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SHORT COMMUNICATION

VIRUS-HOST CELL INTERACTION IN RABBIT FIBROSARCOMA PRODUCED BY SHOPE FIBROMA VIRUS

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It has been shown in previous papers that cells containing multiplying poxviruses including oncogenic poxviruses, show definite suppression of nuclear DNA synthesis and no longer multiply (*7-12*). Modification of the immunological response of the rabbits in various ways often causes progressive growth of tumors after Shope fibroma virus inoculation (*1-6*).

The purpose of this report is to clarify the relationship between virus multiplication and tumor cell proliferation in the fibrosacroma formed in rabbits inoculated with Shope fibroma virus.

Five adult white rabbits weighing about 2

TABLE 1 *Results of experiments*

Rabbit	Treatment	Virus dose inoculated (RTFU)	Onset of tumor formation (days)	Maximum diameter (cm)	Days of maximum size	Onset of secondary tumor formation (days)	Duration of tumor formation (days)	Fibrosarcoma formation	Process of the disease
No. 1	^{60}Co 600 r pds 100 mg	10^6	3	4.5	30	26	>74	+	killed in a moribund state
No. 2	^{60}Co 1200 r pds 100 mg	10^6	3	7.2	30	30	>53	+	killed in a moribund state
No. 3	^{60}Co 600 r pds 100 mg	10^6	4	4.5	31	26	95	+	recovered
No. 4	^{60}Co 1200 r pds 100 mg	10^6	3	3.5	17	—	27	−	died
No. 5	^{60}Co 600 r pds 100 mg	10^6	3	4.5	37	—	64	−	recovered
No. 6	control	10^6	3	3.8	9	—	28	−	recovered
No. 7	control	10^6	4	3.6	8	—	30	−	recovered

pds: predonisolone RTFU: rabbit tumor forming unit

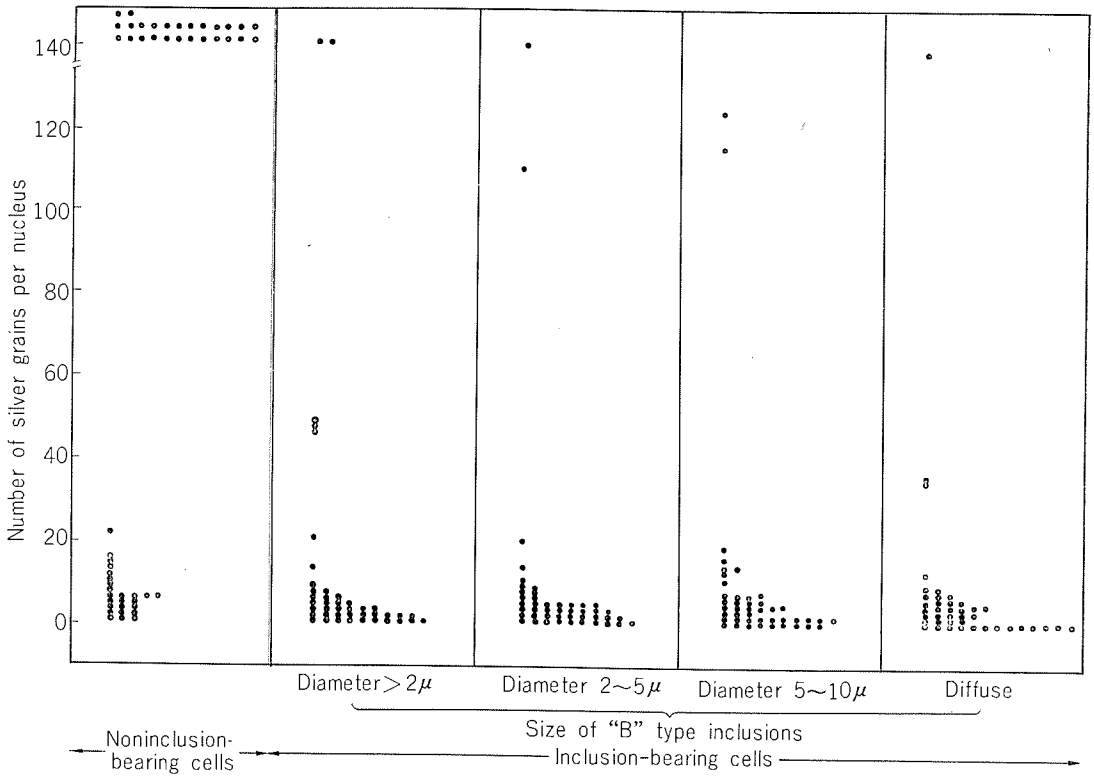

FIGURE 1 Effect of inclusion formation upon nuclear DNA synthesis. Inclusion-bearing cells were divided into 4 groups according to the size of their inclusions. These cells were compared with the cells containing no inclusions. The number of silver grains in the 50 labeled nuclei of the cells of each group are plotted. The dots above the level of 140 show the number of silver grains more than 140.

