Table 1. Hybrid-arrested translation of trypanosome RNA.

Oligonucleotide	Concentration (μM)	Translation products* (10^3 cpm)	Inhibition (%)
(I)	0	295	
	4	46	84
	8	6	98
	20	8	97
(III)	4	292	0
	8	313	−6
	20	259	12

*Samples (5 μl) of the translation mixtures in Figs. 2A and 3 were precipitated with 10% trichloroacetic acid, washed, and counted after being redissolved in 0.1*N* NaOH. The values reported are the average of two separate determinations.

message since it has been shown that oligonucleotides or cDNA fragments complementary to the coding regions do not effectively inhibit translation in rabbit reticulocyte lysates (*21–23*).

Oligonucleotide methylphosphonates, which have an uncharged internucleotide link, have been shown to enter cells and inhibit translation of their corresponding mRNA's (*24*). Capping the 5′- and 3′-hydroxyl groups with apolar substituents may also be sufficient to facilitate transport of the oligonucleotide into the cell and to inhibit degradation by exonucleases (*25, 26*). The high degree of specificity of the oligonucleotide for its target sequence suggests that this approach may be therapeutically useful. An oligonucleotide complementary to a portion of the spliced leader sequence should be lethal to the trypanosome, but would be without effect on host protein synthesis. Recent advances in DNA synthesis make it possible to test this approach.

REFERENCES AND NOTES

1. J. C. Boothroyd and G. A. M. Cross, *Gene* **20**, 281 (1982).
2. L. H. T. Van der Ploeg *et al.*, *Nucleic Acids Res.* **10**, 3591 (1982).
3. S. Sather and N. Agabian, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 5695 (1985).
4. T. De Lange *et al.*, *Nucleic Acids Res.* **12**, 3777 (1984).
5. M. Parsons, R. G. Nelson, K. Watkins, N. Agabian, *Cell* **38**, 309 (1984).
6. C. Tschudi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3998 (1985).
7. J. E. Donelson and M. J. Turner, *Sci. Am.* **252**, 44 (February 1985).
8. J. E. Donelson and A. C. Rice-Ficht, *Microbiol. Rev.* **49**, 107 (1985).
9. P. Borst *et al.*, *Eur. J. Biochem.* **137**, 383 (1983).
10. P. Borst and G. A. M. Cross, *Cell* **29**, 291 (1982).
11. A. Gonzalez *et al.*, *Nucleic Acids Res.* **13**, 5789 (1985).
12. R. G. Nelson *et al.*, *Cell* **34**, 901 (1983).
13. T. De Lange *et al.*, *ibid.*, p. 891.
14. D. A. Campbell, D. A. Thornton, J. C. Boothroyd, *Nature (London)* **311**, 350 (1984).
15. M. J. Lenardo, D. M. Dorfman, J. E. Donelson, *Mol. Cell. Biol.* **9**, 2487 (1985).
16. R. G. Nelson *et al.*, *Nature (London)* **308**, 665 (1984).
17. D. M. Dorfman *et al.*, *Nucleic Acids Res.* **13**, 3533 (1985).
18. M. J. Lenardo, D. M. Dorfman, L. V. Reddy, J. E. Donelson, *Gene* **35**, 131 (1985).
19. W. J. Murphy *et al.*, *J. Protozool.* **31**, 65 (1984).
20. T. De Lange *et al.*, *Nucleic Acids Res.* **12**, 4431 (1984).
21. S. A. Liebhaber, F. E. Cash, S. H. Shakin, *J. Biol. Chem.* **259**, 15597 (1984).
22. K. R. Blake, A. Murakami, P. S. Miller, *Biochemistry* **24**, 6132 (1985).
23. M.-T. Haeuptle, R. Frank, B. Dobberstein, *Nucleic Acids Res.* **14**, 1427 (1986).
24. K. R. Blake *et al.*, *Biochemistry* **24**, 6139 (1985).
25. P. C. Zamecnik and M. L. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 280 (1978).
26. M. L. Stephenson and P. C. Zamecnik, *ibid.*, p. 285.
27. F. T. Silveira *et al.*, *Hileia Med. Belém.* **1**, 61 (1979).
28. E. Pays *et al.*, *Nucleic Acids Res.* **8**, 5965 (1980).
29. U. K. Laemmli, *Nature (London)* **277**, 680 (1970).

