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Effects of *in vivo*-administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on receptor binding of epidermal growth factor in the hepatic plasma membrane of rat, guinea pig, mouse, and hamster

(phosphorylation/hepatic membrane)

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ABSTRACT **The effect of *in vivo*-administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on epidermal growth factor (EGF) receptor activity of the rat hepatic plasma membrane was studied. TCDD causes a significant reduction in EGF binding at an early stage of toxicity (day 2) and at very low doses (1 μg/kg, single i.p., rat). This reduction appears to be due to a decline in the number of receptors. There is a good correlation between levels of decline in EGF binding and loss of body weight among TCDD-treated rats. The reduction in EGF binding occurs at a relatively low dose in the guinea pig (a very sensitive species) and at high doses in the hamster (a tolerant species). Among three mice strains, TCDD (115 μg/kg, single i.p.) caused 98% reduction in EGF binding in the sensitive strains (C57BL/6J and CBA/J) but only a 50% reduction in the tolerant strain (AKR/J). To relate the above biochemical changes to *in vivo* effects, TCDD was postnatally administered (through mother's milk) to mouse neonates. The most prominent toxic manifestations were early eye opening and incisor eruption, loss in body weight gain, and retardation of hair growth. All of these symptoms have been ascribed to EGF effects. TCDD was also found to stimulate phosphorylation of the EGF receptor in the rat hepatic plasma membrane. This phosphorylation effect was observed at day 1 and persisted until the end of the test (day 10). It has long been recognized that agents causing reduction in number of EGF receptors (e.g., phorbol esters) elicit *in vivo* cellular responses that are similar to those caused by exposure to excess doses of growth factors. Accordingly, a hypothesis has been proposed to ascribe some of the EGF-like effects of TCDD, such as fatty infiltration of the liver and hyperplastic proliferation of gastric epithelia and epidermal cells to its action on the EGF receptor.**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an extremely toxic compound. The oral LD_{50} values in the male guinea pig and the rat are cited as 0.6 μg/kg and 22 μg/kg, respectively (1). The action pattern of TCDD is unusual in that animals treated with a single dose steadily lose weight for several weeks, until death occurs, usually in 2–8 weeks. There are marked species differences in sensitivity (2): e.g., as compared to the above species, the Golden Syrian hamster is very tolerant to TCDD; its LD_{50} value is cited as >5000 μg/kg. Furthermore, the toxic manifestations also qualitatively vary from species to species. The basic biochemical cause for such toxic actions of TCDD is unknown (3).

We have recently found that many plasma membrane proteins and functions of the hepatocytes from TCDD-treated rats are quantitatively different from those of untreated rats (4–6).

As a follow-up of such a study, the current investigation was undertaken with the following objectives: (*i*) find several biochemical parameters on the plasma membrane that are severely affected by TCDD, (*ii*) study whether any of the changes occur at low enough doses and at very early stages in susceptible species, and only at high doses in tolerant species, and (*iii*) make an attempt to relate such effects to some of the toxic manifestations *in vivo*.

MATERIALS AND METHODS

Animals and Chemicals. Male Sprague–Dawley rats (150–200 g) and Golden Syrian hamsters (80–90 g) were obtained from Spartan Laboratory Animals (Haslett, MI). Male guinea pigs (200–250 g) were obtained from Michigan Department of Health, Lansing, MI and female BALB/c mice were purchased from Harlan Laboratories (Haslett, MI). Inbred mouse strains C57BL/6J, CBA/J, and AKR/J were obtained from The Jackson Laboratory. Food and water were provided ad lib. All chemicals used *in vivo* were administered to the animals intraperitoneally (i.p.) as solutions in either corn oil/acetone (9:1; TCDD), 0.85% NaCl (phenobarbital), or corn oil (all others). Control animals received an equivalent volume of the vehicle only.

