TERATOLOGICAL STUDIES WITH ALUMINUM IN THE RAT

A Thesis

Presented to

The Faculty of Graduate Studies and Research

The University of Manitoba

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science

by

Ronald Walter Bennett, B.Sc.

August 1974

To my wife, son,

daughters and parents

without whose help this work

would not have been possible.

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ABSTRACT

The effects of aluminum on pregnancy and fetal development were investigated. Aluminum in the form of aluminum chloride was administered intraperitoneally to pregnant rats at different dose-levels and at different stages of gestation. A high incidence of maternal death followed treatment with high dose-levels of the substance. Maternal weight gain during the entire gestational period was less in treated animals, compared to controls. In many cases, maternal liver was severely damaged as a result of the treatment. The offspring of mothers treated with aluminum chloride showed significant growth retardation as well as skeletal defects. In addition the incidence of fetal deaths and resorption was significantly increased.

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LITERATURE REVIEW

LITERATURE REVIEW

1. Teratogenesis: historical background

Monstrous deformities present in children at birth have been known since early times. Undoubtedly, they predated records on clay tablets and appeared in early art forms, for example, a sculpture of a double headed twin goddess, excavated in Turkey, dated approximately 6500 B.C. (Warkany, 1971). The causes of these bizarre phenomena were attributed to astrological influences, divine will, witches, and demons. For many years the study of congenital defects was limited by a lack of understanding of developmental processes and the prevailing influence of superstition.

Harvey, in his book, Exercitatio de generatione animalium, in 1651, stressed the significance of environmental influences on prenatal development and considered important uterine width and maternal posture responsible for some malformations (cited in Persaud, 1970).

It was not until the eighteenth century that a scientific approach to the causes of birth defects was made. Malformations were produced in animals experimentally. The concept of "developmental arrest" was introduced, which explained the fact that in many congenital defects, development appeared to have stopped in some early stage and the primitive features characteristic of that period were retained (Persaud, 1970).

Since the eighteenth century, an ever increasing body of knowledge relating to congenital defects has been accumulating. The literature on environmentally induced defects has become vast (see Shepard, 1973), while investigations on the method of action of teratogens have remained relatively few.

With improvements in general health care, the incidence of several important early childhood diseases, which have contributed greatly to infant morbidity and mortality, has been reduced, and because of this the problems of birth defects have received more attention.

2. Teratogenic Agents

2.1 Drugs and chemicals as teratogens

Advances in technology have led to an increased exposure and uptake of a variety of drugs and chemicals by all living organisms including man. The medical and non-medical use of these substances has in many instances created a variety of socio-medical problems. It has been suggested that many of these agents may be potentially teratogenic when administered under specified conditions to experimental animals (Cahen, 1966; Saxén and Rapola, 1969). Teratogenic potential is apparently related to the metabolic or homeostatic requirement of the developing embryo (Saxén and Rapola, 1969; Wilson, 1965).

Several drugs have been positively implicated as being teratogenic in humans, e.g., thalidomide, steroid

hormones, folic acid antagonists, and antitumor agents (Cahen, 1966; Persaud and Moore, 1974; Wilson, 1973). McBride, in 1961, described the effects of thalidomide given during early pregnancy in women. This provided convincing evidence of a causal relationship between a specific teratogen and human congenital malformations. Many other drugs and chemicals are suspected of being potentially teratogenic in humans, but are as yet to be clearly established in this regard. The majority of these substances taken during pregnancy contribute to a small number of known developmental defects in humans (Wilson, 1973).

On the other hand, many drugs and chemicals have been shown to be teratogenic in different species of experimental animals (Shepard, 1973).

2.2 Maternal infections

Some maternal infections have been positively associated with birth defects in humans. In 1942, Gregg observed cataract, microcephaly, deaf-mutism and heart lesions in infants of mothers who contracted rubella during early pregnancy. Other viral and bacterial infections, e.g., herpes simplex, cytomegalovirus, poliomyelitis, and syphilis have also been reported to be teratogenic (Brown, 1966; Bulova, Schwartz and Harrer, 1972; Dudgeon, 1968; Hardy, 1965).

2.3 Ionizing radiation

Ionizing radiation has been shown to be teratogenic

in humans and experimental animals (Goldstein and Murphy, 1929; Hicks and D'Amato, 1966). Radiation will not only affect development of the embryo during the period of organogenesis, but also after in tissues in the process of cellular differentiation, e.g., central nervous system (Saxén and Rapola, 1969).

2.4 Maternal metabolic imbalances

Certain maternal imbalances, such as, endemic cretinism, diabetes, phenylketonuria, and virilizing tumors may contribute to developmental abnormalities (Wilson, 1973).

Table 1 gives some of the known causes of birth defects in man. It is of particular interest to note that the cause of the majority of congenital defects remains unknown.

3. Biological Properties of Trace Metals

3.1 General remarks

The intensified growth of modern industry has been accompanied by an increased utilization of metals in processing of manufactured goods, food, and drugs. The mobilization of many trace metals has resulted in a growing concern as to the effects of these substances on various biological processes. Many trace elements are recognized as beneficial at particular concentrations and toxic at others (Kazantzis, 1973; Lee, 1973; Maugh, 1973; Mertz, 1974; Widdowson, 1969). There has been, however, insufficient information on the majority of metals with regards to

Table I

Causes of Developmental Defects in Man *

Known genetic transmission	20%
Chromosomal aberration	5
Environmental causes	
Radiations	<1
Therapeutic	
Nuclear	
Infections	2–3
Rubella virus	
cytomegalovirus	
Herpesvirus hominis	
Toxoplasma	
syphilis	
Maternal metabolic imbalance	1–2
endemic cretinism	
diabetes	
phenylketonuria	
virilizing tumors	
Drugs and environmental chemicals	2–3
androgenic hormone	
folic antagonists	
thalidomide	
organic mercury	
some hypoglycemics (?)	
some anticonvulsants (?)	
Combinations and interactions	?
Unknown	65–70%

* Data from Wilson, 1973

their effects on biological systems (Ferm, 1972).

Metals are immutable and do not undergo degradation, but will combine with other elements forming compounds that may also be toxic. The toxicity may not be attributable to the metalic component alone (Hammond, 1973). Certain heavy metals aggregate, for example, mercury, lead, and cadmium, in target organs until threshold levels are reached where structure and function of the cells may be altered (Kazantzis, 1973).

The problem encountered in evaluating the biological effects of trace metals involves the determination of accumulation and distribution in the organism, interaction with other substances and whether metabolic processes have been affected.

3.2 Metals during pregnancy

There is sufficient evidence to suggest that human maternal intoxication with certain metals may adversely affect pregnancy and the developing conceptus. Mercury, one of the few metals teratogenic in man, severely affected the central nervous system of both mother and offspring (cited in Warkany, 1971).

Lead poisoning in women has been associated with sterility, abortion, high fetal and neonatal losses. There has not been conclusive evidence that this metal is teratogenic in humans (Angle and McIntire, 1964; Ferm, 1972).

These two metals are secreted in the milk of

lactating mothers, and their influence on post natal development of the offspring has received little attention (Task group on metal accumulation, 1973).

It is not known whether other metals (e.g., zinc, manganese, cadmium, copper, molybdenum) which may accumulate are teratogenic in humans (Ferm, 1972).

Metals administered to experimental animals produced both general and special toxic effects on male and female reproductive organs as well as the central nervous system (Task group on metal accumulation, 1973; Ferm, 1972; Gale, 1973; Mansour et al., 1973; Parizek et al., 1969; Shephard, 1973).

The hemochorial placenta of primates and rats permits the passage of metals to the fetus more easily than other experimental animals following maternal administration. After the metal reaches the fetus it may influence metabolic processes that are highly active during critical stages of prenatal development, such as, enzymatic activity which controls protein synthesis, cell division, and differentiation (Ferm, 1972).

Teratological effects have been observed in rodents following administration of toxic quantities of cadmium (Barr, 1973; Chernoff, 1973), lithium (Wright, Hoffman and Davies, 1971), lead (Ferm, 1972), and mercury (Spyker and Smithberg, 1972) to the mothers. It has been found that maternal deficiencies of essential metals, for example, manganese and zinc, are also teratogenic (Hurley, 1968; Hurley, Gowan and

Swenerton, 1971).

There is voluminous literature on the adverse effects of metals (excesses and deficiencies) in the avian embryo, in particular the chick (Ferm, 1972; Romanoff, 1972).

4. Aluminum

The earth's surface is abundant with aluminum compounds and it has been suggested that living organisms have evolved in the presence of appreciable quantities of these substances (Crapper, Krishnan and Dalton, 1973). It is of immediate importance to study the influence of aluminum on living organisms due to its increased utilization in recent years (Campbell et al., 1957), and its potential harmful effects when present in food and drugs in unusually large quantities.

4.1 Chemical and physical properties

Aluminum does not occur in its elemental form, but as compounds of other substances. It has an atomic number of 13 and an atomic weight of 26.97. The valency of aluminum is 3. It is not heavy, having a density of only 2.7, and a melting point of 660.2°C.

Aluminum combines with acids and bases, thus producing salts and aluminates. The aluminum ion is rather small and has a high charge. This property enables it to combine readily with small negative ions. These combined forms behave as covalent compounds and do not readily ionize.

4.2 Aluminum compounds

Aluminum ions readily combine with chloride ions to produce $AlCl_3$. In water $AlCl_3$ forms a hydrate, $AlCl_3 6H_2O$ or $Al(H_2O)_6Cl_3$. Ionized chloride reacts with water to form oxychloride and HCl, thus the resulting solution is acidic. $AlCl_3$ will also dissolve in organic solvents.

Solutions of aluminum salts when treated with OH ions form aluminum hydrogels ($Al_2O_3 nH_2O$) which are precipitates. These occur in crystalline and noncrystalline forms. Aluminum hydrogels adsorb acids, bases and salts from solution.

Aluminum hydroxide, $Al(OH)_3$, is formed when aluminum ions occur in a solution of a metal hydroxide, sulfide or carbonate. It is insoluble in water between pH 4 and 8 up to 10.5. In water the solution is acidic. $AlOH_3$ readily dissolves in cold HCl, H_2SO_4, and solutions of fixed bases, the solubility being adversely affected by drying. It neutralizes alkalies by its affinity for hydroxyl ions, forming complex anions.

$$Al(OH_3) + OH \rightleftarrows Al(OH)_4^-$$

Aluminum ions in solution may be predicted to form phosphate compounds, e.g., orthophosphate, primary, secondary, and pyrophosphate. These compounds are relatively insoluble in water, but readily dissolve in aluminum salt solutions or aluminates between pH 3.9-5.5 and 8.6.

Aluminum sulphate $Al_2(SO_4)_3 \cdot 18H_2O$ is soluble in water and is used to prepare some aluminates. In water it is converted by alkalies to $Al(OH)_3$.

Aluminum salts will form binary salts or aluminates, e.g., aluminum sulphate forms a series of salts $R'_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ or $R'Al(SO_4)_2 \cdot 12H_2O$, R' representing the univalent radical.

Aluminum may also combine with proteins and other biologically important substances. The degree of formation of these complexes may be affected by the reactivity and solubility of aluminum that changes with varying physiological pH values, by enzymes or other substances in tissues, the digestive tract and food (Campbell et al., 1957; Ondreicka, Kortus and Ginter, 1971).

4.3 Aluminum distribution

4.3.1 In soil

The quantity of aluminum occurring in the soil is dependent upon the level of mineral material. Its compounds are found in the earth's crust in undecomposed rocks, aluminosilicate clays, hydrosol hydrated oxides, as phosphates, and in ionic form. Aluminum compounds are very insoluble, the solubility being affected by the presence of hydrogen ions. The presence of the ionic form is believed to exist only in areas of low pH levels (Campbell et al., 1957).

