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FOCUS ON

Life-cycle of adenovirus: an ultrastructural study

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RIASSUNTO

Gli adenovirus umani sono membri della famiglia degli Adenoviridae, genus Mastadenovirus, ed includono 51 sierotipi che vengono classificati in 6 differenti specie (dalla A alla F), in accordo con le loro proprietà biologiche.
I virioni icosaedrici, con circa 90 mm di diametro, hanno 240 copies di exoni trimerici sulle facce e un complesso pentonico, costituito da una base pentamerica e di una fibra trimerica a guisa di antenna-like a livello di ciascuno dei 12 vertici.
Il riconoscimento della cellula ospite, da parte della partisella virale avviene in due steps: il domain C-terminale della fibra lega il recettore Coxachie-Adenovirus Receptor (CAR), e la stessa pentonica mediante il motivo RCID interagisce con le integrine alfa-ni-beta 3 e alfa-ni-beta 5 inserite sulla membrana plasmatica cellulare, determinando l'internalizzazione della particella virale via recettore mediato dalla endocitosi.
Dopo l'acidificazione dell'endosoma le integrine la base pentonica coadiuvano al rilasciamento della particella virale dall'endosoma nel cytosol. Il capside dell'adenovirus interagisce con le proteine motrice ed è trasportato mediante microtubuli in prossimità della membrana nucleare, dov'è il DNA viene riversato attraverso i pori della membrana nucleare nel nucleo. Le proteine virali sintetizzatie nel citoplasma vengono trasportate all'interno del nucleo e la progenie virale viene assemblata.
Nel nucleo i virioni "neo sintetizzati" si rinvengono come arrangiamenti paracristallini lattice-like all'interno dei quali alcuni virioni hanno un core elettron-denso, mentre altri virioni evidenziano un core elettron-trasparente. Il possibile meccanismo di escape nucleare prevede la rottura dell'envelope nucleare e contestuale passaggio dei virioni nel citosol. Nel citosol i virioni si rinvengono come formazioni para-cristalline con evidente degradazione del citoplasma adiacente alla loro presenza. A questo punto inizia la necrosi cellulare. Il nucleo si disintegra e porzioni "condensate" di cromatina si dirigono verso la membrana cellulare. I vilioni abbandono la cellula, ormai in avanzata fase di necrosi, attraverso le lesioni della membrana plasmatica.

Human adenoviruses are members of the Adenoviridae family Mastadenovirus genus and include 51 serotypes, which are classified into six different species (from A to F) according to their biological properties. Characteristic features of each species determine cell tropism and pathogenesis, which can result in ocular, respiratory, enteric and urinary tract (1, 2). All adenoviruses are non-enveloped, double-stranded DNA viruses.

The icosahedral virions, of approximately 90 nm in diameter, have 240 copies of trimeric hexons on the facets and a penton complex, composed of a pentameric base and an antenna-like trimeric fibre, at each of 12 vertices. Key to infection efficiency is the outer protein coat or capsid, of the virus itself, as realized during the development of targeted gene delivery systems. Adenovirus infection is mediated predominantly by the penton and fiber capsid proteins, these proteins interact with cellular factors to coordinate key events that permit the passage of viral particles into the cell, ultimately, to the nuclear periphery for DNA transfer. The fiber is responsible for initial cell attachment, and consists of three domains: tail, shaft and knob (3, 4). The tail comprises the first 17 residues from the amino (N)-terminus and is responsible for non covalent attachment of the fi-

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ber to the capsid penton base. The remaining carboxy (C)-terminal segment folds into a globular Knob that contains a receptor-binding domain and a trimerization domain (5, 6). Despite the variety of adenovirus serotypes, the data describing the virus multiplication cycle and intracellular trafficking have been obtained largely from studies of the serotypes 2 and 5, which are both members of the Human adenovirus C species. The host cell is recognised in two steps: the C-terminal domain of the fibre binds to the Coxsachie-Adenovirus Receptor (CAR) and penton base, through its RGD (Arg-Gly-Asp) motif, subsequently interacts with alfa-ni-beta 3 and alfa-ni-beta 5 integrins inserted in the cell membrane, resulting in the internalization of the viral particle via receptor-mediated endocytosis (7). Upon acidification of the endosome, integrins and the penton base assist in release the virus from the early endosome into the cytosol. The adenovirus capsid then interacts with motor proteins, and is transported over microtubules toward the nucleus, where the DNA is delivered and replicated (8). Newly sinthesised viral proteins are carried into the nucleus and virus progeny are assembled. Release of the virus from infected cells occurs through cell lysis.

Given the similarity in the structure of adenovirus capsid across the genus, one migth assume that the pathways of penetration, intracellular trafficking and release of any member will be representative of the group. However this model of viral multiplication cannot be applied to all adenovirus species. Human adenovirus B uses CD46, a protein involved in complement binding, as a primary receptor (9). After internalization via endocytosis, Human adenovirus B accumulates and has a long residence time inside endosomal compartments until it reaches late endosomes or lysosomes (10, 11). Release of capsids into the cytosol occurs in a lower pH enviroment than that of species C adenovirus and is followed by rapid cytosolic translocation to the nucleus through an unknown mechanism (12, 13).

Fiber-CAR interaction is followed by the binding of the penton proteins to alfa-beta 3 or alfa-beta5 integrins (14) through a conserved Arg-Gly-Asp (RGD) consensus motif (15-17). Cryo-EM shows the RGD motifs on protusions that project away from the central axisis of the penton base homopentamer, from which the fiber shaft protrudes (18). Weak densities at these protusions on the adenovirus penton indicate high mobility and suggest that the RGD sites lie at the ends of flexible loops, thus aiding access to integrins. Fiber release triggers structural changes in the penton that are required for proceeding to subsequent step of cell entry or conversely, that penton ligation of integrins induces structural changes that permit fiber

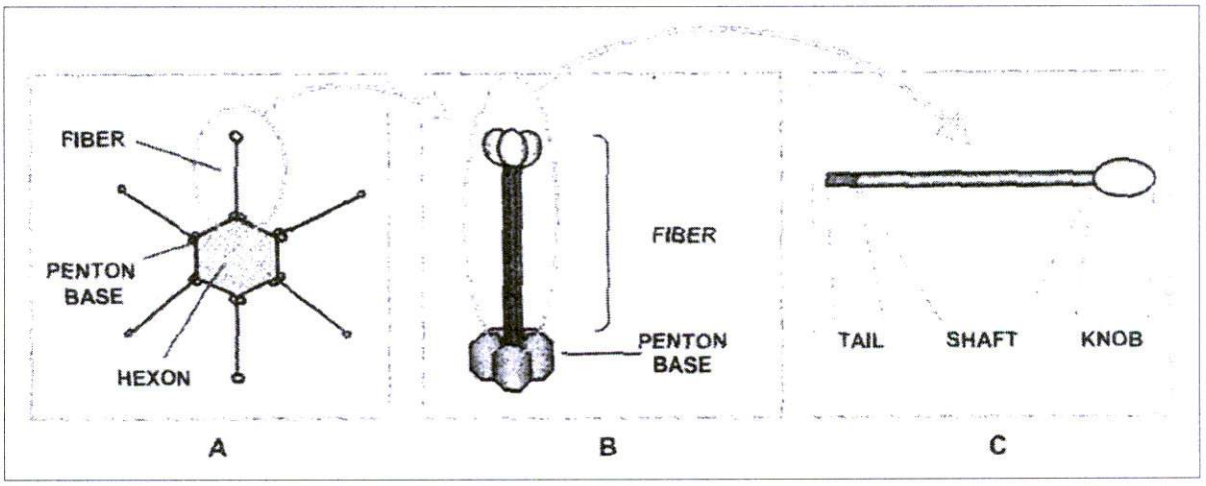

Figure 1 - Schematic of the Ad capsid. (A) Whole capsid identifying fiber, penton base, and hexon. (B) Enlargement of circled region in (A), showing homotrimeric fiber bound to homopentameric penton base. (C) Fiber monomer, identifying tail, shaft, and knob domains. Pictures are not drawn to scale.

