Beach sites for dibenzofurans and PCB's. The concentration of total TCDF in the soil was 40 to 80 ppb and that of PCB's was 3 to 4 ppm. 2,3,7,8-TCDF is approximately one-fifth as efficacious as 2,3,7,8-TCDD in inducing AHH (*24*). As the concentration of total TCDF in the contaminated soil is one-tenth that of TCDD, TCDF should account for less than 2 percent of the observed inductive effect. Although the PCB concentration is five times that of TCDD, the most potent PCB is 1/100 as active as TCDD in inducing AHH (*14, 25*), and this congener is not routinely detected in commercial PCB mixtures. By our estimation, PCB's could account for no more than 0.2 percent of the inductive effect of contaminated soil. However, the possibility exists that other contaminants present in the soil might potentiate the action of TCDD and could be, in part, responsible for the toxic and enzyme induction effects observed in these studies.

From the foregoing it is readily apparent that TCDD in soil is biologically available in two animal species, as measured in terms of a clinicopathologic syndrome in guinea pigs, hepatic enzyme induction in rats, and uptake of TCDD in the livers in both species. Although one has difficulty arriving at an exact percentage for bioavailability, the absorption of TCDD from soil appears to be highly efficient in the guinea pig and rat models. The guinea pig study provides only a crude estimate of bioavailability. The importance of this model lies in its ability to respond to CDD's with a characteristic clinicopathologic syndrome that was duplicated in this study. The induction of AHH in rats is a more quantifiable measure of bioavailability. On the basis of the high bioavailability detected in these two animal species, it seems clear that TCDD-contaminated soil presents a potential hazard to humans if ingested.

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Rat Transforming Growth Factor Type 1: Structure and Relation to Epidermal Growth Factor

Abstract. *The complete amino acid sequence of rat transforming growth factor type 1 has been determined. This growth factor, obtained from retrovirus-transformed fibroblasts, is structurally and functionally related to mouse epidermal growth factor and human urogastrone. Production of this polypeptide by various neoplastic cells might contribute to the continued expression of the transformed phenotype.*

Retrovirus-transformed cells and certain human tumor cells produce transforming growth factor type 1 (TGF-1) (*1*). In contrast, control cells or cells infected with transformation-defective viral mutants (*2–4*) do not produce and release detectable levels of TFG-1. The factor has been purified from serum-free medium conditioned by human melanoma cells (*5*) and by retrovirus-transformed rat and mouse fibroblasts (*6*). The amino-terminal sequences of human, rat, and mouse TGF-1 (hTGF-1, rTGF-1, and mTGF-1, respectively) have been described (*6*). Here we report the complete amino acid sequence of rTGF-1 and compare its biological properties with those of mouse epidermal growth factor (mEGF).

Purification of rTGF-1 from serum-free medium conditioned by Fischer rat embryo fibroblast line C110 (*7*), a subclone of Fischer rat embryo fibroblast line 3A nonproductively transformed by Snyder-Theilen feline sarcoma virus, was monitored in a radioreceptor assay based on receptor cross-reactivity with mEGF (*6*). The amino-terminal sequence of residues 1 to 43, with the exception of residues 26, 30, 36, 40, and 42, was determined by automated Edman degradation of *S*-carboxamidomethylated rTGF-1 in a gas-liquid solid-phase microsequencer (Fig. 1) (*6, 8*). The remainder

