Khera, K.S. 1991

TERATOLOGY 44:259–297 (1991)

Chemically Induced Alterations in Maternal Homeostasis and Histology of Conceptus: Their Etiologic Significance in Rat Fetal Anomalies

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ABSTRACT Possible relationships between maternal acid-base-electrolyte imbalance, histological changes in the maternal/extraembryonic tissues (decidua, placenta, membranes enclosing cavities), and fetal anomalies induced by maternotoxic doses of ethylene glycol, sodium salicylate, and cadmium chloride in rats were investigated. Acid-base-electrolyte, histologic and, teratologic studies were conducted concurrently with, as far as feasible, a similar protocol. Ethylene glycol caused 1) maternal homeostatic changes including metabolic acidosis and hyperosmolality, 2) extraembryonic lesions with degeneration of allantois and reduced villigenesis being more prevalent, and 3) materno-fetal effects such as decreases in fetal and maternal body weights, decreased maternal food intake, and fetal abnormalities (vertebral, rib, and sternebral defects). Few of these changes occurred when $NaHCO_3$, an endogenous agent known to correct metabolic acidosis, was coadministered with ethylene glycol. Ethylene glycol-induced maternal metabolic acidosis, concurrent with hyperosmolality, was suspected to contribute toward reduction in villigenesis and fetal anomalies, including body weight reductions. Sodium salicylate induced the following: 1) mild maternal acidosis, hypokalemia, and hypophosphatemia with no significant change in pH; 2) maternal hemorrhage in extraembryonic cavities, papillary proliferation of the visceral yolk sac endoderm, and failure to form the chorioallantoic labyrinth; and 3) resorptions, hydrocephaly, rib defects, and fetal body weight reduction. Upon simultaneous treatment with sodium salicylate, $NaHCO_3$ significantly reduced, and NH_4Cl enhanced the incidence of the above histologic and teratologic effects, without significantly altering acid-base values. An etiologic association between the above salicylate-induced maternal and extraembryonic lesions and teratogenicity was likely. Cadmium chloride, whether administered by the intraperitoneal (ip) or intravenous (iv) route, caused 1) hydrocephaly, anophthalmia, vertebral and rib defects, reduction in fetal body weight, resorptions and maternal toxicity (acute peritonitis by the ip route only), and 2) extensive necrosis and hemorrhage in the decidua basalis, hemorrhage in the ectoplacental cone and around Reichert's membrane, and absence of chorioallantoic labyrinth. An etiologic relationship between these teratologic and histologic effects seemed probable, since both were dose-related. From the above studies, it was hypothesized that maternal factors—metabolic acidosis, hyperosmolality, hemorrhages in the ectoplacental cone, extraembryonic cavities, and around Reichert's membrane, and necrosis of decidua basalis—may have, directly or indirectly, reduced fetal nutrition and materno-embryonic gaseous exchange, which ultimately altered fetal development.

Received December 4, 1990; accepted March 19, 1991.

The acid-base, electrolyte, and oxy/carboxyhemoglobin equilibria of the maternal organism are among the homeostatic components that can possibly influence the physiology and morphology of embryonic and extraembryonic tissues. As in all rodents, the extraembryonic parts of the rat conceptus consist of the following: ectoplacental cone, chorion, and the enclosed ectoplacental cavity (chorioallantoic placenta); mural trophoblast; Reichert's membrane; visceral and parietal layers of the yolk sac and the enclosed yolk-sac cavity; the coelomic cavity and the amnion and its cavity. Extraembryonic tissue forms an integral part of the life support system essential for the maintenance, nourishment, and protection of the embryo. Accordingly early development mainly is devoted to formation of extraembryonic tissue. As pregnancy proceeds, development of the extraembryonic tissue continues to enlarge the maternofetal link that transmits the increasing O_2 and nutritional demands of the fetus.

A relationship between placental pathology and fetal outcome, mostly investigated in human pregnancies, so far has been controversial (Bennington, '78). This is because of differences in methods of placental examination and definitions of placental lesions and a current opinion that the placenta may conduct its function adequately, if a pathologic conditions is limited in extent ("placental reserve"). However, in well-defined human studies, placental lesions have been associated with perinatal deaths, with implications of a cause and effect relationship (Little, '60; Gruenwald, '63; Singh and Carr, '67; Philippe and Boué, '69; Honoré et al., '76), and with intra-uterine growth retardation (Khong et al., '86). In rat studies, placental necrosis has been reported to cause fetal death (Parizek, '65; Levin et al., '81). It is instructive to note that the lesions have to be sufficiently extensive to countervail the placental reserve and thus impair placental function.

The role of decidual tissue and extraembryonic membranes in fetal development is at present not clear. Decidual tissue of the rat secretes luteotropin hormone (Herz et al., '86) with a prolactin-like activity which presumably is involved in fetal growth of a large number of species (Talamantes et al., '80) and in the salt and water metabolism of human fetus (Tyson et al., '84). Suppression or degeneration of decidualization resulting in arrested pregnancy has been attributed to serotonin in rats (Mitchell et al., '83) and DL III-IT in rats and hamsters (Galliani et al., '86). It is noteworthy that limited necrosis and the presence of inflammatory cells are a normal event in the decidual tissue of humans (McCombs and Craig, '64) and rats (Everett, '35; Bulmer and Dickson, '61). Development of extraembryonic membranes in rats has received limited attention, with only the parietal (Jollie, '68; Clark et al., '75; Jensh et al., '77) and the visceral yolk sac layers (reviewed, Jollie, '90) having been studied. Disturbances in the function of visceral yolk sac layer have been reported to cause fetal malformations (Beck et al., '67; Brent et al., '71).

A vast majority of mechanistic studies in the literature have generally concentrated on embryo examinations with the assumption that the primary lesion that initiated a fetal malformation (including resorption and reduced body weight) almost always is located in the embryo itself. There is no denying that this assumption may be valid for certain teratogenic chemicals. Whether all teratogens would produce malformations only by their direct action on embryonic tissue, exclusive of any other tissue—maternal and extraembryonic, for instance—is an open question.

In the study here reported, three chemicals, ethylene glycol, sodium salicylate, and cadmium chloride, whose teratogenicity has been reported in several in vivo and whole embryo culture studies (for references, Schardein, '85; Shepard, '89), were investigated. Herein reported histologic changes in extraembryonic tissues and maternal pathophysiology were found associated with fetal anomalies. This association introduces a possibility that the histologic/physiologic changes at maternal and extraembryonic locations may contribute toward fetal anomalies.

MATERIALS AND METHODS

Rats

Virgin female Sprague-Dawley rats, weighing 225–275 g, were obtained from Charles River, St. Constant, Quebec. They were paired overnight with proven breeders. The morning of finding a positive vaginal smear was day 1 of pregnancy. All rats had free access to tap water and Purina lab chow 5001. They were housed in a thermostatically maintained room at 22 ± 1°C, il-

luminated with a 12 h per day light cycle regulated by a time switch.

Chemicals

The following test chemicals were used in the investigation of changes in mothers and conceptuses: 1) ethylene glycol, 2) sodium salicylate, and 3) cadmium chloride, containing 2.5% H_2O; the following ancillary chemicals were administered in conjunction with a test chemical: 1) Ammonium chloride (NH_4Cl), lot 15F-0538, and 2) sodium bicarbonate ($NaHCO_3$), lot 104031/13250. The sodium bicarbonate was from BDH, Toronto, Ontario, and the rest were from Sigma, St. Louis, Missouri. The chemicals were either analytical reagents or of an equivalent grade. They were dissolved in distilled water for all routes of dosing.

Type of studies

Homeostasis was determined in cannulated pregnant rats. Blood was measured for acid-base equilibrium and hemoglobin content and serum for osmolality, electrolytes, and other serum components. Since the kidney is the key organ in the maintenance of homeostasis, its function was assessed by conducting urinalysis and monitoring serum for blood urea nitrogen (BUN) and creatinine.

The teratology study was performed by dosing a test agent during pregnancy and evaluation of developmental effects in term fetuses.

Histopathology was on conceptuses in situ, still enclosed in the uterine capsule.

STUDY OF HOMEOSTASIS

Cannulation of carotid artery

The aorta of mated female rats was cannulated with cannulas of about 18 cm in length cut from intramedic polyethylene tubing, PE50 (Clay Adams, Parsipanny, NJ). Their arterial end was slightly tapered and they were filled with sterile solution of calcium salt of heprin (20 IU/ml). The tapered end was placed in the aortic blood stream through the left carotid artery (Popovic and Popovic, '60) of dams while under light anesthesia from inhalation of a halothane-oxygen mixture. The cannula was held in place by ligatures tightly tied around the carotid artery that contained the inserted cannula. The other end of the cannula was tunneled through the subcutaneous tissue of the left side to exit at the back of the neck. The cannula was ligated with the skin at its exit to prevent ejection from the carotid artery. The external free end of the cannula was sealed with heat. The rats were allowed a period of 24–48 h to recuperate from surgery and anesthesia and to resume normal activity before blood was taken. The rats were able to move around freely in their cages and had access to food and water ad libitum.

Collection of blood and urine

Rats were placed in individual metabolic cages. Urine was collected for the time-period between the administration of test drug and withdrawl of the last blood sample for analysis and, quite often for the 24 h predosing interval. For collection of blood samples, conscious rats were placed in a restraining device making every effort to minimize struggling and avoid hyperventilating apnoea, which changes blood gas values. The lumen of the cannula was clamped with a hemostat and then the sealed end was cut. The arterial blood quickly entered the cannula on releasing the hemostat. Under sterile conditions, 1.25–2.0 ml of blood was collected anaerobically in a plastic syringe with a needle, both previously rinsed with calcium heparinate saline solution (1,500 IU/ml). In test rats, the withdrawn blood was replaced, via infusion, with an equivalent volume from similarly pretreated and cannulated nonpregnant donor rats, in an effort to maintain normal extracellular fluid volume and constant concentration of the test chemical in the blood compartment. During the interval between the withdrawl of blood from the donor and transfusion into the test rat, generally requiring less than 10 min, the donor's blood was stored at 37°C. Each time when the blood was withdrawn, the cannula was flushed with heparinized saline (20 IU/ml), and the free end sealed. Blood samples were obtained for three to five of the following postdosing time intervals tested: 1, 3, 5, 6, 7, 9, 24 or 48 h after dosing. The rats were always sampled in the same sequence. The postdosing data are presented as the time-points when the blood collection was started, although, at each time, it took about ½–1 h to process all samples. Analyses, mostly in duplicates, were done using at least 5 dams for each experimental group.

After obtaining the required blood samples and urine, the rats were killed to deter-

mine the presence of pregnancy. Data from nonpregnant rats were discarded.

Analysis of blood and plasma samples

Arterial blood gases

The blood samples were analyzed for pH and partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) within 15 min of collection while stored on ice. The three variables were measured independently of one another by a blood gas analyzer (IL 1306, Instrument Laboratory, Lexington). The blood gas analyzer adjusted blood gas values for changes in total hemoglobin content since it was interfaced to the co-oximeter system (see below) to receive hemoglobin data. The blood gas analyzer was calibrated periodically using commercial standards.

Hemoglobin and its species

The concentration of total hemoglobin and its constituents, oxyhemoglobin, carboxyhemoglobin, methemoglobin, and reduced hemoglobin, were measured with an automated IL482 CO-Oximeter system (Instrument Laboratory, Lexington). The instrument was calibrated in accordance with the manufacturer's instructions at weekly intervals. The co-oximeter data were transmitted to the acid gas analyzer as discussed above.

Osmolality

Osmolality (mOsm/kg H_2O), based on freezing point depression, was measured with a computerized micro-osmometer Model 2430 (Multi-osmette™) (Precision Systems, Natick) using 30 μL serum samples. The Multi-osmette was calibrated with standard control fluids included with each run.

Electrolytes and other serum components

Na^+, K^+, Mg^{2+}, Cl^-, and Creatinine. Na^+ and K^+ (mmol/L) were measured with a Flame Photometer IL 943 (Instrumentation Laboratory S.P.A. Via Socrate, Milano, Italy), using a 20 μL serum sample. Manufacturer's standards were employed for calibration. Concentrations of Mg^{2+} (mmol/L), Cl^- (mmol/L), and creatinine (mg/dL) in serum samples were measured with the synchron CX^R systems (Beckman Instruments, 1989, Brea).

Ca^{2+}, glucose, BUN, phosphate, and total protein. Concentrations of all the above serum components were measured with an ABBOTT chemistry analyser (Abbott Laboratories, Mississauga, Ontario) using 35 μL of serum. Total protein was measured in g/dL, while the remaining values were in mg/dL.

Osmolal gap

The osmolal gap, or the difference between measured and calculated osmolality (Δosm), was determined by the equation:

$$\text{Measured osmolality} - \frac{\text{calculated osmolality}}{0.93} = \text{osmolal gap}$$

The measured osmolality, which represents the number of molecules of a solute dissolved in a solvent, was determined by measuring the freezing point of serum. The calculated osmolality of the serum was determined from known concentrations of the major osmolal constituents of serum, i.e., sodium (mEq/L), glucose ((mg/dL), and urea (BUN, mg/dL):

$$\text{osm (calculated)} = 1.86\ \text{Na} + \frac{\text{glucose}}{18} + \frac{\text{BUN}}{2.8}\ \text{(details: Glasser et al., '73)}$$

The calculated serum osmolality is then converted to osmolality for water (as mOsm per kg H_2O) by dividing by 0.93, since the concentration of water in serum is approximately 93%.

Anion gap

The anion gap, defined as the numerical difference between routinely measured anions and cations, is useful in differentiating various metabolic acid-base disorders (Emmett and Narins, '77). In normal serum, the difference between the sum of cations (Na^+, K^+, Ca^{2+}, and Mg^{2+}) and the sum of anions (Cl^- and HCO_3), all measured in, or converted to, mEq/L, reflects the presence of albumin and inorganic (SO_4^{2-}, PO_4^{2-}) and organic anions which are not routinely measured but are present in the plasma. In the present study, the anion gap, calculated with the above formula, was determined for a treatment group and compared with that of its control.

Urinalysis

The kidney, being a major determinant of bicarbonate concentration in the blood,

plays an essential role in the stabilization of the body's acid-base and electrolyte status. The function of the maternal kidney was assessed indirectly from analysis of urine (quantity excreted, pH, osmolality, Na^+ and K^+ concentrations) and serum (BUN and creatinine concentration) and the quantity of water consumption. The sediment from urine held overnight at 6°C was examined with a microscope under phase contrast illumination.

TERATOLOGY STUDIES

The three test chemicals are known teratogens following single and repeated administration to rats. The extended dosing period proved difficult for conducting acid-base-electrolyte studies, since cannulas failed to function for such periods. Therefore, a similar dose-duration-route combination for each chemical was determined in preliminary experiments and was used in the three types of studies, i.e., acid-base-electrolyte, teratology, and histopathology. The teratology studies were conducted following recognized procedures (Khera et al., '89) with fetuses being recovered on day 22 of pregnancy; two-thirds were examined for skeletal defects and the remainder for visceral anomalies.

The term "fetal anomalies," for the purpose of this paper, is used in a generic sense to denote one or more of the following: visceral and skeletal fetal abnormalities (including variants and aberrations), fetal body weight reduction, and resorptions.

DECIDUAL AND PLACENTAL HISTOLOGY

Dams were killed by asphyxiation in 100% CO_2, generally 24 or 48 h after the single or last dose of the test agent. The uteri from 3–8 test dams/group were perfused with 10% neutral buffered formalin under gentle manual pressure through a 20 ml syringe with a 23 gauge needle inserted in the abdominal aorta. The aorta was clamped close to the diaphragm and the inferior vena cava incised to allow the blood to escape. The entire uterus, with conceptuses in situ, was then removed and immersed in a large volume of 10% neutral buffered formalin (pH 7.0) for 3 weeks. Each conceptus, still enclosed in the uterine capsule, was dehydrated and embedded in paraffin. Serial 5 μm thick sections, perpendicular to the long axis of the uterus, were cut and stained with hematoxylin and eosin. A composite observation of serial sections aided in defining the size and course of allantois and vascular channels, extent of lesions, and interrelation of conceptal tissues.

The terminology and development of rat conceptus already has been well documented (Duval, 1891; Gérard, '25; Bridgman, '48, '49; Wimsatt, '62; Deane et al., '62; Kirby and Malhotra, '64; Wynn, '65; Davies and Glasser, '68; Ramsey, '82; Jollie, '90) and formed the basis of this study. However, the following events, which will be frequently referred to, are defined for clarity. The ectoplacental cavity is delimited (1) mesometrially by the ectoplacental cone, an invasive spearhead of trophoblastic tissue penetrating the decidua basalis of the endometrium, and (2) antimesometrially by the chorion (or "true chorion," or "lamina" of Everett, '35), an inwardly concave epithelioid layer derived from nonvascular extraembryonic ectoderm. The chorion unites with the allantois i.e., the forerunner of the umbilicus. Allantoic villi, each with a fetal capillary in the core, invade chorion, and together with trophoblastic derivatives become the chorionic trabeculae (Everett, '35; Mossman, '37). The whole complex of chorioallantoic trabeculae together with intertrabecular maternal blood spaces constitutes the labryinth of the chorioallantoic placenta.

A new term "conceptal," an adjective of conceptus, is used for convenience of description.

Experimental protocol

Ethylene glycol

Ethylene glycol was tested on the day 11 of pregnancy using the following doses and routes: (1) *acid-base-electrolyte study*—1,250, 2,500, or 5,000 mg/kg orally (po), or 3,333 mg ethylene glycol/kg subcutaneously (sc), either alone or simultaneously with $NaHCO_3$ po; (2) *teratology*—2,800 or 3,333 mg/kg sc, either alone or simultaneously with $NaHCO_3$ po; and (3) *histopathology*—3,333 mg/kg sc, either alone or simultaneously with $NaHCO_3$ po, in a single dose study, and 5,000 mg ethylene glycol/kg po administered alone from day 7 to 13 of pregnancy, in a multiple dose study. $NaHCO_3$, an endogenous substance that corrects metabolic acidosis in humans (Morosetti et al., '86), was tested to determine whether it could reduce the acid-base-electrolyte imbalance and the incidence of fetal anomalies

caused by ethylene glycol. The $NaHCO_3$ was administered po in all ethylene glycol studies, as an initial aqueous bolus of 530 mg $NaHCO_3$/kg body weight by gavage, supplemented with the drinking solution containing 2.65 mg $NaHCO_3$/ml, until 24 h postdosing in both the teratology and single-dose histopathology studies, and until 9 h postdosing, i.e., the end of the acid-base-electrolyte study.

Sodium salicylate

Sodium salicylate, 280 mg/kg/day, as a single sc dose was administered either alone or in combination with 0.473% NH_4Cl (unless stated otherwise) or 1.68% $NaHCO_3$ in a drinking solution as follows.

Acid-base-electrolyte study. In the sodium salicylate + NH_4Cl combination, salicylate was administered on day 8 and 9 of pregnancy together with NH_4Cl solution given from day 7 to 10 of pregnancy. The analyses were done at 3, 7, 9, and 24 h after the first and at 24 h after the second salicylate dose (b) sodium salicylate + $NaHCO_3$ combination. Salicylate was injected in 3 separate experiments on day 8, or 8 and 9, or 8–10 of pregnancy with $NaHCO_3$ solution in these experiments given from day 7 of pregnancy until 24 h after the last salicylate dosing. The 3 experiments gave similar results; therefore combined data are presented.

