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EFFECT OF VIRAL DNA SYNTHESIS OF POXVIRUS UPON NUCLEAR DNA SYNTHESIS OF SYNCHRONIZED FL CELLS

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SUMMARY The relationship between the cell cycle of FL cells and the multiplication of cowpox virus and ectromelia virus was studied in a synchronous cell culture using double thymidine treatment and autoradiography of ^{3}H-thymidine.

Nuclear DNA synthesis of the synchronizing cells showing viral DNA synthesis was definitely, but not completely, suppressed, and occurred exclusively during the physiological " S " phase of these cells. The mitotic figure could also be seen in some cells showing viral DNA synthesis. No disturbance in the chronology of nuclear DNA synthesis in cells showing viral DNA synthesis was observed. The cell cycle did not affect the multiplication of these viruses.

INTRODUCTION

The occurrence of viral DNA synthesis in the cytoplasm of cells infected with poxvirus has been shown by autoradiography of ^{3}H-thymidine. (MAGEE 1960, KATO *et al.* 1960a, b, CAIRNS 1960). For such studies the poxvirus has the advantage that viral DNA synthesis is easily distinguishable from nuclear DNA syntheis in cells at a cellular level by autoradiography of ^{3}H-thymidine. However the interpretations by previous authors of the effect of virus infection upon nuclear DNA synthesis are somewhat conflicting. Thus CAIRNS (1960) reported that at least during the first 9 hours after infection, the cell continues to produce its own nuclear DNA, while Magee (1960) found that infected cells ceased making DNA soon after viral DNA synthesis is complete. KATO *et al.* (1960a, b) concluded that nuclear DNA synthesis is suppressed in virus-infected cells, since the number of silver grains in the nuclei of cells showing viral DNA synthesis is never as high as that in the nuclei of cells without viral DNA synthesis. These observations were qualitative. Quantitative analysis of autoradiograms of cells infected with cowpox virus revealed that immediately viral DNA synthesis begins, nuclear DNA synthesis is suppressed (KATO *et al.* 1962, 1964). Quantitative analysis of autoradiograms of poxvirus infected cells has been extended to the interaction between cells and oncogenic poxviruses such as Shope fibroma virus (KATO *et al.* 1965, 1966a), and molluscum contagiosum agent (TANIGAKI and KATO 1967). It was concluded that nuclear DNA synthesis of cells showing viral DNA synthesis of poxviruses, whether oncogenic or nononcogenic, was definitely suppressed (KATO *et al.* 1966b). KIT *et al.* (1963) also observed suppression of nuclear DNA synthesis in cells infected with vaccinia virus using both auto-

radiographic and biochemical methods.

We have, however, noticed that nuclear DNA synthesis of cells showing viral DNA synthesis did not cease completely, although it was suppressed, as shown in a figure in the paper of KATO *et al.* (1964). In other words, there was some nuclear DNA synthesis in cells showing viral DNA synthesis.

The significance of this nuclear DNA synthesis in cells showing viral DNA synthesis can be explained by studying the following possibilities: (1) Nuclear DNA synthesis proceeded slowly for more than the period of the physiological S phase; (2) Nuclear DNA synthesis occurred at random throughout both the S and G phase, due to disturbance of metabolism induced by virus infection; (3) The degree of nuclear DNA synthesis was lowered, while the cycle of nuclear DNA synthesis was not affected.

With recent improvements in methods for synchronization of cells (TERASHIMA and TOLMACH 1961, XEROS 1962, BOOTSMA *et al.* 1964) more precise analysis of this problem is possible. In the present study, cells synchronized by double treatment with excess thymidine (BOOTSMA *et al.* 1964) were used to clarify the relationship between the cell cycle, nuclear DNA synthesis and viral DNA synthesis.

MATERIALS AND METHODS

1. *Virus*

Cowpox virus (LB red strain carrying the "A"v^- marker) and ectromelia virus (Hampsted strain carrying the "A"v^+ marker) were used. FL monolayer cultures were infected with virus. Twenty four hours later infected cell suspensions were made by policeman and sonicated (Kubota 200 watt, 9KC, 10 min). After centrifugation at 3,000 rpm for 20 min, the supernatant fluids were stocked at $-70°C$ as virus material.

2. *Virus titration*

Cowpox virus and ectromelia virus were routinely assayed on chick fibroblast cultures (CF) with agar overlay using five 50 ml prescription bottles (samll bottle) for each dilution. Plaques were stained with 0.02 per cent of neutral red at 72 hrs for cowpox and at 6 days for ectromelia virus and were visible to the naked eye. The multiplicity of infection (moi) was then determined as the ratio of CF plaque-forming units (PFU) to the number of cells present at the time of infection.

3. *Preparations of cloned cells*

The FL cell line was maintained as monolayer cultures in rubber stoppered 200 ml glass prescription bottles (large bottle). The growth medium consisted of Eagle's minium essential medium (MEM) and 10 per cent calf serum with 100 IU penicillin and 0.10 mg streptomycin per ml. Monocellular suspensions were prepared by 0.02% EDTA (ethylene diamine tetraacetate) in PBS (phosphate buffered saline). The number of cells in the cell suspension to be used was counted in hemocytometer. Five hundred cells per ml of each suspension to be cloned were seeded in Petri dishes. The cells were incubated for 7 days at 37°C in an environment of controlled humidity and CO_2 pressure. During this period the fluid was replaced twice by fresh growth medium. Then each colony was transfered to the small Petri dishes using OKUMURA's method (OKUMURA *et al.* 1964, OKUMURA 1967). These procedures were repeated three times. The clones were transfered to the large bottles.

4. *Treatment with excess thymidine and assay method in Leighton tubes*

The cloned cells were dispersed in 70 Leighton tubes with cover glasses on the bottom.

After one day, growth medium was replaced by medium containing 2.5 mM thymidine (Nutritional Biochemicals Corporation). After 20 hrs, the cultures were washed three times with Hanks' solution and again incubated at 37°C for 20 hrs. Then they were again treated with excess thymidine. After this double treatment fluid was replaced by normal growth medium. Replacement of medium and washing procedures were performed as fast as possible at 37°C.

For assay of synchronization, the percentages of cells synthesizing DNA was determined in cultures by pulse labeling with tritiated thymidine. As shown in Fig. 1, the cover slips of two Leighton tubes were taken out every two hours after removal of the second thymidine solution. One hour before taking out the coverslips, the growth media of the two Leighton tubes were replaced by prewarmed

medium containing 1 μc ^{3}H-thymidine per ml (specific activity 5 c/mM). During exposure of cells to ^{3}H-thymidine solution, for 1 hr, Leighton tubes were kept in a water bath at 37°C. Then cover slips were washed with Hanks' solution and cells were fixed with methanol. These cover slips were autoradiographed.

5. *Virus infection of synchronous cultures in Leighton tubes*

As shown in Fig. 1, another series of Leighton cultures treated with excess thymidine was infected either with cowpox virus or ectromelia virus.

Four cultures were infected with virus every 2 hours after removal of excess thymidine (Tdr). After one hour's adsorption, cells were washed three times with Hanks' solution and incubated with growth medium. Seven and nine hrs after infection, the cover slips of two Leighton tubes were taken out. One hour before taking out the cover slips, the growth medium was replaced by prewarmed medium containing ^{3}H-thymidine. The concentration of the isotope and all subsequent procedures were as in the assay of noninfected synchronous cultures.

6. *Autoradiography*

Cover slips were treated with 2 per cent perchloric acid at 4°C for 40 min to remove unincorporated ^{3}H-thymidine. Dipping autoradiography was carried out with Kodak NTB2 nuclear emulsion. The exposure time was 3 days. Giemsa was employed for post staining to demonstrate the " B " type inclusions of poxvirus. The percentages of labeled cells and mitoses was estimated on at least 500 cells.

