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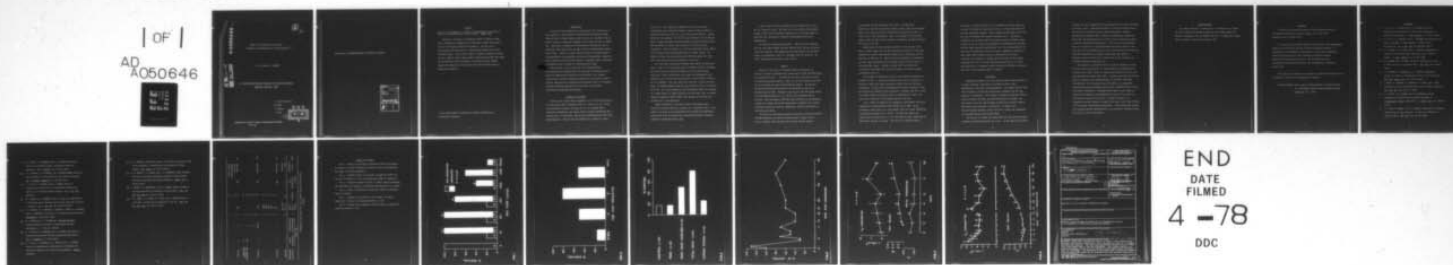
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EFFECT OF X-IRRADIATION ON SURVIVAL OF RABBITS WITH STAPHYLOCOCC--ETC(U)
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Effect of X-Irradiation on Survival
of Rabbits with Staphylococcal B Enterotoxemia^{1,2,3}

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Running head: X-IRRADIATION AND SEB TOXEMIA IN RABBITS

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ABSTRACT

Liu, C. T., and Hilmas, D. E. Effect of X-Irradiation on Survival of Rabbits with Staphylococcal B Enterotoxemias. Radiat. Res.

Nonlethal, total-body, x-irradiation (500 R) of rabbits 4 days before intramuscular staphylococcal enterotoxin B (SEB) inoculation at doses of 10-50 $\mu\text{g/kg}$ prevented the lethality. The same x-ray pretreatment improved survival of rabbits given higher doses of SEB (100-5000 $\mu\text{g/kg}$). Local cardiac region or cephalic irradiation (500 R) failed to improve survival among rabbits inoculated with SEB (100 $\mu\text{g/kg}$). The mechanisms by which total-body x-irradiation protects rabbits against SEB toxemia may be associated with both decreased tissue O_2 demand and leukopenia.

3-5 key words needed: x-irradiation; rabbits; staphylococcal enterotoxin B; survival.

INTRODUCTION

It has been well documented that intravenous (IV) injection of a small quantity of highly purified staphylococcal enterotoxin B (SEB) produces death in rhesus monkeys (1-3), dogs (4, 5), and rabbits (6, 7). Enteric endotoxin release was suggested as the cause of death in rabbits (7). Total-body x-irradiation has been shown to prolong survival of endotoxemic dogs (8) and mice (9, 10), as well as SEB-inoculated rhesus monkeys (11). Further, a highly virulent strain of Venezuelan equine encephalomyelitis virus produced less severe histopathologic changes in brain tissues of mice previously exposed to sublethal (600 R) total-body x-irradiation than it caused in nonirradiated mice (12).

The objectives of this study were 1) to examine whether pretreatment with a sublethal dose of total-body x-irradiation would modify the SEB-induced lethality in Dutch rabbits, 2) to compare localized and total-body irradiation exposures on the basis of rabbit survival characteristics following SEB inoculation, and 3) to determine possible mechanisms associated with protection by total-body x-irradiation during SEB enterotoxemia.

MATERIALS AND METHODS

Healthy, male, Dutch rabbits weighing 1.6 to 2.2 kg were allocated into four groups and 19 subgroups (Table I). Group I (n = 21) served as nonirradiated controls, and group II (n = 31) received 500 R total-body x-irradiation and various doses of highly purified SEB (13) ranging from 5 to 5000 µg/kg. SEB was given intramuscularly (IM) 4 days postirradiation. Survival time was observed for a period of 7 days.

Group III (n = 36) rabbits were exposed to 500 R of total-body x-irradiation and 4 subgroups received a single IM dose of SEB (0.1 mg/kg) was given at 15 min, 3, 4, and 7 days postirradiation. Within group III, two subgroups (15 and 16) were irradiated, but no SEB was given. Oxygen consumption and body weights and blood leukocyte counts were determined at various time intervals for 106 and 46 days, respectively. Group IV rabbits (n = 16) were irradiated locally (500 R) over the head, body (with head shielded), or cardiac region. All rabbits in Group IV were inoculated with SEB (0.1 mg/kg, IM) 4 days after x-ray exposures and were observed for survival.

The total-body x-irradiation exposure dose (500 R) was delivered from a 1 MeV, 3 mA, x-ray generating unit (General Electric, Milwaukee, Wis.) operating at an effective energy of 475 keV without added filtration. Before irradiation, each rabbit was anesthetized with Innovar-vet (0.15 ml/kg, IM) and placed in a Lucite cylinder (length = 49.5 cm; diameter = 13.6 cm), which was rotated at 1 rpm in the x-ray beam. The midline exposure dose rate was 30 R/min in air at a distance of 118 cm. For local irradiation, the anesthetized rabbit was strapped on a wooden board in a supine position, only the head, body, or cardiac region was exposed to 500 R, while other portions of the body were shielded with a 5-mm lead plate.

