tion over the blood vessels of the chorioallantoic membrane and area vasculosa as described elsewhere(5-7). A test period of 24 hours was used during which time the yolk sac tumors nearly doubled in size. The effect on growth was evaluated on the basis of the increased size of embryo and tumor which occurred in the control eggs during the period of the experiment. A total of 320 tumor-bearing eggs involving 12 separate experiments was used in these tests.

Results. Table I records the results with 2 Rain-lily extracts and aminopterin. Aqueous and alcohol extracts of the plant were ineffective. Negative results were also obtained with a solution of liquid pressed from the plant bulbs at high pressure.

It will be noted in the table that the Rain-lily extracts gave about the same order of inhibition of tumor growth as did aminopterin but without the inhibition of the embryo growth which characterized the action of the latter compound.

Discussion. The chemical nature of the effective agent in these Rain-lily extracts will be explored. It is known that an alkaloid with fungicidal action is present in plants of this group. To obtain this material, dry powdered bulbs are extracted with 95% ethanol (8). In the present work ethanol extracts of fresh bulbs were ineffective in the tests on cancer growth. There is apparently a seasonal factor involved since extracts of bulbs collected in the Austin area in the late Spring after the plant was advanced in growth were ineffective.

Summary. Extracts of Rain-lily bulbs have been tested for their effect on the growth of tumor and embryo of tumor-bearing eggs. The results obtained with aminopterin in the same tests are included for comparison. Acid aqueous and acetone extracts of the plant bulb inhibited tumor growth 81% and 94%, respectively, without affecting the embryo. Aminopterin inhibited tumor growth 87% and embryo growth 19%.

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Electronmicrographs of Thin Sections of Molluscum Contagiosum.* (18944)

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The morphology of *Molluscum contagiosum* has been extensively studied under the light microscope. The usual description consists of cells of the epidermis which are altered by the presence of cytoplasmic inclusion bodies (the Henderson-Patterson molluscum body) appearing first in the Malpighian layer and reaching maturity in the lower stratum corneum. Van Rooyen(1) has demonstrated by microdissection that the mature globular inclusion body is filled with elementary bodies lying in a gelatinous matrix. Rake and Blank (2), in tracing the development of the inclusion body, maintained that there is first a

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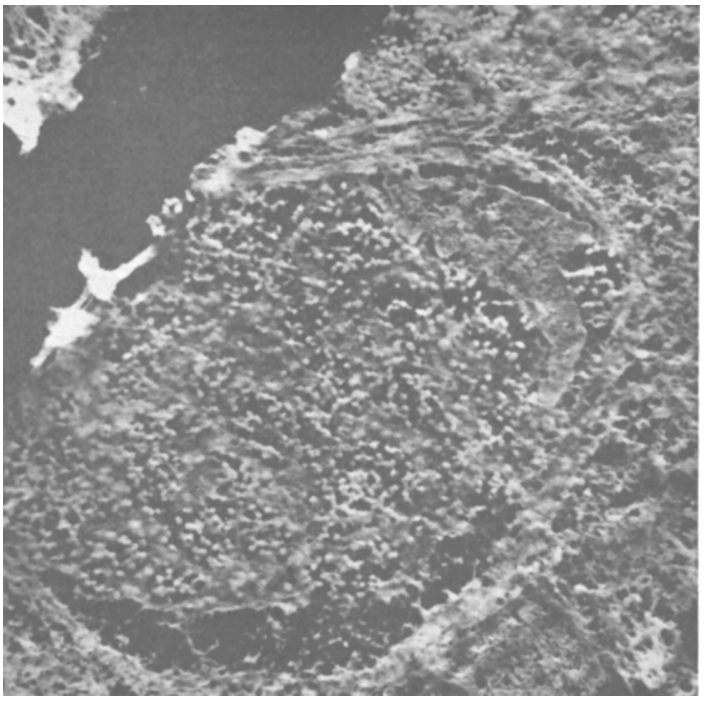

