ation of GGT activities in all groups except 2 suggests that inflammation does not affect the activity of this enzyme. The decreased activity observed at day 8 PI, therefore, is probably a result of mechanical disruption of the intestinal epithelium due to *E. nieschulzi* oocysts being shed since peak oocyst production occurs at this time. Thus, although intestinal inflammation is known to affect absorptive and digestive activities[4], results presented here suggest that malabsorption of amino acids observed during *N. brasiliensis* and *E. nieschulzi* infections is not the result of impaired ability, due to inflammation, of GGT to catalyze reactions involved in amino acid transport and absorption.

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Suppression by the cyclohexanetrione Ro 31-0521 of retinoic acid-induced teratogenicity

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Summary. The cyclohexanetrione Ro 31-0521, which stimulates prostaglandin synthesis, inhibited retinoic acid-induced cartilage degradation in vitro and suppressed the congenital forelimb malformations in rats treated with retinoic acid on day 13 of gestation in a dose-dependent manner.
Key words. Retinoic acid; teratogenesis; cartilage; chondrogenesis; cyclohexanetrione.

Intracellular Ca^{2+} mobilization and protein kinase C activation appear to be cellular mediators of various extracellular informational signals such as those of specific hormones and neurotransmitters[1-3]. In addition, Nishizuka[3] suggested that metabolites of the signal-induced turnover of inositol phospholipids, such as diacylglycerol, arachidonic acid and prostaglandins may be involved in the regulation of other homologous or heterologous cell types. We here report that the cyclohexanetrione Ro 31-0521 (fig. 1) which stimulates prostaglandin synthesis (N. A. Roberts, personal communication), inhibited retinoic acid-induced cartilage degradation in vitro and suppressed the congenital forelimb malformations in rats treated with retinoic acid on day 13 of gestation in a dose-dependent manner.

Materials and Methods. Female Fü-albino rats (outbred stock, Institute of Biological and Medical Research, Füllinsdorf, Switzerland) were mated overnight, and the females which had a vaginal plug the following morning were considered to be at day 1 of gestation.

Humeri from fetal rats at day 20 of gestation were prepared and incubated in Ham's F-10 nutrient mixture (supplemented with 10% fetal calf serum, 60 µg penicillin/ml, 100 µg streptomycin/ml and 10 mM HEPES, pH 7.4) in cell culture dishes in a humidified 5% CO_2 air atmosphere at 36 °C. Retinoic acid (dissolved in ethanol) and the cyclohexanetrione Ro 31-0521 (dissolved in dimethylsulfoxide) were added at the beginning of incubation. Equivalent amounts of the vehicles were added to the control cultures.

The amount of proteoglycans and/or glycosaminoglycans released from the bones into the medium was measured in aliquots of the medium by the alcian blue assay[4] with chondroitin sulphate as a standard. This amount is referred to here as proteoglycan release. The alcian blue-glycosaminoglycan complex was dissociated with 4% (w/v) sodium lauryl sulphate.

To estimate the change in length during the incubation period as the parameter for tissue breakdown[5], the length of the bones was measured under a reversed microscope, using a projecting prism, at a final magnification of 14.1 times, at the beginning and end of incubation.

For the studies in vivo retinoic acid and Ro 31-0521, both suspended in rape seed oil, were administered by oral intubation to pregnant rats using the application volume of 5 ml/kg. Controls received the vehicle only. Fetuses were obtained by laparotomy on day 21 of gestation. Fetuses were prepared for skeletal examination using a NaOH-alizarin red S staining procedure. Malformations of humeri and ulnae/radii were arbitrarily indexed 0–1 and 0–3, respectively, 0 representing controls (fig. 4). The nutrient mixture F-10 and fetal calf serum were obtained from GIBCO Europe, Glasgow, Scotland; all-trans-retinoic acid from F. Hoffmann-La Roche, Basle, Switzerland; the cyclohexanetrione Ro 31-0521 from F. Hoffmann-La Roche, Welwyn,

Suppression by Ro 31-0521 of forelimb malformations induced by retinoic acid on day 13 of gestation

