Relationship Between Oxygen Tension in Tissues and the Protective Action of Para-Aminopropiophenone and of Propylene Glycol. (31742)

A. VACEK* AND T. SUGAHARA (Introduced by Morris Pollard)
Department of Experimental Radiology, Faculty of Medicine, Kyoto University, Kyoto, Japan

It has been shown that para-aminopropiophenone (PAPP) in propylene glycol (PG), injected intraperitoneally before irradiation, can protect mice and rats against lethal doses of X rays (1,2). The protection by PAPP has been assumed to be due to its property of producing methemoglobinemia and temporary tissue anoxia. The protective activity of PAPP could be eliminated only by subjecting the mice to a barometric pressure of 5 or more atmospheres of oxygen(3,4,5). We have no references showing the decline of oxygen tension in tissues after PAPP injection. The optimal time for administration of PAPP before X-irradiation has been assumed to be coincident with maximal methemoglobinemia production.

The present study was undertaken to elucidate the relation between the course and extent of the decrease in oxygen tension in tissues and the protective activity of PAPP in different vehicles against whole-body X-irradiation.

Materials and methods. Experiments were carried out on male mice of strains RF and dd/YF. A total of 250 RF and 150 dd/YF mice, between 20-25 g body weight, were used. Oxygen tensions in tissues were determined by our modification of the covered cathode(6), in the muscle without anesthesia, and in the spleen, liver, testes, and bone marrow under anesthesia induced by intraperitoneally administered Nembutal (0.5 mg/10 g). PAPP was administered intraperitoneally in a single dose of 50 mg/kg, in either 50% solution of propylene glycol (PG), or in saline (0.9% NaCl). The total volume of each inoculum with PAPP was 0.2 ml/10 g. All controls received an equivalent amount of the appropriate solvent.

Blood methemoglobin level was determined by a modification of the method of Evelyn and Malloy(7) described by Storer(1). Two-tenth ml of blood was removed by cardiac puncture and hemolysed in 10 ml M/15 phosphate buffer, pH 7, 4, containing 0.1% saponin. The sample was centrifuged for 5 minutes and poured into each of 2 cuvettes. A drop of 20% potassium ferricyanide was added to the second tube. Extinctions were then read in a Shimadzu Spectrophotometer at 630 mμ. Two drops of 2% sodium cyanide were added to both tubes and the extinctions were again read. The percentage of methemoglobin was then calculated by the following formula:

$$\frac{\text{Drop in extinction of first tube}}{\text{Drop in extinction of 2nd tube (ferricyanide)}} \times 100 = \%\ \text{methemoglobin in sample.}$$

Radiation factors were: 190 kV, 24 mA, 1.0 mm Cu and 0.5 mm Al filters, target distance 50 cm, dose rate measured in air 50 r/min, time of irradiation 15 or 16 minutes. Mice were irradiated in groups of 5 in an acrylite resin box, and the X ray dose was measured during their irradiation.

Results. The intraperitoneal injection of PAPP was followed by a decrease of oxygen tension in all tissues under study. About 90% of the decrease of tension was reached within 20 minutes after PAPP injection (Fig. 1). The decrease of oxygen tension was observed when either PG or saline was used as a vehicle for PAPP, and within 20 minutes reached about 15% of initial value (Fig. 2). When pure oxygen was inhaled for 5 minutes at normal barometric pressure (1 atm.), from 25 to 30 minutes after the injection of PAPP, an increase in oxygen tension was noted when 0.9% NaCl was used as a vehicle; while only a slight elevation of tension was observed when PAPP in PG was used (Fig. 2). The course of the change in the oxygen tension

* Research fellow of Japan Society for the Promotion of Science, on leave from Inst. of Biophysics, Czechoslovak Acad. Sci., Brno, Czechoslovakia.