30. We thank L. V. Kirchhoff for the *T. cruzi* clone. Supported in part by NIH grants HL-33555 (J.W.), AI-18954 (J.D.), and AM-25295 (Diabetes and Endocrinology Research Center Grant), an award from the Burroughs-Wellcome Fund (J.D.), and NIH Medical Scientist Training Program Grant GM-07337 (D.E. and D.D.). J.W. is a recipient of an Established Investigatorship Award from the American Heart Association (82 278).

3 February 1986; accepted 27 March 1986

Regulation by Growth Hormone of Number of Chondrocytes Containing IGF-I in Rat Growth Plate

ANDERS NILSSON, JÖRGEN ISGAARD, ANDERS LINDAHL, ANNICA DAHLSTRÖM, ANNA SKOTTNER, OLLE G. P. ISAKSSON*

Whether growth hormone stimulates longitudinal bone growth by a direct effect at the site of the growth plate or indirectly by increasing the concentration of circulating somatomedins (insulin-like growth factors) has been the subject of controversy. Immunohistochemical methods were used to explore the localization and distribution of insulin-like growth factor I (IGF-I) immunoreactivity in the epiphyseal growth plate of the proximal tibia of male rats. Cells in the proliferative zone of the growth plate of normal rats exhibited a bright immunofluorescence, whereas cells in the germinal and hypertrophic zones stained only weakly. In rats subjected to hypophysectomy, the number of fluorescent cells was markedly reduced. When the hypophysectomized rats were treated with growth hormone, either systemically or at the site of the growth plate, the number of IGF-I–immunoreactive cells in the proliferative zone was increased. The results show that IGF-I is produced in proliferative chondrocytes in the growth plate and that the number of IGF-I–containing cells is directly regulated by growth hormone. These findings suggest that IGF-I has a specific role in the clonal expansion of differentiated chondrocytes and exerts its function locally through autocrine or paracrine mechanisms.

ACCORDING TO THE SOMATOMEDIN hypothesis, growth hormone (GH) does not have a direct effect on cartilage, but instead stimulates chondrogenesis and subsequent growth indirectly through circulating peptide growth factors termed somatomedins (*1*). The insulin-like growth factor I (IGF-I), which is identical with somatomedin C (*2*), is growth hormone–dependent and generally considered to be the main mediator of the stimulatory effect of GH on somatic growth (*3–5*).

Investigators from our laboratory reported earlier that local injection of GH into the epiphyseal growth plate of the proximal tibia of hypophysectomized rats stimulates unilateral longitudinal bone growth (*6–8*). This observation has been confirmed by other investigators (*9*). We have also shown that chondrocytes isolated from rabbit ear and epiphyseal growth plate have specific binding sites for GH (*10*) and that GH stimulates DNA and proteoglycan synthesis in cultured chondrocytes from cartilage of rabbit ear and rat rib growth plate (*11, 12*). These results are inconsistent with an effect of GH mediated through circulating IGF-I.

Although IGF-I was traditionally considered to be produced in the liver, there is now evidence that IGF-I is synthesized in many different organs (*13–15*). We used immunohistochemical techniques to examine the possibility that IGF-I is produced in epiphyseal growth cartilage under the influence of GH. We studied the localization and distribution of IGF-I–like immunoreactivity in sagittal sections of the epiphyseal growth plate of the proximal tibia of normal and hypophysectomized male rats and observed

A. Nilsson, J. Isgaard, A. Lindahl, O. G. P. Isaksson, Department of Physiology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.
A. Dahlström, Department of Histology, University of Göteborg, Box 33031, S-400 33 Göteborg, Sweden.
A. Skottner, KabiVitrum, Stockholm, Sweden.

*To whom correspondence should be addressed.

