Specific Antibody Titer and Survival after Total-Body X-Irradiation in Rabbits and Rats. (20899)

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It has long been known that total body X-irradiation through a wide range of doses, interferes with the immune process(1,2). Irradiation suppresses or abolishes the production of specific antibodies if given just prior to or shortly after the beginning of immunization. Recovery from the effects of irradiation is associated with a restoration of antibody production. Failure to survive after irradiation may in part be due to a relatively low specific antibody titer or to a failure to develop immunity at a sufficiently rapid rate during the post-irradiation period. There is evidence that circulating bacterial antibodies afford protection to experimentally infected rats and mice which have received a low lethal (LD_{5-10}) dose of X-rays(3). In these animals immunization insured a significantly improved chance for survival. The question arises as to whether the capacity of the animal to produce a relatively high titer for specific antibody might be related to its survival following irradiation.

This question appears reasonable in view of the importance of bacteremia in radiation death. Infection has repeatedly been shown to be a major factor in the death of strongly irradiated mice(4-7). Although less definitive, there are data which indicate that infection may play a role in radiation deaths in other species also(5,8-10). The organisms which have been cultured from the blood and certain organs of such animals appear to be of enteric origin and to have flourished in the host as a consequence of its lowered resistance. It seems possible, therefore, that survival in irradiated animals may depend on the efficiency of their immune mechanisms for the production of circulating antibodies at least to the degree that bacteremia affects survival after irradiation. This possibility has been tested by contrasting the pre-irradiation capacity to produce antibodies as measured by specific antibody titer following immunization, with survival following LD_{50-80} doses of X-radiation. The assumption has been made that the specific antibody titer following immunization is a measure of the capacity of the animal to produce antibodies. It is recognized, however, that degree of bacterial immunity may not necessarily be a function of the quantity of circulating antibody.

Experimental. In all experiments described in this paper the animals were immunized and their serums titrated for antibody content prior to irradiation. Male rabbits of the New Zealand strain, 13 to 15 weeks of age were immunized with hens' egg albumin (Exp. 1 and 2, Table I). Ear blood samples were taken for titration 10 days after the final immunizing injection. Serum anti-egg albumin content was quantitatively estimated by the Heidelberger procedure(11). Hemolysin production in rats and rabbits was contrasted with radiation survival in experiments 3, 4, and 5 (Table I). Sprague-Dawley rats, 11 weeks of age and rabbits were immunized with standard injections of sheep erythrocytes. Their serum hemolysin content was estimated 7 days after the end of immunization by the procedure of Taliaferro(12). In other experiments the rates of hemolysin production in 41 rabbits were contrasted with their subsequent survival after irradiation. The rabbits were bled and serums titrated at 1, 4, 6, 7 and 8 days after a single intravenous injection of a 10% suspension of sheep erythrocytes. Rabbits were exposed to 850 r (LD_{62}, 30 days) of X-radiation while one group of rats was exposed to 700 r (LD_{43}, 30 days) and a second group of rats to 800 r (LD_{64}, 30 days). The rabbits were irradiated singly with one-half the total dose delivered to each side of the animal and the rats were irradiated 6 at a time. Irradiation was done with a 200 Kvp X-ray unit operating

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TABLE I. Antibody Production in Rabbits and Rats Contrasted with Survival after X-Irradiation.

Exp. No.	r	Avg survival time (days)	No. of animals	Avg ± SE pre-irrad. serum titer: γ anti egg albumin N/ml	Avg ± SE pre-irrad. serum titer: Dil/ml × 10^3 for 50% hemolysis	Avg wt at r (g)
1*	850	S‡	5	235 ± 81		3314
		14	1	350		3120
		1-2	9	290 ± 130		3384
2*	"	S	4	276 ± 40		3015
		21	3	262		2767
		1	10	568 ± 145		3200
3*	"	S	17		15.5 ± 2.0	2625
		3.2	20		11.7 ± 1.5	2587
4†	800	S	5		1.1 ± 1.5	321
		11.5	9		3.6 ± 2.2	307
5†	700	S	12		2.0 ± 1.7	329
		12.5	9		2.4 ± 1.6	334

* Rabbits in 1 and 2 received 33.5 and 24.6 γ egg albumin N/kg during 10 wk and 6 wk respectively. Rabbits in Exp. 3 received a 10% suspension of sheep erythrocytes 2 ml/kg for 3 i.v. injections at 4-day intervals.

† Rats in 4 and 5 were immunized with 1% and 10% suspension of sheep erythrocytes, 1 ml/kg i.v. and 3 ml/kg s.c. respectively.