kg were irradiated with various doses of ^{60}Co as shown in Table 1. Twenty-four hours later 0.5 ml of Shope fibroma virus (OA strain) material with 10^6 rabbit tumor forming units was inoculated intracutaneously. These rabbits were subsequently treated with 100 mg of predonisolone (doses of 20 mg five times at 4 day intervals). In all irradiated rabbits tumors appeared at the site of virus inoculation about 3 days after virus inoculation. These tumors grew progressively over a 30 day period.

In the untreated controls in the present experiments, fibromata developed normally and reached a maximum size in about 9 days. By the 30th day the tumors had largely disappeared leaving only a scabbed ulcer as described previously.

Three of the five test rabbits had several pea-sized tumors in uninoculated areas of the skin on the 26th and 30th day. These secondary tumors also grew progressively (Fig. 3). On the 40th day histological studies were carried out on biopsy specimen taken from these tumors. These secondary tumors were diagnosed as fibrosarcoma (Fig. 4). Histological observation with Azan-Mallory staining as well as silver staining supported this diagnosis.

Rabbits No. 1 and No. 2 were moribund on the 74th day and 53rd day respectively. Rab-

bit No. 1 was bled to death. At necropsy the rabbit had multiple tumors dependent from the inner surfaces of the ribs and outer surfaces of the scapula and innumerable white foci in the liver. Microscopical examination revealed that these tumors consisted of sarcomatous tissue. In the liver, sarcomatous cells had diffusely infiltrated into the tissue (Fig. 5). Touch preparations of these tumor tissues stained with Giemsa solution, showed that about sixty per cent of the sarcoma cells bore typical " B " type inclusions of poxvirus. Staining of these preparations with fluorescein-isothiocyanate coupled with γ globulin from rabbits which recovered from fibromata produced by Shope fibroma virus, showed many specific fluorescent spots corresponding to these cytoplasmic inclusions. The nuclei of the tumor cells were free from any fluorescence. Rabbit No. 2 was inoculated intravenously with 2 mc of ^{3}H-thymidine solution (specific activity 1.85 c per mM). One hour later it was bled to death. At necropsy two tumors were found on the inner surface of the ribs and, other visceral organs were free from any definite macroscopical foci. However microscopical examination disclosed small sarcomatous foci with three mitoses in the lung (Fig. 6). The subcutaneous sarcoma was excised. Then dipping autoradiography with Kodak NTB2 emulsion was carried out on the scratch preparation and on sections of the tumors. Exposure time was 12 days. About sixty two per cent of tumor cells showed inclusion formation. The sites of these inclusions corresponded exclusively with the accumulation of silver grains (Fig. 7, 8). However only 5 per cent of the nuclei of these cells bearing inclusion were labeled, while about twenty per cent of nuclei of cells with no inclusions were well labeled. Inclusion bearing cells were divided into 4 groups according to the size of their inclusions as shown in Fig. 1. The number of silver grains in the 50 labeled nuclei of the cells of