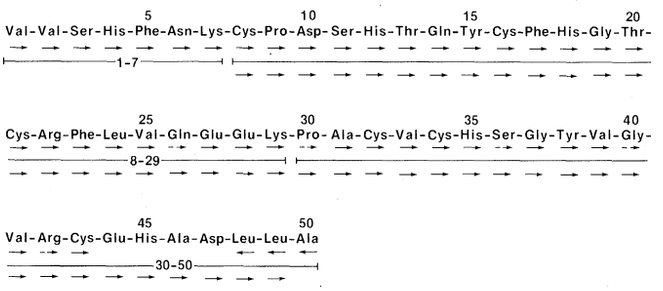

Fig. 1. Amino acid sequence of rTGF-1 and schematic outline of the data supporting the sequence. A sample of rTGF-1 (0.5 nmole) was reduced with dithiothreitol, *S*-carboxamidomethylated with iodoacetamide, and subjected to automated Edman degradation in a gas-liquid solid-phase microsequencer containing Polybrene (*6*). The phenylthiohydantoin amino acids were analyzed by reverse-phase HPLC (detection limit, 1 pmole) (*24*). Sequence assignments for residues based on Edman degradation are indicated by rightward-pointing arrows. Unidentified amino acids in the amino-terminal Edman degradation are indicated by dashed arrows. *S*-Carboxamidomethylated rTGF-1 (0.6 nmole) was digested with the endoproteinase Lys-C (Boehringer) in 50 μl of 0.1*M* tris-acetate (*p*H 8.0) containing 20 percent acetonitrile (substrate-to-enzyme weight ratio, 25 to 1, 16 hours at 22°C). The enzyme cleaves mainly at the carboxyl-terminal side of lysine and, to a lesser extent, arginine residues. The digest was acidified to *p*H 2.0 with trifluoroacetic acid (TFA) and applied to a μBondapak C_{18} column (10-μm particle size; 0.39 by 30 cm; Waters Associates), and eluted (40°C; flow rate, 1 ml/min) with a linear 3-hour gradient of 0 to 60 percent acetonitrile containing 0.05 percent TFA. Ultraviolet-absorbing material was detected at 206 nm. The three main peptides (solid lines under the residues) were identified as peptide 1 to 7 (recovery 58 percent), peptide 30 to 50 (recovery 63 percent), and peptide 8 to 29 (recovery 48 percent) by amino acid analysis (*5*) and Edman degradation. Automated Edman degradations were performed with 20 pmole of peptide 8 to 29 (based on the yield of identified Asp^{10}) and 25 pmole of peptide 30 to 50 (based on the yield of identified Ala^{31}). *S*-Carboxamidomethylated rTGF-1 (140 pmole) was digested with carboxypeptidase A (76 U/mg; Worthington) in 50 μl of 0.05*M* tris-HCl, *p*H 8.0 (substrate-to-enzyme weight ratio, 100 to 1, 10 minutes at 37°C). The digest was acidified by the addition of 10 μl of 6*N* HCl and lyophilized. Released amino acids (leftward-pointing arrows) were quantitatively determined by amino acid analysis. Residues released by carboxypeptidase A digestion were Ala (145 pmole) and Leu (271 pmole).

of the amino acid sequence was deduced from microsequence analysis data obtained from peptide 8 to 29, from peptide 30 to 50 isolated from a digest of *S*-carboxamidomethylated rat TGF-1 with the endoproteinase Lys-C, and from the results of carboxypeptidase A digestion of *S*-carboxamidomethylated rTGF-1. The proteolytic fragments were purified by reverse-phase high-pressure liquid chromatography (HPLC) with volatile solvents (*9*) and identified by amino acid analysis and Edman degradation. The results confirmed the assignments made in the amino-terminal Edman degradation, allowed identification of residues Gln^{26}, Pro^{30}, Ser^{36}, Gly^{40}, and Arg^{42}, and extended the amino acid sequence to Leu^{48} and Leu^{49}. The only residue unaccounted for by the sequence data for peptide 30 to 50 was an additional alanine. Peptide 30 to 50 was assumed to be the carboxyl-terminal peptide of rTGF-1. Carboxypeptidase A digestion of *S*-carboxamidomethylated rTGF-1 released leucine and alanine in a molar ratio of 2 to 1. Thus the residues identified in the Edman degradation of peptide 30 to 50 and the residues identified in the carbox-