Teratology and histopathology. Salicylate was administered on day 8, 9, and 10 of pregnancy in combination with either NH_4Cl (concentration reduced to 0.16% in histopathology) or $NaHCO_3$ given from day 7 to 11 of pregnancy. Appropriate controls were included in each study.

Cadmium chloride ($CdCl_2$)

A bacteria-free aqueous solution of $CdCl_2$, at 1.25 ml/kg body weight was injected on the day 10 of pregnancy; the (1) *acid-base-electrolyte study* was performed only at an ip dose of 5.5 mg/kg; and the (2) *teratology and histopathology studies* were conducted by injecting $CdCl_2$ solution ip in the abdominal region, iv in the jugular vein, or sc in the interscapular neck region. The doses were: 3, 4, 5, or 5.5 mg/kg ip, 2.6 or 4.5 mg/kg iv, and 5.5, 12, 16, or 20 mg/kg sc.

Statistical analysis

The arithmetic mean (M) and standard error (SE) were calculated for each treatment and control group at each postdosing interval. The *t* test was used to compare the results between treatment and control groups for each after-dosing time-point. Variations between successive measurements within each experimental group underwent an analysis of variance and significant differences between means were determined by comparisons. All data were analysed on a Peach IV Executive computer using a commercial programme prepared for the F and unpaired *t* tests for unequal variances. Only test and control differences at $P \leq .05$ are reported.

RESULTS

Effects of inserting cannula and repeated blood sampling

A body weight loss of 17 ± 9 g, attributed to surgical intervention, was observed for the 70 control rats cannulated on the day 12 of pregnancy and weighing 318 ± 25 g (mean ± SE). However, the surgery and indwelling cannula did not cause any significant change in the measured blood values, since the control values were comparable with those for rats cannulated by different techniques (Pepelko and Dixon, '75; Brun-Pascaud et al., '82; Girard et al., 1983) or for a different species (O'Brien et al., '79; Bar-Ilan and Marder, '80).

Blood constitutes about 8% of the body weight and its repeated withdrawal without replacement in conscious rats has been reported to alter both the cardiac index and rate of metabolism (Walsh et al., '80). In our studies, the withdrawl of 1.25–2 ml of blood followed by substitution with an equivalent quantity of whole blood from a donor rat was found to result in a mildly inadequate

Abbreviations

A	allantois
AC	amniotic cavity
AM	angiogenic mesoderm
C	chorion
cc	extraembryonic coelom
DB	decidua basalis
E	ectoplacental cavity
EPC	ectoplacental cone
L	labyrinth
MBS	maternal blood space
R	recess around the Reichert's membrane
RM	Reichert's membrane
T	trophoblast
V	allantoic or chorionic villi
vysl	visceral yolk sac layer
ysc	yolk sac cavity

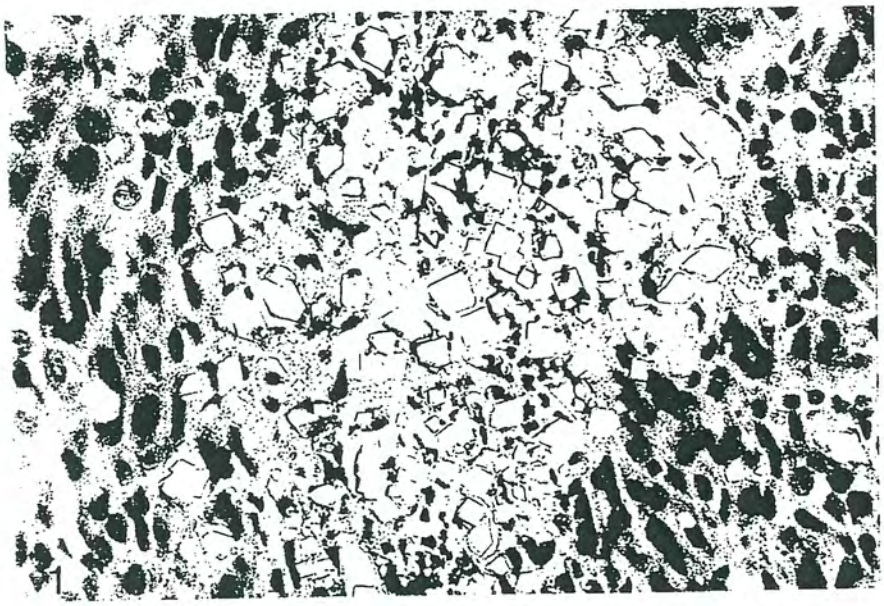


Fig. 1. Square and bipyrimidal crystals in decidua capsularis of a control conceptus. Day 12 of pregnancy in rat. Magnification, ×320.

restitution of hemoglobin and caused a slight rise in blood pH. The latter effect was probably due to an increased HCO_3^- reabsorption by the proximal renal tubules resulting from the initial contraction of the extracellular fluid volume. Our data suggested that obtaining samples of blood at frequent intervals combined with replacement only had a minimal effect on the endpoints measured, and these effects appeared to result from a decreased total hemoglobin.

rystals in urine and histologic sections

Crystals were found in the urine and in histologic sections of conceptuses from control and all treated groups. They were tentatively classified according to shape and arrangement: 1) needles, "dumbell," or "hemp seed," distributed individually or in rosettes and sheaves, were similar to those reported and identified as calcium oxalate monohydrate (Godolphin et al., '80), 2) octahedral, bipyramidal, or envelope (square or rectangular) dispersed individually similar to those described (Hodgkinson, '77; Colando, '79) as calcium oxalate dihydrate, and 3) singly disposed crystals of different shapes and sizes that remained unidentified. All three types occurred in the sediment of urine stored overnight at 6°C and were readily visible with phase contrast illumination. The incidence of type 1, 2, and 3 crystals was 9%, 31%, and 28%, respectively. The first two types of crystal were common in the urine of rats dosed with ethylene glycol: 5,000 mg/kg po and 3,333 mg/kg sc.

In the histologic sections of test and control conceptuses, the unidentified crystals, in varying numbers, were observed in 40 of 124 conceptuses (32%). They were square, rectangular, and bipyramidal in appearance and stained with eosine (Fig. 1). They were surrounded by fibroblastic cells, suggestive of an inflammatory response, and were site specific in occupying the decidua capsularis immediately exterior to the Reichert's membrane at the embryonal pole in proximity to a blood vessel. Their possible relation to fetal anomalies in the control or test groups is not known.

ETHYLENE GLYCOL

Maternal toxicity reduced by $NaHCO_3$

Ethylene glycol, 5,000 mg/kg po or 3,333 mg/kg sc, administered to rats on day 11 of pregnancy depressed reflexes and caused ataxia and lethargy within 25 min of dosing, as previously reported (Winek et al., '78). These symptoms were less marked at

doses of 2,500 and 1,250 mg/kg po and 2,800 mg/kg sc.

$NaHCO_3$ was previously reported to cause a reduction in ethylene glycol-induced acute toxicity in rats (Borden and Bidwell, '68) also had a similar effect in 11 day pregnant rats. All four dams survived following a po $NaHCO_3$ treatment combined with 5,000 mg ethylene glycol/kg sc dose whereas all 4 dams given ethylene glycol alone died.

Homeostasis and teratology

Ethylene glycol alone

Blood samples taken from dams following a single oral dose of 1,250, 2,500, or 5,000 mg/kg on day 11 of pregnancy showed the presence of hyperosmolality at 1, 3, 6, and 9 h postdosing, which was accompanied by metabolic acidosis as evident from the reductions of pH, PCO_2 and HCO_3^- (Fig. 2). The PO_2 values, higher than the minimal normal of 97 mm Hg, suggested that acidosis was not due to a lack of oxygen in the arterial blood.

The osmolal gap, calculated from the measured values of Na^+, glucose, and BUN, was dose-relatedly higher in the ethylene glycol groups than in the control group at 3 h but returned to predosing level by 9 h after dosing (Table 1). This increase in the osmolal gap was not accounted for by ethylene glycol-induced changes in the concentration of sodium, glucose, or urea, as was evident from the calculations. Consequently it may be due to 1) a decrease in the proportion of water in the plasma (Smithline and Gardner, '76), or 2) the presence of ethylene glycol or its metabolites in the plasma.

The anion gaps calculated from mEq/L concentrations of $(Na^+ + K^+ + Ca^{2+} + Mg^{2+}) - (Cl^- + HCO_3^-)$ were as follows:

3 h postdosing
5,000 mg/kg = (127 + 5 + 7 + 0.5)−(109 + 16) = 15.5
control = (132 + 4 + 6 + 0.5)−(108 + 23) = 11.5
9 h postdosing
5,000 mg/kg = (132 + 4 + 6 + 0.5)−(118 + 13) = 11.5
control = (132 + 4 + 5 + 0.5)−(108 + 25) = 8.5

The anion gap, being comparable between the test and control groups, was therefore not effected by ethylene glycol at the 5,000 mg/kg or at the lower doses (data not shown), suggesting that the ethylene glycol-induced metabolic acidosis was a "normal anion gap acidosis."

Urinalysis. Ethylene glycol caused dose-related diuresis and a significant decrease in osmolality ($P < .001$), without causing any important change in pH, Na^+, and K^+ values. The sediment from urine was found to contain tetragonal, octahedral, "envelope," "dumb-bell" monoclinic, or prismatic-shaped crystals. The crystals were more frequent and predominent in the 5,000 mg/kg group, but were also found in the sediment from control and lower dosed animals.

Ethylene glycol combined with $NaHCO_3$

Dams were simultaneously administered $NaHCO_3$ and ethylene glycol in an acid-base-electrolyte and a teratology study. The following results were obtained. The exogenous $NaHCO_3$ significantly ameliorated, but did not completely eliminate the ethylene glycol-induced maternal changes in osmolality, pH, osmolal gap (Fig. 3), and concentrations of HCO_3^-, glucose, and BUN (Table 2). In the teratology study, ethylene glycol alone at 3,333 mg/kg sc killed 3 of the 13 dams tested, but the 2,800 mg/kg sc dose was non-maternolethal. The simultaneous $NaHCO_3$ treatment reduced the ethylene glycol-induced maternal toxicity, loss in fetal body weight (significant at the 3,333 mg/kg, $P < .05$) and the frequency of fetuses with reduced number of ribs (8–12 ribs/fetus) and fused ribs (Table 3). The incidences of retarded ossifications in sternebrae, vertebrae, metacarpals, and metatarsals were reduced and the reduction in some cases was statistically significant ($P < .05$). Higher concentrations of $NaHCO_3$ were not investigated since they are liable to expand the extracellular space (Goidsenhoven et al., '54), increase uterine vascular resistance (Buss et al., '75), and to produce, in conjunction with ethylene glycol, an uninterpretable mixed acidosis-alkalosis disorder.

Histopathology

Ethylene glycol (3,333 mg/kg sc) alone or combined with $NHCO_3$

24 h postdosing. The size of the chorion, its penetration by the proliferating and arborizing allantoic villi (villigenesis), and the erythroblastic population in the allantois were reduced to varying degrees. Lesions that were less frequent, yet perhaps no less important to the final outcome, were karyorrhexis and pyknosis of mesodermal cells in the allantoic bulb (A in Fig. 4), ab-

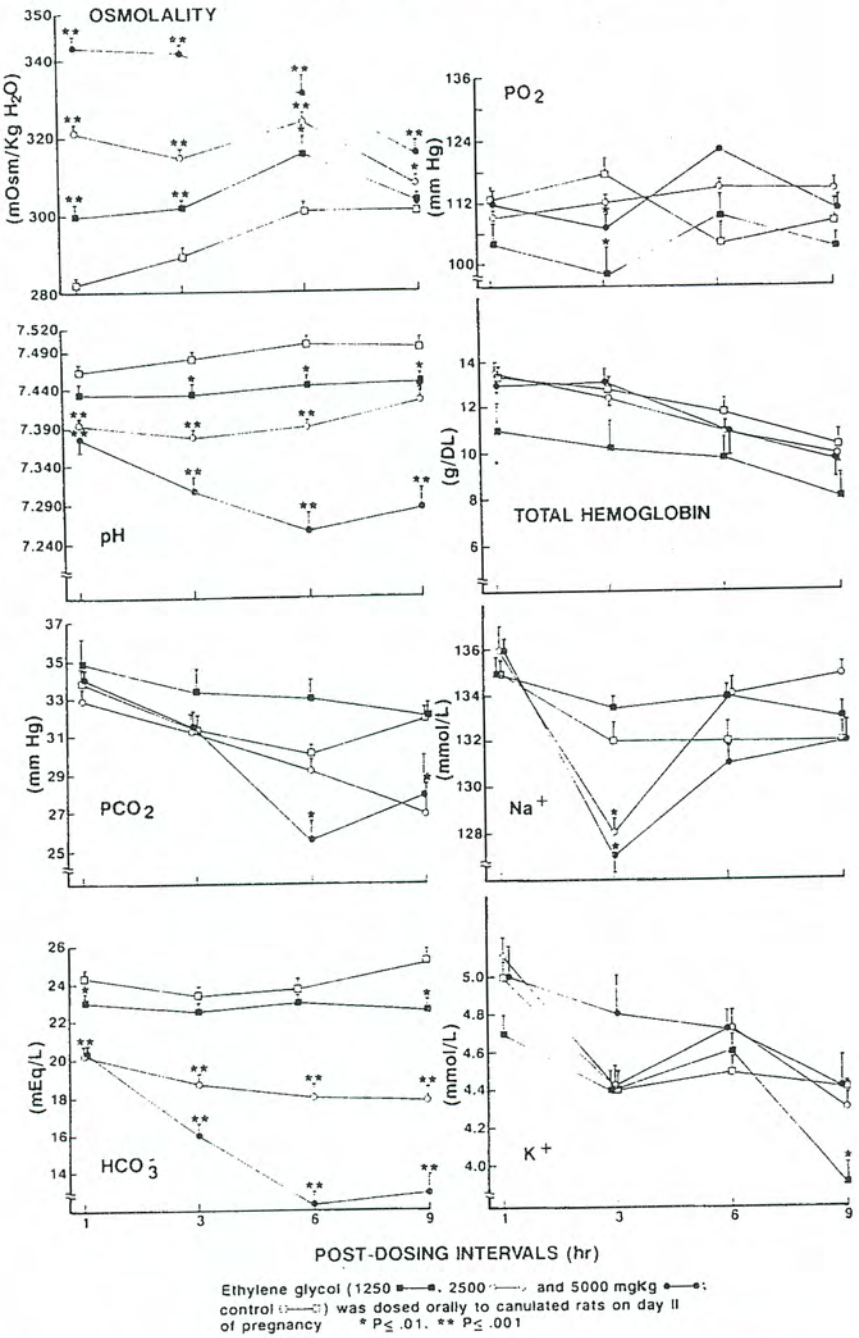


Fig. 2. Ethylene glycol-induced changes in acid-base balance and electrolytes.

sence of chorioallantoic fusion, placental thrombosis, and maternal hemorrhage in the yolk sac cavity (Fig. 5) (Table 4). Necrotic foci were observed in the decidua basalis of dams that received a combined $NaHCO_3$ + ethylene glycol treatment.

TABLE 1. Ethylene glycol-induced osmolal gap in cannulated rats dosed on day 11 of pregnancy (n = 5–8 dams/group)

Postdosing interval (h)	Treatment group (mg/kg)	Measured osmolality mOsm/kg H_2O (mean ± SE)	Na^+, glucose, BUN (mean values)[1]	Calculated osmolality (mean ± SE)[2]	Osmolal gap (measured-calculated osmolality) (mean ± SE)
3	0 (control)	289 ± 2	132, 129, 17.5	278 ± 1	12 ± 1
	1,250	302 ± 2**	133.5, 134, 16	281 ± 1	21 ± 2*
	2,500	315 ± 2**	128, 131, 16	270 ± 1*	44 ± 1**
	5,000	342 ± 2**	127, 140, 19	270 ± 2*	71 ± 3**
9	0 (control)	301 ± 1	132, 137, 14.5	278 ± 1	24 ± 2
	1,250	305 ± 2	133, 144, 16	281 ± 2	23 ± 1
	2,500	308 ± 2*	135, 131, 19	285 ± 1**	23 ± 2
	5,000	316 ± 3**	132, 142, 23	281 ± 5	31 ± 7

[1]Values measured were mmol/L for Na^+ and mg/dl for glucose and BUN.

[2]Calculated osmolality = $\left(1.86\ Na^+ \frac{\text{glucose}}{18} + \frac{\text{BUN}}{2.8}\right) \div 0.93$.

*$P < .01$.
**$P < .001$.

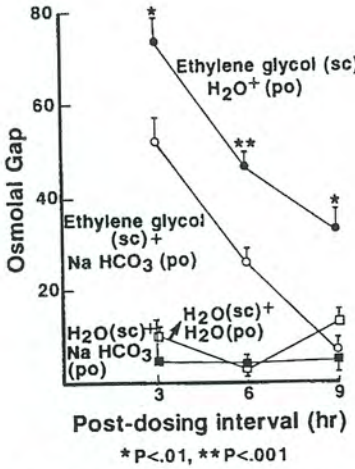


Fig. 3. Reduction of ethylene glycol-induced osmolal gap by simultaneous dosing with $NaHCO_3$ po.

None of the above lesions were observed in conceptuses from dams treated only with $NaHCO_3$ or untreated control.

48 h postdosing. The size of the labyrinth and degree of villigenesis was decreased in conceptuses whose dams were treated with ethylene glycol (Figs. 6, 7). The cross-sectional area of the fetal capillaries, relative to maternal blood spaces in the labyrinth, was quantitated by image analysis with a KONTRON Bildanalysis videoplan workstation (Carl Zeiss, Don Mills). Random fields of the labyrinth from a microscopic section were projected by a television camera on to a video screen at about 50-fold magnification. The magnified images of blood channels, recognized as maternal by the presence of erythrocytes and as embryonal by a content of nucleated erythroblasts, were traced at their perimeters by moving the cursor's highlight, visible on the video screen. A preset software program calculated and analysed the traced mm^2 areas. An imaged area was the total area, 150 mm^2, on the videoscreen that was magnified from 3.026 mm^2 of labyrinth on the microscopic slide.

