reimplanted with *D. cingulatus* CA, the reproduction was inhibited in almost all females when the recipient was *D. cingulatus* and in less than 50% of females when the recipient was *P. apterus*.

Pratt et al[10] suggest that the enzymic competence of CA to oxidise precocene-like molecules to highly reactive epoxides is a basis of their selective cytotoxic action. The lack of such a competence might explain the low sensitivity of *P. apterus* CA to P II. Indeed, the metabolism of *P. apterus* CA seems to be different from other species; none of the classically known juvenile hormones has been found in reproducing females in a measurable concentration (Baehr, personal communication).

The physiological basis of the 'anti-precocene mechanisms' outside the CA can only be guessed at. There is evidence that P II penetrates to the body of *P. apterus* and can reach a concentration high enough to kill the animal. Thus the doses of 800 µg or 1000 µg of P II killed 40.0% (n = 10) or 73.3% (n = 18) of *P. apterus* females respectively within 1 week, although the surviving females still oviposited. In the species sensitive to P II, the concentration below the toxic level is sufficient to inhibit the CA[13]. Such a concentration should be achieved in the body of both *P. apterus* and *D. cingulatus* when the dose of 600 µg of P II (causing mortality in about 10% of individuals of both species) was used in the present experiments (tables 1 and 2, figure).

In *D. cingulatus* females P II suppresses the food intake in addition to its inhibitory effect on CA function[14]. Much smaller egg batches (by about 60%) oviposited by P II-treated *D. cingulatus* reimplanted by *P. apterus* CA (table 2) also indicate suppression of food intake or food utilization. In *P. apterus*, on the other hand, the treatment with P II did not markedly reduce the size of egg batches (tables 1 and 2). Even those *P. apterus* females surviving doses of P II as high as 800 µg or 1000 µg oviposited 47.5 (18-62) or 41.0 (36-45) eggs in the 1st batch respectively - a quantity which is not much lower than the normal egg batches in untreated females (tables 1 and 2). It appears, therefore, that *D. cingulatus* is more sensitive to the antifeedant effect of P II than *P. apterus*. Sláma[15] suggests that the antifeedant property of precocenes is a cause of their interference with the activation of endocrine glands. The inhibition of the implanted CA by P II, however, cannot be attributed only to its antifeedant effect as even completely starved *D. cingulatus* females with the denervated CA oviposited[16]. In contrast, the suppression of feeding by P II might favor its inhibitory effect on the CA. Conversely, a relatively high food intake in P II-treated *P. apterus* might decrease the inhibitory effect of P II on the CA. The CA function is generally stimulated by food intake, either directly or through the neurosecretory cells of the brain[17,18]. Such a stimulation might compete with the inhibition by P II. Although the physiological processes preventing the inhibition of ovarian development by P II in *P. apterus* cannot be precisely described so far, it has been proved that they are not limited to the CA itself.

1 Precocene II was kindly supplied by Zoecon.
2 W.S. Bowers, T. Ohta, J.S. Cleere and P.A. Marsella, Science *193*, 542 (1976).
3 M.H. Landers and G.M. Happ, Experientia *36*, 619 (1980).
4 G.C. Unnithan, K.K. Nair and W.S. Bowers, J. Insect Physiol. *23*, 1081 (1977).
5 H. Schooneveld, Experientia *35*, 363 (1979).
6 H. Schooneveld, Cell Tissue Res. *203*, 25 (1979).
7 M.P. Pener, L. Orshan and J. De Wilde, Nature *272*, 350 (1979).
8 G.E. Pratt and W.S. Bowers, Nature *265*, 548 (1977).
9 P.J. Müller, P. Masner, M. Kälin and W.S. Bowers, Experientia *35*, 704 (1979).
10 G.E. Pratt, R.C. Jennings, A.F. Hamnett and G.T. Brooks, Nature *284*, 320 (1980).
11 R. Socha and M. Hodková, Acta ent. bohemoslov. *76*, 62 (1979).
12 W.S. Bowers and J.R. Aldrich, Experientia *36*, 362 (1980).
13 W.S. Bowers and D.M. Soderlund, Int. Conf. Regulation of Insect Development and Behavior, p.309. Eds F. Sehnal, A. Zabza, J.J. Menn and B. Cymborowski. Karpacz 1980.
14 R. Socha and M. Hodková, Věstník Čs. spol. zool., in press (1982).
15 K. Sláma, Acta ent. bohemoslov. *75*, 65 (1978).
16 M. Hodková and R. Socha, Acta ent. bohemoslov. *79*, 108 (1982).
17 F. Engelmann, The Physiology of insect reproduction. Pergamon Press, London-New York 1970.
18 C.A.D. De Kort and N.A. Granger, A. Rev. Ent. *26*, 1 (1981).

