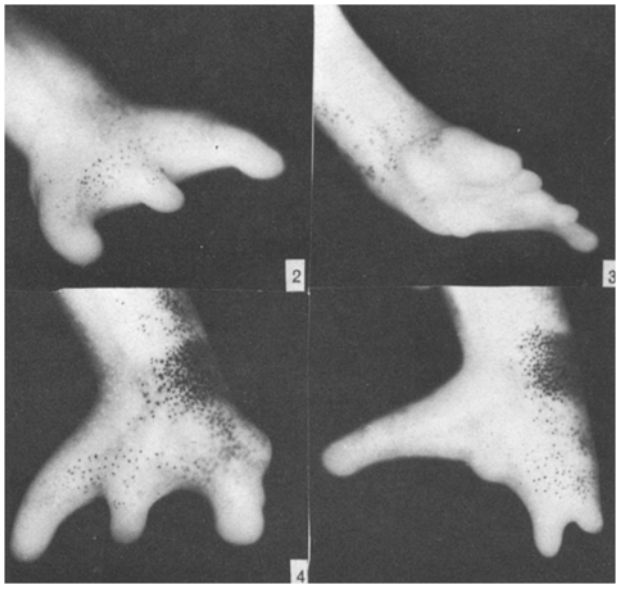
froglets with concentrated vitamin A palmitate for only a few days after limb transection is effective in inducing regeneration, although the regenerates obtained were all hypomorphic and sub-normal. To induce regeneration in adult frogs, Rose[2] had briefly immersed the limb stumps in saturated sodium chloride solution periodically; and Polezhayev[3] had repeatedly traumatized the amputation surface with a needle to keep the wound epithelium of the limb stumps in a juvenile condition. Both these procedures prevented early differentiation of a fibrous dermal pad below the wound epidermis permitting prolonged interaction betweeen the latter and the internal tissues of the stump and resulting in greater dedifferentiation and release of blastemal cells for regeneration. Similar changes in the pattern of wound healing were observed when regeneration was successfully induced in the limbs of adult frogs by implantation of additional adrenal glands in the lower jaw[6].



Figures 2-5. 4 cases of hypomorphic regenerated hands with 3-4 digital structures from vitamin A treated stumps of forelimbs amputated through metacarpal region in froglets of *Rana breviceps*.

It has been found that in tadpoles with amputated limbs vitamin A treatment maintains the epidermis in a larval condition, prevents dermal differentiation below the wound epithelium of the limb stumps and intensifies dedifferentiation as well as proliferation of blastemal cells derived therefrom[7,8]. Vitamin A excess is known to enhance proteolytic activity in cartilage by inducing release of acid proteolases from chondroblasts probably due to its action on lysosome[10]. Increase in proteolysis is normally associated with the phase of dedifferentiation in regenerating limbs[11]. Vitamin A is also said to promote cell proliferation by mitosis[12]. Thus this vitamin promotes all those processes which, during the initial period following amputation, are conducive to regeneration. In fact, results of Niazi and Saxena[9] indicate that this treatment results in the formation of blastema with greater than usual morphogenetic capacities equivalent to those of the original limb bud capable of forming a whole limb instead of only its distal and actually removed part. It may be inferred that vitamin A may have induced regeneration in the forelimbs of froglets due to its above-mentioned properties. It may have modified wound healing and caused sufficient dedifferentiation of the cells derived from stump tissues to enable them to reacquire morphogenetic potentialities to produce regenerates even though of sub-normal morphology.

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Comparison of the in vitro effects of colchicine and its derivative colchiceine on chondrocyte morphology and function[1]

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Summary. Colchiceine is a colchicine-metabolite which has been reported to inhibit axonal transport although not binding to brain tubulin. In the present study, colchiceine was shown not to depolymerize cytoplasmic microtubules, nor to mimic other effects of colchicine on the ultrastructure of cultured chondrocytes. In addition, the synthesis of proteoglycans was inhibited by colchicine but slightly stimulated by colchiceine. These results support the idea that the disturbances in cultured chondrocytes caused by colchicine are specifically related to a loss of cytoplasmic microtubules.

Various biological effects of colchicine, such as arrest of mitosis, loss of cell polarity, inhibition of secretion, and interference with axonal transport are usually attributed to its ability to bind to tubulin with the consequent dissolution of microtubules[3,4]. The specificity of the colchicine action is frequently verified with lumicolchicine, produced by UV irradiation of colchicine and devoid of antimicrotubular properties[5].

