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BIOCHEMICAL MECHANISMS OF SALICYLATE TERATOLOGY IN THE RAT*

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Abstract—Acetylsalicylic acid, salicylic acid and EDTA have been previously reported to be teratogenic in the rat, and such was confirmed in this study. However, neither the *m*- nor the *p*-isomers of salicylic acid, the analogs (neither an SH nor an NH_2 group in place of OH, nor a $CONH_2$ group in place of COOH), nor metabolites of salicylic acid were teratogenic. It is concluded that the teratogenic effect of aspirin is due to salicylic acid, its hydrolysis product, and that addition, shifting or substituting functional groups on the aromatic ring eliminates the teratogenic activity. The possibility that these teratogens act through mineral chelation was considered. The agents were administered by s.c. injection followed by ^{54}Mn, ^{59}Fe and ^{65}Zn on day 9 or 16 of pregnancy in Sprague–Dawley rats. Measurements of urinary excretion and fetal uptake of the mineral isotopes were made at subsequent intervals. EDTA caused a marked increase in urinary excretion and a significant decrease in fetal uptake of all isotopes. Neither aspirin, salicylic acid, nor metabolites of aspirin, nor isomers, nor analogs of salicylic acid produced any significant alterations of mineral excretion or fetal uptake. It is postulated that while neither salicylic acid nor related compounds reduce fetal uptake of the minerals tested, they may still bind them in the maternal–fetal environment so that they are insufficiently available for normal biochemical functions of the developing fetus.

AMONG the known toxic and teratogenic drugs, the salicylates are of particular interest because of their extensive therapeutic use as analgesic, anti-pyretic, anti-inflammatory and anti-rheumatic agents. The salicylates have become by far the most commonly used drugs in the world today.[1] Their teratogenic properties were first discovered by Warkany and Takacs[2] in 1959. They demonstrated that subcutaneous injection of high doses of methyl or sodium salicylate into pregnant rats on day 9, 10 or 11 of pregnancy resulted in a variety of fetal malformations, including gastrochisis, craniorachischisis, hydrocephalus, exencephaly, spina bifida, ocular defects, facial clefts, cardiovascular anomalies, vertebral and costal anomalies. Following this discovery, several investigators studied the effects of various salicylates in different species, and have extended the findings of Warkany and Takacs[2] that the administration of salicylate compounds to the pregnant rodent can result in the production of young with congenital malformations.[3–12]

Since the metal chelating agent EDTA was discovered to be a teratogenic agent in the rat,[13,14] the metal chelating properties displayed by this agent were compared

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with those of aspirin, salicylic acid, its isomers, analogs and metabolites to elucidate possible relationships between chelation of minerals and teratogenesis.

MATERIALS AND METHODS

Chemicals. All drugs and related compounds were obtained from commercial sources, except 2,3,5-trihydroxybenzoic acid, which was kindly supplied by Dr. A. Kreuchunas.* Each reagent was dissolved in deionized water with the aid of a minimum amount of 0·75 N NaOH or 5% $NaHCO_3$ or of both. The solution was brought to volume with water to give a final concentration of 50 or 100 mg/ml.

A 125 mg/ml aspirin solution containing 62·5 μCi/ml of carboxyl-^{14}C-acetylsalicylic acid (86 μCi/mg) and a 177·4 mg/ml sodium salicylate solution containing 10 μCi/ml of carboxyl-^{14}C-salicylic acid (34·8 μCi/mg) were prepared.

Mineral isotope solutions of ^{54}Mn, ^{59}Fe and ^{65}Zn containing 1–2 μCi/ml were prepared in physiological saline. The ^{54}Mn and ^{65}Zn were carrier free and ^{59}Fe had a specific activity of 27 mCi/mg.

Animals. Adult female rats of the Sprague–Dawley strain, 100 days old and weighing between 190 and 220 g, were fed Purina laboratory chow and water *ad lib.* They were placed with males of demonstrated fertility of the same stock overnight, and day zero of pregnancy was designated as the day on which the spermatozoa were detected in the animal's vagina. The presence of a copulatory plug provided further confirmation of pregnancy.

