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TERATOGEN METABOLISM: ACTIVATION OF THALIDOMIDE AND THALIDOMIDE ANALOGUES TO PRODUCTS THAT INHIBIT THE ATTACHMENT OF CELLS TO CONCANAVALIN A COATED PLASTIC SURFACES

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Abstract—Thalidomide metabolites inhibited the attachment of tumor cells to concanavalin A coated polyethylene surfaces. Thalidomide, itself, was non-inhibitory. Thalidomide activation to inhibitory products required hepatic microsomes, an NADPH-generating system, and molecular oxygen. Production of inhibitory metabolites was unaffected by either epoxide hydrolase or 1,2-epoxy-3,3,3-trichloropropane (TCPO), an inhibitor of epoxide hydrolase endogenous to hepatic S9 fraction. Therefore, the attachment inhibitor was probably not an arene oxide. Inhibition was not accompanied by cytotoxicity, as judged by trypan blue exclusion. Although uninduced hepatic microsomes from mice, rats and dogs had similar abilities to activate thalidomide, microsomes from Aroclor 1254 induced rats were relatively inactive in the system. Inhibitory metabolites were generated from the thalidomide analogues EM8, EM12, EM16, EM87, EM136, EM255, E350, phthalimide, phthalimido-phthalimide, indan, 1-indanone and 1,3-indandione. Glutarimide, glutamic acid and phthalic acid did not activate to inhibitory products.

The complexity of the maternal-embryonic system makes *in vivo* studies of teratogen metabolism extremely difficult. However, several studies suggest that metabolic activation of proteratogens occurs. Posner *et al.* [1], to take one prominent example, have concluded that chlorcyclizine teratogenesis is mediated through a metabolite, nor-chlorcyclizine, on the basis of an elegant series of experiments using the cytochrome P-450 inhibitor SKF-525A. Their studies clearly show that unmodified chlorcyclizine has little teratogenic activity. Unfortunately, these studies do not clarify the mechanism of nor-chlorcyclizine teratogenesis nor do they prove that this metabolite is the ultimate teratogenic agent.

In vivo studies with thalidomide are even less revealing. Thalidomide undergoes rapid spontaneous hydrolysis to twelve distinct products, each of which can be hydroxylated to several additional structures [2, 3]. Thus, over 100 different metabolic products can be postulated. There have been conflicting reports as to the teratogenicity of thalidomide hydrolysis products, perhaps due to varying dosages and means of administration. 2-Phthalimido glutaric acid at 150 mg/kg, p.o. was non-teratogenic to pregnant rabbits in one study [4], but teratogenic when administered, i.p. at 200 mg/kg [5]. Similarly, phthalimide was non-teratogenic in rabbits at 150 mg/kg, p.o. [6], but teratogenic in mice at 6.2 mg/kg, i.p. [7].

In a limited number of cases the metabolism of teratogens has been studied *in vitro*. Fantel and coworkers [8] have found that a metabolite of cyclophosphamide, generated with rat liver microsomes, alters the development of whole rat embryos in culture. Similarly, cultured limb mesenchyme cell chondrogenesis is inhibited by cyclophosphamide metabolites but not by cyclophosphamide itself [9]. Klein *et al.* [10] have noted aberrant development of whole rat embryos when cultured in serum from monkeys treated with thalidomide. Thalidomide introduced directly to embryo culture had no effect.

In simpler systems, there are indications that thalidomide metabolites generated by hepatic microsomes are biologically active. Gordon *et al.* [11] have presented evidence that an arene oxide metabolite of thalidomide renders human lymphocytes permeable to trypan blue. In our laboratory, Braun and Dailey [12] found that incubation of thalidomide with C57B1/6 murine liver microsomal enzymes produces a product (or products) that inhibits the attachment of ascites tumor cells to concanavalin A coated plastic surfaces.

Inhibition of attachment in this system has been found to correlate well with teratogenicity [13]. The rationale behind the attachment inhibition assay system has been described elsewhere [13–15]. Briefly, as cell-to-cell and cell-to-extracellular matrix interactions play a major role in determining the direction of morphogenesis, any agent interfering with these interactions will alter the course of development. The attachment of tumor cells to lectin coated surfaces acts as a model for embryonic interactions. Agents interfering with *in vitro* attachment

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can be expected to interfere with embryonic interactions. Hence, agents that inhibit attachment are potentially teratogenic.