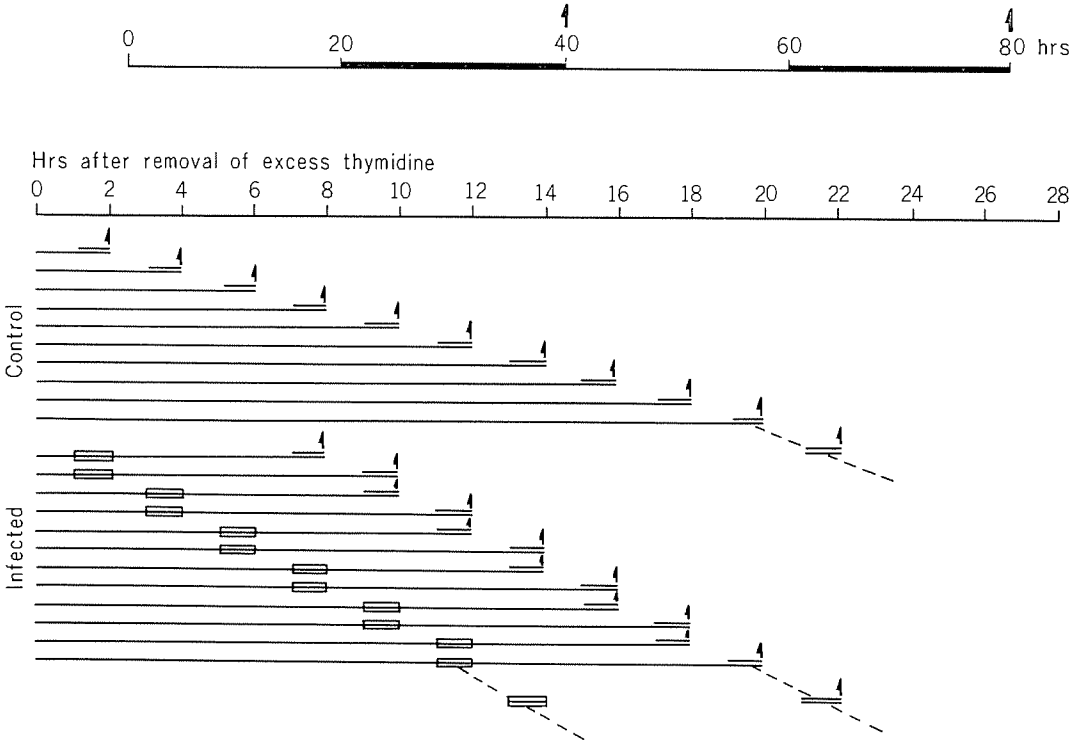


FIGURE 1 Schemes of experiment on double thymidine treatment and virus infection in Leighton tubes. Two Leighton tubes were used at each point.

thymidine 0.625 mg/ml — virus infection — ^{3}H-thymidine 1 μc/ml
growth medium — removal of excess thymidine — removal of sample for assay

7. *Virus titration of infected synchronous cultures in 50 ml prescription bottles (small bottles)*

Cloned cells were dispersed in 16 small bottles. Double thymidine treatment was carried out as described above. Autoradiography of ^{3}H-thymidine in assay of synchronous cultures was carried out as follows. As shown in Fig. 2, one, three, eleven and twenty hours after treatment with excess thymidine, cells from two small bottles were submitted to autoradiography. One hour before removal of cells, the growth medium was replaced by medium containing 1 μc of ^{3}H-thymidine, as mentioned above. Then cells were rinsed twice with PBS and removed from the glass by EDTA treatment. Cells were centrifuged at 1,000 rpm for 10 min and resuspended in acetic-alcohl fixative (one part acetic acid and 3 parts ethyl alcohol) and recentrifuged at low speed. The fixative was decanted and fresh fixative was introduced to wash the cells. The procedure was repeated three times. Then one droplet of this fixed cell suspension was placed on a clean cold cover slip on the surface of which the suspension quickly spread out. Autoradiography was carried out on the cover slip, as mentioned above.

With the other half series of synchronous cultures, experiments were made on virus infection, as shown in Fig. 2. The procedure used for virus infection was the same as that in the Leighton tubes. Nine hours after infection, cells were harvested for virus titration.

RESULTS

1. *Synchronous synthesis of nuclear DNA of FL cells*

Synchronous synthesis of nuclear DNA of cells was similar to that reported by BOOTSMA *et al.* (1964). Pulse labeling with tritiated thymidine showed that after double thymidine treatment, 80–90 per cent of the cells were in the S phase between 3–4 hrs after removal of excess Tdr and less than 10% of the cells were in the S phase between 14–23 hrs after removal of excess Tdr (Figs. 3 and 4). Fig. 3 shows the kinetics of the percentages of uninfected labeled cells and mitoses after removal of excess Tdr. The experiment in Fig. 3 was carried out as a control for cowpox virus in-

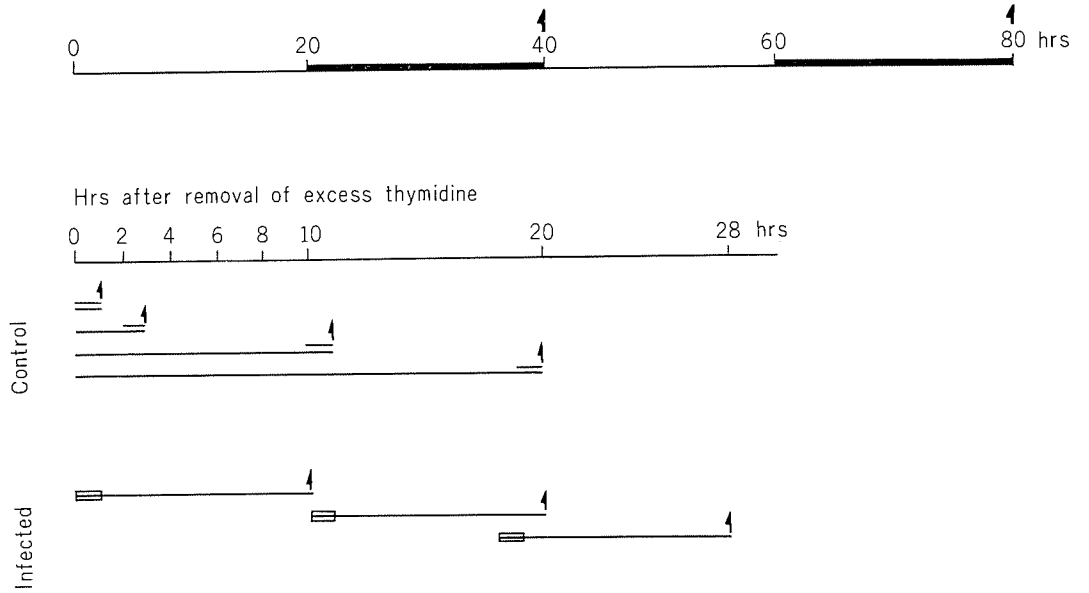
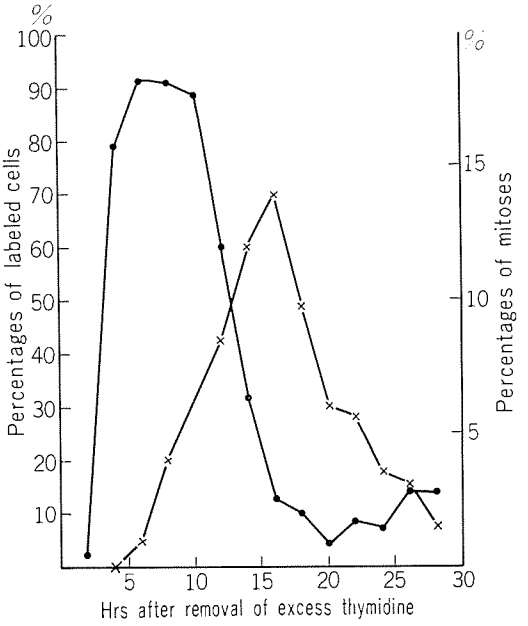


FIGURE 2 Schemes of experiments on double thymidine treatment and cowpox virus infection in 50 ml prescription bottles. Bottles were used for measuring infectivity of progeny virus at each point.
thymidine 0.625 mg/ml ▭ virus infection ═ ^{3}H-thymidine —— growth medium
removal of excess thymidine removal of samples for assay

fection. The experiment in Fig. 4 was carried out as a control for ectromelia virus infection. The peak of the mitotic burst in Fig. 3 occurred between 15 and 17 hrs after removal of excess Tdr and in Fig. 4 between 11 and 13 hrs after removal of excess Tdr. The period of the S phase and the mitotic index in Fig. 3 are slightly different from those in Fig. 4. This may be because the cell line of Fig. 4 was cloned from that of Fig. 3 (which had been cloned once by the method described before). Figs. 13, 14 and 15 show synchronous FL cells in the S phase, in mitosis and in the G_1 period, respectively.

