thology. Preliminary data related to the concentration of [^{3}H]NMU injected in the same fashion as in the present experiments (intraperitoneal) indicate that the amounts of radioactivity counted in the irritated and nonirritated buccal mucosa were not significantly different.

Interpretation of these data provides several arguments favoring, but not proving, the contention that the carcinogenic properties of NMU need not be exerted on the cells that eventually will become tumors. Recently, Sonnenschein and Soto (*9*) have proposed an interpretation compatible with our experimental results. Carcinogens administered intravenously, intraperitoneally, or by mouth affect primarily a central target organ (the liver?), where they have an acknowledged toxic effect (*3*, *7*). This may result in a loss or in a reduction in the ability of that organ to secrete substances that regulate the negative control of cell multiplication. The function of these substances is to prevent the multiplication of their target cells (*10*). Tumors will appear at locations where the proliferative capacity of the cells is increased by normal physiologic processes (skin, digestive system, or mammary glands) or aberrant, pathologic processes (long-term wounds or irritative stimuli) (*7*). They will occur when the balance between the amount of cell multiplication inhibitors and the constitutive property of cells to proliferate is altered, favoring the latter (*9*).

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Growth Hormone Stimulates Longitudinal Bone Growth Directly

Abstract. *Local administration of human growth hormone in vivo to the cartilage growth plate of the proximal tibia of hypophysectomized rats resulted in accelerated longitudinal bone growth. This finding suggests that growth hormone directly stimulates the cells in the growth plate, and does not support the theory that the increase in the plasma concentration of somatomedin that follows growth hormone administration is the cause of this stimulation.*

It is well known that growth hormone (GH) given to hypophysectomized rats results in increased body length and growth (*1–3*). There is controversy, however, about the sequence of events following GH administration that ultimately results in stimulation of body growth. More than 20 years ago Salmon and Daughaday (*4*) demonstrated the existence of GH-dependent serum factors that stimulated in vitro the incorporation of sulfate into cartilage from hypophysectomized rats. Growth hormone, in contrast, produced only small and inconsistent stimulatory effects in vitro. Subsequent studies in vitro revealed that these GH-dependent plasma factors stimulated a number of anabolic processes in cartilage and other isolated tissues (*5*). These findings and others led Daughaday *et al.* (*6*) to propose that the effects of GH on different target tissues were not direct ones, but were mediated by different plasma factors that were given the term "somatomedin."

Although the somatomedin hypothesis of GH action on somatic growth has been accepted by a number of investigators, the evidence for this hypothesis comes mainly from studies in vitro and is therefore circumstantial. Thus, many of the processes that are stimulated by different somatomedin preparations in vitro are stimulated by insulin as well (*7*). However, insulin is unable to promote linear growth when administered to hypophysectomized rats (*8*, *9*). Experiments conducted to validate the somatomedin hypothesis, that is, to produce proportional body growth by administration of different somatomedin preparations in vivo, have given conflicting results. Thus, Fryklund *et al.* (*10*) and Thorngren *et al.* (*11*), using hypophysectomized rats, were unable to detect any stimulatory effect of a partially purified preparation of somatomedin A on longitudinal bone growth. In contrast, van Buul-Offers and Van den Brande (*12*) and Holder *et al.* (*13*) reported a slight increase in body growth in hypopituitary dwarf mice after administration of a crude preparation of somatomedin. We designed the experiments described herein to find out if GH administered locally in vivo could stimulate longitudinal bone growth. To achieve this we injected small doses of human GH (hGH) into the cartilage growth plate of the proximal tibia of one side of hypophysectomized rats, and saline into the tibia of the other side, and determined the effect on accumulated bone growth by using tetracycline as an intravital marker.

Male Sprague-Dawley rats were hy-

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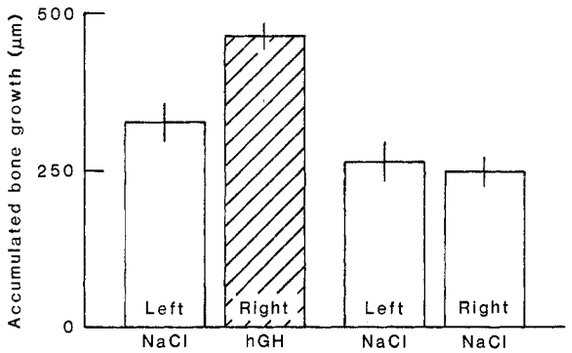

Fig. 1. Effect of local administration of human growth hormone (*hGH*) on longitudinal bone growth. Male rats (36 days old) were hypophysectomized, and 14, 16, and 19 days later 10 μg of hGH was injected into the proximal growth plate of the right tibia of six animals. The contralateral leg received the same volume of saline (first bar on the left). The two bars to the right represent three rats that received saline in both the left and right proximal growth plate of the tibia. Longitudinal bone growth was measured by the tetracycline method as described in the text. Values are means ± standard error. The effect of hGH was highly significant ($P < .001$ by paired *t*-test).