Effect of drug administration on ^{54}Mn, ^{59}Fe and ^{65}Zn urinary excretion. All drugs and isotopes were administered subcutaneously. The dose level of all drugs, except for thiosalicylic acid, was equivalent to 380 mg/kg of aspirin. Thiosalicylic acid was administered at 170 mg/kg, since it is lethal at the higher level. The compounds were injected into pregnant rats in two equally divided doses, 2 hr apart. Immediately following the second dose, 1–2 μCi of the mineral isotope was administered. The rats were then placed into metabolic cages and their urines were collected and assayed for the mineral isotope content in a γ-scintillation counter with a 2-in. NaI (thallium-activated) crystal coupled to a Nuclear-Chicago single-channel analyzer, model 8725.

The rats were periodically weighed. On day 20 of gestation, they were sacrificed. The fetuses were removed and inspected noting death, resorption, as well as external congenital malformations.

Effect of drug administration on maternal–fetal uptake of ^{54}Mn, ^{59}Fe and ^{65}Zn on day 16 of pregnancy. Groups of pregnant rats on day 16 of gestation were given drug and isotope treatments similar to the experimental procedure explained above. Some rats were killed at 6 hr and some at 24 hr after completion of this treatment. Maternal–fetal fluids and tissues, namely maternal whole blood, amniotic fluid, urine, liver, kidney, placenta, yolk sac and fetuses, were weighed and the uptake of the isotopes was determined.

Similar pregnant rats served as controls, receiving physiological saline instead of the drug.

Incorporation of salicylate into the fetus. Groups of pregnant rats, two in each group, were subcutaneously injected with 380 mg/kg of acetylsalicylic acid in two

* For sources and further details, the reader is referred to the first author's dissertation: *Biochemical Mechanisms and the Possible Role of Mineral Chelation in Salicylate Teratology in the Rat*, Vanderbilt University (1972).

equally divided doses, 2 hr apart, on gestation days 12, 13, 14, 15 and 16. At 24 hr after the second dose, the rats were sacrificed by decapitation. The fetuses were then removed for total salicylate determination using the spectrofluorometric method described by Chirigos and Udenfriend.[15]

Another group of pregnant rats on day 16 of gestation were injected subcutaneously with 300 mg/kg of sodium salicylate (177·4 mg/ml) containing 10 μCi/ml of carboxyl-^{14}C-salicylic acid. Also a group of pregnant rats were injected with equimolar amounts of acetylsalicylic acid (125 mg/ml) containing 62·5 μCi/ml of carboxyl-^{14}C-acetylsalicylic acid. One hour later, the rats were sacrificed by decapitation. The fetuses were weighed, lyophilized, dissolved in 1 N NaOH, mixed with scintillation fluid Aquasol, and were counted for total ^{14}C incorporation by the β-scintillation technique, using a Packard Tri-Carb liquid scintillation counter, model 3320.

RESULTS AND DISCUSSION

Of the fifteen compounds listed in Table 1, which were administered to pregnant rats on day 9 of gestation, only salicylic acid, thiosalicylic acid and Na_2-EDTA resulted in marked maternal body weight loss during the advanced periods of pregnancy. Other effects of these three compounds as well as aspirin included loss of appetite, complete relaxation, weakness, drowsiness, muscular limpness, inactivity, accelerated respiration rate, and occasionally elevated water intake and urinary excretion. Also, rats that were treated with Na_2–EDTA exhibited shock, pain and abnormal behavior during and immediately after the injection.

The incidence of fetal resorptions and malformations is shown in Table 1. The former is expressed as the ratio of the fetuses resorbed to the total number of implantation sites on the uterus, and the latter, as the ratio of the living malformed fetuses to the total number of living fetuses. Also the mean fetus weight is shown in Table 1. Aspirin and salicylic acid were the only two drugs in this class of compounds that resulted in high incidence of both fetal malformations and resorption; they also caused abnormally small fetuses. None of the other metabolites, derivatives or analogs of salicylic acid resulted in fetal anomalies. Administration of 2,5- and 2,3-dihydroxybenzoic acids resulted in a high incidence of fetal resorption, but not as high as that of aspirin and salicylic acid. The results also show that, whereas aspirin administration produced a higher incidence of fetal anomalies than did salicylic acid, the latter produced a higher incidence of fetal resorptions. This difference may be explained by the diffusion rates of these two drugs into the embryonic circulation. While salicylic acid may exhibit direct toxic effect on the fetus, resulting in its death, aspirin may not exhibit the same toxic effects because of its slow enzymatic hydrolysis in the fetus to form salicylic acid. Salicylic acid can readily penetrate into the fetal circulation, as revealed by the ^{14}C studies shown in Table 2. Some remained 24 hr after the dose, as shown in Table 3. These assays at 24 hr were performed by the method of Chirigos and Udenfriend,[15] so that they represent salicylate only, and not possible metabolites. Na_2–EDTA administration at this high dose produced no fetal malformations, but a high rate of fetal resorption.