2. *Effect of cowpox virus on nuclear DNA synthesis of synchronous FL cells*

These experiments were designed to compare the nuclear DNA synthesis of synchronous FL cells infected with virus with that of non-infected cells. Cowpox virus was inoculated at 7×10^6 PFU per ml. One half ml of the virus inoculum was introduced into each Leighton tube. There were about 3×10^5 cells in Leighton tube just after removal of excess Tdr and the number increased to about 6×10^5 cells 20 hours after removal of excess Tdr. Therefore, multiplicity of infection was about 10 and decreased to about 5 later. At each point, cells were divided into 3 groups, that is, cells showing cytoplasmic DNA synthesis, cells showing both cytoplasmic and nuclear DNA synthesis and cells showing only nuclear DNA synthesis. Since the areas of cytoplasmic viral DNA synthesis correspond exclusively to the " B " type inclusions of poxvirus (KATO *et al.* 1960a, b, 1964), the term " B " type inclusion-bearing cells (" B "-bearing cells) is henceforth used to represent cells showing cytoplasmic DNA synthesis. The percentages



FIGUR 3 Effect of double thymidine treatment on percentages of FL cells in S phase and in mitosis. This experiment was done as a control of the FL cell-cowpox virus system.
●——● cells with labeled nuclei
×——× cells in mitosis

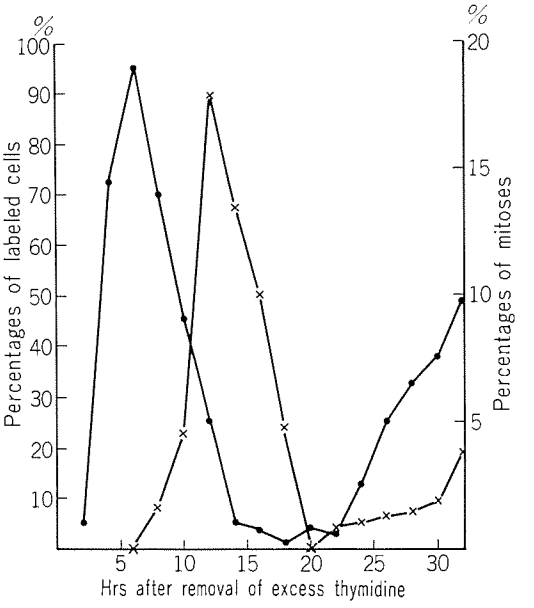


FIGURE 4 Effect of double thymidine treatment on percentages of FL cells in S phase and in mitosis. This experiment was done as a control of the FL cell-ectromelia virus systems.
●——● cells with labeled nuclei
×——× cells in mitosis

of cells in each group were counted as follows. (1) Percentage of " B "-bearing cells among total cells. (2) Percentage of " B "-bearing cells with labeled nuclei in total " B "-bearing cells. (3) Percentage of cells without " B " inclusions but with labeled nuclei in total cells without " B " inclusions. Cells with labeled nuclei with grain counts above ten per nucleus were considered to be labeled. The percentages were calculated from more than 500 randomly selected cells. These percentages are plotted in Figs. 5 and 6. The percentages of cells with labeled nuclei in the control noninfected synchronous culture were also counted and are plotted in these figures.

There were 70% to 90% " B "-bearing cells throughout the experiment, although the percentage was a little higher in Fig. 6 than in Fig. 5. This indicates that the cycle of nuclear DNA synthesis does not affect viral DNA synthesis in the infected cells. On the other hand, the three curves for the kinetics of the percentages of cells with labeled nuclei are almost identical. Thus nuclear DNA synthesis of cells with and without " B " inclusions in infected synchronous FL cells can

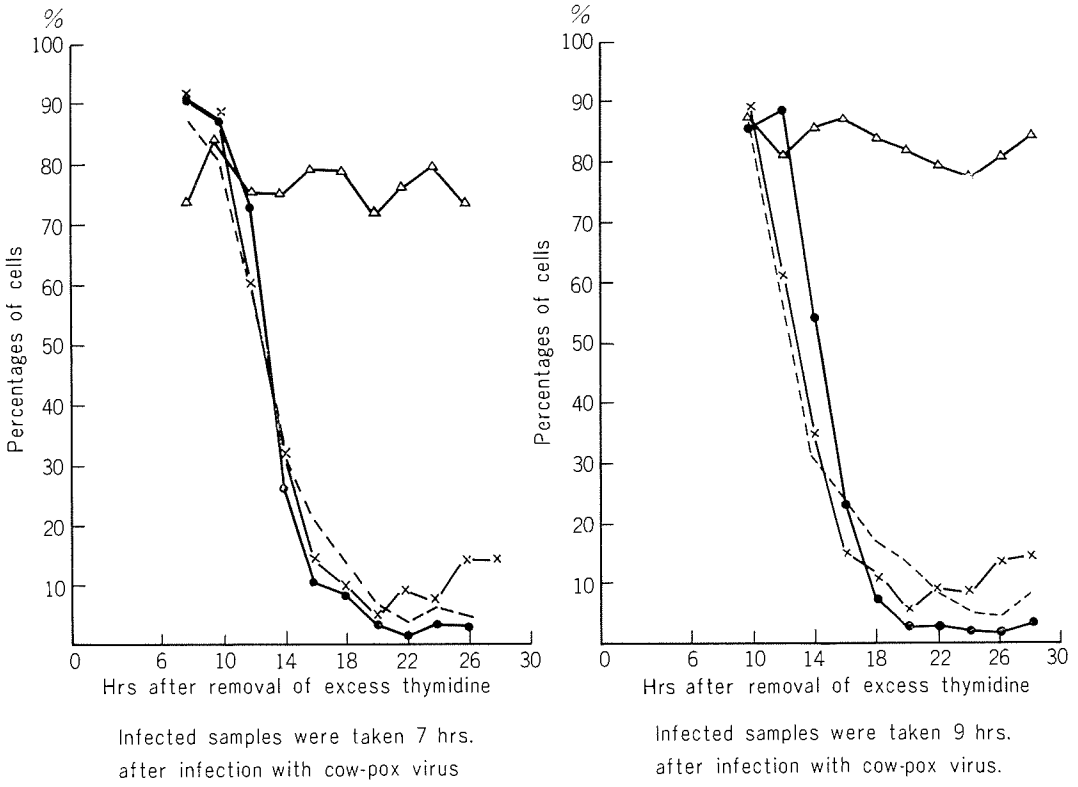


FIGURE 5 and 6 Effect of viral DNA synthesis upon nuclear DNA synthesis of synchronous FL cells infected with cowpox virus. The percentages of " B "-bearing cells in the infected culture are also shown. Multiplicity of infection is between 10 and 5. Percentages of non-infected cells with labeled nuclei are plotted from Fig. 3. Cells with grain counts above 10 per nucleus were taken as labeled.

×——× non-infected cells with labeled nuclei
●——● " B " -bearing cells with labeled nuclei
△——△ " B " -bearing cells
--------- non-" B " -bearing cells with labeled nuclei

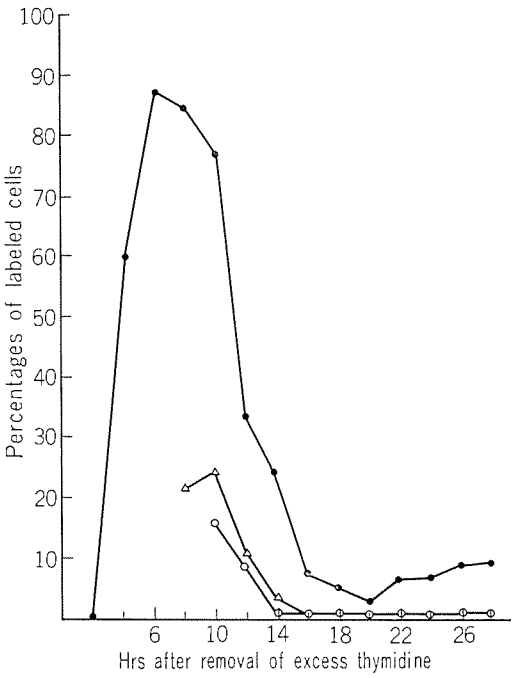


FIGURE 7 Effect of viral DNA synthesis upon nuclear DNA synthesis of synchronous FL cells infected with cowpox virus. Cells with grain counts above 50 per nucleus are considered as labeled.
●——● non-infected cells with labeled nuclei
○——○ " B " bearring cells with labeled nuclei (samples 9 hrs after virus infection)
△——△ " B "-bearing cells with labeled nuclei (samples 7 hrs after virus infection)

proceed in the physiological S phase of these cells.

To compare the number of silver grains per nucleus in cells bearing " B " inclusions with that of control uninfected cells, the cells with labeled nuclei with a grain count above 50 rather than 10 per nucleus, were counted as labeled this time. The kinetics of the percentages of labeled nuclei are shown in Fig. 7. The peak of the percentage of " B "-bearing cells with labeled nuclei became low, while the curve of the kinetics of the percentages of control uninfected cells with labeled nuclei remained almost unchanged. Thus nuclear DNA synthesis of " B "-bearing cells proceeds slowly in the physiological S phase.

3. *Effect of ectromelia virus on nuclear DNA synthesis of synchronous FL cells*

An experiment similar to that with cowpox virus was carried out with the ectromelia virus-synchronous FL cell system. The ectromelia virus was inoculated at 4×10^6 PFU/ml. One half ml of the virus inoculum was introduced into each Leighton tube. There were about 2×10^5 cells per Leighton tube was just after removal of Tdr and the number increased to about 4.5×10^5 cells at the time of the last virus inoculation which was 22 hrs after removal of Tdr. Therefore the multiplicity of infection was about 10 and decreased to about 5 later. Subsequent procedures were the same as those described for the experiment with cowpox virus infection.

There were 70–90% of " B "-bearing cells throughout the experiment, although the percentage was higher in Fig. 9 than in Fig. 8. The cycle of nuclear DNA synthesis does not affect viral DNA synthesis of the infected cells.

