THE EFFECT OF HIGH DOSES OF X-IRRADIATION ON THE PHAGOCYTIC, PROLIFERATIVE, AND METABOLIC PROPERTIES OF THE RETICULO-ENDOTHELIAL SYSTEM*

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The exposure to lethal or near lethal doses of x-irradiation lowers the natural resistance to bacterial infections, experimentally induced (1) or resulting from invasion of the natural flora of the intestinal tract (2). This condition is probably the result of the damaging effect of x-irradiation on various elements which participate in antibacterial defenses: the leukocytes (1), antibody production (3), serum bactericidal action (4, 5), and the reticulo-endothelial system (RES). While the effect of x-irradiation on leukocytes and on antibody production (3) is well established, the effect of x-irradiation on the RES is still a controversial matter. Further attempts should be made to clarify this problem in view of the important role played by the RES in bacterial defenses (6, 7).

On the basis of histological evidence, the macrophages of the RES have been found to be radioresistant (8, 9). Previous studies dealing with the physiological function of the RES in irradiated animals have followed three different approaches: (*a*) the ability of the RES to clear colloidal particles (10–14) or bacteria (17, 18) from the blood; (*b*) the relative distribution of phagocytized material, generally thorotrast in the liver and spleen (16); and (*c*) the capacity of macrophages harvested from the peritoneal cavity to digest phagocytized chicken red cells (19).

Experiments concerned only with the distribution of phagocytized material (16) among various organs provide only limited information on the phagocytic activity of the RES, since the primary change reported, a decrease of uptake by the spleen after heavy doses of x-ray, may be compensated by the phagocytic activity of Kupffer cells and may merely reflect the reduction in size of this organ.

Most authors investigating phagocytic function report that the clearance of colloidal particles from the blood by the RES is unaffected by whole body x-irradiation. However these studies were carried out with very small doses of radioactive colloidal tracers such as P^{32}-labelled chromium phosphate (12, 14) or radioactive gold (10, 11) which are cleared by the Kupffer cells very efficiently and very rapidly. The conclu-

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sions concerning the RES obtained with these techniques should be reevaluated in the light of recent studies of kinetics of phagocytosis of colloidal particles by the RES in relation to dosage which show that unless a sufficient amount of particles is injected the colloidal clearance test measures only liver blood flow (20–22). Phagocytic function of the RES can be explored only when a sufficient dose of particles is injected to challenge all the phagocytic cells. The rate of clearance for this dosage range varies inversely with the dose injected, $K \times D = Ct$ which is a characteristic behavior of substances cleared by the RES (21, 22).

Taplin and his associates, using "prodigiosin" (13), reported that heavy doses of x-irradiation affect to a small extent the clearance rate of this substance by the RES of rabbits.

Studies on the blood clearance of bacteria in irradiated animals showed that the capacity of the RES to clear bacteria is essentially the same as in normal animals (17, 18), but that subsequently increasing numbers of bacteria are released by the liver phagocytes (18), suggesting an inability of the Kupffer cells to destroy phagocytized microorganisms.

Recent experiments on the effect of antibody on the rate at which bacteria are cleared from the blood (23), and on the effect of a heat-stable serum factor presumed to be antibody (24) on the ability of macrophages to destroy bacteria suggest that alterations in bacterial clearance following x-irradiation should also be reevaluated in relationship to the immune response.

In the present study, an attempt has been made to explore by quantitative techniques the phagocytic and metabolic function of the RES in irradiated animals. Following x-irradiation with doses that killed more than half the animals, several lines of approach were followed: (*a*) the rate of clearance of carbon particles from the blood of rats and mice; (*b*) the ability of the RES to recover normal phagocytic activity after blockade (26); (*c*) stimulation of phagocytic function by the yeast extract zymosan (27); (*d*) the ability of the RES to clear P^{32}-labelled *E. coli* from the blood; and (*e*) the capacity of Kupffer cells to digest phagocytized denatured I^{131}-labelled proteins and release the label (22, 25).

Materials

Biological.—The experiments were performed on white male Carworth rats of the Nelson strain and white male Swiss Webster mice. Rats weighed between 150 and 200 gm.; mice between 20 and 30 gm. at the time of x-irradiation. In each case the animals received a dose of x-rays which was somewhat higher than an LD_{50} for the strain and time of the year: 850 r for the rats and 600 r for the mice.

In selected experiments, mice received higher doses, 800 to 1200 r, which were 100 per cent lethal within a week. The animals were exposed to x-rays in the unanesthetized state. Rats were kept in 4 plastic containers arranged at an equal distance from the center of the beam. Mice were x-rayed, in groups of eight, in a plastic container divided radially into eight compartments.

Radiation.—X-irradiation was delivered by a Picker x-ray unit which was run at 220 kvp 20 ma. and at a distance of 50 cm. S.T.D. Inherent filtration of the tube was: glass equivalent

0.25 mm. Cu, oil equivalent 1.0 mm. Al. Added filtration included 1.0 mm. Al and 0.5 mm. Cu H.V.L. at 48 to 50 cm. equivalent to 1.6 mm. Cu or 5 mm. Al.

The output measured before each radiation was delivered averaged about 60 r/minute in air. The total amount delivered was then controlled by time. The general dose was also metered by a Victoreen dosimeter set in the vicinity of the animals.

EXPERIMENTAL METHODS AND RESULTS

The Effect of X-Irradiation on the Phagocytic Activity of the RES.—The phagocytic activity of the RES was investigated by measuring the rate of clearance of carbon particles.

