THE ROLE OF EXTRACELLULAR MATERIALS IN CELL MOVEMENT

I. Inhibition of Mucopolysaccharide Synthesis Does Not Stop Ruffling Membrane Activity or Cell Movement

BRIAN S. SPOONER and GARY W. CONRAD

From the Division of Biology, Kansas State University, Manhattan, Kansas 66506

ABSTRACT

The involvement of mucopolysaccharide synthesis in cell locomotion was investigated by determining the effects of inhibition of synthesis on ruffling membrane activity and cell movement by embryonic heart fibroblasts. Mucopolysaccharide synthesis was inhibited directly by treatment with a glutamine analog, 6-diazo-5-oxo-L-norleucine (DON), and indirectly with cycloheximide. DON treatment reduced synthesis to 20% of control values, and cycloheximide reduced synthesis to less than 10% of control values, as measured by incorporation of [^{35}S]sulfate into mucopolysaccharides. Nevertheless, ruffling membrane activity and cell locomotion continued under both conditions. Cytochalasin B did not inhibit mucopolysaccharide synthesis, although it did stop ruffling and locomotion. These results suggest that if mucopolysaccharides are required for cell movement, they must have long half-lives or represent only a minute fraction of the normal synthetic load.

In recent years, use of refined techniques has provided insight into the mechanism of vertebrate cell locomotion in vitro. Time-lapse cinematography (Ingram, 1969; Abercrombie et al., 1970; Harris, 1973), as well as light and electron microscopy (Buckley and Porter, 1967; Spooner et al., 1971; Goldman, 1972), has revealed several behavioral characteristics of migrating cells and implicated specific subcellular structures in generating the motive forces for movement. Understanding cell movement has been facilitated by recognizing that effective translocation results from three successive events. *First*, the cell extends its leading edge forward into space above the substratum. *Second*, some portion of the extended leading edge is lowered and adheres to the substratum with a net adhesive force greater than that of more posterior points of adhesion. *Third*, a cytoplasmic contraction (or an elastic recoil) takes place between the anterior area of adhesion and more posterior ones, resulting in the cell moving forward as posterior adhesions are broken. This locomotory cycle is repeated as the cell continues to translocate.

A number of investigators are beginning to gain insight into the extension step of locomotion (Ingram, 1969; Abercrombie et al., 1970; Harris, 1973; Wessells et al., 1973), and rapidly accumulating evidence implicates actin and myosin in the contractile phase of the locomotory cycle (Bray, 1973; Spooner et al., 1973; Pollard and Weihing,

1974). However, virtually nothing is known about the mechanism of differentially strong adhesion required for effective translocation. Possibilities include quantitative differences based on a larger area of cell-substratum contact at the leading edge of the cell, and qualitative differences involving different molecular mechanisms. Furthermore, there are, as yet, only hints that glycoproteins (Culp, 1974), calcium ions (Deman et al., 1974), disulfide bonds (Grinnell et al., 1973), and mucopolysaccharides may be involved.

Surface-associated mucopolysaccharides (glycosaminoglycans) and glycoproteins are macromolecules that very well might be involved in cell adhesion. Kraemer (1971), and Kraemer and Smith (1974) have noted, for example, that heparan sulfate occurs on the surfaces of many cells; Pessac and Defendi (1972) have implicated hyaluronic acid in cell aggregation; Terry and Culp (1974) have implicated hyaluronic acid in cell-substratum adhesion; Toole and Trelstad (1971) and Polansky et al. (1974) have suggested the importance of the latter mucopolysaccharide in cell movement in vivo.

The possibility that mucopolysaccharides are involved in cell adhesion was supported by the report (Sanger and Holtzer, 1972) that their synthesis was inhibited by cytochalasin B, a drug that also inhibits cell movement. However, cytochalasin B alters such synthesis only very indirectly by interfering with sugar transport (Cohn et al., 1972; and see below). In this study, we have therefore interfered directly with mucopolysaccharide synthesis by treating a migratory cell-type with the drug 6-diazo-5-oxo-L-norleucine (DON) (Ghosh et al., 1960; Telser et al., 1965; Ellis and Sommar, 1972).

MATERIALS AND METHODS

Cell Culture

Cells were obtained by trypsin dissociation of heart ventricles from chick embryos at 8 days of incubation. Cell suspensions (mixtures of cardiac fibroblasts and muscle cells) were inoculated at approximately 5×10^5 cells per 60-mm diam Petri-style plastic tissue culture dish (Falcon Plastics, Div. of B. D. Laboratories, Los Angeles, Calif.). in 3 ml of nutrient media. Dishes were incubated at 36.5° C in a humidified incubator under an atmosphere of 5% CO_2 and 95% air. Nutrient medium consisted of Ham's F-12 containing 2× amino acids and pyruvate, and *non*dialyzed 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Cells were fed three times per week.

Sternal cartilage was obtained from chick embryos after 14 days of incubation, dissected free of fibrous outer layers, cut into pieces, and incubated in 5 ml of nutrient medium in Erlenmeyer flasks on a gyrotory shaker, gassed with 5% CO_2 and 95% air.

Inhibitors

Cytochalasin B was stored at −20° C as a 1 mg/ml stock solution in dimethylsulfoxide (DMSO). All other inhibitors were stored in the dark as dry powders at −20° C and dissolved immediately before use. A glutamine analog, 6-diazo-5-oxo-L-norleucine (DON), and its *N*-acetyl derivative (Duazomycin A) were used as inhibitors of polysaccharide biosynthesis, whereas cycloheximide was used as an inhibitor of protein biosynthesis. All inhibitors were added as concentrated stock solutions directly to Petri dishes containing nutrient media which had been conditioned by cells for 12–24 h. Cells were preincubated with inhibitors for varying periods of time before addition of isotopic precursors. D-glucosamine hydrochloride and cycloheximide were purchased from Sigma Chemical Co., St. Louis, Mo., and cytochalasin B from Imperial Chemicals, Ltd., London.