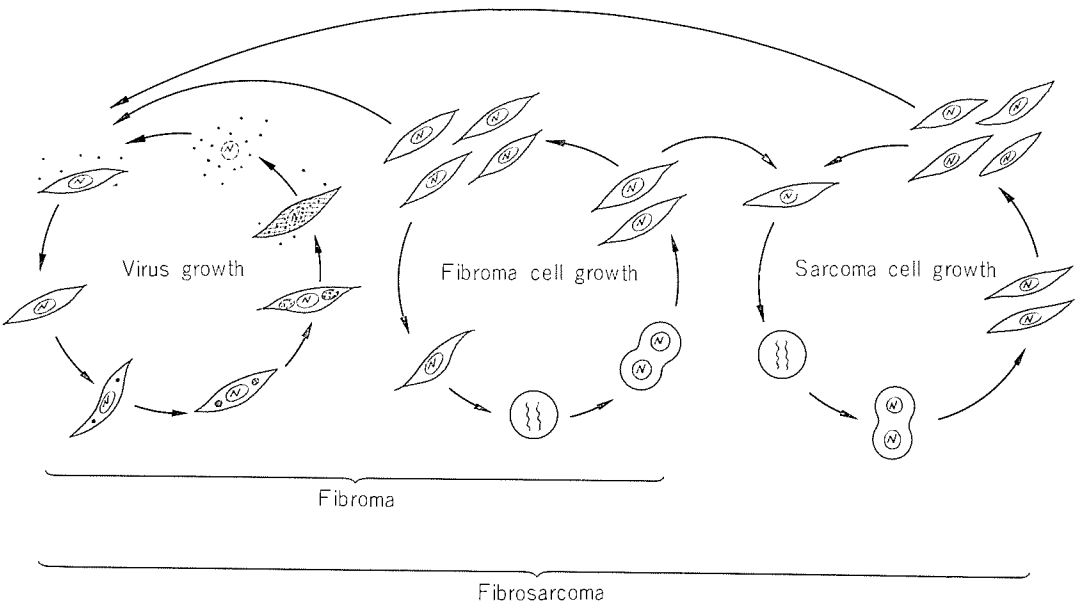

FIGURE 2 Diagram of the relationship between multiplication of Shope fibroma virus and growth of tumor cells.

each group are plotted in the figure. Compared with the nuclei of cells without inclusions, the nuclei of inclusion bearing cells have a definitely lower percentage of labeled nuclei and also fewer silver grains in labeled nuclei. Infectious virus were easily recovered from all fibrosarcoma in these experiments.

From the results fibrosarcoma formation may be explained as follows. Under immunologically modified conditions virus multiplication continued without affecting antibody. Cells in which virus multiplied has definitely suppressed nuclear DNA synthesis and did not proliferate, but degenerated. The virus produced may infect either fibroma cells or sarcoma cells. Thus the cycle of virus multiplication goes on in the tissue. Some factor must make dormant subcutaneous fibroblasts start to proliferate. This factor may be released from degenerating virus-producing cells. The stimulating activity of this factor seems rather transient, since tumor growth ceases soon after the cycle of virus multiplication is blocked by an increase in the amount of neutralizing antibody against the virus (*10*). When rabbits are irradiated, a continuous diffusion of the factor from degenerating virus-producing cells causes the fibroma to continue to grow progressively. As a result continuously and actively proliferating fibroma cells may be transformed into fibrosarcoma cells.

Fig. 2 shows a diagram of this working hypothesis.

Summary

Irradiation with ^{60}Co and subsequent predonisolone-treatment may modify the process of tumor formation in rabbits inoculated with Shope fibroma virus and cause the fibroma to develop the malignant appearance of a fibrosarcoma. In tumor tissue about sixty per cent of tumor cells were virus-producing, as shown by the fluorescent antibody technique and autoradiography with ^{3}H-thymidine as well as by recovery of infectious virus. However these virus-producing cells did not multiply. The mechanism of fibrosarcoma formation by Shope fibroma virus is discussed.

Acknowledgements

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FIGURE 3 A single primary tumor and three secondary tumors of rabbit No. 1.

FIGURE 4 A section preparation of the skin tumor of rabbit No. 1, stained with hematoxylin-eosin showing malignant appearance of fibrosarcoma.