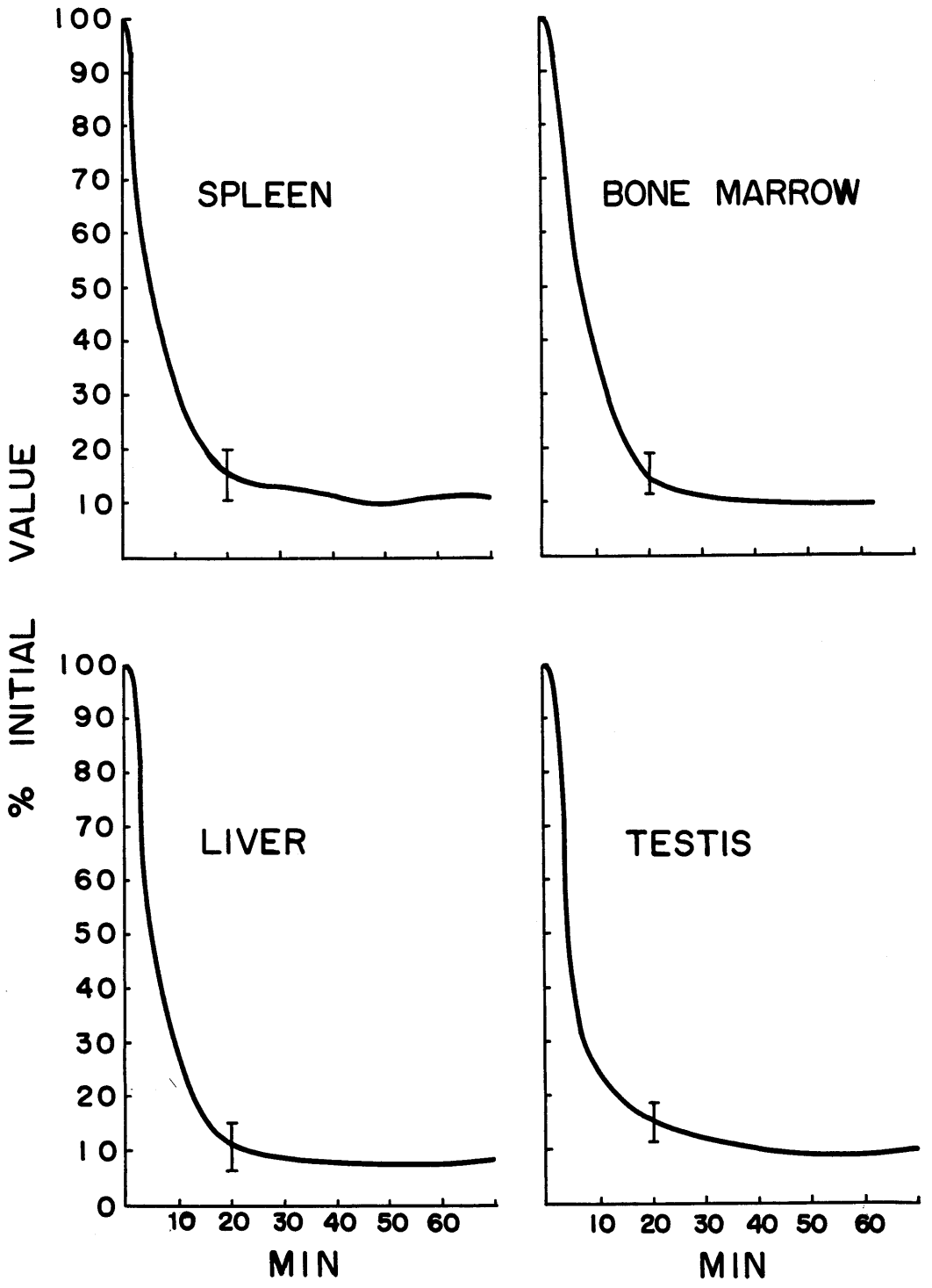

FIG. 1. Oxygen tension in tissues after PAPP-in-PG injection. Y — changes of tension as compared with initial values. X — time after injection.