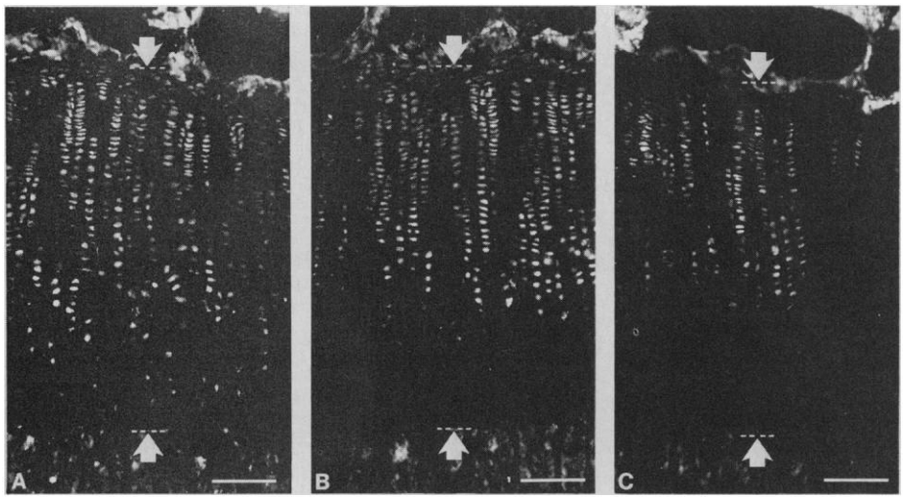

Fig. 1. Immunohistochemical localization of IGF-I in the epiphyseal growth plate. Sagittal sections of the epiphyseal growth plate of the proximal tibia of 33-day-old, normal male rats were prepared for immunohistochemical analysis as described. Three different rabbit antisera (diluted 1:200 in PBS), were used: (A) K624, (B) K1792, and (C) KG96. Scale bar, 100 μm. Distance between vertical arrows indicates the width of the epiphyseal growth plate.

the effect of GH treatment on the hypophysectomized rats.

Male prepubertal rats of the Sprague-Dawley strain were used. They were given free access to tap water and pellet food. Hypophysectomy was performed in 28-day-old rats by the standard parapharyngeal approach. At autopsy the hypophyseal capsule and adjacent tissues were dissected out and the GH content was determined by radioimmunoassay (*16*). None of the animals had detectable amounts of GH after hypophysectomy, as determined by this procedure. In some experiments, hypophysectomized rats were given systemic or local treatment with human growth hormone (approximately 2 IU/mg) produced in *Escherichia coli* (hGH^{bac}) (Somatonorm; KabiVitrum, Stockholm). Rats were anesthetized with a lethal dose of Mebumal and fixed by transcardial perfusion with 4 percent (weight to volume) formaldehyde in phosphate-buffered saline (PBS), *p*H 7.35, for 5 minutes. The tibiae were then excised, immersion-fixed for 4 hours in the same fixative at 4°C, decalcified for 10 days in 10 percent EDTA at 4°C, and washed several times in cold PBS containing 5 percent sucrose. Specimens of the proximal tibiae were rapidly frozen, and thin (10-μm) sagittal sections from the proximal tibia were cut in a cryostat at −20°C.

The sections were thawed onto gelatin-coated glass slides, incubated with 3 percent swine serum to reduce nonspecific protein absorption, then incubated for 18 hours at 4°C with specific antisera. Three different rabbit antisera (all at a dilution of 1:200 in PBS) were used; K624 (KabiVitrum, Stockholm), which is prepared against IGF-I isolated from human plasma, and K1792 (KabiVitrum, Stockholm) and KG96 (KabiGen, Stockholm), both prepared against the synthetic fragment 57–70 of human IGF-I conjugated to bovine serum albumin.

After the specimens were rinsed in PBS, fluorescein-isothiocyanate (FITC)–labeled swine antibody to rabbit immunoglobulin G (Dakopatts A/S, Copenhagen) was applied at a dilution of 1:30, and the specimens were incubated for 1 hour at 37°C. The sections were thoroughly rinsed, mounted in glycerol-containing paraphenylenediamine to reduce fading (*17*), and observed in a Zeiss fluorescence microscope equipped with epi-illumination and appropriate filters for microscopy of FITC fluorescence. The following procedures were used to study the specificity of the immunohistochemical reactions: omission of the primary antibody, absorption of antibodies with human IGF-I before incubation, and use of serum from unimmunized rats instead of specific antiserum.

In normal rats, chondrocytes in the proliferative layer of the epiphyseal growth plate clearly showed specific staining with antibody to IGF-I and also with two different antibodies to the synthetic fragment 57–70 of IGF-I (Fig. 1). The stain was distributed unevenly in the different columns of proliferative chondrocytes that were visible in sagittal sections. Thus, in some columns, most of the cells stained intensely, whereas other columns contained only a few stained cells. Cells in the germinal and hypertrophic zones of the growth plate stained weakly, and no specific stain was visible in the matrix between cells in the growth plate.