Nevertheless, it was recently demonstrated that colchiceine (0^{10}-demethylcolchicine), a derivative of colchicine unable to bind to tubulin, strongly inhibited axonal transport[6]. Furthermore, liver cells have been shown to contain the

Amount of macromolecular-bound ^{35}S-activity present in culture medium and papain-digested cell layer

	Cell layer cpm	SD	Δ%	Medium cpm	SD	Δ%	Total cpm	SD	Δ%
Rat chondrocytes									
Control	34970	1953	-	24421	1457	-	59392	1073	-
Colchicine	29016	510	−17[b]	14686	435	−40[b]	43997	472	−26[b]
Colchiceine	39311	1049	+12[b]	23975	1135	−2	63286	1545	+7[a]
Lumicolchicine	35780	1464	+2	23800	229	−3	59580	1382	+0.3
Rabbit Chondrocytes									
Control	32422	3253	-	13440	1383	-	45863	4532	-
Colchicine	26346	1002	−19[b]	7520	293	−44[b]	33866	929	−26[b]
Colchiceine	36037	1037	+11[a]	12471	662	−7	48508	1360	+6
Lumicolchicine	34535	1460	+7	11697	503	−13	46231	1319	+1

5 dishes were used for each group. Statistical significance was tested with Wilcoxon's rank sum test ([a] $p < 0.05$, [b] $p < 0.01$).

enzymatic machinery necessary to convert colchicine into colchiceine[7]. Conceivably, other cell types may also have this capacity. Some of the diverse biological effects formerly ascribed to colchicine may, therefore, include a colchiceine effect unrelated to an action upon microtubules.

We have previously reported both morphological and functional changes in cultured chondrocytes occurring after colchicine treatment. These changes mainly involved atrophy and dispersion of the dictyosomes of the Golgi complex throughout the cytoplasm[8,9], as well as inhibition of proteoglycan synthesis and secretion[10,11]. Similar morphological effects were also demonstrated in other cell types[12,13]. On the basis of these findings, it was suggested that microtubules are responsible for the maintenance of structural and functional integrity of the Golgi complex.

Decreased secretion of proteoglycans could thus be attributed both to a disturbance in their production due to the alteration of dictyosomes, and to a diminished efficiency in transportation of secretory vacuoles to the cell periphery in the absence of microtubules. In the present study, we have investigated whether any of the above effects could be obtained with colchiceine.

Materials and methods. Colchicine was purchased from Serva Feinbiochemica (Heidelberg, West Germany). Lumicolchicine was prepared as described by Wilson and Friedkin[5], and colchiceine by mild acid hydrolysis followed by chloroform extraction[14]. The crude colchiceine was then purified by chromatography on a 2.6×36 cm column of Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) eluted with 1% (v/v) acetic acid at a flow rate of 40 ml/h, collecting fractions of 8 ml[7]. The absorbance at 254 nm was monitored. The peak corresponding to colchiceine was pooled and lyophilized. Chondrocytes were isolated from the epiphyses of 5-day-old rats and from the auricular cartilage of 28-day-old rabbits, and grown in 35 mm petri dishes (Falcon Plastics, Los Angeles, USA) in supplemented Ham's F-12 medium, as previously described[15,16]. 1 million cells were seeded in each dish and allowed to attach over-night and the culture medium was then changed. The chondrocytes were incubated with 10 μM of the appropriate drug for 5.5 h, the last 4 h in the presence of $^{35}SO_4$ (Radiochemical Centre, Amersham, England). The amount of proteoglycans synthesized was estimated by gel chromatography of both the culture medium and the papain-digested cell layer on disposable columns of Sephadex G-25 (Pharmacia) and determination of macromolecular-bound ^{35}S-activity[17]. For ultrastructural investigation, cultures were fixed in situ with 2% glutaraldehyde and prepared for electron microscopy as described earlier[8].

Results and discussion. In both species of chondrocytes, colchicine produced a moderate inhibition of the sulfate incorporation (table). This effect was most pronounced in the culture medium indicating an inhibition both of proteoglycan synthesis and secretion, as previously described[10]. Neither lumicolchicine nor colchiceine showed such an inhibitory effect. On the contrary, colchiceine caused a small increase in synthesis of proteoglycans and a distinct retention of such molecules in the cell layer (table). It is not known at present if this latter effect is due to true intracellular accumulation of proteoglycans. However, previous work has shown that only a minor portion of the proteoglycans in the cell layer of chondrocyte cultures is intracellular[10,11]. The ultrastructural changes produced by colchicine were as expected[8,9]. Colchiceine and lumicolchicine, on the other hand, did not cause a clear effect on the number of microtubules. Neither did these latter drugs cause any alteration in the organization or structure of the Golgi complex.

As colchiceine does not mimic the effects of colchicine on cultured rat and rabbit chondrocytes, it is likely that the morphological and functional effects of colchicine in these cell types are due to the disappearance of microtubules and, thus, that our previous hypothesis about the role of microtubules remains valid.

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