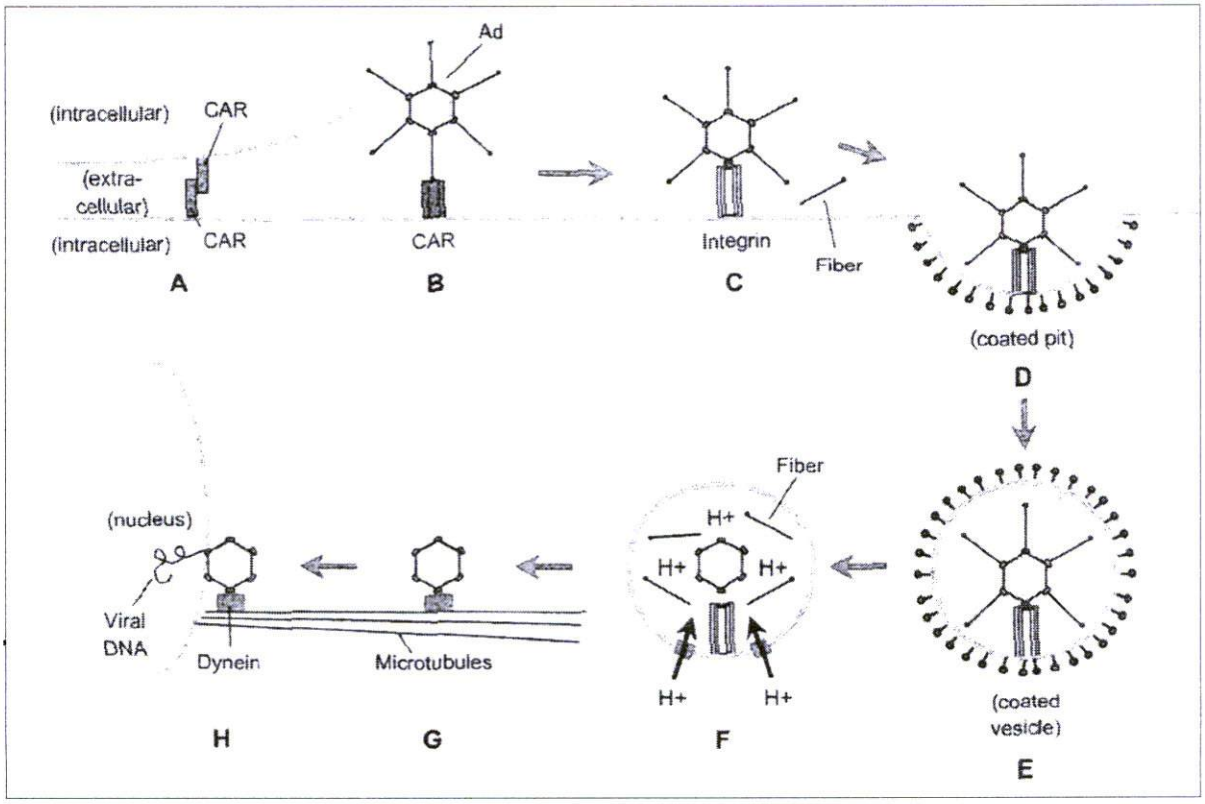

Figure 2 - Schematic of the Ad infection pathway. (A) Homotypic binding of CAR at tight junctions. (B) Interaction of fiber knob with CAR trimer. (C) Interaction of penton base with integrins. Fibers have already begun to be released from the capsid at this step. (D) Clathrin-coated pit formation at integrin Ad binding sites. (E) Clathrin-coated vesicle formation containing Ad. (F) Further dismantling of capsid and acidification of endosome. (G) Interaction of naked capsid with microtubules and dynein motors. (H) Docking at nuclear pore complexes and passage of viral DNA through nuclear pores. Objects in drawing are not drawn to scale.

release. The lower affinity interaction of pentons with integrins is compensated by increased avidity due to pentamer formation. This promotes integrin clustering and enhances downstream cellular signals through enhanced integrin ligation (19).

The integrin receptor clustering resulting from penton binding enhances intracellular signaling anf local formation of clatrin coated pits. The capsid continues to dismantle during viral entry, shedding more fiber proteins and exposing more of the penton protein (8). As the virus containing endosome matures, the vescicular pH drops due to acidification by proton pumps. At pH 6 the virus penetrate endosomal membrane end escape to cytosol, thus evading degradation by lysosomal enzymes (20).

The fiber also influences cytosolic entry by regulating the timing of endosome escape. Adenovirus 5 is released from endosome early after endocytosis at pH 6.0, when the vesicle is still near the cell periphery, while Adenovirus 7 is retainer longer in the endosome and released when the vesicles is near the nucleus, at pH 7.5 (21). These findings suggest that the fiber may act as a pH sensor during endocytic progression, thus dictating the temporal and spatial localization of the capsid upon vesicle exit. The Adenovirus protease greatly influences viral cell entry and endosome escape. The L3/p23 protease cleaves six viral proteins, an activity required to produce mature infectious. The protease is packaged into the capsid and inactivated after capsid release and into an oxidizing environment, but reactivated after entry into acidified endosome (8, 22). Dismantling of the vi-

ral capsid, especially fiber release, appears to play a significant role in cell entry and endosomal lysis.

After cell entry and endosome escape, adenovirus capsid translocate toward the nucleus and accumulate at the nuclear periphery. Motilities are mediated by at least two signaling pathways (23). The first is an integrin-dependent pathway mediated by c-Amp-dependent PKA. Intracellular c-Amp levels regulate the microtubule-dependent vesicle transport (24, 25), and are critical for regulating viral traffic. Integrin binding is required to activate PKA in HeLa cells, and PKA inhibition prevents nuclear targeting of adenovirus and enables net movement toward cell periphery. The second pathway is integrin-independent and requires the activation of P38/MAPK to suppress motility directed toward cell periphery. Thus, both pathways appear to work in concert to promote movement toward the nucleus.

After endosome escape, the naked virus particle rapidly translocates along microtubule tracks at rates of up to 2 um/sec in saltatory movements with a net motility toward the nucleus (26, 27). The microtubule-dependent motor, dynein, which typically supports minus-end directed movement, is a likely candidate for adenovirus translocation. Particles navigating along intact microtubules eventually accumulate at the MTOC (Microtubules Organizing Center) which is perinuclear in cultured cells (28). After endosome escape, 80% of the capsid is still intact (3) and still contains hexon and penton proteins. Thus the hexon, penton, or both may mediate translocation toward the nucleus. While direct interaction of the penton with microtubule-associated motors remains to be seen, indirect findings suggest that the penton may sequester host trafficking machinery after internalization. Upon convergence at the perinuclear envelope, capsids dock at the nuclear pore complex (NPC) and undergo further dismantling. Final dissociation of the capsid, and subsequent nuclear import, rely on the L3/p23 protease to degrade internal protein VI. The majority of capsid proteins, including the hexon and penton, remain at the perinuclear envelope (29). After complete dismantling, viral DNA and protein VII are extruded through the nuclear pores (30). Nuclear import of viral DNA and protein VII is prevented when internal calcium stores are depleted after endosome escape (31).

The viral endocytosis into cells or virions surrounded by the endosomal membrane were observed very rarely. Instead, after the endocytosis the individual virions were often found in the cytosol of cells, and are found in the late endosomes or sporadically scattered in the in the cytosol. After the cell internalization, the virions are found in the nucleus, organized in large paracrystalline arrays or spread throughout the nucleus in smaller clusters. When found in the nucleus, virions were usually arranged in the lattice-like paracrystalline arrays, within which some of them had an electron-dense while others an electron-lucent core. Commonly the condesed chromatin at the nuclear envelope of infected cells is noted. The possible mechanism of nuclear escape includes the separation of nuclear envelope and merger of the nucleus full of virions with the cytosol, or maybe even the individual release of virions throughout the nuclear pore.

In the cytosol, virions were usually arranged in the parcrystalline arrays, although often noted also randomly scattered throughout the cytosol, with frequently observed degradation of the of the cytoplasm at the site of their presence. At this point the, the initiation of cell necrosis can be observed. With the progress of cell lysis, the nucleus disintegrates and condesed is moved near the plasma membrane. At final stage of necrosis, virions leave the cell through the lysed plasma membrane.

SUMMARY

Human adenoviruses are members of Adenoviridae family, Mastadenovirus genus, and include 51 serotypes which are classified into six different species (from A to F), according to their biological properties.