4.3.2 In atmosphere

Aluminum is present in the atmosphere as airborne particulate matter. The amount present at any given time is influenced by meteorological conditions, population density, and vehicular traffic. Large amounts exist in areas where coal is used as a fuel (Campbell et al., 1957; Natusch, Wallace and Evans, 1974).

4.3.3 In plant material

The amount of aluminum contained in plant material varies from trace to high values; generally, the concentrations are quite low. The amount varies with the soil type the plant habitates. Higher values are found in the leaf than the stem. The mean value of aluminum content is estimated to be 200 ppm in herbaceous vegetation (Hutchinson, 1945).

4.3.4 In water

The aluminum content of water in general is insignificant, due to its precipitation or adsorption on sediments. Higher values may occur in specific situations, such as strong alkaline or acidic conditions. Polluted water may give rise to high concentrations due to pH and other qualities that keep aluminum complexes in solution.

4.4 Ingested aluminum

4.4.1 In food

The aluminum content of food varies with the geographic

area the food is obtained from. Food of animal origin contains up to one hundred times less aluminum than plant material (cited in Ondreicka, Kortus and Ginter, 1971).

Aluminum compounds are added in pickling processes, are used in brewing and sugar industries, and present in many products to which baking powder, containing aluminum, is added. The estimate of the aluminum content of food is 10-30 p.p.m. (Calvery, 1942), with a daily intake of between 5 and 135 mg per day (Schlettwein-Gsell and Mommsen-Straub, 1973). Variations in the amount of aluminum ingested may occur in particularly abnormal dietary habits.

The utensils, constructed of aluminum and its alloys used in the food processing industry, contribute little of the metal to food (Lewis, 1954; Poe and Cason, 1951).

4.4.2 Medical use of aluminum

The medical use of substances containing aluminum may allow greater than normal amounts of the metal to gain entrance into the "internal milieu" of the body. This may be in the form of prescribed or non-prescribed medication.

Aluminum compounds are administered therapeutically in the following conditions: peptic ulcers (Fishman, 1945), heartburn and esophagitis (Hansky, 1973), renal failure (Goldsmith and Johnson, 1973), preventing formation of kidney stones (Shorr, 1945), and urogenital trichomoniasis (Karnaky, 1973). Dry particulate forms have been administered in the treatment of silicosis. Other aluminum compounds

may be used as astringent preparations, to control excessive sweating, as deodorants, and antiperspirants.

In addition, aluminum compounds, e.g., aluminum chloride, may also be used to counteract the toxic effects of sodium fluoride in cattle and sheep (cited in Campbell et al., 1957).

4.5 Aluminum metabolism

The low ionizing properties of aluminum compounds make it unlikely that under normal conditions large quantities of the ionized form will be present in the gastrointestinal tract (Myers and Killian, 1928).

In a recent study, however, it was shown that part of the ingested aluminum is absorbed and transported to the tissues (Perry et al., 1962). Results of studies carried out in rats have shown that with increasing aluminum content of food fecal elimination increases, but tissue levels do not rise. With further increases there is significant accumulation of aluminum in the tissues, and it appears in the urine (see also Ondreicka, Kortus and Ginter, 1971). Large quantities of aluminum are not tolerated; the metal is deposited in certain tissues, particularly the skeleton and liver (Ondreicka, Ginter and Kortus, 1966).

Aluminum is secreted by the lactating human mammary gland (Lewis, 1931).

The exact role the urinary system plays in the excretion of aluminum has not yet been resolved. However,

recent evidence (Berlyne et al., 1970; Berlyne et al., 1972) points out that excretion by this route may be more important than was previously suspected in both animals and man.

The physiological requirements of aluminum regarding its influence on growth and metabolism remain to be determined (Ondreicka, Kortus and Ginter, 1971).

4.6 Aluminum toxicity

Studies carried out on the toxic effects of aluminum have largely been incomplete and inconclusive. Some investigators reported that aluminum produced no adverse reactions when administered over extended periods (Hove, Elvehjen and Hart, 1938; Myers and Mull, 1928; Mackenzie, 1932), while others have observed a wide spectrum of toxic effects following administration of high doses (Berlyne et al., 1972; Hara et al., 1959; Kortus, 1967; Ondreicka, Ginter and Kortus, 1966).

Generally the LD_{50} for most common aluminum compounds falls between 1-5 g/Kg of body weight following peroral administration (Seibert and Wells, 1929; Hara et al., 1959). For mice, the LD_{50} of $AlCl_3$ given orally was 770 ± 120 mg/Kg body weight (Ondreicka, Ginter and Kortus, 1966), and for rats, following intraperitoneal injection of $Al(HO_3)_3 9H_2O$ was 327 mg/Kg body weight (Hart and Adamson, 1971). Berlyne et al. (1972) found that a dose of 150 mg/Kg body weight of $Al(OH)_3$ given daily showed high mortality within five

days, when administered intraperitoneally.

It would therefore appear that the toxicity of aluminum is dependent on several factors which include the route of administration, the species of animal, and aluminum compound involved. There is as yet scant information on the chronic toxicity of these compounds.

4.7 Effects of aluminum on phosphate metabolism

It has been predicted that aluminum may combine with phosphates in the gastrointestinal tract according to the formula:

$$Na_2HPO_4 + NaH_2PO_4 + 2AlCl_3 \rightleftarrows 2AlPO_4 + 3NaCl + 3HCl$$

forming non-absorbable phosphorous compounds and increasing phosphorous elimination by the feces. Studies with rats and mice, using large doses of aluminum in food, confirmed this reaction (Ondreicka, Ginter and Kortus, 1966).

Further evidence is available indicating that aluminum may influence phosphate metabolism in tissues other than the gastrointestinal tract. Following oral administration of aluminum chloride in rats, there is a decrease in the incorporation of labelled phosphate into phospholipids, ribo- and desoxyribonucleic acids of certain tissues (Table 2).

In rats, acute and chronic intoxication with aluminum chloride administered orally, reduces the adenosine triphosphate, and increases the adenosine di- and monophosphates

TABLE 2

INFLUENCE OF CHRONIC AND ACUTE ALUMINUM TRICHLORIDE INTOXICATION ON THE INCORPORATION OF ^{32}P INTO TISSUE FRACTIONS IN THE LIVER, SPLEEN, AND KIDNEYS OF RATS *

Fraction	Specific activity (counts/min/100 μg P)			Significance against control	
	Control	Chronic intoxication	Acute intoxication	Chronic intoxication	Acute intoxication
Liver					
Acid-soluble P	1944±96	1806±81	2244±132	$P > 0.05$	$P > 0.05$
Lipid P	2139±108	1720±132	1625±61	$P < 0.05$	$P < 0.01$
RNA	1234±65	928±40	870±207	$P < 0.01$	$P > 0.05$
DNA	176±16	172±23	95±15	$P > 0.05$	$P < 0.01$
Spleen					
Acid-soluble P	2395±177	1997±112	1736±356	$P > 0.05$	$P > 0.05$
Lipid P	1294±176	986±55	744±271	$P > 0.05$	$P > 0.05$
RNA	1671±504	1242±119	358±131	$P > 0.05$	$P < 0.01$
DNA	2285±1324	1750±227	252±62	$P > 0.05$	$P < 0.01$
Kidneys					
Acid-soluble P	1994±57	1801±83	2238±95	$P > 0.05$	$P > 0.05$
Lipid P	1743±194	1190±77	1013±61	$P < 0.05$	$P < 0.05$
RNA	834±65	696±57	602±69	$P > 0.05$	$P < 0.05$
DNA	128±28	334±152	44±8	$P > 0.05$	$P < 0.05$

Average values ± S.E. are given.

* Data from Ondreička, Kortus, and Ginter, 1971

content of blood.

Intoxication with aluminum salts in rats may also affect carbohydrate metabolism. Following oral administration of 200 mg aluminum/Kg body weight there is a decrease in liver and muscle glycogen, increase in the lactic acid content of liver and muscle, and an increase in liver pyruvic acid levels (Kortus, 1967). These responses probably arise secondarily to changes in the absorption of phosphates (Ondreicka, Kortus and Ginter, 1971).

4.8 Aluminum and bone formation

Aluminum may influence bone formation. This may be due in part to binding of aluminum with phosphates in the gastrointestinal tract. In fact, the mechanism involved may be more complicated (Berlyne *et al.*, 1972). Concentrations as low as 1 μ M of aluminum ions can initiate hydroxyapatite precipitation (Bachra and van Harskamp, 1970). Aluminum containing antacid compounds can produce in man increased reabsorption of skeletal calcium, failure of bone formation, even in the absence of the parathyroid gland (Bloom and Flinchum, 1960; Lotz, Zisman and Bartter, 1968).

4.9 Other effects of aluminum

Aluminum has been shown to influence other metabolic processes and its toxicity may not be due only to phosphate depletion (Berlyne *et al.*, 1972). These workers have

reported that uraemic rats with aluminum poisoning showed slightly raised phosphate levels.

Further studies on oxygen uptake of rat liver homogenates showed that oxygen consumption decreased by 25% in treated animals, indicating a direct toxic action on the cells.

Aluminum chloride exerts an inhibitory effect on cytochrome c-oxidase activity of rat lung *in vitro* (Engelbrecht and Jordaan, 1972).

Hara *et al.* (1959) reported that aluminum has an inhibitory action on surgically removed hearts of various animals, which stopped in diastole. Intra-venous administration of aluminum in rabbits resulted in a fall in blood pressure and abolished respiration. No significant action on blood or liver function was detected.

Aluminum may also be toxic to the nervous system. High concentrations have been found in various regions of the brains of patients with Alzheimer's disease.

In cats, subarachnoid injection of aluminum chloride produces neurofibrillary degeneration followed by alterations in short term retention, conditioned avoidance response acquisition, and motivation. Patients with senile and presenile dementia of the Alzheimer type also show neurofibrillary degeneration (Crapper, Krishnan and Dalton, 1973).

4.10 Teratological studies with aluminum

Placental mammals have evolved a method of reproduction

whereby the young develops during a specific gestational period within the uterine cavity of the mother. The mother provides a protective environment for her young, serving as a buffer between the conceptus and external surroundings.

The placenta establishes communication between the mother and offspring, allowing passage of certain substances and restricting the transfer of others. There is good evidence suggesting that a wide spectrum of chemical substances, including drugs, are capable of influencing metabolic processes in the developing offspring following placental transfer.

Trace metals, and in particular aluminum, have been reported to cross the placental barrier; significant concentrations have been detected in the fetus (Rusoff and Gaddum, 1937). However, the effects of these substances on developmental processes have not been fully investigated.

Rats receiving 2 mg of aluminum as potassium aluminum sulphate in the diet, showed no abnormalities for four generations (Myers and Mull, 1928). Some growth retardation was found in mice receiving 19.3 mg of aluminum/Kg/day in their drinking water. This appeared only after the first generation and was more pronounced in succeeding generations (Ondreicka, Ginter and Kortus, 1966). Rats receiving $Al(SO_4)_3$ at dose levels of 10 and 30 micrograms per day for six weeks, showed neither impairment of reproduction nor abnormal offspring (Hove, Elvehjen and Hart, 1938).

Dihydroxy-aluminum-natrium carbonate administered

to pregnant women for the treatment of gravid pyrosis produced no adverse effects (Dordevic and Beric, 1972).

In view of the foregoing it was considered important to determine the effects of aluminum on pregnancy and fetal development.

SECTION 5

MATERIALS AND METHODS

MATERIALS AND METHODS

5.1 Experimental animals

Female albino rats, of the Holtzman strain, weighing 200-240 grams, were obtained from three sources; Bio Breeders, Ottawa; Holtzman Co., Wisconsin; and the breeding colony of the Faculty of Dentistry, Winnipeg. These are descended from the Sprague-Dawley strain.

5.2 Environmental conditions

All animals were kept in environmentally controlled rooms at 72°F±5°; relative humidity at 50%±20.