Retinoic acid mg/kg	Compound	mg/kg	Number of dams	Number of fetuses	Malformation index Humerus	Ulna/radius
120	None		18	209	0.80 ± 0.07	1.24 ± 0.14
120	Ro 31-0521	20	9	108	0.42 ± 0.09*	0.38 ± 0.13*
120	Ro 31-0521	60	9	85	0.54 ± 0.13	0.24 ± 0.14*
120	Ro 31-0521	180	7	77	0.29 ± 0.14	0.02 ± 0.02*

Retinoic acid and Ro 31-0521, both suspended in rape seed oil, were administered orally to pregnant rats once on day 13 of gestation. Controls received rape seed oil alone. Fetuses were obtained by laparotomy on day 21 of gestation and processed for visualization of the skeleton with alizarin red S. Malformations of humeri and ulnae/radii were arbitrarily indexed from 0–1 and 0–3, respectively (fig. 4), and a mean malformation index was estimated per litter. No malformations of long bones were observed in controls and in the groups treated with Ro 31-0521 alone. Results are mean ± SE. For statistical analysis of the malformation indexes the U-test was used. * $p < 0.01$.

U.K. and cell culture dishes from Falcon Plastics, Oxnard, Calif. All other materials were of reagent grade.

Results and discussion. In fetal epiphyseal cartilage retinoic acid induces loss of metachromatic staining seen in sections stained with toluidine blue (fig. 2) which correlates with the loss of proteoglycan from cartilage[5-7]. In addition, retinoic acid causes a marked shortening of the bones (fig. 2) representing cartilage cell degradation expressed in the loss of DNA, RNA and protein[5]. Both the release of proteoglycan into the medium and the change in length of the bones during the incubation period can be used to quantify the retinoic acid effects. The cyclohexanetrione Ro 31-0521 inhibited both parameters in a dose-dependent manner (figs 2 and 3).

The presence of retinoic acid in the culture medium for as short a period as 1 day is sufficient to induce cartilage resorption[7,8]. In contrast, Ro 31-0521 inhibited the retinoic acid effects only as long as it was present in the culture medium. Humeri were incubated in the presence of both retinoic acid and Ro 31-0521 for 2 days and were then transferred into control medium and incubated for a further 5 days. Under these conditions Ro 31-0521 inhibited the retinoic acid-induced proteoglycan release during the first 2 days but then cartilage resorption started, as manifested by the release of proteoglycans and the bone shortening (data not shown). Thus, removal of the cyclohexanetrione abolished its suppression effect, demonstrating that the inhibition was reversible and not due to cytotoxicity.

Because the teratogenic effect of retinoic acid and its effect on fetal cartilage may be related, we tested whether Ro 31-0521 can interfere with the teratogenic effect of retinoic acid. Excess retinoic acid is embryotoxic and teratogenic, and affects almost all body systems in animals[9]. Depending on the time of administration, different morphogenetic systems are affected[10,11]. Marked forelimb malformations are induced when a high dose of retinoic acid is administered to pregnant rats on day 13 of gestation[11] (fig. 4). Ro 31-0521 administered along with retinoic acid on day 13 of gestation suppressed these retinoic acid-induced forelimb malformations in a dose-dependent manner (table). Furthermore, the cyclohexanetrione relieved the decrease in the mean fetal body weight induced by retinoic acid (data not shown). Ro 31-0521 given alone on day 13 of gestation was not teratogenic and did not affect the reproductive parameters.

above receptor cascade system may mediate retinoid action. Effects of retinoids in various systems, which support such an alternative mechanism, were reviewed recently[12].

In conclusion, further exploration of the inhibitory effect of Ro 31-0521 on retinoic acid-induced teratogenicity or other retinoid effects may provide clues for understanding the mechanism

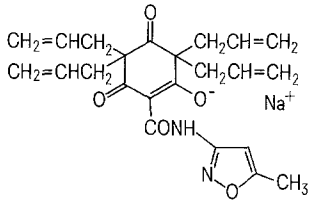

Figure 1. Chemical structure of the cyclohexanetrione Ro 31-0521, 3,3,5,5-tetraallyl-2-hydroxy-N-(5-methyl-3-isoxazolyl)-4,6-dioxo-1-cyclohexene-1-carboxamide sodium salt.