Effect of insulin-like growth factor on collagen and glycosaminoglycan synthesis by rabbit articular chondrocytes in culture

H.L. Guenther, Hannelore E. Guenther, E.R. Froesch and H. Fleisch[1]

Department of Pathophysiology, University of Berne, Murtenstrasse 35, CH-3010 Berne (Switzerland), and Department of Medicine, Kantonsspital, CH-8006 Zurich (Switzerland), 1 October 1981

Summary. Rabbit articular chondrocytes cultured in the presence of insulin-like growth factor I (IGF I) increased significantly the synthesis of both collagen and glycosaminoglycans. The increase in the ratio of $^{35}SO_4^{2-}$ to [^{3}H]glucosamine observed in glycosaminoglycans synthesized in the presence of IGF I seems to indicate that IGF I affects sulphation and synthesis of these polyanionic macromolecules to a different extent.

Insulin-like growth factor I (IGF I) a growth hormone dependent single-chain peptide was recently isolated from human plasma[2-5]. Its growth promoting activity on various tissues and cell systems[6-10], and its mol.wt of 7500 daltons, have assigned IGF I structurally and functionally to the class of the somatomedins[11]. In addition to its growth promoting abilities, IGF I was found to stimulate strongly the incorporation of sulphate into glycosaminoglycans of cartilage from various animal species[12,13]. Recently, it was shown that the synthesis of bone collagen was likewise increased by IGF I[14].

The purpose of the present study was to examine whether IGF I affects the rate of collagen synthesis by rabbit articular chondrocytes. Furthermore, based on previous findings[15] that brachymorphic mice, which have a mutation resulting in disproportionate dwarfism, preferentially synthesize low sulphated glycosaminoglycans, experiments were carried out to determine whether glycosaminoglycans synthesized by articular chondrocytes in the absence or presence of IGF I differ in their sulphate incorporation.

Materials and methods. Chondrocytes were prepared from the proximal femoral surfaces of 3 months old KUN FI

Hybrid Rex rabbits (supplied by Kunath Futter AG, Aarau, Switzerland) as previously described[16]. In experiments designed to study the biosynthesis of collagen in the presence of IGF I, (kindly supplied by Dr R.E. Humbel, Zurich) chondrocytes grown to confluence in Ham's F-12 media (3.5 ml) were incubated for 24 h in medium containing 80 ng/ml IGF I (IGF I was added dissolved in 0.01 N HCl/saline supplemented with 0.2% (w/v) human serum albumin) 0.5% (w/v) bovine serum albumin or 10% (v/v) foetal calf serum, 100 µg of ascorbic acid/ml, 100 µg of β-aminopropionitrile fumarate/ml, 10 µCi of L-[2,3-^{3}H] proline/ml and 1% penicillin/streptomycin solution. The synthesis of glycosaminoglycans was studied with medium that contained 80 ng/ml IGF I, 2 µCi of Na_2 $^{35}SO_4$/ml, 3 µCi of D-[6(n)-^{3}H]-glucosamine hydrochloride/ml and antibiotics. The incubation was carried out under CO_2/air (1:19) at 37 °C. The determinations of collagen and glycosaminoglycan synthesis were performed as described in detail elsewhere[16,17].