Using the attachment inhibition assay, we have continued our study of thalidomide metabolism. We found that the production of an inhibitory metabolite required oxygen, that the metabolite was probably not an arene oxide, and that inhibitory products were formed from compounds containing either a phthalimide or isoindole structure.

MATERIALS AND METHODS

Thalidomide and thalidomide analogues E350, EM8, EM12, EM16, EM87, EM136, EM255 and phthalimido-phthalimide were gifts of Drs. L. Flohe and E. Frankus of Grunenthal GMBH, Stolberg, F.D.R. Epoxide hydrolase was the gift of Dr. A. Y. H. Lu of Hoffmann–LaRoche (Nutley, NJ). Concanavalin A (conA)*, bovine serum albumin (BSA) phthalimide, phthalic acid, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, dimethyl sulfoxide (DMSO) and NADP were purchased from the Sigma Chemical Co. (St. Louis, MO). Indan (97%), 1-indanone (99+%), 1,3-indandione (97%), glutarimide (98%), glutamine (99%) and 1,2-epoxy-3,3,3-trichloropropane (TCPO) were from the Aldrich Chemical Co. (Milwaukee, WI). Uninduced, Aroclor 1254 induced and 3-methylcholanthrene induced rat liver S9 fraction were purchased from Litton Bionetics (Kensington, MD). Tritiated thymidine and Aquasol scintillation fluid were from the New England Nuclear Corp. (Boston, MA). Eagle's minimum essential medium (E-MEM) and fetal calf or donor calf serum were purchased from GIBCO (Grand Island, NY). MOT ascites cells [13] were grown intraperitoneally in C3H/HeJ mice† obtained from the Jackson Laboratory (Bar Harbor, ME).

Polyethylene disks were cut from 1.5 mil thick specimen bags (Scientific Products No. B-1218-2) using a 1.25 cm diameter arch punch purchased from a local machinists' supply company. To coat the disks they were floated overnight on a solution of 2.5% glutaraldehyde, 100 μg/ml protein. For disks coated with less than 100 μg/ml conA, the remainder of the protein was made up with BSA. The following morning the disks were washed in PBS and stored in 0.3 M glycine, 1 mM $MgCl_2$, 1 mM sodium azide, 10 mM Tris–HCl, pH 7.4.

MOT ascites tumor cells [16] were labeled *in vitro* with tritiated thymidine at 2.5 μCi/ml for 1 hr at 37° in E-MEM–5% serum containing medium. Following incubation, the cells were washed four times in PBS and resuspended to 10^8/ml in PBS. Viability as judged by trypan blue exclusion generally exceeded 90%.

Microsomes were obtained from fresh canine liver samples supplied by the laboratory of Dr. Norman Hollenberg, Department of Radiology, Harvard Medical School. The liver samples were immediately cut into small fragments, washed in 0.15 M KCl, and homogenized using a loose fitting, motor driven, glass–Teflon homogenizer. Excellent yields also were obtained from large liver homogenates produced by a Waring blendor. The homogenates were centrifuged at 9000 *g* for 10 min at 4°. The supernatant (S9 fraction) was then centrifuged at 105,000 *g* for 1 hr at 4°, and the resultant pellet was resuspended in 0.15 M KCl. Protein concentrations of the S9 and microsomal fractions were determined using the Lowry assay with BSA as a standard. Microsomal suspensions were immediately frozen in a dry ice–ethanol bath and stored at −80°. There was no difference in the thalidomide activation characteristics of fresh or frozen microsomal preparations. Microsomes prepared from commercial preparations of rat S9, as described above, were used immediately.