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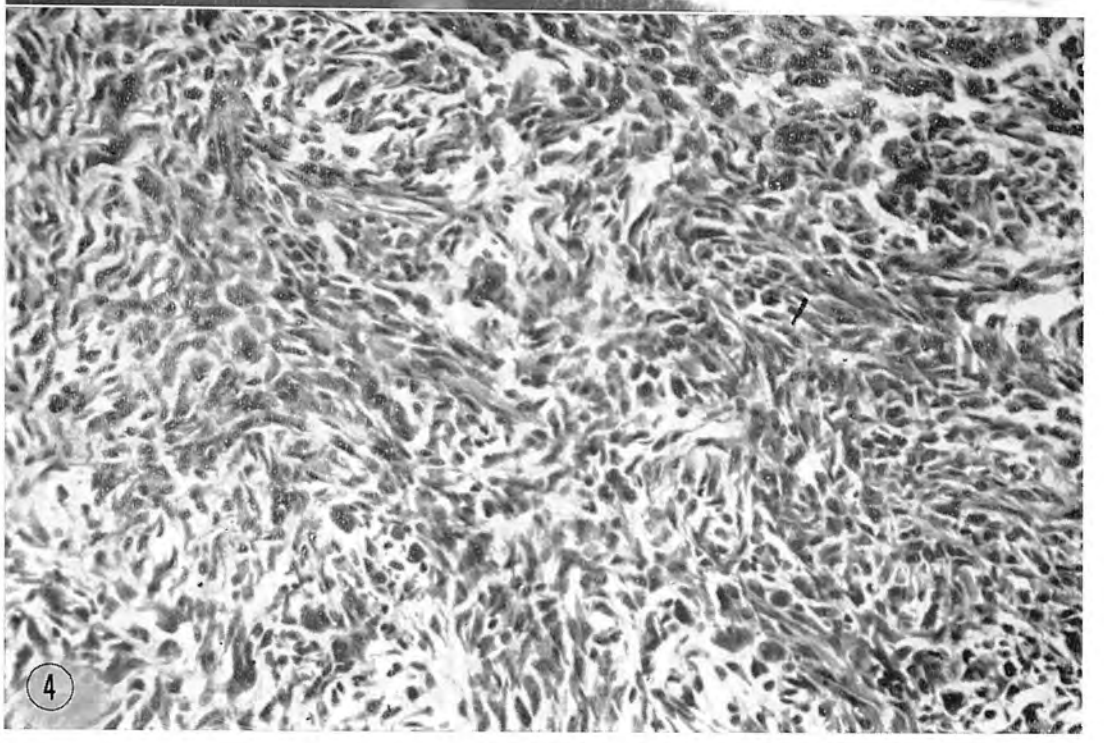

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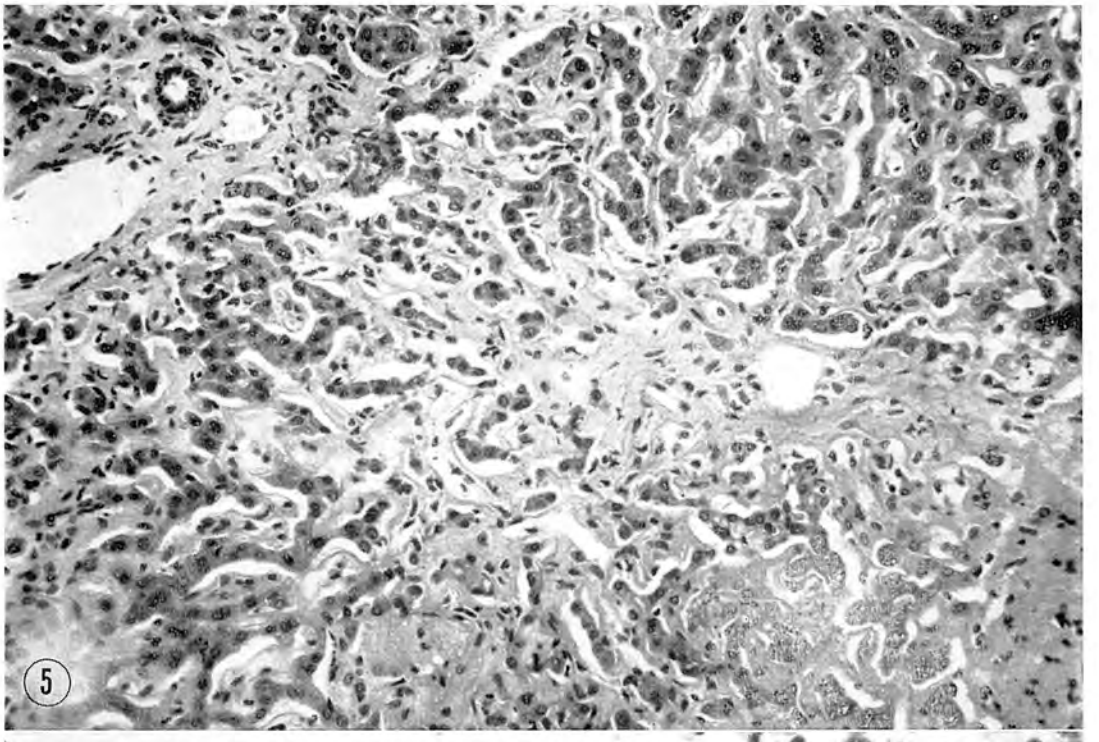
FIGURE 5 A section preparation of the liver of rabbit No. 1, stained with hematoxylin-eosin showing infiltration of sarcomatous cells into liver cells.