Measurement of Sulfated Mucopolysaccharide Biosynthesis

Concentrated solutions of [^{35}S]sulfate ($H_2{}^{35}SO_4$, carrier-free, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) were added to cell cultures at a final concentration of 2–7 μCi/ml. Cultures were preincubated with inhibitors for varying periods of time and then incubated in the presence of radioactive precursor for a subsequent 4-h period. At the end of the labeling period, cells were released from the dish surfaces by a 10-min incubation at 37° C with 0.5% trypsin (Difco Laboratories, Detroit, Mich., 1:250)-0.1% ethylenediaminetetraacetic acid (EDTA), counted in hemocytometer counting chambers, and pooled with the labeling medium. Samples were stored at −20°C until analyzed.

Mucopolysaccharide was isolated in the following way. Samples were denatured at 100° C and digested with predigested pronase (0.17 mg/ml final concentration, Sigma) in 0.2 M Tris-HCl at pH 8.0 for 48 h at 50° C (de la Haba and Holtzer, 1965), with fresh enzyme added after the first 24 h. Enzyme digests were treated with cold 5% trichloroacetic acid for 30 min and centrifuged (15 min, 12,000 *g*); pellets contained negligible radioactivity and were discarded. Supernates were extracted with 4 vol of chloroform-methanol (2:1, vol/vol) (Folch et al., 1957). The aqueous layers, together with material at the interface, were first dialyzed overnight against cold running tap water. They were then dialyzed in the presence of chloroform against several changes of 0.05 M Na_2SO_4 (4 days), and finally against distilled water (1 day). Dialyzates were lyophilized to dryness and redissolved in 5 ml of distilled water.

Radioactivity in aliquots of these solutions was measured in a liquid scintillation spectrometer (Packard 3320, Packard Instrument Co., Inc., Downers Grove, Ill.) with an ethanol-toluene scintillation fluid (Stoolmiller and Dorfman, 1969). Background was automatically subtracted. All data were corrected for ^{35}S decay and normalized to an isotope concentration in the nutrient medium of 10 μCi/ml.

Observation of Ruffling and Cell Movement

Cell movements were observed continuously and photographed intermittently by culturing cells in gassed, sealed, plastic T-style culture flasks (Falcon) with an inverted phase-contrast microscope whose stage was kept at 37° C with a Sage air-curtain incubator (Sage Instruments Div., Orion Research, Inc., Cambridge, Mass.). The behavior and morphology of cells under these conditions, in both the presence and absence of inhibitors, appeared identical in all respects to those of cells grown in plastic tissue culture Petri dishes.

TABLE I

*Effect of DON and Duazomycin A on Incorporation of [^{35}S]Sulfate into Mucopolysaccharides by Sternal Cartilage In Vitro**

	Control	DON + Glucosamine	DON
Total cpm per sample‡	822,385	391,701	250,614
Percent of control	100	48	30
	Control	DUA + Glucosamine	DUA
Total cpm per sample§	449,358	425,880	386,568
Percent of control	100	95	86

*Tissues were preincubated in nutrient media alone or in media containing inhibitors and/or glucosamine for 30 min at 0°C, and then incubated in the presence of radioactive precursor for a subsequent 3.5 h at 37°C.
‡ Each sample consisted of two minced sterna.
§ Each sample consisted of one and one-half minced sterna.
DUA = Duazomycin A.

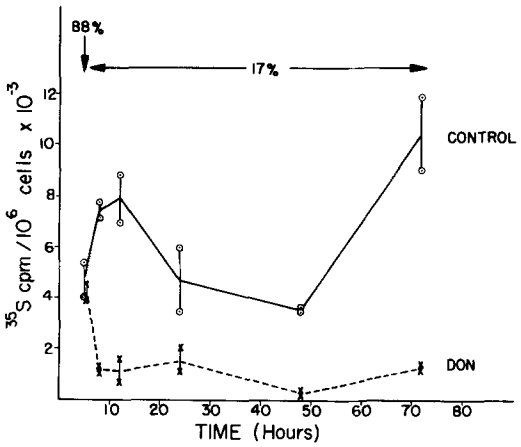


FIGURE 1 The inhibition of mucopolysaccharide synthesis in heart cell cultures during increasing time in DON. For each time point, [^{35}S]sulfate labeling was carried out during the final 4 h. DON concentration was 15 μg/ml in all experimental cultures. Note that a 4-h preincubation with DON, followed by a 4-h labeling period (8-h point), was sufficient to reduce mucopolysaccharide synthesis to 17% of control values. See Fig. 2 for morphological effects.

TABLE II

*Effect of DON on Incorporation of [^{35}S]Sulfate into Mucopolysaccharides by Heart Cells In Vitro**

	Control	DON + Glucosamine	DON
Average number of *attached* cells per dish	804,400	581,200	531,000
Average number of *nonattached* cells per dish	268,800	395,000	390,000
Average *total* number of cells per dish	1,072,800	976,200	921,000
Cpm/10^6 *attached* cells	3,075 3,420 } 3,027 2,586	1,346 1,307 } 1,655 2,313	1,066 723 } 769 517
Percent of control	100	55	25
Cpm/10^6 *total* cells	2,343 2,606 } 2,307 1,972	808 784 } 993 1,388	617 419 } 445 300
Percent of control	100	43	19

* Cells were preincubated with drugs for 12 h at 37°C. Drugs remained present during the 4-h labeling period. Samples were analyzed in triplicate. Both individual and average values are shown.