Activation mixtures generally consisted of 0.15 mg S9 or 0.32 mg microsomes, 10^7 labeled cells, 5 mM NADP, 1.5 mM $MgCl_2$, 20 mM glucose-6-phosphate, 10 mM sodium phosphate buffer, pH 7.4, 3.5 units glucose-6-phosphate dehydrogenase (for mixtures using microsomes) and drug in a final volume of 1 ml. Care was taken to titrate the final pH to 7.4. Drugs were generally dissolved in DMSO. The final concentration of DMSO in the reaction mixture was held to less than 1%, a concentration not affecting attachment. The reaction mixture was incubated at 37° for 1 hr, and the suspension was poured into a 35 mm plastic petri dish in which three or four derivatized disks had been placed with the protein side facing up. Treated cells were permitted to sediment and attach for 20 min at room temperature. The disks were removed with fine forceps, washed in PBS, and counted in Aquasol in a liquid scintillation counter.

Data analysis. Counts from replicate disks were averaged, and non-specific attachment, measured with BSA coated disks, was subtracted. Samples lacking NADP and glucose-6-phosphate, microsomes, or drug served as controls. Attachment by treated cells was then plotted as a percentage of controls, and the drug concentration required to inhibit attachment by 50% was determined. Drugs inhibiting attachment by more than 50% without associated cytotoxicity, as judged by trypan blue exclusion, are considered to have teratogenic potential.

RESULTS

Thalidomide does not inhibit the attachment of tumor cells to concanavalin A (conA) coated surfaces. However a metabolite, generated by hepatic microsomes from C57B116 mice, was found to be inhibitory (Fig. 1). Cell viability, as judged by trypan blue exclusion, was unaffected. Activation of thalidomide required incubation at 37°, liver S9 or microsomes, and an NADPH-generating system con-

* Abbreviations: conA, Concanavalin A; BSA, bovine serum albumin; PBS, phosphate buffered saline; MOT, ascitic mouse ovarian tumor; TCPO, 1, 2-epoxy-3,3,3-trichloropropane; and S9, post-mitochondrial supernatant fraction of homogenized liver.

† Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council [DHEW (now DHHS) publication No. (NIH) 78–23, revised 1978].

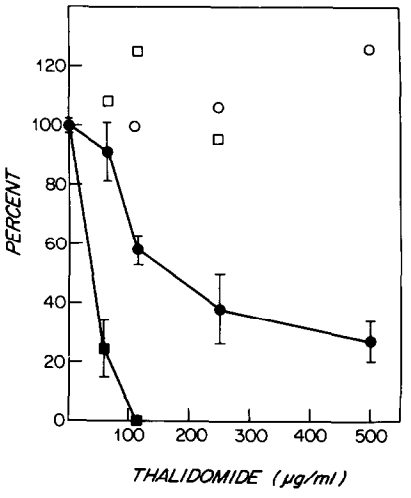
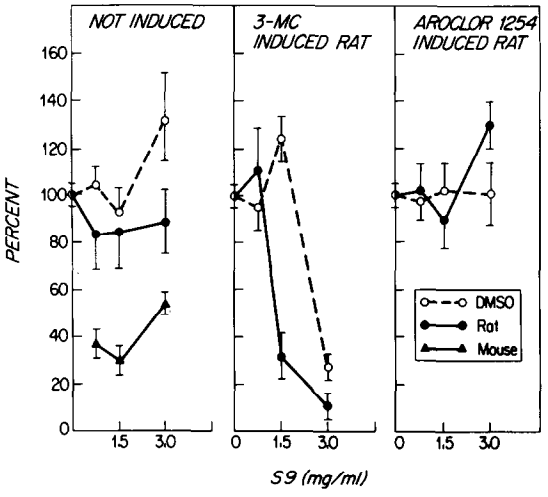


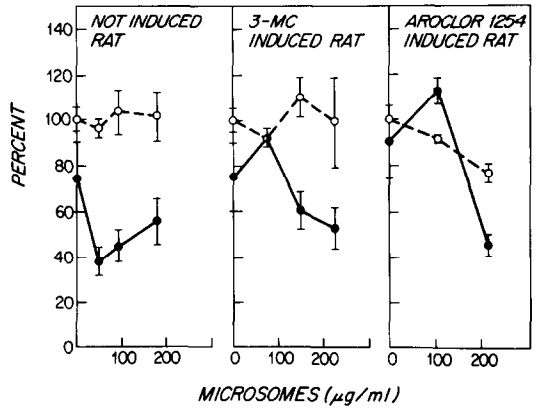
Fig. 1. Attachment inhibition by activated thalidomide. Suspensions (1 ml) of MOT cells, microsomes, cofactors and the indicated concentration of thalidomide were incubated as described under Materials and Methods for 1 hr at 37° and attachment to high and low density conA disks was determined. One hundred percent attachment was determined in the absence of thalidomide. Closed symbols: complete incubation mixtures. Open symbols: control incubations lacking cofactors. Key: (○, ●) attachment to disks coated in 100 μg/ml conA; and (□, ■) attachment to disks coated in 15 μg/ml conA. S.E.M. are shown.

