Br. J. exp. Path. (1987) **68**, 215–223

In-vitro teratogenicity of retinoids

C.E. Steele, R. Marlow, J. Turton* and R.M. Hicks*
*SK&F Research Ltd, The Frythe, Welwyn, Herts AL6 9AR, and *School of Pathology, Middlesex Hospital Medical School, London W1P 7LD, UK*

Received for publication 4 August 1986
Accepted for publication 28 October 1986

Summary. Mid-gestation rat conceptuses were cultured for 48 h in serum containing the retinoids all-trans-retinoic acid (TRA), 13-cis-retinoic acid (13-CRA), etretinate (ETR), etretin or one of six retinamides at concentrations ranging from 0.5 to 400 μg/ml. TRA was toxic at a concentration of 0.5 μg/ml. 13-CRA and etretin caused abnormal development at 1.0 μg/ml. However, the six retinamides were less toxic and adverse developmental effects were only evident at concentrations of 50 or 100 μg/ml. ETR was without effect at 100 μg/ml, the highest dose level of this compound tested. *In vivo*, TRA, 13-CRA and ETR are highly teratogenic. In this culture system, TRA and 13-CRA caused abnormal development at very low concentrations but in contrast, ETR was non-toxic at 100 μg/ml. Therefore these findings indicate that *in vivo*, maternal pharmacokinetics, and bioactivation in particular, play a major role in inducing abnormal development. Cis/trans isomerization was not a major determinant of toxicity. However, there appeared to be a relationship between abnormal development and the actual or estimated pK_a values of the 10 retinoids tested.

Keywords: teratology, *in vitro*, retinoids, rat embryo culture

Retinoids (structural analogues of vitamin A) are used for the treatment of a variety of dermatological disorders including skin cancer, and have chemo-preventive activity in several experimental carcinogenesis models (Peck 1981; 1984; Hicks *et al.* 1982; 1985; Bollag 1983; Sporn & Roberts 1983; Berretti & Grupper 1984; Moon & Itri 1984). They are teratogenic in animals (Elias & Williams 1981; Cunningham & Ehmann 1983; Kamm & Ashenfelter 1984) and drugs such as 13-cis-retinoic acid (13-CRA) (Accutane, Roaccutan, Roaccutane) and etretinate (Tigason) are contra-indicated in pregnancy (Chen 1985). Despite warnings that women of childbearing potential should practice effective contraception during and after treatment with 13-CRA and etretinate (ETR), there have been reports of spontaneous abortion and infants with birth defects following exposure to these two retinoids during pregnancy (Rosa 1984; Chen 1985; Lammer *et al.* 1985; Rosa *et al.* 1986). These defects were of a distinctive pattern involving the central nervous system (hydrocephalus, microcephaly etc), cranio-facial malformations, the heart (septal defects, transposition of the great vessels etc) and the thymus.

Analysis of the teratogenicity of retinoids in experimental animals has shown that structural modification of the retinoid molecule at C15 can produce compounds that retain cancer chemo-preventive activity, but are less teratogenic (Willhite & Shealy 1984;

Correspondence: C.E. Steele, SK&F Research Ltd, The Frythe, Welwyn, Hertfordshire AL6 9AR, UK.

Willhite *et al.* 1984). However, such experiments are complicated by differing species-related and compound-related rates of retinoid absorption, binding and excretion and their relevance to the clinical situation is difficult to assess. This study was designed to investigate the in-vitro teratogenicity to the rat embryo of a number of retinoids by adding them directly to the embryo culture medium.