TCDD was a gift from Dow and was >99% pure (GLC examined); 3,4,3′,4′-tetrachloroazoxybenzene was generously given to us by M. T. Stephen Hsia (University of Wisconsin, Madison, WI) and was >99% pure; sodium phenobarbital was purchased from Mallinckrodt; 3-methylcholanthrene, and epidermal growth factor (EGF) were from Sigma; Aroclor-1242, a polychlorinated biphenyl mixture containing 42% chlorine, was a gift from Monsanto; 3,4,3′,4′-tetrachlorobiphenyl (>99% pure) was obtained from Analabs, North Haven, CT; and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane >99% pure was a gift from Montrose Chemical (Torrance, CA). Firemaster BP-6 was given to us by Matthew J. Zabik (Michigan State University).

Radiochemicals and Biochemicals. ^{125}I-labeled insulin (specific activity, 80–103 μCi/μg; 1 Ci = 37 GBq), ^{125}I-labeled EGF (specific activity, 161–174 μCi/μg), and ^{3}H-labeled Con A (specific activity, 25–50 Ci/mmol) were purchased from New England Nuclear. [^{32}P]ATP (Tris salt, specific activity, 3000 Ci/mmol) was obtained from Amersham. α-Methyl D-mannopyranoside and insulin (porcine) were purchased from Sigma. Receptor grade EGF was obtained from Collaborative Research (Waltham, MA). All other biochemicals and chemicals used were of the highest purity available.

Biochemical Assays. Liver plasma membrane from normal or TCDD-treated animals was prepared as described (7, 8), and preparations were periodically examined by electron microscopy. Binding of either ^{125}I-labeled EGF or ^{125}I-labeled insulin to liver plasma membrane was assayed according to the method of O'Keefe *et al.* (9) and ^{3}H-labeled Con A binding was determined essentially as described by Chandra-

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Abbreviations: TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EGF, epidermal growth factor.

mouli *et al.* (10) with minor modifications as described (4, 5). Phosphorylation assay was done essentially as described by Rubin *et al.* (11). Other details are given in Fig. 5 legend (12, 13).

Growth Characteristics of Neonatal BALB/c Mice Exposed to TCDD Through Mother's Milk. Females of BALB/c mice (15–18 days pregnant) were housed in plastic cages individually, and food and water were provided ad lib. The time of delivery was closely followed and within 3 hr, the dams were treated i.p. with a single dose of either TCDD in corn oil/acetone (9:1) at 10 μg/kg or with the vehicle alone (0.5 ml per 100 g of body weight). Prior to treatment, two littermates from each litter were exchanged and marked to identify them from the original littermates. Body weights of the neonates were recorded daily and were checked for incisor eruption and eyelid opening twice daily (between 8 and 9 a.m. and between 5 and 6 p.m.). The day of delivery was considered as day 0. Hair diameter and length were measured on day 14 from samples taken from the mid-right dorsal region. They were mounted on glass slides with glycerol and measured under a microscope. EGF in 0.85% NaCl was injected subcutaneously to newborn mice daily at 2 μg/g of body weight. Body weights and other developmental parameters were measured as described above. All animals were sacrificed at 22 days of age and thymus weights were recorded.

RESULTS

Time Course of Biochemical Changes. First, we have examined the effects of *in vivo*-administered TCDD on three receptors of the rat hepatic plasma membrane at various doses to assess which of these is most sensitive (Fig. 1). It is clear from the results that the effect of TCDD on EGF binding was most pronounced, followed by that of Con A and insulin. It is significant that the effect on EGF binding is observable at a dose as low as 0.1 μg/kg. It was also noted that insulin binding was significantly lower at the highest dose (115 μg/kg) and higher at low doses as compared to the corresponding control preparation at 10 days after treatment.