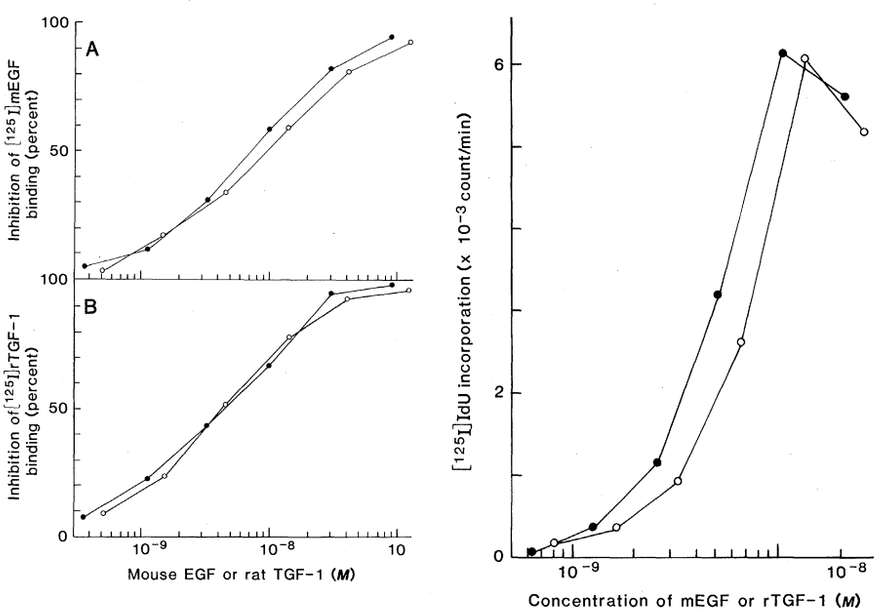

Fig. 2 (left). Inhibition of binding of labeled mEGF and labeled rTGF-1 by mEGF and rTGF-1. Quantitation was based on amino acid analysis of a companion sample. (A) Inhibition of binding of [^{125}I]mEGF to the EGF receptor of A431 human carcinoma cells (*25*) by mEGF (●) and rTGF-1 (○). Purified mEGF (*26*) was labeled with Na^{125}I by a modification of the chloramine-T method (*27, 28*). The binding assay (*6*) was performed on subconfluent monolayers of Formalin-fixed A431 cells. The cells (1×10^4) were incubated with [^{125}I]mEGF (4 ng/ml; 74 μCi/μg) and unlabeled peptides at the indicated concentrations. The amount of labeled EGF bound was determined; nonspecific binding (binding in the presence of 2 μg of mEGF per milliliter) was 1.8 percent and has been subtracted. One hundred percent binding corresponds to 14.6 percent of the input radioactivity. (B) Inhibition of binding of [^{125}I]rTGF-1 to the EGF receptor of A431 cells by mEGF (●) and by rTGF-1 (○). Purified rTGF-1 was labeled (*27*) by dissolving 1 μg of rTGF-1 in 10 μl of 0.1*N* acetic acid, adding 40 μl of 0.4*M* phosphate buffer (*p*H 7.5) containing 1 mCi of ^{125}I as Na^{125}I (carrier-free; Amersham) and then 10 μl of a freshly prepared solution of chloramine-T (50 μg/ml in water). The reaction was allowed to proceed at 22°C for 2 minutes and then stopped by the addition of 25 μl of an aqueous solution of sodium metabisulfite (50 μg/ml). The iodination mixture was immediately transferred to a 0.7 by 14 cm column of Sephadex G-15 Fine that had been equilibrated and eluted with 0.01*M* phosphate-buffered saline containing 0.1 percent bovine serum albumin at 22°C; 0.5-ml fractions were collected. A specific activity of 120 μCi/μg was obtained. The binding assay was performed on subconfluent A431 cells as described above, except that the cells were not fixed with Formalin. The cells (5×10^3) were incubated with [^{125}I]rTGF-1 (4 ng/ml; 120 μCi/μg) and unlabeled peptides at the indicated concentrations. The amount of labeled rTGF-1 bound was determined; nonspecific binding (binding in the presence of 2 μg of mEGF per milliliter was 2.3 percent and has been subtracted). One hundred percent binding corresponds to 6.4 percent of the input radioactivity. Fig. 3 (right). Effects of mEGF (●) and rTGF-1 (○) on DNA synthesis in serum-deprived normal rat kidney cells. Some 4×10^4 normal rat kidney cells (clone 49F) (*29*) were plated in flat-bottomed 96-well tissue culture trays (Costar) in 100 μl of Dulbecco's modified Eagle medium with 10 percent heat-inactivated fetal bovine serum for 5 hours and then maintained in medium containing 0.1 percent fetal bovine serum for 6 days before the assay. Samples of mEGF or rTGF-1, in 50 μl of Waymouth medium (*30*), were added for 30 hours and [^{125}I]IdU (2.5 μCi/ml; Amersham) was added for the final 24 hours of culture (*31*). Cells were washed three times, harvested with a multiple sample harvester, and radioactivity was counted in a gamma counter. The cells in this experiment produced half-maximum stimulation at mEGF concentrations of 30 ng/ml and at rTGF-1 concentrations of 36 ng/ml. Incorporation of [^{125}I]IdU was 940 count/min per dish for unstimulated cultures; maximum [^{125}I]IdU incorporation was seven times greater than control values.

