with some smaller activity due to cartilage cell degeneration.

Summary. A study has been made of the role of alkaline phosphatase in the osteogenesis of 7-day embryonic chick femora during 21 days in organ culture. The femora were analyzed for their content of inorganic P, acid-soluble ester P, lipid P, total nucleic acid P and residual P, after increasing culture periods. The results indicate that there is a close parallel between inorganic P content and alkaline phosphatase activity. Acid soluble ester P content shows a roughly reciprocal relationship to phosphatase activity, while the other P fractions do not appear to be related.

1. Robison, R., *Biochem. J.*, 1923, v17, 286.
2. Fell, H. B., Robison, R., *ibid.*, 1929, v23, 767.
3. Moog, F., *Biol. Bull.*, 1944, v86, 51.
4. Siffert, R. S., *J. Exp. Med.*, 1951, v93, 415.
5. Moog, F., Wenger, E., *Am. J. Anat.*, 1952, v90, 339.
6. Gutman, A. B., Yu, T. F., in *Metabolic Interrelations,* Trans. 2nd Conf., E. C. Reifenstein, Jr., Ed., Josiah Macy Foundation, New York, 1950, p167.
7. Fleisch, H., Neuman, W. F., *Am. J. Physiol.*, 1961, v200, 1296.
8. White, P. R., *Cultivation of Animal and Plant Cells,* Ronald Press, New York, 1954, p125.
9. Parker, R. C., *Methods of Tissue Culture,* 2nd Ed., Paul B. Hoeber, Inc., New York, 1950, p77.
10. Lowry, O. H., Roberts, N. R., Leiner, K. Y., Wu, M., Farr, A. L., *J. Biol. Chem.*, 1954, v207, 1.
11. Lowry, O. H., Bessey, O., *ibid.*, 1946, v163, 633.
12. Bessey, O. A., Lowry, O. H., Brock, M. J., *ibid.*, 1946, v164, 321.
13. Harris, E., Mehl, J. W., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 521.
14. Lang, C. A., *Anal. Chem.*, 1958, v30, 1692.
15. Alder, H. L., Roessler, E. B., *Introduction to Probability and Statistics,* W. H. Freeman & Co., San Francisco, 1960, p129.
16. Pearse, A. G. E., *Histochemistry, Theoretical and Applied,* 2nd Ed., Little, Brown & Co., Boston, 1960, p872.
17. Gutman, A. B., Warrick, F. B., Gutman, E. B., *Science,* 1942, v95, 461.
18. Follis, R. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 441.
19. Whitehead, R. G., Weidmann, S. M., *Nature,* 1957, v180, 1196.
20. ———, *ibid.*, 1959, v183, 876.
21. Fell, H. B., Robison, R., *Biochem. J.*, 1934, v28, 2243.
22. Henrichsen, E., *Acta Orthopaed. Scand.*, 1958, v27, 173.

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Viruses and Mammalian Chromosomes. III. Effect of Herpes Zoster Virus on Human Embryonal Lung Cultures.* (29633)

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Evidence accumulated during the past years supports the concept that some viruses can induce chromosome aberrations(1-4) in host cells; this often leads to formation of new cell types with abnormal chromosome complements(5-8). However, our knowledge of this problem is still fragmentary. It is not known whether the ability of a virus to induce chromosome damage is correlated with its composition, structure, site of multiplication, virulence, toxic products, or with other aspects of virus-cell interaction. It is, therefore, important to survey the virus-chromosome relationships of a variety of virus-host cell systems in order to elucidate these possibilities. The present paper describes several cytological and chromosomal aberrations observed in diploid human cells infected in

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tissue culture with herpes zoster virus (HZV).

