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Histologic Changes Produced by 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Skin of Mice Carrying Mutations That Affect the Integument

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) produces epidermal hyperplasia and hyperkeratosis, squamous metaplasia of the sebaceous gland, and keratinized cyst formation in 8 strains of mice with the recessive mutation, hairless (hr/hr). The extent of these histologic changes is dependent on the genetic background. No cutaneous lesions are produced in haired (hr/+) mice. In examination of mice with 7 other mutations affecting the integument, TCDD produced similar histologic skin changes in cryptothrix, nude, plucked, and atrichosis; a marginal squamous metaplasia of sebaceous glands in Repeated epilation, and had no effect in fur deficient and Naked mutants. These genetically determined epidermal responses are discussed in light of the mechanism of action of TCDD.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) serves as the prototype for a large group of halogenated aromatic hydrocarbons, a class of toxic chemicals in the environment which all evoke a similar and characteristic pattern of biochemical and toxic responses [1–3].

All of the biologic responses produced by TCDD and congeners appear to result from the stereospecific, reversible binding of these compounds to a receptor protein, and the coordinate gene expression initiated by this ligand-receptor complex [3]. In mice, there is a genetic polymorphism in the locus (Ah locus) which determines this receptor† [5]. Inbred strains with the Ah^b allele have a high affinity receptor and are sensitive to TCDD, while inbred strains carrying the Ah^d allele have a lower affinity receptor and are less sensitive to TCDD, i.e., a higher concentration of TCDD is required to produce equivalent biochemical and toxic effects in these mice.

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† Ah *locus*: Mice carrying the Ah^b allele, have a high affinity receptor (K_D for TCDD = 0.3–1.0 × 10^{-9} M) and are *sensitive* to TCDD (and congeners), as shown by the TCDD concentration which induces hepatic AHH activity (ED_{50} = 1 × 10^{-9} mol/kg) and which produces other biologic responses. Mice homozygous for the Ah^d allele are *less sensitive* to TCDD, e.g., the ED_{50} for induction of AHH activity is 1 × 10^{-8} mol/kg. The presence of the TCDD receptor has been demonstrated in these less sensitive mice [4], but the K_D has not been determined, presumably because the K_D exceeds the limited aqueous solubility of [^{3}H]TCDD. Thus, the "defect" in mice homozygous for the Ah^d allele is presumed to be a receptor with a lower affinity, and not a reduced concentration of receptor.

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Abbreviations:
AHH: aryl hydrocarbon hydroxylase
βNF: β-naphthoflavone
ED_{50}: the dose that produces one-half the maximal response
K_D: equilibrium dissociation constant
n: binding sites
TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin

The most well studied response to TCDD is the induction in rodent liver of cytochrome P-450-mediated microsomal monooxygenase activities, e.g., aryl hydrocarbon hydroxylase (AHH) activity, and other coordinately expressed enzymes primarily involved with drug metabolism [6]. TCDD induces AHH activity and/or other drug metabolizing enzymes in virtually all tissues that possess the receptor. In contrast, TCDD and congeners produce a second pleiotropic response, which is far more limited in distribution and shows specificity with respect to tissue and animal species. Included in this second response is the hyperplasia and/or altered differentiation of some epithelial tissues, e.g., skin, gastric and intestinal mucosa, and urothelium [2,3]. While these two pleiotropic responses appear to be expressed independently, both are mediated through the same receptor, as indicated by two lines of evidence: (1) For a large series of halogenated aromatic hydrocarbon congeners, the structure-activity relationship for receptor binding corresponds to that for induction of AHH activity and epithelial proliferation and/or differentiation [7–9]. (2) In mice, both responses produced by TCDD segregate with the Ah locus [9,10].

