monary arterial system. This event is signalled by a further rise in pressure to approximately double that of the mean right ventricular pressure. The balloon can now be deflated and the three-way stop cock turned from the manometer to the saline drip to prevent clotting. The entire catheterization procedure takes approximately 5 minutes. The maximum time of passage through the right heart is usually no more than a few seconds.

For acute occlusion of the pulmonary arteries or the chambers of the right heart, the balloon may be reexpanded. Right or left pulmonary artery occlusion is usually obtained with 5-7 cc of saline. If specific placement of the balloon is required, this system may be filled with a radio-opaque liquid instead of saline and placed by means of fluoroscopy.

Discussion. The success with which these catheters are placed is due mainly to their complete flexibility. In contrast to the usual stiff radio-opaque catheters, these cannot be "guided" at all and depend entirely for their directional movement on the flow of blood pushing the partially inflated balloon through the chambers of the heart—hence "self-guiding". It should therefore be emphasized that the much more flexible polyvinyl plastic is necessary and that substitution of the stiffer polyethylene plastic for the outside tube is not recommended.

If the catheter is truly self-guiding, no skill should be necessary for its placement once it has been inserted into the jugular vein. This has been repeatedly verified by asking medical students and other staff members to place this catheter into the pulmonary artery merely by watching the pressure rise in the saline manometer as they slowly advance the catheter into the jugular vein. The branching of the two main pulmonary arteries is such that this catheter will in most instances enter the left pulmonary artery.

One disadvantage of this catheter is the clots which frequently develop along the folds of the balloon. These, however, are minimal in size and number and have not interfered with the experimental procedures.

Summary. 1. A self-guiding catheter has been described for placement in the right heart or pulmonary arteries of dogs. 2. This technic does not require a fluoroscope and localization is determined from the pressure recording. 3. The same catheter has likewise been used routinely for occlusion of the left pulmonary artery.

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Influence of Colchicine on the Form of Skeletal Muscle in Tissue Culture.* (20746)

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When mature mammalian striated skeletal muscle is cultured *in vitro,* as shown by Pogogeff and Murray(1), there ensues intensive regeneration of muscle with the formation of long continuous sarcoblastic ribbons or fibers. In the rat these may reach lengths approaching 3 mm, and widths of 6 to 15 μ (Fig. 1). Their cytoplasm is highly refractile, firm, and viscous or gelated(2); they exhibit a faint positive birefringence; they often show strong contractile activity (fibrillation and pulsation) and many subsequently differentiate, developing longitudinal and cross-striations.

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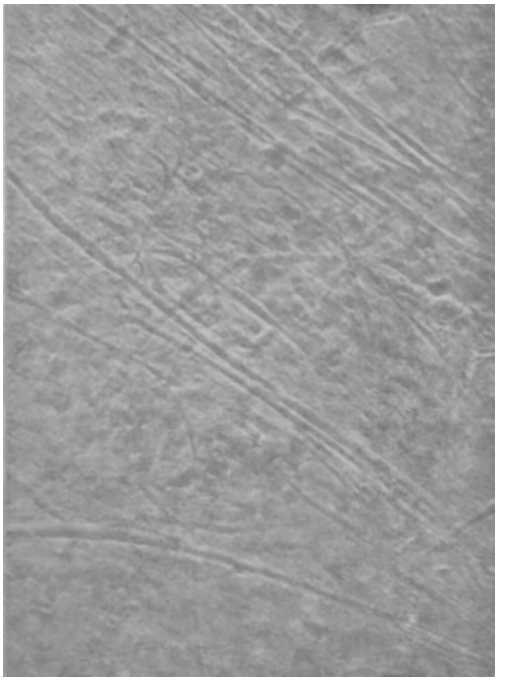

FIG. 1. Control culture, at 17 days. Long slender sarcoblastic fibers. × 100.

In the course of investigations of the mechanisms of nuclear division in sarcoblasts, tissue cultures were treated with colchicine and certain other agents, The remarkable change in the form and structure of the muscle ribbons, leading to their dismemberment, which was observed consequent on administration of colchicine is the subject of this communication.