pophysectomized by the standard parapharyngeal approach (*1*) when they were 36 days old. The rats were housed under controlled conditions with a cycle of 14 hours of light and 10 hours of darkness. They were given free access to tap water and pellet food. At autopsy the hypophysial capsule and adjacent tissues were dissected out and the GH content was determined by radioimmunoassay as described earlier (*14*). None of the animals used in the present study had detectable amounts of GH after hypophysectomy, as determined by this procedure. Fourteen days after hypophysectomy the animals were anesthetized with ketamine hydrochloride (Ketalar) and a small incision was made in the skin at the medial part of the knee joint on both sides. The cartilage growth plate of the proximal tibia was identified under a stereomicroscope and the tip of a chromatographic syringe was gently inserted into the central portion of the cartilage. Then, 10 μg of hGH (Crescormon, 1.8 IU/mg, Kabi AB, Stockholm) in a volume of 2 μl was slowly injected into the growth plate. The same volume of saline was injected into the growth plate of the proximal tibia of the contralateral leg. While the animals were still anesthetized they were also given an intraperitoneal injection of oxytetracycline (OTC; 10 mg/kg; Terramycin, Pfizer). On days 16 and 19 after hypophysectomy the same amount of hGH or saline as was injected previously was injected into the proximal growth plate of the right and left tibiae, respectively. Thus, the total dose of hGH injected was 30 μg.

The hGH-injected animals did not significantly increase in body weight compared to the saline-injected rats. On day 28 after hypophysectomy the rats were killed by decapitation and the accumulated bone growth of the proximal tibia of both sides was determined by measuring the distance between the cartilage and the tetracycline band as previously described (*15, 16*). To determine the GH-independent longitudinal bone growth, which is known to occur to some extent after hypophysectomy (*17*), and to find out the variance in growth rate between the right and left tibiae, we examined the effects in three hypophysectomized animals of injecting saline into the growth plate of the proximal tibia on both sides using the same injection protocol.

As shown in Fig. 1, hGH produced a significant stimulatory effect on the accumulated longitudinal bone growth of the right tibia. The growth response of the left (saline-injected) tibia of the GH-treated animals was not increased compared to rats receiving saline only, suggesting that the total dose of hGH used in the present study was too low to produce an effect on the linear growth of the whole animal.

Since hGH also contains lactogenic activity it was of interest to determine whether the observed local effect of hGH could also be produced by prolactin. Therefore, ovine prolactin (oPrl-P-B4-NIH) in a total dose of 30 μg was injected into the proximal tibial growth plate of the right tibia according to the same protocol as that used for the experiment illustrated in Fig. 1. As shown in Fig. 2, longitudinal bone growth was the same in the right and left proximal tibia, which had received prolactin and saline, respectively. The low accumulated bone growth in this experiment compared to that in Fig. 1 is explained by the fact that the injections started 34 days after hypophysectomy.

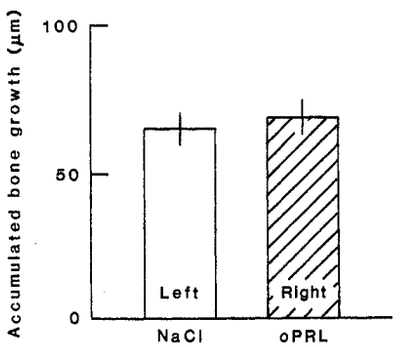

Fig. 2. Effect of local administration of ovine prolactin (*oPRL*) on longitudinal bone growth. Six hypophysectomized rats received three injections of prolactin (10 μg per injection) or saline in the proximal growth plate of the right and left tibia, respectively, according to the same protocol as that in Fig. 1, except that injections started 34 days after hypophysectomy. Values are means ± standard error.

Measurement of longitudinal bone growth by using tetracycline as an intravital marker is a specific and sensitive method for determination of GH activity in vivo (*18*). Furthermore, the increased longitudinal bone growth after GH treatment is caused by increased cell production in the growth plate (*19*). The present results demonstrate that local injection of GH into the cartilage growth plate stimulates longitudinal bone growth in vivo. Thus, it is likely that GH directly stimulates the production of new cells in the growth plate. These results cast some doubts on the somatomedin hypothesis of growth hormone action—that is, the theory that an increased plasma concentration of somatomedin(s) should be a prerequisite for stimulated longitudinal bone growth. However, these results should not be interpreted to mean that somatomedins were not involved in cartilage growth. It is possible that GH stimulates the production of somatomedins locally and that these peptides are important as permissive factors for the integrated tissue response following GH action.

Thus our results do not support the theory that increased plasma concentrations of somatomedins cause accelerated longitudinal bone growth after GH administration. Rather, it seems that somatomedins are produced as a consequence of GH action on various tissues as suggested earlier (*20*).