yl-terminal sequence of rTGF-1 accounted for all the residues in the amino acid composition of peptide 30 to 50.

The structure of rTGF-1 (Fig. 1) and compositional data on the isolated Lys-C peptides are in agreement with the amino acid compositional data on the whole peptide. The proposed primary structure of rTGF-1 is supported by the finding that native rTGF-1 and its synthetic replicate (*10*) do not differ significantly in their biological activities in vitro. Rat TGF-1 is a single-chain polypeptide of 50 residues with a calculated molecular weight of 5616. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of this material, of rTGF-1 isolated from Abelson murine leukemia virus–transformed Fischer rat embryo fibroblasts (*3*), and of human melanoma–derived hTGF-1 (*5*) gave an apparent molecular weight of 7400. The discrepancy between molecular weight and mobility is not understood. The previously reported amino acid composition of hTGF-1 (*5*) was based on an assumed molecular weight of 7400, and thus the number of residues per mole was overestimated. However, the amino acid composition of hTGF-1 agrees well with the expected values (*6*) by assigning a total of 50 residues.

The binding properties of rTGF-1 were compared with those of mouse EGF in radioreceptor assays (Fig. 2). Portions of rTGF-1 and mEGF, added simultaneously with [^{125}I]mEGF, inhibited binding of the labeled EGF to Formalin-fixed A431 human carcinoma cells in a dose-dependent manner (Fig. 2A). The concentrations of mEGF and rTGF-1 that inhibited 50 percent of the binding of [^{125}I]mEGF to the EGF receptors were 7.0 and 9.0 n*M*, respectively. Rat TGF-1 bound directly to A431 cells (Fig. 2B). Both rTGF-1 and mEGF reduced binding of [^{125}I]rTGF-1 to A431 cultures in a dose-dependent fashion. An excess of unlabeled mEGF completely blocked the binding of [^{125}I]rTGF-1 to the EGF membrane receptors. The concentrations of mEGF and rTGF-1 required for half-maximum binding were both approximately 4.5 n*M*. Scatchard analysis of the binding of [^{125}I]mEGF and [^{125}I]rTGF-1 showed a single class of binding sites with an apparent dissociation constant of $2.7 \times 10^{-9} M$ and an average of approximately 2.8×10^6 EGF receptors per cell, confirming earlier results (*11, 12*).

Binding of mEGF or rTGF-1 to the EGF receptor triggers specific effects, including tyrosine phosphorylation of the EGF receptor (*13*) and phosphorylation by A431 and mouse 3T3 cell membranes of a synthetic tyrosine-containing peptide (*14*). Moreover, rTGF-1 stimulates DNA synthesis in serum-deprived fibroblasts. Figure 3 shows the effect of increasing concentrations of rTGF-1 and mEGF on the incorporation of labeled 5-iodo-2′-deoxyuridine (IdU) into DNA of normal rat kidney cells. A sevenfold enhancement of IdU incorporation was induced. Maximum stimulation occurred at lower concentrations of rTGF-1 and mEGF than those required to saturate cellular binding sites on normal rat kidney cells. At higher concentrations the mitogenicity of rTGF-1 and mEGF decreased slightly. These observations indicate that ligands other than mEGF that bind to the EGF receptor can act as an agonist for the early responses (tyrosine phosphorylation) and delayed responses (DNA synthesis, cell division) of mEGF.