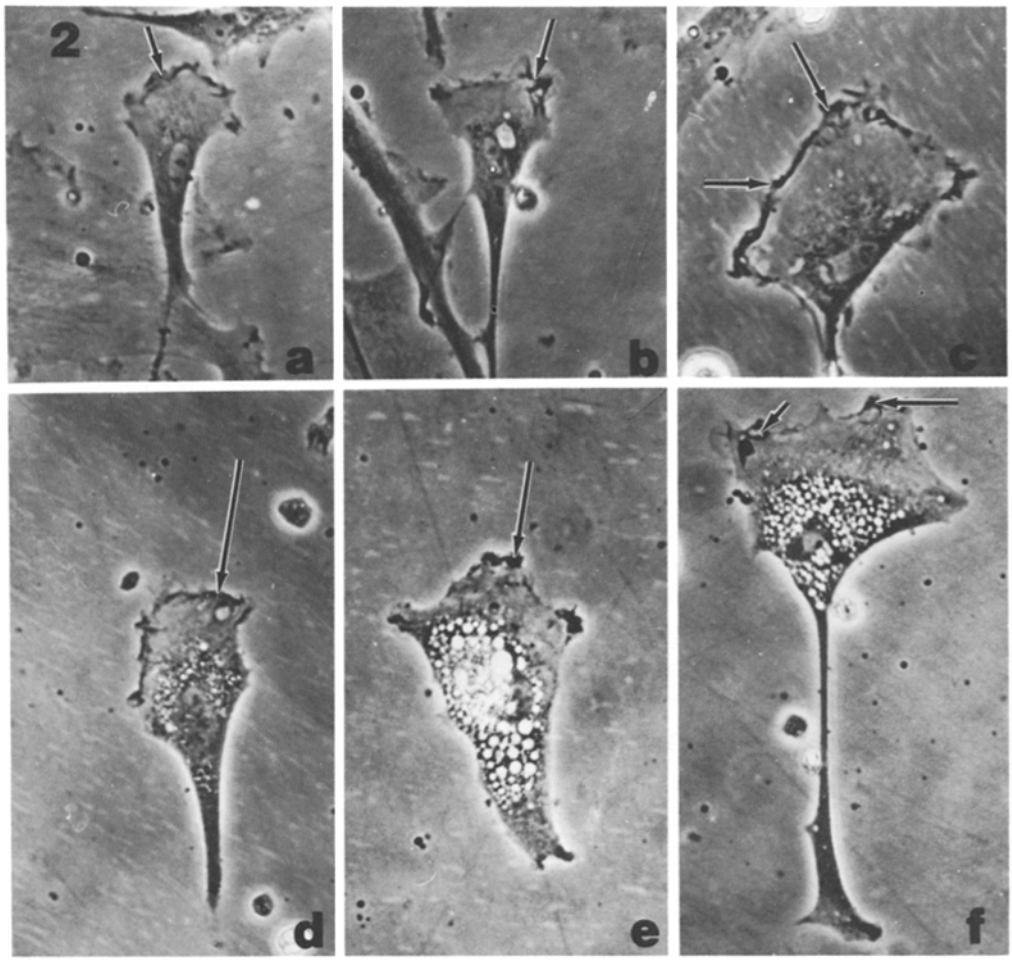


FIGURE 2. Morphological effects of increasing time in DON (15 μg/ml) on heart fibroblasts. See Fig. 1 for DON effects on mucopolysaccharide synthesis at increasing time points. (*a*) DON, 4.5 h; (*b*) DON, 6.5 h; (*c*) DON, 11 h; (*d*) DON, 33 h; (*e*) DON, 58 h; (*f*) DON, 76 h. Note that at all time points the cells exhibit actively ruffling leading edges (arrows). Note also that prominent cytoplasmic vacuoles are present in the cells at 33 h, 58 h, and 76 h of treatment. Phase contrast. × 400.