Materials and methods. Tissue cultures consisted of cells obtained by trypsinizing human embryonic lungs. The nutrient fluid consisted of 80% Eagle's basal medium, 20% calf serum, 100 units of penicillin, and 100 μg of streptomycin per ml. The pH was adjusted to 7.1 with 7.5% $NaHCO_3$. The strain (EY) of HZV employed has been described(9,10). The inoculum consisted of 10^5 or 10^6 cells, of which approximately 10^3 cells were infected. These cells were plated onto lung monolayers growing in 1 oz bottles. Titers were calculated by a plaque method previously described(9). After an adsorption time of 1-2 hours, a fluid with reduced serum (5-10%) was added and the cultures incubated at 37°C. Under the conditions of this experiment, it was known that 48 hours after inoculation, more than 50% of the cells in the cultures contained HZV.

For cytological examinations, monolayers grown on coverslips were inoculated in the same fashion. The coverslips were rinsed twice in saline, fixed in Bouin's solution for 15 minutes, and stained with hematoxylin and eosin (H & E).

Chromosome preparations were made from cultures without treatment with colchicine or colcemid. Cells were dislodged with trypsin, suspended in 1% sodium citrate solution for approximately 5 minutes, and fixed in 50% acetic acid for a minimum of 20 minutes. The cells were then stained with acetic orcein and squashed.

Results. The most striking effect of HZV on human cells appeared to be the interruption of the normal mitotic cycle. Following infection, anaphase and telophase figures gradually disappeared. Cytological observation of H & E stained preparations revealed the typical intranuclear inclusions of HZV and, in contrast to the controls (Fig. 1) harvested simultaneously with the infected cultures, a high number of cells in metaphase with many cells containing abnormally clumped chromosomes (Fig. 2). Another characteristic cytological feature was the formation of multinucleated cells; many contained several large nuclei with inclusions and in addition, contained numerous micronuclei (Fig. 3).

The reduction of anaphases and telophases and the increase of metaphases indicated that the mitotic process was blocked at the metaphase stage. The chromosomes were scattered in the cells and were highly contracted (Fig. 2, 4), as if they were under the influence of colchicine.

Apparently, chromosomes of many cells blocked at metaphase were able eventually to uncoil (Fig. 5) and enter interphase. This change from metaphase to interphase, without anaphasic movement, resulted in formation of many micronuclei of varying sizes (Fig. 3, 6). The very small ones may contain only one chromosome and the larger ones may contain several chromosomes (Fig. 6). It is of interest that the entrance into interphase was asynchronous, which again was analogous to the cellular response following prolonged treatment with colchicine.

In addition to the anomalies of the mitotic cycle, many metaphase plates in the infected tissue cultures showed chromosome aberrations. In 4 samples taken 24 hours after infection, the incidence of chromatid and chromosome breaks ranged from 26 to 45% as compared to an average of 2.1% in the control cultures. In samples taken 48 to 72 hours after infection, metaphase plates with highly contracted chromosomes prevailed.

Between 68 and 81% of the blocked metaphase plates showed aberrations superficially resembling chromosome fragmentation (Fig. 4 and 5). However, the possibility cannot be excluded that at least some of the "fragments" represented uncoiled chromosomes which were still connected by fibrous structures but which could not be resolved by microscopic observation.

Discussion. Our observations show that a variety of abnormalities were induced in the human lung cells infected with HZV: 1) a colchicine-like effect(3), including metaphase arrest and overcontracted chromosomes, 2) formation of multinucleated cells and formation of micronuclei, 3) chromatid and chromosome breaks, and 4) "fragmentation" and possible uncoiling of chromosomes. The ab-

normalities were quite different from those induced by herpes simplex virus(3) which causes chromatid gaps and breaks localized on two chromosomes, or by adenovirus type