sisting of NADP, glucose-6-phosphate and, if microsomes were used, glucose-6-phosphate dehydrogenase. If any of these constituents is omitted or if incubation is at 20°, no inhibitory product is formed [12].

Inhibition is dependent on thalidomide concentration, length of 37° incubation, and the surface density of conA [12]. Assay sensitivity can be increased substantially through the use of disks derivatized in low concentrations of conA. Figure 1 shows that disks coated in 15 μg/ml conA–85μg/ml BSA were 5-fold more sensitive to the thalidomide metabolite than were our standard disks derivatized at 100 μg/ml conA. Measurements using ^{125}I-labeled conA indicated that disks coated at 100 μg/ml had a surface conA density of 724 ng/cm^2 while those coated at 15 μg/ml had a density of 49 ng/cm^2.

Microsomes prepared from adult mouse, rat, rabbit, cat, dog and Rhesus monkey had similar abilities to activate thalidomide (unpublished data). We were interested whether hepatic microsomes from animals pretreated with inducers of cytochrome P-450 isozymes had increased activity against thalidomide. The experiments shown in Figs. 2 and 3 indicate that hepatic cytochrome P-450 isozyme induction did not increase significantly the ability of rat S9 or microsomes to activate thalidomide despite increased levels of benzo(a)pyrene hydrolase. Uninduced S9 fraction had an activity of 0.96 nmole hydroxybenzepyrene per 20 min per mg protein, the 3-methylcholanthrene induced S9 an activity of 1.7 nmoles, and the Aroclor 1254 induced S9 an activity of 14.8 nmoles. Aroclor 1254 induction reduced or eliminated all activity, possibly due to the presence of competing, inactivating, pathways.





Figs. 2 and 3. Effects of S9 (Fig. 2, above) and microsomes (Fig. 3, below) from uninduced, 3-methylcholanthrene induced and Aroclor 1254 induced rat liver. Commercial S9 preparations and microsomes derived from S9 were assayed for their abilities to activate thalidomide as described in Materials and Methods. One hundred percent attachment was determined in the absence of S9 or microsomes. Symbols: (○) control incubations without thalidomide; (●) incubations with 500 μg/ml thalidomide, extracts from rat liver; (▲) 500 μg/ml thalidomide with S9 from mouse liver for comparison (Fig. 2). Left panel: uninduced liver; middle panel; 3-methylcholanthrene induced liver; and right panel: Aroclor 1254 induced liver. S.E.M. are shown.

The inhibitory product may be extracted from the reaction mixture with chloroform. Figure 4 shows the results of an experiment in which thalidomide was incubated with uninduced microsomes and cofactors for 1 hr and extracted with chloroform. The chloroform layer was then removed and dried under nitrogen. Dried extract was added to a cell suspension, and the ability of treated cells to attach was measured. Extract from an activation mixture incubated at 37° was inhibitory while an extract from a mixture incubated at 20° was not. We have shown previously that detectable thalidomide activation does not occur at 20° [12]. Efforts to purify and characterize the inhibitory metabolite have been hampered by the instability of this product in aqueous solution.