Cells with labeled nuclei with grain counts above ten per nucleus were considered as labeled. The three curves of the kinetics of percentages of cells with labeled nuclei are again similar (Figs. 8 and 9).

The inhibition of nuclear DNA synthesis by virus infection was studied (Fig. 10). The highest percentage of cells showing nuclear DNA synthesis were observed 7 hrs after removal of excess Tdr in the control uninfected FL cells and 8 hrs after removal of excess Tdr in infected FL cells taken out 7 hrs after infection, and 10 hrs after removal of excess Tdr in infected cells taken out 9 hrs after infection. Grain counts were made on these samples, on 50 randomly selected nuclei. As shown in Fig. 10, nuclear DNA synthesis of " B "-bearing cells in the samples examined both 7 hrs and 9 hrs after infection, was definitely suppressed.

4. *Effect of cell cycle upon multiplication of cowpox virus*

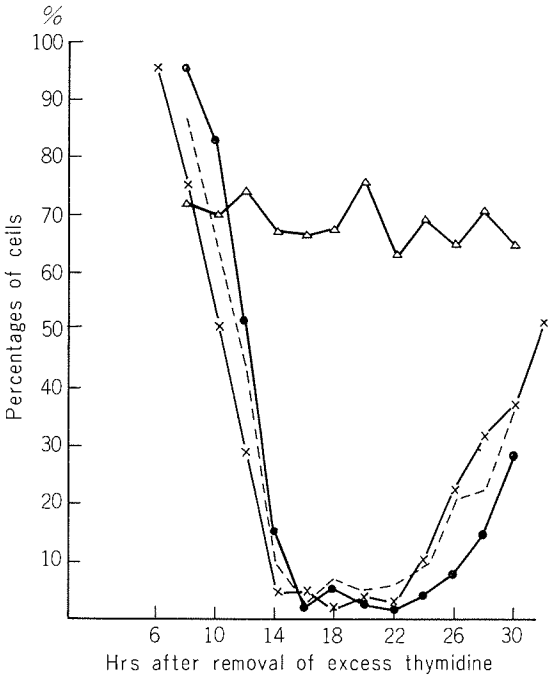
The above experiments show that the cell

cycle does not affect viral DNA synthesis. Next, the infectivity of virus in synchronous FL cells infected with cowpox virus was studied using 50 ml prescription bottles (small bottles). The experimental procedure was described in methods. The inoculum was 0.3 ml of virus of 1.5×10^6 PFU/ml. After the second thymidine treatment there were 1×10^6 cells per small bottle. As shown in Fig. 11, the titer of the progeny virus was $2.4\text{-}6.8 \times 10^6$ PFU/ml, regardless the stage in the cell cycle.

Thus, the stage of the cell cycle has no effect on either viral DNA synthesis or the yield of progeny virus.

5. *" B "-bearing cells with mitotic figures*

The mitotic index of infected cultures was less than 1%. However, " B " inclusions showing active viral DNA synthesis can be seen in some mitotic cells including cells in prophase, metaphase, anaphase and even telophase. The frequency of appearance of " B "-bearing mitotic cells in metaphase seems very high. Since the number of mitoses encountered in the sample is very small, statistical analysis was difficult. Table 1. shows the ratio of " B "-bearing mitotic cells to total mitotic cells seen in the cowpox virus-synchronous FL cell system. The percentages calculated from Table 1, are plotted in Fig. 12. The time of



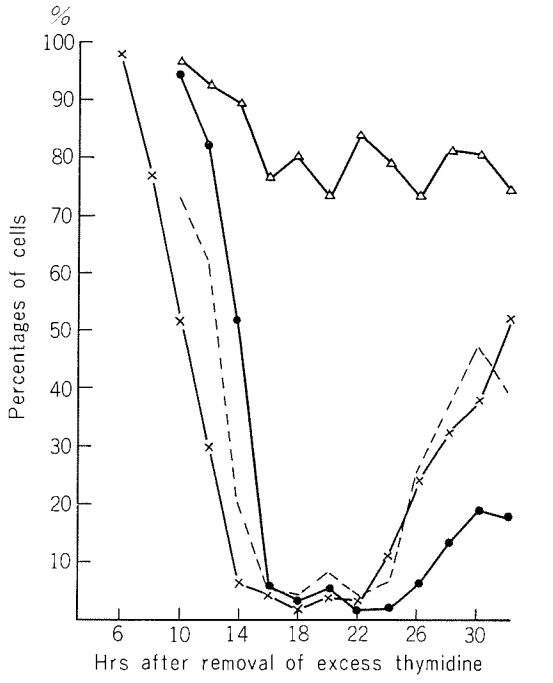


FIGURE 8 and 9 Effect of viral DNA synthesis upon nuclear DNA synthesis of synchronous FL cells infected with ectromelia virus. The percentages of " B "-bearing cells in the infected culture are also shown. Multiplicity of infection is between 10 and 5. Percentages of non-infected cells with labeled nuclei are from Fig. 3. Cells with grain counts above 10 per nucleus are taken as labeled.
×——× non-infected cells with labeled nuclei ●——● " B "-bearing cells with labeled nuclei
△——△ " B "-bearing cells --------- non-" B"-bearing cells with labeled nuclei

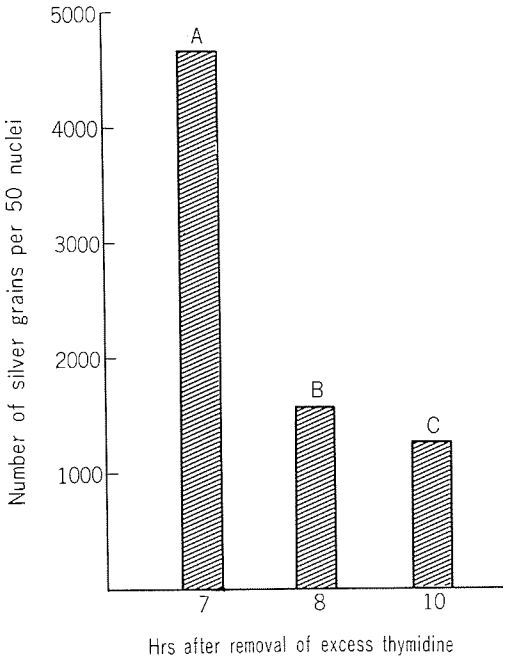


FIGURE 10 Effect of viral DNA synthesis upon nuclear DNA synthesis of synchronous FL cells infected with ectromelia virus. Silver grains per 50 randomly selected nuclei were counted in the sample with the highest percentage of nuclear DNA synthesis.

A: cells in non-infected culture

B: "B"-bearing cells in the sample 7 hrs after infection

C: "B"-bearing cells in the sample 9 hrs after infection

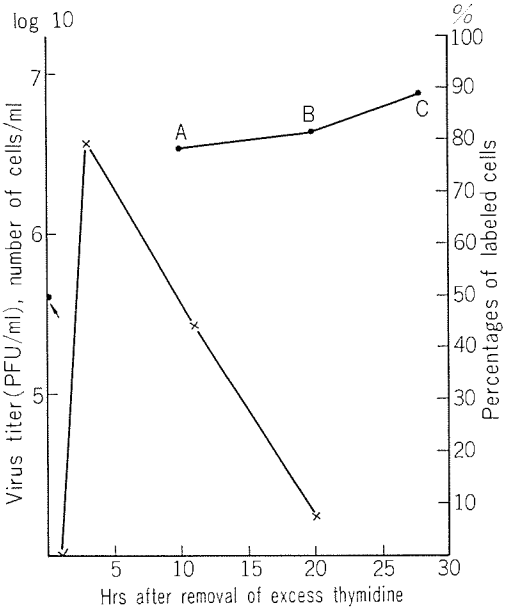


FIGURE 11 Effect of stage of cell cycle upon virus multiplication in cowpox and synchronous FL cell system.

Virus titer: cells infected in A, early S phase, B, early G_2 phase and C, early G_1 phase of the cell cycle. Non-infected control culture cells were synchronized under suitable conditions for this experiment (Cell count: 10^6/bottle $\longrightarrow 2\times10^6$/bottle, percentage of labeled nuclei: ×——×).