A cycle of 12 hours of light from 0700 to 1900 hours, and dark from 1900 to 0700 hours was maintained at all times.

5.2.1 Food and water

All animals received a diet of 6% mouse and rat pellets supplied by Teklad, Winfield, Iowa. Both food and water were available ad libitum.

5.3 Breeding

Females were housed three per cage during mating. Albino Holtzman males were placed with the females at 1700 hours and separated from them at 0900 hours the following morning.

5.4 Determination of pregnancy

Vaginal smears were obtained at 1000 hours±1 hour. Estimated time of coitus could be placed within the preceding 17 hour ± 1 hour period. The day on which spermatozoa were

found in the vaginal smear was designated day one of pregnancy.

Vaginal smears were obtained with a cotton swab, which was inserted into the vagina and rotated along the walls. This was then spread on a glass microscope slide which was subsequently examined under a light microscope at low power. Females indicating the presence of sperm were considered pregnant. They were weighed and marked with an ear clip for identification.

5.5 Experimental conditions

No more than three pregnant females were housed together during the experiment. Larger cages were used for more than one animal. The gravid females were removed during the experiment only for treatment and weighing.

5.6 Treatment

5.6.1 Experimental and control substances

All experimental animals were treated with a solution of aluminum chloride. This was prepared by dissolving two grams of aluminum chloride crystals (Fisher Scientific Co., New Jersey) in 10 ml of sterile distilled water. The final solution showed a pH of $2.35 \pm .05$ at 25°C.

Control animals were treated with sterile distilled water.

5.6.2 Administration of solutions

All solutions were administered by the intraperitoneal

route with a 1 ml plastic disposable syringe fitted with a 25 G 1/2 hypodermic needle.

5.6.3 Acute treatment

Two groups of animals (Group I and II) were administered aluminum chloride intraperitoneally at a dose-level of 40 mg/Kg body weight on different gestational days. Group I of nine animals were treated on day 9. Group II of eight animals were treated on the 13th day of gestation.

The corresponding control animals were treated with sterile distilled water in a similar manner.

5.6.4 Chronic treatment

Experimental animals were divided into six groups (Groups I-VI). Each was administered aluminum chloride solution intraperitoneally daily for five consecutive days. Groups of animals were treated with aluminum chloride at one of the following dose-levels: 75, 100 or 200 mg/Kg body weight. The period of treatment was either days 9-13 or 14-18 of gestation.

Control animals received sterile distilled water in the same manner as described in the experimental groups (Table 3).

5.7 Post natal physical and neuromotor development

Four pregnant females, acutely treated, two from Group I (experimental) and two control mothers, that

TABLE 3

CHRONIC ALUMINUM TREATMENT DURING PREGNANCY

Group	No. of Animals	Treatment	9	10	11	12	13	14	15	16	17	18
I	6	$AlCl_3$ 75 mg/Kg body weight	+	+	+	+	+					
	6	Control	+	+	+	+	+					
II	5	$AlCl_3$ 75 mg/Kg body weight						+	+	+	+	+
	6	Control						+	+	+	+	+
III	8*	$AlCl_3$ 100 mg/Kg body weight	+	+	+	+	+					
	8	Control	+	+	+	+	+					
IV	10*	$AlCl_3$ 100 mg/Kg body weight						+	+	+	+	+
	10	Control						+	+	+	+	+
V	6*	$AlCl_3$ 200 mg/Kg body weight	+	+	+	+	+					
	5	Control	+	+	+	+	+					
VI	5*	$AlCl_3$ 200 mg/Kg						+	+	+	+	+
	6	Control						+	+	+	+	+

*Not all animals survived the treatment.
Day 1: Day sperm found in vaginal smear.

had become pregnant on the same evening, were allowed to progress to term. Following parturition the young were weighed and each litter reduced to 10 offspring±1 offspring. One litter from group I was cross fostered with a litter from a control mother as described by Joffe (1969). The remaining two mothers retained their litters. All mothers and offspring were housed in individual large plastic breeding cages.

Three physical developmental signs, unfolding of the external ear flap, eruption of upper and lower incisors, and opening of eyes, were observed daily on all pups to assess gross morphological development (Cowley and Griesel, 1963).

Neuromotor development was tested daily on all pups according to the method of Hurley and Everson (1959). The pups were placed on their backs on a table and the time required for them to turn over with all four paws flat on the table was measured with a stop watch. Response to the "righting reflex" was considered positive when the animal was able to turn from its back to its feet in 2.5 seconds or less.

A weight record was kept on all animals for a period of twenty-one days.

5.8 Caesarean section

The pregnant females were anesthetized with sodium pentobarbitol on the twentieth day of gestation and a "Caesarean section" performed in order to recover the fetuses.

The two uterine horns were fully exposed. The fetuses were removed by making an incision along the antimesometrial border of the uterus. The fetuses were removed from their amniotic sac following incision of the walls. Each fetus was separated from its placenta by clamping the umbilicus close to the body wall, and severing the cord between clamp and placenta.

The fetuses were weighed and examined for external malformations. Random fetuses from each litter were eviscerated and placed in 100 percent alcohol; the remaining litter was placed in Bouin's fluid for fixation.

Placentas were removed randomly and maternal liver biopsies were taken. These were fixed in Bouin's fluid for histological examination.

5.9 Examination of specimens

5.9.1 Fixation in Bouin's fluid

Following two weeks fixation in Bouin's fluid, fetuses were measured for the crown-rump length.

Free-hand sections were made with a razor blade and these were examined for visceral malformations under a dissecting microscope (Wilson, 1965).

Fetal liver and kidney were obtained following sectioning, and together with maternal liver and the placentas were routinely processed for histological studies. Staining was carried out with haematoxylin and eosin.

5.9.2 Alizarin red S staining technique

Following a one week period of dehydration, fetuses were cleared in 2 percent NaOH solution and stained with alizarin red S. After staining, the specimens were stored in glycerin (Dawson, 1926). These were then examined under a dissecting microscope for skeletal abnormalities.

5.10 Statistical analysis used

The data on the number of malformed fetuses, resorptions, and live born were analyzed using a chi square. All other data was analyzed using the Student "t" Test.

SECTION 6

RESULTS

6.1 Acute treatment

6.1.1 Weight gain of dams

The mean percentage weight gain over the entire gestational period of dams (Group I), treated at a dose-level of 40 mg/Kg body weight on day 9 of gestation, was not significantly different from the corresponding controls (Table 4).

6.1.2 Fetal weight

The mean weights of fetuses, recovered from mothers (Group I and II) treated at a dose-level of 40 mg/Kg body weight on either day 9 or day 13 of gestation, were not significantly different from those of the corresponding control fetuses (Table 5).

6.1.3 Affected fetuses

The number of dead fetuses, recovered from animals (Group I and II) treated with aluminum chloride at a dose-level of 40 mg/Kg body weight on either day 9 or day 13 of gestation, was low and not significantly different from the controls (Table 6).

The incidence of resorption in these two groups (Groups I and II) of animals was not significantly increased compared to that of control animals (Table 6).

Fetuses recovered from aluminum-treated and control animals showed no apparent abnormalities (Table 6).

TABLE 4

MATERNAL WEIGHT GAIN IN $AlCl_3$ TREATED RATS OVER 20 DAYS OF GESTATION

Dose Level	Day of Gestation	Mean Weight Gain (gm)	S.E.
40 mg/Kg body weight	9	112.85	9.09
Control	9	128.00	16.01

TABLE 5

FETAL WEIGHT AFTER MATERNAL TREATMENT WITH $AlCl_3$

Dose Level	Day of Gestation	Mean Fetal Weight (gm)	S.E.
40 mg/Kg body weight	9	2.51	0.04
Control		2.41	0.05
40 mg/Kg body weight	13	2.21	0.04
Control		2.15	0.03

TABLE 6

EFFECTS OF ADMINISTERING $AlCl_3$ TO PREGNANT RATS

No. of Implantations	Treatment* Day	Resorptions	Fetuses Recovered		
			Dead	Normal	Abnormal
106	9	6	0	100	0
87	9 Control	12	1	74	0
90	13	7	0	83	0
67	13 Control	2	3	62	0

* 40 mg/Kg body weight i.p.

6.2 Physical and neuromotor development

6.2.1 Righting reflex

The neuromotor development of offspring from four litters, two from control mothers and two from mothers, treated with aluminum chloride at a dose-level of 40 mg/Kg body weight on day 9 of gestation (Group I), was measured by a test of righting reflex. The response was considered positive when a pup was able to turn over in 2.5 seconds or less.

Some pups in three of the litters were able to turn over in the required time on post-natal day one (Table 7). In one of the four litters no pups were able to accomplish the test in the required time. However, by the sixth day most pups were considered positive and by day seven all the pups in all the groups were able to perform the test in 2.5 seconds or less. Similar results were obtained on day eight.

6.2.2 Physical development

The four litters were observed daily for three signs of physical development (Table 8). Unfolding of the external ear began in all animals on day three and was completed one day later. Eruption of the incisors occurred in three litters on day fourteen and on day thirteen in one litter. Opening of the eyes began in all litters on day fourteen and all the pups had both eyes open by day sixteen. One control group of pups completed opening of their eyes by day fourteen.

TABLE 7

RIGHTING REFLEX IN RAT OFFSPRING AFTER MATERNAL TREATMENT WITH $AlCl_3$

Days After Birth	% of offspring in litter showing a positive response			
	Experimental* I	Experimental* II	Control I	Control II
1	18	0	10	33
4	45	33	40	77
5	90	33	40	100
6	90	100	90	100
7	100	100	100	100
8	100	100	100	100

* Mothers treated with 40 mg/Kg body weight $AlCl_3$ on day nine of gestation.

TABLE 8

PHYSICAL DEVELOPMENT OF RAT PUPS AFTER MATERNAL TREATMENT WITH $AlCl_3$

Treatment		Unfolding of External Ear		Eruption of Incisors		Opening of Eyes	
Dose Level	Day	Onset a	Completion b	Onset a	Completion b	Onset a	Completion b
40 mg/Kg body weight	9	3	4	14	14	14	16
	9	3	4	14	14	14	15
Control	9	3	4	14	14	14	14
	9	3	4	13	13	14	16

a-Day characteristic first observed in litter.
b-Day when all animals in litter exhibited the characteristic.

One experimental group was completed by day fifteen. The eyes were opened on day sixteen in two litters, one experimental and one control group.

6.2.3 Post natal weight gain

The weight of each pup was recorded in the four litters. At birth and seven days later the average weights of the pups of dams, treated with aluminum chloride at a dose-level of 40 mg/Kg body weight on day nine of gestation, showed no significant differences from that of control pups. However, by day fourteen the mean weight of pups of the treated dams (Table 9, Figure 1) was significantly less than control animals ($P < 0.005$).

6.3 Chronic treatment

6.3.1 Maternal deaths

There was a high incidence of maternal deaths following treatment with aluminum chloride at the highest dose-level (Table 10, Figure 2). All mothers treated at a dose-level of 75 mg/Kg body weight on days 9-13 (Group I) or 14-18 (Group II) of gestation survived treatment. However, some animals did not survive treatment at a dose-level of; 100 mg/Kg body weight on days 9-13 (Group III) and 14-18 (Group IV) of gestation; 200 mg/Kg body weight on days 9-13 (Group V) and 14-18 (Group VI) of gestation.

Maternal deaths occurred during the treatment period and also at varying intervals following termination of treatment (Table 10). Autopsy of the mothers revealed abundant

TABLE 9

AVERAGE BODY WEIGHT PER GROUP OF RAT PUPS OF MOTHERS TREATED WITH $AlCl_3$

Treatment of Mothers	Post Natal Age in Days		
	1	7	14
Aluminum	6.24 ± 0.13 *	12.48 ± 0.20	21.34 ± 0.34 **
Control	6.05 ± 0.14	13.10 ± 0.37	24.44 ± 0.82

* Mean $\pm$ S.E.M.