RESULTS

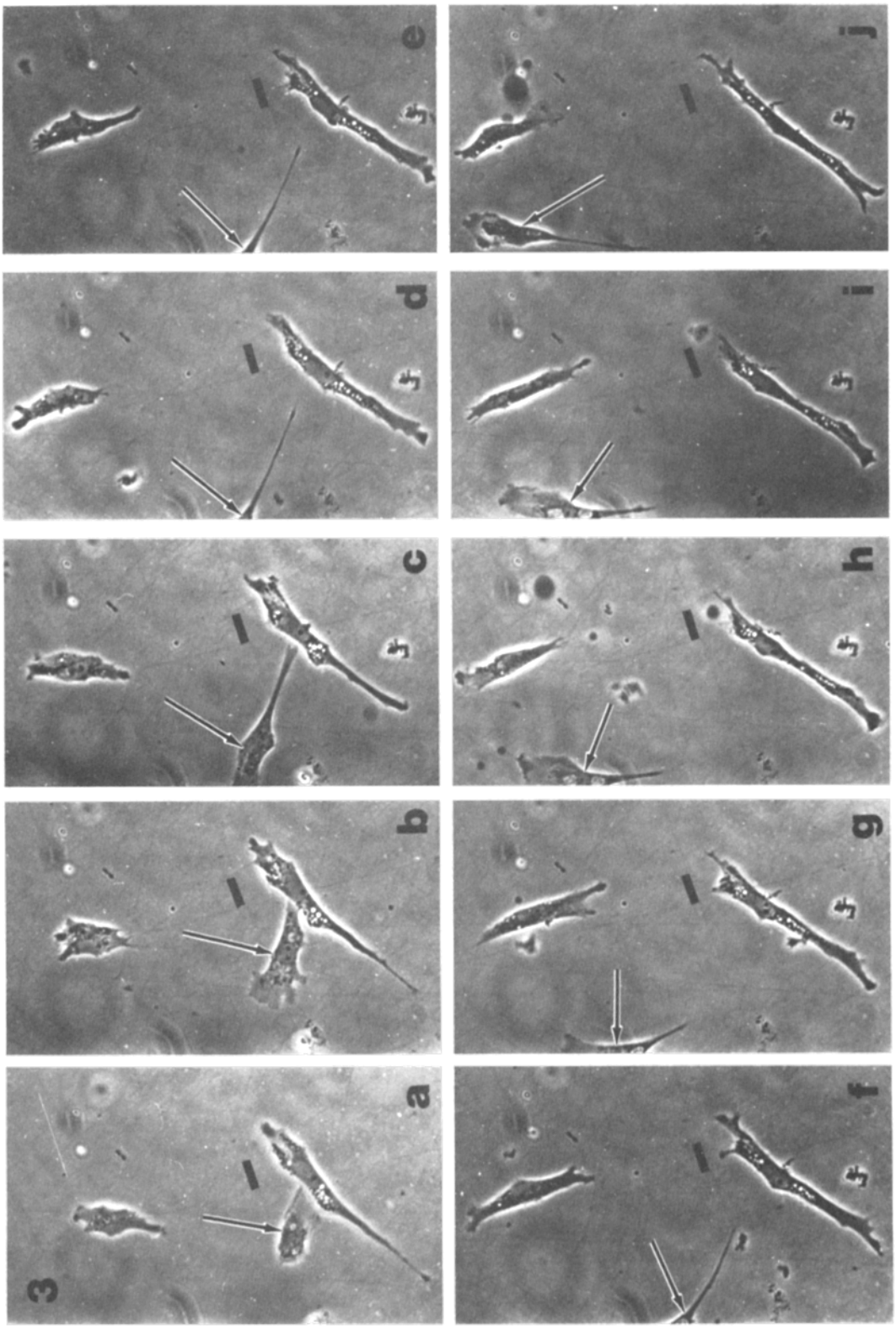
Effects of DON on Mucopolysaccharide Biosynthesis in Sternal Cartilage

The ability of DON to inhibit mucopolysaccharide biosynthesis was confirmed by assessing the effect of the drug on sulfate incorporation by embryonic chick sternal cartilage. The results are shown in Table I. Pretreatment with 15 μg/ml (0.088 mM) DON for 30 min at 0°C followed by a 3.5-h incubation with [^{35}S]sulfate reduced the incorporation of radioactivity into mucopolysaccharides to 30% of controls. The concomitant presence of glucosamine (1 mg/ml, 5 mM) in the incubation medium raised the incorporation of [^{35}S]sulfate to 48% of controls.

Under identical experimental conditions, the *N*-acetyl derivative of DON, Duazomycin A, caused no significant inhibition of mucopolysaccharide biosynthesis (Table I).

Effects of DON on Mucopolysaccharide Biosynthesis by Cultures of Embryonic Heart Cells

Monolayer cultures of embryonic chick heart cells incorporated substantial amounts of [^{35}S]sul-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fate into mucopolysaccharide. Pretreatment with 15 μg/ml DON for 4–68 h at 37°C followed by a 4-h incubation with [^{35}S]sulfate reduced the incorporation of radioactivity to 9%–33% of controls (average = 17%). Higher concentrations of DON (up to 60 μg/ml) did not further reduce the levels of incorporation (see also Bhatnagar and Prockop, 1966). Although DON depressed mucopolysaccharide synthesis during this culture period, the increase in cell numbers in the DON-treated cultures did not differ significantly from the increases in control cultures. Pretreatments with drug for periods of time shorter than 4 h resulted in incorporation rates progressively more similar to those of control cultures (Fig. 1). For most experiments, heart cells therefore were preincubated with DON or DON + glucosamine for 12 h, before addition of [^{35}S]sulfate and further incubation for 4 h. The results of a typical experiment of this sort are given in Table II. The data are given on the basis of numbers of attached cells as well as numbers of total cells, because in the presence of DON or DON + glucosamine there appeared to be a slightly larger proportion of floating cells than in control dishes. As indicated earlier, however, the total number of cells per dish in the DON and DON + glucosamine groups was only slightly below that of control dishes. The data suggest that regardless of whether the attached cell number or total cell number was used in these

TABLE III

*Effects of Cytochalasin B on Incorporation of [^{35}S]Sulfate into Mucopolysaccharide by Heart Cells In Vitro in the Absence of Serum and Presence of Glutamine**

	cpm/10^4 cells	% of control
Control	2,797	100
DMSO	2,300	82
CB + DMSO	2,592	93

* Dimethylsulfoxide (DMSO) controls contained 1% DMSO, and cytochalasin B (CB) cultures contained 10 μg CB/ml medium and 1% DMSO. Cultures were preincubated with drugs for 30 min at 37°C before introduction of radioactivity, and the drugs remained present during the 4-h labeling period.