●——● virus titer

×——× cells with labeled nuclei

↖ input virus titer

TABLE 1 *Ratio and percentages of "B"-bearing cells with mitotic figures to mitotic cells in cowpox virus synchronous FL cell system*

		Hrs after removal of excess thymidine										
		8	10	12	14	16	18	20	22	24	26	28
9 hrs after infection	Ratio		(1/4)	(8/28)	26/51	40/120	51/123	30/91	28/82	2/53	3/55	5/67
	Percentage			(28.6)	50	33	41.5	33	34	3.8	5.5	7.5
7 hrs after infection	Ratio	(0/4)	(0/7)	19/79	7/45	21/126	6/78	9/100	1/73	2/48	1/52	
	Percentage			19	15.6	16.7	7.7	9	1.4	2.5	1.9	

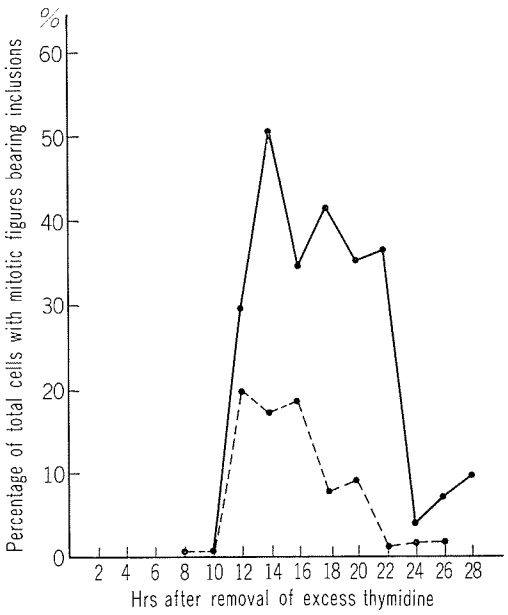


FIGURE 12 Change with time in percentage of cells with " B " inclusions among those with mitotic figures in cowpox virus-synchronous FL cells.
●——●: samples 9 hrs after infection
●------●: samples 7 hrs after infection

highest frequency of appearance of " B "-bearing mitotic cells seems similar to that of the mitotic cells in control uninfected cells shown in Figs. 3 and 4.

Thus some " B "-bearing cells can undergo mitosis.

DISCUSSION

1. *Effects of viral DNA synthesis upon nuclear DNA synthesis in poxvirus-infected cells*

As mentioned in the introduction of this paper, at present autoradiography of ^{3}H-thymidine seems to be the best technique to study the relationship between viral DNA synthesis and nuclear DNA synthesis in poxvirus-infected cells and biochemical assays which only give information on infected cells as a whole, only reveal the general tendency of metabolism. Furthermore, biochemical findings depend largely upon the state of nuclear DNA sysnthesis in the cells before virus infection. The effects of proliferation of poxvirus upon uninfected cells should also be considered. Quantitative analysis of autoradiograms of poxvirus-infected cells revealed that nuclear DNA synthesis in cells in which viral DNA synthesis has begun is suppressed (KATO *et al.* 1964). The present work confirms this with cowpox virus and ectromelia virus.

2. *Cycle of nuclear DNA synthesis in cells showing viral DNA synthesis*

There are no previous reports on this problem. The present experiments showed that slow nuclear DNA synthesis occurred in cells showing viral DNA synthesis, but only in the physiological S phase and it was never initiated in the G phase. Thus there is no chronological disturbance in nuclear DNA synthesis in cells showing viral DNA synthesis.

3. *Effect of the stage of the cell cycle upon virus multiplication*

Preliminary experiments using cold shock suggested that viral DNA synthesis of cells infected with either ectromelia virus or herpes virus occurs regardless of host nuclear DNA synthesis (KATO *et al.* 1961). Cytoplasmic viral DNA synthesis of poxvirus was found to occur when nuclear DNA synthesis of the host cells had been inhibited by Mitomycin C (KATO *et al.* 1961, MAGEE and MILLER 1962), by low temperature (KATO and MIYAMOTO 1964) or by physiological regulation in liver in vivo (KATO *et al.* 1963). The present work showed that neither viral DNA synthesis nor viral multiplication was affected by the stage of host nuclear DNA synthesis.

4. *Relationship between cellular mitosis and poxvirus infection*

The effect of poxvirus infection upon mitosis has been studied by many investigators with contradictory results. Poxvirus is known to cause marked proliferation of cells. Some poxviruses even produce tumors. Therefore,

the problem of the effect of poxvirus on mitosis should be divided into two cases, that is; 1) the case of animal tissue or cell populations infected with poxvirus in which not all cells are infected with poxvirus, at least not all at once, 2) the case of single cells infected with poxvirus. The former case, is discussed in the paper of KATO *et al.* (1965). With regard to the latter case, it is unlikely that cell division proceeds normally in cells showing viral DNA synthesis in which nuclear DNA synthesis is definitely suppressed. In fact, a decrease in the incidence of mitoses after poxvrirus infection has been reported by several investigators (KAMAHORA *et al.* 1957, KIT *et al.* 1963, KOZIOROWSKA and WLODARSKY 1966). The present work confirmed that there was a decrease in the incidence of mitoses in cells infected with either cowpox virus or ectromelia virus.

In spite of the decrease of the incidence of mitoses in cells infected with poxvirus, it has been reported that some mitotic cells bear viral inclusions (KIT *et al.* 1963, GROYON and KNIAZEFF 1967). In our experiments, viral DNA synthesis was seen in the cytolpasm of cells in prophase through telophase, and especially in metaphase. Presumably, viral DNA synthesis started at the end of the S period of the cell cycle of these cells. The question of whether viral DNA synthesis proceeds in mitotic cells, remains for our next experiments.

REFERENCES

BOOTSMA, D., BUDKE, L. and VOS, D. (1964). Studies on synchronous division of tissue culture cells initiated by excess thymidine. *Exptl Cell Res.* **33**, 301–309.

CAIRNS, J. (1960). The initiation of vaccinia infection. *Virology* **11**, 603–623.

GROYON, R. M. and KNIAZEFF, A. J. (1967). Vaccinia virus infection of synchronized pig kidney cells. *J. Virology* **1**, 1255–1264.

KAMAHORA, J., IKEGAMI, N., KAMEYAMA, S., and TAKAHASHI, M. (1957). The effect of infection of ectromelia virus on mitosis of Ehrlich ascites tumor cells. *Med. J. Osaka Univ.* **7**, 687–700.

KATO, S., KAMEYAMA, S. and KAMAHORA, J. (1960a). Autoradiography with tritium labeled thymidine of poxvirus and human amnion cell system in tissue culture. *Biken's J.* **3**, 135–138.

KATO, S., KAMEYAMA, S. and KAMAHORA, J. (1960b). Autoradiography of cells infected with variola and cowpox viruses with ^{3}H-thymidine. *Biken's J.* **3**, 183–189.

KATO, S., KAMEYAMA, S., YANAGIDA, T. and IWASE, M. (1961). Nucleocytoplasmic relationship in virus DNA synthesis (autoradiographic study with ^{3}H-thymidine). *Symposia. Cell Chem.* **11**, 75–89.

KATO, S., KURISU, M. and KAMAHORA, J. (1962). Effects of cytoplasmic DNA synthesis upon nuclear DNA synthesis in poxvirus-infected cells. *Biken's J.* **5**, 127–131.

KATO, S., AOYAMA, Y. and KAMAHORA, J. (1963). Autoradiography of tbe tissues of mice infected with ectromelia virus using ^{3}H-thymidine. *Biken J.* **6**, 9–16.

KATO, S., OGAWA, M. and MIYAMOTO, H. (1964). Nucleocytoplasmic interaction in poxvirus-infected cells. 1. Relationship between inclusion formation and DNA metabolism of the cells. *Biken J.* **7**, 45–56.

KATO, S. and MIYAMOTO, H. (1964). Nucleocytoplasmic interaction in poxvirus-infected cells. *Protein Nucleic Acid Enzyme* **9**, 92–102. (in Japanese).

KATO, S., MIYAMOTO, H. and TSURU, K. (1965). Nucleocytoplasmic Interaction in poxvirus infection-oncogenesis of poxgroup virus. *Symposia Cell Chem.* **15**, 75–96.

KATO, S., ONO, K., MIYAMOTO, H. and MANTANI, M. (1966a). Virus-host cell interaction in rabbit fibrosarcoma produced by shope fibroma virus. *Biken J.* **9**, 51–61.

KATO, S., MIYAMOTO, H., ONO, K., TSURU, K., MANTANI, M., and TANIGAKI, T. (1966b). Virus-host cell interaction in cellular proliferation induced by poxvirus. *Int. Symp. for Cellular*

Chem. Pergamon Press. 433–442.

KIT, S., DUBBS, E. R. and HSU, T. C. (1963). Biochemistry of vaccinia-infected mouse fibroblast strain L-M. III. Radioautographic and biochemical studies of thymidine-^{3}H uptake into DNA of L-M cells and rabbit cells in primary cultures. *Virology* **19**, 13–22.

KOZIOROWSKA, J. and WLODARSKI, K. (1966). The effect of crude preparations of vaccinia on mitosis and DNA synthesis of KB cells. *J. Exptl. Med.* **124**, 199–209.

MAGEE, W. E. and MILLER, O. V. (1962). Dissociation of the synthesis of host and viral deoxyribonucleic acid. *Biochem. Biophys. Acta.* **55**, 818–826.