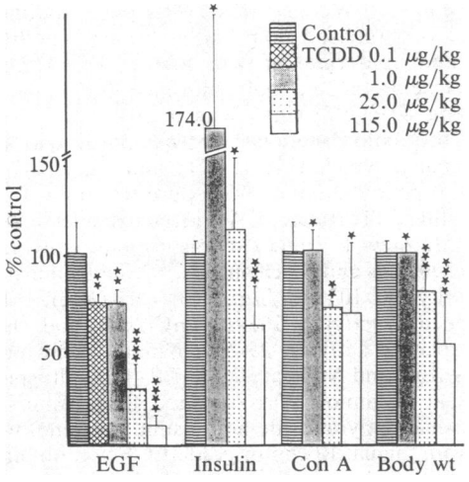

FIG. 1. Dose–response relationship of specific binding of ^{125}I-labeled EGF, ^{125}I-labeled insulin, or ^{3}H-labeled Con A to liver plasma membrane from control and TCDD-treated (single i.p.) rats. Ligand binding was studied in the plasma membrane prepared 10 days after treatment. Data are expressed as percent of control values. Specific binding in control for EGF, insulin, and Con A are 24.5 ± 3.6 pg, 8.4 ± 0.5 pg per 50 μg of protein, and 15.2 ± 2.5 ng per 25 μg of protein, respectively. Body weight gain or loss at these doses are also represented and are expressed as percent of final body weight of the control rats. Mean ± SEM of 4–8 rats. Asterisks indicate P values of Student's t test. ****, $P < 0.005$; ***, $P < 0.05$; **, $P < 0.01$; and *, $P > 0.1$.

In view of the sensitivity of EGF binding to TCDD treatment, we examined the time course of TCDD effect after a single i.p. dose (25 μg/kg; Fig. 2). During the 40-day observation period, TCDD-treated rats gained consistently less body weight than did control rats. During the same period, the level of EGF binding was continuously suppressed. The decline was noticeable on the second day, reached a maximum on day 20, and by day 40 a trend of apparent recovery was observed. At this dose and treatment, this apparent recovery is believed to result from mortality of the susceptible population. The average mortality was 0 at day 20, but reached 20%–30% by day 40; therefore, the data at this time point represent the value from surviving animals.

To study whether the reduction in EGF binding *in vitro* is generally related to *in vivo* TCDD effects, the body weight changes of individual TCDD-treated rats at two different doses (25 and 115 μg/kg) were compared with the levels of ^{125}I-labeled EGF binding to the hepatic hepatic plasma membrane isolated from each rat on day 20 after treatment. Body weight levels (expressed as % initial body weight) of individual rats were plotted against specific EGF binding. The curve was generated by computer, using the points of all preparations (i.e., 25 μg/kg, 115 μg/kg). Data were analyzed using least-squares regression statistics. The coefficient of correlation (r) was 0.9051 among treated rats (data not shown). No correlation between body weight levels and ^{125}I-labeled EGF binding was seen in control rats.

The nature of the changes in the EGF receptor was studied by Scatchard analysis of ligand–receptor binding (Fig. 3). The results indicate that EGF binding generally shows a biphasic relationship, as shown by Ivanovic and Weinstein (14, 15), and that the number of high as well as low affinity receptors in the TCDD-treated rats was reduced without any apparent changes in the receptor affinity.

To study whether such biochemical changes are also evoked by other toxic chemicals or only by TCDD, we assessed the effect of various xenobiotics *in vivo* on EGF binding *in vitro* (Table 1). Among the nine chemicals tested, only TCDD and Aroclor-1242 caused a significant decrease in EGF binding. In both cases, the effects appear to be dose related. It must be noted that rather high doses were used for other chemicals, resulting in the manifestation of toxicities in most cases. Yet, even under these conditions no reduction in EGF binding was observed.

Interspecific Comparison of Biochemical Changes. To further understand the relationship between these biochemical changes and susceptibility of these species to TCDD (see ref. 2 for discussion), the dose response of EGF binding was ex-

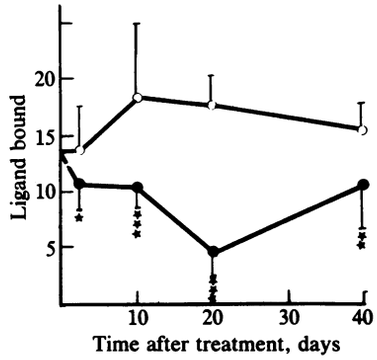

FIG. 2. Time course of changes in specific binding of EGF to liver plasma membranes of TCDD-treated rats (●) relative to untreated control rats (○). Rats were given a single i.p. dose of TCDD at 25 μg/kg and plasma membranes were prepared 2, 10, 20, and 40 days after treatment. Binding was expressed as pg of ligand bound per 50 μg of protein for ^{125}I-labeled EGF. Each point is the mean ± SEM of 4–8 animals. Asterisk designations are as in Fig. 1.