L-[2,3-^{3}H]proline (20–40 Ci/mmole), D-[6(n)-^{3}H]glucosamine hydrochloride (5–15 Ci/mmole) and Na_2 $^{35}SO_4$ carrier-free (100 µCi/mmole), were purchased from New England Nuclear Corp., Dreieichenhain, FRG. Ham's F-12 medium, foetal calf serum, bovine serum albumin (fatty acid free) and antibiotics were obtained from Seromed, Fakola AG, Basel, Switzerland. All other chemicals and reagents were of analytical grade and were purchased from E. Merck AG, Darmstadt, FRG.

Results and discussion. The results presented in table 1 show that when articular chondrocytes, cultured in 0.5% bovine serum albumin, were exposed to IGF I, collagen synthesis was increased as shown by the enhanced incorporation of [^{3}H]proline into proteins resistant to pepsin digestion. Interestingly, when chondrocytes were cultured in the presence of 10% foetal calf serum and without any added IGF I, the rate of collagen synthesis was likewise enhanced, reaching levels equal to those where IGF I and 0.5% bovine albumin were used. This increased collagen synthesis in cells cultured in the presence of 10% serum as opposed to 0.5% bovine serum albumin, could in part be explained by the IGF present in foetal calf serum. As the concentration of total IGF in human serum is of the order of 1 µg/ml, this would amount to a level of approximately 100 µg/ml in the culture medium, if foetal calf serum contains similar amounts of IGF-like material. Furthermore the data show that the addition of IGF I to serum containing cultures did not increase the synthesis of collagen beyond values that were obtained with serum alone. This may indicate that the IGF concentrations in serum were already sufficient to saturate a limited number of available IGF receptors on the chondrocytes.

The data presented above not only complement data previously reported for bone[14], but they also show that IGF I, regardless of species origin, acts as promotor of general tissue collagen synthesis.

The effect of IGF I on the incorporation of radiolabeled sulphate into cartilage glycosaminoglycans has been described in detail by several investigators[12,13]. However, the possibility that growth promoting factors such as IGF I support proper sulphation of glycosaminoglycans in addition to total synthesis has not been investigated. For this purpose chondrocytes were pulsed simultaneously with radiolabeled sulphate and glucosamine. To use glucosamine as precursor to examine total glycosaminoglycan synthesis became feasible[19] after it was found that chondrocytes have the enzyme UDP-n-acetylglucosamine-4'-epimerase, which catalyses the interconversion of glucosamine into galactosamine[20].

Table 2 shows that in the presence of 0.5% bovine albumin in increase of glycosaminoglycan synthesis had occurred as the result of IGF I. However, the increment of [^{3}H]glucosamine incorporation, although quite sizeable, was not as pronounced as the incorporation of radiolabeled sulphate. The increase of [^{3}H]glucosamine incorporation amounted to roughly 75% above values obtained from cultures that had not been stimulated by IGF I. The increase of sulphate incorporation, however, reached values of the order of 170% above base line levels. Chondrocytes, cultured in the presence of foetal calf serum and without IGF I, synthesized similar amounts of glycosaminoglycans to the cells that were treated with IGF I in the presence of 0.5% bovine albumin. As already observed with collagen, the addition of IGF I to chondrocytes grown with foetal calf serum failed to increase further the synthesis of glycosaminoglycans. The

Table 1. Incorporation of [^{3}H]proline into collagen synthesized by chondrocytes in culture in the presence and absence of IGF I

	Radioactivity incorporation (cpm/µg DNA)			
	Cell layer		Medium	
	0.5% BSA	10% FCS	0.5% BSA	10% FCS
No IGF I	514± 13 (5)	1010±14 (5)	1704±22 (5)	3445±106 (5)
With IGF I	1065±112 (3)*	1047±23 (5)	2803±26 (5)*	3394± 99 (5)

Cells at confluence were treated for 24 h either with or without IGF I in the presence of 0.5% bovine serum albumin (BSA) or 10% foetal calf serum (FCS). Results are expressed as means ± SEM, the number of dishes being given in parentheses. * $p<0.001$ (control vs IGF I-treated) by Student's t-test.