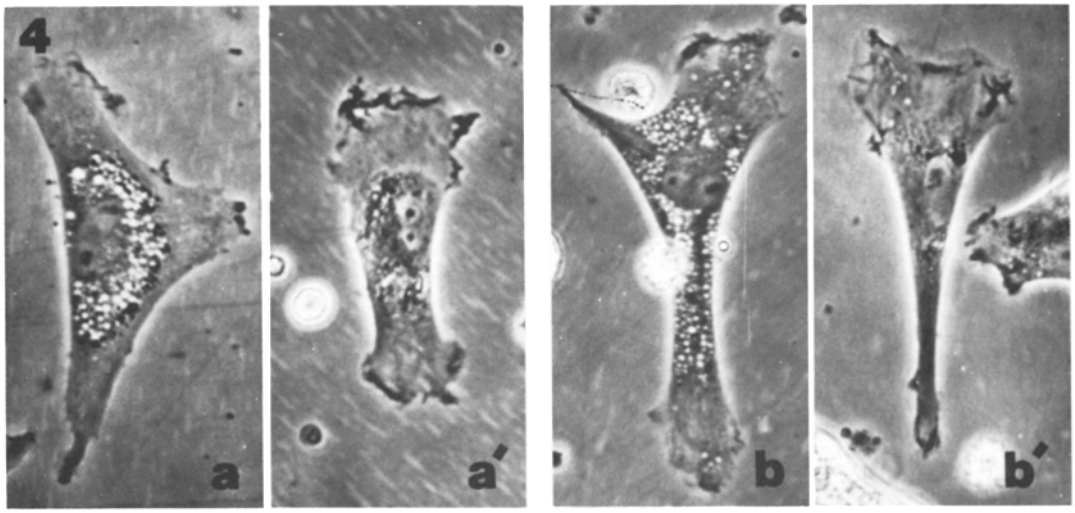

FIGURE 4 The effect of simultaneous presence of glucosamine on DON-induced cytoplasmic vacuoles in heart fibroblasts. (*a*) DON, 58 h; (a') DON + glucosamine, 58 h. (*b*) DON, 76 h; (*b'*) DON + glucosamine, 76 h. Note that the cytoplasmic vacuoles do not appear when glucosamine is present. Phase contrast. × 440.

FIGURE 3 Heart fibroblast translocation in the presence of DON. This culture was exposed to DON (15 μg/ml) for 25 h at the time of the first photograph, and the drug continued to be present throughout this photo sequence. Photo timing with (*a*) arbitrarily 0 min. (*a*) 0 min; (*b*) 42 min; (*c*) 70 min; (*d*) 130 min; (*e*) 215 min; (*f*) 225 min; (*g*) 320 min; (*h*) 340 min; (*i*) 355 min; (*j*) 370 min. The cell marked with the arrow translocates to the left and then to the top of the field. A fixed reference point in the field (black line) emphasizes the distance moved. Phase contrast. × 250.

calculations, the presence of DON in the medium reduced the incorporation of [^{35}S]sulfate to 19%–25% of controls, whereas in the presence of DON + glucosamine, incorporation was reduced to 43%–55% of controls. These data are therefore very similar to those obtained from the cartilage preparations discussed above, and suggest that DON does provide a method for inhibiting mucopolysaccharide biosynthesis in cultured embryonic heart cells.

Partial Characterization of the [^{35}S]Sulfate-Labeled Material Synthesized by Cultures of Embryonic Chick Heart Cells In Vitro

Cultures of embryonic chick heart cells were labeled for 4 h with [^{35}S]sulfate after a 12-h preincubation with DON. [^{35}S]sulfate-labeled material was isolated from these cultures as described above and compared to that isolated