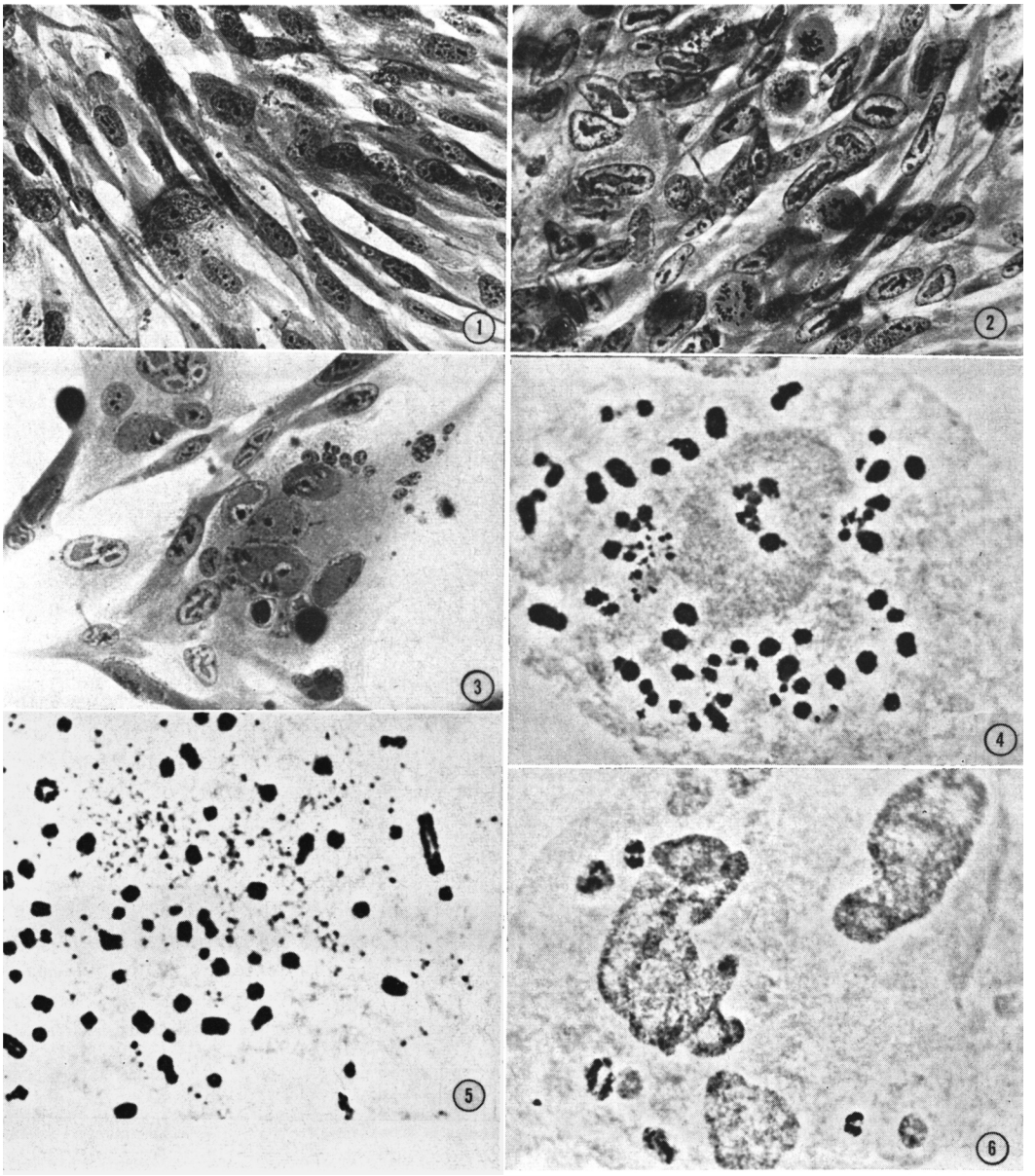

FIG. 1. Normal human embryo lung fibroblasts. Hematoxylin-eosin. 540 ×.

FIG. 2. Human embryo lung fibroblasts 48 hours after infection with HZV. Hematoxylin-eosin. 540 ×. The majority of the cells contain inclusions in their nuclei. Note 4 cells in metaphase with overcontracted chromosomes.

FIG. 3. Same as Fig. 2; a multinucleated cell with at least 5 large, inclusion bearing, nuclei and numerous micronuclei.