Oxygen consumption of the intact rabbit was measured with a one-liter spirometer (Warren E. Collins, Inc., Braintree, Mass.). The volume of utilized O_2 was standardized with pressure and temperature. Body surface areas of rabbits were calculated according to the data compiled by Altman and Dittmer (14).

A small volume of blood was taken from the marginal vein of the ear for leukocyte counts. The blood (20 μ l) was diluted with Unopette reagent (1:100, Becton-Dickinson, Rutherford, N.J.) and the number of leukocytes was counted with a Neubauer hemocytometer under 100 x magnification.

All data were analyzed statistically. When values were compared from the same rabbit between the base-line and later values, the paired t-test was employed. When comparisons were made between two different groups, an independent t-test or a chi-square test was utilized. The "null" hypothesis was rejected at the 5% level.

RESULTS

Effects of total-body x-irradiation (500 R) pretreatment on survival of rabbits challenged with various doses of SEB (100-5000 μ g/kg) are summarized in Fig. 1. A single dose of SEB 5 μ g/kg was not lethal to normal nonirradiated rabbits. There was only 20% survival, when SEB (10 to 100 μ g/kg) was given to nonirradiated rabbits. The survival incidence was between 80 and 100% in the irradiated groups given identical doses of SEB. Although a large dose of SEB (500 μ g/kg) killed all nonirradiated animals, 2 of 5 (40%) of the irradiated rabbits survived. Even with doses of SEB that would ordinarily be overwhelming, 1 mg/kg and 5 mg/kg, 60% and 20% respectively of rabbits that received total-body irradiation survived, suggesting a continued expression of the protective effects.

The effect of time interval between total-body x-irradiation (500 R) and SEB inoculation on survival characteristics is shown in Fig. 2. Five of 6 rabbits (83%) survived when the time interval between

x-irradiation and SEB inoculation was 4 days. No significant difference in survival was observed between control (20%) and irradiated groups (50%), when total-body x-irradiation was applied on either 3 or 7 days prior to SEB inoculation. Only 1 of 6 rabbits (17%) survived, however, when the time interval between x-irradiation and SEB inoculation was reduced to 15 min.

Applications of local irradiation (500 R) to the regions of the head or cardiac region did not alter the percent survival, 17-25%, when compared with control nonirradiated rabbits (17%), inoculated with the same dose of SEB (Fig. 3). When the head was shielded, the irradiation of "body" alone increased survival slightly from 17 (control data) to 50% during SEB toxemia. However, survival (83%) with total-body x-irradiation was higher than with any treatment of selected regional x-irradiation (Fig. 3).

The response of leukocytes to 500 R of total-body x-irradiation is presented in Fig. 4. Using the leukocyte count before irradiation as a baseline of 100%, the counts increased to 130% by the day following irradiation. By days 4 and 5 postirradiation, however, leukocyte counts were drastically reduced to 30% of baseline. Although there was a trend for gradual return of leukocyte counts to preirradiation levels, the return only reached 60 to 70% during a period of 46 days.

Fig. 5 shows the changes in O_2 consumption, body weight, and rectal temperature of nonirradiated control rabbits for 22 days. The O_2 consumption varied from 6 to 7 liters/hr/m², while the rectal temperature was maintained between 39.2 to 39.6°C. The mean body weight was significantly increased from 2.2 to 2.4 kg within 22 days, indicating an approximate increase of 9 g/day. The effect of a sublethal dose of

total-body x-irradiation (500 R) on O_2 consumption and body weight for a period of 106 days is illustrated in Fig. 6. Rectal temperature did not show significant changes. Oxygen consumption decreased on the first day after irradiation and reached a minimum on day 4. Although O_2 consumption of irradiated rabbits fluctuated and had a tendency toward recovery on day 16, reduced O_2 consumption was maintained for 106 days. The body weight decreased significantly 3 to 4 days postirradiation and there was no significant growth for 16 days. Significant increase in body weight above baseline values was observed by day 20 after total-body x-ray exposure. Between days 20 and 106, the body weight increased from 1.95 to 2.15 kg, indicating an increase of 2.3 g/day. The growth rate of irradiated rabbits was greatly inhibited as compared with that of nonirradiated rabbits (9 g/day).

DISCUSSION

SEB-induced lethality (10 to 50 μ g/kg) in rabbits was prevented by a single, nonlethal, total-body, x-irradiation dose of 500 R administered 4 days prior to SEB inoculation. With higher doses of SEB (0.1-5.0 mg/kg), survival was significantly increased with the same amount of x-irradiation. Local cardiac or cephalic irradiation (500 R) failed to prevent lethality after SEB inoculation (0.1 mg/kg), and body exposure with the head shielded provided less protection against SEB toxemia than total-body x-irradiation. It appears that radiation-induced systemic, rather than local, effects are responsible for increased survival during SEB toxemia.

