75% survival in 12 treated and 10% survival in 20 control rats 30 days after irradiation.

Discussion. It seems logical to suppose that PAPP exerts its prophylaxis against the lethal effects of X-ray by the hypoxia resulting from the severe and relatively prolonged methemoglobinemia. The possibility, however, that PAPP may exert its prophylactic effect by some mechanism other than the production of methemoglobinemia is being investigated by testing other unrelated compounds which are known to produce methemoglobinemia. Preliminary studies on sodium nitrite have shown that a dose of 100 mg/kg given intraperitoneally 10 minutes before irradiation with 800 r is without effect on subsequent mortality. This has been confirmed by other workers(13). Determination of the degree of methemoglobinemia produced by this dose of sodium nitrite showed an average of 52% for 4 mice 10 minutes after injection. By 30 minutes, the end of the X-ray exposure period, the level had fallen to 46%. While this level is slightly less than that produced by the lowest dose of PAPP tested, it does not seem likely that this would explain the marked difference in results. The reason for this inconsistency is being investigated further. The use of PAPP to produce hypoxia offers several distinct advantages over previous methods. No cumbersome procedures are needed, the extent of methemoglobinemia and the degree of hypoxia can be easily controlled by varying the dose of PAPP, and considerably less than lethal doses produce the desired results. Doses as low as 20 or 30 mg/kg shifted the 30-day LD_{50} from approximately 515 r(14) to about 800 r of X-radiation. Higher doses of PAPP produced an even more pronounced shift.

Summary. Para-aminopropiophenone (PAPP) when injected intraperitoneally in mice produces a maximum methemoglobinemia in 30 minutes with only a slight decrease in the methemoglobin level after 60 minutes. Of 180 mice given a single exposure of 800 r total-body X-radiation 100% of 60 controls died within 30 days. Pre-treatment of groups of the remaining 120 mice with PAPP in doses of 20, 30, 40, and 50 mg/kg, 30 minutes before X-ray exposure, resulted in 30-day survival rates of 45, 50, 60, and 72.5%, respectively. Injection of 50 mg/kg of PAPP after irradiation did not affect mortality. In a preliminary test pre-treatment with PAPP also reduced the 30-day mortality in rats exposed to X-radiation.

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Effect of 4-Amino-Pteroylglutamic Acid (Aminopterin) on Early Pregnancy.* (17855)

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Recent studies have shown that lesions which are characteristic of severe folic acid deficiency can be rapidly induced in mammals by administration of the potent antagonist, 4-amino-pteroylglutamic acid (4-amino-PGA)(1,2). In work with dogs, a

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TABLE I.
The Effect of 4-Amino-PGA on Pregnancies of Rats.

Group	No. of rats	Dosage, mg/kg	Days after mating: Injected	Days after mating: Sacrificed	Rats with litters	No. of implantations	No. of feti, natural	Damaged	Disintegrated	Reabsorbed
A	23			14	17	180	180			
B	20	3 × .1	5,6,7	8	3	19		14	5	
C	10	.1	5,6,7	14	5	39		10	5	24
D	21	.15	7,7,8	14	17	130		29	51	50
E	15	.15	7,7,8	23		1				all

bitch, sacrificed several days after treatment with the antagonist, was found to have a dead and macerated litter *in utero*. The observation stimulated further studies of possible effects of 4-amino-PGA on fetal development.

Procedure. In the following experiments Sprague-Dawley and Wistar rats and CFW mice were used. The animals were mature, had previously littered, and weighed between 200 and 250 g (rats) and over 25 g (mice). The animals were separated in individual cages, when pregnant. Their weight was recorded daily while under treatment. Animals were sacrificed, while under ether anesthesia, to avoid artefacts. Uteri were inspected *in situ,* placental implantations counted and measured, and abnormal conditions recorded. Uterine segments with placenta or areas of increased vascular supply were embedded in paraffin, sectioned and stained with Harris hematoxylin and eosin. The sternum of all sacrificed animals was fixed in Vandegrift's and sectioned. Fresh solutions of 4-amino-PGA‡ were prepared just prior to their intraperitoneal injection. Three consecutive doses of 0.15 mg/kg in mice and 0.1 or 0.15 mg/kg in rats were given at intervals of either 12 or 24 hours.

Exp. I. Twenty-six female mice and 39 female rats (Sprague-Dawley) were mated for a week. The females were then separated from the males. Seven days later the animals were started on a course of 3 daily injections of 4-amino-PGA. They were then permitted to litter. According to the time of mating a week's range of littering was expected. However, counting from the time of the first injection of the drug, all mice born were delivered between the eighth and tenth days, and all rats, between the tenth and twelfth day. Thus, litters were produced only by animals which had been pregnant 8 or more days at the time of the first injection and it appeared that pregnancies younger than 8 days were not maintained. In a second series of 30 female rats (Sprague-Dawley) treated in the same way, only 14 animals littered between 10 and 12 days after the first injection with 4-amino-PGA. However, 14 of 16 control animals, which had been mated for 7 days, littered over a period of one week.

