degenerating cells. Because group differences in the incidence of degenerating cells so accurately predict group differences in the relative magnitude of SNB motoneuron loss, we believe that these degenerating cells reflect the death of SNB motoneurons.

Normally occurring motoneuron death is a common feature of the developing spinal cord, and the extent of this death is influenced by interactions between motoneurons and their target muscles (*19*). Androgens promote the survival of both SNB motoneurons and their target musculature, the LA-BC complex. It is possible that androgens act directly on SNB motoneurons to promote their survival, indirectly preventing the involution of the LA-BC complex, since normal muscle differentiation and survival require proper innervation (*20*). Although SNB motoneurons in females do send axons to the periphery before the period of SNB motoneuron death, it is not known if these axons form functional synapses with LA-BC muscle fibers (*18*). Another possibility is that androgens prevent SNB motoneuron death indirectly, by ensuring the survival of their target musculature. However, certain androgenic manipulations can prevent LA-BC involution in females without resulting in male-typical SNB motoneuron numbers (*21*), suggesting that regulation of the number of motoneurons in the SNB region does not depend simply on muscle survival.

It seems likely that androgens prevent the death of SNB motoneurons by interacting with factors normally important for motoneuronal survival. For example, androgens might alter the growth of motoneuronal axons or render axons better able to synapse with their target musculature. Androgens may also regulate the availability of postsynaptic target sites, neurotrophic factors secreted by muscle, or synaptic activity. Establishing which cellular mechanisms are regulated by androgens in this simple neuromuscular system will further our understanding of how these mechanisms contribute to the regulation of cell death throughout the developing nervous system.

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11. The day a copulatory plug was observed was designated E1. By this nomenclature, the day of birth is both E23 and postnatal (P) 1.
12. All sections were counted, and neurons with multiple nucleoli were extremely rare. Corrections were made by the Abercrombie method [B. W. Konigsmark, in *Contemporary Research Methods in Neuroanatomy*, W. J. H. Nauta and S. O. E. Ebbesson, Eds. (Springer-Verlag, New York, 1970), pp. 315–340].
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21. Females treated with dihydrotestosterone propionate during the late prenatal period (E17–E22) retain the LA-BC complex, yet in adulthood have the number of motoneurons in the SNB region typical of females (*7*).

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Physiological Effects of Transforming Growth Factor in the Newborn Mouse

Abstract. *Transforming growth factor–type α accelerated incisor eruption and eyelid opening in the newborn mouse and also retarded the growth rates of hair and body weight when administered in high dosage (0.7 to 4 micrograms per gram of body weight). The results of whole animal studies indicate that transforming growth factor–type α and epidermal growth factor do not differ significantly in these effects and suggest that transforming growth factor–type α may be important in immature animals.*

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Transforming growth factor–type α (TGF-α)—also called type I (*1, 2*)—was first isolated from media conditioned by virally transformed cells. It is a mitogenic peptide containing 50 amino acid residues and is operationally defined by its ability to confer reversible phenotypic transformation on NRK fibroblasts (*3*). However, this transforming activity of TGF-α is modulated by another growth factor, transforming growth factor–type β (*4*). Present together, these two transforming growth factors provide for maximal phenotypic transformation. TGF-α displays 32 percent sequence homology with mouse epidermal growth factor (mEGF) (*5*) and exhibits similar activities in competition for binding to the EGF receptor, stimulation of DNA synthesis, and cell growth.

Table 1. Effect of synthetic TGF-α on eyelid opening and incisor eruption in the newborn mouse. Daily subcutaneous injections were made into newborn mice (NCS strain) with 20 μl per gram of body weight. The injections were started with the day of birth (day 0) and given at 24-hour intervals. Daily dosage varied according to body weight, and dosage ranged from 0.03 to 4 μg per gram of body weight. Two animals received injections at each dosage. Control animals received either no injection or dilute phosphate-buffered saline solution. The effects of doses greater than 0.3 μg of both TGF-α and EGF are significantly different from that of the controls (rank sum test, $P < 0.0001$).

