Results. The objective was to determine whether cells infected with the labeled virus at the beginning of S, M and G_1 phases retained the labeled virus particles when allowed to proceed to enter in other phases of the cell cycle. Cells infected with labeled virus at M and G_1 did not contain any significant number of grains. Cells infected at S but harvested after 4 and 6 h from the onset of infection period retained grains primarily in the cytoplasm (Figure 1). Cells which were harvested 8 h or later showed intranuclear accumulation of labeled virus particles (Figure 2). It was also observed that cells infected by labeled SV40 at S phase and then arrested at metaphase retained grains primarily on the chromosomes (Figure 3).

Discussion. It has been shown that the anti-viral action of interferon could not protect cells from transformation by SV40 if a cycle of cellular DNA synthesis had occurred in the infected cells prior to the addition of interferon[10]. In addition it has been suggested that non-dividing cells are resistant to virus induced transformation[11]. Our results suggest that cells in the DNA synthetic phase of the cell cycle are the only ones which interact with viral DNA and that viral genomes retained by these cells as they proceed further in the cell cycle. The association of grains with the metaphase chromosomes, after infection of cells at S phase, indicates that viral DNA localizes on the chromosomes. It should be pointed out that retention of labeled viral DNA on the chromosomes may not mean that the entire genetic material of the virus is integrated into host DNA. However, it has been shown that a portion of viral DNA is integrated in the host genome in viral transformed cells[3–5]. Localization of tritium-labeled adenovirus 12 on metaphase chromosomes has been demonstrated using autoradiographic methods[12–14]. It has recently been demonstrated that rat embryo cells synthesizing DNA are preferentially infected in vitro by rat virus[15].

From these observations it has become evident that infection of cells by SV40 is influenced by the physiological state of the host cell and that cells engaged in DNA synthesis appear to be the only ones vulnerable to viral action. These observations further suggest that chromosomes are primary sites of viral action. It would be interesting to see whether selective distribution of virus DNA on the chromosomes synthesizing DNA can be achieved by infecting cells at different stages in the progression of DNA synthesis[16].

Zusammenfassung. Autoradiographische Untersuchungen an menschlichen, diploiden, synchronisierten Zellen, in der S-Phase mit Tritium markiertem SV40 infiziert, zeigten intranukleären Einbau von markierter DNS (in Metaphase vor allem Chromosomenmarkierung). Keinerlei Kernmarkierung wurde beobachtet, wenn die Zellen in anderen Phasen ihres Zyklus infiziert wurden.

D. MUKERJEE and J. M. BOWEN

Department of Pathology, The University of Texas Medical Branch, Galveston (Texas 77550, USA), and Department of Virology, The University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston (Texas 77025, USA), 13 October 1970.

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Two Types of Viruslike Particles in *Drosophila* Midgut

The midgut of the adult, wild type, Canton-S strain *Drosophila melanogaster* was the subject of electron microscopic investigations concerning the nature of submicroscopic alterations, studied at graded intervals of time, subsequent to ^{60}Co γ-radiation. During these investigations hexagonal, viruslike particles (VLP) of 2 different sizes were observed, 1 in the nucleus and the other in the cytoplasm of some cells (Figure 1).

Lightly etherized flies were dissected in cold, 5% glutaraldehyde, and the midgut was immediately transferred to a cold, one per cent solution of osmium tetroxide[1] buffered at a pH of 7.4. Subsequent to dehydration procedures in a graded series of alcohols and finally propylene oxide, the tissue was embedded in Epon 812, sectioned either on a Porter Blum hand ultramicrotome or on an LKB automatic ultramicrotome, and mounted on bare copper grids. The sections were stained with uranyl acetate[2] for 10 min, followed by lead citrate[3] for 6 min, and then viewed with an RCA EMU-3F electron microscope.