Table 3. Effect of IGF I on cell proliferation

	DNA (µg/dish)	
	0.5% BSA	10% FCS
No IGF I	34.95±0.85 (10)	46.91±1.45 (3)
With IGF I	43.17±0.81 (3)*	46.79±2.38 (3)

DNA determinations[18] were carried out after 24 h exposure of cells to 80 ng/ml IGF I. Results are expressed as means ± SEM, the number of dishes given in parentheses. * $p<0.005$ (Student's t-test).

Table 2. Effect of IGF I on the incorporation of [^{3}H]glucosamine and Na_2 $^{35}SO_4$ into isolated glycosaminoglycans synthesized by chondrocytes in culture

	Radioactivity incorporation (cpm/µg DNA)					
	BSA			FCS		
	[^{3}H]glucosamine	Na_2 $^{35}SO_4$	$^{35}S/^3H$	[^{3}H]glucosamine	Na_2 $^{35}SO_4$	$^{35}S/^3H$
No IGF I	506± 9	450± 9 (5)	0.889±0.028	1282± 55	1900±85 (5)	1.482±0.092
With IGF I	893±12*	1221±102 (3)*	1.367±0.037*	1407±120	2240±70 (5)	1.592±0.145

Cells at confluence were treated for 24 h either with or without IGF I. The treatment was carried out either in the presence of 0.5% bovine serum albumin (BSA) or 10% foetal calf serum (FCS). Results are expressed as means ± SEM, the number of dishes being given in parentheses. * $p<0.001$ (control vs IGF I-treated) by Student's t-test.

$^{35}S/^{3}H$ ratios of 0.87 and 1.37 for untreated and treated cultures respectively and a similar ratio of 1.48 obtained from cultures containing foetal calf serum and without any added IGF I, tend to suggest that glycosaminoglycans synthesized without serum or IGF I appear to be less sulphated in addition to being synthesized at a lower rate. The remarkably similar effects of foetal calf serum and IGF I on chondrocytes, causing them to synthesize increased amounts of glycosaminoglycans with similar $^{35}S/^{3}H$ ratios, demonstrates not only that IGF I, which is contained in foetal calf serum, supports the synthesis of collagen and glycosaminoglycans; the data also indicate that IGF I may play an important role in the sulphation of cartilage glycosaminoglycans.

Interestingly, in the case of the chick embryo cartilage, incorporation of $^{35}SO_4$ is not as markedly stimulated by IGF I as are other processes[12]. These species differences of the response of cartilage to growth factors are remarkable and yet unexplained.

Finally, when IGF I was added for 24 h to already confluent chondrocytes, a modest but statistically significant rise in cell proliferation was noted (table 3). These data re-emphasize the potency of IGF I on cell growth, shown previously with fibroblasts[12].

1 Acknowledgments. We thank Mrs Nelli Borel and Mrs Brunhilde Meier for preparing the manuscript. This work has been supported by the Swiss National Science Foundation (grants 3.824.79 and 3.380-0.78), and by the Ausbildungs- und Förderungsfonds der Arbeitsgemeinschaft für Osteosynthese (AO), Chur, Switzerland.