Dose (μg/g per day)	Number of animals	Day of	
		Incisor eruption	Eyelid opening
	Control		
No injection	10	9–11	12–14
Saline	14	9–11	12–14
	EGF		
2.7–4	4	6–7	7–8
	TGF-α		
0.03–0.3*	20	9–10	12–13
0.35–0.65†	8	7–8	9–11
0.7–1.4‡	6	6–7	8–10
2.7–4	4	6–7	6–7

* Dose: multiples of 0.03 μg (0.03, 0.06, 0.09, ...), with two mice given each dose. † Dose: 0.35, 0.45, 0.55, and 0.65 μg. ‡ Dose: 0.7, 1.05, and 1.4 μg.

Table 2. Effect of synthetic TGF-α on body weight and hair growth on newborn mouse. For method and dosage, see Table 1. The rate of body growth was compared with that of littermate controls. Monotrichs plucked from the midgut section of the coats of these animals on day 8 were examined by light microscopy, with dilute iodine solution to provide contrast. The effects of doses greater than 0.3 μg of TGF-α are significantly different from those of the controls (Student's t-test, $P < 0.005$). There are statistically no differences between the effects of TGF-α and EGF at the high doses.

Treatment (μg/g per day)	Number of animals	Mean compared to control (%)	
		Body weight	Hair growth
	Control		
No injection or saline	22	100	100
	EGF		
2.7–4	2	75	63
	TGF-α		
0.03–0.3	20	102	101
0.35–0.65	8	94	87
0.7–1.4	6	85	76
2.7–4	4	76	65

Although TGF-α is structurally and biochemically similar to EGF, the biosynthesis and expression of the two growth factors are different. Whereas EGF is derived from a precursor protein of about 1200 amino acids (*6*, *7*), the prepro-TGF-α is a protein of 160 amino acids (*8*). Furthermore, EGF is synthesized in the submaxillary gland while TGF-α is difficult to detect in healthy animals in the normal state. Because of the scarcity of TFG-α from natural sources, many of the in vivo functions of TGF-α remain unexplored. By duplicating the protein sequence of TGF-α from rat embryo fibroblasts, we prepared TGF-α in a highly purified state by the solid-phase method (*9*, *10*) and have shown that the synthetic growth factor exhibits chemical and biochemical properties indistinguishable from those of natural TGF-α. We report here the physiological relation between TGF-α and EGF in incisor eruption, eyelid opening, and growth rates of hair and body weight in the newborn mouse.

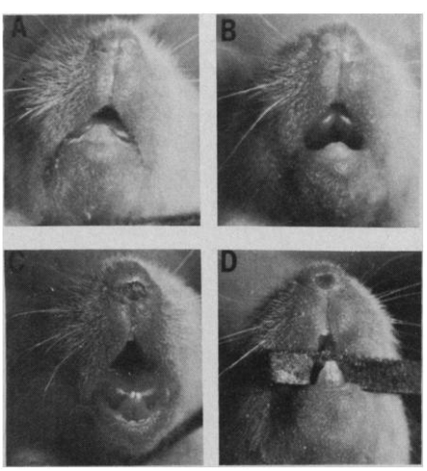

Fig. 1. Extent of incisor eruption in control and treated 8-day-old mice. (A) Control mouse (no injection). (B) Control mouse (saline injection). Neither (A) or (B) shows incisor eruption. (C) Mouse injected daily with EGF (2.7 μg/g). (D) Mouse injected daily with TGF-α (2.7 μg/g). Both (C) and (D) show upper and lower incisor eruption.

When injected into newborn mice, mEGF causes marked effects in somatic development. Daily subcutaneous administration of microgram quantities of mEGF accelerates tooth eruption and eyelid opening (*11*), but retards the rate of body growth and inhibits hair growth (*12*). The possibility that rat TGF-α has the same physiological effects on the newborn mouse was tested by treating newborn NCS mice with the synthetic TGF-α (0.03 to 4 μg per gram of body weight per day injected subcutaneously in the nape of the neck).

