in the recipient embryos points strongly to the development of qualitatively different protein complexes in the embryo concurrent with the embryo's growth.

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Close-Packed Array of Virus-Like Particles within Cells of a Human Skin Papilloma.* (20636)

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A distinctive type of wart found on the plantar surface and elsewhere on the skin of man has been described previously(1-3). In contrast to the more common wart this papilloma is characterized by certain cytological features including an intranuclear inclusion body and cytoplasmic masses. In addition, virus-like spherical particles measuring 68 μ in diameter were demonstrated in suspensions of ground tissue examined with the electron microscope. This report is concerned with the location and arrangement of these particles in the papilloma cells as seen in thin sections.

Methods. The present report is based upon a study of 3 papillomas. The excised warts were fixed either in osmic acid after the method of Palade(4) or in 10% formalin if the tissue was not available for the former.

Dehydration in alcohol was followed by imbedding in butyl methacrylate with or without added methyl methacrylate (10%) and polymerization by 2-4 dichlorobenzoyl peroxide (2%) by placing in an oven at 60°C for 18 hours. Sections were cut using a glass knife(5) in a Servall cantilever microtome.† They were examined without removal of plastic using an RCA electron microscope model EMU 2E. Sections from the same ribbon were mounted on albumin-coated glass slides, soaked in xylol to remove the plastic, and stained with Weigert's iron hematoxylin or methylene blue to aid in orientation by light microscopy. Staining was carried out by leaving the slide in either dye for one to 2 hours and washing in water. It was then dehydrated in alcohol, cleared in xylol and mounted. Ground suspensions of papillomas were prepared for electron microscopy and shadowed by palladium as previously described(1).

Results. Cells of the different epidermal layers constituting the papilloma are distinguishable in thin sections under the electron microscope since they present about the same features as in conventionally stained paraffin sections and hematoxylin-stained methacrylate sections under the light microscope. The cells in the Malpighian layer of this type of papilloma contain a spheroidal intranuclear inclusion body as well as a few irregular cytoplasmic masses.

Particles resembling the virus-like particles obtained from ground suspensions of these warts have been seen in electron micrographs in the nuclei of cells of the Malpighian layer (Fig. 1-3). These particles are small points at low magnification and spherical at sufficiently high magnification, and generally are arranged in regular rows. The nuclear membrane often appears double and may be separated, apparently by artefact, from the mass of virus-like particles within. It has not been observed to contain virus-like particles. The particles are in straight rows sometimes at right angles to one another, while at others

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† Manufactured by Ivan Sorvall, New York City.

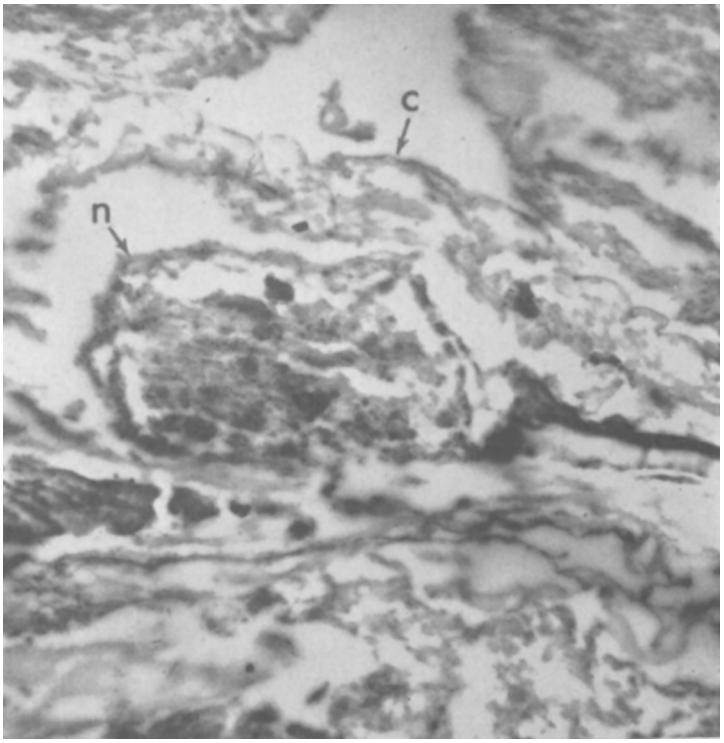

FIG. 1. Nuclear membrane of cell in stratum Malpighii indicated by n, cytoplasmic border by c. Intranuclear inclusion body not present in this particular section through this cell. Gaps in section are due to artefact of preparation, methacrylate not removed. Osmic acid fixation. ×7700.