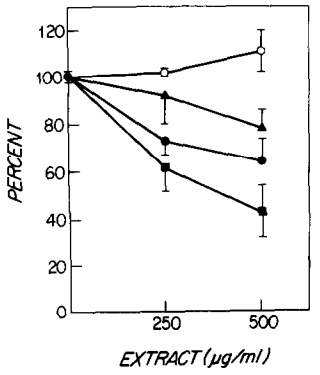


Fig. 4. Extraction of activated thalidomide and E350. Thalidomide and E350 at 500 μg/ml were incubated, as described in Materials and Methods, with microsomes and cofactors in a total volume of 10 ml for 1 hr at 20° or 37°, 10 ml chloroform was added, and the mixture was shaken for 1 hr at room temperature. The chloroform layer was collected and dried under nitrogen, and the residual white powder was assayed for inhibitory activity without the addition of microsomes or cofactors. The indicated amount of extract was dissolved in PBS, 10^7 labeled cells were added and immediately poured over conA coated disks, which incubated for 20 min and adherent counts were measured. One hundred percent attachment was defined as that obtained in the absence of added extract. Symbols: (○) control using untreated thalidomide; (▲) thalidomide activation at 20°; (●) thalidomide activation at 37°; and (■) E350 activated at 37°. S.E.M. are shown.

Efforts in many laboratories to understand the mode of action of thalidomide have focused on structure function relationships of thalidomide analogues. Many of these agents have been tested for teratogenicity in animals. Any hypothesis concerning the mechanism of thalidomide teratogenicity must take this large body of data into account. Accordingly, we have examined a group of compounds related to thalidomide for their ability to inhibit attachment with and without microsomal activation. The results are presented in Table 1. Figure 5 shows the structures of the compounds tested. All drugs containing a phthalimide or phthalimide-like structure were metabolized to an inhibitory product. The glutarimide moiety and its hydrolysis product, glutamic acid, did not activate to inhibitory products. While phthalimide could be activated, its hydrolysis product, phthalic acid, was inactive. Overnight incubation of phthalimide in PBS also eliminated activity.

The experiment shown in Fig. 6 demonstrates that activation requires molecular oxygen. We have used 1,3-indandione rather than thalidomide in this experiment because of its greater stability in aqueous solution. A mixture of drug, hepatic microsomes and cofactors was purged of oxygen with nitrogen gas, incubated at 37° for 1 hr, and cooled to 20°. Samples were added to labeled cells at room temperature, and attachment to concanavalin A coated disks was determined. Elimination of oxygen prevented the

Table 1. Inhibitory activity of thalidomide analogues with and without microsomal activation

Drug	Inhibition* − Microsomes	Inhibition* + Microsomes	Teratogen?†
Thalidomide	−	+	+[6]
EM8	−	+	+−[17]‡
EM12	−	+	+[17]
EM16	−	+	−[18]
EM136	−	+	+[17]
EM255	−	+	+[17]
E350	−	+	−[18]§
EM87	−	+	−[18]
Phthalimido-phthalimide	−	+	+[20]
Phthalimide	−	+	+[7]
Indan	−	+	?
1-Indanone	−	+	?
1,3-Indandione	−	+	+[21]
Phthalmic acid (1400 μg/ml)	−	−	−[5]
"Hydrolyzed" phthalimide‖ (1000 μg/ml)	−	−	?
Glutarimide (2500 μg/ml)	−	−	−[6]¶
Glutamic acid (2500 μg/ml)	−	−	−[5]

* Tested to indicated concentration, solubility limit or 500 μg/ml. A "+" indicates more than 50% inhibition; a "−" indicates no inhibition at the highest concentration tested.

† Key: (+) teratogenic in one or more *in vivo* studies; (−) non-teratogenic; and (?) no data on teratogenicity. References to teratogenicity are listed.

‡ EM8 is very slightly teratogenic in mice.

§ E350 is teratogenic in chick [19].

‖ Phthalimide was incubated overnight at room temperature in PBS.

¶ Alpha amino glutarimide is not teratogenic. We were unable to find a reference to the teratogenicity of glutarimide.