Our results provide histochemical evidence for localization of endogenous IGF-I–like immunoreactivity in the epiphyseal growth plate. It is therefore important to establish the authenticity of the antisera used. As shown in Fig. 2A, antiserum K1792 bound to human IGF-I on sheets of nitrocellulose in a dose-dependent manner when studied after electrophoresis and immunoblotting. Prior absorption of the antibodies with IGF-I in excess completely neutralized the detection of 50 ng of IGF-I and

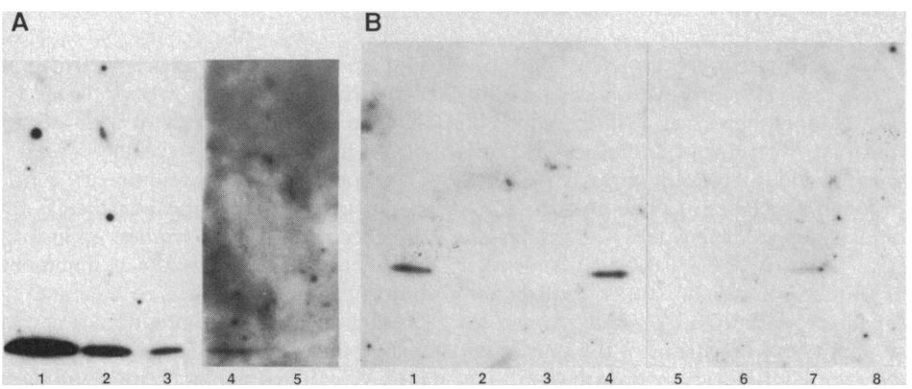

Fig. 2. Evidence for specificity of antiserum K1792 to IGF-I. Biosynthetically produced human IGF-I (Met-IGF-I; KabiVitrum, Stockholm, Sweden), human IGF-II (preparation 9, kindly provided by R. E. Humbel, Zurich, Switzerland), porcine relaxin (NIH, Bethesda, MD), and porcine insulin (Novo, Copenhagen, Denmark) were subjected to electrophoresis on a 10% polyacrylamide gel in the presence of sodium dodecyl sulfate according to the method of Laemmli (*26*). The proteins were electrophoretically transferred to nitrocellulose sheets, as described by Towbin *et al.* (*27*). After being blocked and washed, the sheets were incubated with antiserum at a dilution of 1:200. The antibodies were subsequently visualized and quantitated with ^{125}I-labeled protein A and autoradiography. (A) (Lanes 1 and 4) 200 ng of IGF-I, (lanes 2 and 5) 50 ng of IGF-I, and (lane 3) 25 ng of IGF-I. In lanes 4 and 5 the diluted antibodies were incubated (overnight in a refrigerator) with PBS containing biosynthetically produced human IGF-I (KabiGen, Stockholm, Sweden) (20 μg/ml). (B) (Lane 1) 50 ng of IGF-I, (lane 2) 500 ng of insulin, (lane 3) 50 ng of insulin, (lane 4) 50 ng of IGF-I, (lane 5) 500 ng of relaxin, (lane 6) 50 ng of relaxin, (lane 7) 500 ng of IGF-II, and (lane 8) 50 ng of IGF-II.

partially that of 200 ng. The antibodies showed no cross-reactivity to the IGF-I–like molecules porcine insulin or porcine relaxin at loads of 500 ng per lane of these peptides (Fig. 2B). The antibodies showed a weak immunoreaction to 500 ng of human IGF-II, suggesting that there was a slight cross-reactivity between IGF-I and IGF-II. The specificity of the K1792 antiserum to IGF-I was also tested immunohistochemically. Prior incubation of antiserum K1792 with biosynthetic human IGF-I, but not with the structurally related porcine proinsulin molecule, blocked the immunohistochemical staining (Fig. 3). Thus, the staining in Fig. 1 and Figs. 3 to 5 is probably a result of the interaction of antibodies to IGF-I with an endogenous IGF-I–like molecule.

In hypophysectomized rats the width of the growth plate decreased, as expected, and the number of cells in the growth plate that fluoresced brightly was markedly smaller than that in normal rats (Fig. 4). However, the distribution of IGF-I–immunoreactive material in the different cell zones was approximately the same as in growth plates from normal rats (Figs. 1 and 3). Administration of 200 μg of hGH^{bac} subcutaneously daily for 2 days increased the width of the growth plate, and concomitantly the number of positively stained cells in the proliferative cell layer increased. This observation shows that the number of cells in the growth plate containing IGF-I–immunoreactive material is GH-dependent.