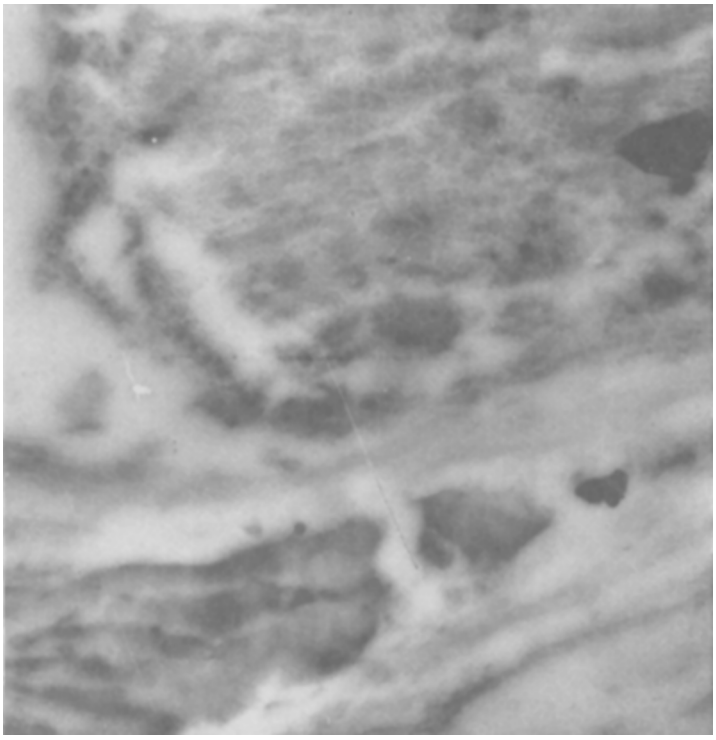

FIG. 2. Further enlargement of negative of Fig. 1. Nuclear material can be seen to be particulate in contrast to the adjacent cytoplasmic substance. Osmic acid fixation. ×21000.

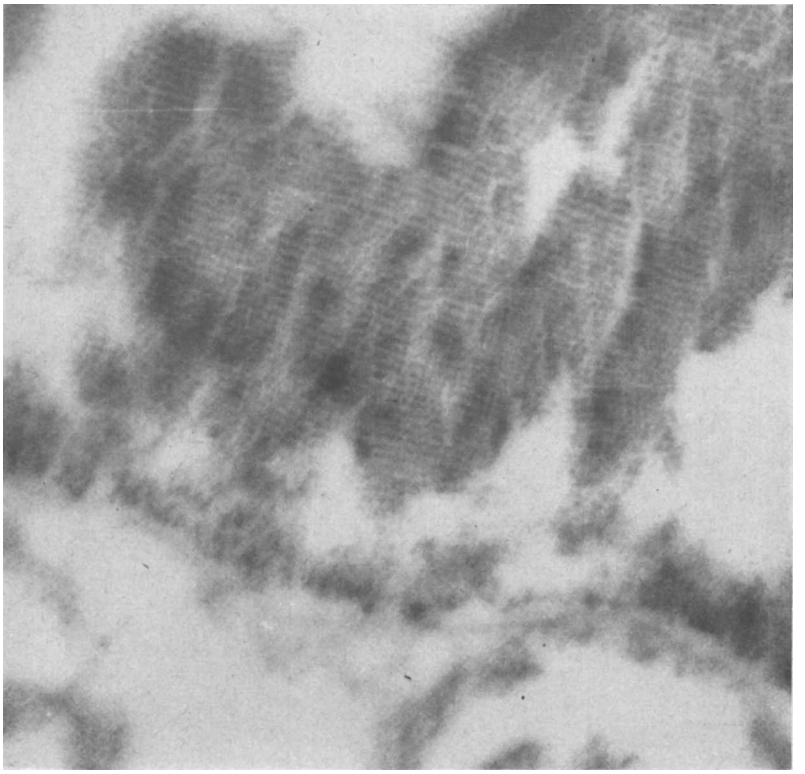

FIG. 3. Small portion of nucleus with nuclear membrane evident and a few adjacent tags of cytoplasm. Gaps are due to artefact of preparation, methacrylate not removed. Nuclear substance composed entirely of particles arranged in rows. Osmic acid fixation. ×33700.