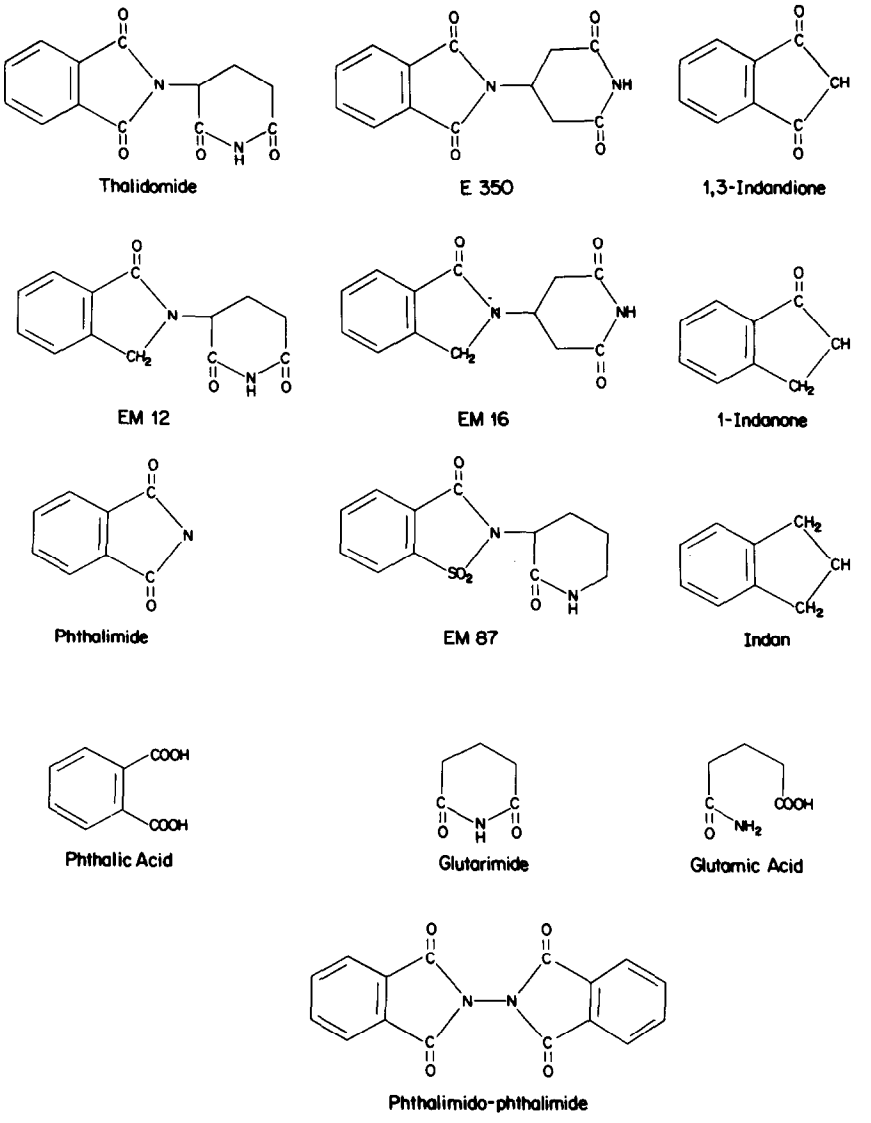


Fig. 5. Structure of drugs used in this study. EM8, EM136 and EM255 (not shown) differ from EM87, EM12 and thalidomide (respectively) in a glutarimide ring carbonyl group. EM136 and EM255 lack the 6′-carbon carbonyl while EM8 has a 6′-carbon carbonyl.

formation of an inhibitory metabolite. Reoxygenation of the purged mixture restored its ability to activate 1,3-indandione to an inhibitory product. In a separate control experiment, the pH of the mixture following gas treatment was found to change by less than 0.1 pH unit.

These data suggest that thalidomide activation involves an oxidative transformation. However, there is evidence that the inhibitory metabolite is not an arene oxide. Addition of epoxide hydrolase (EC 3.3.2.3) to a complete thalidomide activation reaction mixture did not reduce the level of inhibitory activity generated (Fig. 7). The experiment in Fig. 7 is representative of eight similar experiments in which epoxide hydrolase did not affect thalidomide activation. It is possible, however, that an arene oxide refractory to the action of this specific enzyme is the inhibitory product. Inhibition of epoxide hydrolase endogenous to mouse hepatic S9 fraction with TCPO did not increase significantly the level of inhibitory activity (Fig. 8). This experiment is representative of four independent experiments in which TCPO did not affect thalidomide activation significantly.