Administration of 2 μg of hGH^{bac} through an implanted cannula into the epiphysis of the right proximal tibia of hypophysectomized rats, daily for 5 days (*8*), increased the number of cells containing IGF-I–like immunoreactivity in the growth plate of the GH-treated right leg in comparison with the saline-injected left leg (Fig. 5). In a separate experiment the plasma level of IGF-I was measured in hypophysectomized rats that received daily local treatments with 2 μg of hGH^{bac}. The plasma level of IGF-I in these rats was not different from that of the saline-treated group of rats 3 and 5 days after the start of treatment (Table 1). This finding suggests that the increased number of IGF-I–immunoreactive cells in the epiphyseal growth plate, following local GH treatment, was not caused by an increased plasma level of IGF-I but was due to an effect of GH at the site of the injection.

The chondrocytes in the growth plate are strictly organized in a columnar pattern according to the stage of maturation. The germinal zone bordering the bony epiphysis is the stem cell area of the growth plate. Cells from this area differentiate and enter the proliferative zone where the cells undergo limited clonal expansion during the process of longitudinal bone growth (*18*). Longitudinal bone growth is thus the result of cell differentiation and of clonal expansion of cells in the growth plate.

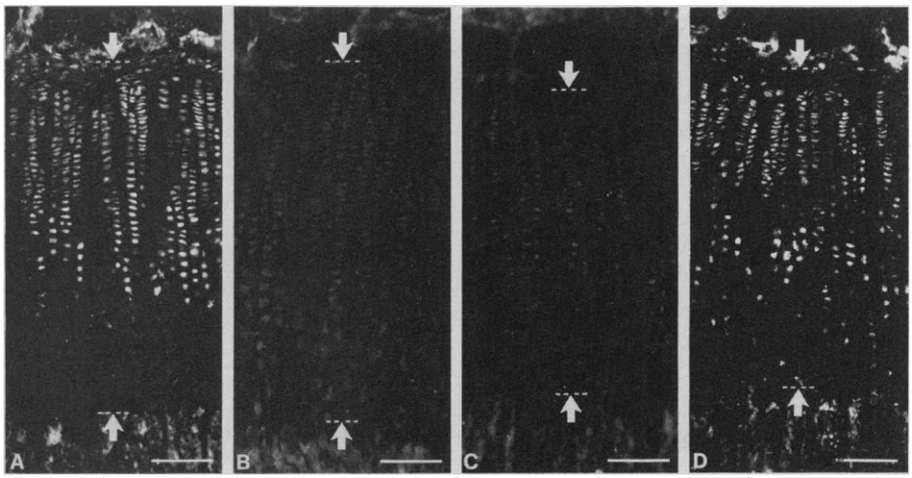

Fig. 3. Evidence for specificity of antiserum (K1792) to IGF-I in tissue. Sagittal sections of the epiphyseal growth plate of the proximal tibia of 33-day-old normal male rats were prepared for immunohistochemical analysis. (A) Sections were stained as in Fig. 1B. (B) Omission of the primary antibody. (C) Absorption of antibodies by incubating the diluted antibodies (1:200) with PBS containing biosynthetically produced human IGF-I (Met-IGF-I; KabiVitrum, Stockholm, Sweden) (1 μg/ml) for 15 minutes at 24°C before applying the antibodies to tissue specimens. (D) Failure of porcine proinsulin (Novo, Copenhagen, Denmark) at a concentration of 100 ng/ml to absorb IGF-I immunoreactivity. Scale bar, 100 μm. In (B) and (C), exposure times were longer than for (A) and (D) in order to avoid a completely black picture. Vertical arrows indicate the width of the epiphyseal growth plate.

Our finding that chondrocytes in the proliferative zone, but not in germinal or hypertrophic zones, contain IGF-I–like immunoreactivity suggests that IGF-I has a specific role in the clonal expansion of differentiated chondrocytes in the growth plate. Schoenle *et al.* (*19, 20*) demonstrated that continuous subcutaneous infusion of highly purified IGF-I into hypophysectomized rats increased tibial cartilage width as well as thymidine incorporation into costal cartilage, providing evidence for a functional role of IGF-I in the growth plate in vivo.

