C164 and New York A are indistinguishable from *L. pomona*. These findings stress the need for reclassification of the American strains. They also indicate that *L. pomona* or an antigenically indistinguishable organism is present in North America.

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Protective Effect of Para-Aminopropiophenone Against Lethal Doses of X-Radiation. (17854)

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Yeasts(1), plants(2,3) and both normal tissues and tumors of mammals(4-9) have been protected against radiation injury by reducing the oxygen tension during the period of exposure to radiation. The reduction of oxygen tension has been accomplished in a variety of ways. Tumors have been rendered hypoxic by *in vitro* enclosure in a nitrogen atmosphere during radiation(6), and portions of intact animals have been made hypoxic by occlusion of blood vessels by either pressure or ligation(4,5,8). Hypoxia during the period of irradiation has been produced in the entire animal by strapping the chest to interfere with respiration(8), by bleeding the animal immediately prior to irradiation (7), and by enclosure in a low oxygen, high nitrogen atmosphere(9). These methods are somewhat cumbersome and with one exception the evaluation of the degree of protection afforded has depended on estimations of radiation damage based on gross or histologic observations. In the instance where animals were exposed in a low oxygen atmosphere weight changes and 30-day mortality were used as criteria for the evaluation of protection. Cyanide has been shown to exert some protective action against X-radiation in mice, but this effect was not necessarily attributed to the histotoxic anoxia produced(10). The present study was undertaken to determine whether or not hypoxia produced by other chemical means during the period of exposure to X-radiation would protect intact animals against radiation damage. Para-aminopropiophenone (PAPP),† a compound shown to produce methemoglobinemia of relatively long duration(11), was

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TABLE I.
30-Day Survival in Control and Para-aminopropiophenone (PAPP) Treated Mice Following 800 r Total-Body X-ray.

Dose of PAPP intraperitoneally mg/kg	% methemoglobin 30′ after injection (Avg for 4 mice)	Time of injection of PAPP	30-day survival	% survival
20	55	30′ before X-ray	9/20	45
30	59	30′ " "	10/20	50
40	65	30′ " "	12/20	60
50	75	30′ " "	29/40	72.5
50	—	10′ after "	0/20	0
0	—	—	0/60	0

chosen as the chemical agent for producing hypoxia.

Materials and methods. A total of 180 female CF_1 mice, weighing 21-26 g, were given 800 roentgens of X-ray in a single total-body exposure (250 kv, 15 ma, ¼ mm Cu, 1 mm Al, 50 cm distance, and 35-40 r/min). Control and experimental animals were irradiated simultaneously in groups of 20. Each mouse was enclosed in an individual compartment of a cylindrical cage, each compartment being equi-distant from the center of the field, and the cage rotated continuously during the exposure period. Preliminary toxicity studies showed the LD_{50} for mice injected intraperitoneally with a 1% solution of PAPP in propylene glycol to be approximately 65 mg/kg. Doses of 20, 30, 40, and 50 mg/kg, which killed no animals in the toxicity studies, were chosen to test for prophylactic effect against radiation damage. A total of 100 mice received these dosages 30 minutes before exposure to radiation. Twenty mice received 50 mg/kg 10 minutes after exposure and 60 were irradiated as controls.

Blood methemoglobin levels for each dose of PAPP were determined by a modification of the method of Evelyn and Malloy(12). One-tenth ml of blood was removed by cardiac puncture and laked in 5 ml M/20 phosphate buffer, pH 7.4, containing 0.1% saponin. The sample was then centrifuged for 3 to 5 minutes and samples were poured into each of 2 cuvettes. A drop of 20% potassium ferricyanide was added to the second tube. Extinctions were then read in a Coleman Spectrophotometer at 630 mu. A drop of 2% sodium cyanide was added to both tubes and the extinctions were again read. The percentage of methemoglobin was then calculated by the following formula:

$$\frac{\text{Drop in extinction of first tube}}{\text{Drop in extinction of second tube (ferricyanide)}} \times 100 = \%\ \text{methemoglobin in sample}$$

Results. Determination of methemoglobin levels in mice following the intraperitoneal injection of PAPP showed that maximum levels were reached in about 30 minutes with a subsequent slow drop in the level. At 60 minutes the level was never more than 10% lower than the 30 minute value. Since the mice were irradiated during this interval the exposure took place when methemoglobin levels were at or near the maximum.

The mortality data are summarized in Table I. Eight hundred roentgens killed 100% of the control mice. The presence of high levels of methemoglobinemia during the time of irradiation exerted an appreciable protective action. Levels of approximately 75% methemoglobin reduced the mortality from 100% to less than 30%. Lower levels of methemoglobin produced less profound but appreciable protection. Administration of PAPP 5 minutes after irradiation was without effect on the 30-day mortality or death time. At the higher doses of PAPP the mice became extremely cyanotic and prostrated but could be aroused by stimulation. These doses did not produce any mortality *per se*.

A similar test on Sprague-Dawley rats given 30 mg/kg of PAPP 30 minutes before exposure to 800 r total body X-ray showed

12. Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, v126, 655.

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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