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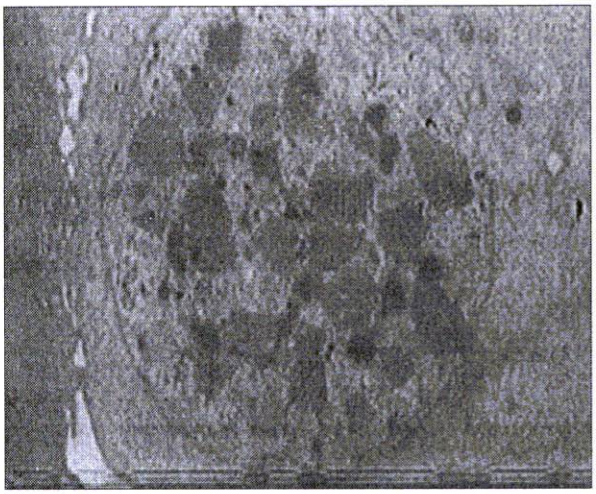

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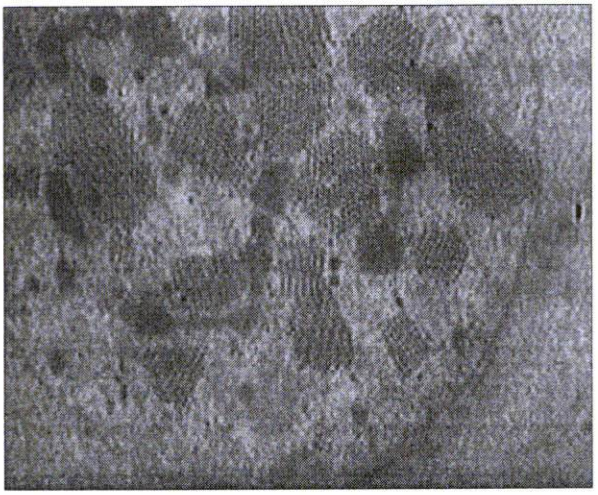

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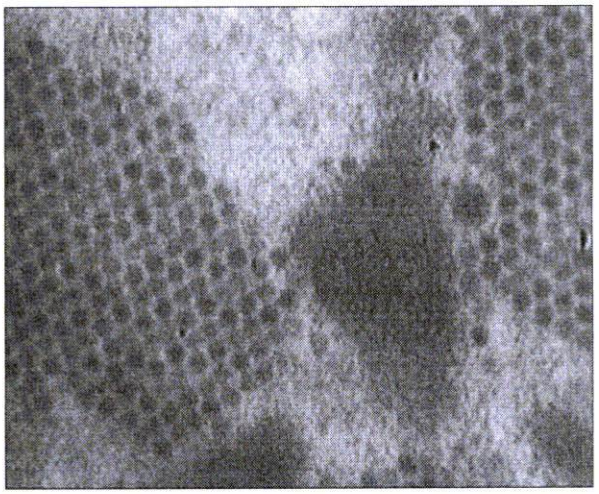

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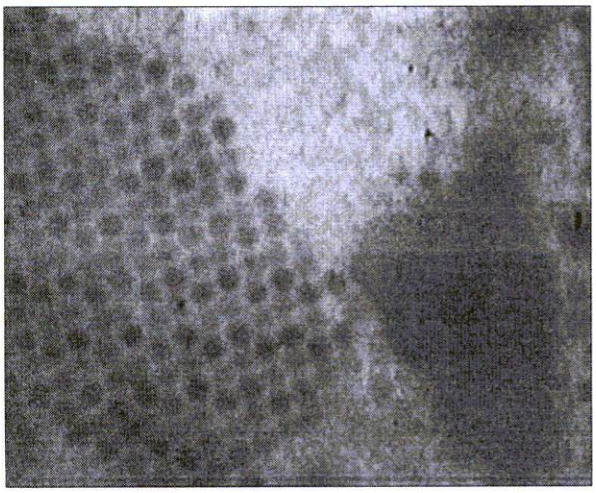

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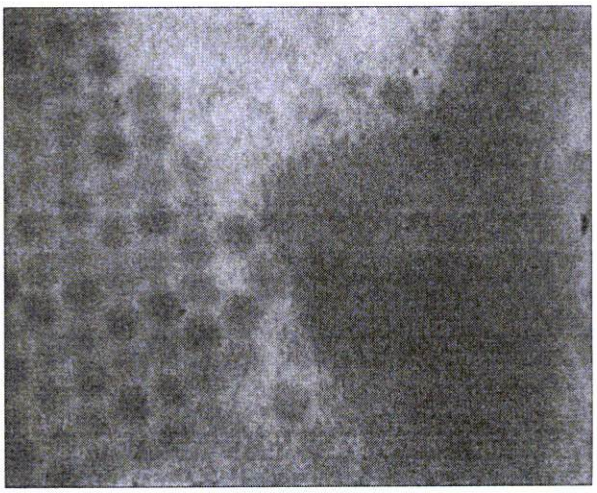

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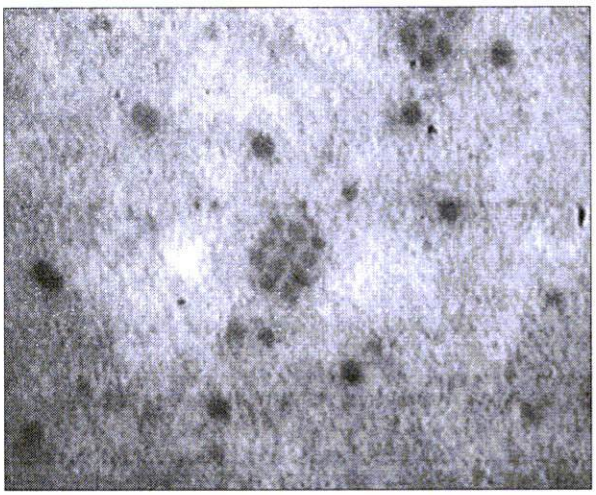

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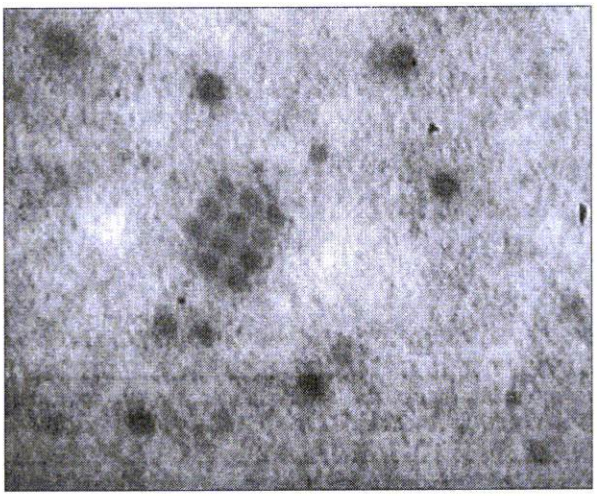

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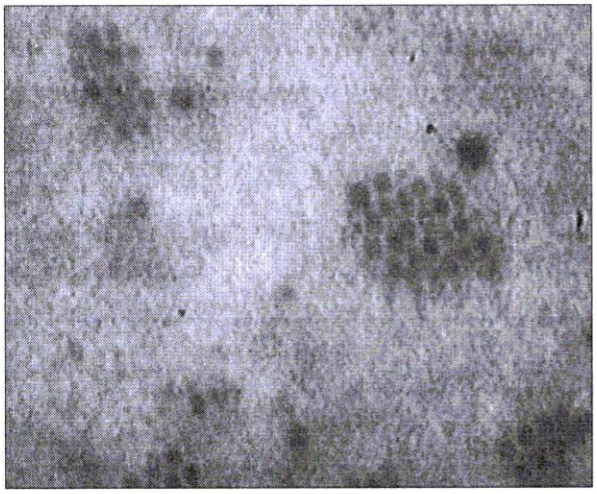

