15785 P

Effect of Colchicine on Resting Cells in Tissue Cultures.

B. MISZURSKI AND L. DOLJANSKI. (Introduced by L. Olitzki.)

From the Department of Experimental Pathology (Cancer Laboratories), The Hebrew University, Jerusalem, Palestine.

During our investigations on the effects of colchicine on cells growing *in vitro* we found that this drug, in addition to its action on the division mechanism of cells, also has a definite effect on the cytoplasm of resting cells.

The design of the experiments was as follows: Forty-eight-hour-old hanging drop cultures of chick embryo heart fibroblasts were immersed in a 10^{-4} or 10^{-5} M solution of colchicine in Ringer. The effect of colchicine was studied from the start of the experiment up to a period of 8 hours in the living state on the warm stage, and in preparations fixed in Carnoy's fluid and stained *in toto* with Giemsa's stain. In another series of experiments the colonies of fibroblasts were cultured for 24-48 hours in media containing similar concentrations of colchicine and were examined in the living state, as well as after fixation and staining.

In a culture of fibroblasts immersed in a colchicine solution of one of the above concentrations, it can be ascertained that, after 5-15 minutes the surfaces of the individual cells forming the zone of outgrowth display an extraordinary activity. The cell processes which connect neighboring cells or extend into the medium are withdrawn and the cells become independent of each other. Such a detached cell sends out pseudopodia in all directions; these pseudopodia are in constant motion, being rapidly projected and retracted. This motion is so rapid and so violent, that the peripheral portions of pseudopodia are often torn off. The cells tend to assume a polygonal or spherical shape and their cytoplasm becomes increasingly basophilic. The superficial cell layers remain active for many hours, short, broad, bleb-like pseudopodia, appearing and disappearing with great rapidity from all sides of the cell body. This picture closely resembles the behavior of the cytoplasm of dividing cells during anaphase and telophase. Often the cells become detached not singly, but as small groups of 2, 3 or more. They remain together as a small clump and this unit shows at its periphery all the phenomena described for the surface of the individual cells (Fig. 1, A and B).

The cell colony treated with colchicine soon loses its regular texture and is composed not of radially arranged spindle cells but of separated single cells and small cell conglomerates (Fig. 1, C). The process of disruption of the texture of the culture is initiated at the periphery and spreads towards the center. Usually within an hour the whole cell colony looks as if it were torn to shreds.

Most of the loosened and rounded fibroblasts do not show any signs of degeneration during the period of observation and the structure of the resting nuclei remains completely unaltered. Only occasionally there is a moderate hydropic vacuolization of the cytoplasm. Even prolonged treatment with colchicine in the above mentioned concentrations has no lethal effect on the cells. Cell colonies cultured for 48 hours in medium containing a colchicine solution 10^{-5} M show an abundant outgrowth after being transferred to normal medium.

The view has often been expressed[1-3] that the essential point in the action of colchicine on the mitotic process is the suppression of the gelation of the spindle. Our observations suggest that colchicine is able to affect not only the spindle substance but also the sol-gel equilibrium of the cortical layer in

