Early hemoglobin-containing precursors of mature erythrocytes are first seen in control cultures after about 42 h of incubation. Prior stages of erythropoiesis appear to occur largely, or even entirely, in cells which attach to the substratum within 18 h of culture, but are stimulated by non-erythroid cells free in suspension (unpublished experiments and below). We therefore examined the effects of pre-treatment with *Ricinus communis* agglutinin upon erythropoiesis in the cells which attached to the substratum within 18 h by 2 approaches (table 2).

A series of cultures with and without lectin were incubated for 18 h and the free cells in suspension were then removed. The attached cells were carefully rinsed with 1 ml of warm BCM, supplemented with 1 ml of warm BCM containing varying concentrations of the agglutinin, and re-incubated. Attached cells from control cultures yielded fewer mature erythrocytes and late reticulocytes (table 2, b) than corresponding undisturbed control cultures (table 2, a). However there was no inhibition of erythropoiesis in the former cultures by 0.1–0.2 µg/ml of *Ricinus communis* agglutinin. In contrast neither the attached cells from treated cultures supplemented with normal BCM medium nor the unattached cells from any 18 h culture yielded any erythroid cells (data not shown).

In a concurrent series of experiments erythropoiesis by control attached cells supplemented with free cells from cultures containing up to 0.1 µg/ml of agglutinin was equal to that in undisturbed control cultures (table 2, c). Erythropoiesis in cultures containing the converse combination of cells was markedly impaired (table 2, d).

The mode of action of the lectin in inhibiting erythropoiesis in our cultures is not yet known. However our data clearly indicate that cell surface receptors with specificity for *Ricinus communis* agglutinin 120 are involved in an early reaction essential for erythropoiesis by our chick blastodisc cells.

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## Biochemical and autoradiographical distribution of hyaluronic acid in calf rib cartilage[1]

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*Summary.* In calf rib cartilage, about one half of total hyaluronate is soluble with guanidinium hydrochloride, the other half only after collagenase treatment. Evidence is presented for its pericellular and intracellular distribution.

In hyaline cartilage, small amounts of hyaluronic acid (0.4–0.8%, w/w) interact with proteoglycans and link proteins to form large salt-soluble aggregates[2,3] in the extracellular matrix. In calf rib cartilage, hyaluronic acid represents 1.0–1.5% of total uronate[4,5]; however most hyaluronate is found in the salt-insoluble residue[4,6]. Moreover, small concentrations of hyaluronate inhibit proteoglycan biosynthesis of cultured chondrocytes[7–10], whereas exogenous proteoglycans in the medium stimulate it[7]. Thus, hyaluronic acid appears to have important biological properties for regulating proteoglycan biosynthesis. Its localization in vivo needs further clarification in hyaline cartilage.

*Materials and methods.* For the investigation of biochemical distribution of hyaluronate, 2 g of fresh cartilage slices (50–100 µm thick) from calf ribs were successively extracted with 0.4 M and 4.0 M guanidinium hydrochloride (GuHCl) in 0.05 M sodium-acetate buffer, pH 5.8[11]. The insoluble residue, dialyzed free of salt, was treated twice with proteinase-free collagenase (EC 3.4.4.19, 20 U/mg, Calbiochem A grade)[12] yielding collagenase extracts I and II and cartilage cells which appeared bare and well-shaped in the light microscope. The 4 extracts, as well as the cells, were digested exhaustively by papain[11] before hyaluronate fractions were isolated from them by applying Ecteola cellulose chromatography[4]. In these fractions, hyaluronate was degraded specifically with leech hyaluronidase (EC 3.2.1.36) and analyzed[4,13]. No hyaluronate-degrading activities could be observed with cartilage slices.

For autoradiographical investigations, cartilage slices (100 µm thick) were incubated for 300 min anaerobically with D-glucosamine $-6\text{-}^3H$ (N)[5] and washed with a solution containing formaldehyde (40 g/l) and cetylpyridinium chloride (5 g/l). Labelled slices were embedded in paraffin and cut to 7 µm thick slices at right angles to their surface. After removing paraffin with xylene from the mounted slices, the tissue sections were treated with leech hyaluronidase[4] followed by the stripping film procedure with Kodak AR 10 film[14]. Silver grains were counted over those cartilage cells, which show clear-cut boundaries, no unspecific background in their surroundings and their nuclei situated in the plane of section.