DISCUSSION

The thalidomide metabolite responsible for attachment inhibition appears to differ from the lymphotoxic thalidomide metabolite described by Gordon *et al.* [11] in three respects. First, generation of the toxic thalidomide metabolite was eliminated by coincubation with epoxide hydrolase and stimulated by TCPO, an indication of arene oxide involvement. In our studies, neither epoxide hydrolase nor TCPO

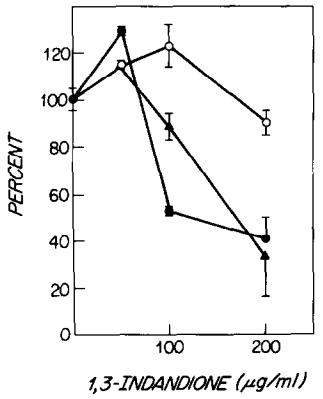


Fig. 6. Effect of anoxia on 1,3-indandione activation. A mixture of canine microsomes at 0.6 μg/ml (protein), cofactors, buffer, drug at 125 μg/ml, but no cells was placed into 5 ml flat bottomed counting vials tightly stoppered with a rubber septum. Two needles were introduced through the septum, and gas was bubbled into the solution through one needle, the other acting as a vent. pH is altered by less than 0.1 unit in this procedure. After treatment the needles were removed, the tubes were incubated for 1 hr at 37°, cooled to room temperature, and samples added to suspensions of labeled cells. The suspensions were immediately poured into petri dishes containing conA coated disks, and attachment was measured as described under Materials and Methods. One hundred percent attachment was determined in the absence of added incubation mixture. Symbols: (▲) control tube—no gasses bubbled through solution; (○) nitrogen bubbled through solution for 10 min then incubated at 37° for 1 hr and assayed for inhibitory activity; and (●) nitrogen bubbled for 10 min followed by 10 min of room air, then incubated and assayed. Note that room air restored the ability to the microsomes to activate the drug following deoxygenation. S.E.M. are shown.

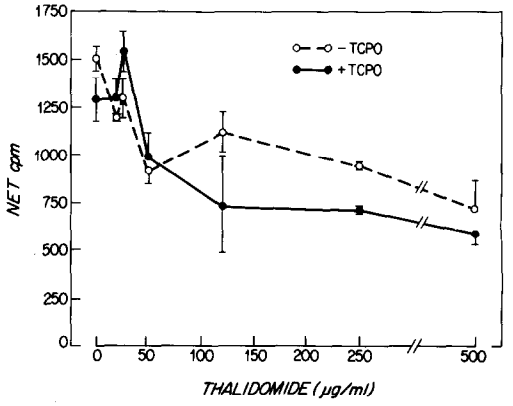


Fig. 8. Effect of TCPO on yield of inhibitory thalidomide metabolite. TCPO was added to a standard reaction containing murine S9, incubated for 1 hr at 37° and assayed as described under Materials and Methods. One hundred percent attachment was determined in the absence of TCPO and thalidomide. Symbols: (○) no TCPO added; and (●) TCPO at 5 μg/ml added. S.E.M. are shown.

significantly altered the production of inhibitory metabolite. Second, the arene oxide metabolite rendered lymphocytes permeable to trypan blue. The attachment inhibitor did not alter trypan permeability of MOT, CHO or V79 cells. However, it is possible that the inhibitory product identified in the present study is more toxic to lymphocytes than to other cell types. Until the product is purified it will not be possible to determine its lymphotoxicity. Third, Gordon *et al.* found that Aroclor 1254 induced rat hepatic S9 fraction was unable to activate thalidomide to a lymphotoxic product while hepatic S9 from Aroclor induced maternal rabbit was competent. We find little difference between uninduced hepatic S9 fraction among the species. Aroclor 1254 induced rat S9 was nearly inactive in the attachment system (Fig. 2). Hence, it is possible that species differences in the activity of Aroclor 1254 induced liver microsomes may account for the apparent disagreement between our data and that of Gordon *et al.*