** $P < 0.005$

Figure 1

POST NATAL GROWTH CURVES OF PUPS
FROM ALUMINUM TREATED MOTHERS

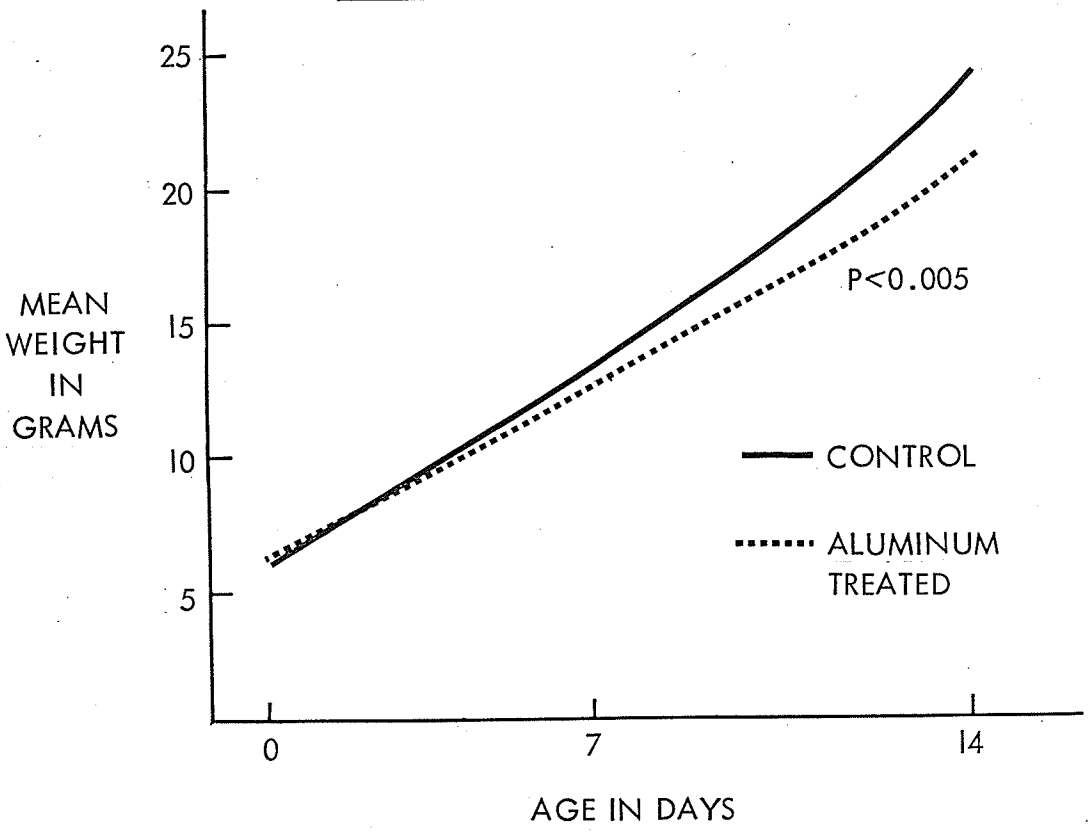


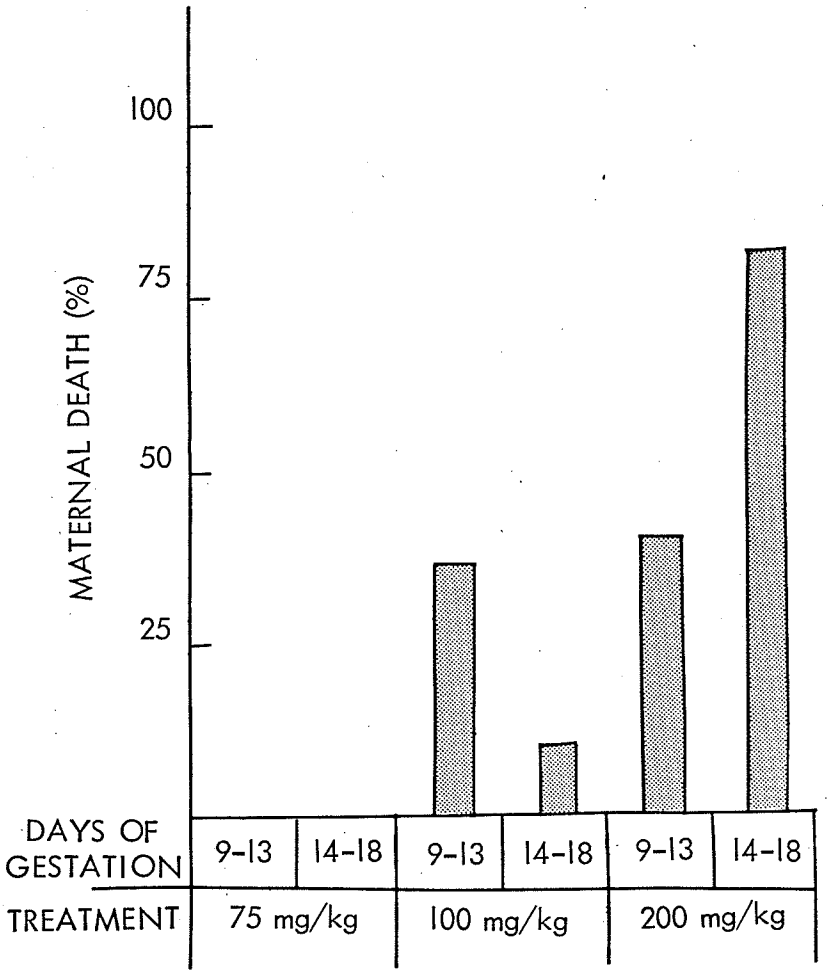
TABLE 10

DATE OF DEATH OF RAT MOTHERS TREATED WITH $AlCl_3$

Treatment		No. of Deaths	Gestational Days										
Dose Level	Days of Gestation		9	10	11	12	13	14	15	16	17	18	19	20
100 mg/Kg body weight	9-13	3					+		+				+	
	14-18	1										+		
200 mg/Kg body weight	9-13	3				+				+				+
	14-18	4									++	+	+	

Figure 2

MORTALITY OF PREGNANT RATS TREATED WITH $AlCl_3$



ascites, extensive adhesions between organs (Figure 3) and perihepatic granulomas (Figure 4).

There were no maternal deaths in any of the control groups.

6.3.2 Maternal weight gain

The mean percent weight gained by the treated mothers over the twenty day gestational period was less compared to the corresponding controls, except that of mothers treated at a dose-level of 100 mg/Kg body weight on days 9-13 (Group III) of gestation (Table 11, Figure 5). Maternal weight gain was apparently negatively related to the dose-level of aluminum chloride. The difference in maternal weight gain between treated and control animals was statistically significant only in animals treated at a dose-level of 75 mg/Kg body weight on days 14-18 (Group II) of gestation ($P < 0.05$), and 100 mg/Kg body weight on days 14-18 (Group IV) of gestation ($P < 0.005$).

6.3.3 Fetal weight

The mean weight of fetuses of mothers treated with aluminum chloride was found to be lower in four groups of animals (Groups I, II, V and VI) compared to the controls (Table 12, Figure 6). The lowest mean values were observed in fetuses of mothers treated at the highest dose-levels. These mean weight differences were statistically significant in fetuses of mothers treated at dose-levels of 75 mg/Kg body

Figure 3

Pregnant rat treated with aluminum chloride at a dose-level of 200 mg/Kg body weight on gestational days 9-13; abundant ascites and extensive adhesions between organs were found at autopsy.

3

Figure 4

Perihepatic granulomas induced in pregnant rat treated with aluminum chloride at a dose-level of 200 mg/Kg body weight on gestational days 9-13.

4

TABLE 11

MATERNAL WEIGHT GAIN AFTER TREATMENT WITH $AlCl_3$
(Total weight gain over 20 day gestational period)

Treatment		Mean Weight Gain/Group (%)	S.E.
Dose Level	Days of Gestation		
75 mg/Kg body weight	9-13	39.16	7.68
Control		49.00	3.79
75 mg/Kg body weight	14-18	35.50	1.19 *
Control		48.66	2.02
100 mg/Kg body weight	9-13	49.33	1.45
Control		46.00	2.48
100 mg/Kg body weight	14-18	30.44	3.87 **
Control		48.00	3.48
200 mg/Kg body weight	9-13	22.00	8.14
Control		45.20	1.98
200 mg/Kg body weight	14-18	18.00 ***	
Control		49.66	8.35

* $P < 0.05$
** $P < 0.005$
*** One sample only.