FIG. 1. Mature inclusion body in cell. The nucleus is shrunken and displaced to the side. × 4000.

general increase in cytoplasmic ribonucleic acid which is followed by the appearance of diffusely scattered islands of desoxyribonucleic acid. The latter enlarged to form the characteristic inclusion body within which these authors described septa containing ribonucleic acid. The elementary bodies from the supernatant portion of centrifuged material from the lesions of molluscum contagiosum have been found by electron microscopy to be brick-shaped(2-5) with average measurements of 330 mμ by 230 mμ(5). The present report extends the observations on the morphology and suggests possible stages in the development of the virus by the examination of thin sections of molluscum lesions with the electron microscope.

Methods. Four separate lesions of *Molluscum contagiosum* freshly removed with a curette were fixed in 4% buffered formalin for 2-3 hours. Pieces of tissue no larger than 1-2 mm on an edge were dehydrated in a graded series of alcohols and embedded in methacrylate (1 part methyl and 8 parts butyl), using 2-4 dichloro-benzoyl peroxide as a catalyst and incubation for 18-24 hours at 60°C(6). Sectioning was performed on a Spencer microtome adapted with a 1:20 wedge(7). The wedge and flywheel were counterbalanced and the microtome was driven by a motor(8). Sections from 0.05 to 0.2 μ thick were cut with a glass knife(9), mounted on collodion-covered grids, and, after removal of the methacrylate by xylol, were shadowed with palladium at an angle of approximately 1:7 before examination with an RCA electron microscope, model EMU. The illustrations

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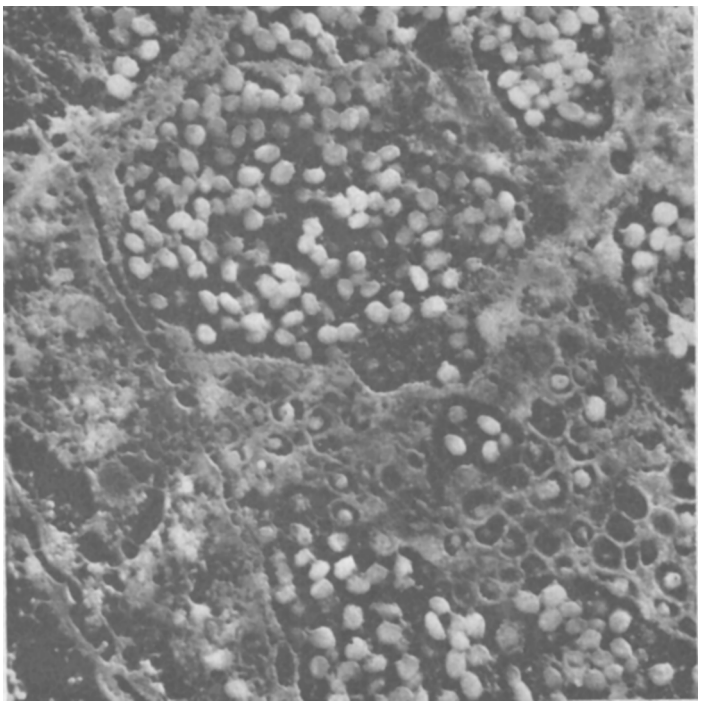

FIG. 2. Small portion of inclusion body. Locules containing discrete elementary bodies. Apparent segmentation within the matrix of septa with variation in form and size of the contained elementary bodies. × 11,000.

are enlargements from negatives made by contact with the original plates.