The finding that GH increases the number of IGF-I–immunoreactive cells in the proliferative zone suggests that the chondrocytes themselves produce IGF-I and that IGF-I stimulates clonal expansion of the chondrocytes in the proliferative zone through autocrine or paracrine mechanisms, as suggested earlier (*6, 11, 15, 21*). When

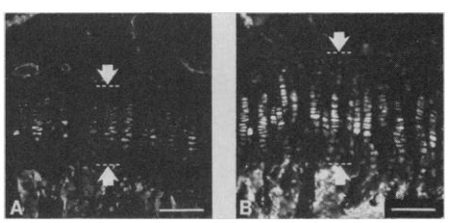

Fig. 4. Effect of systemic administration of hGH^{bac} on IGF-I–like immunoreactive cells in the epiphyseal growth plate of hypophysectomized rats. Sagittal sections of the epiphyseal growth plate of the proximal tibia were prepared for immunohistochemical analysis with antiserum K1792. (A) Epiphyseal growth plate of a 42-day-old male rat that was hypophysectomized at 28 days of age. (B) Effect of daily subcutaneous administration of 200 μg of hGH^{bac} for 2 days to a hypophysectomized rat. The rat was killed 15 hours after the last injection of GH. Scale bar, 100 μm. The same exposure time was used for (A) and (B). Vertical arrows indicate the width of the epiphyseal growth plate.

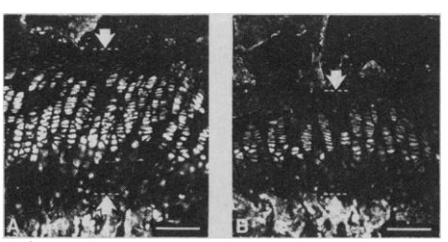

Fig. 5. Effect of local administration of hGH^{bac} on IGF-I–like immunoreactive cells in the epiphyseal growth plate of hypophysectomized rats. Two micrograms of hGH^{bac} was injected daily for 5 days through a cannula implanted in the epiphysis (*8*) of the right tibia of 37-day-old rats that had been hypophysectomized 9 days earlier. The left leg was injected with saline. The rats were killed 18 hours after the last injection. Sagittal sections of the epiphyseal growth plate of the proximal tibia were prepared and analyzed immunohistochemically for IGF-I, with antiserum K1792. The same exposure time was used for both photographs. (A) GH-treated right leg. (B) Saline-treated left leg. Note the difference in growth plate width and immunofluorescence of chondrocytes in the two photographs. Scale bar, 100 μm. Vertical arrows indicate the width of the epiphyseal growth plate.

endogenously produced somatomedin-like peptides in cultured porcine smooth muscle cells and human fibroblasts are neutralized with monoclonal antibodies, DNA synthesis is inhibited, giving direct experimental support for an autocrine or paracrine role of somatomedin-like peptides (*22*). Our study strongly supports earlier observations that administration of GH in vivo stimulates the production of IGF-I in a number of tissues of hypophysectomized rats (*15*). Furthermore, our finding that local administration of GH increased the number of IGF-I–immunoreactive cells on the side injected, without a concomitant rise in the plasma level of IGF-I, shows that the effect of GH was not mediated by circulating somatomedins, but probably was due to a direct effect on cells in the growth plate.

The precise cellular mechanism by which GH acts to increase the number of IGF-I–containing chondrocytes is unknown. The discovery of Morikawa *et al.* (*23*) and Nixon *et al.* (*24*) that GH stimulates differentiation of cultured preadipose cells and myoblasts in tissue culture suggests that stem cell chondrocytes might be the target cells for GH in the growth plate and that GH stimulates the differentiation of these cells. We have found that ^{125}I-labeled hGH preferentially binds to cells in the germinal zone of the growth plate, giving support to this hypothesis (*25*).

We suggest the following hypothesis for the stimulatory effect of GH on longitudinal bone growth. During the process of cell differentiation, directly stimulated by GH, genes that code for growth factors of the somatomedin class, such as IGF-I, are expressed. This gene activation results in an increased local production of IGF-I, which promotes the clonal expansion of chondrocytes through paracrine or autocrine mechanisms. Thus, GH stimulates cell differentiation directly and clonal expansion indirectly through the local production of insulin-like growth factors.

Table 1. Effect of local administration of hGH^{bac} on plasma levels of IGF-I in hypophysectomized rats. Rats were bled through the tip of the tail before treatment, and on the third and fifth days of treatment 18 hours after local injections. Plasma levels of IGF-I were determined in plasma samples extracted with acid ethanol by radioimmunoassay using the antiserum K624. There were three rats in each group. Analysis of variance showed no difference between the groups. The plasma level of IGF-I in a group of six normal rats was 2.81 ± 0.15 U/ml. In the radioimmunoassay, 1 U/ml corresponds to 0.2 μg/ml of highly purified human IGF-I standard (supplied by R. E. Humbel, Switzerland).