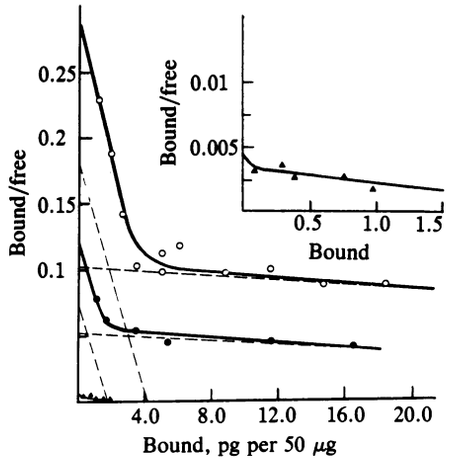

FIG. 3. Scatchard plots of ^{125}I-labeled EGF binding to liver plasma membrane from control and TCDD-treated rats. TCDD was administered as a single i.p. dose of either 25 or 115 μg/kg. Plasma membrane was isolated 20 days after treatment. ^{125}I-labeled EGF specific binding was carried out as described. ○, Control binding. With TCDD-treated rats, the experiments were done using animals that showed modest levels of body weight loss (●) and those that showed a severe degree of weight loss (▲). Each point is the mean of at least two determinations of different membrane preparations. The data indicate two types of EGF binding sites, the high affinity sites with a K_{d_1} and N_1 of 2.7×10^{-11} mol/liter and 1.4 fmol/mg for control and 2.4×10^{-11} mol/liter and 0.7 fmol/mg for treated; and the low affinity sites with K_{d_2} and N_2 of 6.95×10^{-10} mol/liter and 0.27 pmol/mg for control and 9.3×10^{-10} mol/liter and 0.19 pmol/mg for treated. (*Inset*) Binding kinetics of ^{125}I-labeled EGF to liver plasma membrane from TCDD-treated rats that showed severe weight loss and very low EGF binding (▲).

amined. Ten days after single i.p. treatments, plasma membranes were isolated from each animal, and the levels of EGF binding were quantified. The results (Fig. 4) indicate that the guinea pig system was most sensitive, followed by that of the rat and the hamster. If one adopts the I_{50} (i.e., the dose of TCDD to suppress EGF binding to 50% of the control level) as a critical dose, the rat and the hamster may be considered to be ≈14 and ≈32 times less sensitive, respectively, than the guinea pig in this regard. At I_{75}, the species difference was much greater.

Next, inbred strains of mice (C57BL/6J, CBA/J, and AKR/J) known to show different degrees of tolerance to TCDD were examined (16). The results (Table 2) clearly indicate that reduction in EGF binding was more severe in the two sensitive strains (C57BL/6J and CBA/J) than in the resistant strain (AKR/J). Although the CBA/J strain is known to possess a high affinity cytosolic TCDD receptor, it is tolerant to TCDD in terms of cleft palate formation [i.e., a teratogenic effect (16)]. It is interesting that this strain is sensitive to TCDD, as judged by EGF receptor assay as well as body weight loss and thymic involution.

Comparison of TCDD Effects to Those Caused by EGF. To study whether some of the TCDD-caused toxic effects are similar to those produced by excess EGF (see *Discussion*), mouse neonates were treated postnatally with TCDD and various developmental parameters were examined.

The most recognized *in vivo* effects of EGF are early eye opening and tooth eruption in mouse neonates (17, 18). The action of TCDD in this regard was clear (Table 3) in that both events occur at an earlier age in the treated ones than in the control. Other parameters examined also show that the lesions caused by TCDD are remarkably similar to those occurring in EGF-treated animals (see ref. 19).