OKUMURA, H., KOMIYA, K. and IKEGAMI, M. (1964). *18th Meeting of Japanese Tissue Culture Association.*

OKUMURA, H. (1967). Spontaneous malignant transformation of hamster lung tissues in culture. *Cancer cells in tissue culture.* Tokyo Univ. press.

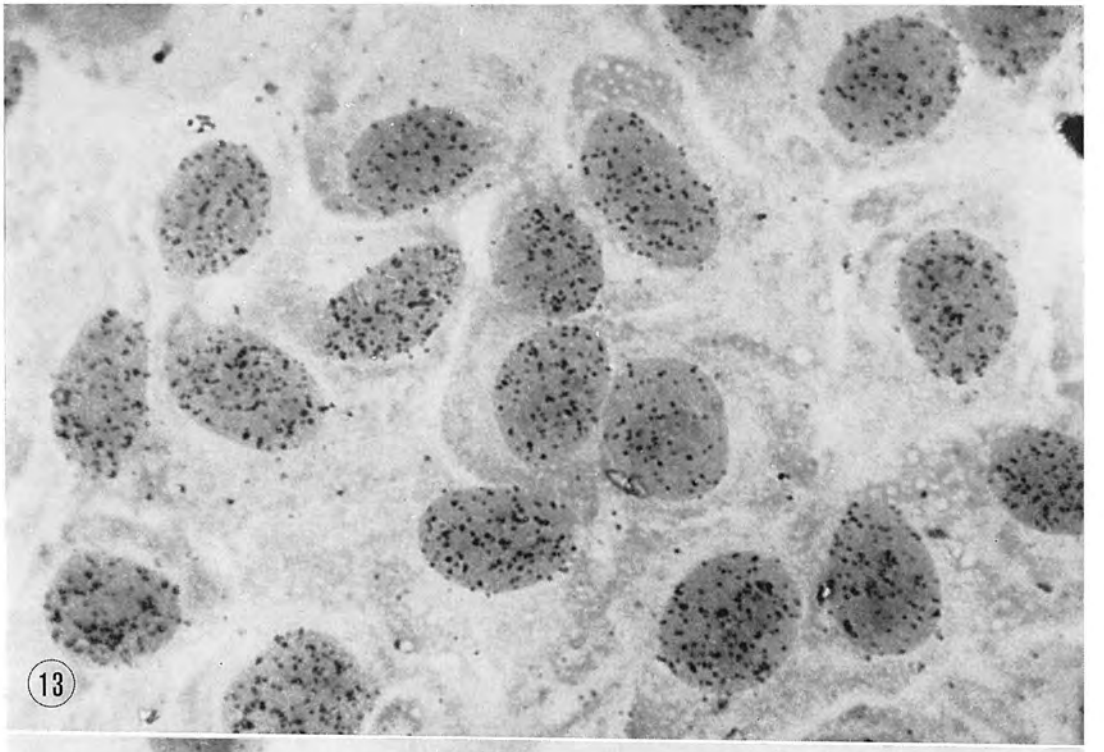
TANIGAKI, T. and KATO, S. (1967). Autoradiographic studies on molluscum contagiosum using ^{3}H-thymidine. *Biken J.* **10**, 41 54.

TERASHIMA, T. and TOLMACH, L. J. (1961). Changes in X-ray sensitivity of Hela cells during the division cycle. *Nature* **190**, 1210–1211

XEROS, N. (1962) Deoxyriboside control and synchronization of mitosis. *Nature* **194**, 682–683.

FIGURE 13 Autoradiogram of non-infected synchronous FL cells. Six hrs after removal of excess thymidine. All FL cells have labeled nuclei (S phase).

FIGURE 14 Autoradiogram of non-infected synchronous FL cells 12 hrs after removal of excess thymidine. Various stages of mitotic figures are seen. Cells are not labeled. Cells in interphase are also seen (G_1, S and G_2 phase).



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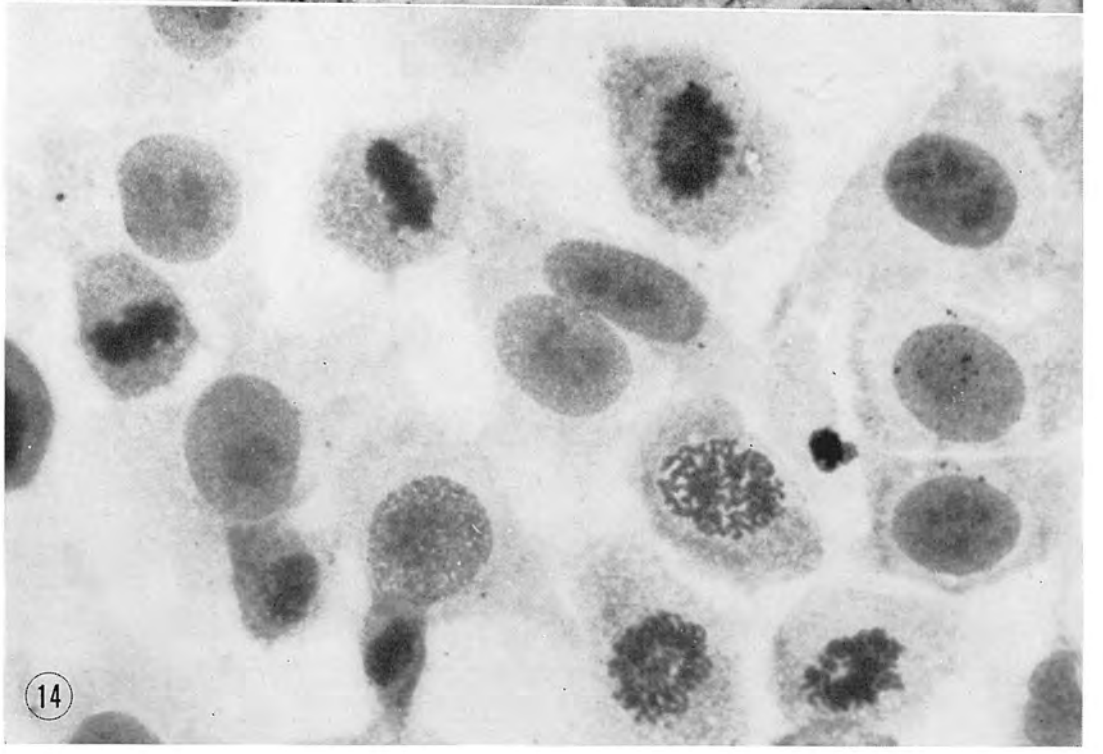

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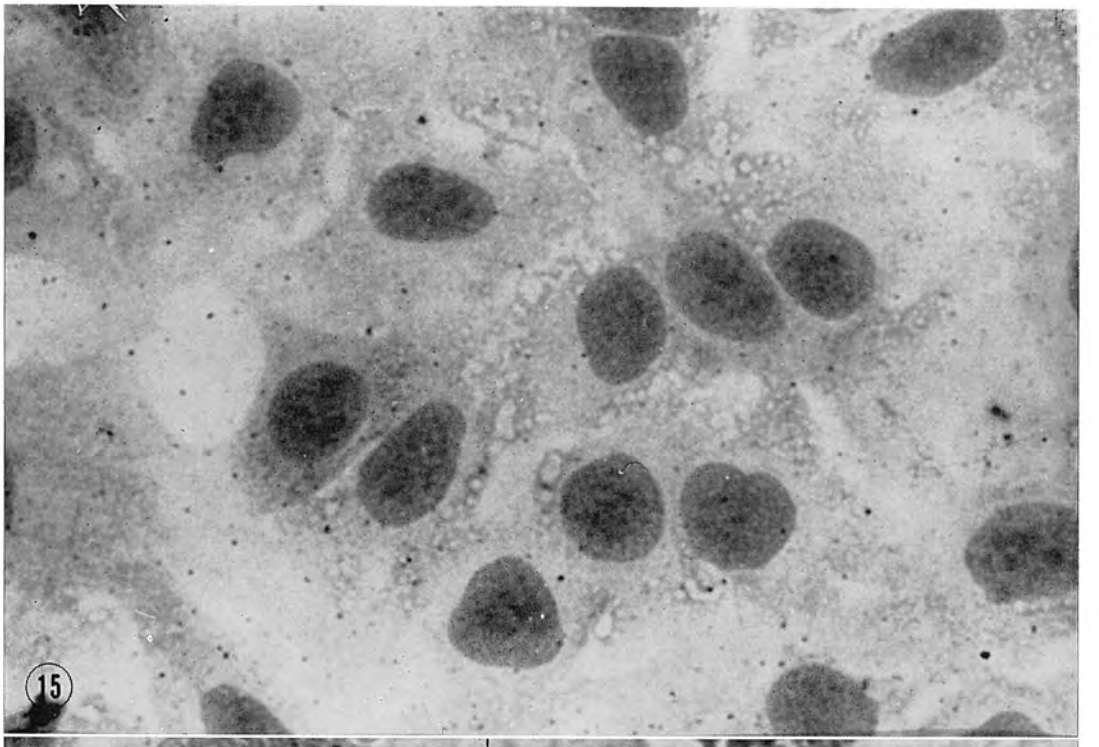
FIGURE 15 Autoradiogram of non-infected synchronous FL cells 20 hrs after removal of excess thymidine. No nuclei are labeled with ^{3}H-thymidine (G_1 phase).