The study of the proliferation and/or differentiation of epithelial tissues produced by TCDD is made more difficult by the species and tissue specificity of this response and especially by the restricted expression of this response in most tissues of normal laboratory rodents. One experimental model for this phenomenon of restricted expression is provided by HRS/J mice, an inbred strain segregating for the hairless (hr) locus [11]. HRS/J hr/hr (hairless) and hr/+ (haired) mice are congenic, differing only at the hr locus (or closely linked loci) on chromosome 14; however, TCDD produces no morphologic changes in the skin of hr/+ mice [or mice that are wild type (+/+) at the hr locus], while in hr/hr mice TCDD produces a hyperplasia and hyperkeratosis of the epidermis and squamous metaplasia of the sebaceous glands. HRS/J hr/hr and hr/+ mice both have the Ah^b allele at the Ah locus, with the same receptor concentration and binding affinity for TCDD, and both respond to the application of TCDD to the skin with a similar dose-related induction of epidermal AHH activity [9]. Thus the response produced by halogenated aromatic hydrocarbons in mouse skin is controlled by the interaction of two genetic loci: (a) the Ah locus, which determines the receptor and hence the *sensitivity* to TCDD, and (b) the hr locus, which determines the *extent* of the response, either limiting it to the induction of drug metabolizing enzymes, or permitting the more extensive response involving epidermal proliferation and keratinization.

In this report, we further examine the effect of genetic constitution on TCDD-induced skin changes in (a) mice carrying the hr mutation on several different genetic backgrounds, and (b) mice carrying other mutations that affect the integument.

MATERIALS AND METHODS

Animals

Hairless locus: Mice carrying the hr locus were obtained from the following sources: HRS/J, The Jackson Laboratory; C57BL/6 (hr/hr) N_2F_{18} and WLHR/Le, Priscilla Lane, The Jackson Laboratory; C3H/HeN (hr/hr), Dr. Carl Hanson, National Institutes of Health; HRA/Skh, Skh/hr-1, and HR/DeHflcr, Dr. Donald Forbes, Skin and Cancer

Hospital, Temple University Medical School. DBA/2J (hr/hr) $N_{12}F_{12}$ were derived in our colony by inbreeding the hairless mutant provided by Dr. Allan Gates, University of Rochester Medical School into DBA/2J mice.

C3H/HeN, HR/DeHflcr, WLHR/Le, and HRS/J mice were maintained by brother-sister mating of hr/+ haired female mice and hr/hr hairless male mice, because in these strains the hairless female while fertile, nurses poorly. In contrast, HRS/Skh (inbred) and Skh/hr-1 (a noninbred strain) were maintained by matings of homozygous hr/hr females and males. C57BL/6J and DBA/J hairless mice were maintained by outcrossing of hr/hr males to the parent strain, and then intercrossing the F_1 (hr/+) progeny. The C57BL/6J (hr/hr) mice used in this study were $N_2F_{18}\cdot N_8F_8$ and the DBA/J (hr/hr) $N_{12}F_{12}$.

Other mutant animals: Naked (N/+, B6C3 background N_{15}), fur deficient (fd/fd, F_{49} on its background) and atrichosis (at/at F_{24}, on its background) were obtained from Priscilla Lane. Repeated epilation (Er/+—C57BL/6BY $N_{28}F_3$) and plucked (pk/pk—C57BL/6J $N_{12}F_{23}$) were obtained from Dr. Eva Eicher, the Jackson Laboratory. Cryptothrix (crh/crh, on an outbred Skh background) were provided by Dr. Donald Forbes. Nude (nu/nu—on a BALB/c background) were obtained from Dr. Robert DeMars, University of Wisconsin. Naked rats (n/n) were provided by Dr. Theopolis Peace, Division of Laboratory Animal Medicine, Armed Forces Institute of Pathology, Washington, D.C. [12]. For a full description of these mouse mutations the reader is referred to [11]. The rabbits were New Zealand White obtained from Kluhertanz's, Edgerton, Wisconsin.