In Fig. 1, fetal abnormalities resulting from treatment with aspirin, salicylic acid and EDTA are illustrated. It is observed that there are very close similarities between the 20-day malformed fetus whose mother received 380 mg/kg of aspirin and a 20-day

TABLE 1. EFFECTS OF DRUGS AND RELATED COMPOUNDS ON THE RAT FETUSES

	Dose (mg/kg)	No. of rats	Dead mothers after treatment	Total no. of implantations	Mean fetus wt (g)	No. of fetuses (alive)	Resorption		Malformed fetuses	
							No.	Per cent of total implantations	No.	Per cent of total living fetuses
Control	380	15	0	172	3·89 ± 0·44	169	3	1·7	0	0
Aspirin	380	10	0	114	3·02 ± 0·70*	93	21	18·4	18	19·7
Salicylic acid	380	17	1	178	3·09 ± 0·93*	95	83	46·6	5	5·3
m-Hydroxybenzoic acid	380	10	0	123	3·96 ± 0·43	120	3	2·4	0	0
p-Hydroxybenzoic acid	380	10	0	106	3·85 ± 0·31	103	3	2·8	0	0
Anthranilic acid	380	10	0	117	4·02 ± 0·47	112	5	4·2	0	0
Thiosalicylic acid	170	10	0	89	3·84 ± 0·24	84	5	5·6	0	0
Salicylamide	380	10	0	116	3·71 ± 0·53	113	3	2·6	0	0
2,3-Dihydroxybenzoic acid	380	10	0	127	4·12 ± 0·52	117	10	7·8	0	0
2,4-Dihydroxybenzoic acid	380	10	0	115	3·76 ± 0·46	111	4	3·4	0	0
2,5-Dihydroxybenzoic acid	380	12	2	107	3·88 ± 0·61	95	12	11·2	0	0
2,3,4-Trihydroxybenzoic acid	380	10	0	106	3·82 ± 0·44	103	3	2·8	0	0
2,3,5-Trihydroxybenzoic acid	380	10	0	104	4·13 ± 0·35	101	3	2·9	0	0
2,4,6-Trihydroxybenzoic acid	380	11	1	113	3·72 ± 0·70	109	4	3·5	0	0
Salicyluric acid	380	10	0	124	3·98 ± 0·56	121	3	2·4	0	0
Na_2–EDTA	380	10	0	115	3·99 ± 0·33	30	85	73·0	0	0

* Significantly different from control, $P < 0·01$.

TABLE 2. INCORPORATION OF ^{14}C-ACETYLSALICYLIC ACID AND ^{14}C-SALICYLIC ACID INTO 16-DAY RAT FETUSES 1 hr AFTER DRUG ADMINISTRATION

	Dose (mg/kg)	No. of rats	% Injected ^{14}C dose/g dry wt of fetal tissue
Aspirin	391	5	3·11 ± 0·94
Salicylic acid	300 (equimolar)	4	4·06 ± 0·87

