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Suppression of Normal Bactericidal Action of Rabbit Serum Following Whole Body X-Irradiation.* (20303)

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The observations that x-irradiation injury results in the development of bacteremia(1), that the x-irradiated animal is more susceptible to infection(2), and that antibiotic therapy affords partial protection following mid-lethal to lethal exposure to x-irradiation(3) emphasize the significance of infection among the pathological processes which follow irradiation. Increase in susceptibility to infection and increased incidence of bacteremia are primarily due to the deleterious action of x-irradiation on the host defense mechanisms. Among the suppressed host defense mechanisms which have been implicated are: depressed antibody formation(4), depressed phagocytosis, and destruction of the normal mechanical fascial barriers against infection(5). Even with depression of the previously mentioned host defenses however one would expect neither the large incidence nor the degree of bacteremia encountered if the sera of x-irradiated animals maintained normal bactericidal activity. This latter function has been studied in rabbits; the present report is concerned with the suppression of the normal bactericidal action of rabbit serum following acute whole body x-irradiation.

Materials and methods. Albino rabbits weighing approximately 3 kg were irradiated in air by means of a Westinghouse Quadrocondex x-ray machine. Radiation factors were: 250 KV; 15 ma; 1.0 mm Al and 0.5 mm Cu filters in addition to an inherent filtration of 2.5 mm Al and 0.25 mm Cu; distance from focal point to surface on which rabbits were standing was 105 cms; the dose rate was approximately 25r per minute as measured by a Victoreen r meter. The whole body x-irradiation doses in these experiments varied from 500 to 725r with a 30-day mortality of 675 $\pm$ 11.3r as calculated by the method of Miller and Tainter(6). Group III (Table I) received 2 doses of 650r whole body x-irradiation with a 28-day interval between exposures. Blood was withdrawn by cardiac puncture or via the marginal ear vein from an equal number of x-irradiated and normal control rabbits at various intervals. The serum was separated, stored at 4°C and the serum bactericidal test performed within 30 hours. Bactericidal activity was tested in the following manner: 0.2 ml of blood serum was added to 1.8 ml of 0.9% NaCl solution (saline) and 0.25 ml of 1:100 dilution of an 18-hour nutrient broth culture of *Bacillus subtilis* (PCI 220) was added to the 1:10 dilution of serum. The serum, saline, culture suspension was placed in a 37°C water bath for 2 hours before the nutrient agar pour plates were prepared from various dilutions of the suspension. The plates were then incubated at 37°C for 48 hours and colony counts were made. Because of variation in the number of viable bacteria in the original inoculum it was neces-

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TABLE I. Effect of X-Irradiation Dose and Post-Irradiation Time on the Bactericidal Activity of Rabbit Serum.

Group No.	X-ray dose, r	Days after x-ray	Surviving rabbits	No. of sera with bactericidal activity: Normal	Depressed*
I	500	1	2	2	0
		7	2	2	0
		12	2	2	0
		16	2	2	0
II	650	1	6	6	0
		7	6	0	6
		13	6	0	6
		19	6	6	0
III	"	1	6	6	0
		5	6	3	3
		9	6	0	6
		15	6	0	6
		20	6	6	0
IIIA†	"	1	6	3	3
		5	6	0	6
		10	6	0	6
		15	3	0	3
		21	2	0	2
		25	2	0	2
		31	2	2	0
IV	"	1	6	6	0
		7	2	0	2
V	700	1	5	3	2
		7	5	2	3
		14	5	1	4
		19	5	5	0
VI	725	1	6	6	0
		5	6	2	4
		11	4	0	4

* Serum was considered to exhibit depressed bactericidal activity when the No. of viable organisms, following bactericidal test, was more than 3 times control group avg.

† Same animals as used in Group III exposed to 650 r 28 days after first x-irradiation exposure; total for this group 1300 r.

sary to test a control group of sera from normal rabbits at the same time as the x-irradiated group was being tested. As a check for bacteremia, 1.0 ml of whole blood was spread on a nutrient agar plate at the time blood was withdrawn for bactericidal testing. Whenever bacteremia was observed the same procedure used in the bactericidal test was carried out with the contaminated serum except that sterile saline was added in place of the culture dilution. With this technic the number of organisms of bacteremic origin was obtained. This number was subtracted from the number obtained following incubation of the test serum, saline, *B. subtilis* suspension to yield the number of viable organisms derived from the *B. subtilis* inoculum. In later experiments this control was abandoned since organisms of bacteremic origin were numerically insignificant by comparison with the test inoculum.

Results. With the technic described for measuring bactericidal activity of sera no difference was observed between sera from non-irradiated control and x-irradiated rabbits following exposure to 500r of whole body irradiation (Table I). After a single exposure to 650r or more of whole body x-irradiation, a loss of the bactericidal component was observed in all but one of the rabbits which lived 5 days or longer. Following the initial whole body x-irradiation the bactericidal activity of the sera of 2 rabbits was depressed on the first day following x-irradiation and the sera from the other 29 rabbits exhibited normal bactericidal activity at this time. When animals were exposed to a second 650r dose 28 days following the first 650r exposure, it was noted

TABLE II. Bactericidal Action of the Serum from X-Irradiated and Control Rabbits.