For an experimental group, at least 8 fields per conceptal labyrinth from 3–4 conceptuses, each from a different dam, were measured (Table 5). Parts of the labyrinth at the periphery (being too small to cover the entire imaged area) and at the center (if a maternal blood space was too big to occupy most of the imaged area) were not included. It was conservatively concluded that, in the ethylene glycol-dosed group, the total cross-sectional area of fetal vascular spaces and the number of fetal vascular spaces in each imaged area were significantly reduced and the cross-sectional area of maternal vascular spaces had increased. The simultaneous $NaHCO_3$ treatment (Table 5) increased the ratio of the total vascular area/imaged area for both the dam and fetus. The significant reduction in maternal/fetal vascular ratio suggested that $NaHCO_3$ had increased the villous area at the expense of maternal blood space area, i.e., fetal capillaries or the villigenesis in the maternal blood spaces increased with a concomitant reduction in the size of the maternal spaces. As a consequence, the surface area of exchange be-

TABLE 2. Acid-base and electrolyte changes following combined or individual dosing with ethylene glycol (E.G.) (3,333 mg/kg, SC) and $NaHCO_3$ (530 mg/kg PO) on gestation day 11 in rats[1]

	Treatment groups															
	Time interval post-dosing															
	1 h				3 h				6 h				9 h			
Indices measured	H_2O + H_2O[2]	H_2O + $NaHCO_3$	Ethylene glycol + H_2O	Ethylene glycol + $NaHCO_3$	H_2O + H_2O	H_2O + $NaHCO_3$	Ethylene glycol + H_2O	Ethylene glycol + $NaHCO_3$	H_2O + H_2O	H_2O + $NaHCO_3$	Ethylene glycol + H_2O	Ethylene glycol + $NaHCO_3$	H_2O + H_2O	H_2O + $NaHCO_3$	Ethylene glycol + H_2O	Ethylene glycol + $NaHCO_3$
mosmo kg-H_2O	285 ± 3	283 ± 6	359 ± 2***	346 ± 6*	283 ± 3	276 ± 1	347 ± 3***	333 ± 7*	281 ± 3	278 ± 3	330 ± 5***	308 ± 5*	283 ± 4	278 ± 2	307 ± 3*	288 ± 7
pH	7.472 ± .002	7.544 ± .017	7.409 ± .008***	7.474 ± .018**	7.481 ± .009	7.522 ± .004	7.262 ± .020***	7.411 ± .017**	7.479 ± .003	7.497 ± .007	7.143 ± .022***	7.499 ± .047**	7.482 ± .005	7.481 ± .003	7.117 ± .047***	7.437 ± .015***
PCO_2 (mm Hg)	31.0 ± .2	34.7 ± 1.3	29.2 ± .2	33.5 ± .4**	29.3 ± .7	34.3 ± .6	26.7 ± 1.1	29.8 ± 1.3	30.6 ± .7	34.7 ± 1.2	24.3 ± 1.3*	25.9 ± 2.2	30.6 ± 1.1	31.6 ± .6	19.1 ± .6***	26.9 ± .4***
PO_2 (mm Hg)	113 ± 1	106 ± 1	111 ± 1	102 ± 6	114 ± 1	110 ± 2	132 ± 2*	123 ± 8*	98 ± 2	101 ± 3	135 ± 2	123 ± 5*	98 ± 2	102 ± 3	139 ± 3***	112 ± 4**
HCO_3 (meq-L)	23.6 ± .3	30.1 ± .2	18.5 ± .3**	24.9 ± 1.1***	22.8 ± .3	28.5 ± .4	12.2 ± .5**	19.1 ± .6*	23.4 ± .4	27.1 ± .5	8.5 ± .6***	17.9 ± .3***	24.2 ± .6	23.8 ± .3	6.5 ± .8***	19.9 ± 1.2***
Total Hb (g/dl)	14.2 ± .2	13.7 ± .3	14.5 ± .9	14.3 ± .5	13.2 ± .1	12.3 ± .3	13.8 ± .5	13.0 ± 1.7	11.7 ± .2	11.8 ± .4	12.8 ± .9	11.7 ± 1.7	10.7 ± .1	10.4 ± .5	11.7 ± .9	10.1 ± 1.9
Na^+ (mmol-L)	132 ± 1	135 ± 1	134 ± 1	136 ± 1	130 ± 1	129 ± 1	128 ± 1	132 ± 1	132 ± 1	132 ± 2	132 ± 1	133 ± 1	127 ± 1	130 ± 1	127 ± 2	132 ± 2
K^+ (mmol-L	5.1 ± 0.2	3.9 ± .1	5.0 ± 1.0	4.9 ± .40	4.4 ± .02	3.7 ± 1.0	4.3 ± .20	3.8 ± .20	4.6 ± .2	4.1 ± .1	4.4 ± .2	4.2 ± .3	4.6 ± .14	3.9 ± .04	4.6 ± .1	4.0 ± .4
Ca^{2+} (mg dl)	—[3]	—	—	—	12.7 ± 0.6	12.1 ± .2	12.2 ± .2	12.3 ± .2	11.9 ± .2	11.5 ± .1	12.1 ± .3	12.5 ± .3	10.3 ± .5	10.4 ± .4	11.8 ± .2*	12.0 ± .2*
Mg^{2+} (mmol L)	—	—	—	—	0.82 ± .02	0.77 ± .02	0.90 ± .04	0.83 ± .02	0.85 ± .03	0.70 ± .02*	0.86 ± .05	0.80 ± .04	0.74 ± .03	0.70 ± 0	0.90 ± .07	0.78 ± .03
Cl^- (mmol L)	—	—	—	—	102 ± 4	100 ± 1	102 ± 2	102 ± 1	105 ± 2	98 ± 1	107 ± 2	108 ± 2	106 ± 2	100 ± 3	115 ± 4	109 ± 2
Glucose (mg dl)	—	—	—	—	136 ± 6	132 ± 3	169 ± 8*	168 ± 4	140 ± 5	126 ± 1	182 ± 7**	171 ± 6**	144 ± 11	132 ± 3	189 ± 17*	150 ± 5
Phosphate (mg dl)	—	—	—	—	6.2 ± .5	6.3 ± .4	6.1 ± .4	5.6 ± .4	6.4 ± .5	6.2 ± 1	6.6 ± .2	5.7 ± .2	6.4 ± .3	6.1 ± .2	6.9 ± .6	5.7 ± .4
Total protein (g dl)	—	—	—	—	5.79 ± .20	5.83 ± .14	6.34 ± .77	5.75 ± .41	6.11 ± .262	5.41 ± .49	7.10 ± .44	6.55 ± .24	6.725 ± .756	6.43 ± .41	7.65 ± .86	5.94 ± .29
BUN (mg-dl)	—	—	—	—	15.0 ± 1.5	13.8 ± .6	18.5 ± .6*	15.3 ± .5*	14.7 ± 1.2	11.6 ± .8	21.7 ± 1.5**	16.0 ± .4*	15.8 ± 1.3	13.2 ± 1.0	23.3 ± 1.2**	20.3 ± 0.9
Creatinine (mg/dl)	—	—	—	—	0.52 ± .04	0.45 ± .02	0.54 ± .02	.41 ± .02	.48 ± .06	.48 ± .02	.50 ± .0	.43 ± .02	.48 ± .03	.47 ± .02	.59 ± .02	.41 ± .02

[1]Additional $NaHCO_3$ consumption via drinking water in the 9 h experimental period for two groups, i.e., ethylene glycol + $NaHCO_3$ and H_2O + $NaHCO_3$, was, respectively, 51 and 7 mg/kg body weight.
[2]H_2O, being solvent for both EG and $NaHCO_3$, was administered sc and po, and in quantities equivalent to those used for dissolving EG and $NaHCO_3$, respectively.
[3]— represents analysis not done.
*$p < .05$.
**$p < .01$.
***$p < .001$ (Statistical comparisons: EG + H_2O versus either H_2O or EG + $NaHCO_3$).

TABLE 3. Teratology study[1]

	Treatment groups (mg/kg)					
	H_2O po + Ethylene glycol (2,800) sc	$NaHCO_3$ (530) po + Ethylene glycol (2,800) sc[2]	H_2O po + Ethylene glycol (3,333) sc	$NaHCO_3$ (530) po + Ethylene glycol (3,333) sc[2]	$NaHCO_3$ (530) po + H_2O sc[2]	H_2O po + H_2O sc
Maternal data						
No. of dams at term/dead	15/0	14/0	10/3	10/0	12/0	12/0
Body weight: loss/dam at 24 h postdosing (g), mean	8	11	15	8	7	6
Fluid (water or 0.265% aqueous $NaHCO_3$) intake/dam for 24 h postdosing (ml) mean ± SE	44.2 ± 2.5	46.6 ± 4.6	47.8 ± 5.9	51.9 ± 6.2	44.6 ± 1.1	57.2 ± 7.2
Food consumption/dam for first and 2nd day post dosing (g), mean ± SE	Not done	Not done	7.5 ± 1.8 15.6 ± 2.9	9.2 ± 1.5 23.1 ± 0.9*	24.7 ± 1.0 21.8 ± 1.5	23.0 ± 1.6 23.6 ± 1.2
Fetal data						
Live fetuses/pregnancy, mean ± SE	13.1 ± 0.9	12.9 ± 1.2	11.9 ± 1.1	11.9 ± 0.7	12.8 ± 0.4	13.2 ± 0.6
Resorptions per litter, mean	0.4	0.4	1.0	0.6	0.7	0.4
Fetal weight (g), mean ± SE	4.8 ± 0.2	5.1 ± 0.1	4.6 ± 0.1	4.9 ± 0.1**	5.2 ± 0.1	5.2 ± 0.1
(i) Visceral examination						
No. showing hydroureter/ No. examined	5/61	5/64	6/37	3/36	4/48	0/48
(ii) Skeletal examination						
No. with skeletal anomalies/ No. examined (fetuses)[3]	55/136	20/128*	70/82	46/83*	11/106	4/110
Anomalies, percent						
Ribs						
Reduced No. (8–12)	25	1	30	6	0	0
Fused	27	9*	35	13	1	0
Supernumerary	5	4	13	16	4	4
Wavy	0	1	4	1	3	0
Sternoschisis	0	0	2	1	0	0
Retarded ossification, percent (0/0)						
Sternebrae	10	0	26	5*	4	1
Vertebrae: centrum						
Cervical	71	51*	82	54	14	29
Thoracic	60	32*	67	29*	3	0
Lumbar	4	1	18	11	0	0
Metacarpal	28	16	32	23	18	19
Metatarsal	28	16	35	24	18	21

[1]Ethylene glycol-induced maternal and fetal effects and their reduction by simultaneous $NaHCO_3$ treatment administered on the 11th day of pregnancy in rats.
[2]$NaHCO_3$ dosing consisted of an initial po bolus of 530 mg/kg, and additional consumption via drinking water during 24 h postdosing period, which can be calculated from "fluid intake" data given in the third row of this table.
[3]Fetuses showing only retarded ossifications were not included.
*$P < .05$.
**$P < .01$ (only comparisons of H_2O + ethylene glycol group with $NaHCO_3$ + ethylene glycol group are shown).

tween the apposing maternal and fetal vascular channels had significantly increased.

Ethylene glycol (500 mg/kg/day po)

Twelve conceptuses from dams dosed with 500 mg/kg/day for days 7–13 of pregnancy, and collected 24 h after the last dose were studied. Except one which had no lesions, all other conceptuses consistently showed a marked reduction in the width of the labyrinth and a small fetus. The labyrinth consisted of larger maternal spaces and proportionately smaller and fewer allantoic villi. The basal zone of the placenta (outer placental layer of spongioblasts) was reduced. An intraplacental hematoma from a maternal hemorrhage was observed in 2 conceptuses. One of the hematomas contained sheaves or tufts of crystals morphologically similar to those of calcium oxalate (mono- and dihydrate, Fig. 8).

SODIUM SALICYLATE

Homeostasis

Sodium salicylate, administered alone, decreased PCO_2 and HCO_3^- concentrations at 3, 7, and 9 h postdosing in one experiment

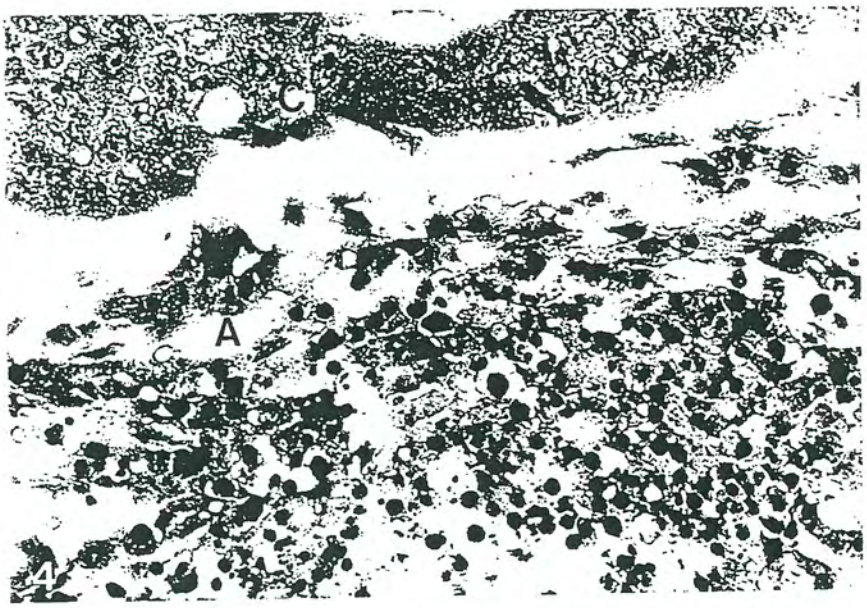


Fig. 4. Ethylene glycol, 3,333 mg/kg administered sc on day 11 of pregnancy. Karyorrhexis and pyknosis of allantois (lying opposite to the chorion) 24 h postdosing. Magnification, ×320.

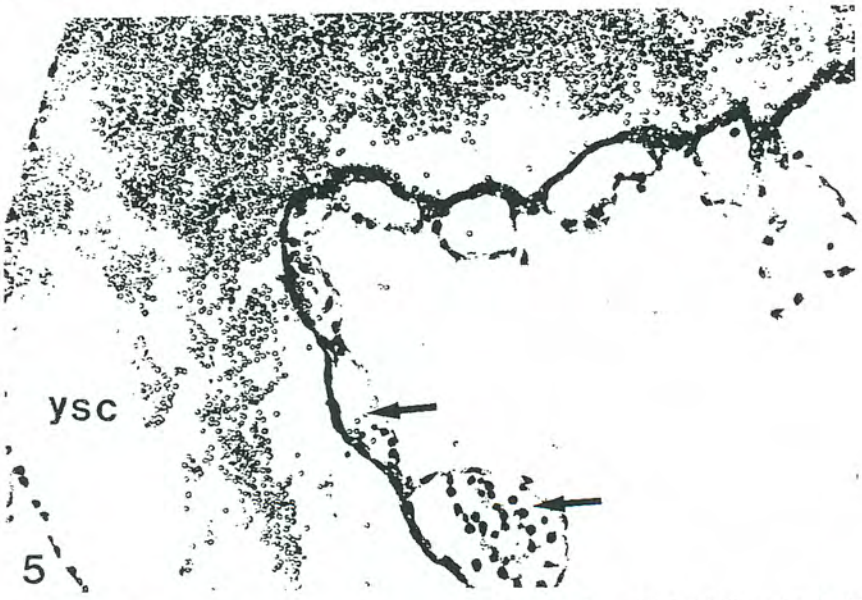


Fig. 5. Ethylene glycol, 3,333 mg/kg administered sc on day 11 of pregnancy. Maternal hemorrhage in the yolk sac cavity, 24 h postdosing. Vitelline vessels (arrows). Magnification, ×56.

($P < .05$–$.001$, Table 7) and, in addition, at 24 and 48 h postdosing in the second experiment ($P < .001$, Table 6). The reduced HCO_3^- concentration suggested a mild metabolic acidosis. Whether the accumulated anions that reduced the HCO_3^- were derived from metabolism of sodium salicylate or from an increased endogenous metabolism owing to salicylate-induced uncoupling of oxidative phosphorylation remains specula-

TABLE 4. Lesions in the conceptus 24 h postdosing[1]

Treatment groups	Ethylene glycol + H_2O	Ethylene glycol + $NaHCO_3$	H_2O + $NaHCO_3$	H_2O + H_2O
A. No. of conceptuses effected/No. examined	7/8	4/12	0/7	0/6
B. No. of conceptuses showing lesions				
1. Chorioallantoic labyrinth:				
(a) Small-sized containing fewer erythroblasts	7	0	0	0
(b) Reduced villigenesis	7	0	0	0
(c) Presence of thrombus	2	1	0	0
2. Allantois (chorionic end):				
(a) Karyorrhexis and pyknosis	2	0	0	0
(b) Absence of fusion with chorion	1	0	0	0
3. Yolk sac cavity and coelome:				
Presence of maternal erythrocytes	1	0	0	0
4. Decidua basalis: Necrotic foci	0	4	0	0

[1]Ethylene glycol, 333 mg/kg sc, alone or combined with $NaHCO_3$ po administered to rats on day 11 of pregnancy. $NaHCO_3$ dosing consisted of an initial bolus of 530 mg/kg and additional consumption by drinking 0.265% aqueous $NaHCO_3$ solution for 24 h.

tive. The absence of a significant change in pH in both experiments indicated that metabolic production and renal excretion of H^+ have fortuitously balanced the blood pH within the range of control rats.

The serum analysis showed the presence of a mild but statistically significant hypokalemia (Tables 6, 7), which in the absence of an increased urinary excretion of K^+ indicated a shift of K^+ from the extracellular fluid into the cells. Hypokalemia induces metabolic alkalosis in an effort to alleviate acidosis, and is a common complication of systemic acidosis. A slight, but statistically significant hypophosphatemia was observed at 7, 24, and 48 h postdosing. Acidosis is known to decompose intracellular organic compounds with the release of inorganic phosphate which is subsequently excreted in the urine (Guest, '42). There are a number of other statistically significant values in Tables 6 and 7 that were nonreproducible or isolated; their teratologic significance, if any, is unknown.

The calculated anion gap revealed no salicylate-related gap and the acidosis was therefore hyperchloremic. No salicylate-related osmolal gap was found when measured osmolality was compared with the osmolality calculated from concentrations of Na^+, glucose, and BUN (Tables 6, 7) (calculations not shown).

The NH_4Cl, in the combined NH_4Cl + sodium salicylate study, intensified the salicylate-induced hypocapnia at 24 h postdosing and HCO_3^- deficit at all postdosing intervals (Table 6). Further, it caused a reduction in pH at 24 and 48 h postdosing and alleviated hypokalemia. The salient influence of $NaHCO_3$ when administered simultaneously with sodium salicylate was mitigation of hypocapnia and HCO_3^- deficit at 3, 7, and 9 h postdosing (Table 7).

Urinalysis

Sodium salicylate caused a significant reduction in both pH ($P < .001$) and osmolality ($P < .05$), but no significant change occurred in urinary volume or in the urinary concentrations of Na^+ and K^+. The serum concentration of BUN was raised at all test intervals in the two experiments (Tables 6, 7) and the rise was quite often, but not always, statistically significant ($P < .05$). The serum creatinine level was enhanced at 7 h postdosing in the two experiments and at 48 h postdosing in one experiment. The sodium salicylate-induced urine and serum changes were not influenced by the NH_4Cl treatment administered simultaneously with sodium salicylate dosing. Compared to the sodium salicylate alone, the combined $NaHCO_3$ + sodium salicylate had no effect other than increasing the volume of urine excreted ($P < .001$) and, as expected, raising the urinary Na^+ concentration ($P < .05$) in urine.