TABLE 3. SALICYLATE UPTAKE BY RAT FETUSES 24 hr AFTER ADMINISTRATION OF 380 mg/kg ASPIRIN

Day of treatment	Day of sacrifice	Av wt of fetus (g)	Uptake (μg/g)	Uptake (μg/fetus)
12	13	0·07	23·51 ± 14·64	1·64 ± 1·02
13	14	0·15	9·60 ± 3·92	1·44 ± 0·59
14	15	0·30	8·39 ± 3·92	2·52 ± 1·17
15	16	0·48	7·44 ± 3·76	3·57 ± 1·17
16	17	0·84	5·44 ± 4·18	4·57 ± 3·51

malformed fetus whose mother received 291 mg/kg of salicylic acid. This amount of salicylic acid is equimolar to the larger quantity of aspirin. A 20-day malformed fetus whose mother received 350 mg/kg of Na_2–EDTA is also shown. It is observed that there are distinct differences between this malformed fetus and that of the aspirin-treated fetus. In Fig. 1 is also shown a 20-day fetus whose mother received 100 mg/kg of aspirin daily from day 4 through day 11 of pregnancy. The size and weight of the fetus were significantly less than those of the control 20-day fetus. Other investigators

TABLE 4. EFFECTS OF DRUGS AND RELATED COMPOUNDS ON URINARY EXCRETION OF ^{54}Mn, ^{59}Fe AND ^{65}Zn IN THE RAT ON DAY 9 OF PREGNANCY

	No. of rats	^{54}Mn	^{59}Fe	^{65}Zn
Control	15	0·067 ± 0·08	0·33 ± 0·23	0·42 ± 0·26
Aspirin	10	0·078 ± 0·04	0·49 ± 0·33	0·67 ± 0·27
Salicylic acid	16	0·150 ± 0·16	0·37 ± 0·16	0·54 ± 0·29
m-Hydroxybenzoic acid	10	0·142 ± 0·08	0·39 ± 0·16	0·65 ± 0·30
p-Hydroxybenzoic acid	10	0·091 ± 0·06	0·62 ± 0·36	0·44 ± 0·23
Anthranilic acid	10	0·050 ± 0·04	0·43 ± 0·20	0·38 ± 0·19
Thiosalicylic acid	10	0·058 ± 0·05	0·55 ± 0·19	0·53 ± 0·28
2,3-Dihydroxybenzoic acid	10	0·102 ± 0·11	0·47 ± 0·20	0·40 ± 0·28
2,4-Dihydroxybenzoic acid	10	0·059 ± 0·06	0·32 ± 0·13	0·30 ± 0·19
2,5-Dihydroxybenzoic acid	12	0·081 ± 0·07	0·38 ± 0·21	0·51 ± 0·28
2,3,4-Trihydroxybenzoic acid	10	0·141 ± 0·14	0·38 ± 0·17	0·50 ± 0·31
2,3,5-Trihydroxybenzoic acid	10	0·069 ± 0·06	0·50 ± 0·23	0·46 ± 0·23
2,4,6-Trihydroxybenzoic acid	11	0·100 ± 0·09	0·33 ± 0·17	0·41 ± 0·22
Salicyluric acid	10	0·087 ± 0·08	0·44 ± 0·17	0·37 ± 0·14
Na_2–EDTA	10	40·483 ± 7·74*	46·55 ± 5·87*	69·78 ± 7·85*

* Significantly different from control, $P < 0·01$.

TABLE 5. ^{54}Mn CONTENT IN DIFFERENT MATERNAL–FETAL ORGANS AND URINE 6 hr AFTER INJECTION OF DRUGS AND RELATED COMPOUNDS IN 16-DAY PREGNANT RATS

