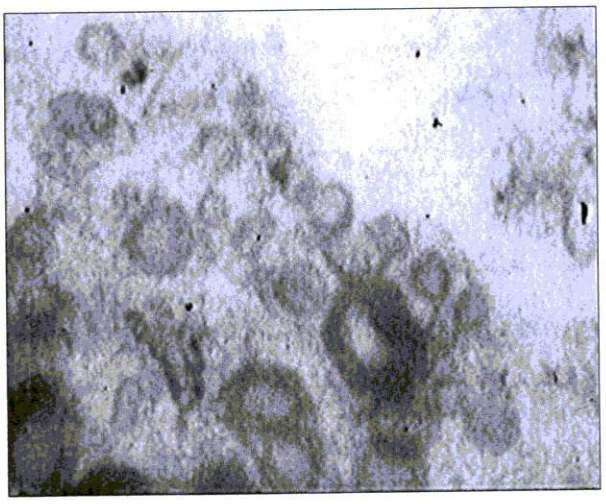

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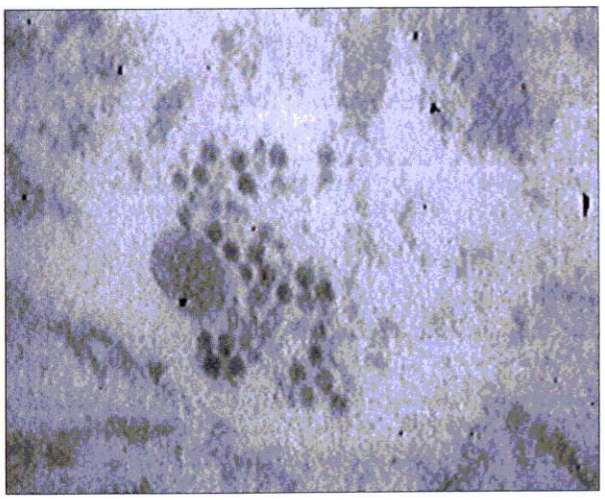

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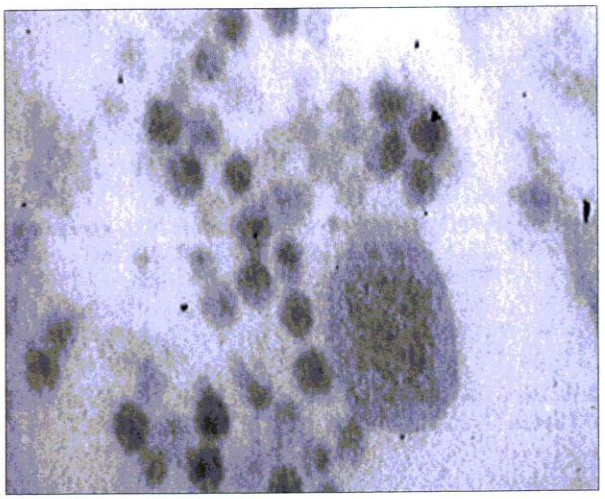

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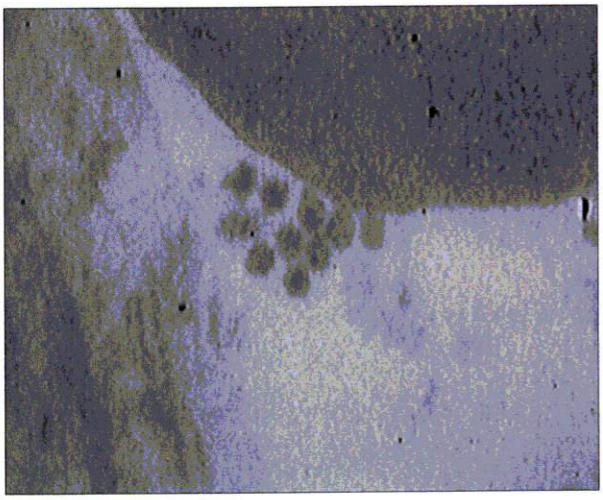