at an angle of approximately 120°. This orientation has been seen to disappear under slight distortion as for example may result from a tear in the section due to heat from the beam, even though this may be at some distance from the cell under observation.

The particles are uniform and spherical (Fig. 6) both in ground suspension of the papillomas and as they lie in the cells. Their diameter measures 56-80 mμ, averaging 68 mμ, in preparations from suspensions when they are separated, and 50-53 mμ averaging 52 mμ when packed(1). In the sections their diameter measures in different preparations from 20-38 mμ. The shrinkage during fixation and dehydration perhaps accounts for the smaller size of the particles in sectioned material. There is, of course, no assurance that the diameter obtained from suspensions is not also distorted somewhat due to dehydration. The surface of the particles is smooth or at times slightly rough or pitted in the shadowed suspension preparations while in sections their outline is uniformly smooth and circular. In preparations from suspensions the particles have not been seen unless shadowed. In sections the particles diffuse electrons more completely than the substance between them. All of the material within the nucleus of the affected cells except for the inclusion body consist of clumps of regularly arranged particles. The intranuclear inclusion body has little discernible internal structure other than an occasional faint irregular granularity. It is sharply demarcated as are also the cytoplasmic masses which have about the same appearance in section. Both of these structures have greater electron scattering power than the rest of the cytoplasm, whether fixed in osmic acid or formalin. In the examples thus far studied virus-like particles have not been recognized in the cytoplasm of the cells of the Malpighian layer although they fill the nuclei of these cells as

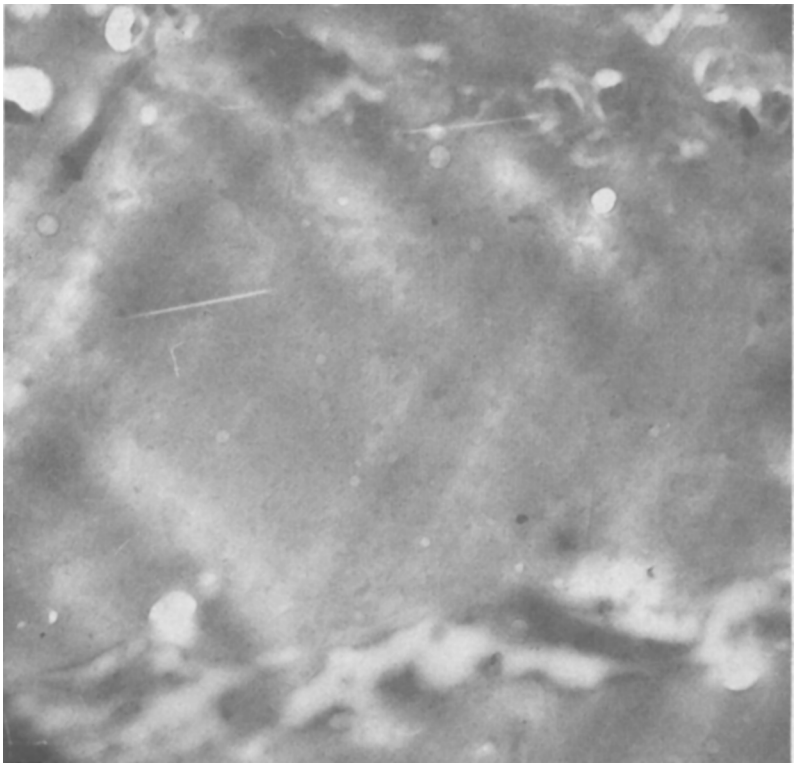

FIG. 4. Cell from lower stratum corneum with interdigitations between adjacent cells evident at its upper and lower border. Nucleus not identifiable in cells of this layer. All of the cytoplasm but the peripheral zone contains regularly arranged particles visible as a meshwork of straight lines. Methacrylate not removed. 10% formalin fixation. ×14500.