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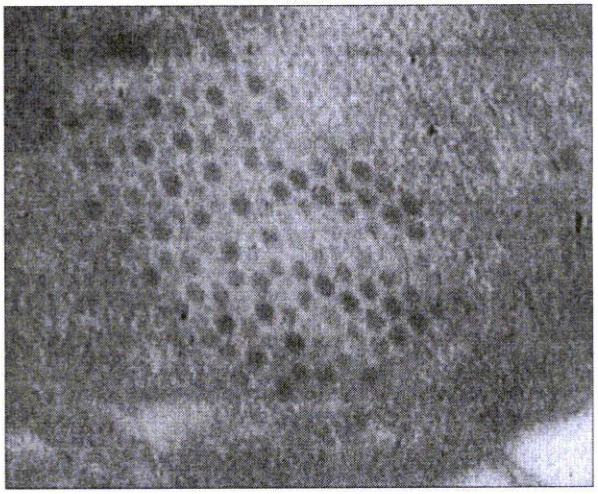

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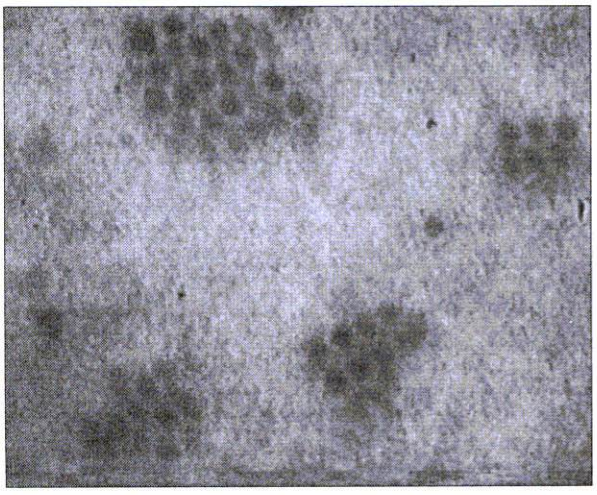

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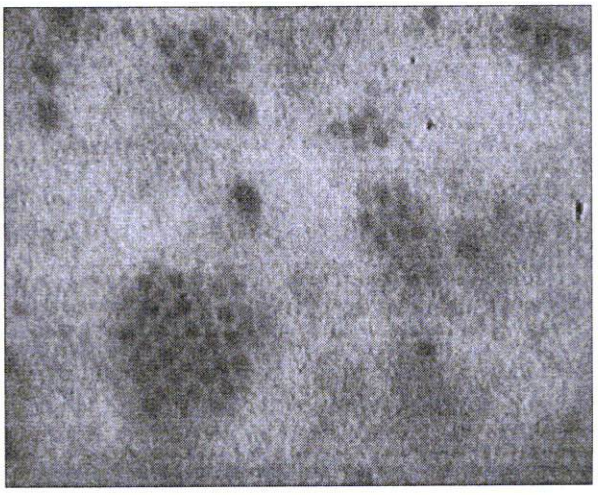

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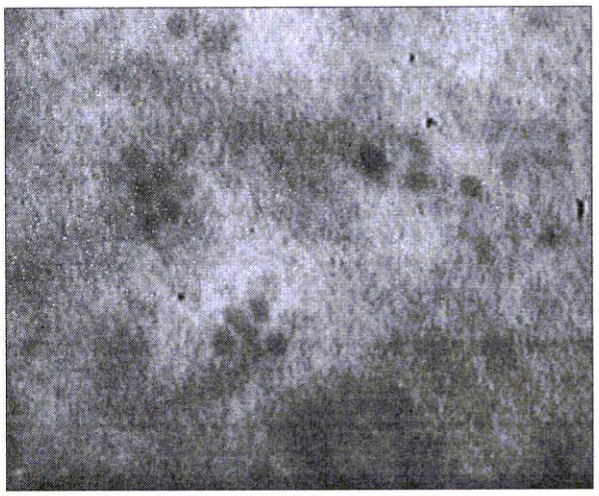

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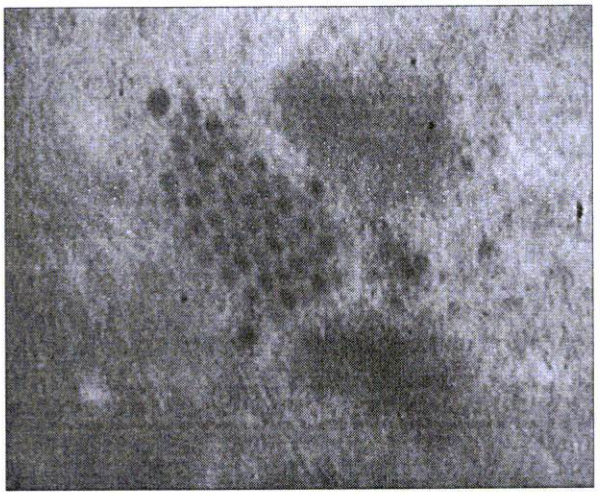

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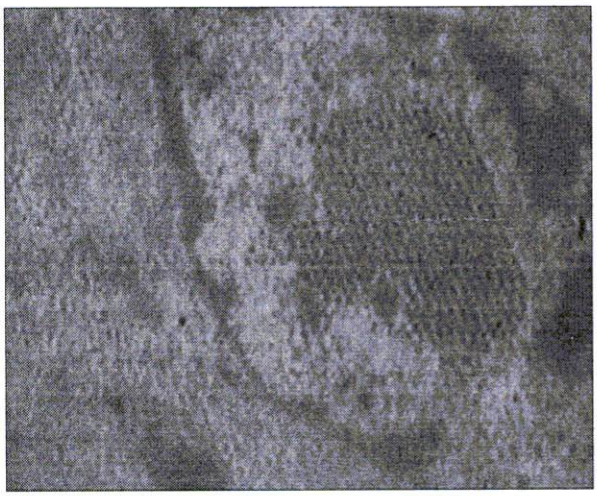

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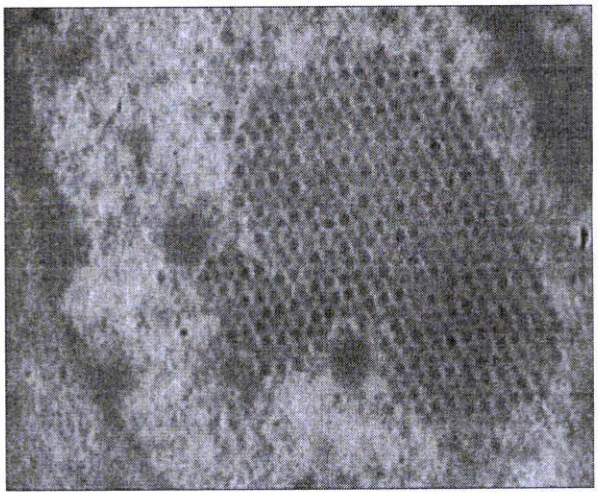

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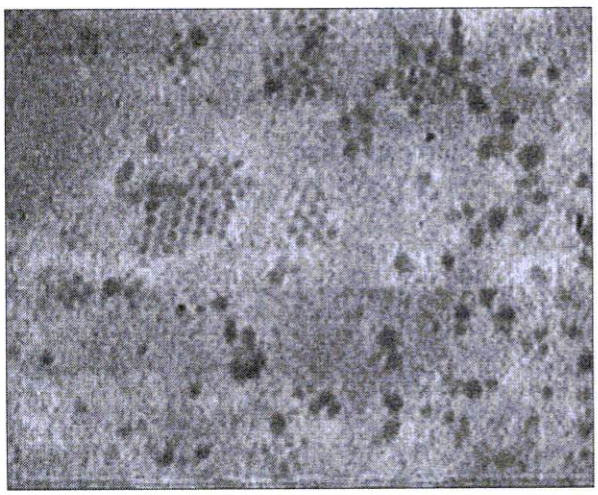

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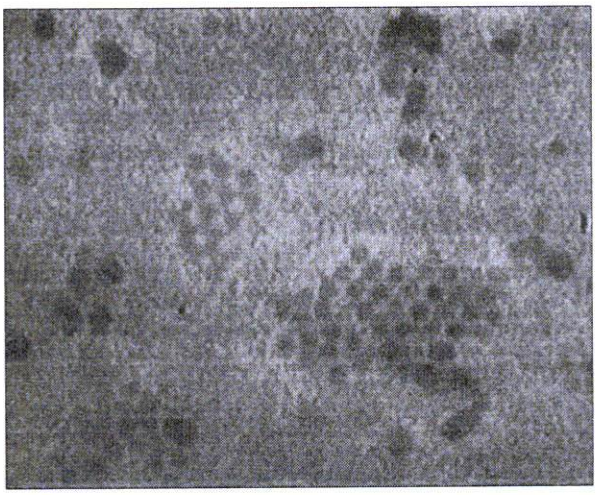