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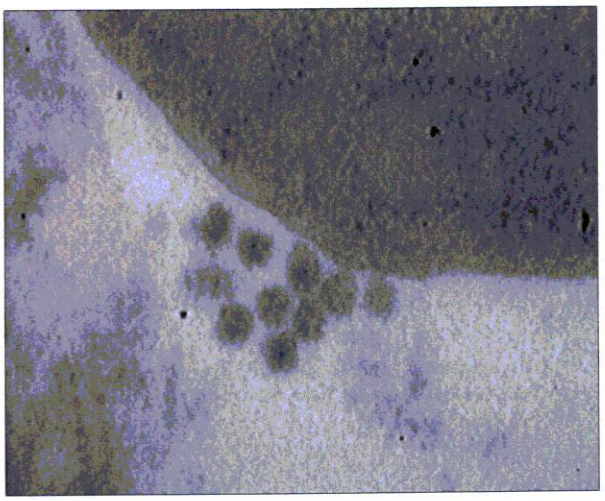

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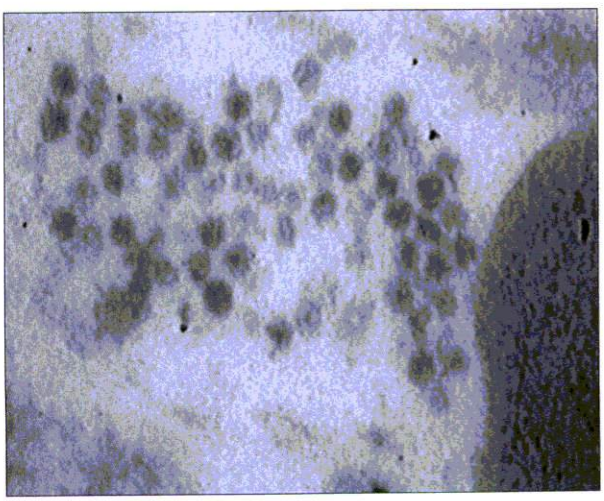
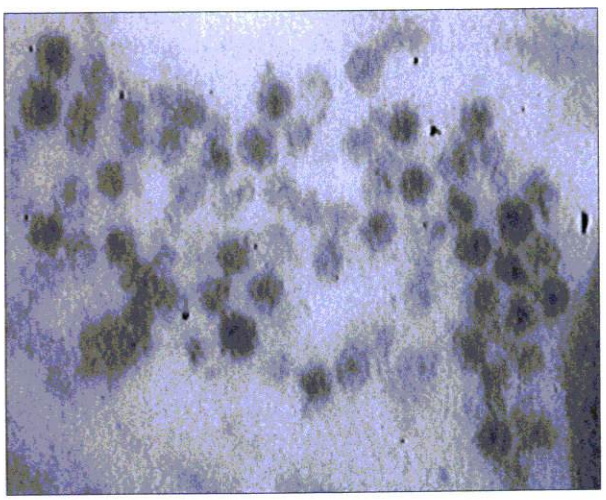
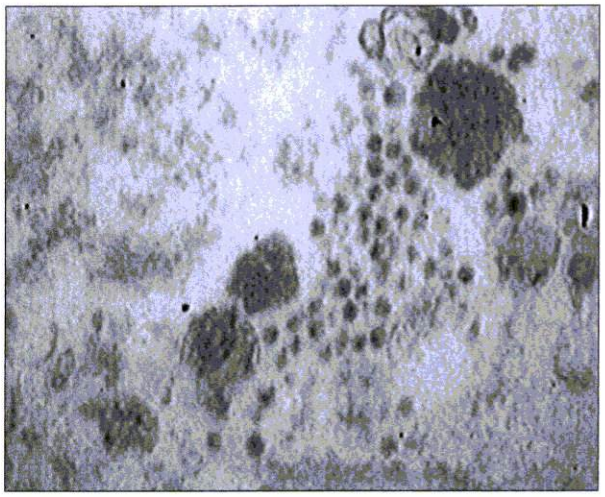