The survival of rabbits was highest when the time interval between irradiation and SEB inoculation was 4 days. At this particular interval

(4 days), both O_2 consumption and circulating leukocyte counts decreased to minimal levels. Simultaneous changes of these two variables may be associated with increased survival during SEB toxemia. However, decreased O_2 consumption alone, induced by thyroidectomy, dehydration, starvation, and propylthiouracil, failed to show any beneficial effect on survival (unpublished observation). If it is true that leukocytes serve as "carriers" for transporting SEB from various tissues to the lung (11) and that leukocytes play a role in the formation of kinins and increased permeability of pulmonary capillaries (15) removal of leukocytes from the blood would be beneficial for the prevention of SEB-induced pulmonary edema (16, 17).

The change in O_2 consumption after total-body irradiation varies with dose and animal species. In rhesus monkeys, O_2 consumption was increased with 1,000 R, showed no significant change with 2,500 R, and was suppressed with larger doses (18). In guinea pigs (19) and fed rats (20), O_2 consumption was unchanged after 250 and 800 R total-body x-irradiation. In the present study with rabbits, O_2 consumption decreased and there was impaired growth after a nonlethal dose of 500 R total-body x-irradiation. Although rabbits began to gain weight at 3 weeks after irradiation, the hypometabolic values did not return to preirradiation levels for 106 days. A similar reduction of O_2 consumption has been observed in rabbits following a lethal dose (1200 R) of ^{60}Co -exposure (unpublished observations). These observations suggest that radiation-induced hypometabolism is not related to irradiation dose, but rather to species differences.

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FOOTNOTES

¹ A portion of the work was reported at the Federation Meeting of the American Physiological Society, Chicago, Ill., April 1977 (Federation Proc. 36, 610, 1977).

² In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

³ The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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REFERENCES

1. C. T. LIU, M. J. GRIFFIN, and R. T. FAULKNER, Effect of staphylococcal enterotoxin B on body fluid compartments in conscious rhesus monkeys. J. Med. Primatol. 5, 336-344 (1976).
2. C. T. LIU, R. D. DELAUTER, and R. T. FAULKNER, Cardiovascular and hepatic responses of rhesus monkeys to staphylococcal enterotoxin B. Am. J. Vet. Res. 38, 1849-1854 (1977).
3. D. D. ELSBERRY, D. A. RHODA, and W. R. BEISEL, Hemodynamics of staphylococcal B enterotoxemia and other types of shock in monkeys. J. Appl. Physiol. 27, 164-169 (1969).
4. D. G. SKINNER and M. A. HAYES, Effect of staphylococcal toxin on renal function: irreversible shock. Ann. Surg. 162, 161-180 (1965).
5. J. V. PROHASKA, V. KOCANDRLE, and E. HOUTTUIN, Hemodynamic changes in response to staphylococcal exotoxin and enterotoxin. Bull. Soc. Intern. Chir. 25, 727-736 (1968).
6. J. ISRAEL, M. OLDSTONE, S. LEVENSON, E. D. FRANK, and J. FINE, Mechanism of action of staphylococcal toxin in rabbits. Proc Soc. Exp. Biol. Med. 108, 709-711 (1961).
7. G. W. PETTIT, M. R. ELWELL, and P. B. JARHLING, Possible endotoxemia in rabbits after intravenous injection of Staphylococcal aureus enterotoxin B. J. Infect. Dis. 135, 646-648 (1977).
8. J. A. VICK, R. DEGRAAF, and C. C. BERDJIS, Interaction of ionizing radiation and E. coli endotoxin. 1. Effect of radiation on endotoxin shock. Mil. Med. 133, 387-390 (1968).

9. W. W. SMITH I. M. ALDERMAN, and R. E. GILLESPIE, Increased survival in irradiated animals treated with bacterial endotoxins. Am. J. Physiol. 191, 124-130 (1957).
10. P. V. VITTORIO, E. A. WATKINS, and S. DZIUBALO-BLEHM, Changes in the sensitivity of x-irradiated mice to bacterial endotoxin. Can. J. Physiol. Pharmacol. 45, 395-400 (1967).
11. C. T. LIU, M. J. GRIFFIN, and D. E. HILMAS, Effect of staphylococcal enterotoxin B on cardiorenal functions and survival in x-irradiated rhesus macaques. Am. J. Vet. Res. Submitted.
12. D. E. HILMAS, R. E. WHITMIRE, and G. S. DILL, JR., Modification of Venezuelan equine encephalomyelitis virus infection in mice by x radiation. Am. J. Vet. Res. 38, 1413-1419 (1977).
13. E. J. SCHANTZ, W. G. ROESSLER, J. WAGMAN, L. SPERO, D. A. DUNNERY, and M. S. BERGDOLL, Purification of staphylococcal enterotoxin B. Biochemistry 4, 1011-1016 (1965).
14. P. L. ALTMAN and D. S. DITTMER (Ed.), Biology Data Book. Federated American Societies of Experimental Biology, Washington, D. C., 1964, pp. 120-121.
15. R. L. MILLER, M. E. WEBSTER, and K. L. MELMON, Interaction of leukocyte and endotoxin with the plasmin and kinin system. Eur. J. Pharmacol. 33, 53-60 (1975).
16. C. T. LIU, R. D. DELAUTER, M. J. GRIFFIN, and C. L. HADICK, Effects of staphylococcal enterotoxin B on functional and biochemical changes of the lung in rhesus monkeys. Toxicon Submitted.