Eruption of lower incisor teeth and opening of eyelids occurred earlier in the rat TGF-α–treated mice than in littermate controls. The extent of the response was related to the dose of TGF-α administered (Table 1). Doses smaller than 0.3 μg of TGF-α per gram of body weight produced no significant effects. At 2.7 μg of TGF-α per gram of body weight, incisor tooth eruption and eyelid opening were observed on days 6 to 7, instead of days 9 to 10 for tooth eruption and days 12 to 14 for eyelid opening as seen in control animals. When mEGF was compared with rat TGF-α on both of these effects at a dose of 2.7 μg/g, no significant difference between the two growth factors was observed. The eruption of the upper incisor usually occurred a day after the eruption of the lower incisor. Thus, on day 8, eruption of both incisors was visible in mice treated with EGF or TGF-α, but neither incisor was observed in the controls (Fig. 1).

In these experiments, the effects on tooth eruption and eyelid opening are reported as a range of days instead of mean averages, and the day of birth is counted as day 0. The slight variability in these effects was dependent on the size of the litter and on the number of pregnancies of the mother. Small litter size (fewer than five) tended to accelerate these effects by 1 or 2 days. However, the responses of the newborn mouse to TGF-α are clear and significant even after these variabilities are taken into account.

Treatment with rat TGF-α, like treatment with mEGF (*12*), produced inhibition of hair growth that was correlated with the dose administered (Table 2). Examination of the overall rates of hair growth visually and by scanning electron microscopy of gluteraldehyde-fixed skin revealed that EGF or TGF-α at 2.7 to 4 μg per gram of body weight produced significantly fewer monotrichs as well as a finer and shorter coat than those found in control animals. With TGF-α at 2.7 μg daily, hair length and diameter were reduced approximately 30 percent when compared with these measures in control animals (Fig. 2).

Finally, rat TGF-α retarded the overall growth rate of newborn mouse (Table 2). The growth rates during the first 10 days after birth were examined, and in control animals, doubling and tripling of body weights were usually seen on days 5 and 9 respectively. In contrast, growth was

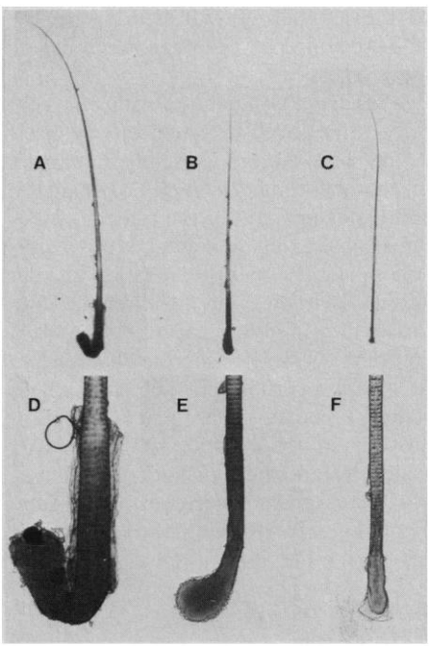

Fig. 2. Extent of inhibition of hair growth in control and treated 8-day-old mice. (A) and (D) Control mouse (saline injection). (B) and (E) Mouse injected daily with EGF (2.7 μg/g). (C) and (F) Mouse injected daily with TGF-α (2.7 μg/g). (D), (E), and (F) are shown at magnifications 6.4 times those of (A), (B), and (C) to show differential follicle developments affected by TGF-α. Both TGF-α (C and F) and EGF (B and E) showed striking inhibition of hair growth.

stunted in animals treated with TGF-α at doses higher than 0.3 μg/g per day. Again, the inhibition of growth rate correlated well with the dose administered. At 4 μg/g per day, the growth of animals treated with either rat TGF-α or mEGF was 25 percent slower than that of the control animals.

Although the biochemical events leading to these effects of TGF-α on the newborn mouse remain unresolved, the results clearly support those obtained from studies (*1–3*) in vitro and provide direct evidence in vivo that TGF-α is a member of the EGF family. Preliminary data from other physiological studies of TGF-α on whole animals or tissues also support the conclusion that TGF-α is physiologically similar to EGF. For example, TGF-α stimulates ornithine decarboxylase activity and protein synthesis (*13*) but significantly inhibits histamine-induced gastric acid secretion (*14*). These and other results suggest that TGF-α, although originally found in malignancy, might be physiologically as important as its normal counterpart, EGF. One possibility is that EGF is required to maintain the normal state of the animal, whereas TGF-α, which is found in states of rapid growth such as pregnancy and malignancy, perhaps serves as the additional growth factor required to meet the needs for such rapid growth.