The presence of slightly elliptical[4,5] and spherical[6] viruslike particles of uniform size have already been reported in larval and in adult tissues, as well as in cell cultures of *Drosophila*. None of the previous reports indicated possible size differences between nuclear and cytoplasmic VLP, and all 3 groups of investigators showed similar electron micrographs, for the spherical VLP of one[6] were slightly elliptical in shape. Such variations in the morphology of these particles were also evident in this study, but were attributed to the plane of sectioning, for in some instances clearly hexagonal shapes were observable (Figures 2 and 3), each containing a dense, central core. The nuclear VLP were 565 ± 21 Å in dimension and those in the cytoplasm were 727 ± 23 Å ($P \ll 0.001$), when the distances between opposing sides were measured. The dimensions evident in the present material are considerably larger than those previously reported[4–6]. The VLP may be found

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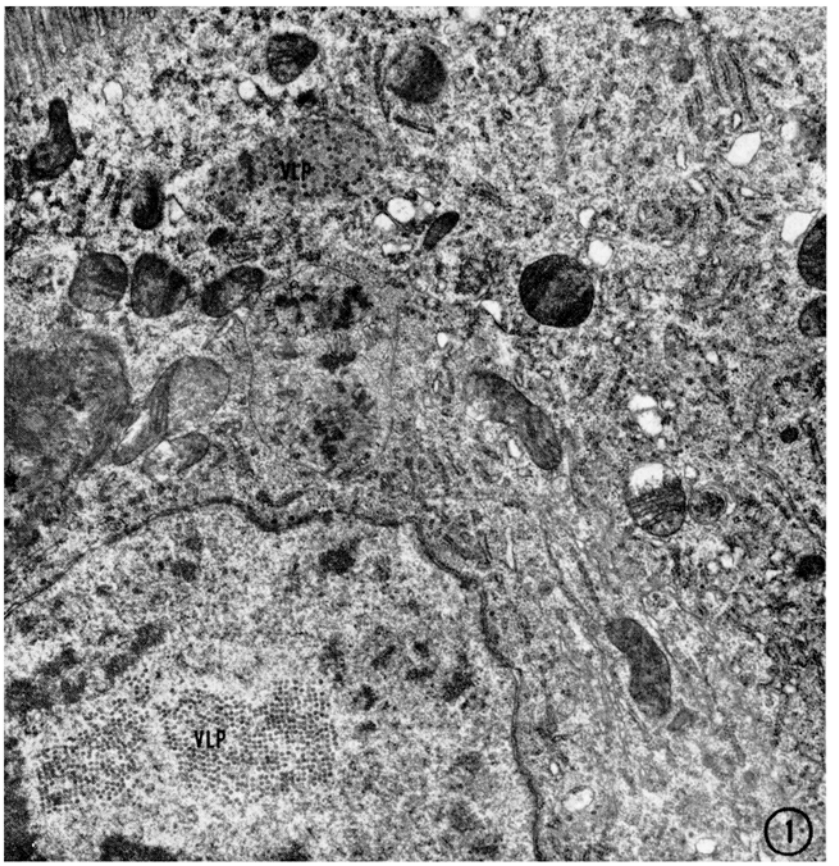

Fig. 1. Electron micrograph of a portion of a cell of *Drosophila* imago midgut. Viruslike particles (VLP) are present in the nucleus as well as in the cytoplasm. ×13,000.