17. M. J. FINEGOLD, Interstitial edema. An electron microscopic study of the pathology of staphylococcal enterotoxemia in rhesus monkeys. Lab. Invest. 16, 912-924 (1967).
18. P. M. BROOKS, E. O. RICHEY, and J. E. PICKERING, Prompt pulmonary ventilation and oxygen consumption changes in rhesus monkeys associated with whole-body gamma-irradiation. Radiat. Res. 6, 430-449 (1957).
19. F. SMITH, W. G. BUDDINGTON, and M. M. GRENAN, Oxygen consumption following whole-body x-irradiation of guinea pigs. Proc. Soc. Exp. Biol. Med. 81, 140-141 (1952).
20. D. E. SMITH, E. B. TYREE, H. M. PATT, and E. JACKSON, Effect of total-body x irradiation on metabolism of the rat. Proc. Soc. Exp. Biol. Med. 78, 774-777 (1951).

TABLE I
Allocation of 104 Dutch Rabbits into four Groups and 19 Subgroups

Group	Site of irradiation	Dose of x-irradiation (R)	Subgroup	IM Dose of SEB (g/kg)	n	Time (days)		Distinctive Measurement
						SEB-inoculation postirradiation	Observation period	
I	None (control)	0	1-4	5, 10, 50 & 100	21	-	7	Survival
II	Total-body	500	5	10	5	4	7	Survival
			6	50	5			
			7	100	5			
			8	500	5			
			9	1000	5			
			10	5000	6			
III	Total-body	500	11	100	6	15 min	7	Survival
			12		6	3		
			13		6	4		
			14		6	7		
			15		6	-	106	
			16		6	-	46	
IV	Head only	500	17	100	6	4	7	Survival
	Body only		18		6			
	(head shielded) Cardiac region		19		4			

LEGENDS FOR FIGURES

Fig. 1. Effect of total-body x-irradiation (500 R) pretreatment on survival of rabbits challenged with SEB IM 6 days postirradiation. The number in the bar represents n.

Fig. 2. Temporal effect of total-body x-irradiation (500 R) on survival of rabbits (n = 6 for each group) given SEB (0.1 mg/kg) IM.

Fig. 3. Comparison between survival of rabbits locally irradiated and total-body x-ray exposure 4 days before SEB inoculation (0.1 mg/kg).

Fig. 4. Effect of total-body x-irradiation (500 R) on leukocyte counts of rabbits.

Fig. 5. Changes in O_2 consumption, body weight, and rectal temperature of control, nonirradiated rabbits (n = 6).

Fig. 6. Changes in O_2 consumption and body weight in sublethally irradiated rabbits (n = 6).