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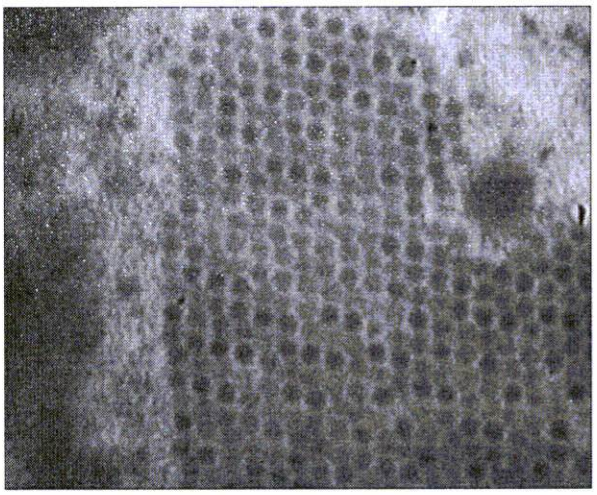

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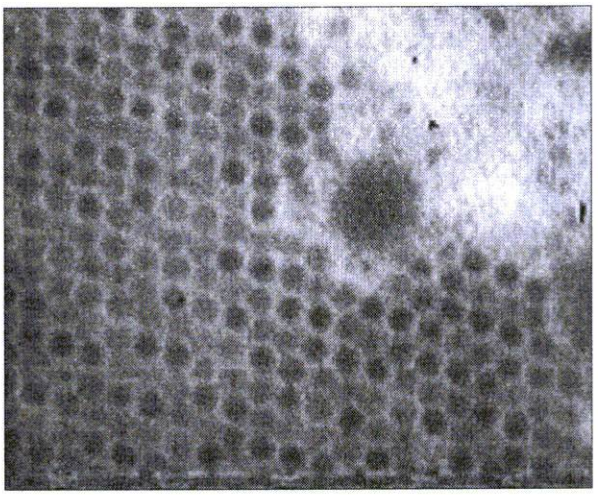

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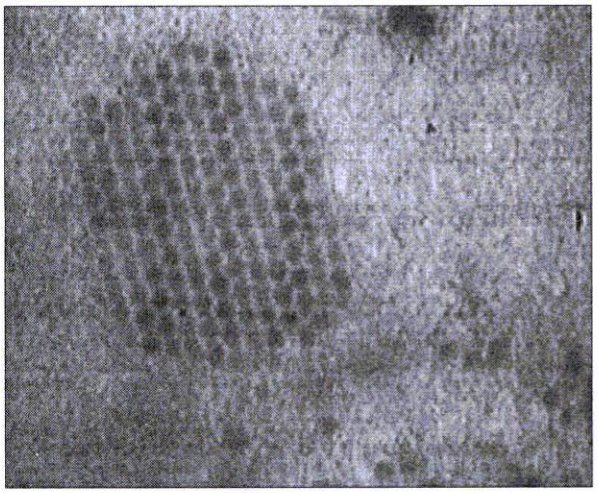

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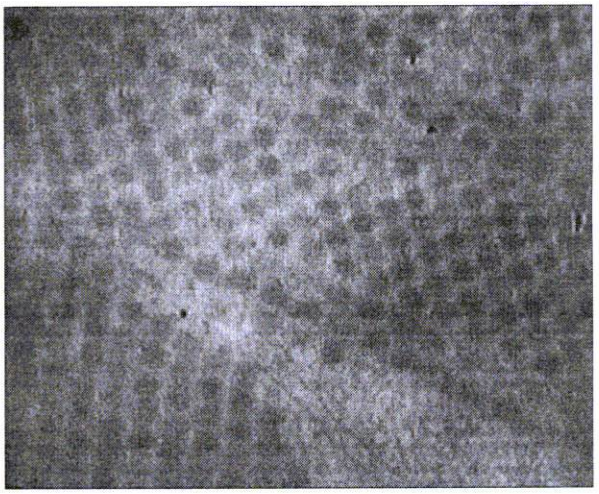

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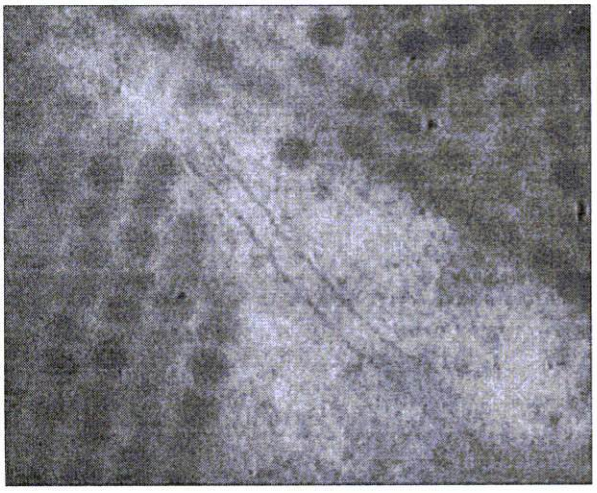
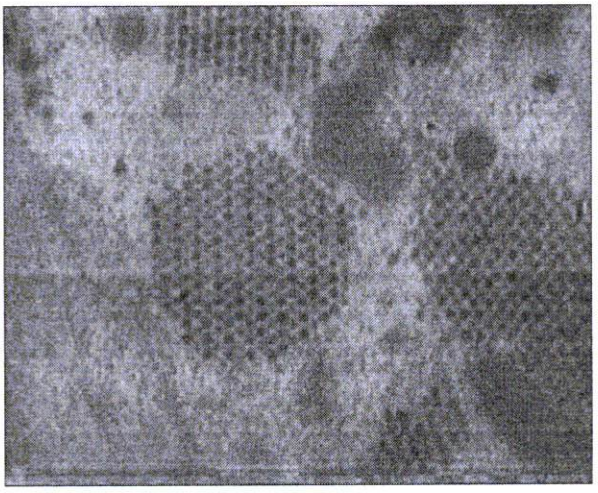


Adenovirus x 30.000



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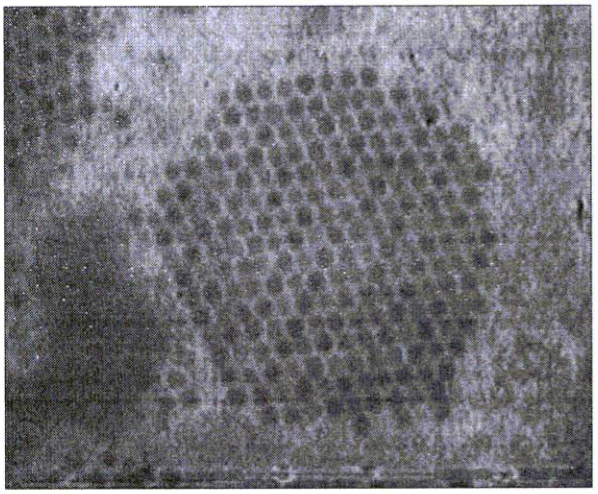

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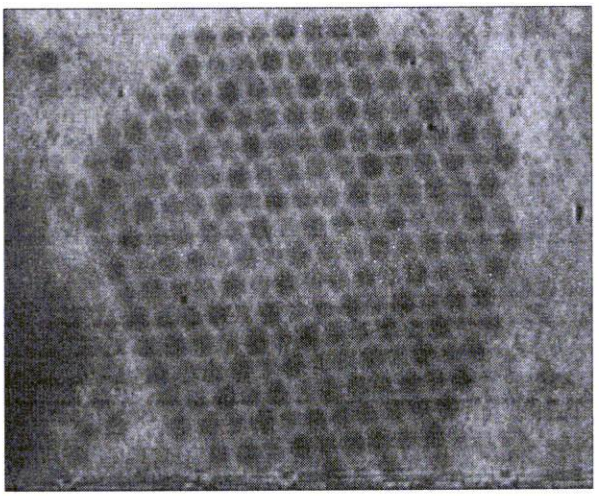