Teratology

Sodium salicylate, 280 mg/kg/day, administered on days 8–10 of pregnancy decreased maternal body weight gain and food intake during the dosing period and caused resorptions, fetal weight reduction, and fetal malformations (Table 8). All of these effects were significantly ($P < .05$) reduced by a

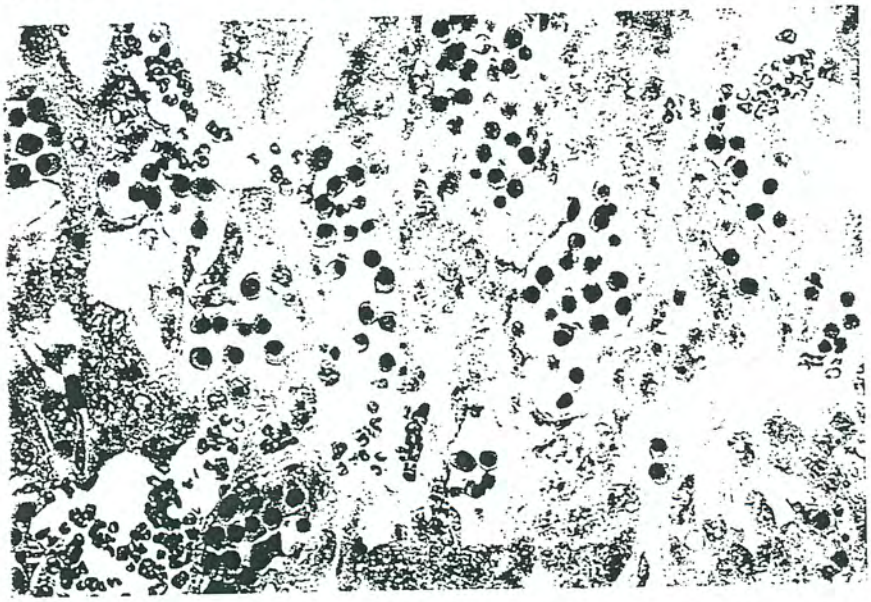


Fig. 6. Ethylene glycol 3,333 mg/kg administered sc on day 11 of pregnancy. Fewer fetal capillaries (small arrows) and more frequent maternal blood spaces (spaces mostly open because of vascular perfusion with the fixative) (large arrows) in the labyrinth, 48 h post-dosing. Magnification, ×320.

Fig. 7. Control, (day 13 of pregnancy) for Figure 6. Relative size and distribution of fetal capillaries (villi) and maternal blood spaces in the labyrinth. Magnification, ×320.

simultaneous treatment with $NaHCO_3$ in the drinking water given from 24 h before to 24 h after salicylate dosing (day 7–11 of pregnancy). In contrast, the NH_4Cl concurrently administered with sodium salicylate enhanced the salicylate-induced maternotoxic effects and increased the resorptions, from 5.1 to 12.9 per litter. The incidence of

TABLE 5. Ethylene glycol: relationship between maternal and fetal vascular areas in the labyrinth, 48 h following maternal dosing on the 11th day of pregnancy (mean ± standard error)

Dose groups[1]	Number of vascular spaces/imaged area		Cross-sectional area of an individual vascular space[2]		(Total cross-sectional area of vascular spaces ÷ imaged areas) × 100		Vascular ratio, maternal/ fetal area
	Maternal	Fetal	Maternal	Fetal	Maternal	Fetal	
A. H_2O + H_2O (control)	6.5 ± .5	21.8 ± 1.3	765 ± 63	298 ± 25	14.9 ± 1.3	18.3 ± 1.3	2.79 ± .29
B. $NaHCO_3$ + H_2O	5.9 ± .5	21.5 ± 1.3	1,463 ± 160***	533 ± 46**	13.5 ± .8	20.5 ± 1.0	3.02 ± .26
C. Ethylene glycol + H_2O	6.8 ± .6	15.2 ± 1.2**	2,108 ± 375***	293 ± 36	20 ± 1***	8 ± 1***	11.21 ± 2.62***
D. Ethylene glycol $NaHCO_3$	6.1 ± .4	19.6 ± 1.5	1,628 ± 163***	489 ± 61	19 ± 1***	17 ± 1*	4.15 ± 0.57*

[1]Dosing schedule same as in Table 4.
[2]Measurements on the videoscreen following 50-fold magnification (in mm unit).
*$P < .05$.
**$P < .02$.
***$P < .001$ (group comparisons: A versus C, and C versus D shown in C and D rows, respectively).

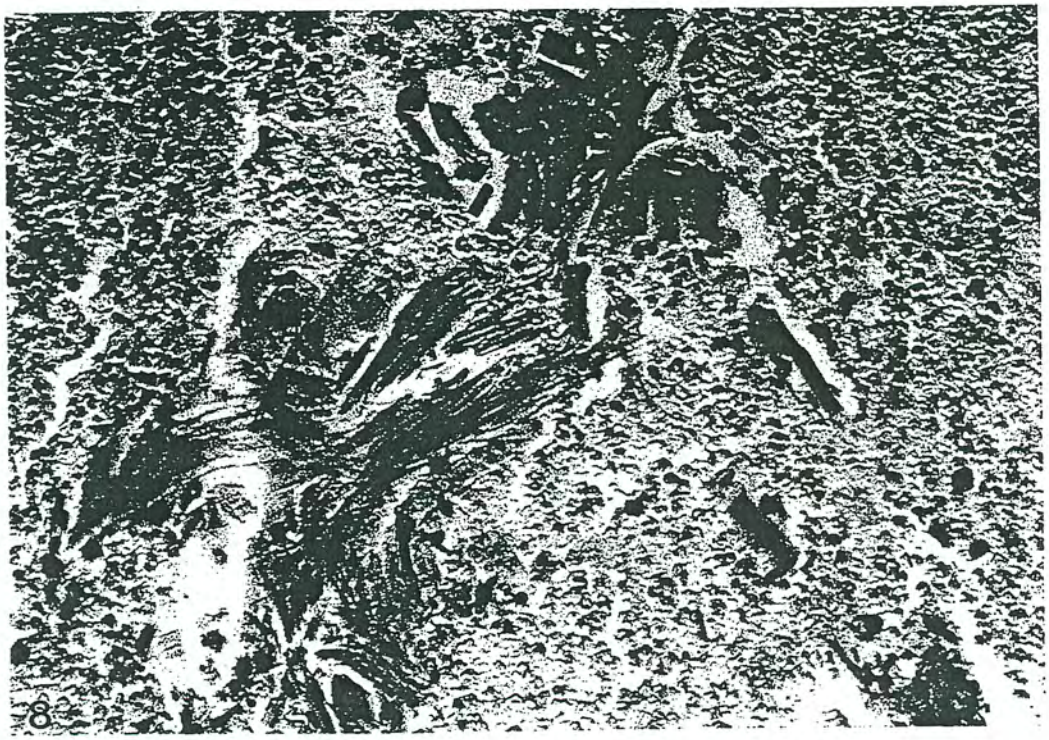


Fig. 8. Ethylene glycol, 500 mg/kg/day administered po on days 7–13 of pregnancy. Conceptal hematoma containing needle-shaped crystals arranged in sheaves, 24 h postdosing. Magnification, ×670.

anomalies and mean fetal weight in the salicylate + NH_4Cl group were based on 10 live fetuses, all from one dam.

Histopathology

24 h after dosing

All 13 conceptuses studied from eight sodium salicylate-treated dams had lesions (Table 9).

Control conceptuses on day 11 of pregnancy had three separate vascular systems with apparent or imminent influence on embryonic development: (1) maternal channels; (2) embryonic or vitelline system—both were seemingly functional; and (3) chorioallantoic system, still developing but not yet functional. The channels of the maternal system were ill-defined. Maternal ar-

TABLE 6. Acid-base and electrolyte data from cannulated rats dosed with sodium salicylate (NaSal) either alone, or in combination with NH_4Cl during pregnancy[1]

First treatment sc	3 h				7 h			
Second treatment in drinking water	H_2O + H_2O (control)	H_2O + NH_4Cl	NaSal + H_2O	NaSal + NH_4Cl	H_2O + H_2O (control)	H_2O + NH_4Cl	NaSal + H_2O	NaSal + NH_4Cl
mosm kg H_2O	280 ± 1	281 ± 1	276 ± 1	277 ± 1	276 ± 5	280 ± 2	276 ± 2	279 ± 1
pH	7.501 ± .009	7.492 ± .009	7.519 ± .005	7.469 ± .023	7.493 ± .009	7.479 ± .014	7.494 ± .011	7.468 ± .019
PCO_2 (mm Hg)	32.5 ± 1.1	31.7 ± .5	26.2 ± .5***	24.9 ± 1.0	34.7 ± 1.1	31.9 ± .8*	27.0 ± .8***	24.4 ± .9
PO_2 (mm Hg)	97 ± 11	109 ± 2	127 ± 1**	126 ± 2	101 ± 3	104 ± 2	111 ± 2*	116 ± 2
HCO_3 (mEq/L)	25.6 ± .9	24.5 ± .4	21.5 ± .2***	18.4 ± 1.1**	26.8 ± .4	24.0 ± .8**	20.9 ± .6***	18.0 ± .9*
BE ec F (mEq/L)	2.3 ± .7	0.9 ± .4	−1.5 ± .2***	−4.8 ± 1.7*	3.3 ± .4	0.3 ± .1***	−2.5 ± .6***	−5.8 ± 1.2*
Total Hb (g/dl)	16.6 ± .6	14.9 ± .8	15.4 ± .5	15.6 ± .6	15.7 ± .7	15.0 ± .2	14.3 ± .2	15.2 ± .7
Na^+ (mmol/L)	136.3 ± .6	136.7 ± .6	134.4 ± .5	132.7 ± .5	128.8 ± 2.2	133.6 ± 1.4	132.8 ± .7	132.2 ± .4
K^+ (mmol/L)	5.1 ± .1	4.7 ± .1**	4.0 ± .1***	4.4 ± .1***	4.9 ± .1	4.9 ± .1	4.0 ± .1***	4.8 ± .2*
Mg^{2+} (mmol/L)	1.6 ± .2	1.6 ± .2	1.6 ± .2	1.7 ± .2	1.5 ± .2	1.5 ± .2	1.7 ± .2	1.7 ± .2
Ca^{2+} (mg/dl)	13.7 ± .3	12.8 ± .4	11.5 ± .4**	12.1 ± .3***	12.7 ± .2	13.0 ± .3	12.3 ± .3	12.9 ± .7
Cl^- (mmol/L)	109 ± 1	109 ± 1	109 ± 1	109 ± 1	107 ± 1	107 ± 2	108 ± 2	109 ± 2
Glucose (mmol/dl)	132.8 ± 2.2	137 ± 3	143 ± 6	145 ± 7	134.8 ± 2.9	134.3 ± 3.9	136.3 ± 5.9	140.8 ± 5.6
Phosphate (mg/dl)	5.67 ± .40	5.46 ± .35	5.73 ± .28	4.64 ± .24	6.30 ± .21	4.99 ± .26***	5.55 ± .22*	5.48 ± .45
Total protein (g/dl)	6.15 ± .11	6.63 ± .16*	6.12 ± .11	6.47 ± .25	5.89 ± .16	6.58 ± .31	6.09 ± .19	6.59 ± .42
BUN (mg/dl)	14.4 ± .7	16.3 ± .9	23.0 ± 1.5**	23.4 ± 1.6	12.7 ± .6	14.7 ± .9	28.4 ± .6***	33.2 ± 4.0
Creatinine (mg/dl)	0.173 ± .053	0.154 ± .042	.0381 ± .059	0.276 ± .038	0.163 ± .051	0.194 ± .064	.459 ± .084**	0.434 ± .054

First treatment sc	9 h				24 h				48 h			
Second treatment in drinking water	H_2O + H_2O (control)	H_2O + NH_4Cl	NaSal + H_2O	NaSal + NH_4Cl	H_2O + H_2O (control)	H_2O + NH_4Cl	NaSal + H_2O	NaSal + NH_4Cl	H_2O + H_2O (control)	H_2O + NH_4Cl	NaSal + H_2O	NaSal + NH_4Cl
mOsm kg H_2O	291 ± 9	278 ± 1	277 ± 3	282 ± 3	276 ± 3	281 ± 1	281 ± 2	281 ± 2	282 ± 1	282 ± 1	278 ± 2	278 ± 1
pH	7.491 ± .003	7.497 ± .008	7.479 ± .008	7.413 ± .067	7.487 ± .004	7.466 ± .010	7.469 ± .004	7.357 ± .042**	7.488 ± .016	7.435 ± .009**	7.501 ± .013	7.357 ± .049**
PCO_2 (mm Hg)	34.8 ± .6	33.8 ± 1.4	26.3 ± 1.3***	21.6 ± .2	34.2 ± .6	32.5 ± .9	29.6 ± .5***	24.0 ± 1.9**	33.1 ± .5	35.2 ± .8*	28.3 ± .9***	26.2 ± 1.1
PO_2 (mm Hg)	106 ± 7	97 ± 2	109 ± 2	120 ± 1***	109 ± 1	113 ± 1	112 ± 1	115 ± 2	110 ± 2	106 ± 2	111 ± 1	109 ± 3
HCO_3 (mEq/L)	26.9 ± .4	26.4 ± .8	19.7 ± .6***	15.9 ± 1.7*	26.2 ± .3	24.3 ± .6**	21.8 ± .3***	14.9 ± 1.9***	25.5 ± .6	24.3 ± .6	22.9 ± .3***	15.9 ± 2.1**
BE ec F (mEq/L)	3.4 ± .4	2.9 ± .8	−4.0 ± .6**	−10.4 ± 3.4*	2.6 ± .3	−.27 ± .2**	−2.1 ± .4**	−8.9 ± 2.5**	1.9 ± .5	0 ± 0	−0.4 ± .3**	−5.9 ± 1.3**
Total Hb (g/dl)	16.4 ± .4	14.6 ± .2	13.5 ± .6	13.5 ± 1.0	13.8 ± .4	13.6 ± .5	13.6 ± .4	13.2 ± 1.0	13.0 ± .7	12.9 ± .8	14.5 ± .2	13.1 ± 1.3
Na^+ (mmol/L)	134.9 ± .4	135.1 ± .5	134.8 ± .9	134.3 ± 1.6	133.7 ± .4	134.6 ± .5	134.6 ± .7	133.8 ± 1.4	135.3 ± .9	136.8 ± 1.2	136.1 ± .9	133.2 ± .6
K^+ (mmol/L)	4.8 ± 1.7	4.7 ± .0	4.1 ± .1**	4.2 ± .2	4.9 ± .2	4.9 ± .1	4.0 ± .2**	4.5 ± .2	5.1 ± .1	4.7 ± .1	4.1 ± .3**	4.3 ± .1
Mg^{2+} (mmol/L)	0.80 ± 0	0.83 ± .04	0.97 ± .04	0.87 ± .08	1.5 ± .2	1.5 ± .2	1.8 ± .2	1.7 ± .2	1.9 ± .1	1.8 ± .03	2.5 ± .1	2.2 ± .1
Ca^{2+} (mg/dl)	12.5 ± .3	12.7 ± .2	12.3 ± .5	11.1 ± .4	11.9 ± .4	11.9 ± .3	11.2 ± .4	11.2 ± .6	11.4 ± .5	11.2 ± .4	10.3 ± .2	10.4 ± .4
Cl^- (mmol/L)	107 ± 1	112 ± 1	108 ± 2	109 ± 1	108 ± 1	109 ± 1	109 ± 1	118 ± 2**	108 ± 2	108 ± 1	107 ± 1	111 ± 2
Glucose (mmol/dl)	133 ± 3	141 ± 8	155 ± 15	147 ± 6	139 ± 5	134 ± 3	132 ± 14	120 ± 7*	143 ± 4	139 ± 3	126 ± 4*	114 ± 7**
Phosphate (mg/dl)	6.7 ± .4	5.9 ± .3	5.6 ± .7	4.9 ± .4	5.8 ± .3	5.9 ± .3	3.8 ± .3***	3.5 ± .2	6.7 ± .4	6.5 ± .8	3.5 ± .5***	3.3 ± .4
Total protein (g/dl)	6.2 ± .2	6.2 ± .2	6.2 ± .2	5.6 ± .2	5.9 ± .1	6.2 ± .1	5.8 ± .1	5.7 ± .2	5.9 ± .3	6.1 ± .1	5.8 ± .1	5.9 ± .2
BUN (mg/dl)	13.5 ± .6	19.4 ± 1.0***	32.1 ± 2.5***	30.6 ± 5.1	16.8 ± .9	19.5 ± 1.0	23.8 ± 1.6	25.4 ± 1.7	18.5 ± 1.4	20.5 ± .5	22.6 ± 3.6	25.2 ± 3.6
Creatinine (mg/dl)	ND[3]	ND	ND	ND	.286 ± .087	.334 ± .070	.539 ± .139	.447 ± .053	0.269 ± .08	0.393 ± .06	.602 ± .11*	.613 ± .09

[1]Sodium salicylate, 280 mg/kg sc, was administered as first dose on day 8 of pregnancy and a 2nd dose 24 h later. The 3, 7, 9, 24, and 48 h represent intervals after the first salicylate dosing.
[2]The NH_4Cl was dispensed in drinking water from day 7 of pregnancy until the end of the experiment. The average daily NH_4Cl intake, given either alone or combined with sodium salicylate, was 421 and 448 mg/kg body weight, respectively, with respective average daily intake of drinking fluid of 25 and 24 ml/dam.
[3]Not done.
*P < .05.
**P < .01.
***P < .001: Group comparisons: H_2O + H_2O versus H_2O + NH_4Cl; NaSal + H_2O versus NaSal + NH_4Cl.

