(Fig. 2). Exposure to higher concentrations of hemin (50 μM) increased the proportion of labeled cells (up to 80 percent), but was accompanied by increasing signs of toxicity (decrease in proliferative rate and proportion of live cells).

The pattern of globin synthesis by HEL cells raises several questions. It is unclear at present why only the γ chains are expressed and why the β chains are absent. Further studies in which molecular approaches are used could provide insights regarding the expression of genes of the β-globin genomic region as well as the molecular basis of the severe α-thalassemia phenotype in HEL cells.

It is also of special interest that this new human erythroleukemic line expresses characteristics associated not only with erythroid lineage but with other, nonerythroid, lineages (*9*). Whether this is a consequence of neoplastic transformation or whether it suggests that these particular cells are multipotent remains to be established. Further studies on the HEL cells may elucidate the relation between the phenotypic characteristics of these cells and differential globin gene expression.

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Tumorigenesis at a Predetermined Oral Site After One Intraperitoneal Injection of *N*-Nitroso-*N*-Methylurea

Abstract. *Tumors in the soft tissues of the oral cavity of rats developed at predetermined sites as a result of a combination of an intraperitoneal injection of a direct-acting carcinogen,* N-*nitroso*-N-*methylurea, and a continuous irritation of the buccal mucosa by a stainless steel wire. The incidence of histologically malignant tumors was significantly higher in the irritated area than in any other area of the body. These results constitute evidence for a carcinogenic mechanism whereby the cells that develop into tumors may require the promotional effect of a nonspecific, nonmutagenic stimulus.*

Several investigators have reported on the production of tumors in the soft tissues of the oral cavity by carcinogenic agents applied topically for extended periods of time (*1*). The fundamental premise on which these experiments are based is the requirement that the carcinogen be in direct contact with the cells that will eventually form the tumor. Although supporting evidence in this regard has been published, absolute certainty for this inference is lacking (*2*). The purpose of our study was to determine if a single intraperitoneal injection of a carcinogen could predictably stimulate tumor formation at a predetermined site on the buccal mucosa that was continuously irritated by a stainless steel wire.

We used 116 male Wistar/Furth (W/F) rats, 6 to 8 weeks old, in these experiments (Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts). The carcinogen *N*-nitroso-*N*-methylurea (NMU) (ICN Pharmaceutical, Inc., Plainview, New Jersey) was selected because its half-life has been estimated to be between 2 and 15 minutes (*3*). In addition, no intracellular or extracellular metabolite of NMU has been shown to have the carcinogenic property generally attributed to this drug (*3, 4*). The area selected for prospective tumor formation was the right buccal mucosa. The stimulus used to induce long-term cell proliferation was a 0.025-cm stainless steel wire that was ligated around the upper right second molar and projected into the buccal mucosa. The wire served as a continuous, nonspecific, mechanical irritant which resulted in a sustained, local proliferative reaction. It has been shown that this kind of irritation results in hyperplasia, which, however, does not lead to tumor formation (*5*). We assessed the tumor incidence and latency by varying the time at which the NMU injection was administered in relation to the initiation of the mechanical irritation. Therefore, NMU was injected 7 days after (group 1) as well as 7 days before the mechanical irritation was applied (group 2). The left buccal mucosa, where no stainless steel wire was placed, was considered a control for the targeted area of prospective tumor development in the same animal. Other controls included animals injected with NMU in which no wire was placed (group 3), animals in which the wire was placed but no carcinogen was injected (group 4), and animals that were neither injected with NMU nor irritated with the wire (group 5).

Table 1 shows the incidence of tumors in the five groups of rats. In group 1, the mean time for tumor production in the irritated area of the buccal mucosa calculated from the day of carcinogen injection to the day on which the animals were killed was 131 ± 20.69 days, with a median of 105 days. In group 2, the

Table 1. Location and incidence of soft tissue tumors in experimental and control animals. The numbers in parentheses indicate the number of animals in each group. All lesions of the oral cavity that are listed as tumors were histologically malignant (see Fig. 1).