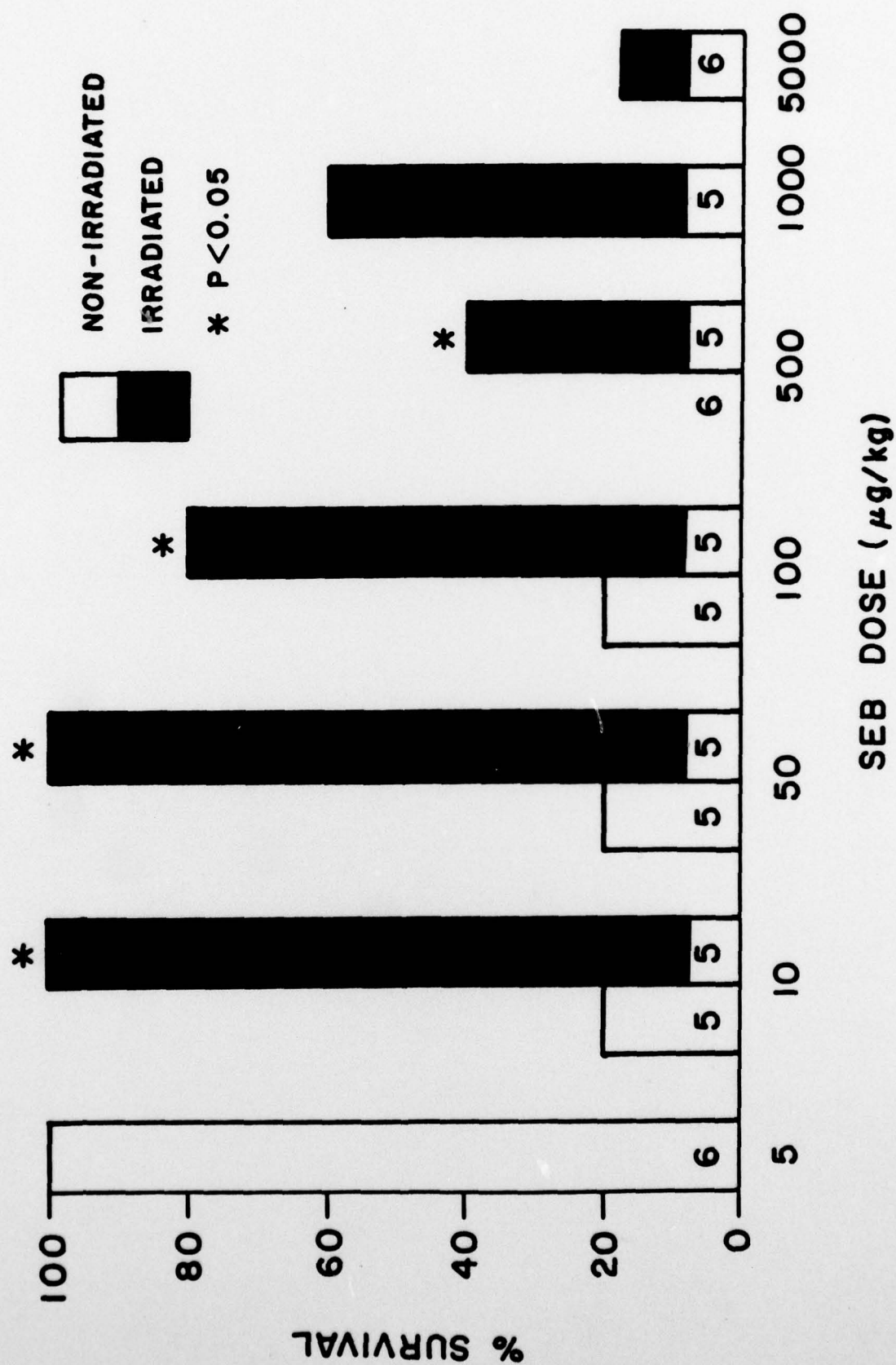


FIG. 1

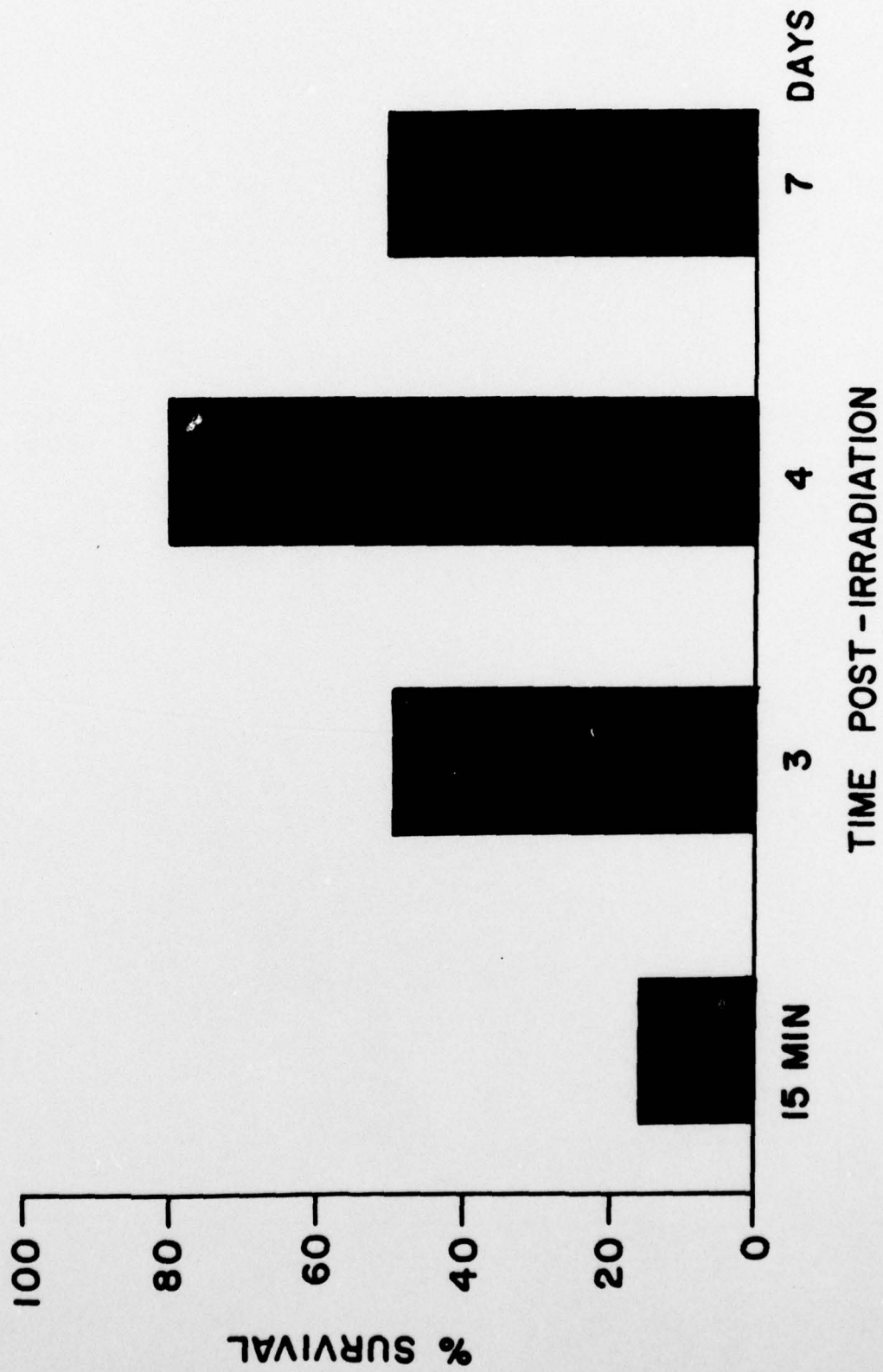