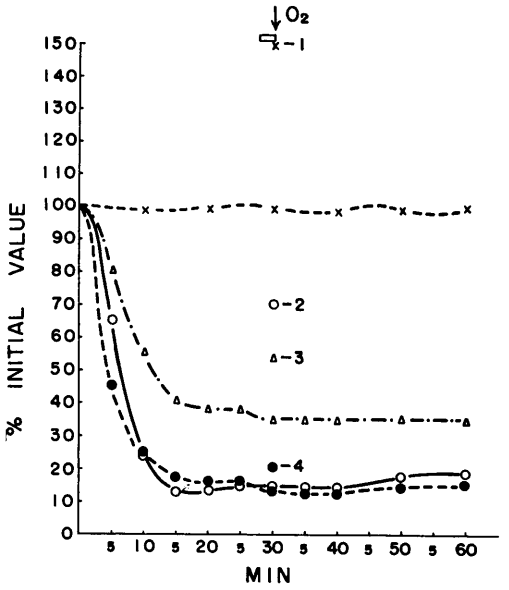

FIG. 2. Role of vehicles for PAPP in the decrease of oxygen tension in muscle. Y — changes of initial value (100%) = level of the tension before injection); X — time after injection; x - - - - x injection 0.9% NaCl; Δ - - - - Δ injection 50% PG; O—O injection PAPP in 0.9% NaCl; ● - - - - ● injection PAPP in 50% PG; □ O_2 — time of breathing of pure O_2 (1 atm.) and increase of tension. x — 1 after 0.9% NaCl; O — 2 after PAPP in 0.9% NaCl; Δ — 3 after PG; ● — 4 after PAPP in PG.

after PAPP was similar in both strains of mice.

Data on the level of produced methemoglobinemia showed that the maximal level was reached in about 30 minutes after injection of PAPP, 15 minutes after injection, methemoglobinemia was about 70-80% of maximal value (Table I).

The mortality data are summarized in Tables II and III. All mice without treatment with PAPP or PG died, but from 50 to 78% of mice of both strains survived if PAPP in either vehicle or PG alone were administered 15 minutes before irradiation in air. The difference in percent survival among the 3 groups was not statistically significant. No

TABLE II. Effect of Time of Administration of PAPP in PG on Protection of RF Strain Mice Against 750 r Total-Body X-Irradiation.

	Time of injection	30-day survival	% Survival
Control S	15′ before	0/11	0
" PG	15′ "	8/16	50
PAPP	15′ "	14/22	63
"	30′ "	14/24	58
"	60′ "	8/20	40

S = saline (.9% NaCl).
PG = 50% propylene glycol.

statistically significant differences were observed in 30-day survival, if mice were irradiated 15, 30 or 60 minutes after the PAPP-in-PG injection. Irradiation in pure oxygen at 1 atm. pressure did not change the protective activity of PAPP in PG, or PG alone, but decreased that of PAPP in 0.9% NaCl. The course of protection with different vehicles for PAPP was similar in both strains of mice.

Discussion. Intraperitoneal injection of PAPP resulted in methemoglobinemia and decrease of oxygen tension in tissues. The decline of tension was correlated with the protective effect of PAPP. The maximal level of methemoglobinemia after administration of PAPP, as used in the present experiment, was reached 30 minutes after injection. However, we observed the same level of protection if mice were irradiated 15, 30 or 60 minutes after PAPP administration. According to the data on the decreased O_2 tension, we believe that the extent of the drop of the tension in tissues, necessary for the radioprotection, was reached earlier than the maximal reduction of the capacity of the blood to carry O_2 to the tissues(5).

PG appears to have protective value(2,8, 9,10). According to our data the protection by PG can be correlated with a decrease of oxygen tension in tissues. Intraperitoneal injection of 2% PG induced no change in

TABLE I. % Methemoglobin After Intraperitoneal Injection of PAPP or Its Vehicle.