Administration of TCDD and Skin Histology

TCDD dissolved in acetone (0.3 μg/100 μl for mice carrying the Ah^b allele and 1.0 μg/100 μl for mice homozygous for the Ah^d allele) or acetone alone was applied to the dorsal skin of mice once a week for 4 weeks. Mice with significant hair coats (hr/+ or some of the mutants) were shaved prior to treatment. In most cases, the treated and control animals were matched for sex and age (4–8 weeks old at the time of treatment), but the scarcity of some mutants precluded precise matching. One week after the last dose of TCDD, the animals were killed; a full-thickness piece of skin from the dorsal midline was removed, flattened, and pinned with the epidermal side up to a Styrofoam block which was floated with the skin submerged in 10% buffered formalin overnight. The samples were processed in an autotechnicon, embedded in paraffin, cut in 5-μm sections and stained with hematoxylin and eosin. The sections were scored by 2 independent observers for 4 histologic changes. Sebaceous gland metaplasia was scored "0" if numerous lipid-filled sebaceous cells were evident, or "+" if the lipid-filled cells were absent and in their place were cells resembling keratinocytes. Scores from "0" to "3+" were given for the comparative increases in epidermal thickness (hyperplasia), in stratum corneum thickness and amount of keratinaceous material on the skin surface (hyperkeratosis), and in the number of dermal cysts containing keratinaceous material (keratinaceous cyst formation). The scores (shown in Tables I and II) represent the overall impression of the average histologic change from viewing several sections, comparing the skin of TCDD-treated mice to their respective acetone controls. In Figs 1, 2, and 3 we have tried to select representative tissue sections; however, a single section may not adequately convey the average change for all 4 histologic phenotypes.

Esterase 10

Blood was drawn from the orbital sinus and the red cells assayed by gel electrophoresis for esterase 10 isozymes [13].

The Ah Locus

The phenotype of the Ah locus was determined by 2 methods: (a) induction of hepatic AHH activity by β-naphthoflavone (βNF), a weak agonist for the receptor, and (b) determination of hepatic cytosol receptor binding affinity for TCDD.

Mice were administered corn oil (8 ml/kg) or βNF (100 mg/kg) dissolved in corn oil, i.p. for 2 days. Twenty-four hours after the second injection the animals were killed and their livers removed, weighed, and homogenized. Hepatic AHH activity was determined in the 9000 *g* supernatant fraction as previously described [14]. Enzyme activity is expressed as pmol 3-hydroxybenzo[a]pyrene formed/min/mg wet weight of liver. The values in Table I and II are the mean of 2 or 3 animals (occasionally only a single animal). In mice with the Ah^b allele, βNF induces hepatic enzyme activity 3-fold or more above the activity in control animals, while in mice homozygous for Ah^d allele, βNF produces less than a 50% increase.

The receptor binding was determined in the 100,000 *g* supernatant fraction of mouse liver by incubation with [^{3}H]TCDD ($1–15 \times 10^4$ dpm/ml = $0.16–2.3 \times 10^{-9}$ M) in the absence or presence of a 200-fold molar excess of 2,3,7,8-tetrachlorodibenzofuran for 30 min at 20°C [15]. Unbound ligand was adsorbed by the addition of charcoal/dextran (final concentration, 1.0/0.1%), and the total and bound radioactivity determined by liquid scintillation spectroscopy. Specific binding was calculated as the total binding minus nonspecific (nondisplaceable) binding and the data plotted according to Scatchard [16]. The slope of the line ($-1/K_D$) was calculated by least mean square regression line from all 10 ligand concentrations (or in a few cases after discarding aberrant binding values at the lowest and highest ligand concentration) and had a correlation coefficient of 0.93 or better.

RESULTS

We examined 8 strains of mice carrying the hairless allele for their phenotypes at the Ah and Esterase 10 loci and their epidermal histologic response to TCDD (Table I). The pheno-

TABLE I. *Mouse strains carrying the hairless mutation: phenotypes of the Ah and Es 10 loci, and the epidermal histologic response to TCDD*