TABLE 7. Acid-base and electrolyte data from cannulated rats following treatments with either sodium salicylate (NaSal) 280 mg/kg sc alone, or combined with $NaHCO_3$[1]

	Post dosing interval (h)							
	3				7			
Treatment groups	Control H_2O + H_2O	H_2O + $NaHCO_3$	NaSal + H_2O	NaSal + $NaHCO_3$	H_2O + H_2O	H_2O + $NaHCO_3$	NaSal + H_2O	NaSal + $NaHCO_3$
mOsm/kg H_2O	289 ± 0.1	284 ± 3	287 ± .5	285 ± 3	290 ± 3	288 ± 3	289 ± 2	287 ± 3
pH	7.486 ± .005	7.497 ± .004	7.533 ± .017	7.595 ± .011**	7.509 ± .01	7.499 ± .01	7.514 ± .00	7.569 ± .007
PCO_2 (mm Hg)	34 ± .8	34 ± .8	28 ± .7***	35 ± 1**	34 ± 1	33 ± 1	30 ± 2**	37 ± 1*
PO_2 (mm Hg)	102 ± 2	101 ± 1	126 ± 6***	114 ± 2	104 ± 2.6	106 ± 3	115 ± 2**	109 ± 2
HCO_3^- (mEq/L)	26 ± .5	26 ± .5	23 ± .4***	32 ± 1***	27 ± 0.6	26 ± .4	25 ± .8*	34 ± 1***
Total Hb (g/dl)	13.0 ± .4	13.8 ± .1	14.0 ± .2*	13.8 ± .2	12.3 ± .5	12.9 ± .4	13.2 ± .2*	12.7 ± .2
Na^+ (mmol/L)	135.5 ± 1.1	133.1 ± .3	132.5 ± 0.4**	136.8 ± .8**	135 ± 1	136 ± 1	133 ± 1	134 ± 1
K^+ (mmol/L)	5.6 ± .2	4.8 ± .1	4.1 ± .1***	4.1 ± .1	4.67 ± .1	4.52 ± .1	3.99 ± .1***	3.87 ± .1
Ca^{2+} (mg/dl)	12.3 ± .6	11.9 ± .5	10.3 ± .4*	10.5 ± .4	11.2 ± .4	11.1 ± .5	10.3 ± .2	11.1 ± .2
Cl^- (mmol/L)	105 ± 2	103 ± 1	102 ± 1*	95 ± 2**	103 ± 1	106 ± 1	103 ± 1	99 ± 1*
Mg^{2+} (mmol/L)	—	—	—	—	.70 ± .04	—	.91 ± .03	.89 ± .04
Glucose (mg/dl)	134 ± 10	132 ± 3	156 ± 14	148 ± 7	137 ± 5	127 ± 5	148 ± 13	138 ± 3
Phosphate (mg/dl)	—	—	—	—	5.8 ± .3	—	4.0 ± .5**	3.9 ± .4
BUN (mg/dl)	15.8 ± 2.9	17.8 ± 1.9	18.3 ± .1	16.5 ± 1.1	14 ± 1	18 ± 2	19 ± 2	21 ± 2
Creatinin (mg/dl)	—	—	—	—	.48 ± .03	—	.62 ± .05*	.64 ± .03

	Post dosing interval (h)							
	9				24			
Treatment groups	H_2O + H_2O	H_2O + $NaHCO_3$	NaSal + H_2O	NaSal + $NaHCO_3$	H_2O + H_2O	H_2O + $NaHCO_3$	NaSal + H_2O	NaSal + $NaHCO_3$
mOsm/kg H_2O	284 ± 2	283 ± 3	286 ± 3	285 ± 6	292 ± 4	294 ± 8	297 ± 3	304 ± 5
pH	7.504 ± .014	7.508 ± .011	7.498 ± .019	7.543 ± .008	7.499 ± .020	7.531 ± .022	7.489 ± .008	7.536 ± .009
PCO_2 (mm Hg)	36 ± 1	33 ± .4	30 ± 1***	37 ± 1***	34 ± 2	32 ± 2	33 ± 1	36 ± 1
PO_2 (mm Hg)	104 ± 3	104 ± 3	118 ± 3***	104 ± 2***	107 ± 4	111 ± 4	106 ± 2	101 ± 2
HCO_3^- (mEq/L)	29.3 ± .7	26 ± .4**	24 ± 1***	33 ± 2***	27 ± 1	27 ± 1	27 ± 1	32 ± 2*
Total Hb (g/dl)	11.5 ± .4	12.3 ± .4	12.6 ± .2**	12.1 ± .2	9.8 ± .5	—	10.4 ± .4	10.6 ± .3
Na^+ (mmol/L)	134.6 ± .7	134.8 ± .1	133.3 ± .9	134.8 ± .6	136.8 ± .4	137.2 ± .4	135.4 ± .6	137.6 ± .5
K^+ (mmol/L)	4.85 ± .1	4.70 ± .1	4.29 ± .2*	3.80 ± .1	5.13 ± .2	4.74 ± .2	5.24 ± .2	5.02 ± .2
Ca^{2+} (mg/dl)	11.7 ± .3	—	10.9 ± .4	10.9 ± .4	11.2 ± .2	11.0 ± .1	10.4 ± .2**	10.9 ± .2
Cl^- (mmol/L)	94.9 ± 9	—	105 ± 1	100 ± .9	106 ± 1	106 ± 2	106 ± 1	104 ± 2
Mg^{2+} (mmol/L)	—	—	—	—	—	—	—	—
Glucose (mg/dl)	141 ± 6	135 ± 3	158 ± 13	145 ± 5	148 ± 6	149 ± 5	142 ± 2	141 ± 3
Phosphate (mg/dl)	—	—	—	—	6.7 ± .2	5.9 ± .4	5.1 ± .4**	4.7 ± .3
BUN (mg/dl)	14.5 ± 2	—	22.5 ± 3	20 ± 2	—	—	—	—
Creatinin (mg/dl)	—	—	—	—	—	—	—	—

[1]$NaHCO_3$ administered in drinking water from day 7 of pregnancy until 24 h after NaSal dosing, i.e., the last acid-base analysis. NaSal, 280 mg/kg/day sc, was dosed on day 8 (Exp. I), or 8 and 9 (Exp. II), or 8–10 (Exp. III) of pregnancy in separate experiments. The data from the three experiments are compiled. Average daily $NaHCO_3$ intake for the groups administered $NaHCO_3$, either alone or in combination with sodium salicylate, was 2,860 and 4,050 mg/kg body weight, respectively, with respective average daily fluid consumptions of 46 and 64 ml per dam. — represents analysis not done.
*$P < .05$.
**$P < .01$.
***$P < .001$; group comparisons: H_2O + H_2O versus NaSal + H_2O; NaSal + H_2O versus NaSal + $NaHCO_3$.

terial blood circulated, from out toward the embryo, through intricate vascular spaces in the decidual tissue, between mural trophoblasts and ultimately in the vascular network adjacent to the surface of the Reichert's membrane. The maternal blood in the network and the Reichert's membrane are separated by the fenestrated and attenuated processes of trophoblasts (Jollie, '90). The trophoblasts provide a structural support, and regulate the volume of blood circulating in the network (Welsh and Enders, '87). In the embryonic or vitelline vascular system, vessels containing nucleated erythroblasts lined the interior surface of the visceral layer of the yolk sac. The peripheral vitelline vascularization occurred in all control conceptuses by day 10 of pregnancy, as previously reported (Lambson, '66). In the chorioallantoic system, the fusion of allantois with chorion was complete and the allantoic villi had started penetrating the chorion.

TABLE 8. Teratology study: sodium salicylate (NaSal)-induced maternal and fetal effects and their reduction by $NaHCO_3$ and enhancement by NH_4Cl following maternal treatments administered on days 7–10 of pregnancy in rats

Treatment groups	H_2O po + H_2O sc	H_2O po + NaSal sc[1]	$NaHCO_3$ po + NaSal sc[2]	NH_4Cl po + NaSal sc[3]	$NaHCO_3$ po + H_2O sc[2]	NH_4Cl po + H_2O sc[3]
Maternal data						
Dams (Nos.) at term/dead	15/0	20/0	22/0	22/0	14/0	13/0
Body weight, difference, day 11-day 8 of pregnancy, g (mean ± SE)	15.1 ± 3.0	−12.8 ± 4.4	−7.5 ± 3.4*	−40.1 ± 4.9**	12.7 ± 3.3	15.4 ± 4.5
Food intake per day for 8, 9, & 10 of pregnancy, g (mean ± SE)	20.8 ± 0.6	8.9 ± 1.6	12.3 ± 1.1**	4.0 ± 1.0**	18.9 ± 0.7	21.3 ± 0.8
Consumption of water or aqueous solution ($NaHOC_3$ or NH_4Cl) per day for days 7–11 of pregnancy, g (mean ± SE)	39.9 ± 1.8	48.6 ± 4.1	72.4 ± 5.5**	33.8 ± 3.7	60.2 ± 3.7**	41.7 ± 2.8
Fetal data						
Live fetuses, No. (mean ± SE per litter)	14.1 ± 0.6	5.1 ± 1.2	12.0 ± 0.8**	0.5 ± 0.5**	11.6 ± 0.9	11.9 ± 0.9
Resorption + dead fetuses, No. (mean ± SE per litter)	0.5 ± 0.2	7.9 ± 1.3	1.1 ± 0.3**	12.9 ± 0.8**	0.5 ± 0.2	0.8 ± 0.3
Fetal body weight, g (mean ± SE)	5.5 ± 0.1	4.2 ± 0.2	4.5 ± 0.1	3.9 ± 0.1	5.4 ± 0.1	5.3 ± 0.1
Anamolies, incidence percent						
Hydrocephalus	0	27	3*	30	0	0[4]
Exencephaly	0	6	0*	0	0	0
Spina bifida	0	3	0	0	0	0
Fused ribs	0	6	1	60	2	0
Wavy ribs	1	22	2	0	1	6
Supernumerary rib	1	20	7	20	0	2

[1]280 mg/kg/day administered sc on days 8, 9 and 10 of pregnancy.
[2]$NaHCO_3$, 16.8 mg/ml, and [3]NH_4Cl, 4.73 mg/ml, were administered in drinking water from days 7 to 11 of pregnancy. Their average daily intake can be calculated from the consumption of aqueous solution, given in this table.
[4]Two fetuses malformed, one had cleft upper lip and another umbilical hernia.
*$P \leq .01$.
**$P \leq .001$; groups compared: H_2O + NaSal versus either $NaHCO_3$ + NaSal or NH_4Cl + NaSal.

TABLE 9. Conceptal lesions 24 h after maternal dosing with sodium salicylate (NaSal), alone and in combination with either $NaHCO_3$ or NH_4Cl[1]

First treatment as drinking fluid + second dosing, sc	H_2O + NaSal	Na_2CO_3 + NaSal	NH_4Cl + NaSal	H_2O + H_2O
I. Conceptuses (No.) showing lesions/total examined	13/13	4/12	9/9	0/6
II. No. of embryos that showed:				
1. Hemorrhage in the yolk sac, amniotic, coelomic, and ectoplacental cavities	7	3	6	0
2. Ectoplacental cone: necrosis and hemorrhage	3	0	8	0
3. Ischemia of maternal vascular network around Reichert's membrane	4	0	0	0
4. Plasma-containing recess surrounding Reichert's membrane	5	1	4	0
5. Visceral yolk sac endoderm: papillated and hypertrophic	11	2	8	0
6. Vitelline vessels absent	6	1	8	0
7. Allantois absent	13	1	8	0
8. Fetus: growth retarded	10	3	8	0

[1]Dosing schedule was the same as in teratology study (Table 8, footnote), except that NH_4Cl concentration in drinking solution was reduced to 0.16% to reduce incidence of resorption.

In salicylate-treated conceptuses, all three vascular systems were affected in a distinct pattern. Maternal erythrocytes in varying numbers were found in the yolk sac and ectoplacental, exocoelomic (Fig. 9), and amniotic cavities, indicating a breakdown of

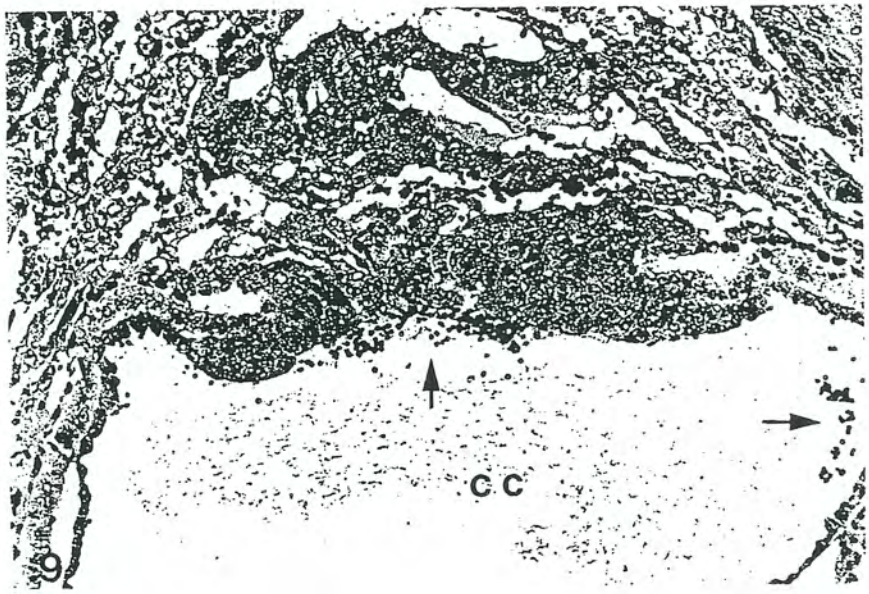


Fig. 9. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. Maternal hemorrhage (arrows) in the extraembryonic coelom, 24 h after the last dose. Magnification, ×120.

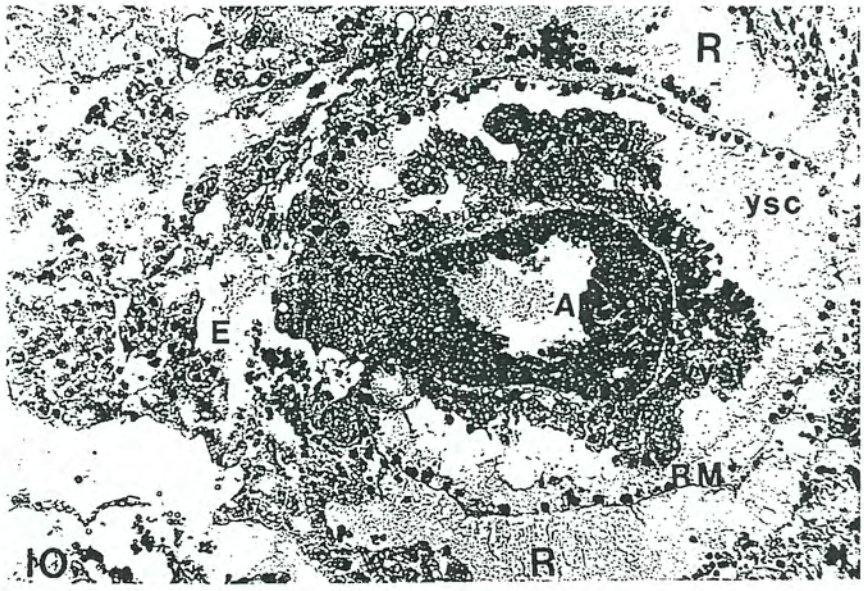


Fig. 10. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. Recess filled with plasma surrounding the Reichert's membrane, 24 h after the last dose. Magnification, ×120.

the maternal vascular system in areas around the conceptus. The maternal vascular network at the Reichert's membrane was either ischemic or had, together with giant trophoblasts, receded from the Reichert's membrane thus creating a recess filled with plasma that had few erythrocytes (Fig. 10). The visceral yolk sac endodermal cells in the salicylate group were tall and columnar and had vesicular nuclei, basal in

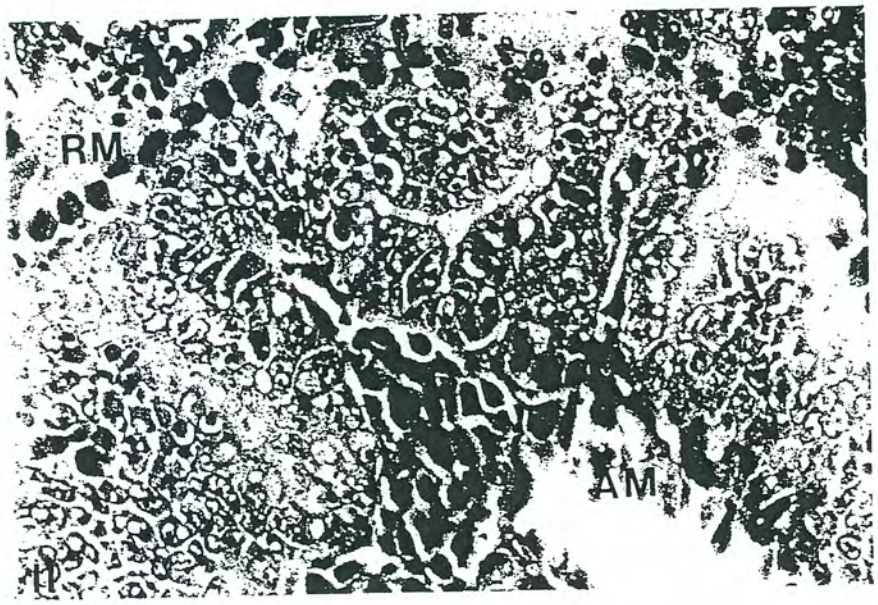


Fig. 11. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. Papillary proliferation of visceral endodermal cells, 24 h after the last dose. Magnification, ×330.

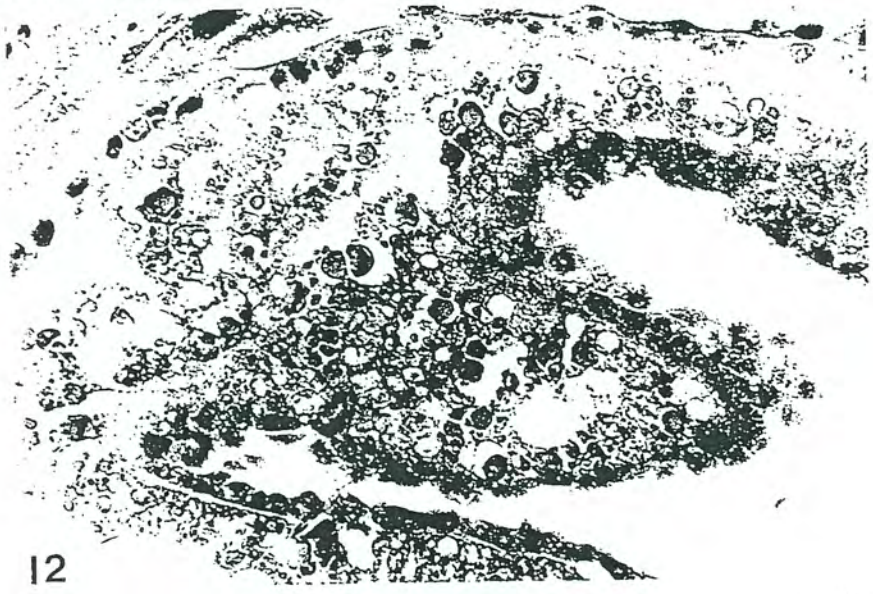


Fig. 12. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. Acidophilic globules in the hypertrophic visceral endodermal cells, 24 h after the last dose. Magnification, ×320.

position, compared to cuboidal cells in controls, which had round or oval nuclei, central in position. The visceral endodermal cells from salicylate-treated conceptuses proliferated as papillary projections into the yolk sac cavity (Fig. 11). They contained supranuclear acidophilic globules of different sizes (Fig. 12); some resembled maternal erythrocytes in shape and staining, while others were like the lysosomes in the apical region of aging endodermal cells (Jollie, '84). The angiogenic mesoderm at the inte-

rior surface of visceral yolk sac layer was mostly undifferentiated, and vitelline vessels were usually absent. The allantois in all salicylate-treated conceptuses was absent, and chorion was markedly reduced in size. In three salicylate-treated conceptuses, the ectoplacental cone and the adjoining decidua basalis were degenerating, hemorrhagic, and contained unidentified crystals different from those described above. Most of the embryos were retarded in development.

In all conceptuses whose dams were exposed to a simultaneous $NaHCO_3$ + sodium salicylate treatment, the incidence and severity of changes in the maternal vascular channels around Reichert's membrane, the vitelline circulation, and embryonic development were attenuated compared to those in conceptuses from sodium salicylate-treated dams (Table 9). Although the chorioallantoic fusion was delayed, the allantois was present in the extramebryonic coelom. The endodermal cells showed slight-to-moderate tallness, fewer acidophilic globules, and limited proliferation.