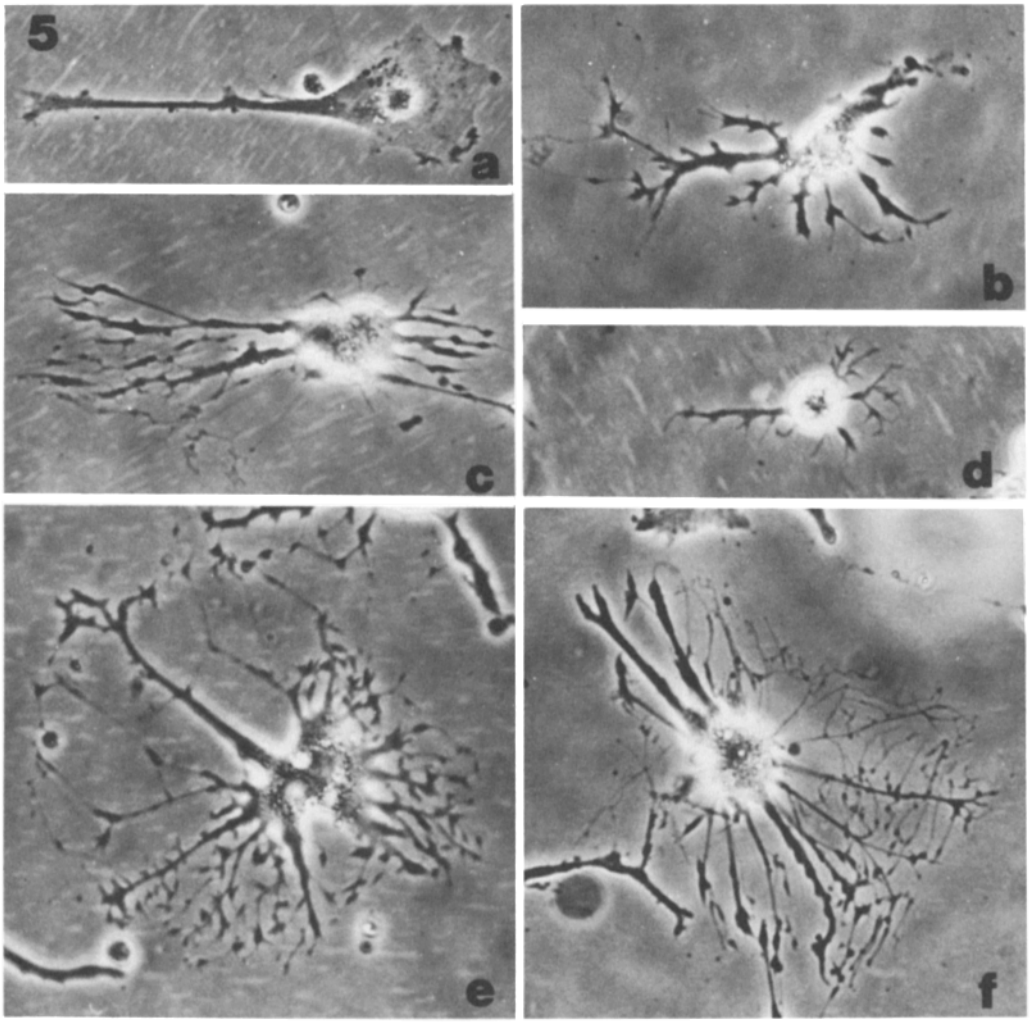

FIGURE 5 Effects of Don and cytochalasin B (CB) on the morphology of heart fibroblasts. See Table III for cytochalasin effects on mucopolysaccharide synthesis. Treatment and magnification for each photo listed below. All phase contrast. (*a*) DON, 17 h, × 360; (*b*) DON, 24 h, CB, 12.5 h, × 400; (*c*) DON, 13 h, CB, 1.5 h, × 450; (*d*) DON, 17 h, CB, 5.5 h, × 500; (*e*) DON, 24 h, CB, 12.5 h, × 400; (*f*) DON, 17 h, CB, 5.5 h, × 400. Cytochalasin B produces severe morphological alterations, while DON has no discernible effect.

from control cultures. When applied to columns of *O*-(diethylaminoethyl)cellulose (DEAE)-Sephadex A-25, the ^{35}S-labeled material from both cultures eluted with solutions of 1.0 M NaCl at a position coincident with chondroitin sulfates. Insufficient radioactivity was present in material from DON cultures to allow further analysis. 13% of the labeled material in control cultures was degraded by nitrous acid treatment (Cifonelli and King, 1973) and, to that extent, was heparan sulfate-like. The remainder eluted from columns of Dowex-1 (Cl^-) (AG 1-×2, 200–400 mesh; Bio-Rad Laboratories, Richmond, Calif.) with 1.6 M NaCl, again coincident with chondroitin sulfates.

Effects of DON on Cell Movement and Ruffling Membrane Activity of Heart Cells In Vitro

Concentrations of DON (15 μg/ml) that depressed biosynthesis of sulfated mucopolysaccharides to 19%–25% of controls in cultures of embryonic chick heart cells did not inhibit ruffling membrane activity in the same cells (Fig. 2). Cells treated with DON for as long as 76 h possessed typical migratory shapes and actively ruffling leading edges. The drug did have a morphological effect on the cells, however. Beginning at about 24 h of drug treatment, cytoplasmic vacuoles began to accumulate in the cells. These vacuoles increased in numbers with increasing times in DON (Fig. 2). Timed photomicrographs of fixed microscopic fields established that cells continued to translocate even in the presence of DON (Fig. 3). Even after 43 h of treatment with DON, a time when the cells had become greatly engorged with large vacuoles or droplets, cell movement and ruffling membrane activity continued unabated.

Cultures incubated from the beginning of the experiment in the presence of DON + glucosamine never developed the large accumulations of cytoplasmic vacuoles or droplets, and in all morphological respects were indistinguishable from control cells (Fig. 4). Such cells continued to move and form ruffling membranes throughout the course of the experiment.

Effects of Cytochalasin B on Mucopolysaccharide Biosynthesis and Cell Movement

The effects of DON on cultured heart cells were in sharp contrast to the effects of cytochalasin B on these cells. Although DON directly inhibited mucopolysaccharide synthesis, cytochalasin B did not (Table III). Cytochalasin B-treated and DMSO-treated cells incorporated radioactive sulfate into mucopolysaccharides at rates not significantly different from those of control cells (Table III). Furthermore, cytochalasin B inhibited ruffling membrane activity, stopped cell locomotion, and severely altered cell shape (Fig. 5). Thus, while DON inhibited mucopolysaccharide biosynthesis, cytochalasin B did not, and while cytochalasin B inhibited cell locomotion, DON did not.

Effects of Glutamine-Free Medium on Mucopolysaccharide Biosynthesis by Embryonic Heart Cells In Vitro

Glutamine absence should be equivalent to DON presence. We therefore tested the effect of glutamine-free medium on [^{35}S]sulfate incorporation into mucopolysaccharides. Serum was also eliminated in this experiment to assure the glutamine-free condition. The result is shown in Table IV. Incorporation was reduced to 20% of the control value, i.e. levels of inhibition no greater than those in the presence of DON.

Effects of Cycloheximide on Mucopolysaccharide Biosynthesis and Cell Locomotion by Embryonic Heart Cells In Vitro

A nonspecific approach to achieve greater inhibition of mucopolysaccharide biosynthesis was successful through use of the protein synthesis inhibitor, cycloheximide. A 12- to 16-h preincubation with cycloheximide (20 μg/ml, 7×10^{-5} M) reduced [^{35}S]sulfate incorporation into mucopolysaccharides during a subsequent 4-h labeling pe-

TABLE IV

*Incorporation of [^{35}S]Sulfate into Mucopolysaccharides by Heart Cells In Vitro in the Presence and Absence of Glutamine**

	Cpm/10^6 cells	% of control
With glutamine	2,797	100
Without glutamine	530	19

* Cells were rinsed and fed with Eagle's minimum essential medium (Grand Island Biological Co.) without serum, and with or without glutamine, 24 h before the 4-h labeling period.