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Single-Neuron Labeling in the Cat Auditory Nerve

Abstract. *Single auditory nerve fibers in the cat were labeled intracellularly with horseradish peroxidase. The sample of fibers was selected to represent different response types over a wide range of characteristic frequencies. All 56 labeled neurons were found to be radial fibers innervating inner hair cells, suggesting that none of the single-unit data reported to date has been from the outer hair cell innervation. Differences in rates of spontaneous discharge and thresholds to tones among these labeled neurons were closely correlated with morphological differences in the caliber and location of their unmyelinated terminals on the body of the inner hair cell.*

The auditory nerve bundle contains primary neurons connecting cochlear sensory cells with the cells of the cochlear nucleus. The classic morphological studies of cochlear innervation (*1*) suggested that there are at least two fundamentally different types of afferent fibers in the auditory nerve: radial fibers innervating inner hair cells (IHC's) and outer spiral fibers innervating the outer hair cells. Recent morphological studies suggest that radial fibers can be further divided into three distinct types on the basis of caliber, mitochondrial content, and synaptic morphology of the peripheral terminals (*2*) and that radial fibers outnumber outer spiral fibers by 20 to 1 (*3*).

In the years since the first measurements of spike discharges from auditory nerve fibers (*4*), a number of single-unit classification schemes have been devised (*5*, *6*), each suggesting possible correlations with the morphological data available at the time. In the work reported here the validity of such schemes was tested by intracellular labeling of functionally identified neurons with standard horseradish peroxidase (HRP) techniques (*7*).

Injections of HRP were made iontophoretically through beveled micropipettes filled with a 10 percent solution of Sigma type VI HRP in 0.05*M* tris buffer (*p*H 7.3) containing 0.15*M* KCl. The electrodes yielded resting potentials in the auditory nerve as high as −60 mV and passed currents of 4 to 5 nA (50-msec pulses, 50 percent duty cycle, electrode positive). Injection of HRP was initiated only if the unit's resting potential was more than −30 mV and was terminated when the potential became smaller than −15 mV. An injected unit was visible as a labeled fiber only if the product of nanoamperes and minutes of injection exceeded 11 (*8*). For each single unit selected, a tuning curve (tone threshold as a function of stimulus frequency) and a measure of spontaneous discharge rate (SR) were obtained prior to HRP iontophoresis. For almost all units selected, additional tuning curves (typically three or four) and measures of SR (typically one or two) were obtained after the injection was terminated.

Roughly 30 hours after the last injection, the cochlea was fixed (*9*), reacted in toto by perfusion of solutions containing cobalt chloride and diaminobenzidine (*10*) through the oval and round windows, and embedded in Epon. The organ of Corti was then dissected out and mounted on glass slides so that all the sensory cells could be examined by light microscopy with an oil-immersion, phase-contrast objective lens at ×2600 (*9*).

In each experiment the aim was to label four or five fibers sufficiently separated along the length of the cochlea to allow unambiguous correlation between physiology and histology. The overall sample (from 14 cats) included fibers with characteristic frequencies (CF's) evenly distributed from 0.16 to 36 kHz. Within each CF region, units were selected to represent the full spectrum of SR's. The SR ranges from 0 to over 100 spikes per second and is strictly correlat-

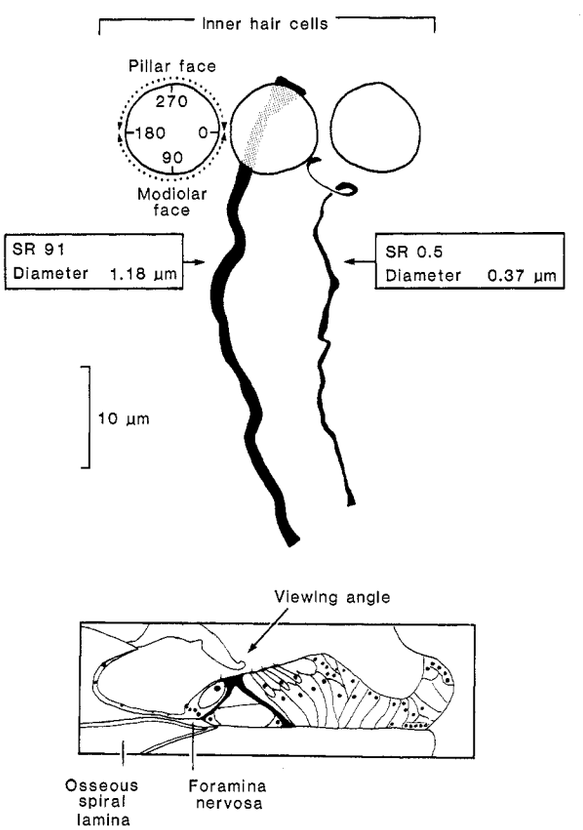

Fig. 1. Tracings of three IHC's and two radial fiber terminals as they appear when viewed from the endolymphatic surface of the organ of Corti. Outer hair cells would be toward the top of the figure. The conventions for dividing the hair cell circumference (to quantify radial fiber position) are shown on the IHC at the left. The two fibers did not innervate the same IHC, although both were from the same cochlea.

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