FIG. 2

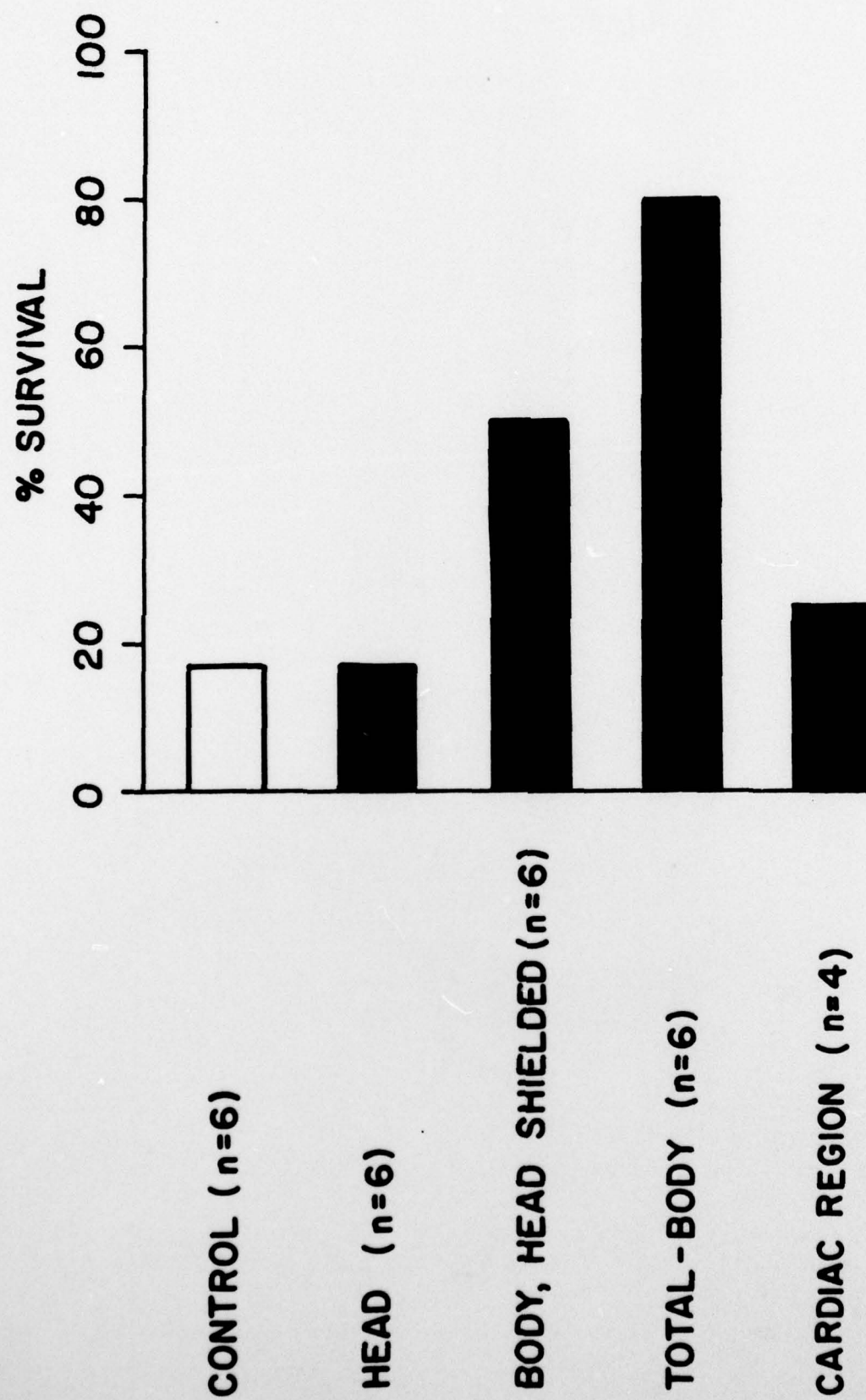


FIG.3

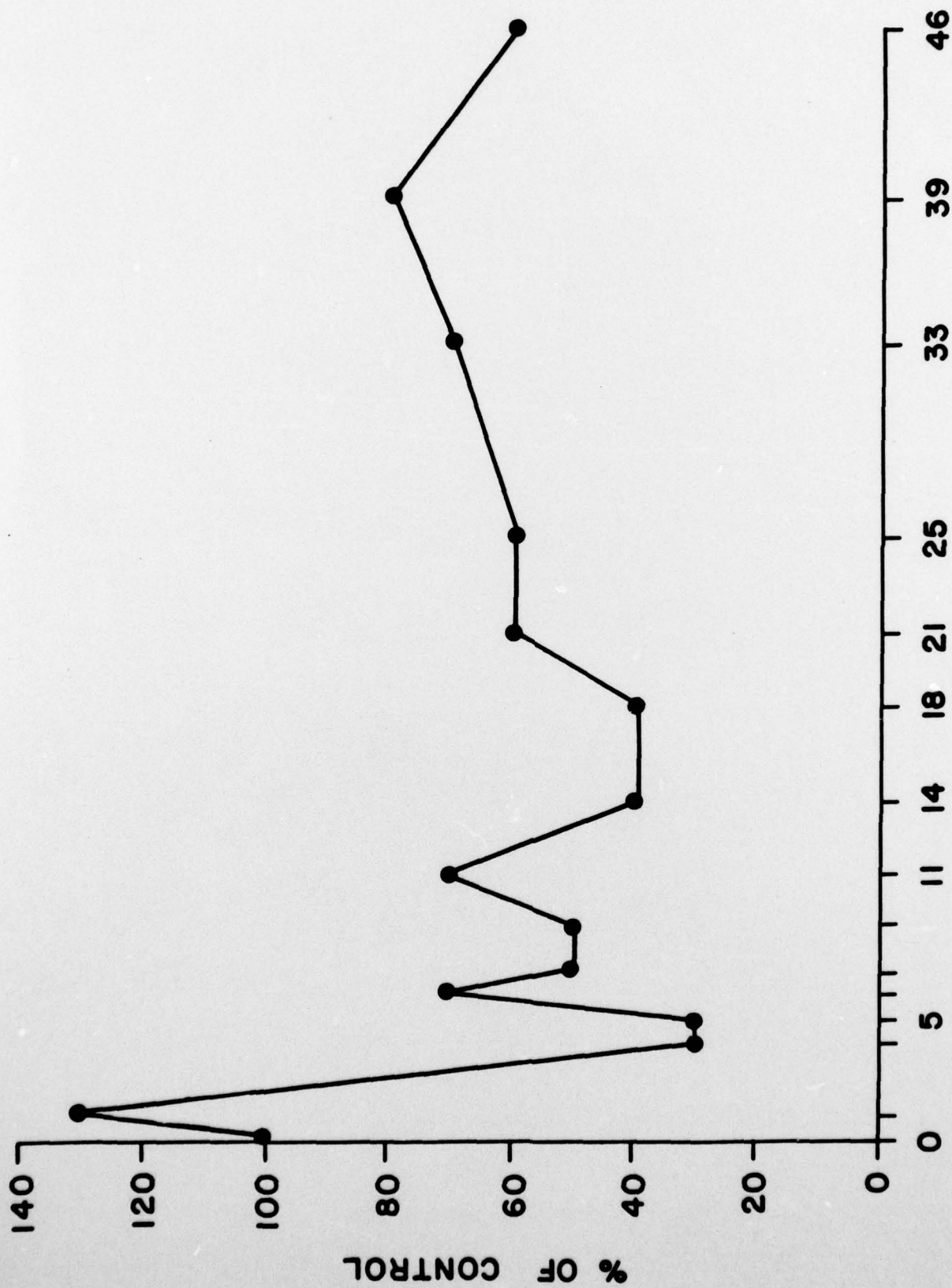