Results. In the prickle-cell layer of the epidermis, cells of the molluscum lesion contained within their cytoplasm varying numbers of elementary bodies which did not appear to be confined within a membrane and were fewer in number than in the mature inclusion bodies. In these cells the virus particles seemed to have no particular arrangement or localization, but were distributed at random within the cytoplasm. The shape of the elementary bodies, when only a few were present in the cell, often differed from that found in the mature inclusion bodies. Some were thinner in proportion to their length with typical measurements of 338 by 105 mμ, and at times were even lancet-shaped. The completely formed elementary bodies found in the more mature inclusions were generally smooth and more ovoid in outline than the early forms, with average measurements of 360 by 210 mμ. These are similar in appearance and size to elementary bodies obtained from suspensions of ground molluscum lesions.

The largest inclusion bodies are divided into compartments by septa with mature elementary bodies filling the locules between the septa (Fig. 1, 2). In some instances areas of the matrix composing these septa appear to be cut out into rounded or indented structures (Fig. 3). Some of these bodies are distinctly contoured and separated from the surrounding matrix by a more electron-translucent zone as seen in shadowed preparations (Fig. 3). All gradations in size and density can be found between these structures and the mature elementary bodies which would suggest that the latter may be formed by a segmentation and condensation of this matrix. In those early infected cells, where only a very few elementary bodies are present, no segmentation of matrix in the cytoplasm has been seen.

In several instances mature elementary bodies were found associated with smaller particles. In one preparation these particles closely resembled those pictured by Rake and Blank(2) but were somewhat smaller, measuring 76 x 61 mμ.

Further observations have been made on

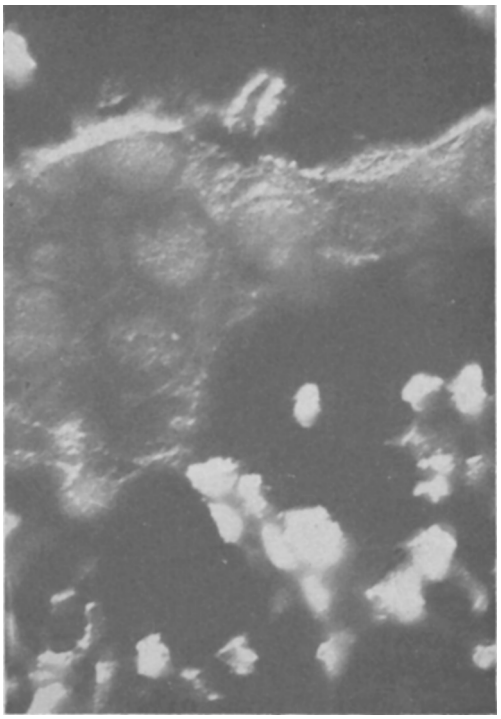

FIG. 3. Portion of septum with a few elementary bodies in adjacent locule. Segmentation of matrix of septum is evident. × 28,000.

the elementary body itself. In Fig. 4, there is a cluster of elementary bodies most of which have been cut twice leaving only a central segment which appears as a dense ring of material with an empty center. That this is a real observation appears certain from the many sections in which both intact and sectioned elementary bodies are seen. It is possible, then, that the elementary bodies of *Molluscum contagiosum,* at least in these instances, possess a formed cortex and a less dense interior.

Discussion. Thin sections of molluscum lesions examined with the electron microscope have suggested new concepts of the morphology and development of *Molluscum contagiosum.* The elementary bodies, in some instances, appear to have a dense wall and a less dense interior. The wall is of uniform density, of varied thickness and without demarcation between cortex and what might be adherent contents. Though the central portion, in some instances, appears empty, in others, apparently sectioned, no hollow is seen. It is possible then that there is a difference in the central portion of elementary bodies which may depend upon their stage of development. This is further suggested by the fact that hollow central areas are seen consistently in some groups of elementary bodies and not in others. It must be kept in mind, however, that it may also be possible for the bodies either to be cut or pushed to one side depending upon the sharpness of the knife and their orientation to the cutting edge.

Epidermal cells with only a few elementary bodies in their cytoplasm may be considered to represent an early phase of infection. There are all gradations from these to other cells that are almost completely filled by a well-defined inclusion body. When an abundant matrix separates the elementary bodies, it is often segmented into small areas of varying sizes and densities approaching that of the mature elementary body. In the larger inclusions, this matrix remains as thin septa yet within them the same process of segmentation and condensation can be observed.