FIGURE 6 A section preparation of the lung of rabbit No. 2, stained with hematoxylin-eosin showing microtumor formation with three mitoses.



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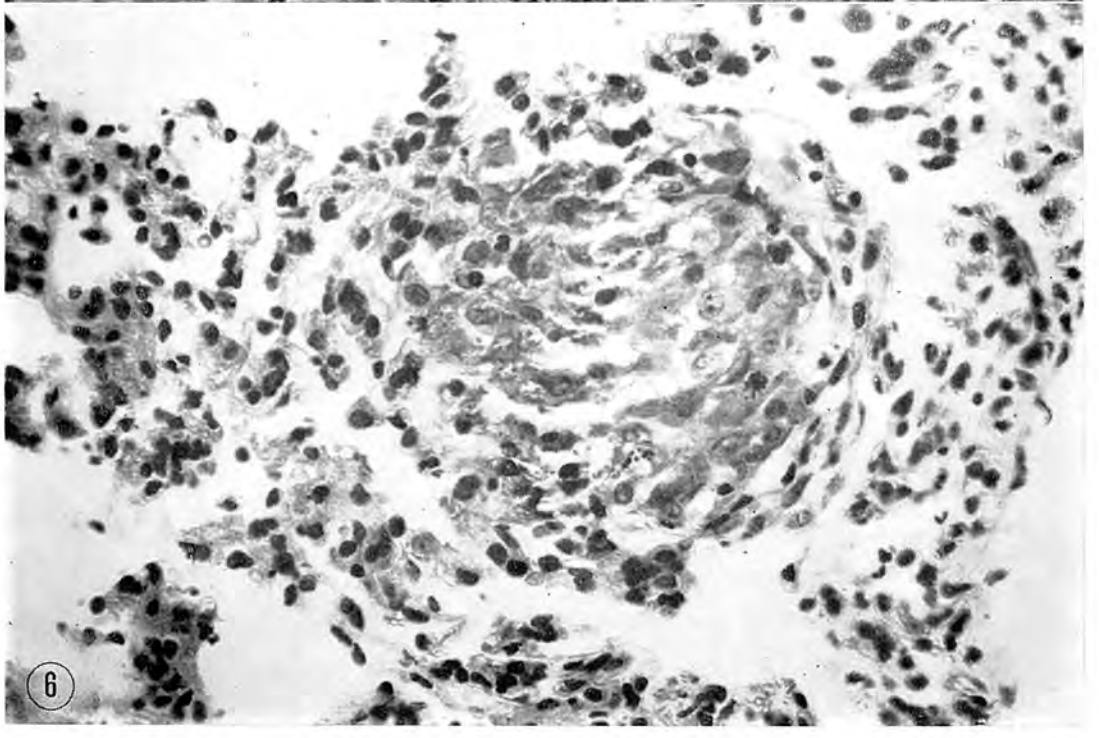