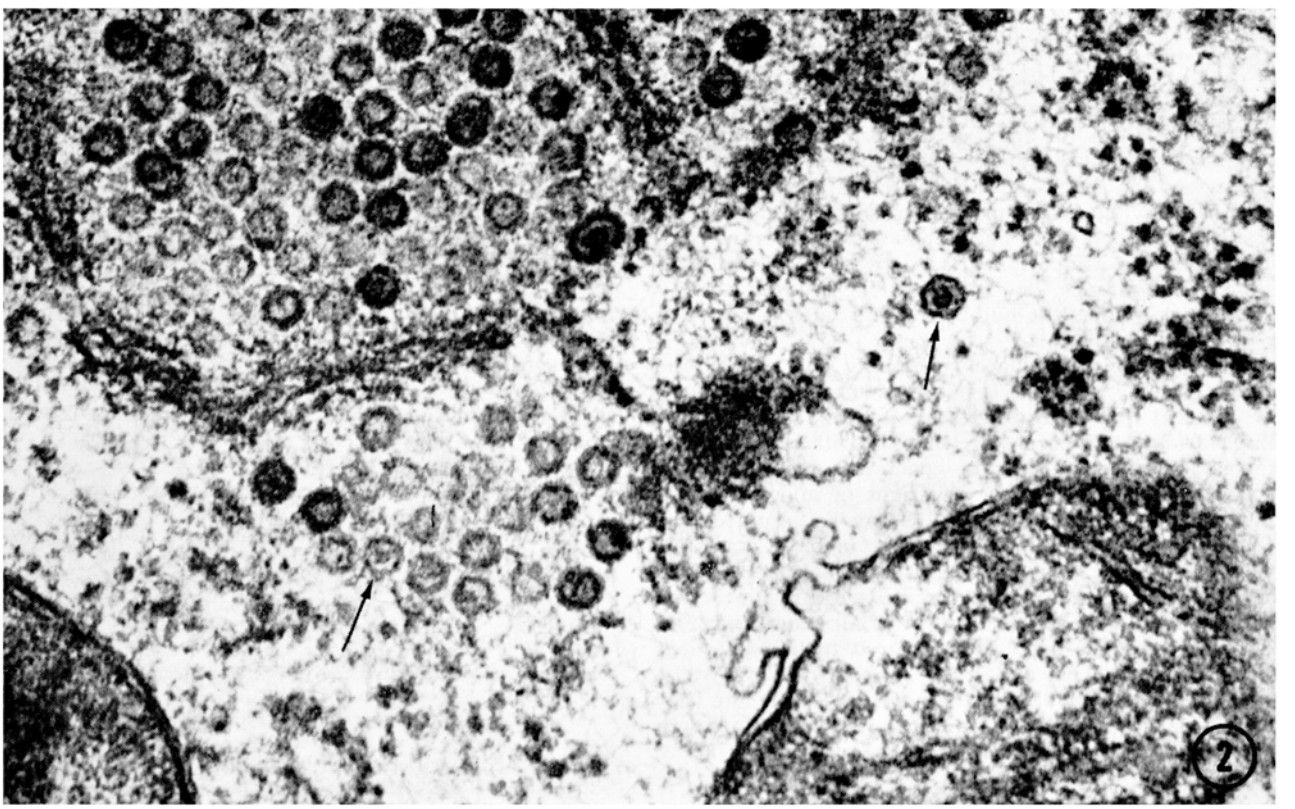

Fig. 2. Electron micrograph showing viruslike particles in the cytoplasm of adult *Drosophila* midgut cell. Note hexagonal VLP and its central core. ×84,800.