Treatment	Serum* dilution	Rabbit No.	Avg bacteria/ml†	Group avg
650 r	1:10	B–60	15200	12760
		61	16850	
		62	16500	
		63	9580	
		64	262	
		65	18160	
"	undil.	60	16630	6760
		61	3430	
		62	1450	
		63	148	
		64	726	
		65	2244	
0	1:10	C–60	6	6
		61	5	
		62	4	
		63	11	
		64	7	
		65	5	

* Serum withdrawn 9 days after a single 650 r x-irradiation exposure.

† Prior to 2 hr incubation period, each tube contained approximately 4840 bacteria/ml.

that the sera of 3 of the 6 animals in the group (Group IIIA) lost bactericidal activity within 24 hours whereas the sera of all 6 of these rabbits had exhibited normal bactericidal activity one day after initial x-irradiation exposure. The sera from all animals that lived 20 days following initial x-irradiation with either 650 or 700 r regained normal bactericidal action. The sera from the 2 rabbits which survived the second 650r dose had not regained normal bactericidal activity 25 days after the second exposure. This function was found to be normal on the thirty-first day.

It was frequently noted that the bactericidal action of sera from x-irradiated animals was depressed to the extent that multiplication of the bacteria took place during the 2-hour incubation period. This is shown in Table II and indicates that the bactericidal function of the sera was lost and that the organisms were multiplying during the incubation period. The second table also illustrates the differences observed between the bactericidal action of the sera from x-irradiated and normal rabbits and demonstrates that the bactericidal action of sera was quantitatively suppressed. It can be seen from Table II that a 1:10 dilution of serum from an x-irradiated rabbit may allow multiplication of bacteria during the incubation period while undiluted serum retains definite bactericidal action. The results show that undiluted sera from this group of x-irradiated rabbits were not as bactericidal as 1:10 dilutions of normal rabbit sera. It has been noted that with the described method for testing bactericidal action, counts of less than one organism per ml were obtained with undiluted normal rabbit sera.

Discussion. The results demonstrate that acute whole body x-irradiation in doses of 650r or more cause a depression in the bactericidal action of rabbit serum. It is possible that this loss or depression of serum bactericidal activity plays a major role in the increased susceptibility to infection which follows irradiation injury. The suppressed bactericidal activity following x-irradiation may be as detrimental to the host as the x-irradiation effects on other host defense mechanisms. Although the number of circulating phagocytes is reduced to about 10% of normal, it has been shown that whole body x-irradiation (350r) in the mouse does not alter the uptake of ThO_2 by the fixed phagocytes of the spleen and liver and that this treatment results in an increase in the per cent of peritoneal phagocytes containing engulfed bacteria following an intraperitoneal injection of *Micrococcus pyogenes* var. *aureus*(7).

The mechanisms involved in the loss of bactericidal activity from the sera of irradiated rabbits are as yet unknown but preliminary evidence indicates that the normal antibody or complement concentrations are not significantly altered following x-irradiation and a substance that inhibits the normal bactericidal component can not be demonstrated in the sera of x-irradiated rabbits. Buchner's (8) theory that leucocytes secrete the bactericidal substance found in normal serum could account for the loss of bactericidal action of serum following irradiation injury.

Summary. 1. Whole body x-irradiation of 650 to 725r results in depression of the normal bactericidal activity of rabbit serum. 2. With the x-irradiation doses employed, the bactericidal action of serum from rabbits is normal on the first post-irradiation day, is depressed by the fifth day following whole body

x-irradiation and returns in surviving animals by the twentieth post-irradiation day. 3. Following a second dose of 650r, serum bactericidal activity is depressed earlier and remains depressed for a longer time than after the first 650r x-irradiation exposure.

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Succinylmonocholine Iodide: Its Enzymatic Hydrolysis and Neuromuscular Activity.* (20304)

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In the past two years, succinylcholine has been used with increasing frequency for the production of muscular relaxation in the anesthetized patient. It was shown by Glick(1), Bovet-Nitti(2), and Castillo and de Beer(3), that succinylcholine is hydrolyzed by the plasma cholinesterase of various mammals. It was suggested by Ginzel *et al.*(4), by Whittaker and Wijesundera(5), and recently demonstrated by the latter(6) that the hydrolysis of succinylcholine by concentrated horse serum cholinesterase occurs in two steps. First, succinylcholine is hydrolyzed to succinylmonocholine and choline; succinylmonocholine is then very slowly hydrolyzed to succinic acid and choline. Because of its more rapid enzymatic hydrolysis in the human plasma(7), the mg/kg dose of succinylcholine necessary for the production of neuromuscular blockade, and the mg/kg/min dose used for the maintenance of muscular relaxation in anesthetized man is considerably greater than in other mammals(8).

Since under these circumstances the possibility of the accumulation of succinylmonocholine in anesthetized patients cannot be excluded, it seemed worthwhile to investigate the hydrolysis of succinylmonocholine in human plasma and to reinvestigate its neuromuscular effect in laboratory animals.

Methods. The hydrolysis of succinylmonocholine iodide† and succinyldicholine dichloride by normal heparinized and heat-inactivated heparinized human plasma was measured with Warburg's manometric technic at 37°C and pH 7.4 using a bicarbonate-carbonate buffer system. 0.4 ml of plasma or heat-inactivated plasma diluted to 1.6 ml with 0.025 M $NaHCO_3$ was placed in the main chamber of the Warburg vessels and 2 mg of succinyldicholine dichloride or succinylmonocholine iodide dissolved in 0.2 ml and titrated to pH 7.4 with NaOH was placed in the sidearms of the vessels. After the systems were brought into equilibrium with a 5% CO_2-95% O_2 gas mixture, the substrates were added to

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