Exp. II. In order to study the effect of the drug during the first week of pregnancy in rats, experiments were carried out with more accurate knowledge of conception time. For this purpose Wistar female rats were permitted to litter. Immediately thereafter, the newly born were removed and the mother remated for 48 hours. A high percentage of conception was anticipated from the ovulation which usually follows within a short time after delivery. Indeed, of 23 control rats sacrificed on the fourteenth day after the beginning of such mating 17 were found to be pregnant with a total of 180 feti (group A, Table I).

In a series of 30 experimental rats, mated as above and injected with the drug on the fifth, sixth, and seventh day following the previous littering, only 8 showed evidence of pregnancy with 58 sites of implantation (groups B and C, Table I). The uterine notches in the treated rats were only 3 to 4

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‡ The authors are indebted to the Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y., for supplies of 4-amino-PGA.

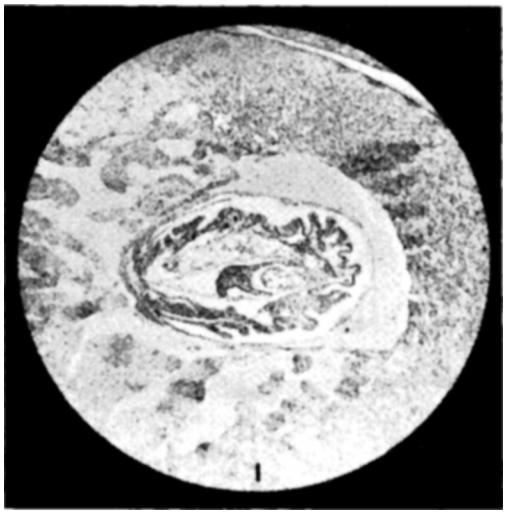

FIG. 1.
Control 8-day rat embryo.

FIGS. 2, 3, 4.
Rat embryos 14 days old; mothers injected 3 times with 0.15 mg/kg of 4-amino-PGA on 7th, 7th, and 8th day of pregnancy.
FIG. 2. Intact amnion surrounds damaged fetus. Organ structures still recognizable, mesenchyme damaged.

mm in diameter irrespective whether the animals were sacrificed at 8 or 14 days. The notches of control rats (Group A) sacrificed on the fourteenth day, were 1 cm in diameter. Two uteri of the experimental group were filled with a dark bloody fluid between the hemorrhagic implantation sites.

Of a further series of 21 rats, injected on the seventh and eighth day after the previous littering, 17 showed evidence of pregnancy when sacrificed with 130 sites of implantation (Group D, Table I). Again the uterine notches were approximately 4 mm in diameter and no whole feti could be found. Two animals of group D had distended uteri filled with a thick bloody fluid between 4 sites of implantation.

A further series of 15 rats injected on the 7th, 7th and 8th day after the previous littering (group E, Table I) and sacrificed

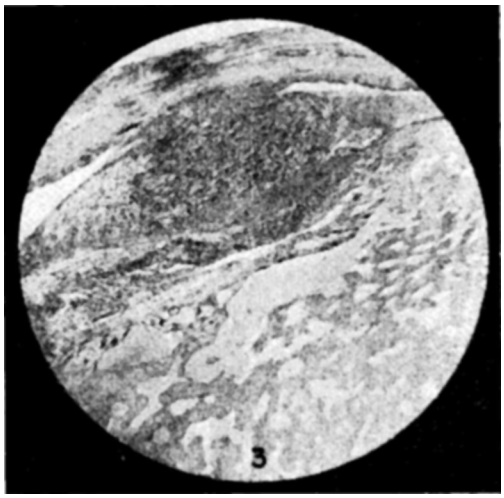

FIG. 3. Necrotic fetus infiltrated with leucocytes.

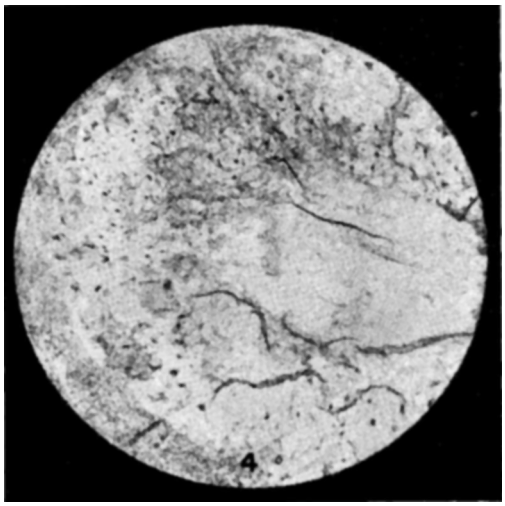

FIG. 4. Necrotic fetus resorbed, central area filled with blood. Early involution of placenta.

after 23 days had no litters at all. In only one rat one previous site of implantation was found consisting of a small blood filled notch in the uterus 3 mm in diameter.

Microscopical examination. Previous studies had shown 4-amino-PGA to have a depressing effect on the bone marrow(1). The same lesion was found in the present animals. When examined 24 hours after the last injection of 4-amino-PGA, the bone marrow was depleted approximately 50 per cent with respect to myelo- and erythropoietic elements. Within one week after the last injection, the marrow was completely recovered and showed normal cellularity.

