Inhibitory Action of Colchicine on Cell Movement in Organ Culture* (34131)

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(Introduced by K. C. Swan)

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This study reports the development of an organ culture system using wholly synthetic incubation media as a model system for the investigation of connective tissue cell responses to standard injuries. The system maintains its morphological integrity in culture up to 10 days (the longest time period studied) without microscopic evidence of necrosis. In this study the system was used to investigate connective tissue cell migration after wounding. When the cells in a central zone of the cornea were killed by freezing, this acellular zone was gradually, but sparsely, replaced with cells which migrated from a zone of mobilized connective tissue cells at the periphery of the injury. In the course of using colchicine, a well-known inhibitor of mitosis, to evaluate the role of cell division in the repopulation of the frozen, acellular zone, a unique inhibitory effect of colchicine on cell movement was observed. The experiments reported here describe the organ culture system, the sequence of cell replacement and the inhibition of cell migration by colchicine.

Materials and Methods. Young adult New Zealand white rabbits, weighing approximately 2 kg, were anesthetized briefly with intravenous injections of Surital (Sodium thiamylal, Parke, Davis and Co.). The corneas were also anesthetized by topical applications of Dorsacaine and the center of each cornea then frozen with the cataract cryoprobe (tip approximately 2 mm square) of the Linde CE-3 cryosurgery unit. All freezes were for 10 sec at −48°. The rabbits were allowed to awaken from the anesthetic and were then killed by air injection. The eyes were enucleated and the entire corneas removed with a 1–2-mm tag of sclera attached, for handling, around the entire circumference of the cornea. The lens and iris were discarded and the corneas rinsed in incubation medium for 20 min prior to transfer to 50-ml DeLong culture flasks containing 15 ml of medium. Incubation was carried out at 33.5° in a New Brunswick gyrotory incubator shaker, rotating at minimal speed (188 rpm) and gassed with air-CO_2. Media were changed every 24 hr. Composition of the medium was Eagle's Minimum Essential Medium (Earle's balanced salt solution) supplemented with glutamine to 2 m*M*/liter and MEM nonessential amino acids to 0.1 m*M*/liter. Penicillin (100 units/ml), streptomycin (100 μg/ml), and fungizone (0.25 μg/ml) also were added.

Series of experiments also were done using crystalline bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) at 1 mg/ml and at 5 mg/ml, as well as fetal bovine serum (10 ml FBS/100 ml media). No differences were noted with these different media.

Results and Discussion. At the end of experimental culture periods the corneas were frozen in liquid nitrogen and coronal sections cut. The sections were air-dried, fixed in absolute methanol, and stained with dilute Giemsa (1) for 10 min.

All of the connective tissue cells in the frozen zone were killed. They fragmented and gradually disappeared over a 48-hr period, although in rare cases they became mummified in appearance. However, cells at the edge of the frozen area gradually underwent a series of morphological changes, developed numerous large nucleoli, basophilic cytoplasm, and eventually became elongated (Fig. 1). These changes occurred primarily between 24 and 48 hr after injury (2), al-

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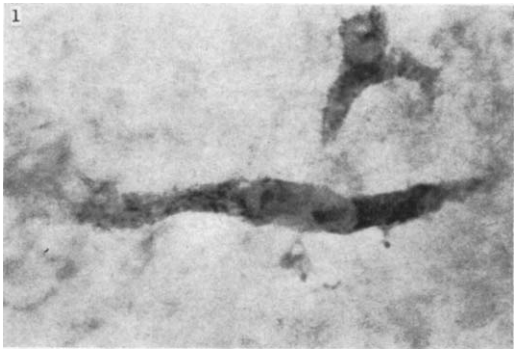

FIG. 1. Elongated migrating fibroblast in 96-hr organ culture of wounded rabbit's cornea; Giemsa stain; ×1275.

though developing nucleoli were usually discernible for 24 hr. By 48 hr these changes appeared to be relatively complete. Migration of these activated cells into the killed zone began between 48 and 72 hr after injury. Fibroblasts sparsely spanned the entire frozen zone at 96 hr. These reactions to this degree of injury were found to be extremely reproducible.