Location	Group 1 (18)	Group 2 (29)	Group 3 (30)	Group 4 (29)	Total* (116)
Buccal mucosa irritation site	7†	7‡			14
Other sites of oral mucosa			1§		1
Abdominal wall	1	1			2
Intestine		3	1		4
Kidney	1	2	1		4
Lung	1	2			3
Vertebrate marrow	1	1			2
Other systemic locations		1‖	2¶		3

*A group of ten male W/F rats not subjected to any treatment and kept for 11 months (group 5) is included in the total. No tumors developed in this group. †Five spindle cell tumors, one undifferentiated sarcoma, one fibrosarcoma. ‡Four spindle cell tumors, two undifferentiated sarcomas, one round cell carcinoma. §Spindle cell tumor between the lower incisors. ‖ Skin tumor. ¶Spleen and skin tumors.

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mean time for tumor formation was 196 ± 26.91 days, with a median of 211 days. Animals receiving the long-term mechanical irritation after the carcinogenic injection required a longer latent period for tumor production in the irritated buccal mucosa. No tumors occurred in the buccal mucosa of animals in group 3; however, one tumor did occur between the lower incisors. This could be the result of the promotional effect of a hair impaction in the periodontal ligament (*6*). No tumors were produced in the left buccal mucosa of animals in groups 1 and 2. No tumors were produced in animals of groups 4 and 5. The study was terminated after 11 months.

The microscopic pictures of the tumors that developed in the irritated buccal mucosa showed no significant differences between groups 1 and 2. Nine of the 14 tumors that developed in the irritated area of the buccal mucosa were classified as spindle cell tumors (Fig. 1, A through D). The remaining five soft tissue tumors included one fibrosarcoma, one round cell carcinoma, and three undifferentiated sarcomas. Animals in groups 1 and 2 that did not develop tumors in the irritated area of the buccal mucosa generally showed hyperplasia and severe inflammatory infiltration around the area where the wire touched the buccal mucosa. A similar inflammatory hyperplastic reaction was observed in the animals in group 4. Animals in group 3 showed no pathologic alterations in the buccal mucosa. Animals in group 5 did not show any abnormal features in the mouth. The systemic tumors that developed in animals injected with the carcinogen included one carcinoma of the small intestine, one kidney adenoma, and one lipoma beneath the skin.