When the hepatic plasma membrane was isolated from the treated and control rats, incubated with [γ-^{32}P]ATP, subject-

Table 1. Effect of *in vivo* treatment with various chemicals on ^{125}I-labeled EGF binding to liver plasma membrane of the rat

Compound	mg or μg/kg × no. of doses	^{125}I-labelled EGF-specific binding, % control ± SEM (no. of animals)
Control (corn oil/acetone; 9:1)	× 1	100.0 ± 10.9 (5)*
Control (corn oil)	× 7	100.0 ± 5.9 (3)*
TCDD	25 μg × 1	16.5 ± 6.0 (4)†
TCAOB	25 mg × 2	101.0 ± 48.0 (3)
Aroclor-1242	350 mg × 1	64.6 ± 11.9 (3)†
	1300 mg × 1	57.9 ± 6.3 (3)†
	1900 mg × 1	50.5 (1)†‡
3,4,3',4'-Tetrachlorobiphenyl	50 mg × 7	105.1 ± 9.8§ (2)
3-Methylcholanthrene	20 mg × 7	103.9 ± 5.5§ (2)
	100 mg × 7	116.8 (1)‡
Firemaster BP-6	50 mg × 7	116.8 ± 4.7§ (2)
Phenobarbital	120 mg × 7	104.3 ± 5.0 (3)
DDT	100 mg × 7	120.7 (1)

Chemicals were administered i.p. with corn oil or with corn oil/acetone (9:1). Phenobarbital was administered as an aqueous solution in 0.85% NaCl. 3,4,3',4'-Tetrachloroazoxybenzene (TCAOB) was administered on days 1 and 5. When the chemical was administered more than once, plasma membrane was prepared 24 hr after the last dose; in the case of TCDD and TCAOB, membrane was prepared 10 days after the first dose.

*Values are 28.5 ± 3.1 and 25.6 ± 1.5 pg per 50 μg of protein for corn oil/acetone (9:1) and corn oil controls, respectively.

†$P < 0.05$.

‡High mortality among the treated animals.

§Ranges of variation.

ed to gel electrophoresis, and autoradiographed, it became evident that the band intensity, representing ^{32}P incorporation into the EGF receptor, increased in plasma membrane from animals sacrificed at days 1, 2, and 10 (Fig. 5). The results agree with the observation made by others (19, 20) that agents affecting EGF receptors cause an increase in phosphorylation of the EGF receptor. Also, it was noted that there are other bands of which intensities were much higher

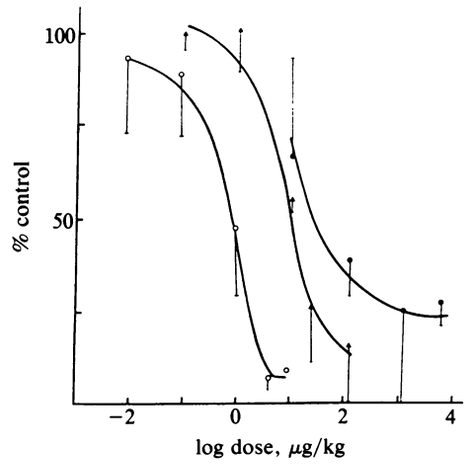

FIG. 4. Dose–response relationship of ^{125}I-labeled EGF binding to liver plasma membrane from TCDD-treated guinea pig (○), rat (▲), or hamster (●). TCDD in corn oil/acetone (9:1) was given as a single i.p. injection at indicated doses. Liver plasma membrane was prepared 10 days after treatment and assayed for ^{125}I-labeled EGF binding. Values are expressed as percent control and mean ± SEM of 3–8 animals. The absolute values for the controls are as follows: guinea pig, 5.8 ± 0.4; rat, 28.5 ± 3.1; hamster, 3.6 ± 1.1 pg per 50 μg of protein.

Table 2. Changes in ^{125}I-labeled EGF binding to hepatic plasma membrane, body weight, and thymus weight in mouse strains 10 days after TCDD treatment

Strain	EGF bound, pg per 50 μg of protein		% initial body weight		Thymus, mg	
	Control (*n*)	Treated† (*n*)	Control (*n*)	Treated† (*n*)	Control (*n*)	Treated† (*n*)
C57BL/6J	102.7 ± 5.8 (7)	7.1 ± 3.3 (3) *****	95.7 ± 2.9 (3)	89.8 ± 4.6 (3) ***	46.0 ± 1.7 (3)	10.7 ± 1.2 (3) *****
CBA/J	122.0 ± 14.0 (7)	2.7 ± 0.1 (3) *****	103.1 ± 2.8 (3)	88.8 ± 3.9 (3) ****	42.7 ± 2.5 (3)	11.0 ± 2.7 (3) *****
AKR/J	102.8 ± 11.9 (4)	55.5 ± 15.2 (3) *****	98.3 ± 1.1 (3)	96.8 ± 5.7 (3)	82.0 ± 7.6 (3)	23.0 ± 0.9 (3) *****