FIG. 4. An arrested metaphase plate showing overcontracted chromosomes and fragmentation of some chromosomes. Sample taken 48 hr after infection with HZV.

FIG. 5. An arrested metaphase plate showing fragmentation and uncoiling of numerous chromosomes. Sample taken 60 hr after infection with HZV.

FIG. 6. A cell with micronuclei of varying size. Note some chromosomes still in colchicine-like mitotic arrest. Sample taken 60 hr after infection with HZV.

12(4) which simulates radiation damage. A preliminary communication(11) has also recently focused attention on the possibility of chromosome breaks in leukocytes of patients with chickenpox, a disease caused by a virus identical with or closely related to HZV. Although chromosome cytology following viral infection is only at its beginning stage of development, it already appears evident that individual viruses may produce specific effects on the nuclei and mitotic cycle of a variety of host cells. Caution is required, however, in interpretation of chromosomal aberrations that are not unique for a specific virus, as a variety of alterations have been reported in actively growing cultures of "normal" cells(12). In the present study, however, parallel control cultures, examined simultaneously, showed none of the marked abnormalities seen in the infected cultures.

Summary. Human embryo lung fibroblasts infected with herpes zoster virus reacted as though they had been treated with colchicine. Most of the infected cells were arrested in metaphase and contained overcontracted chromosomes. Formation of multinucleated cells and micronuclei was also observed. Many chromatid and chromosome breaks were seen during the early stages of infection.

1. Hampar, B., Ellison, S. A., *Nature,* 1961, v192, 145.
2. Nichols, W. W., Levan, A., Hall, B., Östergren, G., *Hereditas,* 1962, v48, 367.
3. Stich, H. F., Hsu, T. C., Rapp, F., *Virology,* 1964, v22, 439.
4. Stich, H. F., Van Hoosier, C. L., Trentin, J. J., *Exp. Cell Res.,* 1964, v34, 400.
5. Hampar, B., Ellison, S. A., *Proc. Nat. Acad. Sci.,* 1961 v49, 474.
6. Koprowski, H., Pontén, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., Saksela, E., *J. Cell. and Comp. Physiol.,* 1962, v59, 281.
7. Shein, H., Enders, J., *Proc. Nat. Acad. Sci.,* 1962, v48, 1164.
8. Moorhead, P. S., Saksela, E., *J. Cell. and Comp. Physiol.,* 1963, v62, 57.
9. Rapp, F., Benyesh-Melnick, M., *Science,* 1963, v141, 433.
10. Rapp, F., Vanderslice, D., *Virology,* 1964, v22, 321.
11. Aula, P., *Hereditas,* 1963, v49, 451.
12. Kleinfeld, R., Melnick, J. L., *J. Exp. Med.,* 1958, v107, 599.

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Pituitary Gonadotropic Inhibitory Activity of Various Steroids in Ovariectomized-Intact Female Rats in Parabiosis. (29634)

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The anti-gonadotropic activity of a considerable number of steroids has been evaluated in castrated male-intact female parabiotic pairs(1,2), but few steroids have been studied in the intact female-ovariectomized female parabionts. Meyer and Hertz(3) determined the dose of estrone required to inhibit completely the post-castration hypersecretion of gonadotropins. Biddulph *et al*(4) have studied the relative potency of estradiol-17β, estriol and progesterone in this assay and Byrnes and Meyer(5) reported on the relative pituitary inhibition and uterine stimulation activity of estrone and estradiol-17β. Dorfman and Dorfman(6) evaluated the reproducibility of the assay procedure employing estrone as the reference standard.

The pituitary gonadotropic inhibitory activity of certain synthetic steroids, mainly ring A phenolic compounds, or derivatives thereof, have now been studied with the objective of finding steroids with clear dissociations of pituitary inhibitory activity and estrogenic potencies.

Method and materials. The method has been detailed elsewhere(7) and will only be