Structurally, rTGF-1 belongs to the mEGF-urogastrone family, a group of polypeptides involved in cell proliferation and differentiation (*15*). Mouse EGF is a 53-residue polypeptide of known sequence (*16*). It has three intrachain disulfide bonds (*17*) and is synthesized as a large protein precursor (*18, 19*). Urogastrone (hEGF) (*20*) is functionally related to mEGF and shares 69.8 percent of its amino acid sequence. Comparison of the rTGF-1 sequence with corresponding sequences of mEGF and hEGF revealed significant homologies (33.3 and 43.8 percent, respectively). Comparison of the rTGF-1 sequence with all protein sequences stored in the *Protein Sequence Database* (*21*) did not reveal any extensive homology with any other known sequence, including the mEGF-like sequence repeat units in the mEGF precursor.

The sequences of rTGF-1, mEGF, and hEGF can be aligned so that cysteine residues display homologous positions by inserting a single deletion between residues 20 and 21 of rTGF-1. The three members of the EGF-like family thus share 16 invariant residues, and two additional residues are structurally conserved (Leu^{24} for Ile and Glu^{44} for Gln) (Fig. 4). No free sulfhydryl groups were found in rTGF-1. The lack of free sulfhydryl groups and the presence of six half-cystine residues suggest three disulfide bridges in this polypeptide. On the basis of the chemical and biological results described, we propose that the placement of disulfide bonds in rTGF-1 is similar to that in mEGF (Fig. 4). Rat TGF-1 contains the residues necessary for maintaining a conformation that is essential for recognizing the EGF receptor.

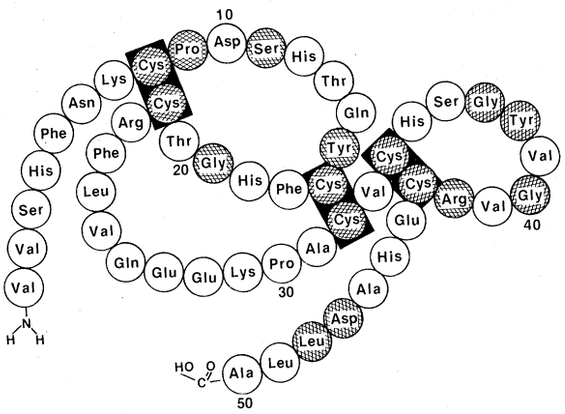

Fig. 4. Amino acid sequence of rTGF-1, with placement of disulfide bonds based on (i) an assumed homology with mEGF (*17*) and (ii) comparison of amino acid sequences of rTGF-1, mEGF (*16*), and hEGF (*20*). Invariant residues in all known EGF-like structures are in shaded circles. One single gap between positions 16 and 21 of rTGF-1 has been introduced for the best alignment with the segments 14 to 20 in mEGF and hEGF and for maintaining the proposed overall disulfide bond configuration of all known EGF-like structures. Abbreviations used for the amino acid residues are as follows: Ala, alanine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Cys, cysteine, Gln, glutamine; Glu, glutamic acid; Gly, glycine; His, histidine; Leu, leucine; Lys, lysine; Phe, phenylalanine, Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; and Val, valine.

Production and release of TGF-1 from neoplastic cells is apparently induced through transformation (*2–4*). The abnormal expression of an EGF-related polypeptide that can utilize the normal EGF receptor to mediate its biological activity could play a role in the growth and maintenance of neoplastic cells (*22, 23*). Available structural data should allow studies of the regulation and physiological function of TGF-1 in normal and transformed cells and may suggest strategies to produce antagonists for EGF actions.