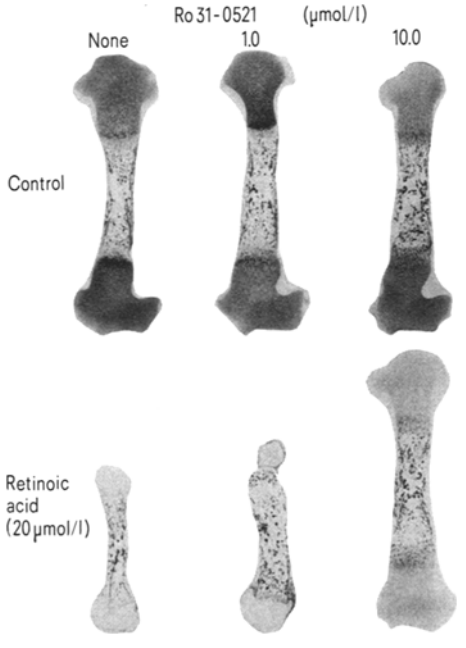

Figure 2. Effect of Ro 31-0521 on loss of metachromatic staining with toluidine blue and on gross morphological changes induced by retinoic acid in cultured fetal rat bones. Humeri from fetal rats at day 20 of gestation were incubated in supplemented nutrient mixture F-10 for 6 days. Retinoic acid and Ro 31-0521 were added at the beginning of incubation. Bones were fixed with neutral buffered 3.5% formol and paraffin sections (4 μm) were stained with toluidine blue. Ro 31-0521 at 10 μM inhibited the loss of metachromatic staining and suppressed cartilage tissue breakdown induced by retinoic acid.

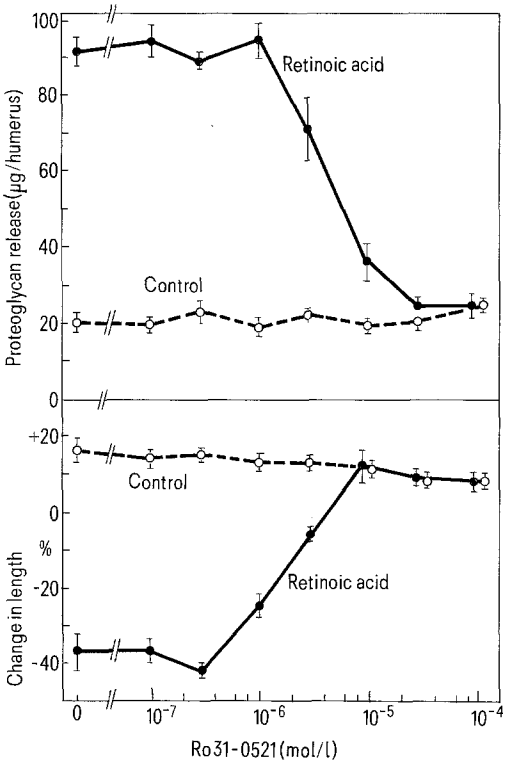

Figure 3. Dose-response curve of the inhibiting effect of Ro 31-0521 on retinoic acid-induced proteoglycan release and change in length in fetal rat bone cultures. Humeri of fetal rats (gestation day 20; 3 × 2 humeri/dish/group) were incubated in supplemented culture medium F-10 for 6 days. Retinoic acid (20 μM), and Ro 31-0521 at the indicated concentration, were added at the beginning of the incubation. The amount of proteoglycan released into the medium, an index of matrix degradation, was determined in aliquots of the medium after 6 days of incubation. The length of the bones was measured at the beginning and at the end of incubation and the change in length estimated. The bone shortening induced by retinoic acid correlates with cartilage tissue breakdown expressed as loss of DNA, RNA and protein[5]. Results are mean ± SD of triplicate determinations.