described. The cells from the lower stratum corneum are often slightly separated making the interdigitations between them conspicuous. These cells no longer contain nuclei; however, they do contain solid areas of regularly arranged rows of particles as in the nuclei of the cells of the Malpighian layer just described (Fig. 4 and 5). These areas of closely packed particles fill the entire cell except for a peripheral zone just within the cell border which includes the interdigitations and a narrow zone within. The peripheral area appears to be without structure. No particles have been identified in the intercellular space nor in the cells of the uppermost layers of the stratum corneum where the cornified cells are present as irregular partly separated strands.

Discussion. Electron micrographs of thin sections of a distinctive type of human skin papilloma reveal a close-packed array of spherical particles within the nucleus of the cells at one stage of development and in the cytoplasm at a later stage of development. These spherical particles are very easily demonstrated in ground suspensions of the tissue of the papilloma(1). The cells of this type of wart differ from those of the more common wart in that they contain an intranuclear inclusion body (as distinct from the nucleolus) and cytoplasmic masses as well as other features of abnormal cellular development(3).

Virus-like particles have been found only in the nucleus of cells of the Malpighian layer. And, indeed, there is nothing other than masses of virus-like particles identifiable in these nuclei, except the intranuclear inclusion body. The virus-like particles are generally arranged in regular rows. The intranuclear inclusion body presents an almost uniform cut surface and a sharp limiting border with no virus-like particles visible. In cells of the lower stratum corneum, where no

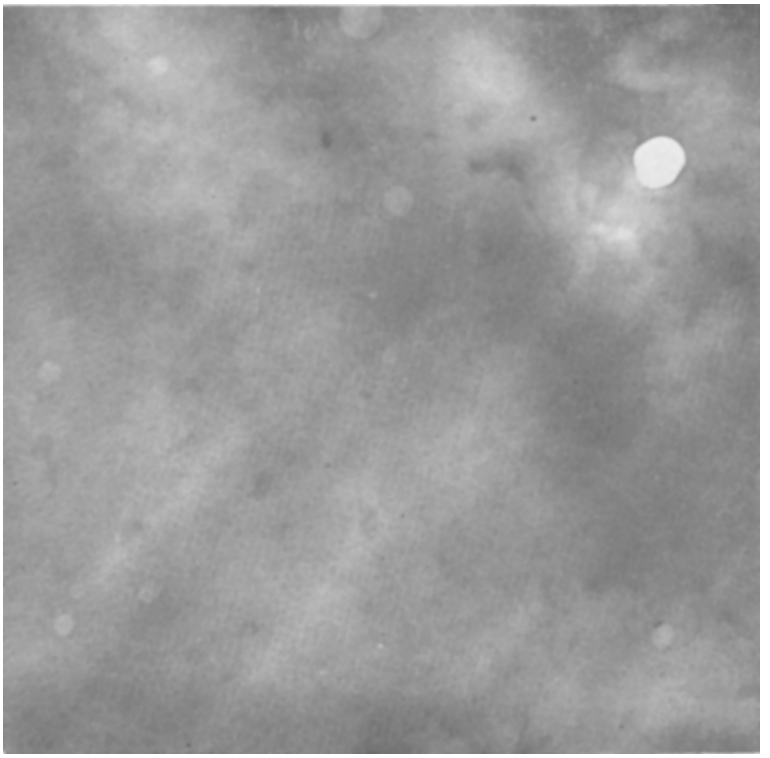

FIG. 5. Same cell as in Fig. 4, higher magnification. Orientation possible from hole in right upper corner. Lines resolvable into particles in close-packed array. 10% formalin fixation. ×30000.