Materials and methods. A total of 210 successful cultures were prepared by explanting gluteal and hamstring muscle of suckling rats by the double coverslip method of Maximow (3). The planting mixture consisted of 1 part chicken plasma, 2 parts human placental serum and 1 part 50% chick embryo extract. Cultures were washed in Simms X-7 balanced salt solution(4) and fed thrice weekly with a mixture in which the plasma of the planting mixture was replaced by ox-serum ultrafiltrate. In subsequent feedings the proportion of embryo-extract was gradually somewhat reduced, to encourage differentiation. Cultures were maintained for periods of time ranging from 15 to 75 days, and two were kept for 185 days. At various ages, groups of 2 to 10 cultures were treated with colchicine or other biologically active substances, included in the feeding mixture to the final concentrations given in the following list: *Colchicine,* from 10^{-5} M in 5-fold dilutions to 10^{-8} M; chloral hydrate, 10^{-3} and 10^{-4} M; benzoquinone, 10^{-3} and 10^{-6} M; sodium barbital, 10^{-5} M; heparin 0.25 mg/ml, quinine dihydrochloride, 2.5 x 10^{-5} M; procaine base 4 x 10^{-5} M; "veratrine" 10^{-5} g per ml; d-tubocurarine hydrochloride, 10^{-5} M; "mapharsen" (3 amino 4 hydroxyphenylarsenoxide hydrochloride) 5 x 10^{-5} M. Untreated controls were also used, but it was thought that an assortment of other drugs which included mitotic poisons and agents which affect muscle contraction should be employed as further control material. The *standard test period* during which each agent was first allowed to act on a group of cultures was the usual interval between feeding, *i.e.* 48 hours. In subsequent experiments, periods of 12, 18, 24 and 36 hours and from 4 to 20 days of continuous exposure to colchicine were tested. Treatment was stopped by thorough washing in two changes of balanced salt solution; cultures were then either fixed, or re-fed and allowed to recover. The observations re-

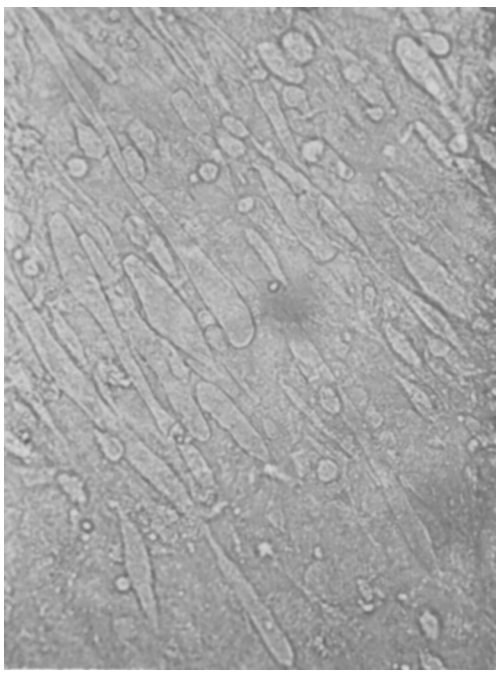

FIG. 2. Culture G 6, treated at age 15 days with 5×10^{-7} M colchicine. Short, widened segments 48 hr later. × 100.