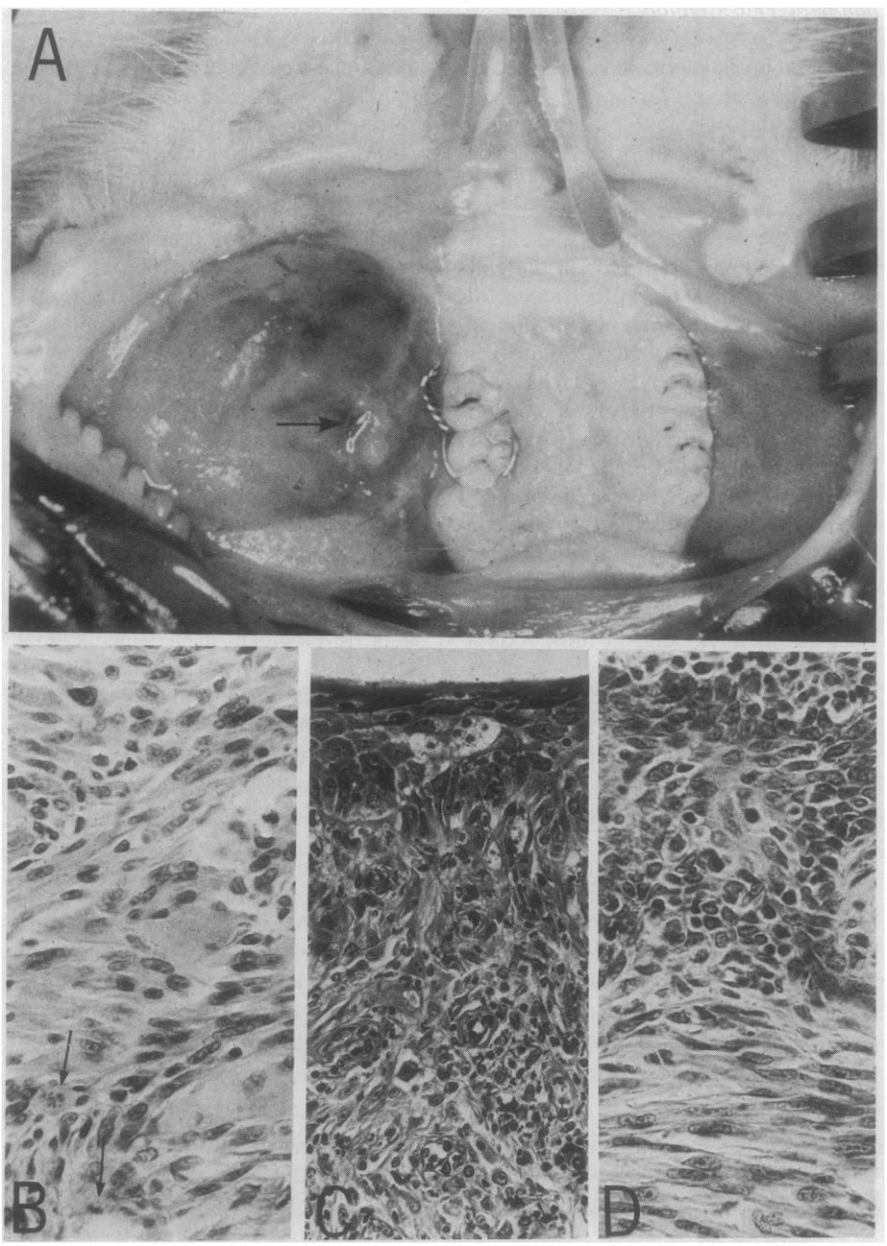

Fig. 1. Macroscopic and histopathologic features in spindle cell tumors in the buccal mucosa of rats injected with NMU (intraperitoneal) and locally irritated with a stainless steel wire. (A) Macroscopic view of the upper palate and the right and left buccal mucosa. Around the second upper right molar a stainless steel wire can be seen. The arrow points to an ulcer with an endophytic tumor. The mucosa and soft tissues on the left side show normal characteristics. (B) Spindle cell tumor showing elongated, bipolar cells with vesicular-like nuclei containing one or more nucleoli. Several mitotic figures and variations in shape, size, and staining intensity of the nuclei are evident (arrows) (×120). (C) Another spindle cell tumor from the irritated buccal mucosa. Increased mitotic figures, variations in shape, size, and staining intensity of nuclei of the spindle-shaped cells, and several giant, multinucleated cells are evident (×76). (D) Another field of the tumor shown in (C). No apparent distinction between the basement membrane and the spindle cells can be seen. A transition of basal cells to spindle cell elements is apparent (dropping-off phenomenon) (×120).

The results of our experiments indicate that a single intraperitoneal injection of NMU is capable of producing tumors in distant areas where active cell proliferation is triggered by a nonspecific, nonmutagenic, long-term mechanical irritant. This irritation is a determining factor in localizing tumors to predetermined areas of the oral cavity, that is, areas where cell proliferation is very active. Our results further suggest that the source of the increased proliferative reaction need not be linked to the effect of the carcinogen on the cells that will eventually develop into tumor cells. Other researchers have shown that circumscribed, increased cell proliferation, caused by a repeatedly applied or continuous nonspecific irritant, may serve as a locus for tumor formation after the systemic administration of a carcinogen (*7*).

The incidence of tumors induced in rats of group 1 was not statistically different from that observed in rats of group 2 ($P < .1$). This result suggests that the timing of the wire insertion was not an essential factor for tumor development. This finding is not compatible with the concept that the carcinogen, to be effective, should be administered at peak periods of DNA synthesis (*8*) because only in rats of group 1 was local cell proliferation increased when the carcinogen was injected. However, the latent period for tumor induction in the irritated buccal mucosa was shorter when the irritation was applied before the NMU injection. It should be noted that the opposite, nonirritated buccal mucosa of the carcinogen-treated animals did not reveal any pa-

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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