Strain	hr locus	Esterase 10 locus	Ah locus	Liver receptor		Hepatic AHH activity (pmol/mg/min)		Sebaceous gland metaplasia	Histologic changes		
				K_D (nM)	n (fmol/mg protein)	Control	βNF		Keratinization	Hyperplasia	Keratinized cyst formation
HRS/J	hr/hr	b/b	Ah^b	0.52[a]	77	2.4	44	+	+++	++	+++
	hr/+	b/a		0.56	62	2.7	42	0	0	0	0
C57BL/6J	hr/hr	a/a	Ah^b	0.38	153	8.1	118	+	±	+	+
	hr/+	a/a						0	0	0	0
C3H/HeN	hr/hr	b/b	Ah^b	1.31	155	1.9	20	+	++	+	++
	hr/+	b/b		1.22	145	4.2	33	0	0	0	0
DBA/2J	hr/hr	b/b	Ah^d	—[b]	—	4.7	5.9	+	±	+	++
	hr/+	b/b						0	0	0	0
HRA/Skh	hr/hr	b/b	Ah^b	0.65	61	5.2	27	+	+	++	++
Skh/hr-1	hr/hr	b/b	Ah^b	0.47	67	3.4	18	+	+	+	++
HR/DeHflcr	hr/hr	a/a	Ah^b	1.09	100	7.4	42	+	++	++	+++
	hr/+	a/b									
WLHR/Le	hr/hr	c/c	Ah^b	1.13	110	11.5	41	+	+	+++	+++
	hr/+	c/c									

Inbreeding and animal husbandry of these strains, and methods used for phenotyping the Esterase 10 and Ah loci are found in *Methods and Materials*. For histologic studies, mice carrying the Ah^b allele were administered 0.3 μg TCDD per mouse, once a week for 4 weeks, and mice homozygous for the Ah^d allele (DBA/2J) were administered 1.0 μg TCDD per mouse, once a week for 4 weeks, applied to the skin. The skin was processed and graded for the degree of histologic changes (0 to 3+) as described in *Methods and Materials*.

[a] Data previously reported in [9]. The equilibrium dissociation constants initially reported as 0.30 and 0.33 nM were incorrect. The concentration of binding sites (n) is the same as originally reported.

[b] Binding not measurable.

type of the Ah locus was determined by measurement of the hepatic receptor and by induction of hepatic AHH activity by βNF. DBA/2J was the only strain with the Ah^d allele, (low affinity receptor) i.e., unmeasurable specific binding and no enzyme induction by βNF. Among the 7 strains with the Ah^b phenotype, there was a 2- to 3-fold variation in the receptor affinity for TCDD and the receptor concentration. No correlation is found among mice with the Ah^b allele between receptor concentration and the extent of AHH induction (induced/control activity) (Tables I and II.)

The hr locus is 1 map unit from the Esterase 10 locus on chromosome 14. In HRS/J mice, after 70+ generations of inbreeding, the hr allele is linked to the Esterase 10^b allele, and the wild type allele (+) is linked to Esterase 10^a [12]. Similarly, HR/DeHfIcr hr/+ mice remain heterozygous at Esterase 10. In WLHR/Le mice, Esterase 10 has the uncommon c allele previously reported only in BUB/BnJ mice and Mus m. molossinus. The Ah locus, presently unmapped, is not linked to the hr locus in a three-point cross between piebald, Esterase 10, and the Ah locus (unpublished data).

The application of TCDD (0.3 μg/mouse for mice carrying the Ah^b allele, and 1.0 μg/mouse for mice homozygous for the Ah^d allele, once a week for 4 weeks) to the skin produced histologic changes in the epidermis of all strains homozygous for the hairless allele, but was without effect in the hr/+ (haired) mice examined. Sebaceous gland metaplasia, i.e., the loss of lipid-filled sebaceous cells, and their replacement with keratinizing cells is the earliest [9] most sensitive and most consistent response in the skin of hairless mice (Table I). In HRS/J hr/hr mice, TCDD produced extensive keratinization (+++) and moderate hyperplasia (++) of the interfollicular epidermis; and marked formation of sebaceous cysts filled with concentric whorls of keratinaceous material (+++) as shown in Table I and Fig 1 (acetone vs TCDD administration). In hairless mice from other strains, the extent of the TCDD-induced hyperplasia and keratinization varied when compared

TABLE II. *Mice bearing various integumental mutations: genetic background, phenotype, of the Ah locus, and histologic skin changes in response to TCDD*