In the combined NH_4Cl + salicylate-treated group, the lesions, although qualitatively similar to those in the salicylate-alone group, were more frequent (Table 9) and severe. The visceral yolk sac layer was thicker as a result of papillary proliferation. The ectoplacental cone was hemorrhagic, structurally disorganized, and contained, in four conceptuses, unidentified crystals similar to those observed in the salicylate group. The embryos showed generalized karyorrhexis and were more severely retarded than those from the salicylate-treated dams.

48 h after dosing

The control chorioallantoic labyrinth on day 12 of pregnancy had a histologic appearance (Figs. 13, 14) suggesting that a materno-fetal vascular exchange may have already been functioning.

The chorioallantoic labyrinth in all 17 conceptuses from the salicylate-treated group was either missing, abnormal, or delayed in development because of the following lesions (number of conceptuses in parantheses): the allantois was either absent (6) or degenerating and reduced in size (3), and the chorion was absent (2) and reduced in size or rudimentary (5). Since the allantoic villi had not penetrated the chorion (16), the labyrinth comprised tortuous maternal vascular spaces separated by trophoblasts (Figs. 15, 16). The allantois arose from angiogenic mesoderm of the vitelline vessels, in addition to its normal origin from the embryonic hind gut (3). The yolk sac cavity as observed 24 h earlier contained maternal erythrocytes, and its visceral endoderm was papillary or villous and had intracytoplasmic acidophilic globules (3). The embryos were growth retarded (4) and showed karyorrhexis in the neuroepithelial layer, connective tissue, or both (6). A large "lake" of maternal blood separated the ectoplacental cone from the decidua basalis (Fig. 17) such that embryonic resorption appeared imminent (3). None of the six control embryos examined contained these lesions.

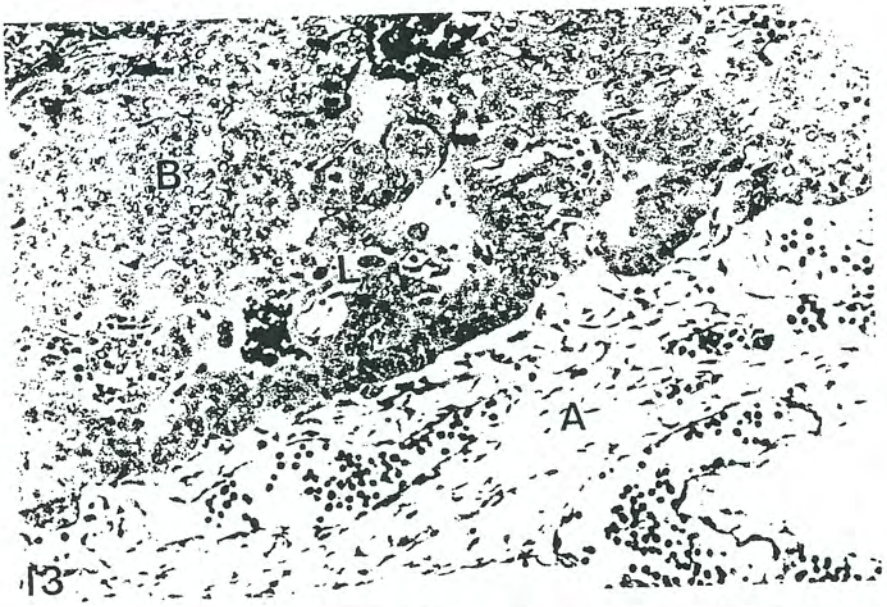
CADMIUM CHLORIDE

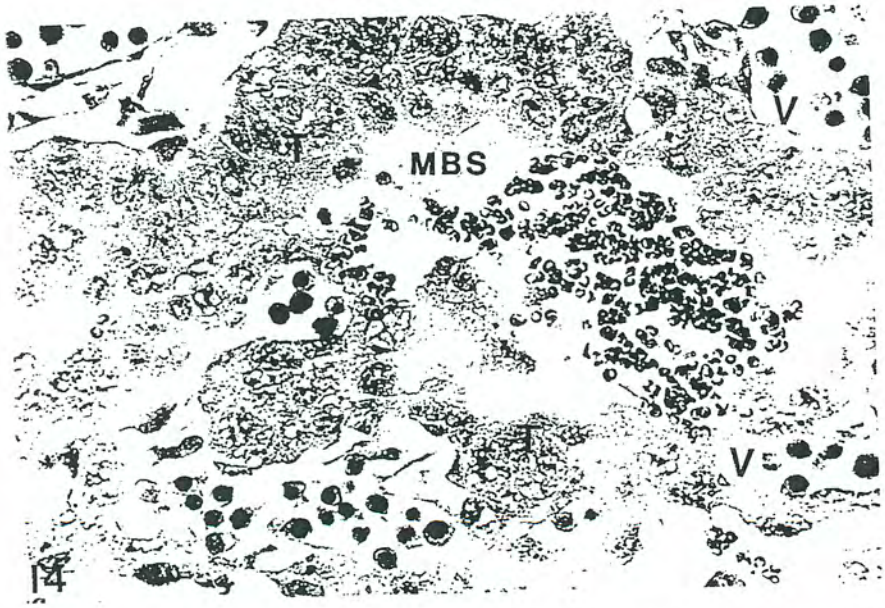
Homeostasis

$CdCl_2$ was only administered ip at a dose of 5.5 mg/kg (Table 10). A reduction in PCO_2 and HCO_3^- 7 and 24 h postdosing, and a slight decrease in urinary pH (data not shown) suggested a mild, transitory, and compensated metabolic acidosis. Other effects were reduced concentrations of Na^+ at all the four test intervals, of Cl^- at 3 and 7 h, and of total protein at 3, 7, and 24 h, as well as a lower osmolality at 3 and 9 h postdosing. The osmolal gap for $CdCl_2$, calculated from values in Table 10, was comparable with that of the control rats at all test intervals. There were increased glucose concentrations at 3, 7, and 9 h postdosing and increased BUN levels at 9 and 24 h postdosing, accompanied by a reduction in urine excretion at 24 h after dosing (data not shown), suggesting renal damage. The teratologic significance of these findings is uncertain.

Teratology: ip, iv, and sc routes

A single dose of $CdCl_2$, 3–5.5 mg/kg ip, or 2.6 and 4.5 mg/kg iv, caused an approximate dose-related increase in mortality and a reduction in dam's body weight, food intake, and water consumption (Tables 11, 12). The 5.5 mg/kg ip dose also caused resorptions, while 5 and 5.5 mg/kg ip and the 4.5 mg/kg iv doses induced fetal weight reduction ($P < .05$). The two dosing routes produced a similar pattern of fetal anomalies with the overall incidence being generally dose-related. At the two lowest doses, i.e. the 3

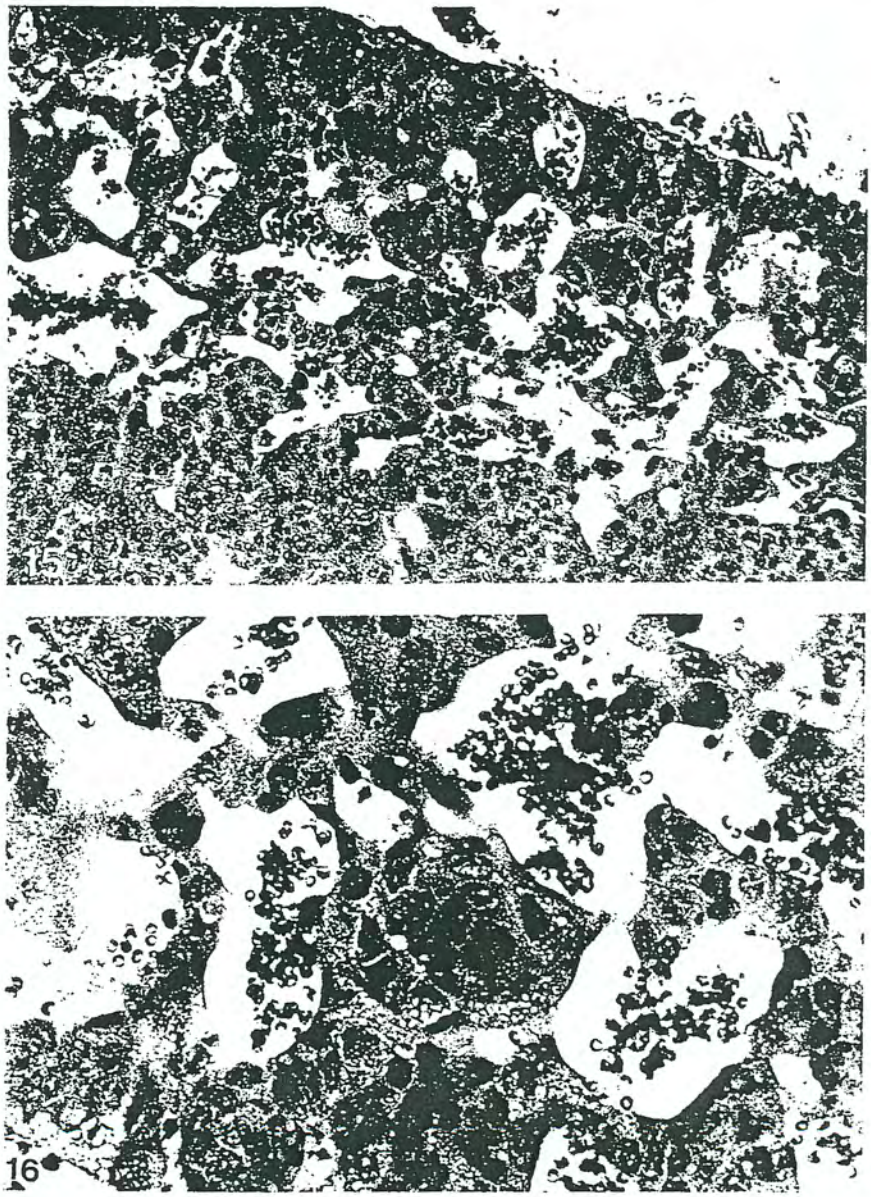




Figs. 13, 14. Control, day 12 of pregnancy, showing allantois, labyrinth containing fetal capillaries (villi) and maternal blood spaces separated by trophoblasts, and basal zone of placenta. Magnification: ×60 (Fig. 13), ×320 (Fig. 14).

mg/kg ip and 2.6 mg/kg iv, a low, yet increased incidence of skeletal anomalies was observed.

A single sc dose of 12, 16, or 20 mg/kg reduced the maternal body weight and food and water consumption (Table 13). The 16 and 20 mg/kg doses also reduced fetal body weight, while the 12, 16, and 20 mg/kg doses caused a dose-related pattern of anomalies resembling those of the ip and iv routes. The 5.5 mg/kg sc dose manifested no adverse effect in the dam or fetuses.



Figs. 15, 16. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. "Labyrinth" comprised only maternal blood spaces and trophoblasts; the villi had no allantoic blood vessels, 48 h after the last dose. Magnification: ×60 (Fig. 15), ×320 (Fig. 16).

Maternal pathophysiology: ip, iv, and sc routes

Within 24 h of the ip dosing (5 rats/group) on the 10th day of pregnancy, $CdCl_2$ led to a peritoneal exudation often hemorrhagic (ml, mean ± SE) of 6.6 ± 0.7, 8.9 ± 1.4, 9.5 ± 1.7, and 6.8 ± 1.2 at the respective doses of 3, 4, 5, and 5.5 mg/kg. The exudate readily coagulated on collection and contained a large number of polymorphonuclear leukocytes. The granulocytic count in blood samples of dams from the 5.5 mg/kg

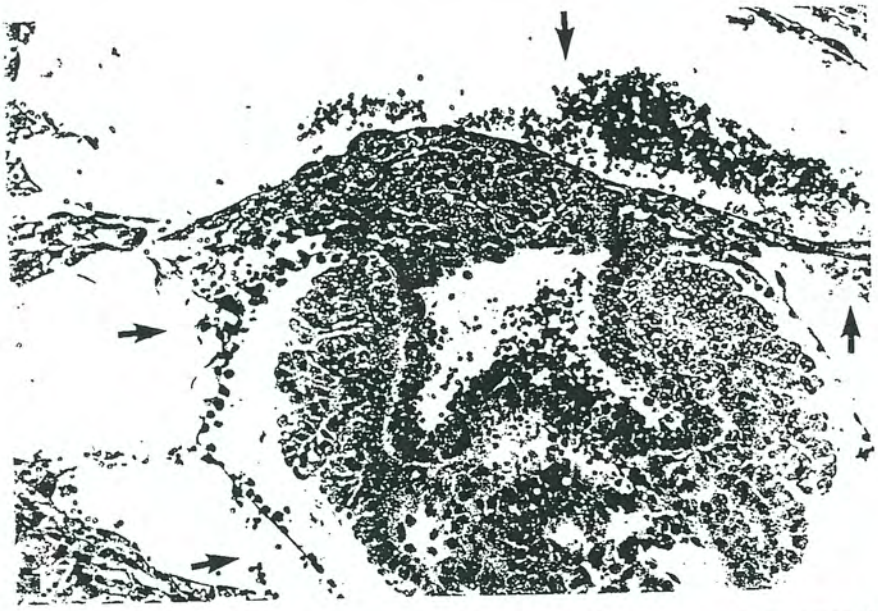


Fig. 17. Sodium salicylate, 280 mg/kg/day administered sc on days 8–10 of pregnancy. Large areas of maternal hemorrhage (arrows) around the conceptus, 48 h after the last dose. Decidual tissue on the lower left corner. Magnification, ×60.

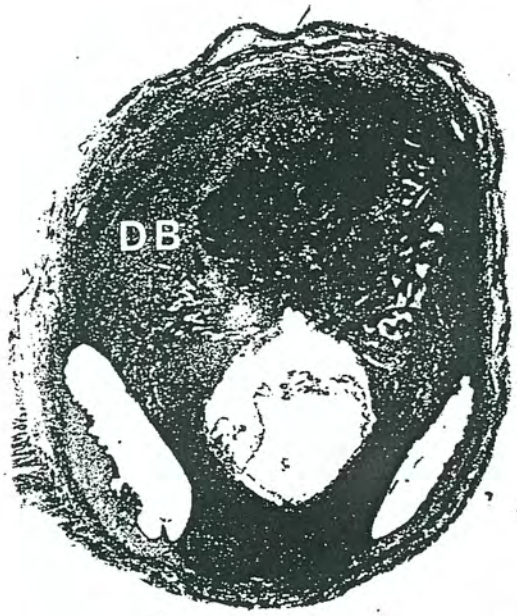


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Fig. 18. Cadmium chloride, 5.5 mg/kg/day administered ip on day 10 of pregnancy. Extensive necrosis and hemorrhage in the mesometrial decidual zone. 12 h after ip dosing. Magnification ×10.

TABLE 10. Acid-base-electrolyte data: Cadmium chloride, 5.5 mg/kg, administered ip to cannulated rats on the 10th day of pregnancy

	Postdosing interval (h)							
	3		7		9		24	
Treatment group	Control	$CdCl_2$	Control	$CdCl_2$	Control	$CdCl_2$	Control	$CdCl_2$
mOsm/kg H_2O	279 ± 2	270 ± 1***	274 ± 2	271 ± 2	274 ± 1	264 ± 1***	285 ± 1	289 ± 7
pH	7.481 ± .06	7.489 ± .01	7.459 ± .01	7.493 ± .01	7.475 ± .004	7.485 ± .004	7.481 ± .01	7.549 ± .02
PCO_2 (mm Hg)	34 ± 1	32 ± 2	35 ± 1	30 ± 1**	34.6 ± .9	32.2 ± 1.8	34 ± 1	25 ± 2**
PO_2 (mm Hg)	103 ± 1	120 ± 3***	104 ± 2	100 ± 9	104 ± 1	114 ± 5*	103 ± 3	109 ± 2
HCO_3^- (mEq/L)	25.6 ± .5	23.9 ± .8	25.3 ± .5	23.0 ± .8*	25.8 ± .5	24.5 ± 1.1	25.2 ± .6	21.7 ± 1.1**
Total Hb (g/dL)	13.3 ± .2	14.8 ± .3	12.7 ± .1	15.6 ± .2***	11.2 ± .9	14.8 ± 1.2	ND	ND
Total protein (g/dl)	6.6 ± .1	5.8 ± .1***	6.4 ± .2	5.6 ± 0.1**	5.9 ± .2	5.5 ± .2	6.4 ± .2	4.5 ± .2***
Glucose (mg/dl)	143 ± 2	158 ± 5***	147 ± 4	164 ± 4**	138 ± 7	162 ± 6*	138 ± 4	131 ± 3
Na^+ (mmol/L)	132.8 ± .8	128.1 ± .8***	133.3 ± .9	127.8 ± .7***	133.9 ± 1.2	125.4 ± 1.4***	132.9 ± .9	95.2 ± 4.5***
K^+ (mmol/L)	5.0 ± .2	5.9 ± .8	4.7 ± .1	4.8 ± .1	4.5 ± .1	4.7 ± .2	5.1 ± .1	5.6 ± 7.9*
Ca^{2+} (mkg/dl)	12.6 ± .3	11.5 ± .2*	12.1 ± .5	12.0 ± .3	11.4 ± .4	11.5 ± .6	11.9 ± .5	10.5 ± .3*
Cl^- (mmol/L)	106 ± 2	100 ± 1**	103 ± 1	103 ± 1	104 ± 1	105 ± 3	105 ± 2	96 ± 3*
Mg^{2+} (mmol/L)	.97 ± .04	.95 ± .02	.88 ± .03	.94 ± .03	.87 ± .02	1.0 ± .07	.82 ± .02	.78 ± .04
BUN (mg/dl)	18.1 ± 1.0	17.4 ± .02	17.1 ± 1.0	18.5 ± 2.5	15.4 ± .8	20.6 ± 1.1**	18.4 ± .5	30.0 ± 2.7***
Phosphate (mg/dl)	6.6 ± .3	7.3 ± .3	7.0 ± .3	6.1 ± .3	6.4 ± .2	6.1 ± .3	6.4 ± .3	5.9 ± .3
Creatinine (mg/dl)	.31 ± .05	.26 ± .02	.26 ± .05	.29 ± .05	.38 ± .03	.31 ± .05	.36 ± .05	.41 ± .06

*$P < .05$.
**$P < .01$.
***$P < .001$.