When colchicine (either 1×10^{-5} *M*/liter or 1×10^{-7} *M*/liter) was added to the medium at any time prior to 48 hr (0–24 hr or 24–48 hr), all of the cells continued to develop nucleoli and deeply basophilic cytoplasm but did not become elongated. If colchicine was added from 48–72 hr, when the cells had already elongated and were migrating in the control tissues, all of the colchicine-treated cells rounded up and did not move (Fig. 2). If colchicine was added at 72 hr, when cells had already moved part way into the killed zone, all of the cells stopped moving and again rounded up. No mitotic figures were seen at any time period. Colchicine was only partly inhibitory at a concentration of 1×10^{-8} *M*/liter and without effect at 1×10^{-9} *M*/liter. The inhibitory effects of colchicine (1×10^{-7} *M*/liter) were completely reversed when the cultures were returned to control media for 48 hr.

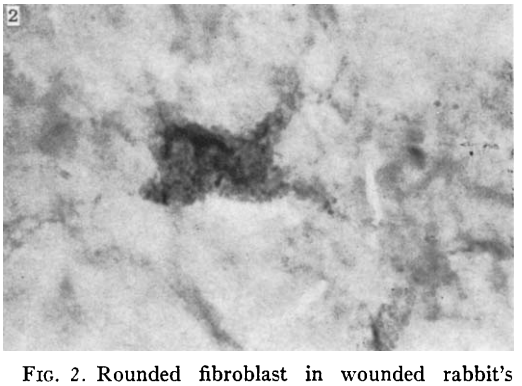

FIG. 2. Rounded fibroblast in wounded rabbit's cornea in which migration has been inhibited by colchicine (1×10^{-7} *M*/liter) added at 72 hr. 96-hr organ culture; Giemsa stain; ×1275.

In addition to its action as an inhibitor of mitosis, scattered reports have appeared in the literature describing the effects of colchine on other cellular activities. In resting stage hanging-drop cultures of chick heart fibroblasts, Miszurski (3) found that colchicine (1×10^{-3} to 1×10^{-7} *M*/liter) caused a withdrawal of cell processes which connected neighboring cells or which extended into the medium. Colchicine diminished phagocytosis of uric acid crystals by polymorphonuclear leukocytes (4), inhibited (1.5×10^{-7} *M*/liter) chemotactic migration of human polymorphonuclear leukocytes (5), and decreased (5×10^{-4} to 1×10^{-6} *M*/liter) the frequency of saltatory movement of fat droplets and phagocytosed carbon particles in cultured HeLa cells (6). Godman (7) has described the disruption by colchicine (1×10^{-5} *M*/liter to 1×10^{-8} *M*/liter) of sarcoblast ribbons grown in tissue culture. He proposed that these changes might be due to the action of colchicine on an oriented system of extended protein micelles. In addition Bischoff and Holter (8) observed a parallel effect of colchicine (1×10^{-6} to 1×10^{-7} *M*/liter) on myotube fragmentation and metaphase arrest in 3-day cultures of chick embryo breast muscle. It would appear that colchicine might provide a very useful tool for studying the mechanisms of cell movement and, further, that such studies might help to further elucidate the mechanism of action of colchicine.

Summary. An organ culture method for studying the migration of connective tissue cells into wounded tissues was described. Cells at the periphery of a frozen, killed zone appeared to undergo a period of mobilization during the first 48 hr after injury. After mobilization, cells began to migrate into the

killed zone, gradually but only sparsely refilling the previously acellular area by 96 hr after injury. Colchicine (1×10^{-7} M/liter) totally inhibited cell movement when applied either before or after cell movement had already begun. The inhibitory effects of colchicine were completely reversible. No mitotic figures were observed at any time period studied (0–96 hr).

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