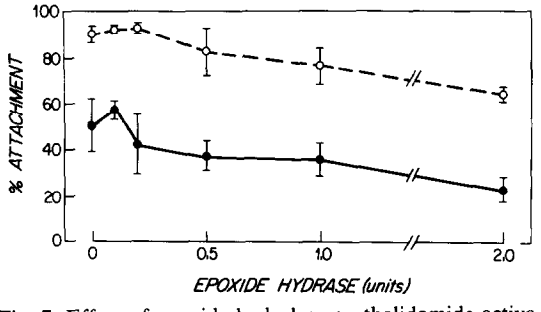


Fig. 7. Effect of epoxide hydrolase on thalidomide activation. Epoxide hydrolase at the indicated concentration was added to a standard reaction, containing murine S9, thalidomide and cofactors, and incubated at 37° for 1 hr; attachment was measured as described in Materials and Methods. One hundred percent as determined in the absence of thalidomide or epoxide hydrolase. Symbols: (○) control incubation with DMSO vehicle only; and (●) 500 μg/ml thalidomide added. S.E.M. are shown.

It is possible that two distinct thalidomide metabolites can be generated by liver microsomes, one lymphotoxic and the other inhibitory. Is either involved in thalidomide teratogenesis? Only isolation and testing of the metabolites themselves, in more elaborate *in vitro* systems and *in vivo*, will clarify whether the toxic metabolite, the inhibitory metabolite, or some third compound is involved in thalidomide teratogenesis.

Neither the lymphotoxic nor the inhibitory product is a completely satisfactory solution to the vexing problem of thalidomide teratogenesis.

It is unlikely that overt cytotoxicity is involved in thalidomide teratogenicity. If significant lymphocyte toxicity followed thalidomide administration, serious lymphopenia would be expected in treated individuals. We know of no evidence that this occurs. Among leprous patients taking thalidomide to alleviate the lepra reaction, serum IgG and IgA levels are not altered although there is a decline in IgM titer [22]. Thus, the lymphotoxic characteristics of the thalidomide metabolite discovered by Gordon *et al.* [11] cannot play an important role in adults. Cytotoxic damage to other tissues should be manifested as acute toxicity. However, thalidomide is an unusually non-toxic substance in adults. Only on prolonged exposure are there any toxic symptoms in humans [23]. If the toxicity of the arene oxide metabolite is involved in teratogenicity, it must be expressed

primarily in the embryo or the embryo must be far more sensitive to this product than the adult.

While the inhibitory metabolite described here is not overtly toxic, there are serious problems in assigning this product a role in thalidomide teratogenicity. First is the lack of a significant difference in the ability of adult hepatic microsomes from thalidomide responsive (rabbits) and non-responsive (rats) species to activate thalidomide (unpublished data). Possible resolutions of this difficulty are (a) detoxification, absorption and distribution differences between species, (b) differences in embryonic metabolism between species or (c) variations in the sensitivity of morphogenic processes to thalidomide metabolites among the species.

A second difficulty in the assignment of the inhibitory product to a role in teratogenesis is the partial lack of correlation between *in vitro* and *in vivo* activities in the thalidomide analogues. The data in Table 1 indicate that a phthalimide or indan structure is necessary for the activation of thalidomide-related compounds to inhibitory products. Thus, four non-teratogenic thalidomide analogues, EM8, EM16, EM87, and E350, are metabolized to inhibitory products. Preliminary studies with EM8 and EM87 indicate that, at pH 7.4, they decay to an inactive product more rapidly than does thalidomide. Thus, it is possible that these two analogues are spontaneously inactivated before they can accumulate at hazardous concentrations in the embryo.

Non-teratogens E350 and EM16, however, are relatively stable at pH 7.4. These analogues are beta isomers of thalidomide and EM12, respectively, and have spontaneous hydrolysis rates identical to their teratogenic isomers. Why should the phthalimide–glutarimide linkage play a prominent role in the teratogenicity of these compounds? The physical structures of the alpha and beta isomers differ significantly. In the non-teratogenic beta isomer, the glutarimide ring rotates freely about the N–C linkage to phthalimide. In the alpha-linked structures, rotation is stearically hindered with the glutarimide 2′-carbonyl oxygen held in apposition to a phthalimide oxygen by van der Waal's forces [24]. The imide bonds of the phthalimide moiety in alpha-linked structures are partially shielded by a carbonyl group in the glutarimide ring. The shielded imide bonds may be more resistant to enzymatic hydrolysis than those of their beta isomers. Studies on the *in vitro* activity of hydrolytic products of thalidomide and its analogues are in progress.

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