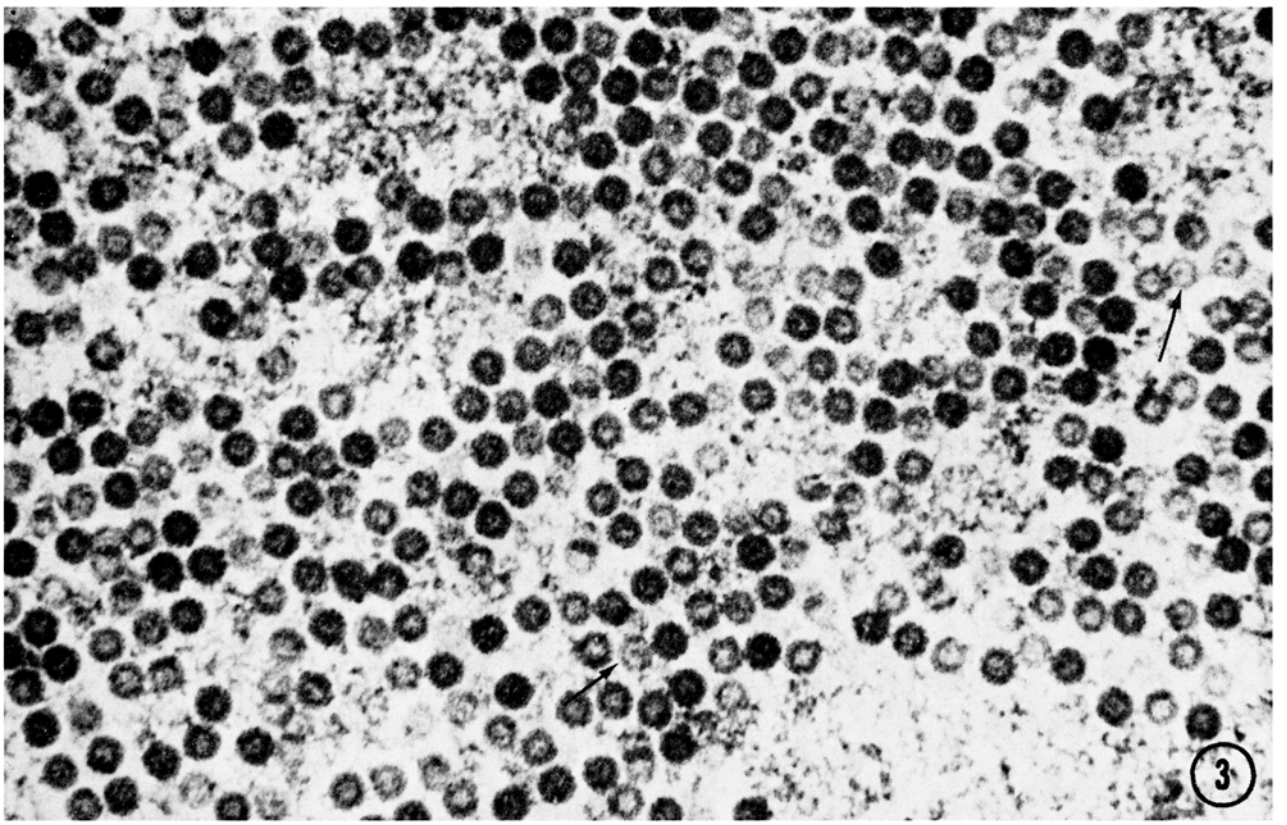

Fig. 3. Electron micrograph of nuclear viruslike particles in the nucleus of the cell in the previous figure. Note the nearly hexagonal VLP and its central core. ×84,800.

in various kinds of arrangements, both in the nucleus and the cytoplasm, ranging from random clusters to very highly organized aggregates.

Interestingly, the VLP were first seen in the nucleus, and as the fruitfly ages they subsequently appear in the cytoplasm. Both the number of VLP per cell and the number of cells containing such particles increase with the age of the fly[7]. Viruslike particles are not normally seen in the midgut of young *Drosophila*, although nuclear VLP were noted in a single instance in a young fly[7]. Exposure to γ-irradiation utilized by us accelerated the time of appearance, and increased the number of VLP per cell, as well as the number of cells infested by these particles.

One can only speculate concerning the nature and origin of these particles. It has been suggested that they are present only in dividing cells[4], and while there are implications in the literature that the cells of *Drosophila* imago midgut might divide, concrete evidence for this is lacking. Not once were any signs of midgut mitosis noted in our material. Hence, it may be assumed that these viruslike particles are present in most organs of the fruitfly. Evidence for this is accumulating[4-7], which suggests that these VLP are transmitted through the egg and perhaps the sperm. Transovarial transmission of insect viruses has been demonstrated to be a frequent phenomenon[8]. Then, one might suppose, these particles have to pass through a period of incubation before they can begin the process of replication. Radiation might act as a trigger which shortens the incubation time of the VLP.

Since a considerable size difference does exist between the nuclear and cytoplasmic viruslike particles, it is improbable that those in the nucleus pass through the nuclear pore, proliferate and give rise to the cytoplasmic VLP. Instead it is simpler to assume the existence of 2 different viruslike particles, one restricted to the nucleus and the other to the cytoplasm[9].

Résumé. On a trouvé des particules hexagonales, comme des virus, de deux grandeurs, dans les cellules de la partie centrale de l'intestin de la *Drosophile*. Les particules les plus grosses étaient dans le cytoplasme et les plus petites dans le noyau. L'apparence de la distribution de ces particules sont en fonction de l'âge de la mouche.

LESLIE P. GARTNER[10,11]

Department of Histology and Embryology,
Baltimore College of Dental Surgery,
School of Dentistry, University of Maryland,
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[9] Work performed during the period of an United States Public Health Service Fellowship in the Department of Zoology and Physiology, Rutgers University, Newark, N.J. The electron microscopy was performed at the Max Wachstein Research Laboratory of the Beth Israel Hospital, Passaic, N.J. I wish to thank Dr. B. P. SONNENBLICK of the University and Dr. H. J. SOBEL and Mr. E. MARQUET of the Hospital for their interest and encouragement.

[10] Present address: Department of Histology and Embryology, Baltimore College of Dental Surgery, School of Dentistry, University of Maryland, Baltimore (Md. 21201, USA).

[11] Part of this work was supported by research grant No. AM 12818 of the National Institute of Arthritis and Metabolic Diseases.