	Dose (mg/kg)	No. of rats	Whole blood	Kidney	Liver	Placenta	Fetus	Yolk sac	Amniotic fluid	Fetal–placental unit	Urine
Control		3	0·0046 ± 0·001	2·5 ± 0·4	2·9 ± 0·6	0·37 ± 0·03	0·35 ± 0·05	0·71 ± 0·17	0·001 ± 0·001	0·34 ± 0·01	0·02 ± 0·01
Aspirin	380	3	0·0033 ± 0·0009	2·0 ± 0·2	2·6 ± 0·5	0·35 ± 0·04	0·31 ± 0·09	0·50 ± 0·27	0·001 ± 0·001	0·34 ± 0·07	0·05 ± 0·04
Salicylic acid	380	3	0·0033 ± 0·0006	1·8 ± 0·2	2·3 ± 0·4	0·34 ± 0·05	0·29 ± 0·05	0·54 ± 0·08	0·001 ± 0·001	0·30 ± 0·08	0·04 ± 0·03
m-Hydroxybenzoic acid	380	3	0·0036 ± 0·0008	2·0 ± 0·5	2·0 ± 0·4	0·41 ± 0·08	0·33 ± 0·04	0·64 ± 0·14	0·002 ± 0·001	0·30 ± 0·05	0·04 ± 0·03
p-Hydroxybenzoic acid	380	3	0·0038 ± 0·0013	2·6 ± 0·5	3·0 ± 0·5	0·36 ± 0·05	0·41 ± 0·10	0·69 ± 0·07	0·002 ± 0·001	0·36 ± 0·10	0·03 ± 0·03
Anthranilic acid	380	3	0·0040 ± 0·0016	2·1 ± 0·4	2·1 ± 0·7	0·40 ± 0·08	0·37 ± 0·07	0·71 ± 0·16	0·001 ± 0·001	0·34 ± 0·03	0·02 ± 0·01
Thiosalicylic acid	120	3	0·0047 ± 0·001	1·9 ± 0·3	1·9 ± 0·4	0·34 ± 0·02	0·28 ± 0·09	0·57 ± 0·21	0·002 ± 0·001	0·29 ± 0·04	0·03 ± 0·02
Na_2–EDTA	200	4	0·0014 ± 0·0016*	0·7 ± 0·09†	0·8 ± 0·2†	0·47 ± 0·06	0·23 ± 0·03*	0·40 ± 0·06*	0·006 ± 0·002*	0·22 ± 0·24*	38·70 ± 3·80†

* Significantly different from control, $P < 0·05$, but not $P < 0·01$.
† Significantly different from control, $P < 0·01$.

have also reported reduced fetal weight as well as maternal body weight loss as a result of high salicylate administration during early stages of pregnancy in the rat.[16,17]

The effects of the salicylates, metabolites, derivatives and analogs as well as EDTA on the urinary excretion of ^{54}Mn, ^{59}Fe and ^{65}Zn are shown in Table 4. The values, expressed as per cent output per 24 hr of the administered dose, are the average for each group of rats with standard deviation. The results indicate that administration of neither aspirin, salicylic acid, its metabolites, derivatives nor analogs resulted in any significant increase in urinary output of these minerals. Na_2–EDTA administration, on the other hand, resulted in marked increase of these minerals in the urine.

The ^{65}Zn, ^{59}Fe and ^{54}Mn contents in different maternal–fetal organs and urine 6 hr after the administration of aspirin, salicylic acid, its derivatives and analogs, as well as Na_2–EDTA in 16-day pregnant rats are shown in Tables 5, 6 and 7. The values, expressed as per cent uptake of the injected dose per g of tissue, are the average for each group of rats with standard deviation. The results indicate that neither aspirin, salicylic acid, its derivatives nor analogs significantly alter maternal–fetal uptake of these minerals. However, Na_2–EDTA significantly lowered maternal–fetal uptake of these minerals with subsequent marked increase in their urinary excretion. Similar results were also obtained when pregnant rats were sacrified 24 hr after this treatment.

It was also observed that administration of 380 mg/kg of salicylic acid to pregnant rats on day 16 of pregnancy resulted in three incidents of hematuria, high rate of fetal mortality, and superficial fetal hemorrhage, which was occasionally observed along the brain and spine. These observations were also noted in fetuses whose mothers received high doses of thiosalicylic acid ranging between 120 and 170 mg/kg. Eriksson[18–21] recently showed that a high dose of sodium salicylate administered on days 16 and 17 of pregnancy induced fetal death and fetal hemorrhage in two mouse strains.

Since EDTA is not metabolized or conjugated in the body but quantitatively recovered unaltered in the urine,[22–25] it seems likely that the teratogenic activity attributed to it may be considered the result of metal chelation. Presumably, the excretion of these chelates from the body would result in metal suppression, and subsequent deprivation of the minerals to the developing embryo.

From this study several conclusions were drawn.