FIG. 4

* $P < 0.05$

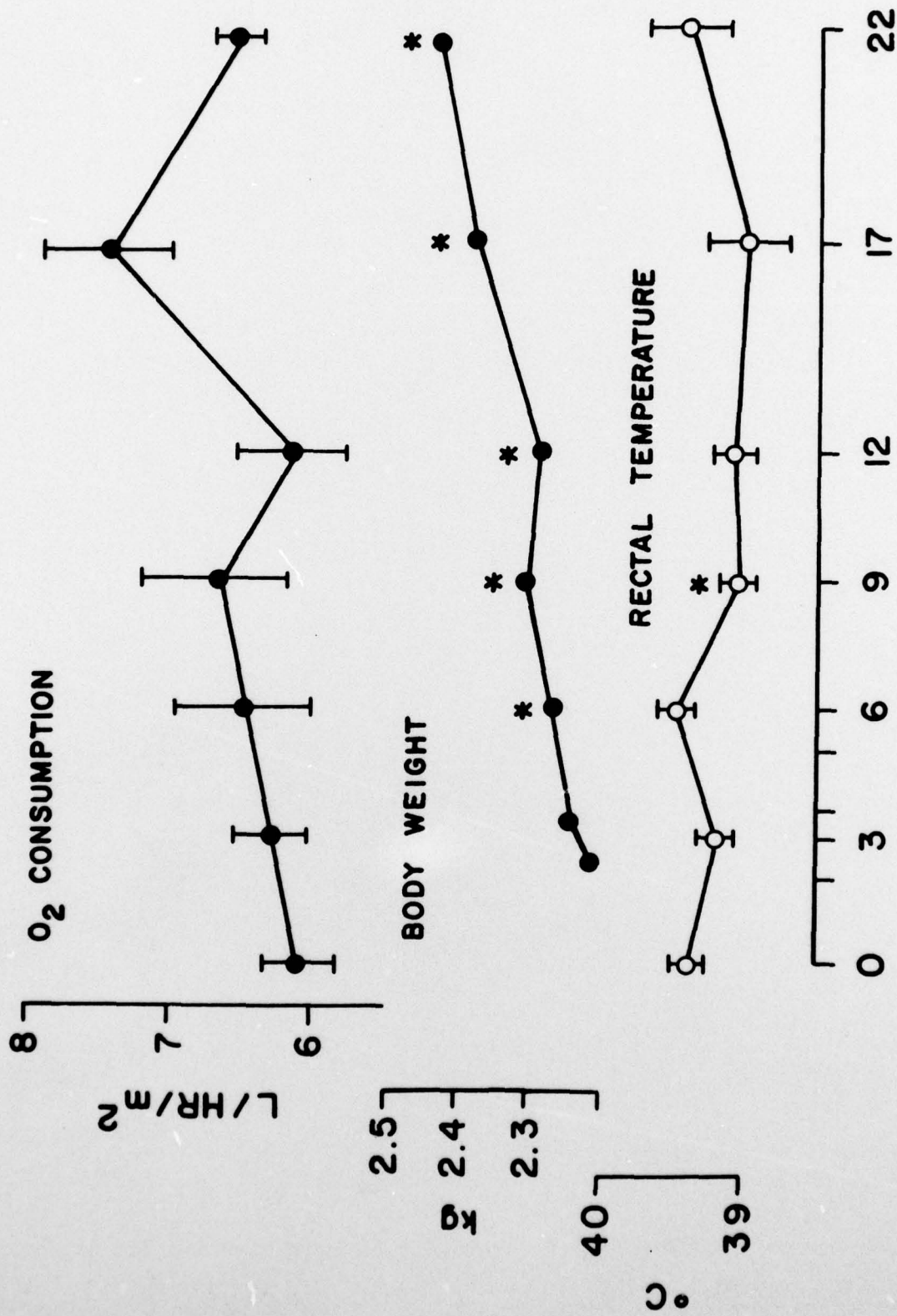


FIG. 5