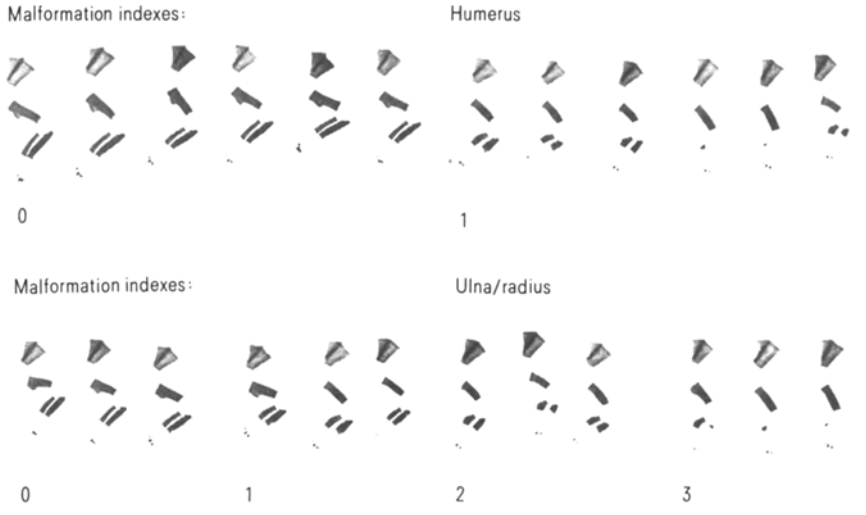

Figure 4. Forelimb malformations induced by retinoic acid. Pregnant female rats were intubated with retinoic acid (120 mg/kg) on day 13 of gestation. Fetuses were obtained by laparotomy on day 21 of gestation and processed for visualization of the skeleton with alizarin red S. Malformations of humeri and ulnae/radii were arbitrarily indexed 0–1 and 0–3 respectively, 0 representing controls.

Recently, Sporn and Roberts[12] reviewed the role of retinoids in differentiation and carcinogenesis and proposed that a molecular hypothesis for the mechanism of retinoid action that is compatible with the broadest range of experimental data is that retinoids modify gene expression. However, little information is available about how they control gene expression. The discovery of specific intracellular retinoid binding proteins[13,14] has led to the proposal that these binding proteins allow specific transfer of retinoids into the nucleus and to interactions with chromatin there.

Control of gene expression via interactions with protein kinases may integrate a receptor cascade system consisting of phospholipid degradation, Ca^{2+}, calmodulin, arachidonic acid, prostaglandins, cyclic AMP and cyclic GMP[2,3]. Biddulph et al.[15] reported changes in concentrations of prostaglandin E_2 and cyclic AMP during various stages of chondrogenesis in chick limb bud cell cultures, supporting a regulatory role for both prostaglandin E_2 and cyclic AMP in the early events associated with chondrogenesis. In this limb bud cell culture system retinoic acid not only inhibits chondrogenesis[16,17] but induces a time- and dose-dependent degradation of newly-differentiated cartilage nodules[17] similar to the degradation of cartilage in cultured fetal bones[5–7]. These effects of retinoids on cell differentiation, particularly in the processes of chondrogenesis, may be related to the mechanisms by which congenital limb defects are produced by retinoids. That the above receptor cascade system may be involved in retinoid action is supported by our findings that retinoic acid-induced cartilage degradation in vitro involves Ca^{2+} and calmodulin[8,18]. The present finding that the cyclohexanetrione Ro 31-0521, which stimulates prostaglandin synthesis, suppressed both cartilage degradation in vitro and teratogenicity in vivo induced by retinoic acid, further supports the view that the of action of retinoids on cell differentiation. In addition, it may help to elucidate the biological activity of cyclohexanetriones.

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Decrease of K^+ conductance underlying a depolarizing photoresponse of a molluscan extraocular photoreceptor

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Summary. An identified neurone in the *Onchidium* abdominal ganglion responds to light with a depolarizing generator potential, so that this neurone functions as an extraocular photoreceptor. The light-evoked depolarizing response is produced by a selective decrease in K^+ conductance.

Key words. Molluscan extraocular photoreceptor; depolarizing photoresponse; decrease in K^+ permeability.

The hyperpolarizing photoresponse of most vertebrate ocular photoreceptors is produced by a decrease in membrane conductance to Na^+ ions[1]. This contrasts with the invertebrates studied to date, in which the hyperpolarizing or depolarizing response to light is associated with an increase in conductance to Na^+ and K^+ ions[2–8]. Thus, a photoreceptor potential produced by a de-