(1) Because acetylsalicylic acid and salicylic acid were the only agents in this class of compounds that induced similar fetal anomalies (Fig. 1), and since aspirin can readily undergo hydrolysis to form salicylic acid[26] which is excreted in large amounts as the unaltered compound, it is concluded that salicylic acid is the teratogenic agent. Kimmel *et al.*[27] showed that salicylic acid was the major metabolite that reached the embryo after oral administration of carboxyl-^{14}C-acetylsalicylic acid into pregnant albino rats. They also reported that when pregnant rats were given 510 mg/kg of benzoic acid followed by 250 or 500 mg/kg of aspirin, salicylic acid was the causative agent in aspirin teratogenesis.

(2) Although it may be argued that intake of large doses of salicylates is an unlikely occurrence, a woman may ingest large doses of salicylate during early pregnancy, as an excessive therapeutic measure. If we interpret the effects of salicylates in rats as described in this study, and relate them to the situation in man, the daily ingestion of large amounts of salicylates (e.g. 100 mg/kg/day, which is a high therapeutic dose

TABLE 6. ^{59}Fe CONTENT IN DIFFERENT MATERNAL–FETAL ORGANS AND URINE 6 hr AFTER INJECTION OF DRUGS AND RELATED COMPOUNDS IN 16-DAY PREGNANT RATS

	Dose (mg/kg)	No. of rats	Whole blood	Kidney	Liver	Placenta	Fetus	Yolk sac	Amniotic fluid	Fetal–placental unit	Urine
Control		5	0·21 ± 0·06	0·08 ± 0·02	0·21 ± 0·03	0·33 ± 0·07	1·03 ± 0·34	1·07 ± 0·18	0·008 ± 0·002	0·79 ± 0·25	0·13 ± 0·06
Aspirin	380	4	0·19 ± 0·04	0·07 ± 0·05	0·25 ± 0·12	0·40 ± 0·29	1·17 ± 0·79	1·10 ± 0·87	0·007 ± 0·004	0·75 ± 0·62	0·21 ± 0·02
Salicylic acid	380	4	0·22 ± 0·08	0·05 ± 0·03	0·19 ± 0·14	0·31 ± 0·19	0·93 ± 0·58	0·89 ± 0·52	0·006 ± 0·004	0·62 ± 0·39	0·22 ± 0·05
m-Hydroxybenzoic acid	380	3	0·26 ± 0·13	0·08 ± 0·05	0·22 ± 0·12	0·34 ± 0·07	0·96 ± 0·24	1·20 ± 0·66	0·007 ± 0·003	0·63 ± 0·14	0·18 ± 0·02
p-Hydroxybenzoic acid	380	4	0·26 ± 0·13	0·12 ± 0·07	0·39 ± 0·30	0·39 ± 0·20	1·13 ± 0·53	1·53 ± 0·95	0·011 ± 0·007	0·84 ± 0·47	0·19 ± 0·02
Anthranilic acid	380	3	0·27 ± 0·09	0·08 ± 0·01	0·24 ± 0·09	0·34 ± 0·08	1·06 ± 0·30	0·91 ± 0·15	0·007 ± 0·001	0·74 ± 0·19	0·19 ± 0·03
Thiosalicylic acid	120	4	0·26 ± 0·11	0·08 ± 0·04	0·25 ± 0·20	0·35 ± 0·29	0·79 ± 0·46	0·88 ± 0·50	0·011 ± 0·009	0·53 ± 0·33	0·19 ± 0·04
Na_2–EDTA	200	4	0·06 ± 0·01*	0·21 ± 0·04*	0·10 ± 0·02*	0·10 ± 0·01*	0·27 ± 0·05*	0·40 ± 0·04*	0·006 ± 0·001	0·17 ± 0·02*	47·77 ± 2·85*

* Significantly different from control, $P < 0·01$.

TABLE 7. ^{65}Zn CONTENT IN DIFFERENT MATERNAL–FETAL ORGANS AND URINE 6 hr AFTER INJECTION OF DRUGS AND RELATED COMPOUNDS IN 16-DAY PREGNANT RATS