Figure 5

MATERNAL WEIGHT GAIN AFTER TREATMENT WITH $AlCl_3$

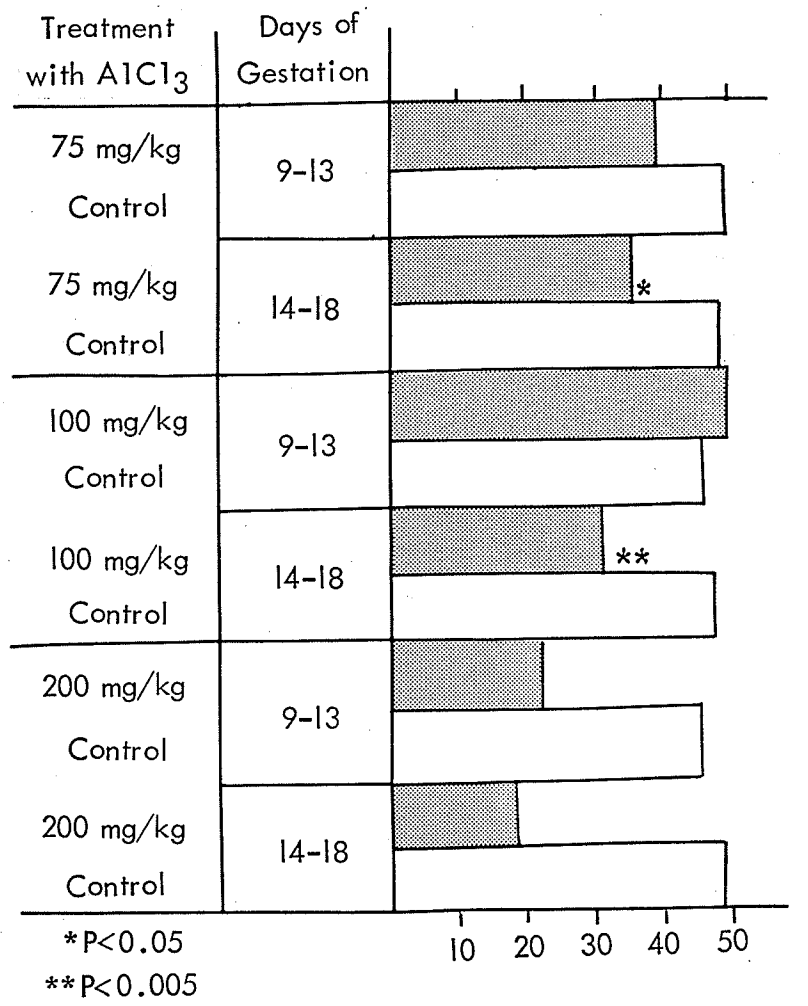


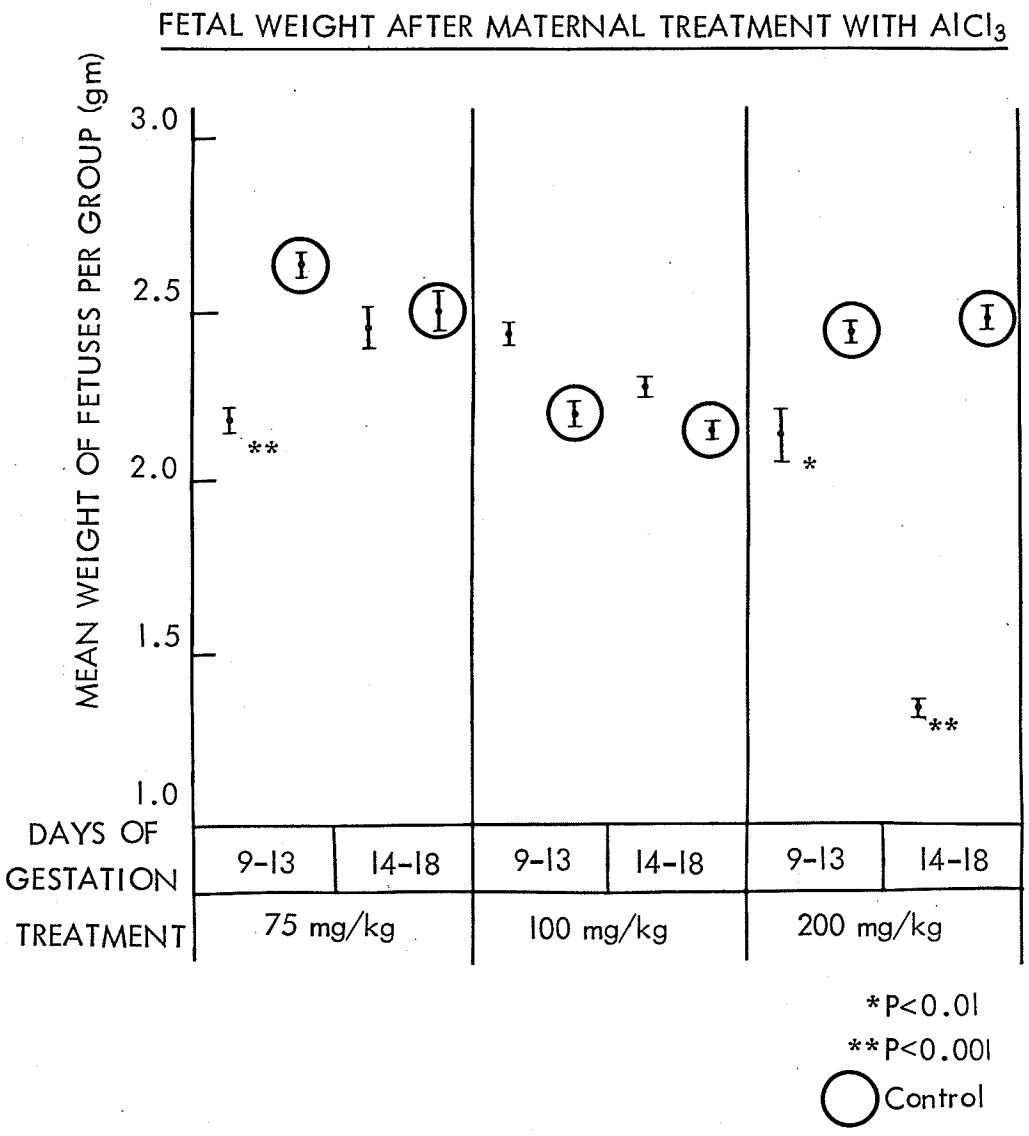
TABLE 12

FETAL WEIGHT AFTER MATERNAL TREATMENT WITH $AlCl_3$

Treatment	Days of Gestation	Mean Weight	S.E.
75 mg/Kg	9-13	2.18 **	0.04
Control		2.63	0.03
75 mg/Kg	14-18	2.46	0.07
Control		2.50	0.06
100 mg/Kg	9-13	2.42	0.03
Control		2.20	0.03
100 mg/Kg	14-18	2.28	0.03
Control		2.16	0.02
200 mg/Kg	9-13	2.13 *	0.09
Control		2.44	0.03
200 mg/Kg	14-18	1.34 **	0.03
Control		2.49	0.07

* $P < 0.01$
** $P < 0.001$

Figure 6


FETAL WEIGHT AFTER MATERNAL TREATMENT WITH $AlCl_3$
MEAN WEIGHT OF FETUSES PER GROUP (gm)
3.0
2.5
2.0
1.5
1.0
**
*
**
DAYS OF GESTATION
9-13
14-18
9-13
14-18
9-13
14-18
TREATMENT
75 mg/kg
100 mg/kg
200 mg/kg
*P<0.01
**P<0.001
Control

weight on days 9-13 (Group I) of gestation ($P < 0.01$); 200 mg/Kg body weight on days 9-13 (Group V) of gestation ($P < 0.01$); and 200 mg/Kg body weight on days 14-18 (Group VI) of gestation ($P < 0.001$).

The mean weight of control fetuses was significantly less than that of fetuses recovered from mothers treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 9-13 and 14-18 of gestation (Groups III and IV).

6.3.4 Fetal length

The mean crown-rump length of fetuses recovered from mothers treated with aluminum chloride was less than the corresponding control values in all six groups of animals. The lowest mean crown-rump length was associated with the highest dose-levels (Table 13, Figure 7).

The mean crown-rump length of fetuses recovered from mothers treated at a dose-level of 75 mg/Kg body weight on days 9-13 (Group I) of gestation ($P < 0.001$); 200 mg/Kg body weight on days 9-13 (Group V) of gestation ($P < 0.05$); and 200 mg/Kg body weight on days 14-18 (Group VI) of gestation ($P < 0.01$), was significantly reduced compared to the corresponding controls (Figure 8).

6.3.5 Resorptions

The incidence of resorptions was found to be significantly higher in three groups of animals (Tables 14, 15, 16) treated with aluminum chloride at dose-levels of 75 mg/Kg

TABLE 13

FETAL LENGTH AFTER MATERNAL TREATMENT WITH $AlCl_3$

Treatment	Days of Gestation	Mean C/R Length	S.E.
75 mg/Kg	9-13	2.92 ***	0.02
Control		3.17	0.01
75 mg/Kg	14-18	3.12	0.13
Control		3.15	0.08
100 mg/Kg	9-13	3.06	0.02
Control		3.11	0.02
100 mg/Kg	14-18	2.78	0.02
Control		2.83	0.01
200 mg/Kg	9-13	2.81 *	0.10
Control		3.09	0.02
200 mg/Kg	14-18	2.10 **	0.12
Control		3.04	0.04

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Figure 7

FETAL LENGTH AFTER MATERNAL TREATMENT WITH $AlCl_3$

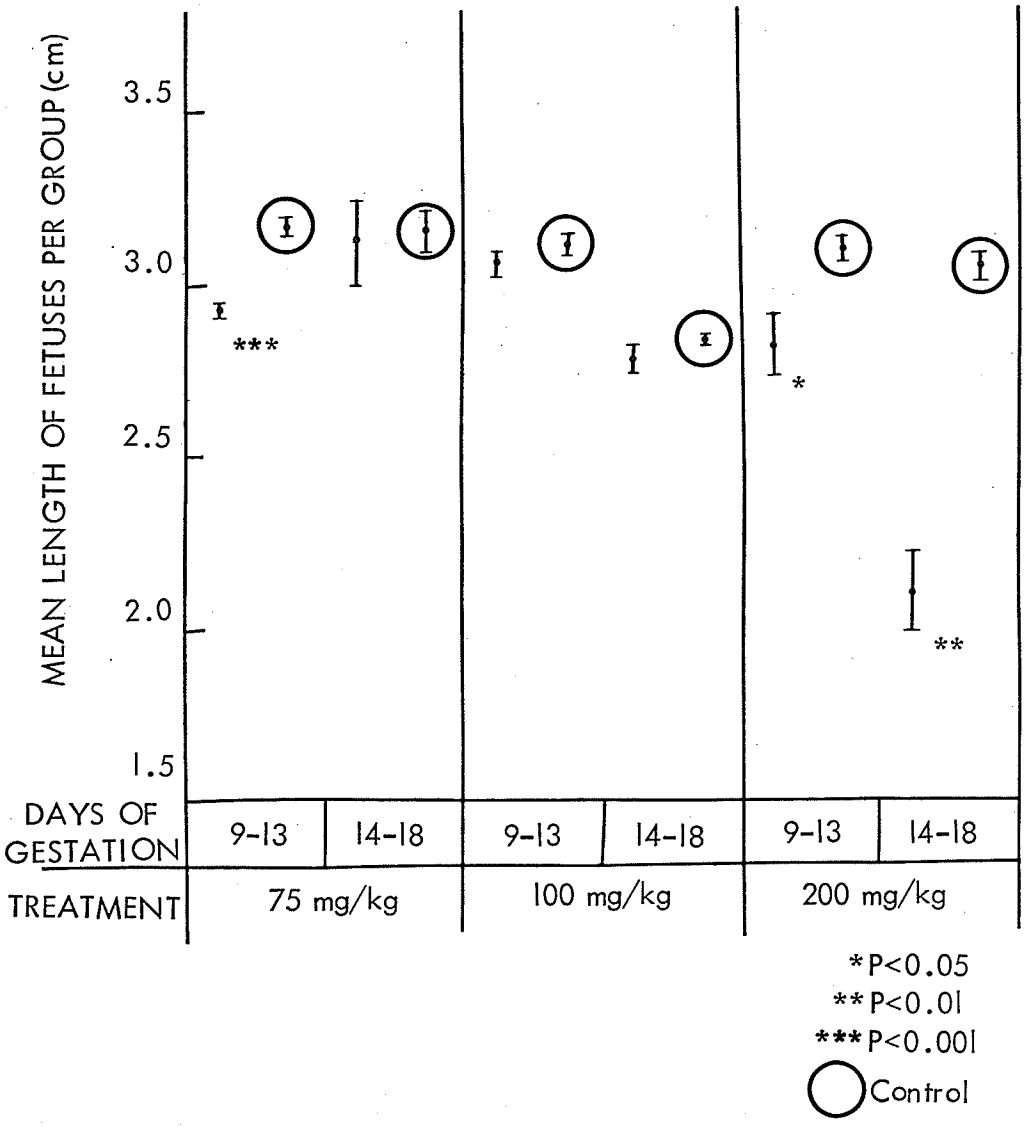


Figure 8

Control fetus on left. The three fetuses on the right are stunted and were recovered from mothers treated with aluminum chloride at a dose-level of 200 mg/Kg body weight on days 14-18 of gestation.