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Transmammary Infection of Newborn by Larval Trematodes

Abstract. *Newborn cats and mice became infected with* Alaria marcianae *if they nursed from females that had been experimentally infected with the parasite. All lactating females showed mesocercarial stages in their mammary glands. This may be the first trematode found to undergo transmission through the mammary glands under experimental conditions. Similarities in the behavior of mesocercariae in humans and in the mouse suggest that an infected human female might infect her infant if she elected to nurse it.*

Infection with the fluke *Alaria marcianae* is recognized as a significant hazard to human health (*1–3*). During our investigations of the larval migration of this parasite in laboratory mice, rats, and cats we noted that the offspring born to infected females also became infected. Although well known in "roundworms" (*4*), maternal transmission is rarely reported in the Trematoda (*5–7*). In this report we outline the route of transmission to the offspring in two mammalian hosts, propose an animal model that would best describe human infection, and discuss the significance of that model in terms of human infection.

Studies of the larval migration of *A. marcianae* in definitive hosts, such as dogs and cats, have demonstrated a complex route in which the passively ingested mesocercarial stage burrows through the stomach wall and diaphragm, enters the lungs, and there develops to the metacercarial stage. After a brief residence in the lungs this stage is coughed up and swallowed; it matures in the small intestine as early as 3 weeks later (*8, 9*). The adult is passed spontaneously from the intestine within 6 months.

Migration of *A. marcianae* in paratenic hosts such as mice and rats does not involve the complex stomach-lung-intestine migration just described for definitive hosts, nor does it result in the development of the mesocercaria to a metacercaria. This lack of further development is a parasitological phenomenon known as paratenicity. In a paratenic host mesocercariae migrate from the stomach to the subcutaneous fat, where they remain as active migratory stages. All reports on human infection have indicated that man is a paratenic host for *A. marcianae* as well. Human infection has resulted from careless handling of contaminated meat during its preparation or through actual consumption of such meat (*10*). In the human the migrating mesocercariae have been found to invade nearly every organ, sometimes with fatal results (*2*).

In our first series of experiments we used laboratory cats as definitive hosts. To determine whether young could be infected prenatally, we orally inoculated two female adults with 200 mesocercariae each and then let them mate. At parturition a total of eight neonates were removed from the two mother cats, killed, and autopsied. None was infected with any stage of *A. marcianae*. Both mother cats were passing eggs in their feces by 3 weeks postpartum.

To determine whether young could be infected postnatally, we allowed four female cats to mate and give birth. Within 24 hours after parturition each mother cat was orally inoculated with 200 mesocercariae, returned to her litter, and allowed to nurse her young. After 21 days of nursing all 14 offspring were passing *A. marcianae* eggs in their feces. Autopsies of ten of these kittens showed metacercariae in the lungs and adults in the intestines of each of them. Apparently the worms were undergoing their typical migration in a definitive host.

Only one mother was allowed to keep her litter. The other three lactating cats were killed and autopsied, and mesocercariae were observed in the mammary glands of all of them.

The surviving mother cat and her infected young were used to determine whether an infected female could transmit *A. marcianae* to a second litter and whether transmammary infection could pass into the third generation. The female was mated again, and she gave birth to a second litter. At 21 days all four offspring had *A. marcianae* eggs in their feces. The first litter was reared to maturity and the females were mated. Only one of the two females gave birth. None of the four kittens in this third-generation litter developed an infection.

In definitive host infection, then, the mesocercariae undergo a stomach-lung-intestine migration; however, if the mammal is lactating the mesocercariae are diverted from their normal migration and toward the mammary glands. The transmammary passage of parasites to the young culminates in a stomach-lung-intestine migration, with eventual elimination of mature worms. Infected females are capable of infecting sequential litters, but passage beyond the second generation does not occur because of the maturation of the worms in the kittens and their subsequent expulsion. The mesocercariae remain in the mammary glands of lactating females, presumably because they no longer receive the cues necessary to lead them to the lung and intestine.

Laboratory mice were used as paratenic hosts in the next series of experiments. To determine whether prenatal infection could occur, we orally inoculated nine female mice with 200 mesocercariae each and let them mate. Their litters were examined immediately before birth (through cesarean section) or