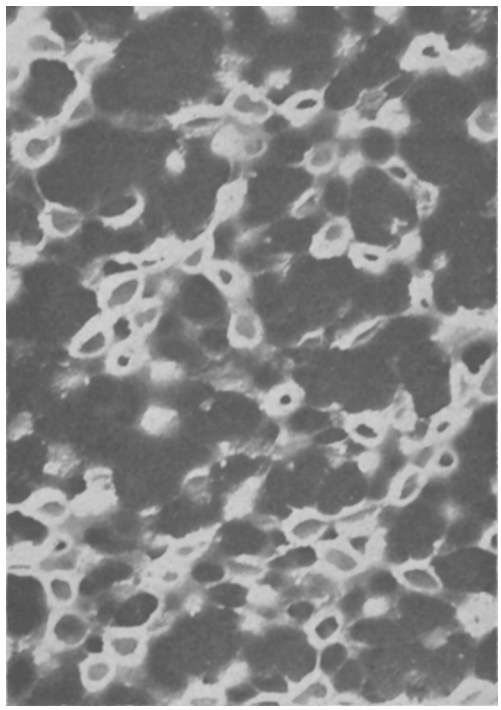

FIG. 4. Elementary bodies that have been sectioned. Note dense wall and apparently empty interior. × 34,500.

The septa are the thin hematoxylin-stained lines that can be seen crossing the inclusion body in hematoxylin-eosin preparations. Rake and Blank(2) have considered these septa to contain ribonucleic acid, basing this conclusion upon staining with pyronin-methyl green. Kurnick(10) has demonstrated that depolymerized desoxyribonucleic acid stains with pyronin similarly to ribonucleic acid. The locules between the septa, where the mature elementary bodies lie, contain desoxyribonucleic acid since these regions are stained by the Fuelgen method(2). It seems apparent, then, that the site of formation of the elementary bodies is in the material which is selectively stained with pyronin.

In the early infected cells, many of the elementary bodies are thinner than most of those found in the mature inclusions and in addition lancet shaped forms are seen. There is also an apparent absence of segmented matrix in the cytoplasm of these early infected cells which leaves the origin and fate of the elementary bodies at the early stage of infection purely speculative. It should be noted that these latter irregularly shaped bodies, which have been considered here to be elementary bodies, differ in appearance from structures that have been interpreted as mitochondria in epidermal cells and further that they have never been seen in thin sections from control material from the epidermis examined with the electron microscope.

Summary. Thin sections of *Molluscum contagiosum* lesions from man have been examined with the electron microscope. Cells were seen in the prickle cell layer of the epidermis which contained only a very few cytoplasmic elementary bodies. These are considered to be early infected cells. Cells were seen with varying numbers of elementary bodies scattered through the cytoplasm while others were entirely filled with elementary bodies forming a mature inclusion. The elementary bodies in the early infected cells differed somewhat in shape from most of the elementary bodies seen in the larger inclusions. The mature inclusion bodies were divided into locules by septa. The locules contained mature elementary bodies. The elementary bodies appear to form from the material composing the matrix of the septa by a process of segmentation and condensation. The elementary bodies themselves were sectioned and appeared in some instances to have a formed cortex with a less dense interior.

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Chemical Differentiation of Cartilage and Bone. II. Sulfatase Activity.* (18945)

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The presence of the polysaccharide, chondroitin sulfuric acid, has been recognized in cartilage for many years. From time to time the possible role of this material in the calcification mechanism of cartilage has been raised. That calcium might be selectively bound to the organic portion of cartilage was suggested a number of years ago by Freudenberg and György(1). More recently the hypothesis(2-4) has been brought forward

* Aided by grants from Mead Johnson and Co., and the Damon Runyon Cancer Fund.

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