The effects of retinol and retinoic acid (natural forms of vitamin A) on rat whole embryo cultures have already been reported (Morriss & Steele 1974; 1977). Embryos cultured during the organogenetic phase in medium containing as little as 0.1–0.5 μg/ml retinol or retinoic acid exhibited typical defects involving the central nervous system, a pattern of malformations later shown to be similar to that observed *in vivo* (Steele *et al.* 1983). Such experiments not only demonstrated that retinol and retinoic acid have a direct effect on the rat conceptus but also raised the possibility that various forms of the vitamin may be teratogenic for the human fetus (Morriss & Thomson 1974). The large difference between *in vivo* and *in vitro* teratogenic doses (60 000 μg/rat and 0.1 to 0.5 μg/ml, respectively) suggest that *in vivo* only a very low proportion of the administered retinoid reaches the embryo in an active form, but that a relatively small increase in plasma concentration may adversely affect embryonic development without causing deleterious effects on the mother. Subsequent experiments using human serum, prepared from volunteers who had ingested a single dose of 100 000 USP units of 'vitamin A' (fish liver oil), as the culture medium for rat embryos suggested that vitamin A would be teratogenic (Steele *et al.* 1982). However, control human sera also produced a high level of neural tube defects in rat embryos (Steele 1985*a*).

For this study, whole rat embryo cultures at a particular phase of embryonic development were used such that the development of the control embryo *in vitro* is equivalent to that observed *in vivo* (New *et al.* 1976). Retinoids were added directly to the culture medium and their effects on the developing embryo assessed. Preliminary results with 13-CRA and ETR have recently been published in summary form (Steele *et al.* 1986).

Materials and methods

Whole embryo culture has been described in detail elsewhere (New 1978; Steele 1985*b*) and only an outline is given here. Headfold stage rat embryos of the Wistar stock (Horlan Olac Ltd, Bicester, Oxon) were explanted in cold Hanks saline during the afternoon of the 10th day of pregnancy and cultured for 48 h in immediately-centrifuged serum (Steele & New 1974).

The structures of the retinoids tested are shown in Fig. 1. Etretin, ETR and 13-CRA were provided by Roche Products Ltd (Welwyn Garden City, Herts) and the retinamides *N*-(4-hydroxyphenyl)retinamide (4HPR), *N*-ethylretinamide (NER), 13-cis-*N*-ethylretinamide (13cisNER), *N*-butylretinamide (NBR), and *N*-(2-hydroxyethyl)retinamide (OHNER) by the National Cancer Institute (courtesy of Dr C. Smith and Dr M. Sporn) and the Southern Research Institute (Dr Y. Fulmer Shealy). *N*-tetrazol-5-ylretinamide (TZR) was supplied by courtesy of Dr F. Frickel (BASF, Aktiengesellschaft, Ludwigshafen, FRG). Retinoids, dissolved in absolute ethanol, were added to the serum (<4 μl alcohol/ml serum) to give final concentrations of 0.5, 1, 50, 100 or 400 μg/ml. All-trans-retinoic acid (TRA), (Sigma Chemical Co., Poole, Dorset) was used in some cultures to compare the effects of synthetic retinoids with that of a natural retinoid; the results were also compared with previously published data (Morriss & Steele 1977).

At the end of culture, the presence or absence of a heartbeat and yolk sac circulation was recorded and the embryo was dissected from the yolk sac and amnion. The morphological parameters listed in Tables 1 and 2 were assessed and the embryos then dissolved in 0.5 M NaOH for protein estimation (growth). Protein was determined by the