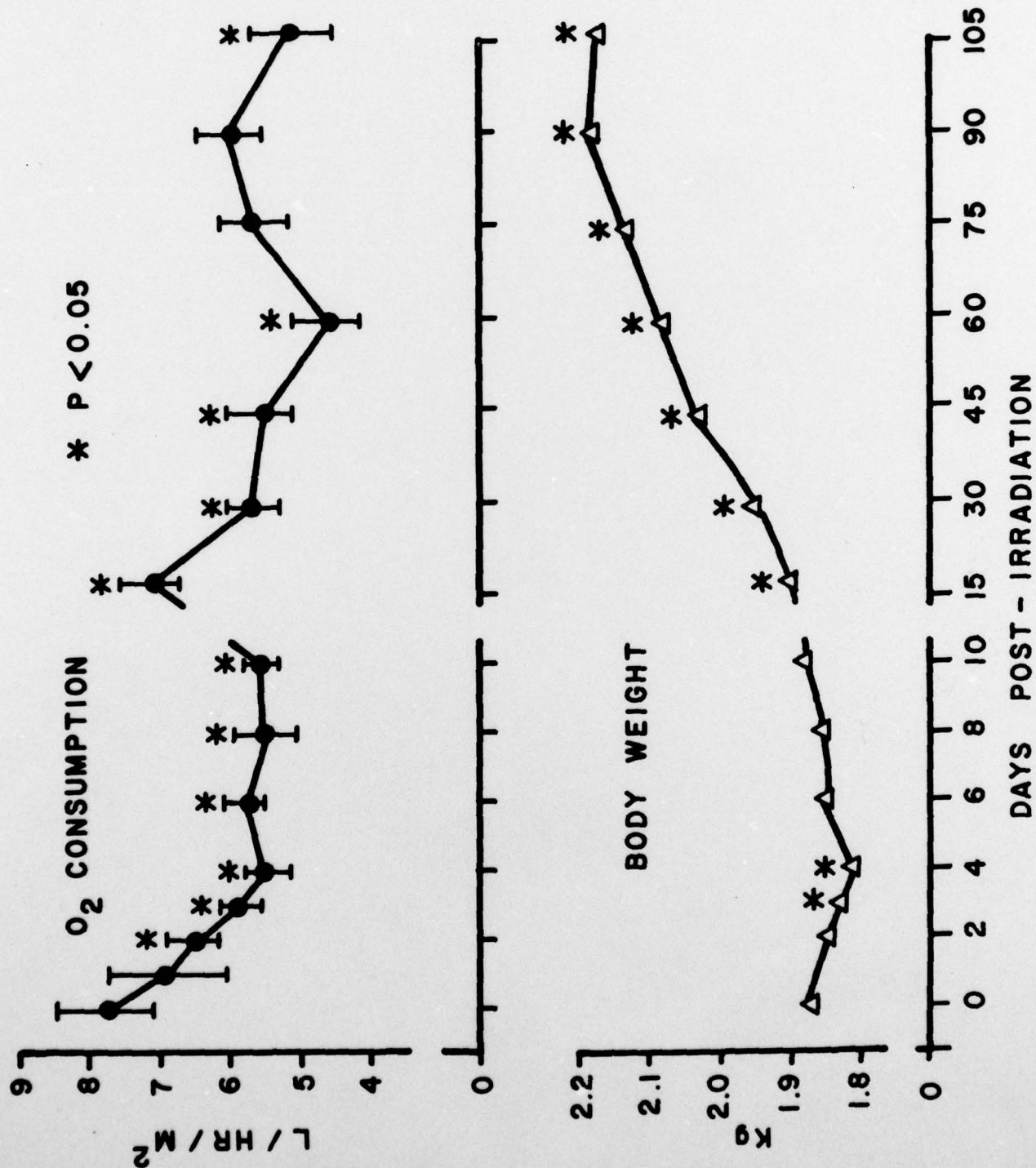


FIG. 6

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