*Results and discussion.* In hyaline cartilage of calf ribs, about 50% of total hyaluronate was solubilized with 0.4 M and 4.0 M GuHCl, the rest could be dissolved only after

Table 1. Biochemical localization of hyaluronate in calf rib cartilage in vivo

| Hyaluronate content (µmoles hexosamine*/mg DNA**) | | | | | |
|---|---|---|---|---|---|
| Total | 0.4 M guanidine extract | 4.0 M guanidine extract | Collagenase extract I | Collagenase extract II | Cartilage cells |
| 8.7 | 2.2 | 1.7 | 2.7 | 0.7 | 0.3 |
| 100% | 25% | 20% | 31% | 8% | 3% |

* Estimated with modified Elson-Morgan reaction[5]. ** Isolated and measured acc. to Kleine[13].

Table 2. Autoradiographical localization of hyaluronate in calf rib cartilage in vitro

| Treatment | Cartilage cells* (total) | Cartilage cells* Cellular body | Perinuclear area + nuclear area | Pericellular area* | Extracellular area** |
|---|---|---|---|---|---|
| Control (n=210) | 59 ±18 | 12 ±6 | 12 ±4 | 17 ±6 | 18 ±9 |
| Hyaluronidase***-treated (n=190) | 55 ±20 | 13 ±7 | 11 ±4 | 14 ±9 | 17 ±9 |
| Student's t-test | $p<0.05$ | $p<0.20$ | $p<0.02$ | $p<0.001$ | $p<0.30$ |

* Silver grains per cell from 15 different cartilage slices, mean values ± SD. ** Area around the cell having its diameter. *** Kindly obtained from Biotrics, Boston, Mass., USA; no proteolytic activity against bovine serum albumin could be detected with the enzyme preparation.

degradation of the network of collagen boundles by exhaustive treatment with collagenase, minor amounts (~3%) were detected in cartilage cells (table 1) which appeared to be intact in the light microscope but were seen to be injured with electron microscopic investigations (Kleine, unpublished experiments). Thus the amount of hyaluronate found in the cells may be higher in vivo.

After incubation of cartilage slices with D-[$^3$H]glucosamine, mainly chondroitin-4−, −6-sulfate chains of proteoglycans were labelled, but also hyaluronate[4,5] which was specifically degraded by leech hyaluronidase in the thin cartilage slices: the difference of silver grains counted over cartilage cells before and after the treatment with the enzyme corresponds to the degraded hyaluronate. Silver grains mainly over the pericellular area became soluble under this treatment (table 2), besides a small amount over the nuclear and perinuclear area where the Golgi apparatus as well as hyaluronate biosynthesis appear to be localized[15, 16]. The data point to the presence of unbound degradable hyaluronate in the pericellular area (probably in the lacuna of cartilage cells), whereas most other hyaluronate appears to be protected against the enzyme effect by proteoglycans or their aggregates: hyaluronic acid and proteoglycans extracted with 4 M GuHCl reassociate to form aggregates under associative conditions[17]. However, constituents of 0.4 M GuHCl extract and of collagenase extracts under these conditions aggregate only to a minor extent if at all. Further experiments are in progress to investigate the regulator role of hyaluronate in hyaline cartilage. At present evidence for separate biosynthesis mechanisms of hyaluronate and proteoglycan in cartilage cells has been obtained[13].

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## Galactose induced Balbiani-ring-like structures in chromosomes I and II of *Chironomus thummi*

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*Summary.* In one strain of *Chironomus thummi*, galactose feeding of the larvae induced the appearance of 2 Balbiani-ring-like structures on salivary gland chromosomes I and II. This phenomenon may be related to the galactose induction of BR6 in *C. pallidivittatus*.

Feeding of *C. tentans* and *C. pallidivittatus* larvae with galactose and other sugars produces characteristic changes in the puffing patterns of polytene chromosomes in the salivary glands[3]. The common effect of such sugar treatments consists of changing the relative puffing situation of Balbiani rings BR1 and BR2; both of which are situated on chromosome IV, by inducing the regression of BR2 and the full expansion of BR1. Santa-Cruz et al.[6] have previously described a similar effect on the Balbiani rings of *C. thummi*. In addition to these modifications, sugar treatments induce the appearance of an exceptional Balbiani ring (BR6) in chromosome III of *C. pallidivittatus*. In *C. tentans*, a homologous structure has not been observed so far.

The purpose of this paper is to give some information about the induction of Balbiani-ring-like structures on polytene chromosomes I and II of *C. thummi* after galactose administration. The notion 'Balbiani ring' represent sites in the chromosome in which there is a high activity in RNA-synthesis. They will be abbreviated as 'BR'.

*Material and methods.* 4th instar larvae of *Ch. thummi* collected near Valencia (Spain) were raised in the laboratory at 18 °C in a salt solution[4] in distilled water under