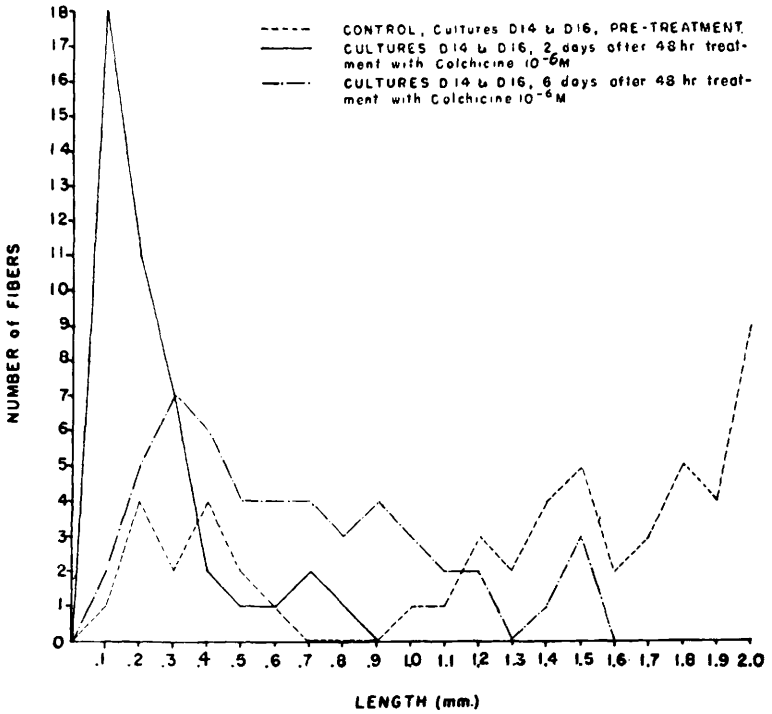

FIG. 3. Frequency polygon showing effect of colchicine on length of sarcoblast fibers at successive intervals. Note recovery phase 6 days after beginning of treatment.

corded in the present report were all made on living material.

Results. Untreated muscle cultures: Outgrowth of muscle slips was established in 7 to 15 days, and continued until the maximal rate was attained, usually in not less than 15-20 days from the time of appearance of the sarcoblasts. By this time some had attained lengths up to 3 mm. Measurements of the lengths of all muscle fibers in representative fields of the outgrowth zone, when plotted as a frequency polygon or histogram, were distributed in a characteristic bimodal curve, reflecting the presence of long, continuous fibers and lesser numbers of short strap forms of from 0.2 to 0.7 mm (Fig. 3).

Colchicine effects. Such cultures, when treated with colchicine in effective dosage, invariably suffered the same striking disruptive changes. At first, the fibers or straps developed focal expansions, ovoid or fusiform bulges, sometimes appearing somewhat haustrated. As these were pulled apart by their shortening and widening, they remained connected only by slender bridges of sarcoplasm. At the same time, sharp diagonal, transverse, tangential, and longitudinal clefts appeared in remaining lengths of the fibers, and effectively completed the process of segmentation which ultimately overtook almost all of the fibers. The developed picture was one of a generalized fragmentation of the muscle slips into short, distinctly widened, somewhat angulated segments, usually still in the linear disposition of their original fibers, and averaging 0.1 to 0.3 mm in length. As the effect of colchicine progressed, the pieces shortened and spread further, most measuring between 0.03 and 0.15 mm in length and 20-24 μ across (about three times the median widths of fibers in control cultures of the same age). They appeared as chunky, separated, sessile masses with rounded contours (Fig. 2), of definitely increased transparency and diminished refractility, usually without vestiges of striation, and apparently of much diminished consistency. It is of particular interest that some rhythmically contracting fibers, usually striated, continued to fibrillate even after segmentation was fairly advanced, the partially separated pieces con-

tracting independently. In the later stages, as the effects of colchicine progressed, contractility was lost. Granules and droplets were not generally prominent, and all except the smallest segments contained one or more nuclei. The latter, typically elongated in the normal sarcoblast fiber, assumed a spherical shape with augmented diameter in the more advanced colchicinized segments, as though released from the constraint of the stiffened sarcoplasm. Anucleate fragments (sequestra) and a few of the other segments degenerated and disappeared. Most segments, however, remained viable, with relatively clear, transparent cytoplasm, or showed only minor toxic effects, even after prolonged continuous exposure to colchicine; for when the drug was removed they regenerated to nearly normal appearance and function.

Colchicine solutions in dilution to about 10^{-8} M elicited the change in the fiber form during the standard 48-hour exposure. In assessing dosage and effects, the principal variants considered were drug concentration, duration of action and age of the culture, or more precisely, the size and degree of differentiation of sarcoblasts. In summary, it may be said that except with the lowest effective concentrations of the drug, the first changes were observed 5 to 9 hours after colchicine administration. With 36- to 48-hour exposures, they reached their maximal extent in 4 to 5 days after administration, regardless of whether the culture was continuously colchicinized, or whether it had been washed and fed after the exposure period; that is, the effect of colchicine continued after it was removed from the medium.

In the course of 18-36 hours exposure to effective concentrations of colchicine, almost all of the slender and more undifferentiated sarcoblastic ribbons segmented; the broader, more differentiated fibers remained as a rule in earlier stages of colchicine alteration, being apparently more resistant to the drug. Cultures of intermediate or older age group, whose population of more differentiated and larger elements was greater, were accordingly slower to respond.

Concentrations of colchicine of 10^{-5} M were somewhat toxic to some cultures; the magnitude of the effects elicited by lower titers on fibers of similar ages was proportional to dosage: concentration x time of exposure.