	Dose (mg/kg)	No. of rats	Whole blood	Kidney	Liver	Placenta	Fetus	Yolk sac	Amniotic fluid	Fetal–placenta unit	Urine
Control		3	0·12 ± 0·02	2·1 ± 0·20	2·4 ± 0·47	0·67 ± 0·06	0·33 ± 0·04	0·93 ± 0·32	0·03 ± 0·005	0·29 ± 0·04	0·08 ± 0·04
Aspirin	380	3	0·10 ± 0·01	1·9 ± 0·08	3·5 ± 0·39*	0·70 ± 0·05	0·32 ± 0·05	0·93 ± 0·09	0·02 ± 0·005	0·28 ± 0·067	0·14 ± 0·07
Salicylic acid	380	3	0·08 ± 0·01	2·0 ± 0·15	3·5 ± 0·38*	0·64 ± 0·06	0·31 ± 0·009	0·88 ± 0·11	0·02 ± 0·007	0·28 ± 0·04	0·13 ± 0·08
m-Hydroxybenzoic acid	380	3	0·10 ± 0·01	2·2 ± 0·20	2·4 ± 0·50	0·60 ± 0·07	0·40 ± 0·08	0·93 ± 0·26	0·03 ± 0·004	0·30 ± 0·10	0·16 ± 0·10
p-Hydroxybenzoic acid	380	3	0·12 ± 0·03	2·3 ± 0·29	2·9 ± 0·47	0·68 ± 0·11	0·40 ± 0·04	1·04 ± 0·29	0·03 ± 0·003	0·30 ± 0·05	0·12 ± 0·05
Anthranilic acid	380	3	0·12 ± 0·01	2·0 ± 0·30	2·7 ± 0·32	0·70 ± 0·14	0·41 ± 0·11	0·90 ± 0·14	0·03 ± 0·001	0·29 ± 0·03	0·09 ± 0·07
Thiosalicylic acid	120	3	0·16 ± 0·03	1·9 ± 0·36	2·9 ± 0·24	0·66 ± 0·11	0·30 ± 0·03	0·99 ± 0·11	0·03 ± 0·007	0·28 ± 0·08	0·14 ± 4·07
Na_2–EDTA	200	4	0·012 ± 0·003†	0·63 ± 0·16†	0·19 ± 0·009†	0·009 ± 0·011†	0·04 ± 0·002†	0·23 ± 0·062†	0·009 ± 0·004†	0·050 ± 0·007†	72·15 ± 5·14†

* Significantly different from control, $P < 0·05$, but not $P < 0·01$.

† Significantly different from control, $P < 0·01$.

for treatment of rheumatic fever) in early pregnancy may have significant diverse effects on the developing embryo. Therefore, what one thinks of as a "safe" drug may in fact not be so innocuous.

(3) Since the *m*- and *p*-hydroxybenzoic acids did not induce fetal malformation, it appears that the COOH and the OH groups must be adjacent to one another for teratogenic and toxic effects to take place. Also, since anthranilic and thiosalicylic acid administration did not induce fetal malformations, it appears that substitution of the OH group by SH or NH_2 eliminates the teratogenic property. Also, since salicylamide administration did not induce fetal anomalies, it appears that substitution of the COOH group for $CONH_2$ eliminates the teratogenic activity. Also since administration of the dihydroxy and trihydroxybenzoic acids did not induce fetal anomalies, addition of OH groups to the salicylic acid molecule eliminates its teratogenic activity.

It is of interest to note that the effect of salicylate on the uncoupling reaction also requires the specific configuration of the molecule. Thus Brostoff *et al.*[28] observed that modification of the OH group or altering its position on the ring results in loss of uncoupling activity. It is not known whether the uncoupling reaction and teratology are related, but the fact that they follow similar patterns suggests that they may be.

(4) Comparison of the effects of salicylates and Na_2–EDTA on urinary excretion of minerals indicates that salicylate teratology cannot be related to direct elimination of minerals from the maternal rat. It may be concluded, therefore, that the stability constant *in vitro* of the salicylate to form a strong metal chelate complex cannot be the primary consideration *in vivo*, because of the many interfering complicating factors and uncontrolled parameters that come into play when the drug is introduced into the living organism. Further studies are required to prove or disprove the possibility of salicylates acting *in situ* by binding temporarily to macro-molecules that require metals for their biological functions, such as those involved in nucleic acid,[29–31] protein[32,33] and mucopolysaccharides.[34–36] Until then the possibility of salicylates and their metal complexes *in vivo* being factors in teratology remains a challenge for further investigation.

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