---

1 Beams, H. W., and Evans, T. C., *Biol. Bull.*, 1940, **79**, 188.

2 Ludford, R. J., *J. Nat. Canc. Inst.*, 1945, **6**, 89.

3 Wilbur, K. M., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 696.

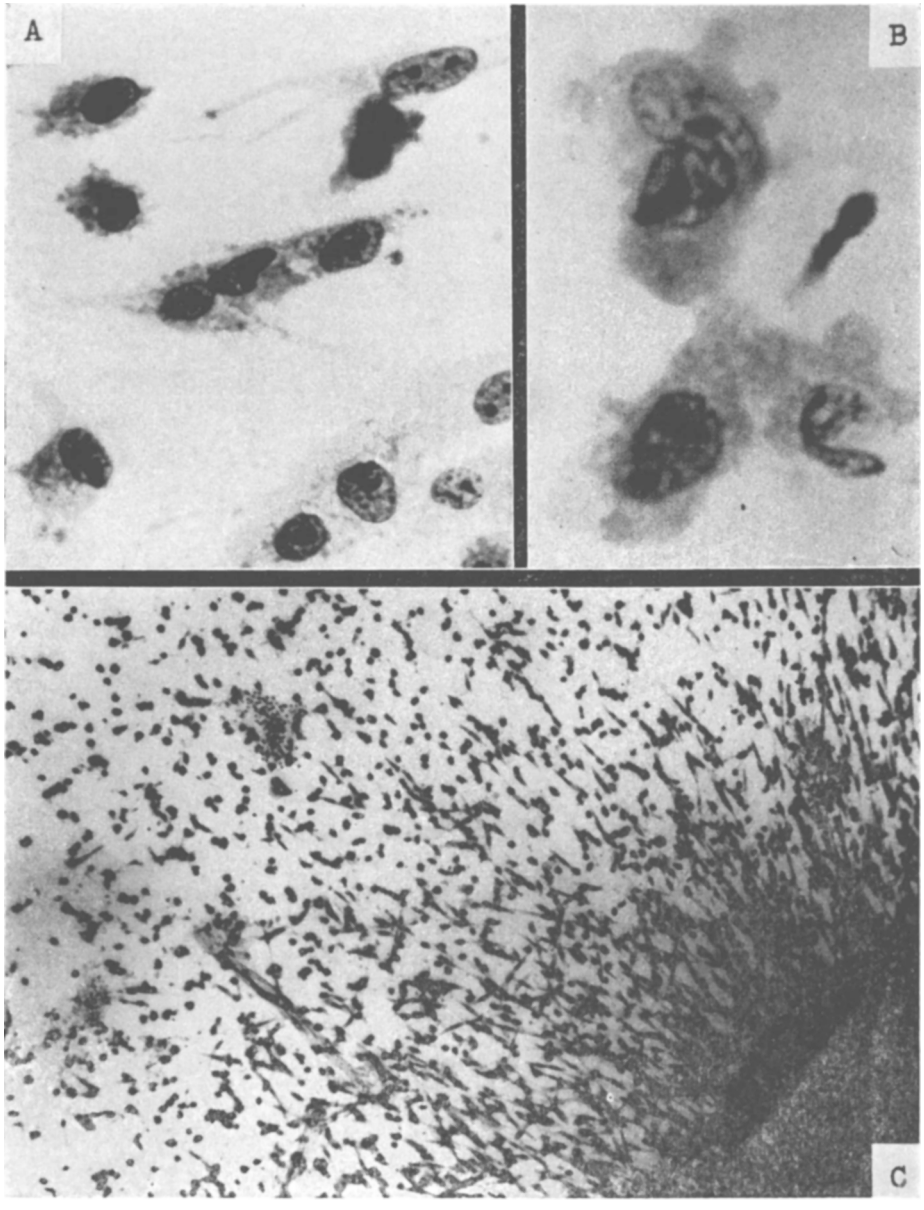

FIG. 1.
Detached and rounded fibroblasts in cultures treated with 10^{-5} M colchicine solution for periods from 15 minutes to 1 hour. Giemsa's stain. A, × 800. B, × 1800. C, × 120.

the resting cell.

As far as can be judged from the illustrations in the paper of Gavrilov and von Bistram,[4] it is possible that these authors have observed in tissue cultures treated with colchicine, the phenomenon described by us. Ludford[5] has noted that resting cells under the influence of weak solutions of colchicine tend to become less spread out and more rounded.

Summary. Chick embryo heart fibroblasts cultured *in vitro,* when immersed in colchicine

[4] Gavrilov, W., and von Bistram, D., *Bull. Assn. franc. p. l'étude du cancer,* 1939, **28**, 319.

[5] Ludford, R. J., *Arch. f. exp. Zellforschg.,* 1936, **18**, 411.

solutions 10^{-4} or 10^{-5} M, show a tendency to assume a spherical shape. Their surfaces display a considerable activity as manifested by rapid protrusion and withdrawal of pseudopodia. Subsequently the zone of outgrowth of the culture splits up into detached elements, comprising one or more cells. This whole process is not lethal to the cells and is, to a great extent, reversible.

15786

Production of Polycythemia in Rabbits by Anoxia and Cobalt.*

JOSEPH GOODMAN (with the technical assistance of Byron Howells.)
(Introduced by D. R. Drury.)

From the Department of Aviation Medicine, University of Southern California, Los Angeles, Calif.

A need for polycythemic rabbits as experimental animals led to the consideration of methods of producing the state of polycythemia in this species. There are at least 2 experimental methods for inducing this condition. The older and better known of these is the response to anoxia induced by high altitude; another is the administration of cobalt salts.

Although the stimulating effect of lowered oxygen tension is well known and has been extensively studied, its mechanism is not as yet clearly understood. The effect has been noted in many animals other than man.[1] These include monkeys,[2] dogs,[3] rats[3] and rabbits.[3-5]

Armstrong and Heim[4,5] reported on the exposure of young male rabbits for 4 hours daily 5 days a week to pressures equivalent to 18,000 feet. In addition to this treatment the animals were subjected to an altitude tolerance test on the 7th day. This tolerance test involved rapid decompression to the point where the animals became unconscious. The equivalent altitude attained was noted and the animal immediately returned to ground level. In the 3rd week of their experiment a deterioration set in and the hemoglobin and hematocrit levels depreciated to lower than the original values. They concluded that rabbits were quite unsatisfactory for this work.

Later Thorn, Jones, Lewis, Mitchell and Koepf[3] conducted a series of experiments in which they exposed rabbits to pressures equivalent to 18,000 and 25,000 feet for 4 hours a day 7 days a week. The 18,000-foot animals were continued for 5 weeks whereas the 25,000-foot experiment was discontinued at the end of 3 weeks because of excessive fatalities. Those exposed to 18,000 feet showed a temporary delay in growth, together with a slight drop in the concentration of serum, sodium chloride and CO_2 combining power and a decrease in the weight of the thymus gland. These changes were accompanied by an increase in O_2 capacity of the blood, in the hematocrit values and in the adrenal gland weight. Seventy-five percent of those exposed to 25,000 feet died of hemorrhage into the lungs and/or herniation of distended loops of the intestines into the thoracic cavity. There were no fatalities in the 18,000-foot group, nor was there evi-

* This work was supported in part by the OSRD and in part by the U. S. Army Air Force, Air Material Command, Wright Field, Dayton, Ohio.

1 McFarland, R. A., *J. Comp. Physiol.*, 1937, **23**, 244.

2 Jasper, H. H., *Canadian National Research Council*, Ottawa, 1942.

3 Thorn, G. W., Jones, B. F., Lewis, R. A., Mitchell, E. R., and Koeff, G. F., *Am. J. Physiol.*, 1942, **137**, 606.

4 Armstrong, H. G., and Heim, J. W., *J. Aviation Med.*, 1938, **9**, 45.

5 *Ibid.*, 1938, **9**, 92.