Recovery. The rapidity and completeness of recovery were inversely proportional to drug dosage and toxicity. After a standard 48-hour treatment with colchicine concentrations of 10^{-5} M or less, recovery occurred in most cultures within a fortnight. Restitution did not occur in the presence of colchicine.

Controls. Changes of the kind described were never seen in the fibers of normally growing untreated muscle cultures. Neither the mitotic poisons nor the alkaloids, nor the organic arsenical listed, administered in concentrations comparable to or greater than the colchicine concentrations, produced an effect in any way similar to that of colchicine. These effects of colchicine on sarcoblastic ribbons were unfailing, and elicited specifically by colchicine, in high dilution.

Discussion. Three points of interest emerge from these observations of the effects of colchicine upon the sarcoblastic cytoplasm: the low concentrations at which they may take place, the length of time required for full effect and for recovery, and the dramatic nature of the changes themselves.

It is apparent that colchicine exerts a profound effect on the structural integrity of the immature muscle fiber, in concentrations considerably below those which inhibit mitosis in fibroblasts of the same species. It is probably significant that concentrations of the same order (about 2.5×10^{-8} M), according to Barany and Palis(5), condition a 25-30% decrease in the rate of the viscosity fall of actomyosin gels induced by ATP. These small concentrations are supposedly without visible effect on adult "muscle fibers"(5,6), although somewhat greater quantities are said to cause the Lundsgaard effect to appear in muscle preparations, owing to an interference with glycolysis(6).

Minimal effective concentrations of colchicine acting reversibly as a mitotic poison on cultures or isolated cells vary in different cell types, but usually exceed 10^{-6} molar. In the case of rat fibroblasts it is about 2×10^{-6} molar (7). But such concentrations may cause other effects such as alterations of cytoplasmic viscosity. Visual evidence of decreased proto-

plasmic viscosity in resting cells treated with colchicine has been recorded(7-10). Colchicine causes Arbacia eggs to stratify more easily when centrifuged, and inhibits the increased gelation that normally occurs during their division or after fertilization(11,12). The reduced cytoplasmic viscosity caused by colchicine in onion root-tip cells is thought to be responsible for "c-tumor" formation(13). Stalfelt(14) however has reported that colchicine increases protoplasmic viscosity.

In regeneration *in vivo* and *in vitro,* skeletal muscle is one of the slowest-growing of the tissue types and is characterized by an extraordinarily long lag-period, in these observations 7-15 days for the rat, with an added 15-20 days before maximal growth is reached. It may be presumed that one factor in this characteristic slow growth is the elaborate differentiation which is proceeding simultaneously. It is interesting that although colchicine may be assumed to penetrate the cell very quickly, since the first changes are noted within 5-9 hours after exposure, its maximal effects are not reached for 4 or 5 days, and are exerted even though the agent might have been withdrawn after 36-48 hours. Recovery occurs in approximately a fortnight. In the rat fibroblast the mitotic effects of colchicine reach their height at 20 hours(7), and recovery occurs within an equivalent time.

It is reasonable to assume that the ribbon-like continuity of the sarcoblast fiber, its gelated condition and positive birefringence are explainable structurally in terms of a parallel arrangement of longitudinally oriented long-chain protein (myosin) micelles. This submicroscopic structural plan of the sarcoblast is re-exhibited in microscopic dimensions with differentiation and the development of longitudinal striation. It is possible that colchicine may interfere with the maintenance of this organized micellar pattern by acting to bring about either a folding of the long protein chains (fibrous to globular)(15) or a dissociation of aggregates of the polypetid micelles (13). The results of such action would be to effect a more random arrangement of protein chains, which might be grossly manifested in shortening, alteration of form and size, and decreased viscosity.

It is suggested that such action by colchicine is visibly expressed here in destruction of the ribbon continuity and in the shortening and expansion of resulting fragments of the sarcoblastic fiber, a protoplasmic mass in which the alignment, length and numbers of oriented protein chains reach extreme development.

Summary. Colchicine administered to tissue cultures of rat skeletal muscle in concentrations of 10^{-8} molar or greater causes a breaking-up of the sarcoblastic ribbons into short, widened segments of apparently decreased consistency. These segments remain viable. 9 other biologically active agents used as controls have no such effect. A possible mechanism of this action of colchicine is discussed.

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