‡ S = Survivors.

at 200 Kv and 20 ma with the following factors: 0.25 mm Cu and 0.51 mm Al added filtration; 70 cm focal distance; 23.5 r per minute.

Results. As was expected, a wide range in values for the anti-egg albumin titers was found (Exp. 1 and 2, Table I). Differences between average anti-egg albumin titers of those rabbits which subsequently survived 850 r X-rays and non-survivors are not significant. Survival for longer than 48 hours was not associated with titers that were significantly different from the average in either experiment. Similar results were obtained in the experiments with multiple injections of sheep erythrocytes. Average pre-irradiation serum hemolysin titers for rats or rabbits which survived mid-lethal irradiation were not significantly different from the averages for the non-survivors (Exp. 3, 4 and 5, Table I).

Pre-irradiation rates of hemolysin production were contrasted with survival after 850 r X-rays in 41 rabbits. In this experiment 63% (26 of 41) of the animals died with a mean survival time of 1.2 days. Average serum hemolysin content and slope values for each day post-immunization for the 26-non-survivors were within limits of variation found for the 15 surviving rabbits titrated at corresponding times. It is noteworthy that peak titer was attained by 19 rabbits in the non-survivor group and only 7 rabbits in the survivor group by 7 days after the single immunizing injection. Average titer on day 7 for the non-survivor group was significantly lower than the average for the survivor group. However, average titers in both groups were similar by day 8.

Conclusions. 1. The capacity to produce specific antibodies as a function of the immune mechanism has been tested before irradiation in rabbits and rats with respect to survival following mid-lethal doses of X-radiation. No direct correlation was demonstrable between survival or survival time after X-ray and pre-irradiation anti-egg albumin or hemolysin titer in 79 rabbits or hemolysin titer in 35 rats. 2. A study of the induction phase of hemolysin production showed that the early attainment (6 or 7 days after immunization) of peak titer could be correlated with non-survival in 41 rabbits subsequently exposed to 850 r. The absence of a striking correlation between pre-irradiation specific antibody titer and X-ray sensitivity in rats and rabbits just described, suggests that the capacity of the immune mechanism for antibody synthesis in these animals is not directly related to their

survival following mid-lethal irradiation.

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Influence of Source of Cholesterol, Grade of Cottonseed Oil, and Breed on Experimental Avian Atherosclerosis. (20900)

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The chicken is particularly susceptible to coronary and aortic atherosclerosis when placed on a high cholesterol diet(1). White Leghorn cockerels are usually used for this purpose and it is reported that nearly 100% incidence of aortic atherosclerosis may be expected when 7- or 8-week-old cockerels are maintained on a diet supplemented with 2% cholesterol and 5% cottonseed oil for a period of 8 weeks(2,3). Achievement of the highest possible incidence of atherosclerosis in the experimental animals represents a great advantage for the investigator. Failure to obtain a consistently high percentage of atherosclerotic animals on an athrogenic diet leads to equivocal interpretations of the experimental results. Therefore, it appears worthwhile to report our incidence data utilizing 3 different strains of chickens, 2 sources of cholesterol and 2 grades of cottonseed oil.

Methods. All strains of cockerels were obtained from the hatchery at one or 2 days of age and reared for 8 weeks in battery brooders on a standard starter mash. The strains were Kerr White Leghorns, Kerr New Hampshires and Hy-Line White Leghorns. U.S.P. cholesterol from 2 suppliers (Merck, Armour) and 2 grades of cotton seed oil (refined, destearinated) were employed in this study. The chickens were kept on the starter mash plus a supplement of 2% cholesterol and 5% cottonseed oil from the 8th through the 16th week of age and sacrificed. The incidence and severity of the aortic atherosclerosis were

TABLE I. Incidence and Severity of Aortic Atherosclerosis in Cockerel Chickens Fed a Diet Supplemented with 2% Cholesterol (Merck) and 5% Refined Cottonseed Oil from 8th through 16th Week of Age.

Strain and breed	No. of chickens	% incidence of aortic atherosclerosis	Avg severity index* Including negatives	Avg severity index* Excluding negatives
Kerr White Leghorns†	24	67	1.08	1.68
Kerr New Hampshires†	36	17	.35	2.10
Hy-Line White Leghorns‡	20	65	1.07	1.65

* Avg severity of aortic atherosclerosis (graded on a 0-4 basis).
† Kerr Chickeries, Inc., Frenchtown, N. J.
‡ Corn Belt Hatcheries, Joliet, Ill.