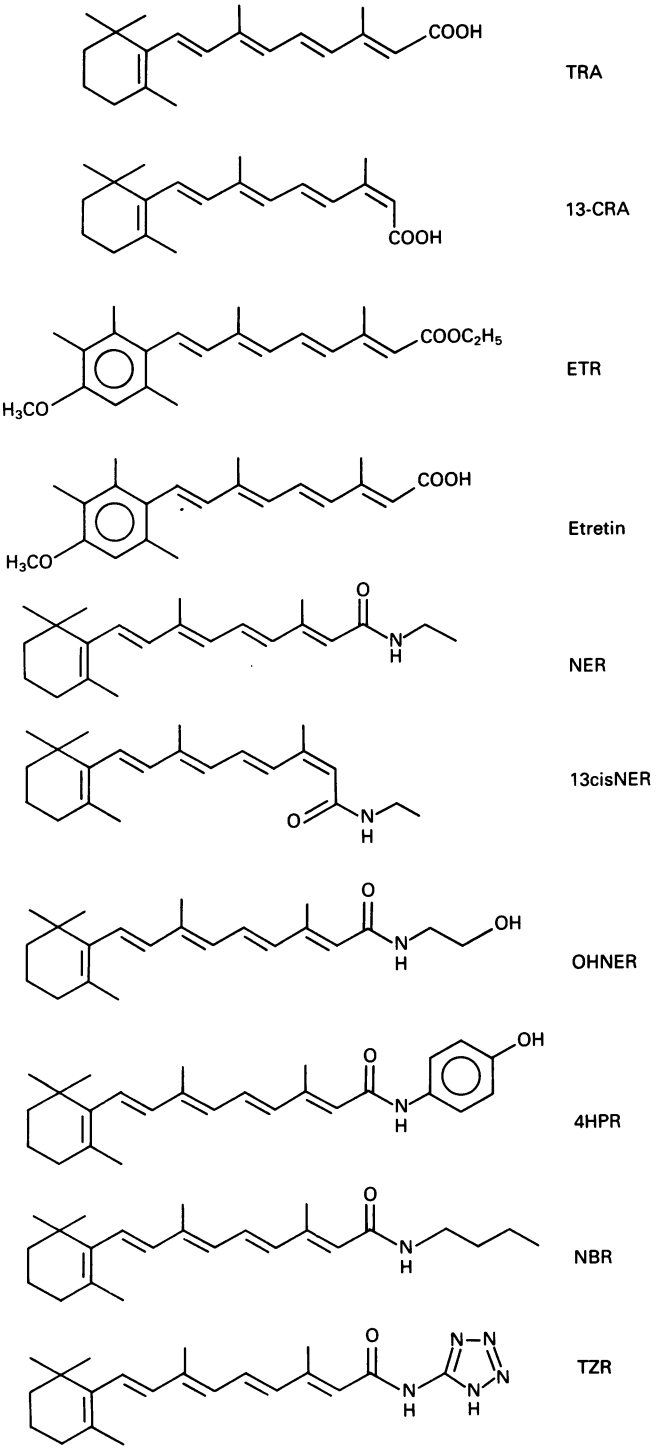

Fig. 1. Structures of the retinoids tested.

Table 1. Effect on rat embryo development of 48 h culture in medium containing TRA

TRA concentration (μg/ml medium)	Anterior neuropore closed[a]	Complete or partial turning[a]	Protrusion of embryonic axis[a]	Somites $\bar{x}\pm$s.e.	Protein (μg) $\bar{x}\pm$s.e.
0[b]	19/19	19/19	0/14	23[c]	164±8
0.5[b]	PF	2/19	10/14	10[c]	67±3
0	5/5	5/5	0/5	26±1	212±5
0.5	3/5	4/5	2/5	18±2	132±29
0	5/5	4/5	0/5	24±2	181±29
1.0	4/5	0/5	5/5	12[d]±1	37±7
0	4/4	4/4	0/4	26±1	nd
10.0	PF	0/4	4/4	PF	nd

[a] No. of embryos with feature/no. of embryos assessed.
[b] Data from Morriss and Steele (1977) included for comparison.
[c] Standard error not given.
[d] Mean of three embryos.
PF Structures too poorly formed to allow assessment.
nd No data available.

method of Lowry *et al.* (1951) and the final value for each embryo was the mean of three estimations.

Results

The effects of TRA on embryonic development were in accord with previously published observations (Morriss & Steele 1977). Abnormal development, e.g. lack of closure of the neural tube and protrusion of the embryo from the yolk sac, occurred in medium containing as little as 0.5 μg/ml TRA (Table 1).