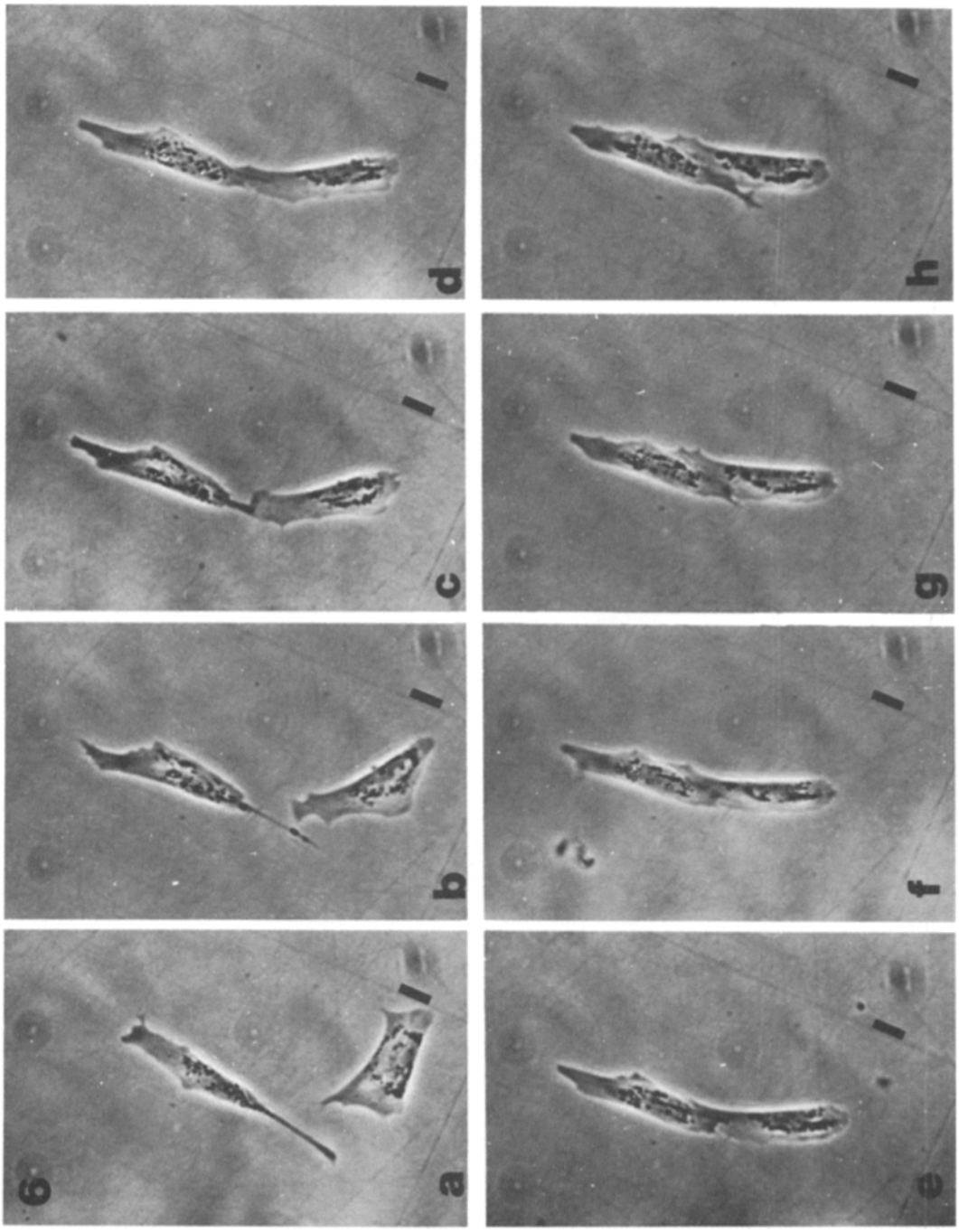
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TABLE V

Effects of Cycloheximide on Incorporation of [^{35}S]Sulfate into Mucopolysaccharide by Heart Cells In Vitro

	Cpm/10^4 cells		
	Control	Cyclo-hexi-mide	Percent of Control
Serum-free media*	2,797	193	7
Serum-containing media‡	2,104	4	<1

* Cultures were preincubated with drug for 12 h at 37°C before introduction of radioactivity, and the drug remained present during the 4-h labeling period.

‡ Cultures were preincubated with drug for 16 h at 37°C before introduction of radioactivity, and the drug remained present during the 4-h labeling period.

riod (cycloheximide continuously present) to less than 10% of control values (Table V). This approach therefore allowed greater than 90% inhibition of synthesis. Nevertheless, the cells in these cultures continued actively to translocate (Fig. 6).

DISCUSSION

The possibility that mucopolysaccharides are involved in some aspect of the locomotory cycle was tested in this study by determining the effects of inhibiting mucopolysaccharide biosynthesis on the ability of cultured heart cells to form ruffling membranes and to translocate in vitro. The results reveal no relationship between mucopolysaccharide biosynthesis and cell movement.