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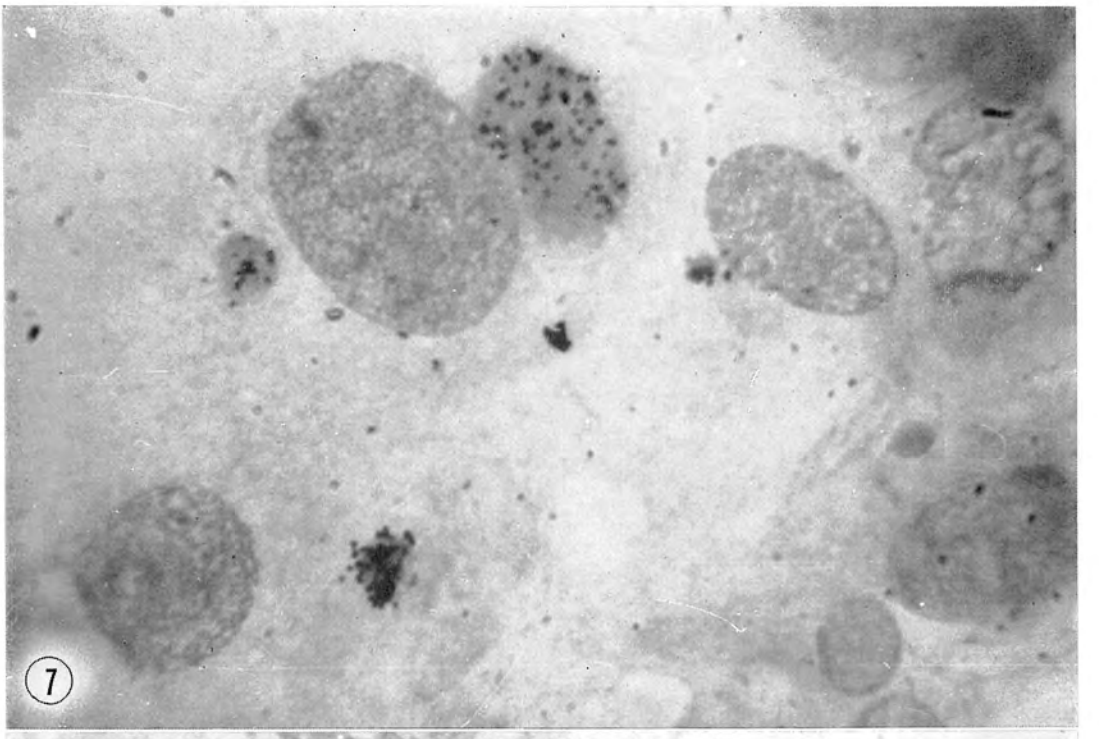
FIGURE 7 and 8 Autoradiograms of scratch preparations of sarcoma tissue of rabbit No. 2, stained with Giemsa solution. Several cells show accumulations of silver grains in the cytoplasm corresponding to the inclusions. Nuclei of these cells were free from silver grains. A cell having no inclusion in Fig. 8 shows nuclear DNA synthesis.



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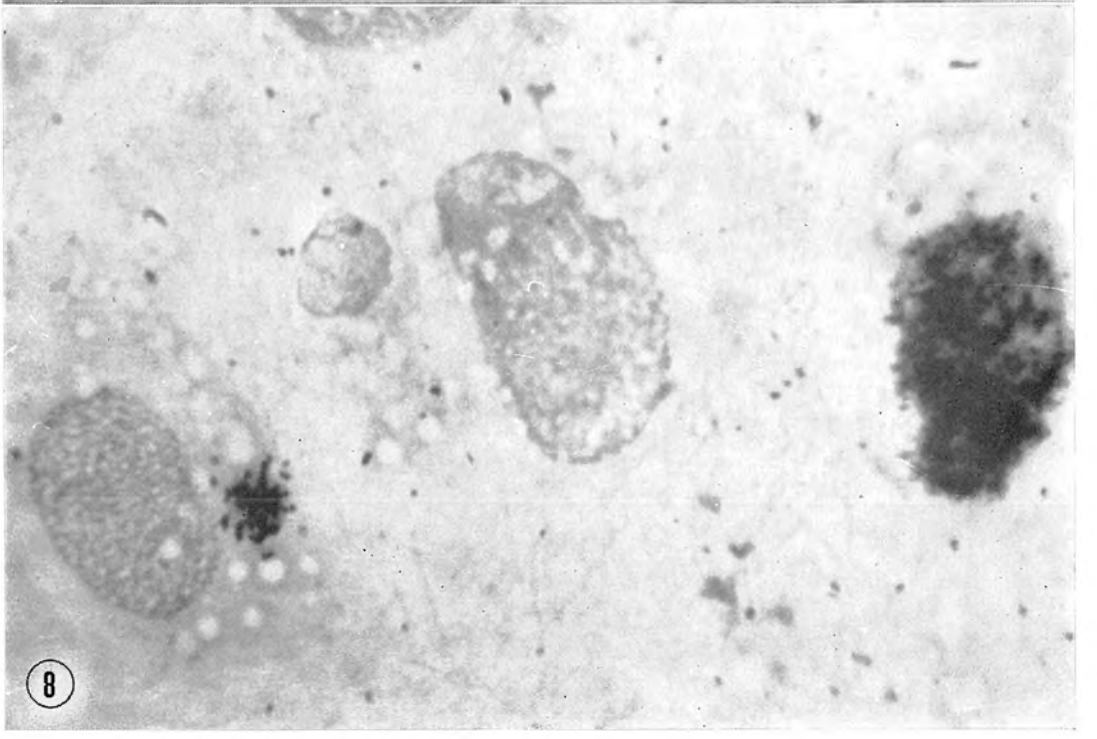

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