No lesions were found in the muscular wall of the uterus in any experimental animal. The rats of group B (Table I), which contained litters, showed decidual reaction of the endometrium with early implantation of small placentas. These implantations filled the lumen of the uterus like small plugs. Deciduas and placentas appeared to be intact but the feti were damaged. The internal organs of the embryos did not stain well and many nuclei had lost good definition.

Rats sacrificed on the fourteenth day of pregnancy and 7 days after the last 4-amino-PGA injection (groups C and D, Table I) provided better evidence of fetal damage and death than did the previous group. Again no lesions were found in the muscular coat of the uterus. The placentas corresponded to those of 7-day embryos and were intact in most cases. In 22 animals evidence of pregnancy existed in the presence of 169 sites of implantation. Undamaged feti, however, were not found. Thirty-nine of these implantations contained dead feti in which organ structure could still be recognized but in which most of the mesenchymal elements had degenerated and disintegrated. The embryos were surrounded in most cases by an intact amnion. In 56 instances the feti had shrunken to a mass of debris which formed a thin thread infiltrated with leucocytes and surrounded by placentas. In the remaining 74 implantations no fetal tissue was detected and it appeared that the embryonic debris had either been resorbed or emptied into the uterine lumen. The latter process was suspected in those cases in which the lumen was filled with blood and adjacent placentas and deciduas were undergoing involution.

Rats sacrificed 23 days after mating (group E) showed complete resorption of feti and placentas. Only in one instance a small placental rest with a central hemorrhage was detected.

Discussion. The findings above indicate that the doses of 4-amino-PGA used were toxic to pregnant rats insofar as they caused a temporary depletion of bone marrow. The treated animals lost a small amount of weight but did not become seriously ill. However, their embryos were irreversibly damaged when treatment was initiated at time of implantation. Histological study indicated the site of action of the drug to be in the embryonic mesenchyme. Decidual and placental tissue appeared to be normal in most cases. From this one would assume a direct toxic action of the compound on the fetus. Embryos of rats and mice older than 10 days proved more resistant to the antivitamin in preliminary experiments. This observation agrees with studies on the chick embryo in which a direct relationship was demonstrated between age of embryo and amount of 4-amino-PGA necessary to effect death(3). The results of the present experiments confirm the observation of Nelson and Evans who studied reproduction in female rats kept on folic acid deficient diets supplemented with crude "x-methyl-folic acid"(4). These authors found complete resorption of litters when the crude antagonist of folic acid was fed from the first day of mating. The experiments described above, however, demonstrate that the potent antagonist, 4-amino-PGA, can destroy a well-established pregnancy when the agent is given in only 3 small doses. If one accepts the hypothesis of the antifolic acid action of 4-amino-PGA, one

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has to conclude that folic acid is necessary for the maintenance of early fetal life in mice and rats. This conclusion is also advanced by Nelson and Evans(4).

Summary. 4-amino-pteroylglutamic acid was found to be lethal to the early embryo of mice and rats. Fetal death was observed with doses which temporarily caused depletion of the bone marrow but no other significant signs of intoxication in mothers.

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Partial Purification of Viruses with an Anion Exchange Resin.* (17856)

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A variety of techniques have been developed for the purification of viruses. Methods which have been applied to poliomyelitis viruses include: adsorption to, and elution from alumina gel(1) or aluminum hydroxide(2); half saturation with ammonium sulfate(3); filtration(4); procedures involving ether extraction, protein precipitation and centrifugation or ultracentrifugation (5-9); methanol precipitation(10); and protein precipitation with protamine sulfate(11). A relatively simple and rapid procedure for the partial purification of the Lansing strain of poliomyelitis virus has been developed in this laboratory. It utilizes a strong-base anion exchange resin. The method is also applicable to the extraction of poliomyelitis virus from human feces and Theiler virus (TO) from mouse feces and presumably may be applied to other viruses as well.

With this method, it is possible to eliminate rapidly the bulk of extraneous material and to obtain a preparation that has not lost a significant amount of virus. Advantages of the method include a considerable saving of time, the requirement of only the simplest equipment, and the elimination of lengthy chemical procedures.

Materials and methods. The Lansing strain of poliomyelitis virus from nervous tissue of mice, poliomyelitis virus from human feces, and Theiler virus obtained from the feces of normal mice were used in these experiments. The Lansing virus and Theiler virus were assayed in 4-5 and 3-4-week-old mice respectively (CFW-albino agouti strain, Carworth Farms), using the intracerebral route of infection. Poliomyelitis virus extracted from human stools was tested intracerebrally in monkeys (*Macaca mulatta*). Of the 3 strong-base anion exchange resins tested (Amberlite XE-56, Amberlite XE-75, and Amberlite XE-67)† only Amberlite XE-67 proved satisfactory for virus purification.

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† Supplied by the courtesy of Rohm & Haas Co., Philadephia, Pa.

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