Mutation	Genotype	Chromosomal location	Genetic background	Ah locus	Liver receptor		Hepatic AHH activity (pmol/mg/min)		Squamous metaplasia	Histologic changes		
					K_D (nM)	n (fmol/mg protein)	Control	βNF		Keratinization	Hyperplasia	Keratinized cyst formation
Cryptothrix	crh/crh	?	Skh/crh outbred	Ah^d	—[a]		8.5	11.9	+	+++	+	++
Plucked	pk/pk	18	B6D2 $N_{12}F_{24}$	Ah^b	0.46	147	2.3	113	+	++	+	+++
	pk/+				0.58	173						
Nude	nu/nu	11	BALB/cBy	Ah^b	0.70	71	8.2	44	+	+	++	++
	nu/+				0.70	81	7.8	47	0	0	0	0
Atrichosis	at/at	10	[b]	Ah^b	0.77	84			+	+	0	+
	at/+											
Fur deficient	fd/fd	9	[b]	Ah^d	—[a]		3.4	3.9	0	0	0	0
	fd/+								0	0	0	0
Repeated epilation	Er/+	4	(B6xB6/By)F_1	Ah^b	0.37	189	6.4	50	±	0	0	0
Naked	N/+	15	(B6C3xB6)F_1	Ah^b	0.84	148	4.4	95	0	0	0	0
Rat, naked	n/n				0.41	138			0	0	0	0
Rabbit ear	+/+								+	++	++	0

The 7 mouse mutants were reared and treated as described in Table I and *Methods and Materials*. Also included in this table is the rat bearing the recessive mutation, naked, and the ear skin of normal rabbit. The rat received a dorsal application of 1.0 μg TCDD once a week for 5 weeks, while in the rabbit, TCDD (50 ng/week for 6 weeks) was applied to the inner surface of the ear.

[a] Binding unmeasurable.

[b] Maintained as an inbred strain on its own background.

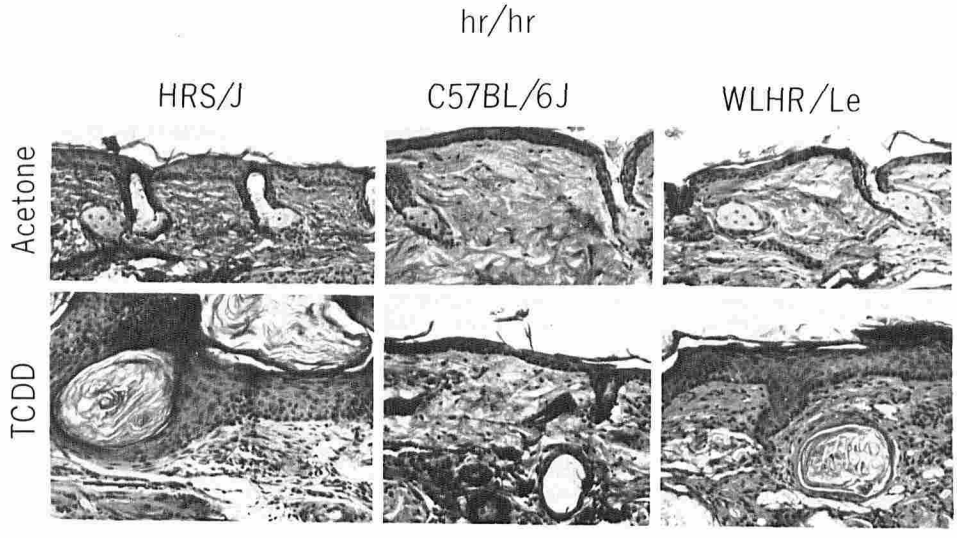

FIG 1. Effect of TCDD on the histology of skin of mouse strains homozygous for the hr allele. Acetone or TCDD dissolved in acetone (0.3 μg/100 μl) was applied to the dorsal skin of HRS/J, C57BL/6J, and WLHR/Le mice homozygous for the hr (hairless) allele once a week for 4 weeks. Sections of full-thickness pieces of skin were prepared as described in *Materials and Methods*. The *bar* in the lower left corner represents 0.1 mm.