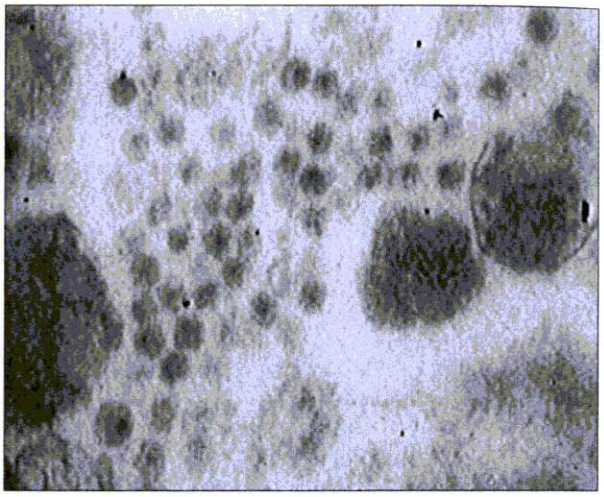
Yellow fever virus x 15.000



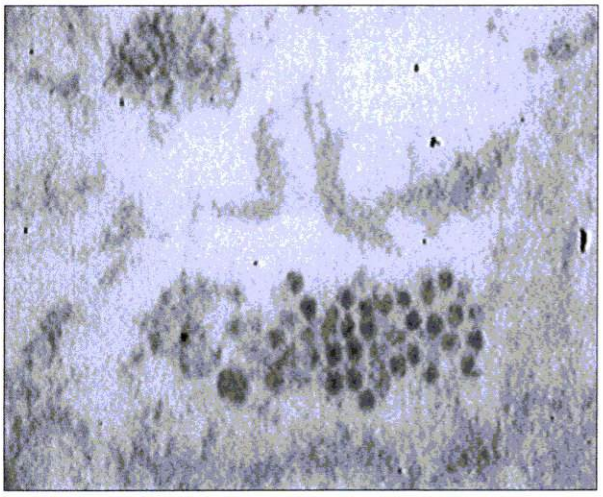
Yellow fever virus x 20.000



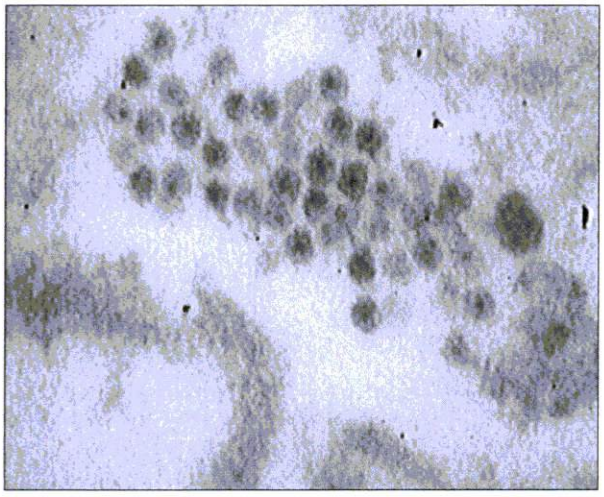
Yellow fever virus x 10.000



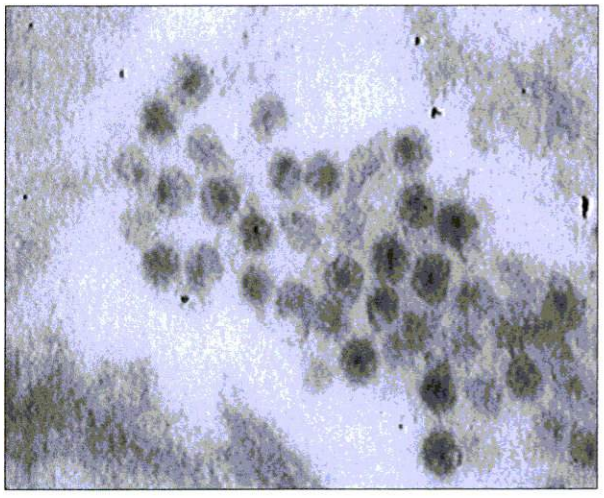
Yellow fever virus x 15.000