FIGURES 16 and 17 Autoradiogram of synchronous FL cells infected with ectromelia virus. Ten hrs after removal of excess thymidine. Fig. 16 shows FL cells 7 hrs after infection. Fig. 17 shows FL cells 9 hrs after infection. All nuclei and also " B " inclusions were well labeled.
Arrow shows " B " inclusions.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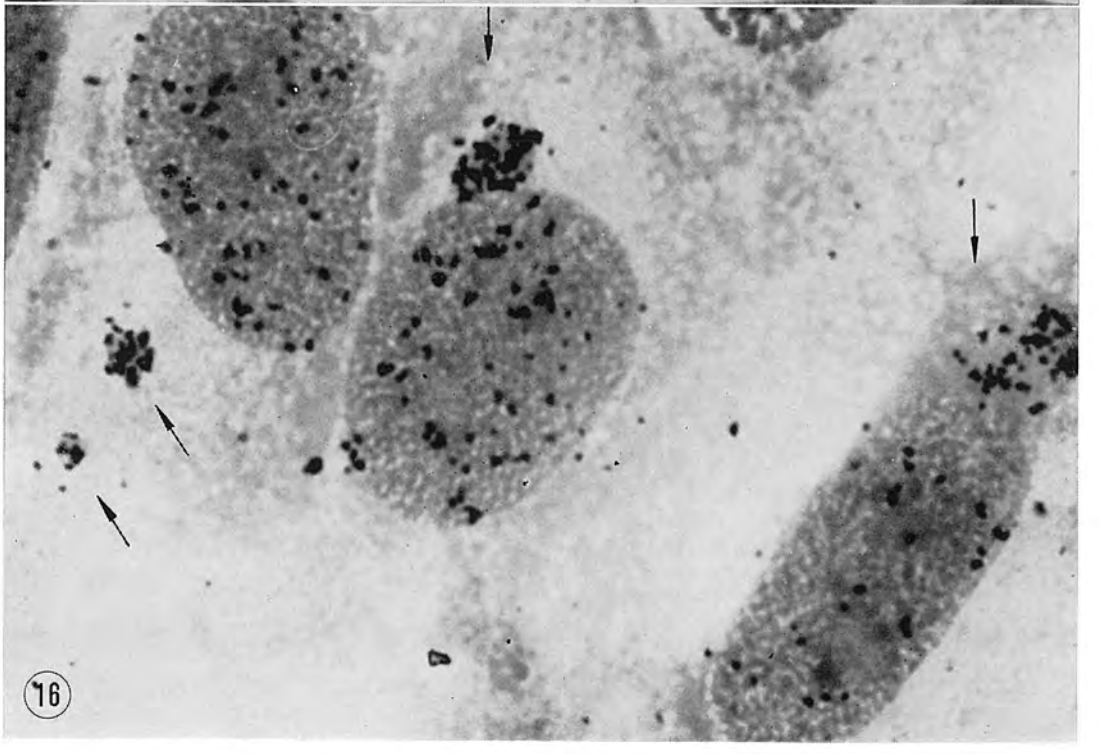
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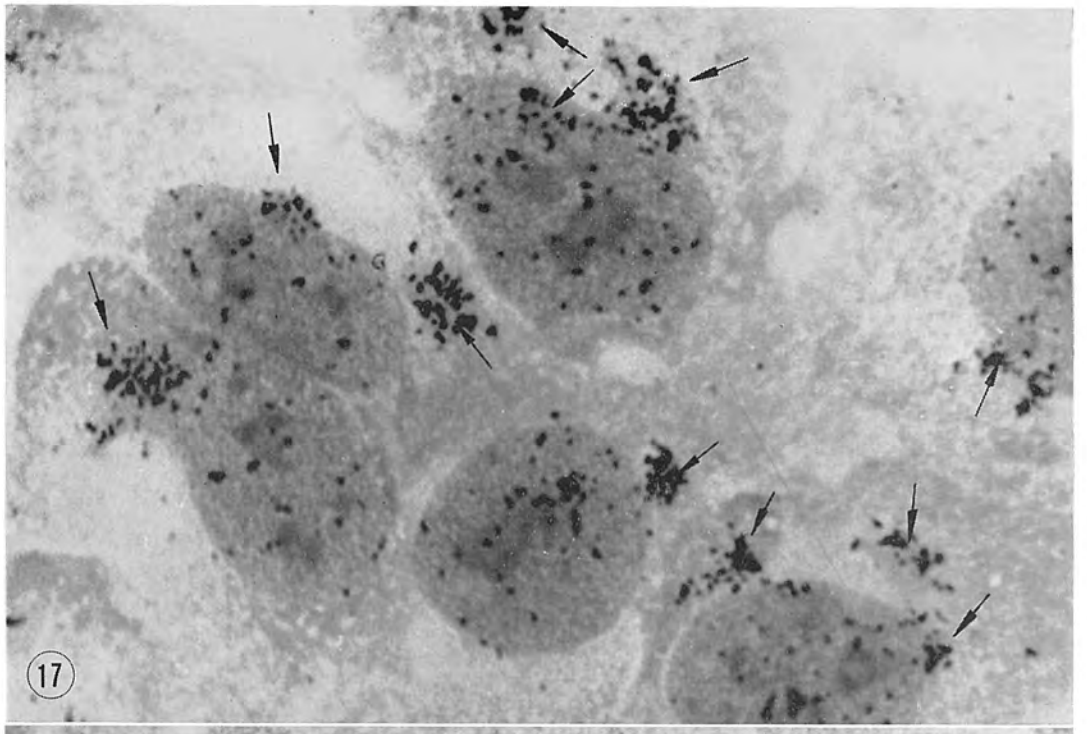
FIGURE 18 Autoradiogram of synchronous FL cells infected with ectromelia virus. Twenty hrs after removal of excess thymidine. Seven hrs after infection. No nuclei were labeled, while " B " inclusions were well labeled.



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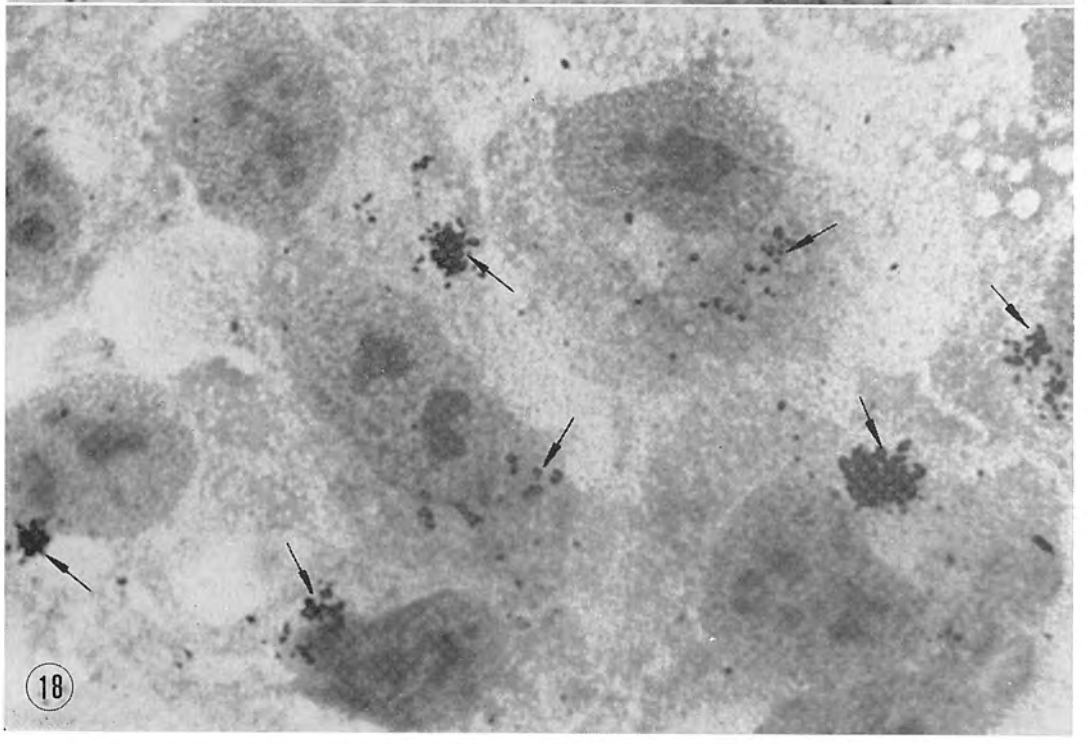