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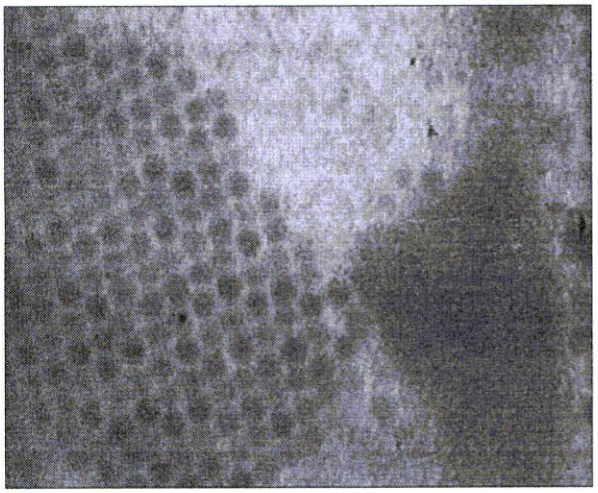

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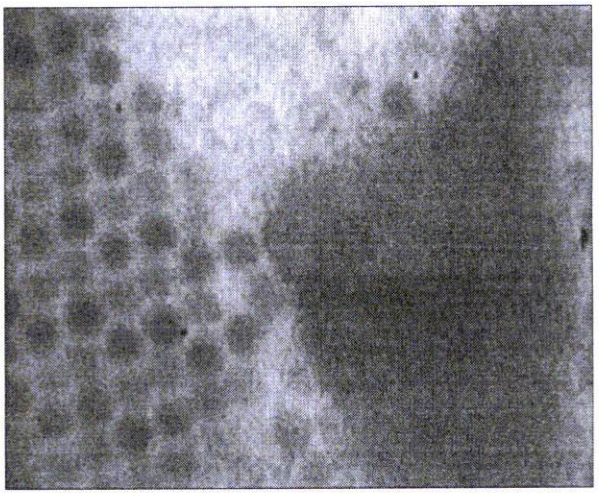

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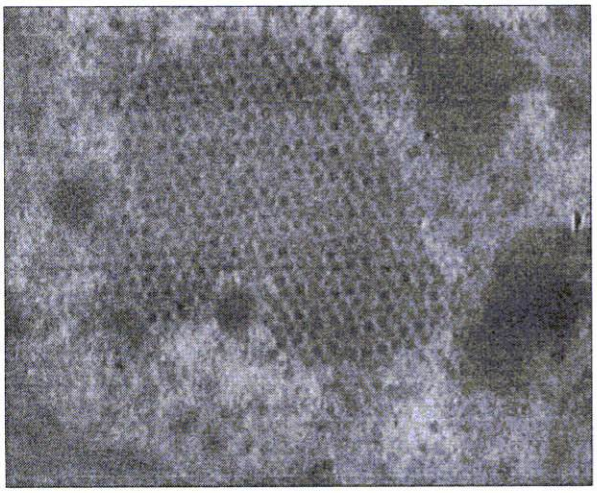

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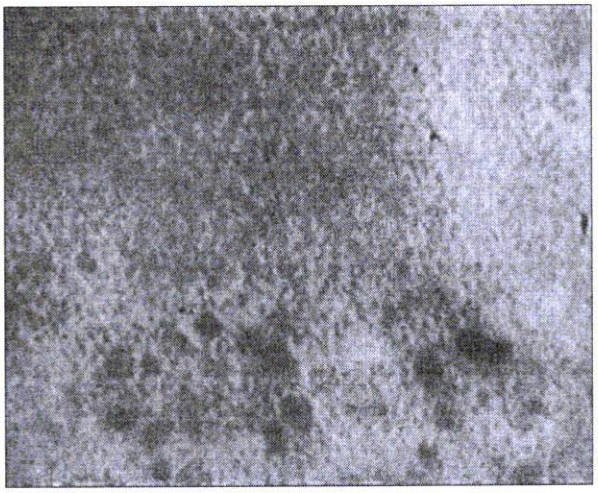

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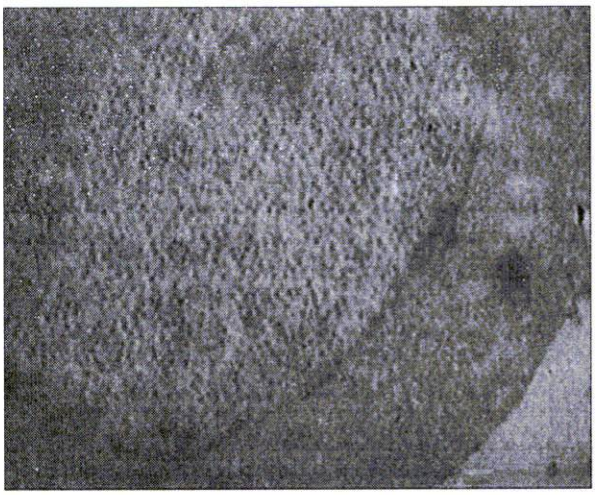
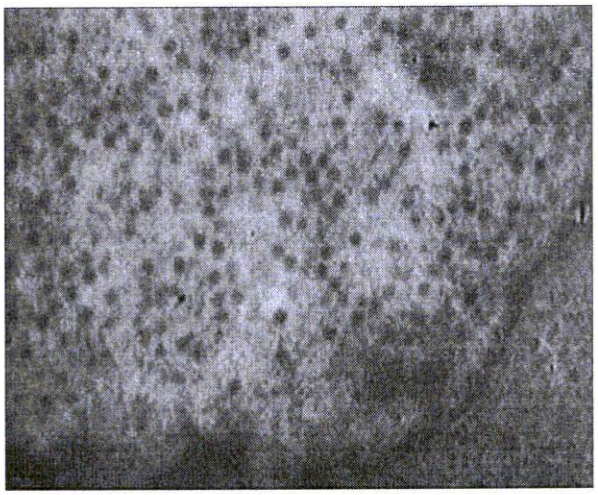
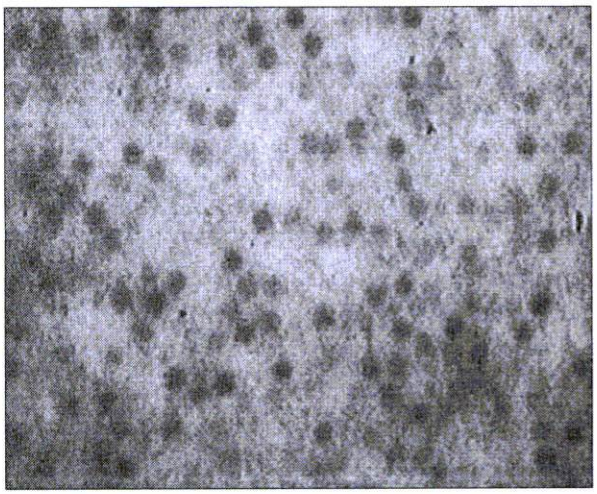


Adenovirus x 5.000



Adenovirus x 7000



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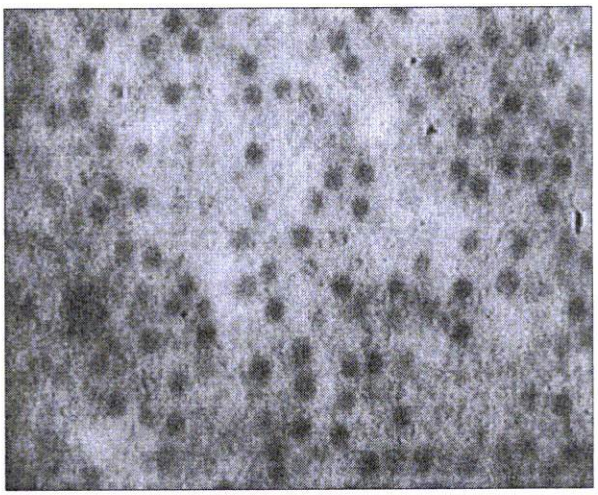