TABLE 11. ip teratology study: salient effects of $CdCl_2$ administered ip on the 10th day of pregnancy to rats

	Control	Dose, mg/kg 3	4	5	5.5
Maternal data					
(i) Dams at term/dead, No.	16/0	5/0	5/0	9/2	21/5
(ii) Body weight change in 2 days immediately after dosing (g, mean ± SE)	+9.9 ± 1.6	+21.4 ± 1.3****	−5.0 ± 2.4	−16 ± 3**	−18 ± 2****
(iii) Intake during 2 succeeding days after dosing (g, mean ± SE)					
Food	25.6 ± 3.9; 22.3 ± .8	1.8 ± 1.9****; 11 ± 3.5**	0.6 ± 0.3****; 1.5 ± 1.1****	2.2 ± 3.9****; 7.8 ± 2.9****	1.8 ± .9****; 3.3 ± 1.2****
Water	40.7 ± 1.9; 42.8 ± 2.3	22.8 ± 3.3****; 51.8 ± 5	14.6 ± 2.7****; 33.6 ± 6.0	16.5 ± 1.7****; 38.2 ± 3.9	13 ± 2****; 31 ± 3
Fetal data					
1. Values per litter (mean ± SE)					
Live fetuses, No.	13.5 ± 1.2	15.2 ± 1.2	8.6 ± 2.7	13.6 ± .9	9.7 ± 1.0
Resorptions + dead fetuses, No.	0.5 ± .2	1.4 ± .3**	0.8 ± .2	0.7 ± .4	4.3 ± 1.0****
Body weight, g	5.2 ± .1	4.9 ± .1	5.3 ± .3	4.8 ± .2**	4.4 ± .2***
2. Incidence (%) of anomalies*					
Anophthalmia	0	0	0	0	21
Hydrocephaly	0	0	0	4	32
Vertebral column, major defect	0	0	0	1	0
Thoraco-lumbar vertebrae, fused	0	0	0	0	28
Ribs					
Fused	0	14	7	26	21
Reduced in No.	0	14	43	36	55
14th rib rudimentary	3	17	30	18	30
Sternebrae					
Sternoschisis	0	0	0	1	3
Missing, fused, or retarded	7	48	36	38	56

*The incidences of retarded ossification of cranium and phalanges, and supernumerary and wavy rib in the test and control groups not being statistically different, are not listed.
**$P < .05$.
***$P < .01$.
****$P < .001$.

group at 24 h and 48 h postdosing was 2.5 and 3.8 × 10^9/L, respectively, compared to the control counts of 0.3 and 0.4 × 10^9/L, or an 8-fold increase at both intervals. A similarly increased granulocytic count was also observed in stained blood smears. These findings suggested that $CdCl_2$ on ip dosing caused an acute exudative peritonitis.

The sc injection in the interscapular region with 12 mg/kg, but not with 5.5 mg/kg, gave rise within 24 h to a diffusely gelatinous swelling involving subcutaneous tissue of the lateral thoraco-abdominal region, generally on the right side. The circumference and thickness of the swelling increased with 16 and 20 mg/kg doses and persisted until term.

A local irritant effect of $CdCl_2$ following ip and sc injection in the present study is consistent with a similar irritant and inflammatory effect on lungs following inhalation of $CdCl_2$ aerosol (Henderson et al., '79).

The iv dosing with 4.5 mg/kg failed to produce exudative peritonitis or subcutaneous swelling.

Histopathology

ip dosing

Conceptuses were investigated at the following postdosing intervals and doses: 12 h and 18 h at 5.5 mg/kg; 24 h at 3, 4, 5, or 5.5 mg/kg; and 48 h at the 5.5 mg/kg dose.

At 12 h postdosing, $CdCl_2$ induced hemorrhages in the ectoplacental cone in all six conceptuses examined. Extensive necrosis, with and without hemorrhages, was observed in the decidua basalis from three conceptuses (Fig. 18) which resembled cadmium-induced testicular edema and ischemic necrosis previously reported to occur 4–6 h postinjection (Mason et al., '64). Five conceptuses examined at 18 h postdosing all had similar hemorrhagic lesions. The maternal blood occupied a wide area surrounding Reichert's membrane and the ectoplacental cone (Fig. 19). Maternal erythrocytes were also found in the ectoplacental cone,

TABLE 12. iv teratology study: salient effects of $CdCl_2$ administered on the 10th day of pregnancy to rats

	Control	Dose, mg/kg 2.6	Control	4.5
Maternal data				
(i) No. of dams: at term/dead	13/0	12/0	8/0	23/14
(ii) Body weight change in 2 days immediately after dosing (g, mean ± SE)	1.5	0.5	+13 ± 1	−12 ± 3***
(iii) Intake during 2 successive days after dosing (g, mean ± SE):				
Food	15.7 ± 1.2; 37.2 ± 15.6	7.6 ± 1.0; 20.1 ± 1.5	17.7 ± 1.9; 23.3 ± 1.2	5.7 ± 1.0***; 12.3 ± 1.5***
Water	37.9 ± 2.5; 47.1 ± 2.8	36.4 ± 3.4; 45.5 ± 2.0	35 ± 3; 43 ± 2	35 ± 4; 36 ± 3
Fetal data				
1. Values per litter (mean ± SE)				
Live fetuses, No.	15.2 ± .5	13.5 ± 1.4	12 ± 1.3	11.9 ± 0.8
Resorptions + dead fetuses, No.	0.7 ± .3	0.3 ± .2	0.5 ± .2	0.6 ± .3
Body weight, g	5.1 ± .1	4.9 ± .2	5.2 ± .1	4.7 ± .1**
2. Percent incidence, anomalies*				
Anophthalmia	0	0	0	56
Hydrocephaly	0	12	0	35
Exencephaly	0	0	0	6
Vertebral column: major defect	0	0	0	22
Thoraco-lumbar vertebrae, fused	0	0	0	58
Ribs				
Fused	0	6	0	35
Reduced in No.	0	33	0	66
Rudimentary 13th	2	26	0	18
Tailless or short tail	0	0	0	6
Sternebrae				
Missing, fused, or retarded	2	66	1	58

*The incidences of retarded ossification of cranium and phalanges and supernumerary and wavy rib in the test and controls not being statistically different, are not listed.
**$P < .01$.
***$P < .001$.

ectoplacental cavity, and exocoelom. The necrosis was generalized in the chorion, ectoplacental cone (Fig. 20), visceral yolk sac endoderm, and embryonal tissues. It was characterised by a two to three cell deep layer of degenerating cells, which were being cast off into the adjoining coelomic, yolk sac, and amniotic cavities. The above changes resembled the occurrence of maternal blood extravasations throughout the trophoblast, extensive degeneration of decidua basalis, absence of labyrinthine development, and fetal degeneration following ovariectomy of rats on day 10 of pregnancy (Peel and Bulmer, '74).

At 24 h postdosing, the incidence of lesions at the 3, 4, 5, and 5.5 mg/kg was dose-related (Table 14). The central region of the decidua basalis was necrotic and hemorrhagic (similar to that shown in Fig. 18) and was separated from the healthy tissue by a sharp boundary. Decidual cells were rounded, individually isolated, and had pyknotic or fragmented intracytoplasmic nuclei within the still intact cell wall (Fig. 21). This necrosis was different from that already reported by Everett ('35), a physiological cell death observed in all test and control specimens in the form of a band of amorphous globular masses at the interface between the decidua basalis and the ectoplacental cone (Fig. 22). The "zone specific" long narrow band of cellular debris was believed to result from degeneration of uterine epithelium in physiologic response to decidual ontogeny. The $CdCl_2$-related necrotico-hemorrhagic decidual area extended, in some cases, to the ectoplacental cone, which was also hemorrhagic and cytoarchitecturally disrupted. The allantois was reduced in size, generally necrotic, and had not yet fused with a relatively small chorion, which itself showed cellular degeneration. The cells in the neuroepithelial plate and its surrounding endoderm showed karyorrhexis.

Eleven conceptuses obtained 48 h after $CdCl_2$ dosing had multiple lesions. The lesions included the following (number of conceptuses in parenthesis): the chorioallantoic placenta appeared non-functional (10) since

TABLE 13. sc teratology data: salient effects of $CdCl_2$ administered on the 10th day of pregnancy to rats

	Control	Dose, mg/kg 5.5	12	16	20
Maternal data					
(i) No. of dam: at term/dead	13/0	10/0	19/0	14/1 (aborted)	13/0
(ii) Body weight change in 2 days immediately after dosing (g, mean ± SE)	15.9 ± 2.4	+14.9 ± 1.1	−0.35 ± 1.5****	−3.9 ± 1.9****	−12.6 ± 1.1****
(iii) Intake during 2 successive days after dosing (mean ± SE)					
Food	17.2 ± 1.1; 22.8 ± .9	18.3 ± 0.9 (Not done)	12.7 ± 1.1***; 14.1 ± 1.0****	8.4 ± 1.5****; 8.3 ± 1.4****	4.7 ± 1.1****; 4.2 ± .9****
Water	34.2 ± 1.1; 42.4 ± 2.5	32.6 ± 2.3 (Not done)	39.2 ± 1.2***; 34.5 ± 1.3**	29.6 ± 2.4; 31.6 ± 2.8***	21.8 ± 2.5****; 22.8 ± 3.1****
Fetal data					
1. Values per litter (mean ± SE)					
Live fetuses, No.	12.4 ± 1.2	10.8 ± 1.2	13.7 ± .4	12.8 ± 1.0	10.0 ± 1.3
Resorptions + dead fetuses, No.	0.2	0.8	0.1	0.4	2.3 ± 1.0
Body weight, g	5.5 ± .2	5.1 ± 1.3	5.2 ± .1	4.8 ± .3**	4.6 ± .1****
2. Incidence (%) of anomalies*					
Anophthalmia	0	0	12	5	12
Hydrocephaly	0	0	5	3	9
Underdeveloped kidneys	0	0	0	4	25
Short tail	0	0	0	7	2
Vertebral column: major defect	0	0	0	3	45
Thoraco-lumbar vertebrae: fused	0	0	1	11	33
Ribs					
Fused	0	0	6	19	34
Reduced in No. (9–12)	0	0	29	55	75
13th rudimentary	4	4	18	18	8
Sternebrae					
Missing, fused, or retarded	1	7	47	70	75

*The incidences of retarded osifications of cranium and phalanges, and supernumerary and wavy rib in the test and control groups not being statistically different, are not listed.
**$P < .05$.
***$P < .01$.
****$P < .001$.

the allantois was either absent (2) or had failed to reach the chorion (9) and was undergoing karyorrhexis (3) (Fig. 23). The chorion was small (5) and necrotic (8); the decidua basalis (7) and the ectoplacental cone (4) were necrotic and hemorrhagic. Embryonic tissues including the neural plate were undergoing karyorrhexis (4).

In control conceptuses, the segment of the central spiral vessel that passes through the decidua basalis and the peak of ectoplacental cone and leads toward the chorionic disc was lined with intraluminal endovascular trophoblasts as previously reported by Legrand ('68). In $CdCl_2$-treated conceptuses, the endovascular trophoblastic lining of the central spiral artery was absent in three conceptuses. The central vessel could not be recognized in seven conceptuses since the decidua basalis was disrupted by necrosis and hemorrhage.

iv dosing

Lesions in conceptuses observed 24 h following iv dosing were morphologically similar to those following ip dosing (Table 14). In addition, edematous distension of the yolk sac cavity with a consequent reduction of the exocoelomic and amniotic cavities was also observed. The incidence and intensity of the lesions were more pronounced at the 4.5 mg/kg than at the 2.6 mg/kg dose.

sc dosing

No lesions were found in the fetal or extraembryonic tissues 24 h following dosing with 5.5, 12, 16, or 20 mg/kg and 48 h following dosing with 20 mg/kg of $CdCl_2$.

DISCUSSION

Ethylene glycol

A connection between ethylene glycol-induced hyperosmolality and its deleterious effect on fetal development is possible. Cellular metabolism and permeability of H^+ across cellular membranes are influenced by intracellular osmolality, which is in dynamic equilibrium with extracellular osmolality (Makoff et al., '70). An extracellular

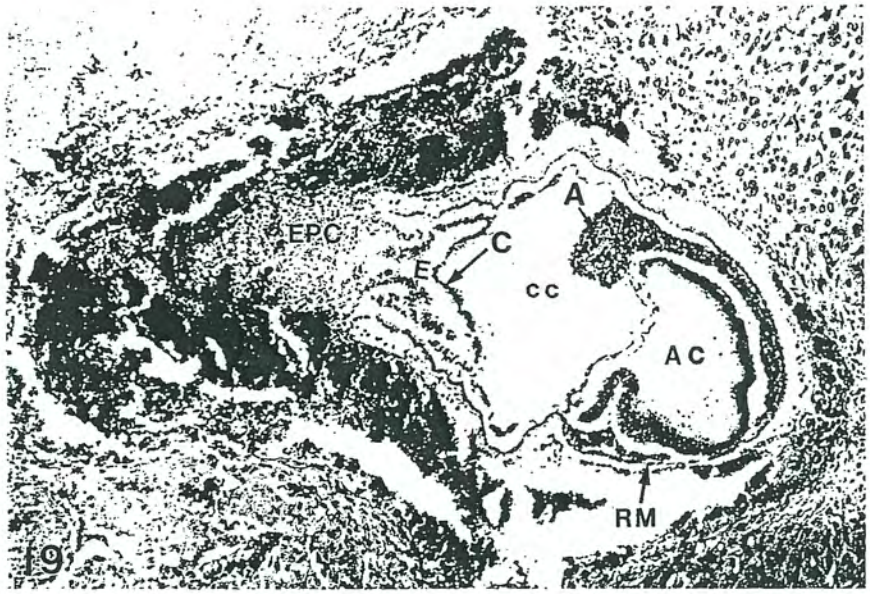

Fig. 19. Cadmium chloride, 5.5 mg/kg administered ip on day 10 of pregnancy. Maternal hemorrhage around the ectoplacental cone and Reichert's membrane, 18 h after dosing. Magnification, ×56.

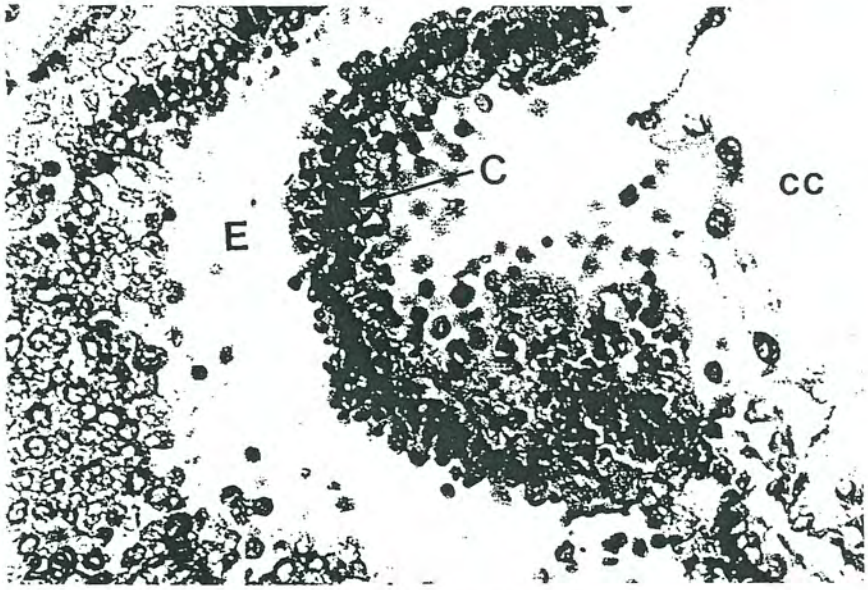

Fig. 20. Higher magnification of chorion shown in Figure 19. Degenerative changes involving chorion and ectoplacental cone. Magnification, ×330.

hyperosmolar condition initiates adaptive mechanisms (Finberg et al., '59; Kregenow, '71; Arieff and Kleeman, '74; Baxter et al., '76) which may lead to functional derangement in the cell (Arieff et al., '72). A rapid rise in osmolality (by > 50 mosm/kg H_2O) may be lethal (Sotos et al., '60; Dodge et al., '62; Stern, '74). Osmotic changes in the mother have been demonstrated to produce similar fetal osmotic changes in rabbits

TABLE 14. Cadmium chloride dosed ip or iv: salient histopathologic changes in maternal and conceptal tissues

Dosing-route	Dose, mg/kg	Conceptuses with lesions/ total examined, number	Decidua basalis: necrosis	Ectoplacental cone: necrosis and/or hemorrhage	Allantois: not fused with chorion	Yolk sac cavity distended with plasma	Chorion: degenerative changes	Fetus: karyorrhexis in neuroepithelium and other tissues
Ip	Control	0/12	0	0	0	0	0	0
	3	4/8	2[1]	1	1	0	0	0
	4	9/15	5	5	1	0	1	1
	5	12/12	12	12	5	0	4	3
	5.5	8/8	8	8	8	0	8	3
Iv	Control	0/7	0	0	0	0	0	0
	2.6	4/12	0	1	2	1	0	0
	4.5	9/18	9	4	8	7	0	7

[1]Effected areas were small in size.

(Dancis et al., '57). For example, within 30 minutes of maternal infusion, a hypertonic mannitol solution raised plasma osmotic pressure in the fetus equivalent to that in the mother causing fetal dehydration by a rapid water transfer from fetal to maternal circulation (Bruns et al., '63). Similar results were obtained in rats (Adolph and Hoy, '63), humans (Battaglia et al., '60), and primates (Bruns et al., '64) with several solutes capable of creating transplacental osmotic gradients.

Neither the cause of maternal acidosis in rats administered ethylene glycol nor the effects of acidosis on embryonic development are known. Glycoaldehyde, glycolic acid, glyoxylic acid (major pathway), and oxalate (minor pathway) are the metabolites of ethylene glycol in the rat (Wiley, '38; Friedmann et al., '56; Gessner et al., '61; Bove, '66), while oxalic acid is an important metabolite in rhesus monkeys (Hodgkinson and Zarembski, '68; McChesney et al., '71). Acidosis in the pigtail monkey and dogs has been attributed to glycolate (Clay and Murphy, '77) and to glycolic and lactic acids in humans (Gabow et al., '86). Oxalate itself is highly toxic and may produce acidosis (Brown and Gottler, '22; Wiley, '38).

Ethylene glycol poisoning in humans causes widespread capillary damage (Hagemann and Chiffelle, '48) characterized by perivascular inflammation and hemorrhages, which were attributed to its metabolite, oxalic acid (Pons and Custer, '46). Somewhat similar hemorrhage in the rat yolk sac cavity in one conceptus and thrombus formation in or adjacent to the placenta in 3 additional conceptuses were observed in the present study.

From the fragmentary evidence in the literature, it is apparent that maternal metabolic acidosis, hyperosmolality, and perivascular hemorrhages may have the potential to disrupt fetal development.

Sodium salicylate

Sodium salicylate-induced acid-base-electrolyte changes were the following: mild acidosis with consequent hypokalemia and hypophosphatemia, with pH being readjusted to the normal range by increased renal excretion of H^+. These changes presented a minimal deviation from normal; consequently, their etiologic involvement in fetal anomalies appeared doubtful. The salicylate-induced acid-base-electrolyte imbalance increased slightly with simultaneous NH_4Cl treatment and decreased slightly with simultaneous $NaHCO_3$ treatment. In contrast, the salicylate-induced resorptions were intensified by simultaneous NH_4Cl dosing, and these resorptions as well as the salicylate-induced fetal abnormalities were markedly alleviated by simultaneous $NaHCO_3$ dosing. Since the effects of the simultaneous treatments with NH_4Cl and $NaHCO_3$ on sodium salicylate-induced acid-base-electrolyte changes were minimal whilst their effects on fetal anomalies were considerable, the pathophysiologic changes attributed to sodium salicylate may not have been so disruptive as to cause adverse effects on embryonic development.