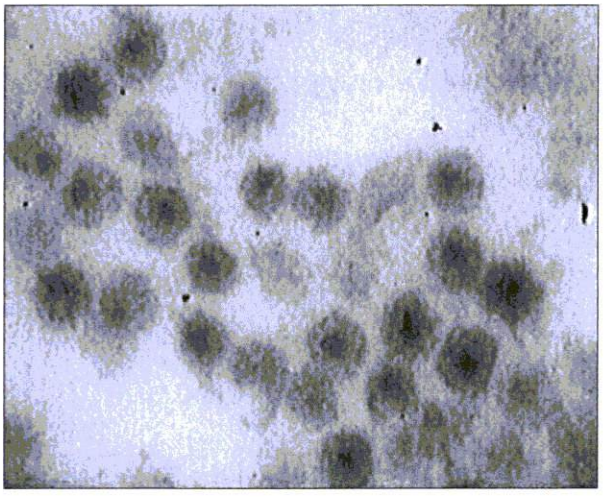
Yellow fever virus x 10.000



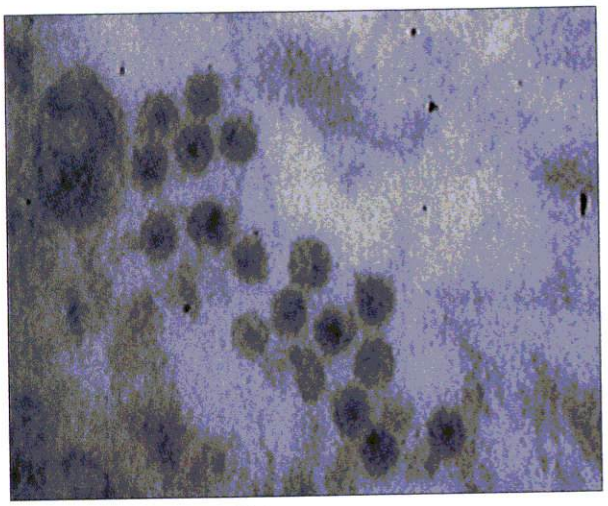
Yellow fever virus x 15.000



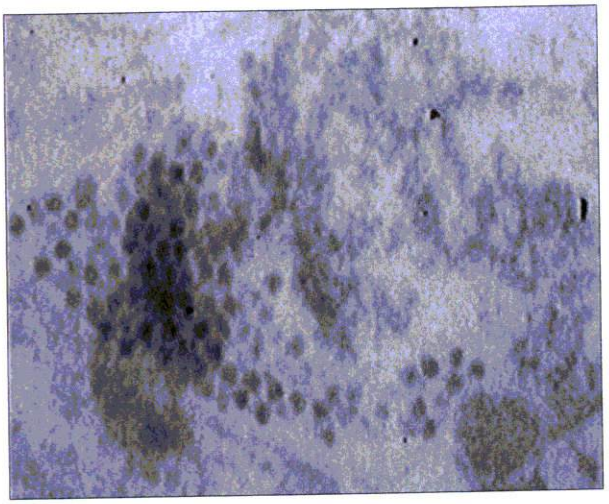
Yellow fever virus x 20.000



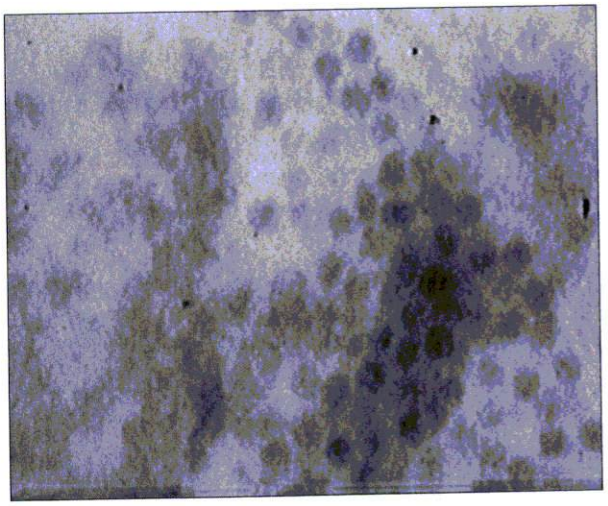
Yellow fever virus x 30.000