The glutamine analog, DON, was selected as an inhibitor of mucopolysaccharide synthesis. DON acts by inhibiting the aminotransferase reaction that converts fructose-6-phosphate to glucosamine-6-phosphate with glutamine as the amino donor (Ghosh et al., 1960; Ellis and Sommar, 1972; Ellis and Stahl, 1973; Trujillo and Gan, 1973). The resultant absence of glucosamine stops the assembly of the carbohydrate chains of the mucopolysaccharide molecules as well as molecules of glycoprotein. The same step is blocked by eliminating glutamine from the culture environment for those cells unable to synthesize this amino acid (Oppenheimer, 1973). Furthermore, the simultaneous presence of DON and exogenous glucosamine should alleviate the drug effect by bypassing the DON block (Telser et al., 1965; Oppenheimer, 1973). Effects not relieved may be due in part to inhibition of 5-phosphoribosyl-pyrophosphate amidotransferase, the first step in purine biosynthesis (Buchanan, 1973), or to toxic effects of glucosamine (Bekesi and Winzler, 1969; Lloyd and Kemp, 1971; Scholtissek, 1972; Kim and Conrad, 1974). Levels of inhibition of [^{35}S]sulfate incorporation by DON, similar to those reported here, have been observed in other systems (Telser et al., 1965 [87%]; Bhatnagar and Prockop, 1966 [72%]; Ellis and Stahl, 1973 [85%]; Pratt et al., 1973 [65%]). As expected, the *N*-acetyl derivative of DON (Duazomycin A) did not significantly depress incorporation, because only cells in the liver apparently are able to convert Duazomycin A to DON by removing the acetyl group (Bates et al., 1966).

Incorporation of [^{35}S]sulfate into mucopolysaccharides by heart cells was consistently reduced to levels about 20% of controls (i.e. 80% inhibition) by DON presence. This effect required a minimum preincubation time of 6 h, although cartilage required only a 30-min preincubation time. Furthermore, DON-treated fibroblasts began to accumulate cytoplasmic vacuoles at about 24 h of treatment and became engorged with these vacuoles with continued culture. Glucosamine presence in the culture medium allowed a partial alleviation of the drug effect on mucopolysaccharide synthesis, and totally prevented the appearance of the cytoplasmic vacuoles. It is possible that the DON-induced vacuoles contain core proteins that cannot be secreted in the absence of glycosylation (Rodén, 1970; Winterburn and Phelps, 1972). If so, they offer a potential tool for investigation of the core proteins of proteoglycan molecules. They also lead

FIGURE 6 Heart fibroblast locomotion in the presence of cycloheximide. This culture was exposed to cycloheximide (20 μg/ml) for 16 h at the time of the first photograph, and the drug remained present throughout this photo sequence. See Table V for drug effects on mucopolysaccharide synthesis. Photo timing with (*a*) arbitrarily 0 min. (*a*) 0 min; (*b*) 40 min; (*c*) 95 min; (*d*) 105 min; (*e*) 130 min; (*f*) 175 min; (*g*) 185 min; (*h*) 205 min. The lower cell in the field translocates toward the upper cell, partially underlaps it, and then begins to pass on the right. A fixed reference point in the field (black line) emphasizes the distance moved. Phase contrast. × 350.

to a prediction that in the presence of *both* DON and bromodeoxyuridine, the vacuoles should not be present, because the latter drug interferes with the synthesis of core proteins, but not of the polysaccharide chains (Levitt and Dorfman, 1973).

The ability of the cells to move in the presence of DON is in dramatic contrast to their behavior in cytochalasin B. DON inhibits mucopolysaccharide synthesis (Telser et al., 1965) but not cell movement, while cytochalasin B inhibits cell movement (Carter, 1967; Spooner et al., 1971) but not mucopolysaccharide synthesis. The failure of cytochalasin B to inhibit mucopolysaccharide synthesis in this study confirms the observations of Cohn et al. (1972). It is expected, of course, that if cytochalasin B were left on cells long enough, reduced intracellular glucose concentrations would eventually affect mucopolysaccharide synthesis as well as many other cellular reactions.

Elimination of glutamine from the culture medium did not allow any more complete inhibition of mucopolysaccharide synthesis than did the presence of DON. We do not have a satisfactory explanation for the inability of either DON presence or glutamine absence to produce greater than 80% inhibition of synthesis. However, we note that similar levels of inhibition have been seen in other systems (Pratt et al., 1973). Although it is conceivable that the cells make a small amount of glutamine, this still would not explain the DON result, because DON competes with glutamine for the aminotransferase (Ellis and Sommar, 1972).

Treatment with cycloheximide resulted in greater depression of mucopolysaccharide synthesis. This nonspecific approach assumed that general inhibition of protein synthesis would include the core proteins of proteoglycan molecules, thereby preventing assembly of the carbohydrate side chains (de la Haba and Holtzer, 1965; Telser et al., 1965). In the presence of cycloheximide, although [^{35}S]sulfate incorporation was reduced to less than 10% of control values (i.e., greater than 90% inhibition), cells still ruffled and translocated. Therefore, the locomotory cycle of vertebrate cells in tissue culture is not perturbed by elimination of 90% of the normal mucopolysaccharide synthetic activity (as measured by [^{35}S]sulfate incorporation) of the cells.

In conclusion, if sulfated mucopolysaccharides are involved in cell locomotion, either they must have extremely long half-lives, or, the minute amounts that escape drug blockage must be sufficient for movement. It also will be of interest to examine the possible role of surface glycoproteins and nonsulfated mucopolysaccharides such as hyaluronic acid in the adhesion step of locomotion, since such molecules are involved in some kinds of cell-to-cell adhesions.

We thank Drs. A. M. Guarino and H. B. Wood, Jr., of the National Cancer Institute for assistance in obtaining DON (NSC-7365) and Duazomycin A (NSC-51097). These drugs were supplied by the Drug Research and Development Branch, Chemotherapy, National Cancer Institute. We also thank K. M. Winkel, G. W. Hart, and Y. F. Chen for expert technical assistance.

This work was supported by grants from the National Institutes of Health (GM 19289 and EY 00952) and in part by a grant to Kansas State University from the National Institutes of Health (FR 7036).

Received for publication 5 August 1974, and in revised form 4 November 1974.

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