Time	Plasma IGF-I (U/ml)	
	Saline-treated rats	hGH^{bac}-treated rats
Before treatment	0.40 ± 0.11	0.36 ± 0.06
Third day of treatment	0.76 ± 0.13	0.62 ± 0.12
Fifth day of treatment	0.53 ± 0.08	0.51 ± 0.10

REFERENCES AND NOTES

1. W. H. Daughaday *et al.*, *Nature (London)* **235**, 107 (1972).
2. D. G. Klapper, M. E. Svoboda, J. J. Van Wyk, *Endocrinology* **112**, 2215 (1983).
3. L. S. Phillips and R. Vassilopoulou-Sellin, *N. Engl. J. Med.* **302**, 371 (1980).
4. W. H. Daughaday, in *Endocrine Control of Growth*, W. H. Daughaday, Ed. (Elsevier, New York, 1981), p. 1.
5. A. C. Herington, H. J. Cornell, A. D. Kuffer, *Int. J. Biochem.* **15**, 1201 (1983).
6. O. G. P. Isaksson, J.-O. Jansson, I. A. M. Gause, *Science* **216**, 1237 (1982).
7. O. G. P. Isaksson *et al.*, in *Human Growth Hormone Symposium*, S. Raiti and R. A. Tolman, Eds. (Plenum, New York, 1986), p. 563.
8. J. Isgaard, A. Nilsson, A. Lindahl, J.-O. Jansson, O. G. P. Isaksson, *Am. J. Physiol.*, **250**, E367 (1986).
9. S. M. Russell and E. M. Spencer, *Endocrinology* **116**, 2563 (1985).
10. S. Edén, O. G. P. Isaksson, K. Madsen, U. Friberg, *ibid.* **112**, 1127 (1983).
11. K. Madsen, U. Friberg, P. Roos, S. Edén, O. Isaksson, *Nature (London)* **304**, 545 (1983).
12. K. Madsen, A.-M. Makower, U. Friberg, S. Edén, O. Isaksson, *Acta Endocrinol.* **108**, 338 (1985).
13. D. R. Clemmons, L. E. Underwood, J. J. Van Wyk, *J. Clin. Invest.* 67, 10 (1981).
14. H. Stracke, A. Schultz, D. Moeller, S. Rossol, H. Schatz, *Acta Endocrinol.* **107**, 16 (1984).
15. A. J. D'Ercole, A. D. Stiles, L. E. Underwood, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 935 (1984).
16. C. Ekholm, T. Hillensjö, O. Isaksson, *Endocrinology* **108**, 2022 (1981).
17. G. D. Johnson, G. M. Araujo, C. Nogueira, *J. Immunol. Methods* **43**, 349 (1981).
18. N. F. Kember, *Cell Tissue Kinet.* **11**, 477 (1978).
19. E. Schoenle, J. Zapf, R. E. Humbel, E. R. Froesch, *Nature (London)* **296**, 252 (1982).
20. E. Schoenle, J. Zapf, C. Hauri, T. Steiner, E. R. Froesch, *Acta Endocrinol.* **108**, 167 (1985).
21. J. L. Kostyo and O. Isaksson, in *Reproductive Physiology II*, R. O. Greep, Ed. (University Park Press, Baltimore, 1977), vol. 13, p. 255.
22. D. R. Clemmons and J. J. Van Wyk, *J. Clin. Invest.* **75**, 1914 (1985).
23. M. Morikawa, T. Nixon, H. Green, *Cell* **29**, 783 (1982).
24. B. T. Nixon and H. Green, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3429 (1984).
25. A. Nilsson *et al.*, in preparation.
26. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
27. H. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* 76, 4350 (1979).

28. Supported by grants 4250 and 2207 from the Swedish Medical Research Council and by KabiVitrum AB, Stockholm. We thank R. E. Humbel, Zurich, Switzerland, for supplying IGF-I and IGF-II. Aspects of these results were presented at the 18th Nordic Congress for Physiology and Pharmacology in Uppsala, Sweden, 10 to 14 June 1985.

15 April 1986; accepted 6 June 1986