Time after inj (min)	PAPP in saline	PAPP in PG	PG alone	Saline alone	Control, no injection
15	52 ± 5.4	50 ± 3	/	/	20.1 ± 2
30	83.4 ± 4	73 ±10	24.4 ± 2.2	20.1 ± 2	/
60	72 ± 4	65 ± 7	/	/	/

Each determination is average from 6 mice.

TABLE III. Protective Effect of PAPP with Different Vehicle, When Administered 15 Minutes Before Irradiation.

	Vehicle	Condit. irrad.	RF—750 r 30-day survival	RF—750 r % Surviv.	dd/YF—800 r 30-day survival	dd/YF—800 r % Surviv.
Control	no	air	0/10	0	0/10	0
	S	air	0/10	0	0/10	0
	S	O_2	0/10	0	0/ 6	0
	PG*	air	0/ 8	0	—	—
	PG	air	5/10	50	13/20	65[b]
	PG	O_2	6/12	50	8/13	62[b]
PAPP, 50 mg/kg	S	air	9/15	60[c]	10/20	50[a]
	S	O_2	3/15	20[a]	2/20	10[a]
	PG	air	15/23	65[b]	14/18	78[b]
	PG	O_2	12/18	67[b]	7/ 9	78[b]

S = saline (.9% NaCl). PG* = 2% propylene glycol, PG = 50% propylene glycol.
Differences in survival between [a] and [b] $P < .01$.
[a] and [c] $P < .05$.

oxygen tension, nor was protection against x-irradiation observed. PG is a compound that requires oxygen for metabolism, and increased O_2 consumption can reduce the oxygen tension within cells, as we have observed after DNP administration(11). PAPP in PG did not show a statistically significant increase in protection as compared with PG alone or PAPP in 0.9% NaCl, which is in accordance with the level of oxygen tension.

We assume that the stability of the protection of PAPP in PG, when pure oxygen at normal barometric pressure was inhaled during irradiation, may be due to the combined effect of both compounds on the oxygen tension in tissues.

Summary. Intraperitoneal injection of PAPP resulted in a decreased oxygen tension in tissues; and 90% of the decrease was reached within 15-20 minutes after injection. The course and the extent of the decrease in oxygen tension after PAPP injection was the same when 0.9% NaCl or 50% PG was used as a vehicle. Oxygen tension in tissue was decreased after injection of PG (50%). The inhalation of pure oxygen at 1 atm. pressure, after injection of PAPP, caused an increase of O_2 tension in tissues when 0.9% NaCl was used as a vehicle; however, when PG or PAPP in PG was injected, only slight elevation of oxygen tension was observed. The mortality data are related to the changes of oxygen tension in tissues.

1. Storer, J. B., Coon, J. M., Proc. Soc. Exp. Biol. and Med., 1950, v74, 202.
2. Gray, J. H., Tew, J. T., Jensen, H., ibid., 1952, v80, 604.
3. Doull, J., Plzak, V., Brois, S. J., Rad. Res., 1959, v11, 439.
4. Plzak, V., Doull, J., ibid., 1963, v19, 228.
5. Salerno, P. R., Friedell, H. L., ibid., 1954, v1, 559.
6. Vacek, A., Physiol. Bohemoslov. (Prag), 1965, v14, 594.
7. Evelyn, K. A., Malloy, H. T., J. Biol. Chem., 1938, v126, 655.
8. Salerno, P. R., Mattis, P. A., Friedell, H. L., Fed. Proc., 1952, v11, 387.
9. Bacq, Z. M., Herve, A., Fisher, P., Bull. Acad. Roy. Med. Belg. VIth series, 1953, v18, 226.
10. Alexander, P., Bacq, Z. M., Consens, S. F., Fox, M., Herve, A., Lazar, J., Rad. Res., 1955, v2, 392.
11. Vacek, A., Rotkovska, D., Int. J. Radiol. Biol., 1964, v8, No. 3, 285.

Received June 20, 1966. P.S.E.B.M., 1967, v124.