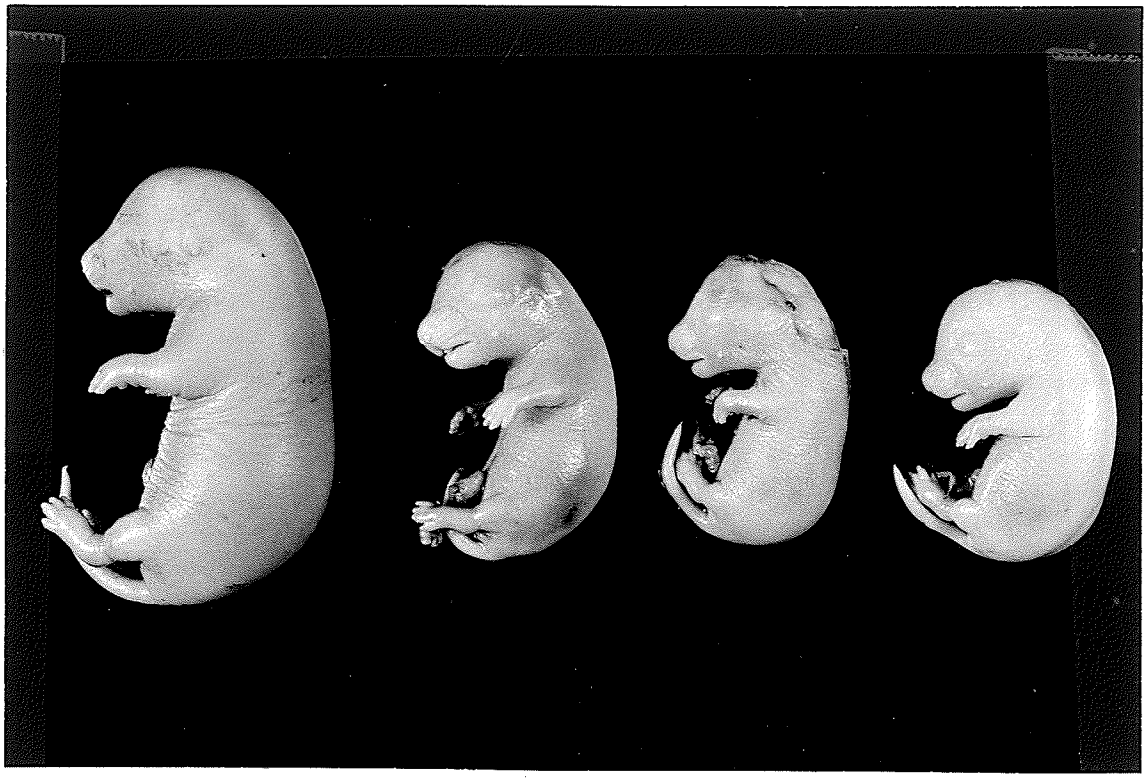


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TABLE 14

EFFECTS OF ADMINISTERING $AlCl_3$ TO PREGNANT RATS

No. of Animals	Treatment	Days of Gestation	Implantations	Resorptions		Malformations	
				No.	%	No.	%
6	75 mg/Kg	9-13	68	12*	17	0	
6	Control		62	0		0	
5	75 mg/kg	14-18	54	2	3	0	
6	Control		67	2	2	0	

* $P < 0.005$

TABLE 15

EFFECTS OF ADMINISTERING $AlCl_3$ TO PREGNANT RATS

No. of Animals	Treatment	Days of Gestation	Implantations	Resorptions		Malformations	
				No.	%	No.	%
8	100 mg/Kg	9-13	54	1	1	1	1
8	Control		90	3	3	0	
10	100 mg/Kg	14-18	112	12**	10	10*	8
10	Control		120	0	0	2	1

* $P < 0.05$
** $P < 0.001$

TABLE 16

EFFECTS OF ADMINISTERING $AlCl_3$ TO PREGNANT RATS

No. of Animals	Treatment	Days of Gestation	Implantations	Resorptions		Malformations	
				No.	%	No.	%
6	200 mg/Kg	9-13	37	14*	37	0	
5	Control		62	0		0	
5	200 mg/Kg	14-18	8	1	12	0	
6	Control		76	4	5	0	

* $P < 0.001$

body weight on days 9-13 (Group I) of gestation ($P < 0.05$); 100 mg/Kg body weight on days 14-18 (Group IV) of gestation ($P < 0.001$); and 200 mg/Kg body weight on days 9-13 (Group V) of gestation ($P < 0.001$).

The incidence of resorptions in other treatment groups (Groups II, III and VI) and in the control groups did not differ significantly.

6.3.6 Dead offspring

A high incidence of dead offspring was recovered from mothers (Group V) treated with aluminum chloride (Table 17) at a dose-level of 200 mg/Kg body weight on days 9-13 of gestation ($P < 0.05$). All dead fetuses were recovered from one litter, and these were at different developmental stages (Figure 9) at day twenty of gestation.

The number of dead offspring recovered from the other treatment groups did not differ significantly from the corresponding controls.

6.3.7 Malformations

Gross fetal abnormalities were detected in two groups of animals receiving aluminum chloride at a dose-level of 100 mg/Kg body weight on days 9-13 (Group III) and 14-18 (Group IV) of gestation (Table 15), and also in the corresponding controls of group IV.

An extra rib was detected on the right side in one fetus of a mother of treatment group III. Two malformed fetuses were found in the control group corresponding to

TABLE 17

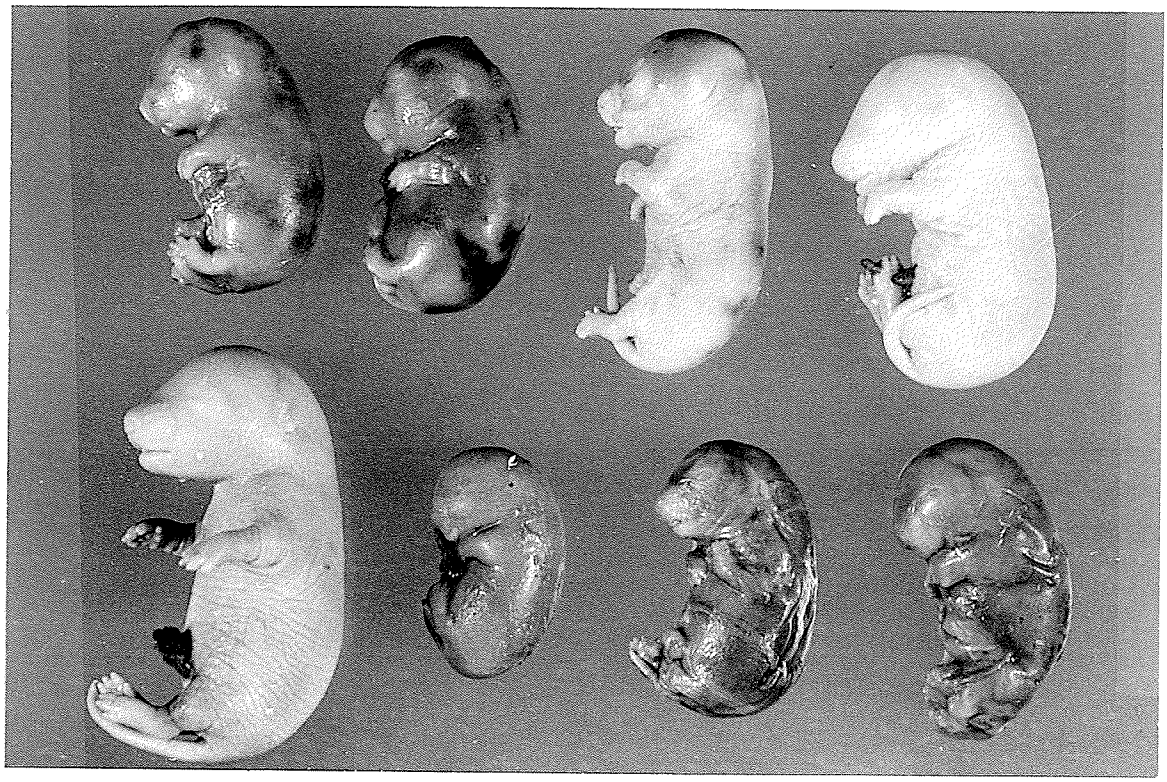
NUMBER OF DEAD OFFSPRING RECOVERED FROM MOTHERS TREATED WITH $AlCl_3$

Treatment		No. of Dead offspring	No. of Live offspring
Dose Level	Days of Gestation		
75 mg/Kg	9-13	2	54
Control		4	58
75 mg/Kg	14-18	0	52
Control		3	65
100 mg/Kg	9-13	2	85
Control		4	136
100 mg/Kg	14-18	6	94
Control		4	116
200 mg/Kg	9-13	5 *	18
Control		2	60
200 mg/Kg	14-18	0	7
Control		5	67

* $P < 0.05$

Figure 9

Control fetus on lower left. Two fetuses on upper right were born alive; the remaining litter were dead and at different developmental stages. The mother was treated with aluminum chloride at a dose-level of 200 mg/Kg body weight on days 9-13 of gestation.



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treatment group IV. One fetus had one extra rib on the left side, while the other was severely malformed.

Because the number of abnormal fetuses recovered was small, and such anomalies do occur spontaneously, they were not attributed to the treatment.

A significantly high incidence of abnormal fetuses was recovered from animals treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 (Group IV) of gestation ($P < 0.05$). Three fetuses (from two litters) were found to have abnormal digits (Figure 10). Seven fetuses (from four litters) showed wavy ribs (Figures 11, 12) and in some cases ribs were missing.

A large number of fetuses recovered from mothers (Group IV) showed poor ossification (Figure 13) as revealed by the alizarin red S staining technique (Dawson, 1926). This was evident particularly in the cranial bones, in the lower part of the vertebral column, as well as the long bones of the limbs.

6.4 Histology: maternal liver

A large number of animals from all six treatment groups (Groups I-VI) were found to have perihepatic granulomas (Figure 4). The number of animals showing this lesion was increased with increasing doses of the test substance (Table 18). Microscopic examination of maternal liver showed signs of centrilobular necrosis (Figure 14) which was characterized by degeneration and destruction of liver cells around

Figure 10

Control fetus on left. Fetuses on right were recovered from mothers treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation and showed abnormal digits.

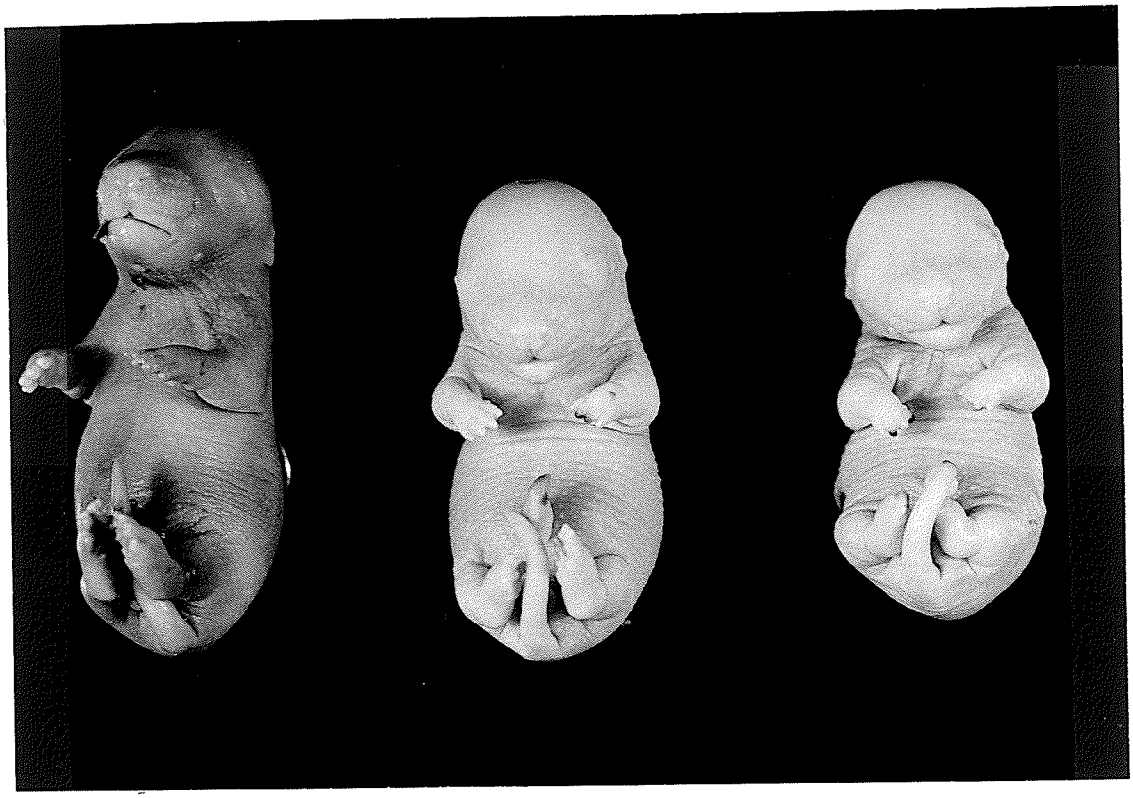

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Figure 11

Skeletal system of fetus stained by the alizarin technique showing wavy ribs. The mother was treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation.