The present results also provide greater insight to the structural relation between TGF-α and EGF. The similar behavior of TGF-α and EGF is remarkable in that only 16 of the 50 amino acids of TGF-α have sequence homology to mouse EGF. Ten of the 16 amino acid residues are related to conformational and structural requirements for the proper refolding of the molecule—such as disulfide formation and β-bends (six cysteines, three glycines, and one proline)—and only six amino acid residues may have functional roles. It is likely that the common secondary structure of TGF-α and EGF has an important role in determining the biological properties of these growth factors. The use of synthetic TGF-α analogs will be useful to test this hypothesis.

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The pX Protein of HTLV-I Is a Transcriptional Activator of Its Long Terminal Repeats

Abstract. *Expression of the pX protein of human T-cell leukemia virus type I (HTLV-I) in animal cells demonstrates that this protein is a specific transcriptional activator of the long terminal repeats (LTR) of HTLV-I. Several other promoters are not affected by pX. No lymphocyte-specific factors are required for this activation. pX can be detected in the nucleus of transfected monkey kidney cells (line CV1) by indirect immunofluorescence. These results indicate that the pX protein is essential for the replication cycle of the virus and that it may be directly involved in the immortalization of human lymphocytes by HTLV-I.*

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The exogenous retrovirus human T-cell leukemia virus type I (HTLV-I) appears to be the etiologic agent of adult T-cell leukemia-lymphoma (*1, 2*). HTLV-I is a member of a family of human retroviruses that display some structural and functional similarities, including the tropism for a restricted cell type, the OKT4 helper T lymphocyte. HTLV-I resembles a chronic leukemia virus in that it is replication competent, causes a monoclonal malignancy after a long latency period, and does not contain any recognizable cell-derived oncogene. However, HTLV-I can efficiently immortalize normal human lymphocytes in vitro, a property previously associated with acute transforming viruses containing oncogenes (*3*). In spite of the monoclonality of HTLV-I associated malignancies, no specific chromosomal sites have been detected for the integration of the provirus (*4*). There is evidence that the HTLV viruses, HTLV-I, HTLV-II, and HTLV-III, together with bovine leukemia virus (BLV), may constitute a group of retroviruses that can be characterized by the way in which they interact with the infected cell. There is a great increase in the level of steady-state messenger RNA (mRNA) directed from the promoter in the long terminal repeats (LTR's) in cell lines infected with HTLV-I compared to uninfected parent cells (*5–7*). It has been postulated that this is a transcriptional activation caused directly or indirectly by a viral product (*5, 8*). In addition to the typical retroviral genes *gag*, *pol*, and *env*, the HTLV-I genome contains four overlapping open reading frames at the 3′ end of the genome (*9*). One of these, the extended X_{IV} (*9*) or LOR (*8*) reading frame (here referred to as the X-LOR) is highly conserved between HTLV-I and HTLV-II (*10*). A splice acceptor at the beginning of this reading frame is also conserved among HTLV-I, HTLV-II (*11*), and probably BLV (*12*). Furthermore, a protein of the expected molecular weight was identified in cells infected with HTLV-I or HTLV-II by immunoprecipitation and partial radiosequencing (*13*). It was proposed that this viral product (here referred to as pX) may be responsible for the observed *trans*-activation. Alternatively, virus-permissive cell lines may constitutively produce specific factors that, upon infection with virus, allow the increased transcription from the LTR (*6, 7*). To distinguish between these explanations, and to understand the role of pX in the life cycle of the virus and its relation to leukemogenesis, we have expressed the pX protein in animal cells and studied its structural and functional properties.

The strategy we used is depicted in Fig. 1A. The promoter and the first structural exon of the mouse metallothionein I (MT) gene were ligated to HTLV-I upstream of the mapped splice