Low concentrations of 13-CRA (1 μg/ml) were directly harmful to the developing embryo (Table 2). So also was etretin (1 μg/ml), the main metabolite of ETR. At this concentration both 13-CRA and etretin retarded growth (protein) and differentiation (somites), and adversely affected the pharyngeal arches (Table 2). Approximately similar effects, but with some variation in intensity, were seen with five retinamides (4HPR, TZR, OHNER, NER and 13cisNER) at a higher concentration (50 μg/ml), but in comparison the retinamide NBR was less toxic and only affected development at 100 μg/ml (Table 2). ETR itself was without effect at 100 μg/ml and was not sufficiently soluble in ethanol to achieve a concentration in the medium of 400 μg/ml.

Statistical analyses of the results are set out in Table 3.

Discussion

The main metabolite of ETR, etretin, when added to the culture medium was teratogenic for rat embryos at low dose (1 μg/ml). Unmetabolised ETR by contrast was without effect at 100 μg/ml (Table 2) despite the fact that it is teratogenic *in vivo* in rodents (Hummler & Schüpbach 1981) and in humans (Chen 1985). This suggests that the parent compound requires bio-activation for its teratogenicity to be expressed, as proposed by Kochhar *et al.* (1985). They indicated that ETR required metabolic conversion to etretin for the suppression of chondrogenesis in a limb bud mesenchymal cell culture system. 13–CRA was also teratogenic at low dose

Table 2. Effect on rat embryo development of 48 h culture in media containing retinoids[a]

Treatment	N[b]	HB[c]	YSC[d]	Complete or partial turning	Two or more pharyngeal arches	Neuropore closed ant.	Neuropore closed post.	Allantois fused with chorion	Limb buds	Somites $\bar{x}\pm$s.e.	Protein (ug) $\bar{x}\pm$s.e.
						1μg/ml					
Control	5	5	5	5	5	5	1	4	5	23±1	167±10
13cisNER	5	4	4	5	5	5	1	4	4	23±1	150±17
NER	5	5	5	5	5	5	3	5	5	25±0	188± 9
4HPR	5	5	5	5	5	5	3	5	5	24±1	174±11
Control	16	16	16	15	16	13	5	16	16	24±0	137± 7
13-CRA	16	16	1	2	3	16	15	8	6	17±1	77± 8
Etretin	16	16	9	15	8	10	8[e]	11	10	19±0	117± 6
TZR	16	16	13	16	16	16	5	13	15	24±0	149± 9
						50μg/ml					
Control	14	14	14	14	14	14	7	13	14	25±0	198± 8
13cisNER	14	14	12	14	14	14	2	11	14	23±0	158± 7
NER	14	14	13	14	14	14	1	14	14	23±0	172± 8
4HPR	14	12	4	9	8	13	6	12	6	19±1	119±10
Control	13	13	13	13	13	13	3	12	13	25±0	219± 8
ETR	13	13	13	13	13	11	1	13	13	24±1	227±12
NBR	13	13	11	13	13	11	3	11	13	24±0	203± 7
OHNER	13	11	11	13	12	11	1	12	13	22±1	202± 9
Control	10	10	10	10	10	10	3	10	10	25±0	195±10
13-CRA	10	10	0	0	0	4	10	2	0	11±0[f]	55± 4
Etretin	10	10	0	0	0	8	10	1	0	12±0[f]	51± 4
Control	15	15	15	15	15	15	9	13	15	25±0	204± 4
TZR	15	12	1	4[g]	1[h]	7[h]	9[h]	10[h]	3[h]	17±3[i]	58±12
						100μg/ml					
Control	11	11	11	11	11	9	5	11	11	25±0	185± 9
13cisNER	11	11	7	9	11	7	8	9	11	21±1	130± 8
NER	11	11	6	5	11	5	2	11	11	20±1[j]	118±11
4HPR	11	6	0	1	1	10	10	11	2	14±1	87± 7
Control	12	12	12	12	12	12	2	11	12	26±0	194±12
ETR	13	13	13	13	13	13	4	13	13	24±0	182± 8
NBR	13	13	13	13	13	13	3	13	13	23±0	159±15
OHNER	13	13	7	10	11	12	10	13	10	20±1	128±11
TZR[k]	15	7	0	3[l]	0[m]	4[m]	1[m]	6[m]	0[m]	14±1[n]	41± 6
						400μg/ml					
Control	5	5	5	5	5	5	0	4	5	26±0	163± 9
NER	5	5	0	1	3	3	3	5	2	16±2	62± 3
13cisNER[o]	5	5	0	0	0	2	2	3	0	*p*	20± 2
NBR	5	5	0	0	1	1	0	2	1	*p*	37± 1