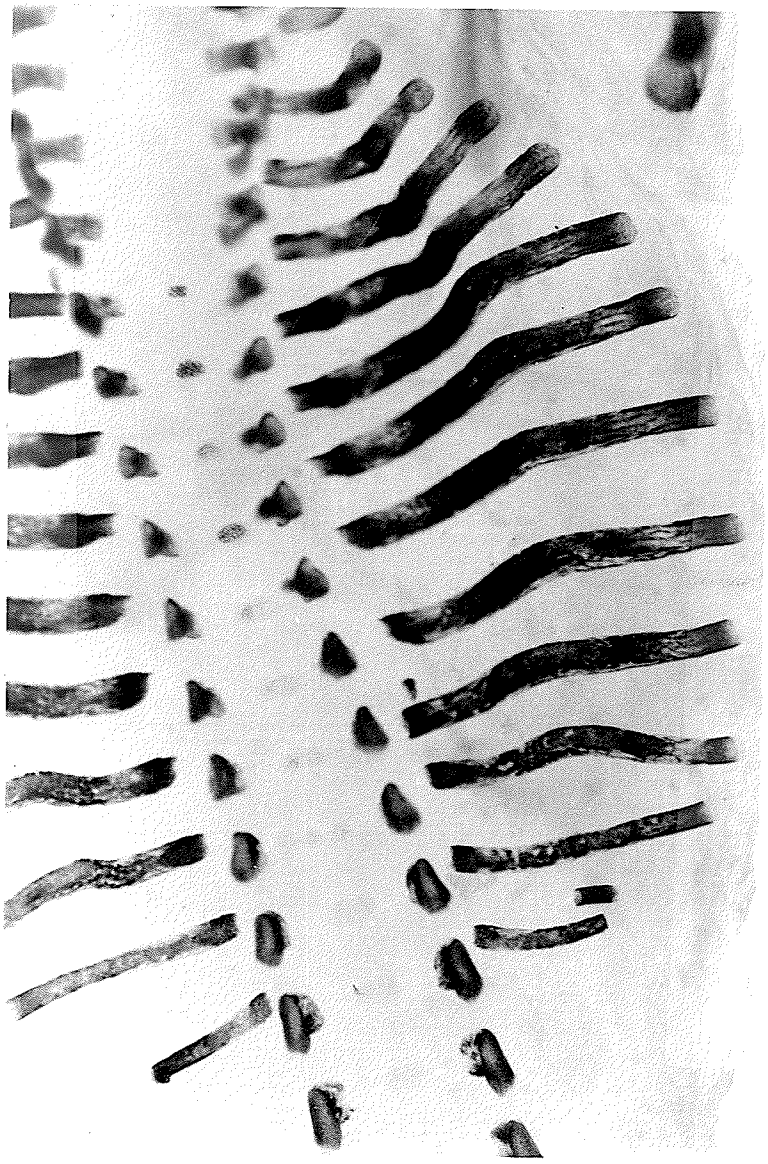

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Figure 12

Effect of aluminum chloride on skeletal development in the rat. Fetus recovered from mother treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation. Note wavy ribs on both sides and missing ribs on the right.

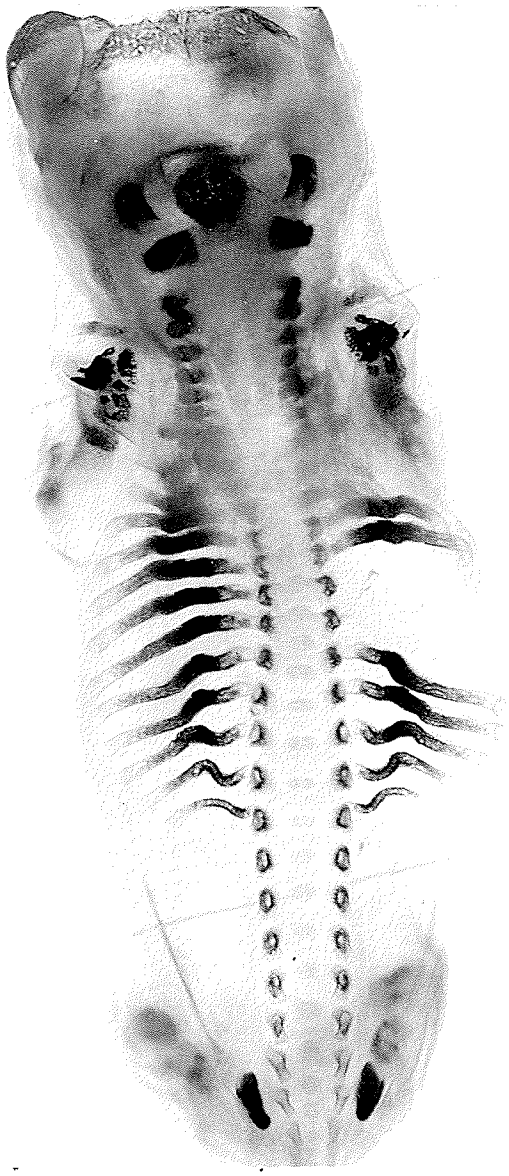

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Figure 13

Effect of aluminum chloride on skeletal development in the rat. Control fetus on the right. Poor ossification particularly in skull, caudal regions, and long bones of limbs were seen in fetus (left) of mother treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation.

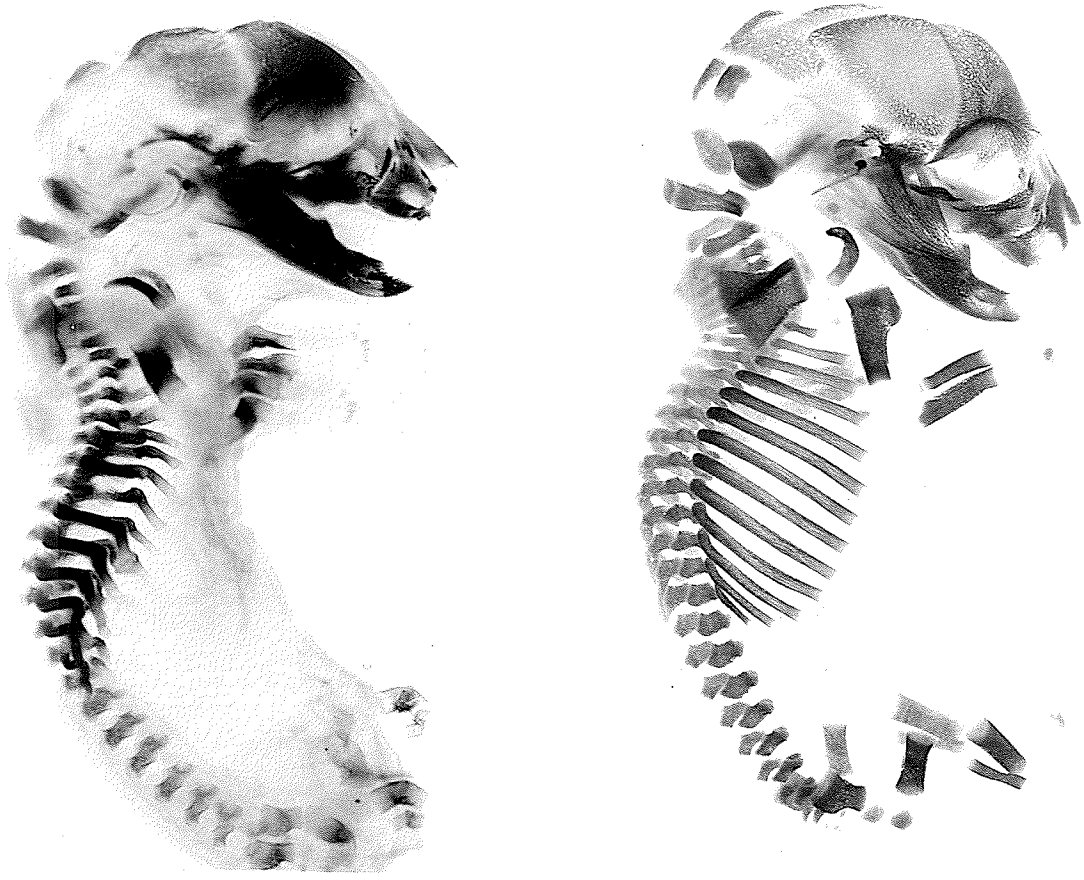

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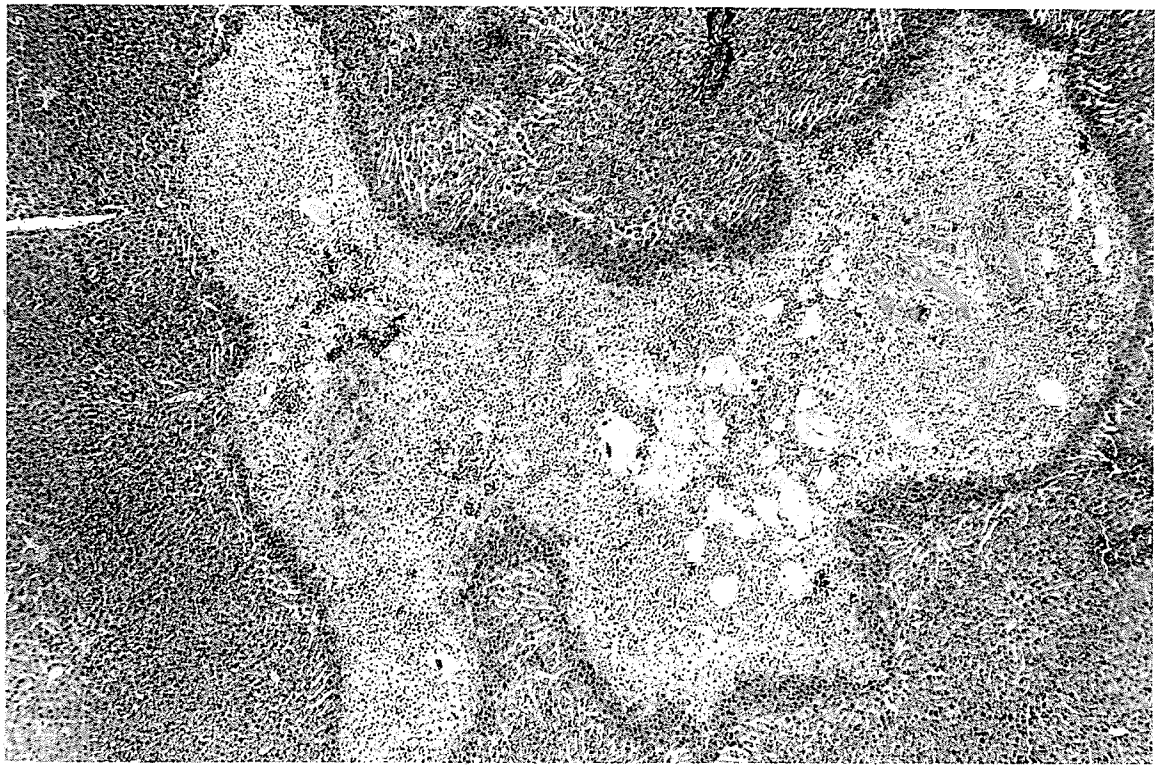
TABLE 18

PREGNANT FEMALE RATS TREATED WITH $AlCl_3$
EXHIBITING NECROSIS OF LIVER

Treatment		No. of Surviving Mothers	Incidence of Necrosis
Dose Level	Days of Gestation		
75 mg/Kg body weight	9-13	6	5
	14-18	5	3
100 mg/Kg body weight	9-13	5	5
	14-18	9	6
200 mg/Kg body weight	9-13	3	3
	14-18	1	1

Figure 14

Centrilobular necrosis in liver of rat treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 9-13 of gestation. 7 µ, Haematoxylin and Eosin, x 10.



14

the central vein. In other regions of the liver lobule, intact cells were found that often contained pyknotic nuclei. Hepatic sinusoids were moderately distended in the mid zonal region of the liver lobule.