Mice were treated with a single i.p. dose of TCDD in corn oil/acetone (9:1) at 115 μg/kg. Controls received an appropriate volume of the vehicle alone (0.5 ml per 100 g of body weight).
†*P* value designations are as shown in Fig. 1. *****, $P < 0.0005$.

in TCDD-treated preparations than in control (bands at 27, 42, 48, 59, 75, 95, 115, and 270 kDa).

DISCUSSION

In the current work we have established that TCDD, when administered *in vivo*, causes changes in receptor activities for EGF, insulin, and Con A on the rat hepatic plasma membrane, and that EGF receptor binding is the most sensitive parameter in this regard. Obviously, the most pressing question is how TCDD causes various toxic symptoms. Thus, the meaning of the above effect of TCDD on EGF receptor must be discussed.

In the past, several compounds have been identified to have a property to reduce EGF receptor binding (19). Examples are as follows: phorbol acid esters such as 12-tetradecanoylphorbol 13-acetate) (15, 20), Rous sarcoma and other viruses causing neoplastic transformation (21, 22), hormones such as vasopressin (23) and thyroxine (24), and a solvent dimethylsulfoxide (25). At least the first three groups of agents are known to cause or promote neoplastic development in various tissues. The most intriguing aspect of this phenomenon is that the tissues and the cells exhibiting loss of EGF receptor activities by these treatments elicit responses characteristic of excess EGF (15, 19, 20). For instance, cells that have been treated with 12-tetradecanoylphorbol 13-acetate, showing decreased number of EGF receptors, exhibit an increase in ornithine decarboxylase, stimulation of plasminogen activator, enhancement of sugar transport, prostaglandin synthesis, and stimulation of mitogenic activities (26). All these cellular changes are observed in cells and tissues receiving excess EGF (15, 20). Also, Rose *et al.* (26) have found that EGF promotes skin tumors in mice treated with methylcholanthrene, as in the case of 12-tetradecanoylphorbol 13-acetate. The tumor-promoting potential of TCDD in mouse skin has also been shown by Poland *et al.* (27) in hairless mice.

The cause of this EGF-like effect by agents that decrease EGF receptor activity as proposed by DeLarco and Tadaro (28) and Weinstein and his associates (15, 20, 29) appears to be due to the production of endogenous growth factor(s) by the affected cells and tissues. In the case of transforming viruses, such growth factors (termed TGF for "transformation growth factors") have actually been found, isolated, and characterized (30, 31). An alternative explanation for these events may be that these agents or treatment ultimately cause phosphorylation of the EGF receptor, as shown in the case of 12-tetradecanoylphorbol 13-acetate (19), dimethylsulfoxide (25), Rous sarcoma virus (22), partial hepatectomy (11, 32), etc., and that this phosphorylation action alone could activate a chain of events that is normally triggered by the binding of the ligand, EGF, to its receptor. In turn, these cells with phosphorylated receptors may no longer require external ligands to process the messages of transaction.

In the current study, we have found that TCDD does indeed increase phosphorylation of the EGF receptor in the rat liver plasma membrane, and that it has the same *in vivo* effects as exogenous EGF with regard to the early eyelid opening and tooth eruption in neonatal mice. The eyelid effect has been cited as the most specific biological index of EGF activity *in vivo* (19).

With this evidence, we propose the hypothesis that some of the toxic manifestations of TCDD, particularly the hyperplastic response among epithelial tissues, is a result of the action of TCDD on the EGF receptor.