[a] With the exceptions of somite number and protein content, the figures in each column represent the number of embryos with the feature at the head of the column. [b] Number of embryos. [c] Presence of heartbeat. [d] Presence of yolk sac circulation. [e] 15/16 embryos in medium containing etretin had stumpy tails. [f] $n=9$, 1/10 too poorly formed to count. [g] $n=12$. [h] $n=10$. [i] $n=6$, remainder too poorly formed to count. [j] $n=7$. [k] Control embryos for this group are those for the 50μg/ml TZR group. [l] $n=13$. [m] $n=10$. [n] $n=3$. [o] One embryo in the 400μg 13cisNER/ml group was too poorly formed to allow assessment of pharyngeal arches, neuropore closure, limb buds and somites. $n=4$ for these parameters. *p* Embryos too poorly formed to count somites.

Table 3. Statistical analysis (Student's *t*-test) of results (Tables 1 and 2)

	Somites	Protein
Control *vs* 0.5μg/ml TRA[a]	$P<0.05$	ns
Control *vs* 1μg/ml TRA	$P<0.01$	$P<0.01$
Control *vs* 10μg/ml TRA	$P<0.001$	$P<0.001$
Control *vs* 1μg/ml 13-CRA	$P<0.001$	$P<0.001$
Control *vs* 50μg/ml 13-CRA	$P<0.001$	$P<0.001$
Control *vs* 50μg/ml ETR	ns	ns
Control *vs* 100μg/ml ETR	$P<0.05$	ns
Control *vs* 1μg/ml etretin	$P<0.001$	$P<0.05$
Control *vs* 50μg/ml etretin	$P<0.001$	$P<0.001$
Control *vs* 1μg/ml NER	ns	ns
Control *vs* 50μg/ml NER	$P<0.01$	$P<0.05$
Control *vs* 100μg/ml NER	$P<0.01$	$P<0.001$
Control *vs* 400μg/ml NER	$P<0.001$	$P<0.001$
Control *vs* 1μg/ml 13cisNER	ns	ns
Control *vs* 50μg/ml 13cisNER	$P<0.001$	$P<0.001$
Control *vs* 100μg/ml 13cisNER	$P<0.001$	$P<0.001$
Control *vs* 400μg/ml 13cisNER	—	$P<0.001$
Control *vs* 50μg/ml OHNER	$P<0.01$	ns
Control *vs* 100μg/ml OHNER	$P<0.001$	$P<0.001$
Control *vs* 1μg/ml 4HPR	ns	ns
Control *vs* 50μg/ml 4HPR	$P<0.001$	$P<0.001$
Control *vs* 100μg/ml 4HPR	$P<0.001$	$P<0.001$
Control *vs* 50μg/ml NBR	ns	ns
Control *vs* 100μg/ml NBR	$P<0.001$	ns
Control *vs* 400μg/ml NBR	—	$P<0.001$
Control *vs* 1μg/ml TZR	ns	ns
Control *vs* 50μg/ml TZR	$P<0.05$	$P<0.001$
Control *vs* 100μg/ml TZR	$P<0.001$	$P<0.001$

[a]Data from present experiment (see Table 1).
ns $P>0.05$.