6.5 Histology: placenta and fetal tissue

The placenta, fetal kidney, and fetal liver of mothers treated with aluminum chloride at a dose-level of 75, 100 and 200 mg/Kg body weight on days 9-13 or 14-18 of gestation (Groups I-VI) appeared normal and showed no significant changes compared to the controls.

SECTION 7

DISCUSSION

Aluminum is found in great abundance on the earth's crust (Campbell et al., 1957; Hutchinson, 1945), and biological systems probably evolved in the presence of appreciable concentrations (Crapper, Krishnan and Dalton, 1973). Many of these biological systems probably had their beginning in the oceans where there is very little aluminum, and were exposed to large quantities only later when their evolution continued.

Experimental evidence has not established what role aluminum may play in biological systems. It seems that aluminum is not required for growth or other vital functions, and deficiencies cause no known diseases (Campbell et al., 1957). For this reason aluminum has been classified as a ubiquitous trace element (Ferm, 1972).

Many organisms, including man, may be exposed to large or abnormal concentrations of aluminum compounds. Although many investigations have been carried out on the toxic effects of aluminum compounds in animals (Berlyne et al., 1972; Hara et al., 1959; Kortus, 1967; Ondreicka, Ginter and Kortus, 1966) their effects during pregnancy have received little attention (Mackenzie, 1932; Myers and Mull, 1928; Ondreicka, Ginter and Kortus, 1966). These studies utilizing low doses of aluminum administered orally, showed adverse effects on neither the mothers nor the offspring.

Animals treated with aluminum at high dose-levels, administered orally, resulted in a phosphate depletion syndrome

(Ondreicka, Ginter and Kortus, 1966). A similar syndrome has been reported in man (Lotz, Zisman and Bartter, 1968). This is due to the binding of aluminum with phosphate in the bowel and the subsequent excretion in the feces.

Berlyne et al. (1972) suggested that oral administration of aluminum compounds, with accompanying phosphate depletion should not be primarily considered a manifestation of aluminum toxicity.

7.1 Maternal toxicity

Previous studies have shown that aluminum compounds administered to experimental animals orally or intraperitoneally produced toxic effects and resulted in the accumulation of aluminum in certain tissues (Berlyne et al., 1972; Myers and Mull, 1928; Ondreicka, Ginter and Kortus, 1966) and it may be responsible for the necrotic lesions produced in the livers of experimental animals treated with aluminum chloride.

The mean percent weight gain of pregnant animals in almost all cases decreased with increasing dose-levels of aluminum chloride. This may possibly be due to maternal liver damage involving derangement of metabolic functions. Kortus (1967) found the weight of aluminum chloride treated rats was significantly less than the controls. Liver glycogen concentrations were markedly decreased. These changes were attributed to disturbances in phosphorous metabolism. Other investigations in animals have shown that intoxication with aluminum compounds produces disturbances of phosphorylating

mechanisms which affect closely related carbohydrate metabolism (Ondreicka, Kortus and Ginter, 1971). Aluminum intoxication has also been associated with depressed liver respiration and low liver protein concentrations (Berlyne et al., 1972).

There were no differences in mean percent weight gain between treated and control animals following acute administration of aluminum chloride at a dose-level of 40 mg/Kg body weight. In addition the treated mothers showed no signs of toxicity, and perihepatic granulomas were absent in these animals. It may be that at this dose-level, the renal clearance of aluminum is sufficient to maintain low tissue levels (Ondreicka, Kortus and Ginter, 1971). In addition, the possibility of low levels of the substance reaching the fetus should be taken into consideration.

7.2 Fetal effects

Metals may cross the placenta and become associated with fetal tissues. These may be teratogenic by interacting with organic molecules or be involved with important enzyme systems (Ferm, 1972). It has been suggested that aluminum crosses the placenta in rats (Rusoff and Gaddum, 1937) and may therefore reach the conceptus in sufficient concentrations to influence development.

7.2.1 Acute treatment

Fetuses of mothers treated with aluminum chloride at a dose-level of 40 mg/Kg body weight on days 9 or 13 of

gestation did not differ significantly from the controls. Maternal clearance, detoxification, or the dose-level may be such that the levels of aluminum reaching the fetus was not high enough to produce significant effects.

Other tests were employed to reveal more subtle changes that may have occurred during fetal development. Two litters of animals treated with aluminum chloride, and two control litters, were allowed to come to term so that post natal developmental processes may be observed. Righting reflexes and the three signs of physical development appeared to be normal in the offspring of treated mothers, compared to control litters. However, the finding of a significantly lower mean weight of offspring of treated mothers 14 days after birth suggests that the aluminum may be influencing fetal development. Due to the small number of treated mothers studied, great emphasis should not be placed on this finding until further studies with more animals was carried out.

7.2.2 Chronic treatment

The age of the fetus determines to a large extent the organs which are susceptible to teratogenesis. The critical periods of development in the rat extend approximately from day 8 through day 17 of gestation. During this period the conceptus is highly susceptible to the damaging action of teratogens. As the period of organogenesis progresses the fetus becomes more resistant to the influence of teratogens

and larger dose-levels are required to produce the same results (Wilson, 1965).

In the present investigation aluminum chloride was administered to pregnant rats during the early (days 9-13 of gestation) or late period (days 14-18 of gestation) of organogenesis. There was no clear dose-response relationship with respect to the mean weight and length of fetuses following maternal treatment with aluminum chloride, although the lowest values obtained for either parameter were found in fetuses of mothers treated with the substance at the highest dose-level (200 mg/Kg body weight).

Aluminum containing compounds inhibited cell division (Gelfant, 1963) and demonstrated antitumor activity in experimental animals (Hart and Adamson, 1971). The possibility, therefore, exists that cellular function in the fetus may be impaired following treatment with aluminum chloride resulting in altered developmental patterns or intrauterine growth retardation. In addition, aluminum may influence phosphate and other closely related metabolic processes such as carbohydrate metabolism.

In two groups of control fetuses mean fetal weight was significantly less than fetuses of mothers (Group III, IV) treated with aluminum chloride at a dose-level of 100 mg/Kg body weight. However, the mean length of treated and control fetuses in the same groups (Group III, IV) was not significantly different.

The mean length and weight of fetuses were significantly lower than the corresponding controls in three groups of mothers treated with aluminum chloride at a dose-level of 75 mg/Kg body weight on days 9-13 of gestation, and 200 mg/Kg body weight on days 9-13 or 14-18 of gestation.

Maternal treatment with aluminum chloride induced fetal resorptions in all groups of animals. However, the incidence of resorptions was significantly greater than the controls in only three groups of mothers. These were treated with aluminum chloride at dose-levels of 75, 100 or 200 mg/Kg body weight. The occurrence of fetal resorptions showed no relationship to the period of gestation during which treatment was administered.

The number of dead fetuses recovered from mothers treated with aluminum chloride was significantly higher compared to the controls following treatment only at the highest dose-level (200 mg/Kg body weight on days 9-13 of gestation). All dead fetuses were recovered from one of the three surviving mothers and showed different developmental stages at the time of recovery near term. Previous studies in newborn mice have indicated that aluminum chloride produced growth retardation (Ondreicka, Ginter and Kortus, 1966) and a similar mechanism may account for the developmental failure observed in these dead fetuses.

A significantly high incidence of developmental defects was present in fetuses whose mothers were treated with aluminum chloride at a dose-level of 100 mg/Kg body

weight on days 14-18 of gestation, compared to controls.

Although the mechanism of action of aluminum in the fetus is not known, further consideration should be given to the effects of this substance on the mother, on the anti-mitotic and antitumor properties of aluminum compounds, and their influence on fetal metabolism.

It has been reported that aluminum compounds affect bone formation (Berlyne et al., 1972; Bloom and Flinchum, 1960; Lotz, Zisman and Bartter, 1968; Parsons et al., 1971). The high incidence of skeletal defects as well as poor ossification in fetuses of mothers treated with aluminum chloride suggest that this substance may have some influence on fetal bone formation.

The incidence of fetal abnormalities following maternal treatment with aluminum chloride at all three dose-levels (75, 100 and 200 mg/Kg body weight) on days 9-13 of gestation was not significantly increased compared to controls. This period of gestation (days 9-13) precedes that of bone formation in the rat which begins on approximately day 17 of gestation (Walker and Wirtschafter, 1957). Aluminum chloride administered to mothers at a dose-level of 75 mg/Kg body weight on days 14-18 of gestation produced no malformations, while the high dose-level of 200 mg/Kg body weight administered over the same gestational period was highly toxic to the mothers. One animal survived the treatment and few fetuses were recovered.

Few fetuses recovered from mothers treated with aluminum chloride during the early period of organogenesis (days 9-13 of gestation) were abnormal. This may indicate that high aluminum concentrations do not persist until gestational day 17 in maternal and/or fetal tissues.

Aluminum chloride administered at a dose-level of 75 mg/Kg body weight during the period of bone formation (days 14-18 of gestation) was apparently inadequate to produce significant changes in the fetus.

However, skeletal defects were induced when the test substance was administered to mothers at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation (period of bone formation).

The mechanism of action of aluminum on the formation of bone is unknown. There is evidence that 1 mg of aluminum can initiate hydroxyapatite precipitation (Bachra and van Harskamp, 1970). This may be due to the binding of aluminum with phosphate in the tissues thus reducing phosphate available for bone formation. Further studies are needed to determine the mode of action of aluminum on fetal osteogenesis.

Examination of fetuses for visceral anomalies revealed no differences between treated and control animals. Aluminum administered to pregnant mothers at all dose-levels (75, 100 and 200 mg/Kg body weight) during the critical period of organogenesis in the rat (days 9-13 and 14-18 of gestation) did not adversely influence the development and organization of fetal organs.

CONCLUSIONS

CONCLUSIONS

Aluminum chloride administered intraperitoneally to pregnant rats adversely affected the mother as well as the development of the offspring. A high incidence of maternal deaths followed treatment with the test substance at a dose-level of 100 and 200 mg/Kg body weight. In addition, maternal weight gain over the entire gestational period was less compared to the controls and showed a dose-effect relationship. In most cases, maternal liver was severely damaged following treatment with aluminum chloride.

Treatment of the mothers with aluminum chloride at a dose-level of 40 mg/Kg body weight on gestational day 9 did not significantly affect the mean weight of fetuses recovered near term (day 20 of gestation). However, the offspring of mothers treated in a similar manner and allowed to come to term, showed a significant lapse in weight gain compared to controls 14 days after birth.

The mean crown-rump length was less than that of the corresponding controls in fetuses of mothers treated with aluminum chloride at dose-levels of 75, 100 and 200 mg/Kg body weight. The mean weight of fetuses of mothers treated at a dose-level of 75 mg/Kg body weight and 200 mg/Kg body weight was lower than the corresponding controls. The lowest mean weight and fetal crown-rump length were observed following maternal treatment at the highest dose-level. A high incidence of fetal resorptions was found in mothers

treated with aluminum chloride at a dose-level of 75 mg/Kg body weight on days 9-13 of gestation, 100 mg/kg on days 14-18 of gestation and 200 mg/Kg body weight on days 9-13 of gestation.

A significant number of dead fetuses was recovered from mothers treated with 200 mg/Kg body weight aluminum chloride on days 9-13 of gestation.

A high incidence of developmental defects was detected in fetuses whose mothers were treated with aluminum chloride at a dose-level of 100 mg/Kg body weight on days 14-18 of gestation. The abnormalities present were restricted to the skeletal system.

This study suggested that aluminum administered to pregnant rats retarded growth and development in the offspring. In addition, treatment of the mothers with aluminum chloride caused fetal death and resorption as well as skeletal defects but no visceral malformations.

Whether the mechanism of action of aluminum chloride in the pregnant rat involves changes in maternal metabolism, such as the coupling of the aluminum with phosphate, or the direct action of the metal on the fetus itself, remains to be determined.

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