Table 3. Effect of TCDD on eyelid opening, incisor eruption, and hair growth on neonatal BALB/c mice

	Treatment		
	Control (*n*)	TCDD (*n*)	EGF (*n*)
Development			
Eyelid opening, days	13.7 ± 0.5 (14)	11.4 ± 0.5 (13) *****	10.7 ± 0.5 (10) *****
Tooth eruption, days			
Lower	9.9 ± 0.5 (14)	9.0 ± 0.4 (13) *****	7.5 ± 0.5 (10) *****
Upper	11.0 ± 0.0 (14)	10.2 ± 0.4 (13) *****	8.2 ± 0.1 (10) *****
Hair			
Length, mm	7.3 ± 0.9 (24)	4.9 ± 0.7 (36) *****	5.1 ± 0.7 (24) *****
Diameter, μm	17.5 ± 0.3 (30)	12.3 ± 0.2 (30) *****	13.2 ± 0.3 (25) *****
Weight			
Body weight, g	10.0 ± 0.7 (10)	7.9 ± 0.8 (10) *****	8.2 ± 0.5 (5) **
Thymus, mg	74.0 ± 12.6 (10)	41.7 ± 6.8 (10) *****	54.5 ± 2.5 (5) **

Mice were treated as described in *Materials and Methods*. *P* values are as in Table 2.

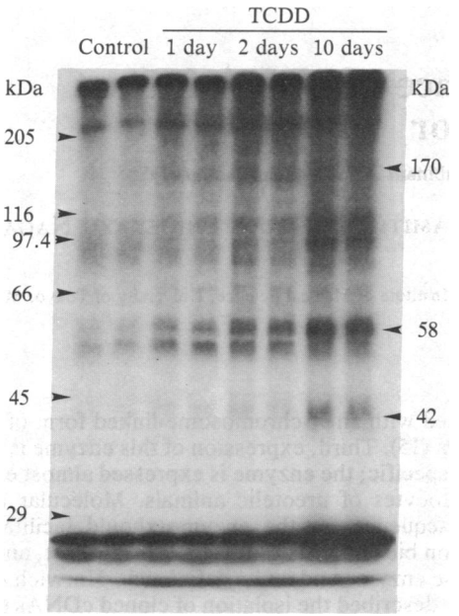

FIG. 5. Autoradiograph of phosphorylated hepatic plasma membrane proteins from control and TCDD-treated (25 μg/kg) rats. Plasma membrane was prepared from control and TCDD-treated rats 1, 2, or 10 days after treatment. Plasma membrane protein (100 μg each) from control or TCDD-treated rat livers was added to each tube, containing 50 mM Pipes/30 mM $MgCl_2$/10 mM 2-mercaptoethanol/0.32 mM EGTA in a total vol of 120 μl. In some cases, 250 ng of EGF in 10 μl of phosphate buffer containing 0.1% bovine serum albumin was added to control tubes. After the tubes were incubated for 15 min at 0°C, phosphorylation reaction was initiated by the addition of 15 μCi of [^{32}P]ATP at a final concentration of 10 μM. The reaction was stopped after 1 min at 0°C by the addition of 80 μl of a solution containing 9% $NaDodSO_4$/20% (vol/vol) glycerol/5% mercaptoethanol/0.005% bromophenol blue. The tubes were held for 5 min in a water bath at 100°C, and a 150-μl aliquot of the final reaction volume was used for electrophoresis on a 7.5% polyacrylamide slab gel, with a 3% stacking gel, using the Laemmli method (12) at 30 mA constant current per slab. After electrophoresis, the gels were stained with Coomassie blue or Biorad silver stain (13), dried, and exposed to Fuji $NIFR_x$-100 medical x-ray film for 2–7 days.

If one examines the toxic manifestations caused by TCDD from such a viewpoint, one cannot help but notice many similarities between them and those caused by EGF administration *in vivo* and *in vitro*. They are, for example, fatty invasion of the liver (17, 33), inhibition of terminal differentiation of keratinocytes (34, 35), skin cancer promotion (26, 27), proliferation of conjunctiva cells (18, 36), inhibition of gastric secretion (30, 36, 37), ornithine decarboxylase changes (38, 39), and serum hypertriglyceridemia (17, 40), in addition to hair growth, early eye opening, etc., as shown in the current work. While in several cases the experimental conditions for TCDD studies differ from those of EGF and caution is needed to draw a direct analogy, these similarities are remarkable and therefore the relationship between TCDD and EGF effects warrants further attention.

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