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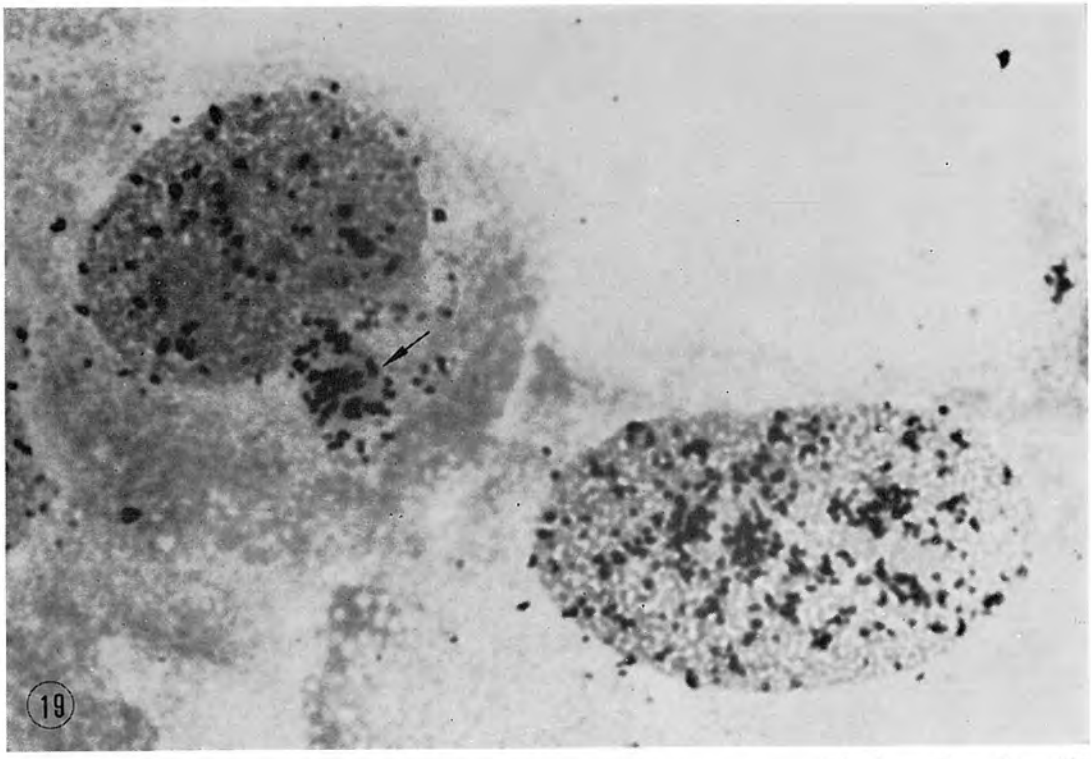
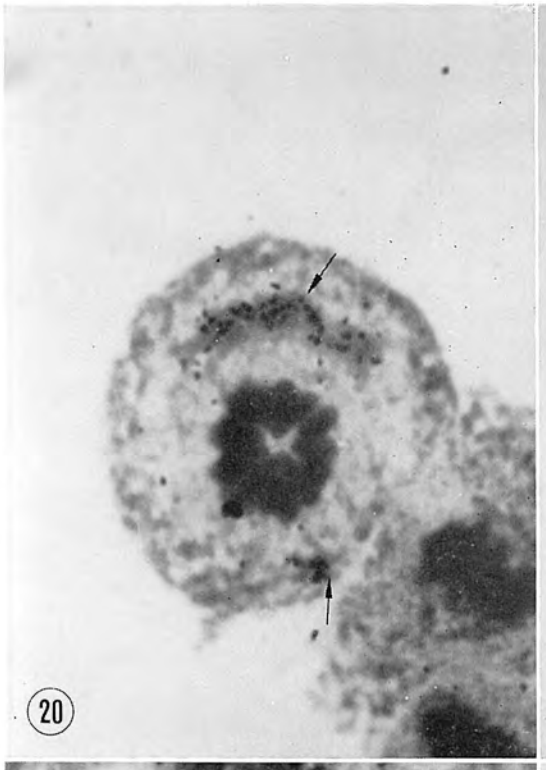


FIGURE 19 Autoradiogram of FL cells infected with ectromelia virus 9 hrs after infection. One of two FL cells has labeled nucleus with labeled " B " inclusion in the cytolpasm. The other FL cells has labeled nucleus without cytoplasmic labeling. (Giemsa Staining). Nuclei of the inclusion-bearing, and non-inclusion-bearing cell were both well labeled, especially the latter.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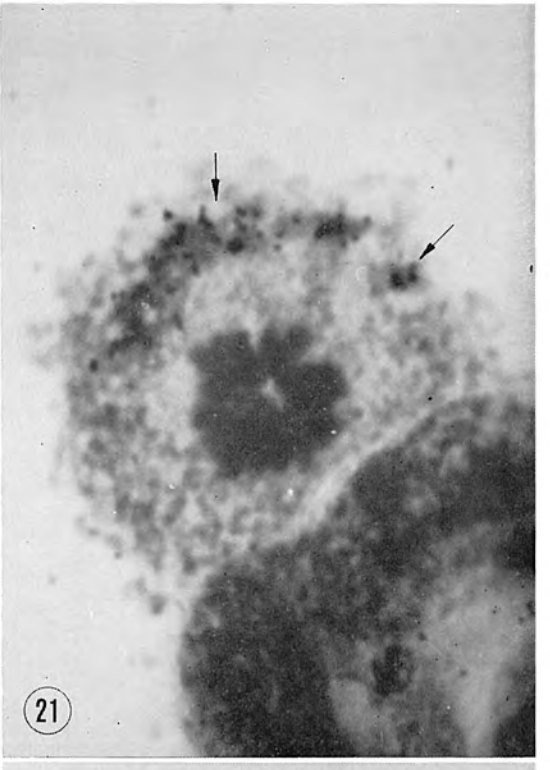
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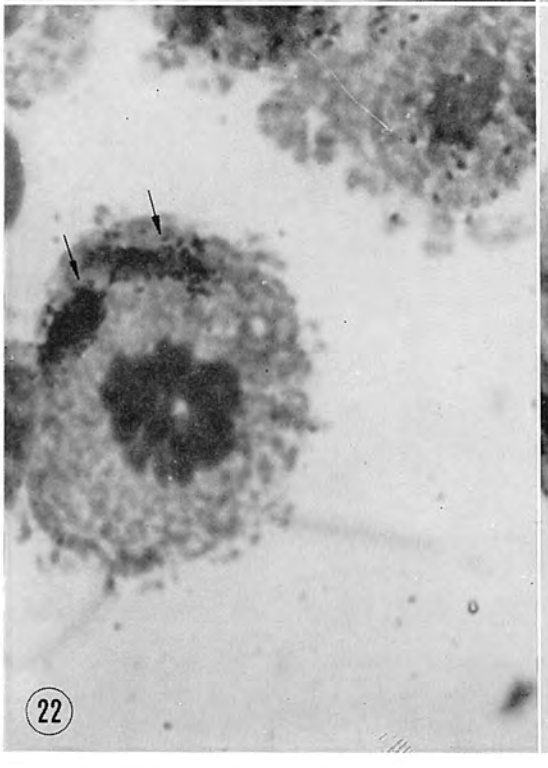
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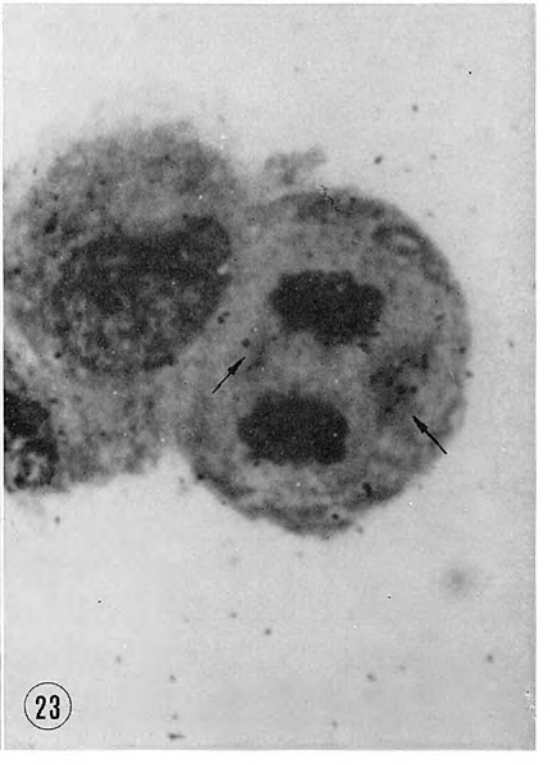
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FIGURE 20, 21, 22 and 23 Autoradiograms of synchronous FL cells infected with cowpox virus. Mitotic figures with labeled " B " inclusions in cytoplasm of cells. FL cells in metaphase with labeled " B " inclusions. (Figs. 20, 21 and 22) FL cells in anaphase with labeled " B " inclusions (Fig .23).