2 E. Rinderknecht and R.E. Humbel, Proc. natl Acad. Sci. USA *73*, 2365 (1976).
3 E. Rinderknecht and R.E. Humbel, J. biol. Chem. *253*, 2769 (1978).
4 E. Rinderknecht and R.E. Humbel, Proc. natl Acad. Sci. USA *73*, 4379 (1976).
5 J.J. Van Wyk, M.E. Svoboda and L.E. Underwood, J. clin. Endocr. Metab. *50*, 206 (1980).
6 B. Morell and E.R. Froesch, Eur. J. clin. Invest. *3*, 119 (1973).
7 E. Schoenle and E.R. Froesch, Eur. J. Biochem. *87*, 285 (1978).
8 G.K. Haselbacher and R.E. Humbel, J. Cell Physiol. *88*, 239 (1976).
9 M.M. Rechler, J.M. Podskalny, I.D. Goldfine and C.A. Wells, J. clin. Endocr. Metab. *39*, 512 (1974).
10 R.H. Chochinov and W.H. Daughaday, Diabetes *25*, 994 (1976).
11 T.L. Blundell and R.E. Humbel, Nature *287*, 781 (1980).
12 E.R. Froesch, J. Zapf, T.K. Audhya, E. Ben-Porath, B.J. Segen and K.D. Gibson, Proc. natl Acad. Sci. USA *73*, 2904 (1976).
13 A.E. Zingg and E.R. Froesch, Diabetologia *9*, 472 (1973).
14 E. Canalis, J. clin. Invest. *66*, 709 (1980).
15 R.W. Orkin, R.M. Pratt and G.R. Martin, Devl Biol. *50*, 82 (1976).
16 H.L. Guenther, H.E. Guenther and H. Fleisch, Biochem. J. *184*, 203 (1979).
17 H.L. Guenther, H.E. Guenther and H. Fleisch, Biochem. J. *196*, 293 (1981).
18 R.T. Hinegardner, Analyt. Biochem. *39*, 197 (1971).
19 L. Hronowski and T.P. Anastassiades, J. biol. Chem. *255*, 9210 (1980).
20 S. Kornfeld and V. Ginsburg, Exp. Cell Res. *41*, 592 (1966).

A histometric analysis of male accessory sex glands after administration of prolactin[1]

J.P. Gunasegaran, S.C. Mitra and M. Kaul

Department of Anatomy, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-605 006 (India), 21 July 1981

Summary. Histometric analysis of accessory sex glands in male albino mice after prolactin treatment revealed stimulatory responses in the epithelium of the dorsolateral lobe of the prostate. This lobe was more sensitive to prolactin than the ventral lobe. This stimulatory effect may be mediated through testosterone rather than being a direct action of prolactin.

Prolactin (PRL) is involved in the mammary function[2] and luteotrophic activity in female mice[3]. It stimulates testicular growth and induces fertility in PRL deficient male mice[4]. Although there is considerable evidence to suggest that PRL can stimulate the growth and development of accessory sex glands in male rats[5,6], its mode and site of action on these glands are not fully understood. Moreover, histometric details of accessory sex glands in male mice under the influence of PRL are not well documented. The present study is aimed at evaluating the site and mode of action of PRL in accessory sex glands of male mice.

Materials and methods. 2 groups (10 in each) of colony-bred adult male albino mice (72 days old; b.wt 25–27 g) were used in the present study. Animals were maintained at

Table 1. Effect of prolactin on histometry of dorsolateral prostate in male albino mice

	Volume/unit volume of tissue			Surface area of sec. epi.	Height of epithelium
	Stroma	Sec. epi.	Sec. alveoli	(mm^2/mm^3)	(μm)
Control	0.446 ± 0.013	0.214 ± 0.007	0.553 ± 0.013	16.86 ± 0.18	8.25 ± 0.07
Experiment	0.363 ± 0.012**	0.322 ± 0.003*	0.636 ± 0.012**	20.52 ± 0.23*	10.6 ± 0.16*

Results are expressed as mean ± SEM. * $p < 0.001$; ** $p < 0.05$. Sec. epi., secretory epithelium; sec. alveoli, secretory alveoli.

Table 2. Weights of accessory sex glands in male albino mice after prolactin treatment

	Seminal vesicle (1 lobe with secretion; mg)	Ventral prostate (mg)	Dorsolateral prostate (both lobes; mg)
Control	56.6 ± 5.47	12.5 ± 1.02	8.6 ± 0.73
Experiment	72.0 ± 7.2	10.4 ± 1.64	11.7 ± 1.78