(1 μg/ml). This is in agreement with similar experiments using 1 μg/ml 13-CRA in rat embryo culture (Webster 1985) and 0.6 to 6.0 μg/ml 13-CRA in mouse embryo culture (Goulding & Pratt 1986). 4HPR and TZR were embryotoxic at 50 μg/ml. By contrast, higher concentrations of the retinamides NER, 13cisNER, NBR and OHNER were required to produce embryotoxicity.

The teratogenicity of retinoids *in vitro* to some extent reflects their activities *in vivo*. For example, *in vivo* the trans isomers of some retinoids are more teratogenic than those with the cis configuration (Willhite & Shealy 1984), although the stereoconformation was not considered to be the major determinant of teratogenicity. In the present *in vitro* experiments (Tables 1 and 2) the cis isomer of retinoic acid (13-CRA) was slightly less toxic than the trans isomer although both were toxic at 1 μg/ml. In contrast, both the 13-cis and all-trans isomer of NER were equally toxic at 100 μg/ml (Table 2).

Willhite *et al.* (1984) showed that structural modifications of the terminal group at C15 did not necessarily prevent teratogeni-

Table 4. Acidic pK_a values of the retinoids tested

	pK_a	Lowest toxic concentration in embryo culture (μg/ml)
TRA	4	$\leqslant 1$
13-CRA	3.8[a]	
Etretin	5.3[a]	
4HPR	10	50
TZR	15	
OHNER	15	
NER	22	
13cisNER	22	
NBR	22	$\geqslant 100$
ETR	—	

[a]Values supplied by Roche Products Ltd; all other figures are estimated values based on model compounds or the Taft equation (Perrin *et al.* 1981).

city. Our experiments confirm this (see Fig. 1) as compounds with a carboxyl (TRA), hydroxyethyl (OHNER), hydroxyphenyl (4HPR) or a tetrazol (TZR) terminal group were all teratogenic.

It is known that the pK_a value is a major determinant of retinoid teratogenicity *in vivo* (Willhite *et al.* 1984); a low pK_a value is necessary for the attachment of the retinoid to a binding protein which is a possible prerequisite for teratogenicity. The actual or estimated pK_a values of the retinoids tested are given in Table 4. The three most toxic compounds (TRA, 13-CRA and etretin) had low pK_a values ranging from approximately 3 to 6. Those retinoids of intermediate toxicity (4HPR, TZR, OHNER, NER and 13cisNER) had higher pK_a values which ranged from 10 to 22. The two least toxic compounds were NBR and ETR; NBR had a pK_a value of 22 and ETR was estimated to have a pK_a value of this order of magnitude although a precise figure could not be obtained.

Metabolism, excretion and placental transfer of retinoids are clearly major factors influencing their teratogenicity in the whole animal, and account for any lack of correlation between the toxicologically active concentrations of individual retinoids reported here and their highest 'no-effect' doses *in vivo*. For example, TRA and 13-CRA adversely affected embryonic development *in vitro* at concentrations of 0.5 and 1.0 μg/ml, respectively (Tables 1 and 2), whereas the highest 'no-effect' doses *in vivo* in the rat using the oral route of administration are 6 and 50 mg/kg/day, respectively (Kamm 1982). The complete lack of teratogenicity of ETR *in vitro* at 100 μg/ml (Table 2), by contrast with the highest 'no effect' dose of 4 mg/kg/day *in vivo* (Kamm 1982), was the most striking discrepancy noted between the response of the rat embryo to a retinoid in culture and *in utero*. Embryo culture therefore provides a sensitive and controllable model in which to compare the toxicity and teratogenicity of active retinoids and their metabolites. In order to take account of the effects of maternal metabolism, further studies with embryos cultured in serum from retinoid-fed rats are in progress.

Acknowledgements

This work was financed in part with Federal funds from the American Department of Health and Human Services under contract numbers NO1 CP 75938 and NO1 CP 05602-56. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does the mention of trade names, commercial products or organizations imply endorsement by the US Government. We thank Mrs M. Fagg for typing the manuscript.