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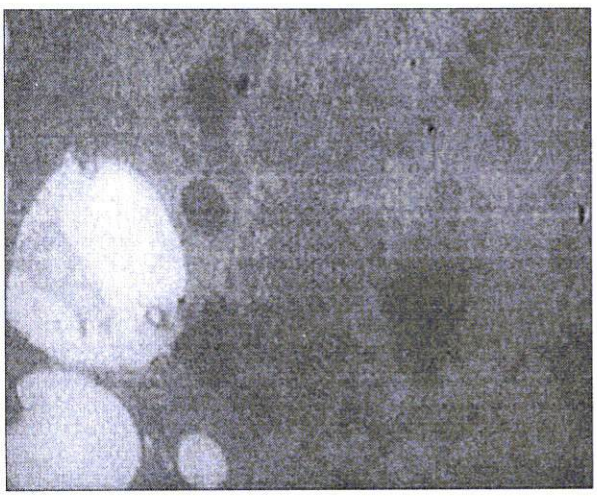

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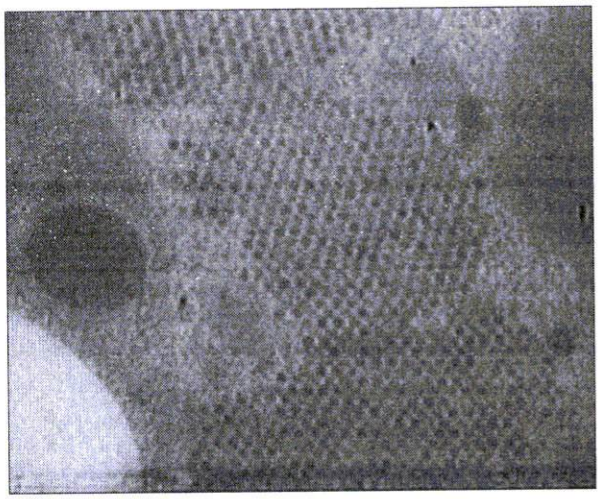
Adenovirus x 7.000



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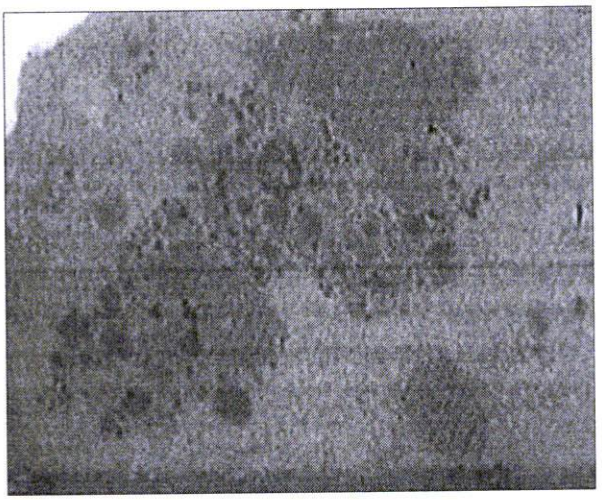

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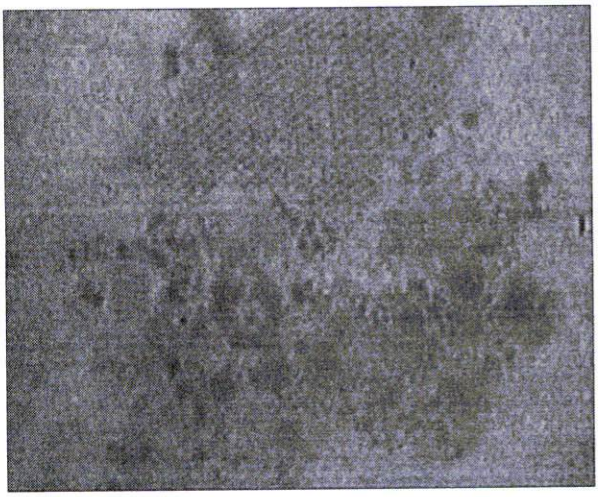

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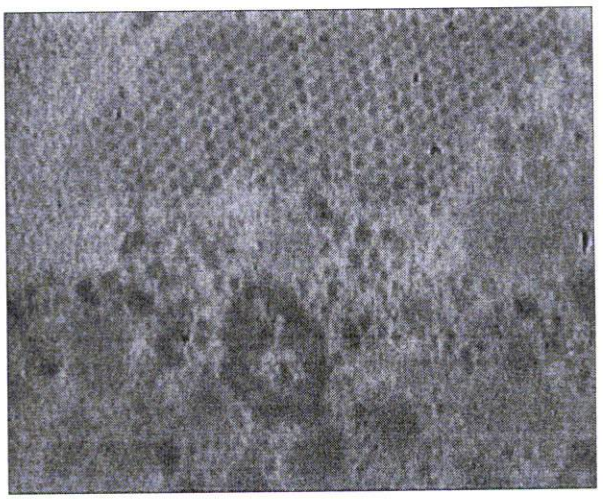

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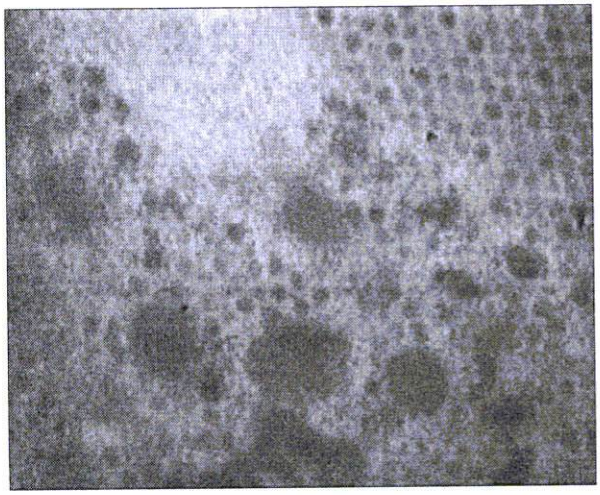

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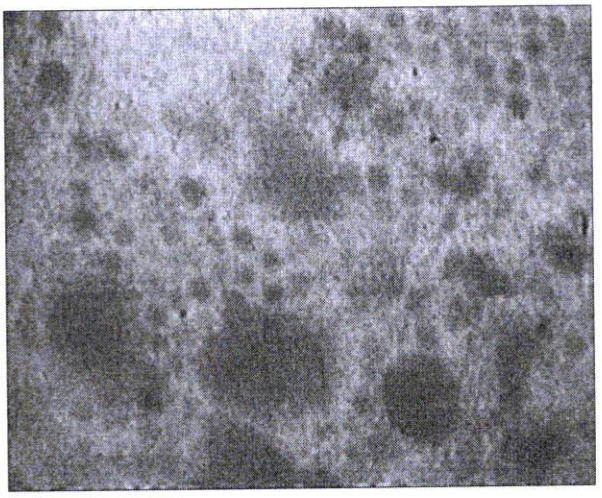

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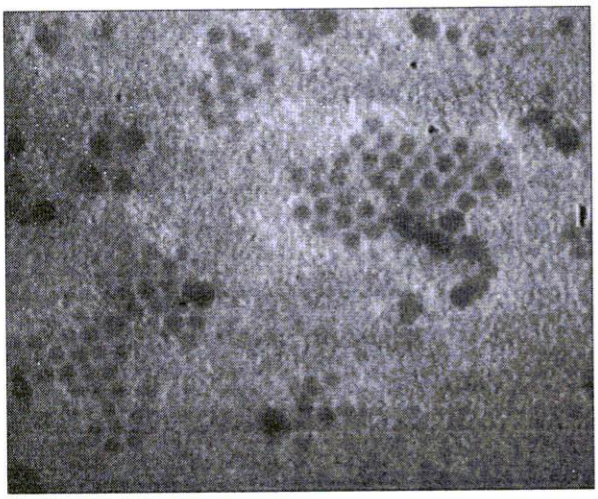

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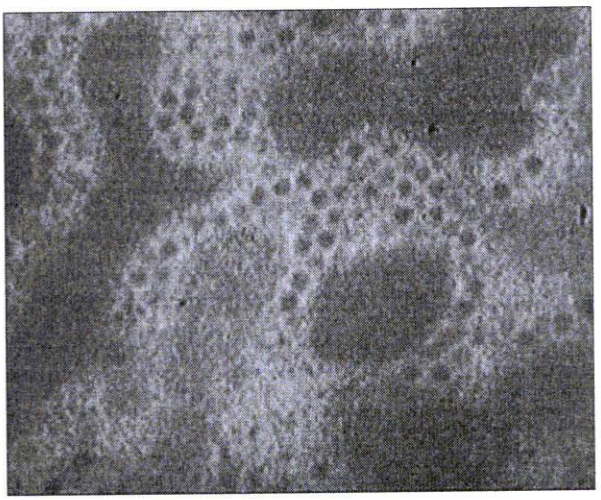