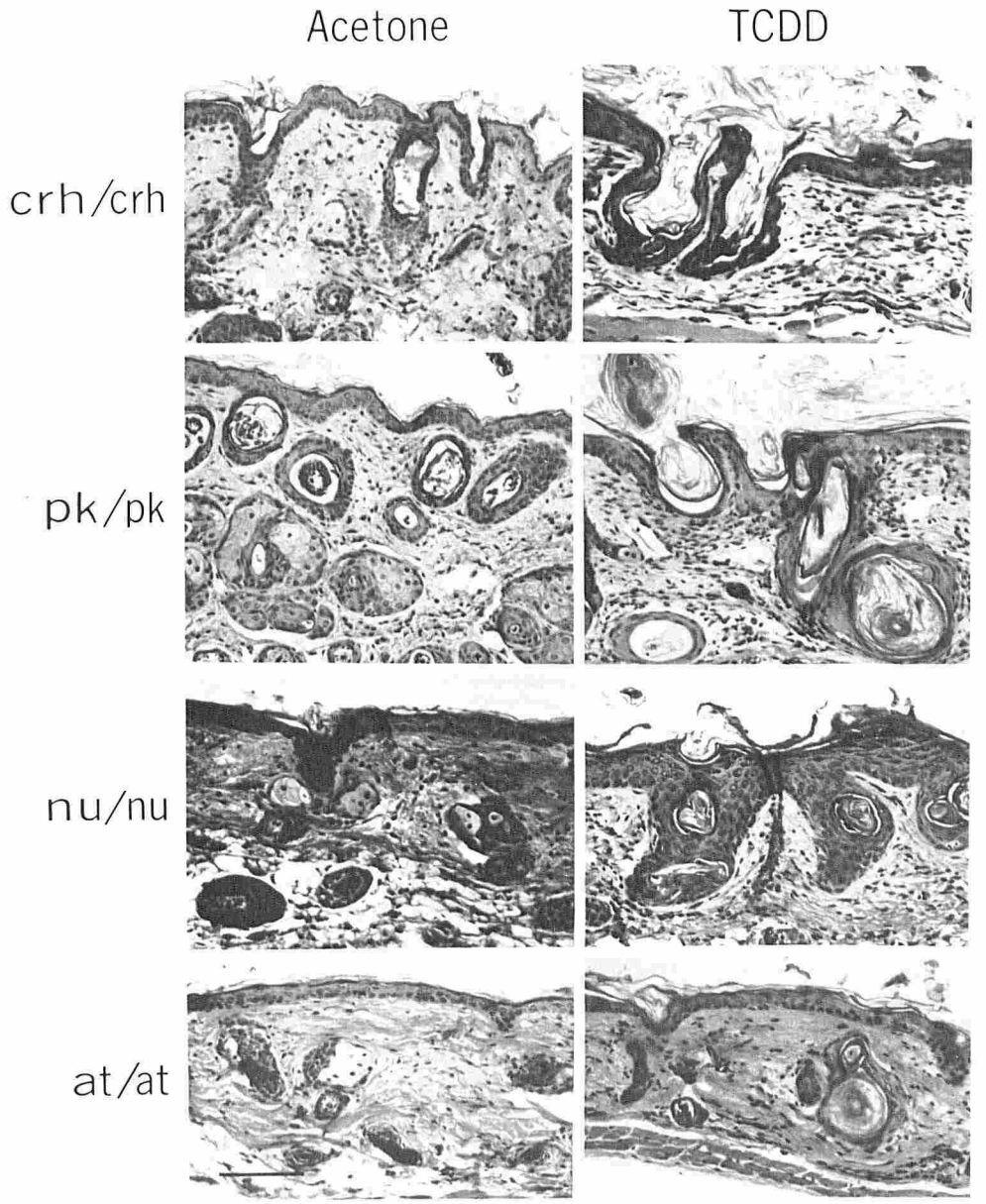

FIG 2. Effect of TCDD on the histology of skin of mice carrying mutations of the integument. Acetone or TCDD was applied to the dorsal skin of mice carrying the mutations cryptothrix (crh/crh), plucked (pk/pk), nude (nu/nu), and atrichosis (at/at) once a week for 4 weeks. The cryptothrix mutant which carries the Ah^d allele received 1.0 μg TCDD/week, the other mutants which carry the Ah^b allele received 0.3 μg TCDD/week. Sections of full-thickness pieces of skin were prepared as described in *Materials and Methods*. The *bar* in the lower left corner represents 0.1 mm.

with their respective acetone controls (Table I, Fig 1). For instance in WLHR/Le mice, the interfollicular epidermis showed minimal keratinization (+) and marked hyperplasia (++++) while in Skh/hr-1, C57BL/6J, and DBA/2J both the hyperplasia and keratinization produced were minimal (+) compared to that in HRS/J mice.