References

BERRETTI B. & GRUPPER CH. (1984) Cutaneous neoplasia and etretinate. In *'Retinoid Therapy: A Review of Clinical and Laboratory Research.'* Eds W.J. Cunliffe & A.J. Miller. Lancaster: MTP press. pp. 187–194.

BOLLAG W. (1983) Vitamin A and retinoids: from nutrition to pharmacotherapy in dermatology and oncology. *Lancet* i, 860–863.

Chen D.T. (1985) Human pregnancy experience with retinoids. In *Retinoids: New Trends in Research and Therapy*. Ed. J.H. Saurat. Basel: Karger. pp. 398–406.

CUNNINGHAM W.J. & EHMANN C.W. (1983) Clinical aspects of the retinoids. *Semin. Dermatol.* **2**, 145–160.

ELIAS P.M. & WILLIAMS M.L. (1981) Retinoids, cancer and the skin. *Arch. Dermatol.* **117**, 160–180.

GOULDING E.H. & PRATT R.M. (1986) Isotretinoin teratogenicity in mouse whole embryo culture. *J. Craniofac. Genet. Dev. Biol.* **6**, 99–112.

HICKS R.M., CHOWANIEC J., TURTON J.A., MASSEY E.D. & HARVEY A. (1982) The effect of dietary retinoids on experimentally induced carcinogenesis in the rat bladder. In *Molecular Interrelations of Nutrition and Cancer*. Eds. M.S. Arnott, J. van Eys & Y. -M. Wang. New York: Raven Press. pp. 419–447.

HICKS R.M., TURTON J.A., CHOWANIEC J., TOMLINSON C.N., GWYNNE J., NANDRA K., CHRYSOSTOMOU E. & PEDRICK M. (1985) Modulation of carcinogenesis in the urinary bladder by retinoids. In *Retinoids, Differentiation and Disease*. London: Pitman (Ciba Foundation Symposium 113). pp. 168–190.

HUMMLER H. & SCHÜPBACH M.E. (1981) Studies in reproductive toxicology and mutagenicity with Ro10-9359. In *Retinoids: Advances in Basic Research and Therapy*. Eds C.E. Orfanos *et al.* Heidelberg: Springer-Verlag. pp. 49–59.

KAMM J.J. (1982) Toxicology, carcinogenicity and teratogenicity of some orally administered retinoids. *J. Am. Acad. Dermatol.* **6**, 652–659.

KAMM J.J. & ASHENFELTER K.O. (1984) Preclinical and clinical toxicology of selected retinoids. In *The Retinoids*. Vol. 2. Eds M.B. Sporn, A.B. Roberts & D.S. Goodman. New York: Academic Press. pp. 287–326.

KOCHHAR D.M., NAU H., PENNER J.D., HICKEY T. & DANSKY L. (1985) Developmental toxicity of retinoids and mode of action. *Teratology* **31**, 25A–26A.

LAMMER E.J., CHEN D.T., HOAR R.M., AGNISH N.D., BENKE P.J., BRAUN J.T., CURRY C.J., FERNHOFF P.M., GRIX A.W., LOTT I.T., RICHARD J.M. & SUN S.C. (1985) Retinoic acid embryopathy. *N. Engl. J. Med.* **313**, 837–841.

LOWRY O.H., ROSEBROUGH N.J., FARR A.L. & RANDALL R.J. (1951) Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

MOON R.C. & ITRI L.M. (1984) Retinoids and cancer. In *The Retinoids*. Vol. 2. Eds M.B. Sporn, A.B. Roberts & D.S. Goodman. New York: Academic Press. pp. 327–371.

MORRISS G.M. & STEELE C.E. (1974) The effect of excess vitamin A on the development of rat embryos in culture. *J. Embryol. exp. Morph.* **32**, 505–514.