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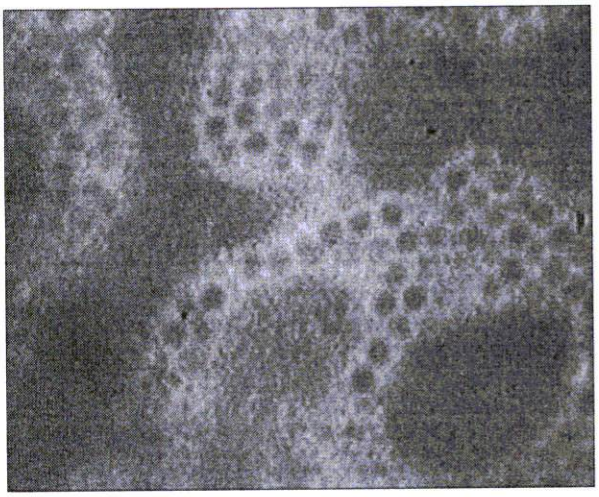

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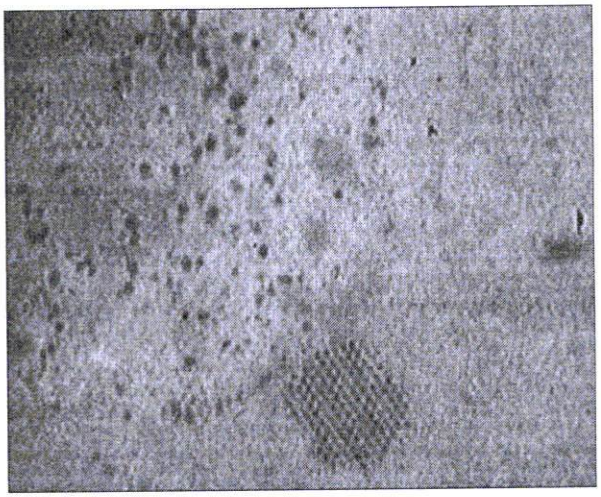

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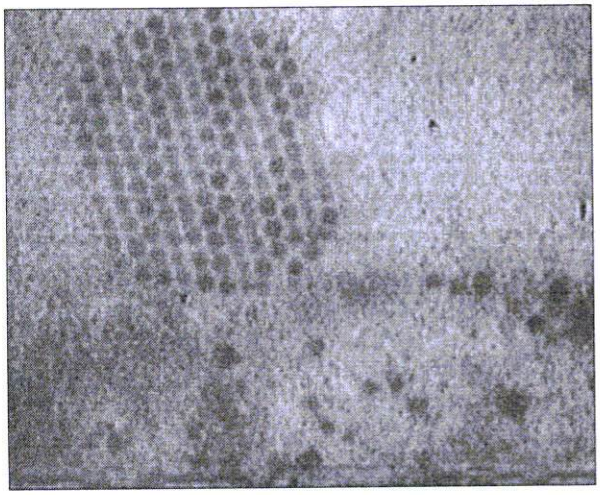

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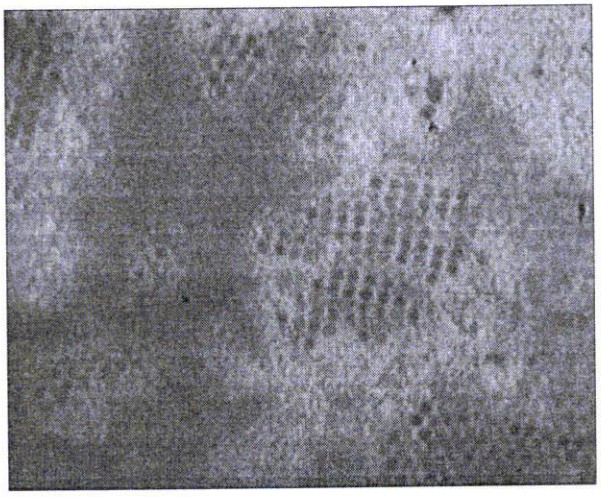

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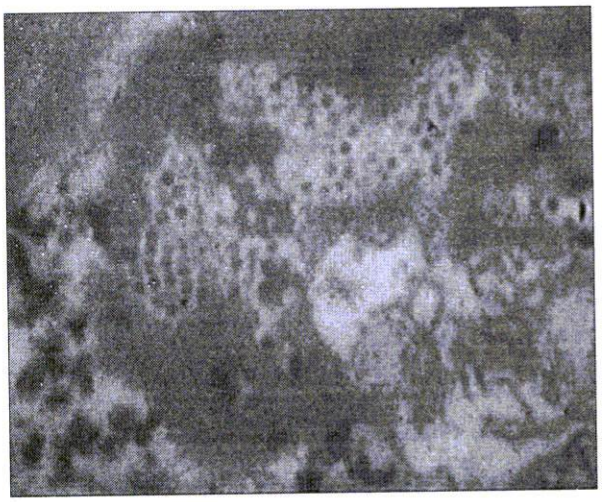

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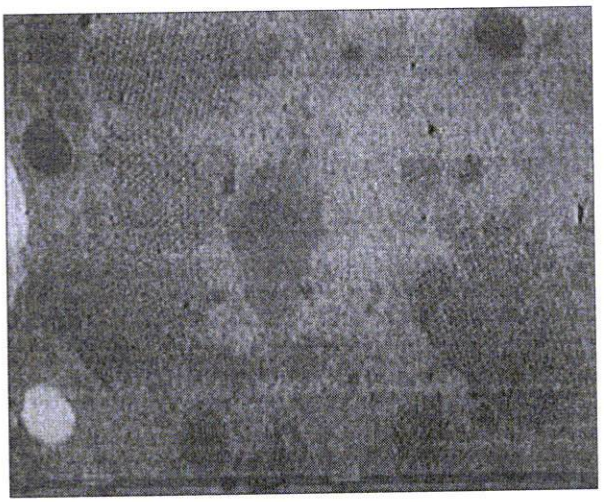

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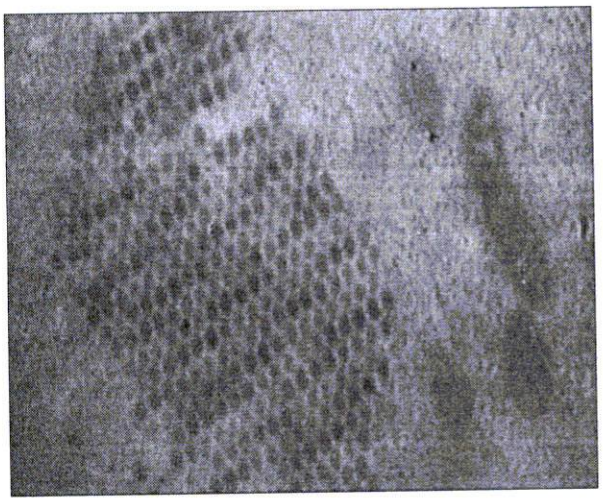

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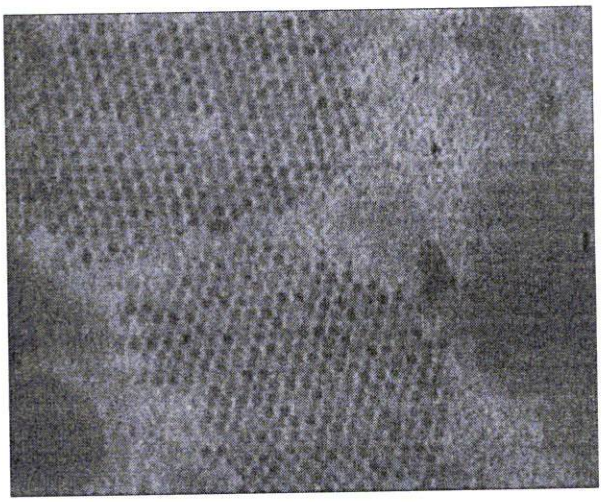

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