Other Mutations Affecting the Integument

We examined the epidermal respone to TCDD in mice carrying 7 mutations that affect skin and hair, and in the rat with the recessive mutation, naked, and in the normal rabbit. The mouse mutants vary greatly in hair coat: cryptothrix and nude which have almost no body hair (comparable to hairless mice), slightly more hair is seen in plucked, Naked has only patches devoid of hair, and Repeated epilation, fur deficient, and atrichosis have more substantial coats (for a description of these mutants see [11]. These mutations were on different genetic backgrounds, 5 mice carried the Ah^b allele and 2 of the mutants, cryptothrix and fur deficient, had the Ah^d allele. The administration of TCDD produced histologic skin changes in cryptothrix, plucked, nude, and atrichosis mutant mice (Table II, Fig 2). In Repeated epilation, the response was marginal with slight squamous metaplasia of the sebaceous gland and no further histologic changes. No histologic changes were observed in fur deficient, or Naked mutants.

The rat bearing the recessive mutation naked, had very little hair, a high affinity receptor, and no epidermal changes induced by TCDD (Fig 3). The response of the inner surface of the ear of a normal rabbit to halogenated aromatic hydrocarbons is quite characteristic (Fig 3), and has been used as a biologic assay for these compounds [17].

DISCUSSION

In this report we have examined the histologic changes produced by TCDD in the skin of mice carrying (a) the hairless mutation of various genetic backgrounds, and (b) other mutations affecting the integument.

Eight strains of mice carrying the recessive mutation hairless, all responded to TCDD with squamous metaplasia of the sebaceous glands, epidermal hyperplasia, hyperkeratosis, and keratinized cyst formation. While all strains displayed comparable sebaceous gland metaplasia, the relative extent of the other histologic changes was dependent on their genetic background. Congenic mice that were heterozygous (hr/+) or homozygous (+/+) for the wild type allele at the hr locus, developed no histologic skin changes after administration of TCDD.