Histopathologic changes due to sodium salicylate were quite specific. They consisted of maternal hemorrhages, particularly in the yolk sac and coelomic cavities, papillary proliferation of the visceral yolk sac endodermal cells which contained acido-

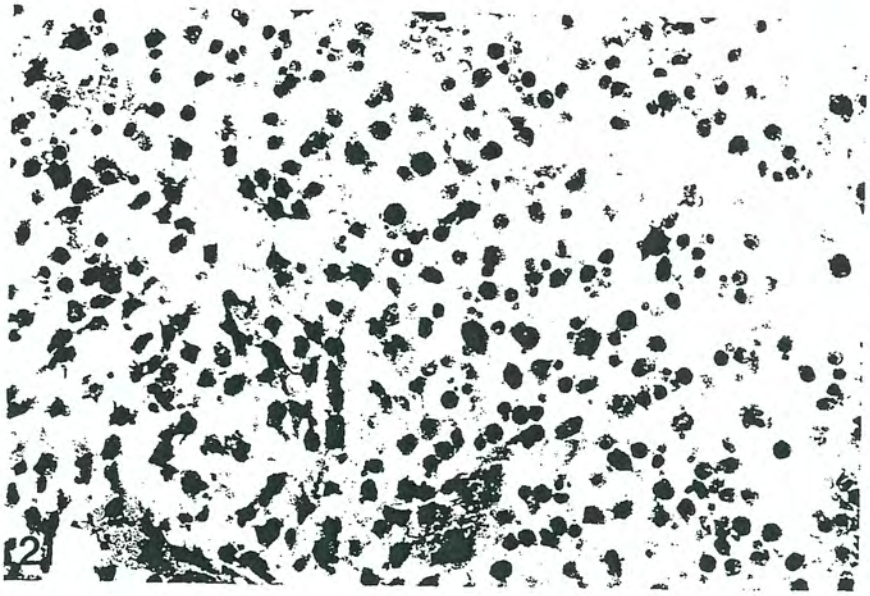

Fig. 21. Cadmium chloride, 5.5 mg/kg administered ip on day 10 of pregnancy. Necrosis of decidual cells, 24 h after ip dosing. Magnification, ×330.

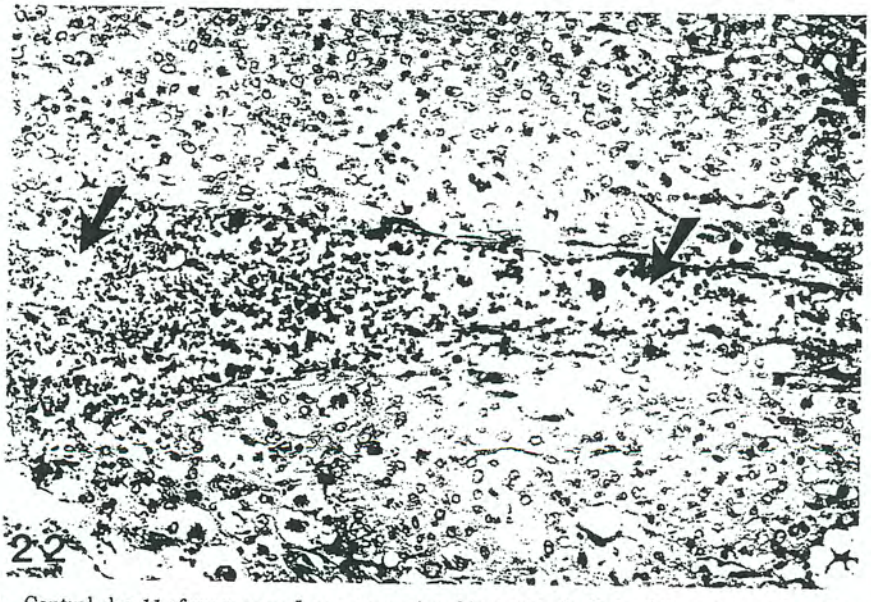

Fig. 22. Control, day 11 of pregnancy. Long narrow band (arrows) of cellular debris observed in all control and test conceptuses. Magnification, ×132.

philic globules resembling maternal erythrocytes, inhibited mesodermal differentiation and vitelline vessel formation, allantoic degeneration, and an absence of the chorioallantoic labyrinth. The pathway of maternal hemorrhage was via ectoplacental cavity and then, through a breach of continuity in the chorion, into the exocoelomic cavity. A pathway of maternal hemorrhage into the yolk sac cavity could not be determined. The

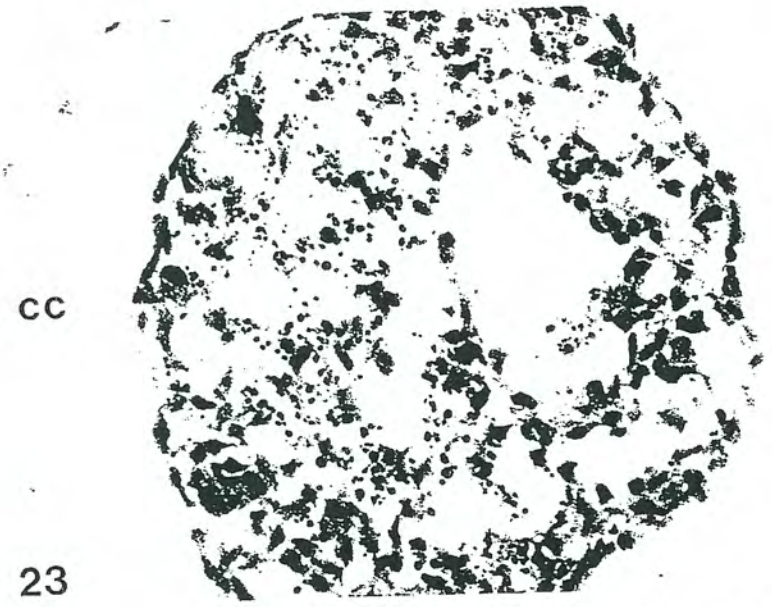

Fig. 23. Cadmium chloride, 5.5 mg/kg administered ip on day 10 of pregnancy. Karyorrhexis of allantois situated in the extraembryonic coelom, 48 h after ip dosing. Transverse section. Magnification, ×320.

incidence and severity of the histologic changes and the incidence of fetal anomalies induced by sodium salicylate were both significantly reduced by simultaneously administered $NaHCO_3$ and significantly increased by simultaneously administered NH_4Cl. The possibility of a contributory role for these lesions in the etiology of fetal anomalies was therefore strengthened.

The maternal erythrocytes in the vascular network on days 11 and 12 of pregnancy were in close anatomic apposition to the Reichert's membrane. The membrane, being semipermeable, is known to permit O_2-CO_2 gaseous exchange between the maternal erythrocytes and the developing embryo. Normally, Reichert's membrane is a major barrier between maternal and fetal environments and would not permit the passage of maternal erythrocytes. In salicylate-treated conceptuses, the maternal vascular network was either ischemic or separated from Reichert's membrane by a wide recess filled with maternal plasma.

The most important functions of endodermal cells of the visceral yolk sac are 1) to capture maternal serum proteins, including IgG, from the transudate filtered through Reichert's membrane and parietal yolk sac, and then either a) to hydrolyse them to amino acids (Jensen et al., '75; Freeman et al., '82; Freeman and Lloyd, '83) which subsequently are synthesized into proteins for embryonic and adnexal tissue or b) in the case of IgG, to translocate unaltered protein to the fetus, and 2) to produce and secrete alpha fetal proteins, transferrin, apolipoproteins, and α_1-antitrypsin. Accordingly, visceral yolk sac endoderm performs functions similar to those of the intestines and liver (Meehan et al., '84; Muglia and Locker, '84). A disturbed visceral yolk sac function has been shown to cause teratogenicity (Slotnick and Brent, '66; Beck et al., '67; Brent et al., '71; Jensen et al., '75; Freeman et al., '82).

The distension in the yolk sac cavity and the thickening of visceral yolk sac endoderm increased the distance (Figs. 10, 12) for the materno-fetal gaseous exchange. It is possible that the increased distance may have reduced O_2/CO_2 exchange and slowed an overall development of the vitelline circulation and thus that of the embryos.

In conceptuses on day 12 of pregnancy and 48 h after salicylate dosing, the chorioallantoic vascularization, compared to the control conceptus, already had been absent for at least 24 h. For normal embryogenesis beyond day 12 of pregnancy, the chorioal-

lantoic vascularization is critical for nutrition and gaseous exchange. Embryos from 12½ day pregnant rats when explanted into in vitro cultures showed no growth or development (New and Coppola, '70).

Cadmium chloride

Cadmium is rapidly cleared from the plasma and becomes associated with erythrocytes, regardless of the route of administration (Shaikh and Lucis, '72). Cadmium has been causally linked to acute necrosis and hemorrhaging in the testis (Parizek, '60), central sensory ganglia, cerebrum, and cerebellum (Gabbiani et al., '67a,b) and to hypertensive and vascular diseases (Schroeder, '64). Cadmium renders blood vessels of the testis and epididymis more permeable and slows testicular blood flow; the resulting hypoxia damages the germinal epithelium and interstitial tissue (Gunn et al., '63; Chiquoine, '64; Mason et al., '64; Waites and Setchell, '66; Kormano, '68).

ip and iv routes of dosing

The occurrence of necrosis and hemorrhage in the decidua basalis and ectoplacental cone and the presence of a hemorrhagic zone around Reichert's membrane were compatible with the cadmium-induced necrosis and hemorrhage in the testicle and other tissues.

On day 10–11 of pregnancy, maternal vascular channels in control conceptuses passed through the central region of the decidua basalis and the peak of the ectoplacental cone to reach the chorionic disc. The blood was then distributed radially in the disc and the venous blood was collected in the lateral sinusoids situated in the lateral decidual zone (Young, '56; Panigel, '59; Leiser and Baier, '88). In cadmium-treated conceptuses, the arterial channel was ruptured since the central region of the decidua basalis and the ectoplacental cone were undergoing necrosis. There was hemorrhage and hemostasis around Reichert's membrane. A reduced materno-fetal O_2/CO_2 exchange leading to fetal hypoxia is expected. The other lesion was an apparent failure to form a chorioallantoic labyrinth. It is hard to believe that in the absence of an integrated chorioallantoic labyrinth, embryonic differentiation will continue with the alacrity that attends the normal rate of development.

Placental transfer of Cd^{2+} occurs in hamsters early in pregnancy (Ferm et al., '69). Radioactivity in the primitive gut of embryos was noticed following iv injection of $^{109}CdCl_2$ to pregnant hamsters and mice on the 8th day of pregnancy (Dencker, '75). However, the gastrointestinal tract has never been a site of fetal malformation in any species so far studied. In another study, embryonic levels of cadmium were about .005% of an intragastric dose of 100 μg ^{109}Cd/rat administered on day 11 of pregnancy (Ahokas and Dilts, '79). However, isolating 11 day-old embryos without Cd^{2+} contamination from the surrounding tissues is technically difficult. There are no convincing in vivo data to show that presence of Cd^{2+} in 10–11 day-old fetal tissues or a direct action of Cd^{2+} on fetal tissue is responsible for fetal anomalies.

sc route

The sc route required higher doses of $CdCl_2$ to produce fetal anomalies (Table 13). The mechanism of teratogenic action by this route was apparently different from the mode of action by the ip and iv routes, and was not pursued further.

Maternal toxicity and maternal factors in fetal anomalies

The doses of the three chemicals in these studies manifested signs of maternal toxicity, i.e., reductions in body weight, food intake, and water consumption, deaths (Tables 3, 8, 11–13), and clinical signs of toxicity. Although materno-toxic signs for the three chemicals were similar, maternal factors relevant to fetal anomalies were different. Ethylene glycol induced metabolic acidosis and hyperosmolality which may have reduced villigenesis in the chorioallantoic labyrinth. Sodium salicylate and cadmium chloride caused maternal hemorrhages in the extraembryonic cavities. In addition, cadmium chloride caused necrosis in the decidua basalis. Interestingly, the 2.6 mg $CdCl_2$/kg iv dose that manifested no apparent sign of maternal toxicity (Table 12) did produce conceptal lesions (Table 14) and fetal anomalies (Table 12). The maternal pathophysiological and histological changes, reported here, seemed to have acted intricately with the extraembryonic tissue to produce extraembryonic lesions.

The maternal toxicity may be categorized into two arbitrary classes: 1) alterations in maternal tissues that are relevant to em-

bryonic development (maternal factors), and 2) alterations in maternal organ-system that have no apparent effect on embryonic development. A distinction between these two classes should be based on changes in maternal homeostasis, physiology, and a primary morphologic site of a chemical's action in maternal and extraembryonic organ-systems. Clinical indicators of maternal toxicity cannot make this distinction since they hardly vary between drugs tested for teratogenicity (Khera, '85) and can arise from a wide variety of conditions. The presence or absence of maternal toxicity, as estimated at present, may have little relevance to fetal health and its absence does not necessarily preclude maternal factors leading to fetal anomalies.

Hypothetically, a chemical may cause an adverse effect on embryonic development 1) by a direct effect on embryonic tissues (primary fetal response), 2) through pathophysiological changes in maternal and extraembryonic tissues, which, in turn, may influence fetal health (secondary fetal response), or 3) by a combination of 1 and 2 (both primary and secondary responses). It is pointed out that in our study, the possibility of a direct effect of the three test drugs on the fetus itself was not explored.

CONCLUSIONS

Ethylene glycol

Ethylene glycol caused metabolic acidosis and hyperosmolality, as early as 1 h after dosing rats on day 11 of pregnancy. This effect was followed by allantoic and circulatory lesions and a reduced chorionic infiltration by the allantoic villi. These lesions implied a decreased surface area between maternal and embryonic circulations and may have limited nutritional and O_2 needs for embryonic development. Labyrinthine lesions were associated with decreased fetal weight and increased frequency of fetuses with fused ribs, reduced number of ribs, and retarded ossifications of sternebrae, vertebrae, and phalanges. The $NaHCO_3$ treatment simultaneous with ethylene glycol-dosing reduced maternal acidosis and hyperosmolality that correlated with similar reductions in both labyrinthine lesions and fetal anomalies. Acidosis and hyperosmolality in the embryo occurring in response to these changes in the mother is possible and was not ruled out.

Ethylene glycol-induced maternal metabolic acidosis, hyperosmolality, and perhaps perivascular hemorrhages were postulated as maternal factors which, by inhibiting villigenesis, reduced embryonic nutrition. Reduced nutrition then may have contributed toward fetal weight reduction, reduced rib number, and retarded ossifications, i.e., delayed development.

Ethylene glycol, 5,000 mg/kg/day po from day 7 to 16 of pregnancy (Price et al., '85) induced a high incidence of rib and vertebral anomalies and fetal weight reduction, such as those reported here, as well as a low, yet significant, incidence of cleft lip and palate, anophthalmia, meningoencephalocele, and other anomalies. A contributory role for maternal acidosis and hyperosmolality in the fetal effects was expected for two reasons: 1) a reduced width in the labyrinth after dosing from day 7 to 13 of pregnancy was similar to, yet more striking than, that observed after a single 3,333 mg/kg sc dose, and 2) acid-base studies, not reported here, showed that a 5,000 mg/kg daily po dosing for days 7–16 of pregnancy produced both maternal metabolic acidosis and hyperosmolality that lasted for at least 9 h and recurred after every dose. These disorders were reversible about 24 h after dosing.

Sodium salicylate

Sodium salicylate, 280 mg/kg/day, administered sc as a single dose on days 8, 9, and 10 of pregnancy caused changes in maternal homeostasis, fetal anomalies, and conceptal pathology. The salient anomalies were resorptions, reduction in fetal body weight, hydrocephaly, exencephaly, spina bifida, and rib defects (fused, wavy, and supernumerary). On coadministration with sodium salicylate, NH_4Cl significantly increased and $NHCO_3$ reduced their incidence. The maternal homeostatic changes were mild-to-moderate PCO_2 reduction, HCO_3^- deficit, hypokalemia, and hypophosphatemia, with pH of the blood staying within the normal range. These homeostatic changes were modified only to a limited extent when sodium salicylate was administered with either NH_4Cl or $NaHCO_3$. Thus, the sodium salicylate-induced maternal changes did not appear to have a role in the observed fetal anomalies.

The pathological conceptal changes consisted of 1) maternal hemorrhage in the cavities (primarily in the yolk sac cavity and

coelom), 2) papillary proliferation of the visceral endodermal cells that contained eosinophilic globules resembling maternal erythrocytes, and 3) marked exudation that distended the yolk sac cavity and created a recess around the Reichert's membrane. These changes were followed by suppressed differentiation of vitelline vessels, degeneration of the allantois, and an absence of a viable chorioallantoic labyrinth, probaby from decreased materno-fetal gaseous exchange and nutrition to the fetus.

A further support that the conceptal lesions might have contributed to salicylate-induced fetal anomalies came from the results of the simultaneous treatment of sodium salicylate with either $NaHCO_3$ or NH_4Cl. $NaHCO_3$ significantly reduced and NH_4Cl enhanced the incidence of both conceptal lesions and fetal anomalies.

Cadmium chloride

$CdCl_2$ administered on day 10 of pregnancy was studied for maternal physiology by the ip route at 5.5 mg/kg, and for teratology and conceptal morphology by the ip route at 3, 4, 5, or 5.5 mg/kg and by the iv route at 2.6 or 4.5 mg/kg dose.

The physiological changes were mild, transitory, compensated acidosis and a minor increase or decrease in the concentration of a number of electrolytes. Since the deviation of these changes from control values was small and without any consistent trend, their relationship to fetal anomalies was considered to be trivial. The teratology studies by the ip and iv routes showed a dose-related resorption, fetal body weight reduction, anophthalmia, hydrocephaly, thoraco-lumbar vertebral fusion, and defects in ribs and sternebrae.

The conceptal lesions related to fetal anomalies in the ip and iv studies of $CdCl_2$ were dose-related and consisted of extensive necrosis and hemorrhage in the decidua basalis and in the ectoplacental cone and a hemorrhagic area that encapsulated the Reichert's membrane. These hemorrhages were sufficiently extensive to prescribe hypoxia by restricting the materno-fetal gaseous exchange and subsequent degenerative changes in the chorion and allantois abolishing their fusion and formation of labyrinth.

The incidence of conceptal lesions in the morphology study and of fetal anomalies in the teratology study by the ip and iv routes was dose-related which strengthened the association of conceptal lesions with fetal anomalies.

By the sc route, the 12, 16, or 20 mg/kg of $CdCl_2$ produced teratogenic effects similar to those by the ip and iv route. However, these doses failed to produce detectable changes in conceptual morphology which suggested that teratogenic mechanisms by the sc route were different from that by the ip and iv routes.

General comment

It is well known that mechanical interruption of uterine blood supply to the pregnant rat uterus leads to fetal pathology and congenital malformations (Brent, '90). The work reported here further stresses the necessity of recognizing maternal and extraembryonic changes as contributors to the formation of fetal anomalies. An understanding of the underlying mechanism of drug-induced fetal anomalies should begin with an understanding of homeostatic disorders in the maternal organism, and morphologic changes in the extraembryonic, placental, and fetal tissues. Studies should first define the initial or primary sites of action of a teratogen as a starting point for molecular analysis.

ACKNOWLEDGMENTS

The author thanks Ms. Carole Whalen and Messrs. George Angers and Pierre Huard for excellent technical assistance and Dr. D. Arnold for discussions and encouragement.

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