MORRISS G.M. & STEELE C.E. (1977) Comparison of the effect of retinol and retinoic acid on post-implantation rat embryos *in vitro*. *Teratology* **15**, 109–120.

MORRISS G.M. & THOMSON A.D. (1974) Vitamin A and rat embryos. *Lancet* **ii**, 899–900.

NEW D.A.T. (1978) Whole-embryo culture and the study of mammalian embryos during organogenesis. *Biol. Rev.* **53**, 81–122.

NEW D.A.T., COPPOLA P.T. & COCKCROFT D.L. (1976) Comparison of growth *in vitro* and *in vivo* of post-implantation rat embryos. *J. Embryol. exp. Morph.* **36**, 133–144.

PECK G.L. (1981) Retinoids in clinical dermatology. In *Progress in Diseases of the Skin*. Vol. 1. Ed. R. Fleischmajer. New York: Grune and Stratton. pp. 227–269.

PECK G.L. (1984) Synthetic retinoids in dermatology. In *The Retinoids*. Vol. 2. Ed. M.B. Sporn, A.B. Roberts & D.S. Goodman. New York: Academic Press. pp. 391–411.

PERRIN D.D., DEMPSEY B. & SERJEANT E.P. (1981) *pK_a Prediction for Organic Acids and Bases*. London: Chapman & Hall. p. 37.

ROSA F.W. (1984) A syndrome of birth defects with maternal exposure to a vitamin A congener: isotretinion. *J. clin. Dysmorphology* **2**, 13–16.

ROSA F.W., WILK A.L. & KELSEY F.O. (1986) Teratogen update: vitamin A congeners. *Teratology* **33**, 355–364.

SPORN M.B. & ROBERTS A.B. (1983) Role of retinoids in differentiation and carcinogenesis. *Cancer Res.* **43**, 3034–3040.

STEELE C.E. (1985*a*) Human serum as a culture medium for rat embryos. *Experientia* **41**, 1601–1603.

STEELE C.E. (1985*b*) The role of post-implantation mammalian embryo culture in the study of teratogenic mechanisms. In *Prevention of Physical and Mental Congenital Defects, Part C: Basic and Medical Science Education and Future Strategies*. New York: Alan R. Liss Inc. pp. 271–276.

STEELE C.E., MARLOW R. TURTON J. & HICKS R.M. (1987) Comparison of the teratogenicity of trans-retinoic acid and synthetic retinoids using whole embryo culture. In *Pharmacokinetics in Teratogenesis*. Eds H. Nau & W. Scott. Florida: Uni-Science Series CRC Press. In press.

STEELE C.E. & NEW D.A.T. (1974) Serum variants causing the formation of double hearts and other abnormalities in explanted rat embryos. *J. Embryol. exp. Morph.* **31**, 707–719.

STEELE C.E., PLENEFISCH J.D. & KLEIN N.W. (1982) Abnormal development of cultured rat embryos in rat and human sera prepared after vitamin A ingestion. *Experientia* **38**, 1237–1239.

STEELE C.E., TRASLER D.G. & NEW D.A.T. (1983) An *in vivo/in vitro* evaluation of the teratogenic action of excess vitamin A. *Teratology* **28**, 209–214.

WEBSTER W.S. (1985) Isotretinoin embryopathy: the effects of isotretinoin and 4-oxo-isotretinion on post-implantation rat embryos *in vitro*. *Teratology* **31**, 59A.

Willwhite C.C., Dawson M.I. & Williams K.J. (1984) Structure-activity relationships of retinoids in developmental toxicology. I. Studies on the nature of the polar terminus of the vitamin A molecule. *Toxicol. Appl. Pharmacol.* **74**, 397–410.

WILLHITE C.C. & SHEALY Y.F. (1984) Amelioration of embryotoxicity by structural modification of the terminal group of cancer chemopreventive retinoids. *J. Natl. Cancer Inst.* **72**, 689–695.