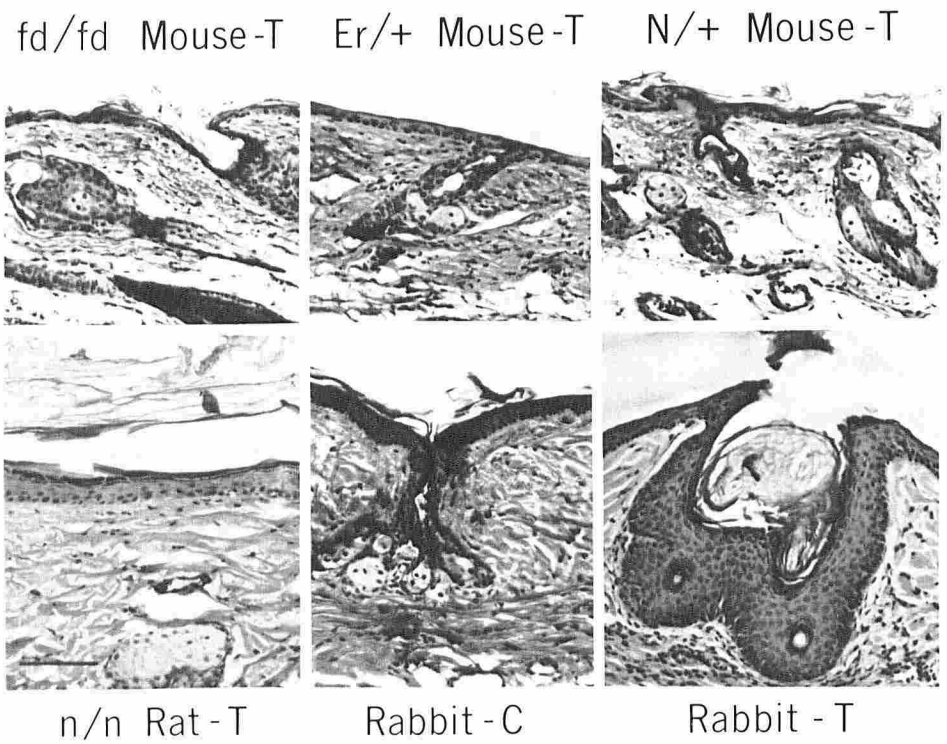

FIG 3. The effect of TCDD on the histology of skin of mice and rats carrying mutations of the integument and of the ears of rabbits. The mouse mutants Repeated epilation (Er/+), fur deficient (fd/fd), and Naked (N/+) were administered TCDD (mouse-T) on the dorsal skin once a week for 4 weeks. The Naked mutant carrying the Ah^b allele received 0.3 μg TCDD/week, the other 2 mouse mutants received 1.0 μg TCDD/week. The histology of the skin of these mice treated with TCDD was similar to that from the acetone-treated control mice. Rats homozygous for the allele naked (n/n) were administered 1.0 μg TCDD/wk for 6 weeks on the back. Histology of skin from naked rats treated with acetone and TCDD were similar. The inner surface of the outer ear of rabbits was treated with acetone (rabbit-C) or TCDD (rabbit-T) 50 ng/week for 6 weeks. Sections of full-thickness pieces of skin were prepared as described in *Materials and Methods*. The *bar* in the lower left corner represents 0.1 mm.

We examined mice with 7 other mutations affecting the integument (Table II). TCDD produced cutaneous changes in 4 of these, cryptothrix, plucked, nude, and atrichosis, comparable to those seen in hairless mice. The biochemical basis for these 5 recessive mutations is unknown. They all map to separate chromosomal locations (cryptothrix is unmapped, but not linked to hr [18]) and presumably code for different gene products. The susceptibility to TCDD-induced skin lesions imparted by these mutations, is apparently not due to a specific gene product coded for by these loci, but rather may be secondary to the altered physiologic state in the skin of these mutant mice. The phenotype of hairlessness or baldness per se, is not responsible for the susceptibility to TCDD-induced skin lesions. As previously noted, the ears of mice are normally not haired, and this skin in hr/+ or hr/hr mice shows no histologic response to TCDD [9]. The atrichosis mutant has a rather full hair coat and is susceptible to TCDD; while the naked rat, has a very sparse coat (comparable to that of the hairless or nude mouse) and is not susceptible.

Many of the toxic responses by halogenated aromatic hydrocarbons in epithelial tissues are species specific. These compounds produce characteristic skin lesions in humans, monkeys, and rabbits, but not in most haired laboratory animals (mice, rats, guinea pigs, and hamsters). The hairless (hr/hr) mouse provides a convenient model in which to study the hyperplastic/metaplastic skin response produced by TCDD and congeners. Comparison of the hr/hr mice and congenic hr/+ or +/+ is useful in the general consideration of the tissue and species-specific nature of many of the toxic responses produced by these compounds. Congenic hr/hr and hr/+ mice both possess the TCDD receptor and respond to TCDD with the induction of epidermal AHH activity. In a two-stage model of skin carcinogenesis, following initiation with 7,12-dimethylbenz[a]anthracene, TCDD promotes tumor formation in hr/hr mice, but not hr/+ mice [19]. Thus the hr locus determines the toxic and tumor-promoting response to TCDD in mouse skin.

All of the biochemical and morphologic effects produced by halogenated aromatic hydrocarbons are thought to result from their binding to the receptor, and the ensuing gene expression. The receptor mediates 2 distinct and dissociable pleiotropic responses: (a) the induction of monooxygenase activity in virtually all tissues in which it is present and (b) proliferation and/or differentiation in some epithelial tissues, a response which shows species and tissues specificity. Understanding this latter pleiotropic response, and the controls which restrict its tissue expression are a central problem in the mechanism of toxicity of halogenated aromatic hydrocarbons. Mice carrying the recessive mutations of the integument (hairless and the other recessive which impart epidermal susceptibility) provide useful models in which to study this restricted pleiotropic response as well as the biochemical changes which TCDD produces and which eventually result in skin lesions and/or tumor promoters.

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