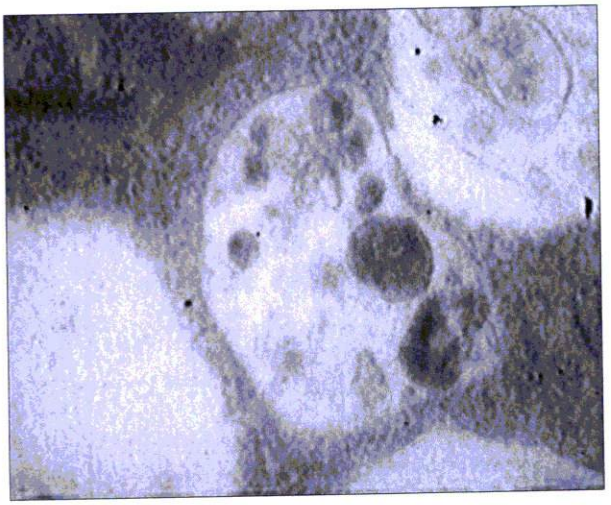
Yellow fever virus x 20.000



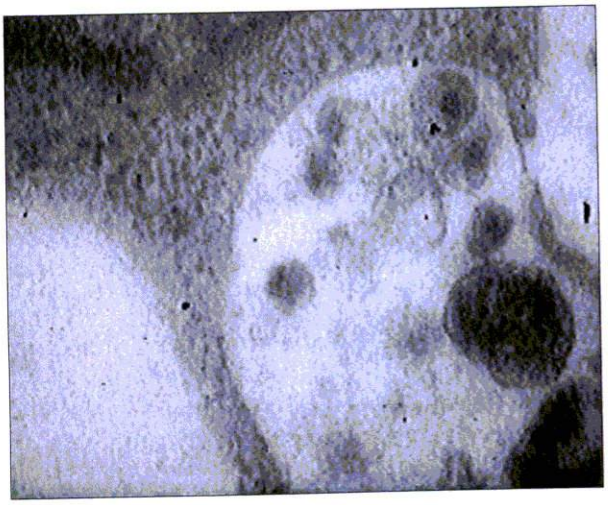
Yellow fever virus x 10.000



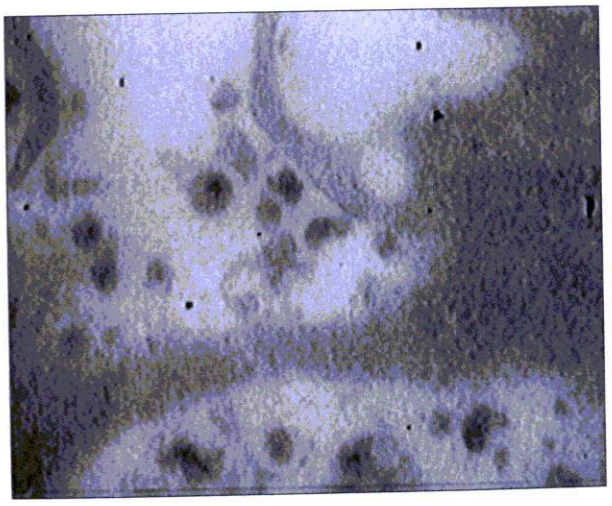
Yellow fever virus x 15.000



Yellow fever x 20.000



Yellow fever virus x 30.000



Yellow fever virus x 15.000