nuclei can be seen the entire cytoplasm, except the outer rim, consists of a solid mass of particles with an arrangement similar to that in the nuclei from the Malpighian stratum. The possibility exists that the virus-like particles here have been derived from the nucleus which as a recognizable structure has disappeared. The appearances suggest, however, that the amount is greater than merely that which had previously filled the nucleus. Further examples of consecutive stages are necessary to determine this as well as to correlate the development of virus-like particles with the cytological and histochemical features already established. There are certain implications, however, inherent in the finding that the particles are generally present only in a close-packed array throughout a large portion of the volume of the cell; such an arrangement could result from the formation of particles *in situ* by a process of crystallization or by their alignment under restricting or confining pressure. Such structure is seen in preparations of macromolecular solids consisting of plant virus proteins which form true crystals(6,7). In preparations of virus-like particles from ground suspensions from these papillomas, the particles being spherical and of uniform size, aggregate in bunches at times quite apparently through the drying forces of the surface film. This is illustrated in Fig. 6, where they have been caught on one side of a collagen fibril. Although here and there a few particles appear to be grouped in a close-packed array the bulk do not. The preparation illustrated in Fig. 1A in the first publication on these papillomas(1) shows a close-packed array with at least 2 layers, suggesting a crystal-like formation. The perfection of structure here resembles that observed in the sections of cells. It is to be interpreted perhaps as an actual portion of a cell which had been incompletely ground and dispersed, although there has not been opportunity to verify this.

It is our belief that the particles actually

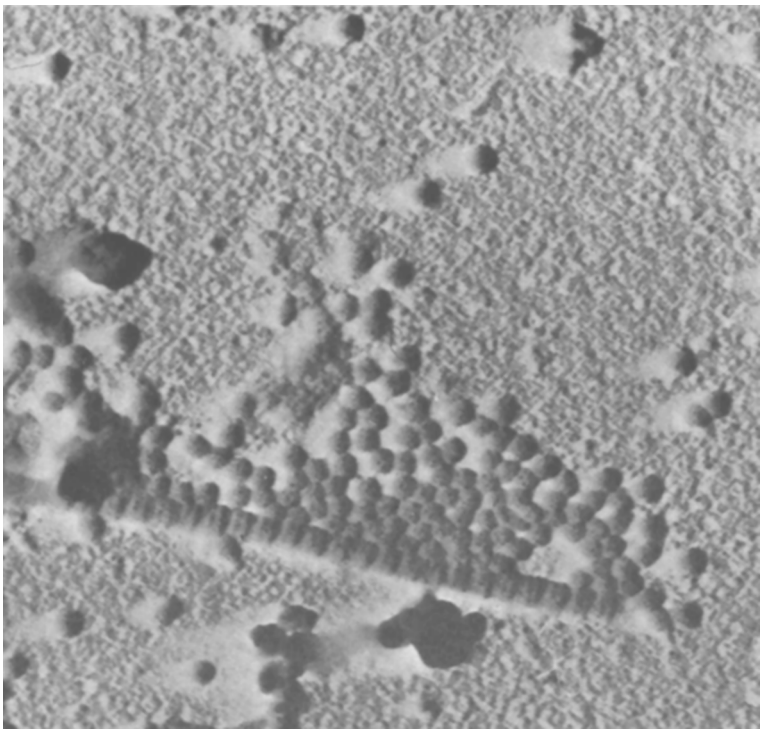

FIG. 6. Preparation of ground suspension of fresh papilloma tissue shown in section in Fig. 3. Shadowed with palladium (angle 1:7). Particles have come to lodge during drying on one side of collagen fibril contaminant from dermis. Loose packing is seen. ×50000.

represent virus particles and are the cause of the type of papilloma described. Inasmuch as this has not yet been established the term virus-like has been used for the particles.

Summary. Intracellular spherical particles have been identified under the electron microscope in thin sections of a particular type of human wart which yields similar particles in ground suspension. In cells of the Malpighian layer of the neoplastic epidermis constituting the papilloma, the particles lie in the nucleus, but have not been recognized in the cytoplasm or in the intranuclear inclusion body or in the discrete cytoplasmic masses characteristic of this type of wart. In cells of the lower stratum corneum of the wart where the nucleus is not recognizable the particles occupy all of the cell except for a narrow peripheral border. The arrangement of the particles is generally regular and like that of close-packed spheres.

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