Carbon suspension C_{11}-1431a manufactured by Gunther Wagner, Hannover, Germany, containing carbon particles measuring about 250 A homogeneous in size, suspended in gelatin, was used in all the experiments. The stock solution was diluted to the proper concentration with a solution of gelatin as described to ensure stability (28). When such a suspension was injected intravenously, the carbon particles were cleared predominantly by the phagocytes of the liver and spleen according to an exponential function of the time: $\frac{\text{Log } C_1 - \text{Log } C_2}{T_2 - T_1} = K$, (Equation I), in which C_1 and C_2 were the carbon concentrations at time T_1 and T_2, and K (the phagocytic index) was a measure of the rate of phagocytosis of carbon particles by the RES for a given dose of carbon. K varied with the injected dose according to the relationship $K \times D$ = constant, in the range of dosage suitable to explore RES phagocytic activity.

8 mg. of carbon per 100 gm. of body weight was injected into rats and 16 mg. per 100 gm. into mice. The rate of clearance of carbon from the blood was measured by drawing 0.025 ml. blood samples from the retroorbital venous plexus with a calibrated glass pipette previously washed with heparin (29). The blood samples were lysed in 2 ml. of 0.1 per cent Na_2CO_3 and the concentration of carbon measured electrophotometrically at 675 mμ.

Usually 5 samples were sufficient to draw the clearance curve, from which phagocytic index K could be calculated using Equation I. The animals were sacrificed by decapitation and the liver and spleen weighed in the wet state. The ratio of the combined weights of the liver and spleen to body weight $\frac{W}{WLS}$ was calculated. In previous experiments it had been found that in rats and mice, the rate of clearance depended not only on the dose but also on the size of the liver and spleen (28). There is a third power relationship between K and the relative weights of the liver and spleen which allow the calculation of a corrected index $\alpha = \frac{W}{WLS}\sqrt[3]{K}$ measuring the phagocytic activity of the tissue itself, independent of variations in size of the liver and spleen.

The data presented in Table I show that the phagocytic activity of the RES towards carbon particles is not affected in rats by 850 r, a dose of radiation about 50 per cent lethal in our experimental conditions. Values of K and α measuring phagocytic activity are not significantly different from normal values in irradiated rats at any time from the 2nd to the 10th day after radiation.

In mice also (Table II) a dose of x-irradiation (600 r) calculated to be about 50 per cent lethal, did not alter the ability of the RES to clear colloidal particles from the blood, 7 days after radiation. However when 100 per cent lethal doses (800 to 1200 r) were administered, and the mice tested on the 2nd day because

of the early mortality, a significant, although not spectacular decrease of the phagocytic function of RES was observed.

Effect of X-Irradiation on the Recovery of Phagocytic Function after RES "Blockade".—Studies were also carried out on the ability of the RES of the rat to recover its phagocytic activity after the induction of a state of blockade by a large dose of carbon.

TABLE I

Effect of Whole Body X-Irradiation (850 r) on the Rate of Clearance of Carbon Particles by the RES, as Measured by the Phagocytic Indices K and α for a Dose of 8 Mg. of Carbon per 100 Gm. of Body Weight in the Rat

Days post radiation	K	$\frac{W}{WLS}$	α
2	0.016	22.4	5.6
	0.021	24.4	6.7
	0.020	25.1	6.8
	0.021	24.0	6.6
4	0.015	24.8	6.1
	0.014	24.2	5.8
	0.014	24.5	5.9
	0.015	20.4	5.1
	0.022	21.4	6.0
6	0.016	20.2	5.1
	0.047	17.0	6.1
	0.029	20.2	6.2
10	0.017	24.2	6.2
	0.020	21.6	5.8
	0.023	24.1	6.9
	0.015	20.6	5.1
Mean values	0.020	22.4	6.0
Mean values controls	0.018	24.5	6.4

Twenty-four hours after exposure to 850 r the rats were injected intravenously with either 16 or 48 mg. of carbon per 100 gm., amounts known to depress significantly the phagocytic function of the RES (21). A total of 17 x-rayed rats and 11 controls were examined. The rats were then allowed to recover for 4 days, a period known to be sufficient for complete recovery after the smaller blocking dose and for partial recovery after the higher blocking dose. The phagocytic function of the RES was investigated on the 4th day by measuring the rate of clearance from the blood of 8 mg. of carbon per 100 gm., (testing dose). The values of K, $\frac{W}{WLS}$, and α were calculated in each case.

While a dose of 850 r did not affect the ability of the RES in the rat to clear colloidal carbon from the blood this amount of x-irradiation effectively inter-

TABLE II

Effect of Whole Body X-Irradiation, 600 to 1200 r, on the Rate of Clearance of Carbon Particles by the RES as Measured by the Phagocytic Indices K and α for a Dose of 8 Mg of Carbon per 100 Gm. of Body Weight in the Mouse

	K	$\frac{W}{\sqrt[3]{WLS}}$	α	
16 controls	0.024 ± 0.010	15.0	4.3 ± 0.5	
X-irradiated 7 days after 600 r	0.024	15.1	4.3	Mortality 50 per cent in 30 days
	0.013	16.9	4.0	
	0.032	14.3	4.5	
	0.049	14.0	5.1	
Mean values per 600 r.....	0.030	15.1	4.5	
2 days after 800 r	0.010	19.0	4.1	Mortality 92 per cent 4th to 9th day
	0.012	13.5	3.1	
	0.015	16.5	4.1	
	0.013	17.5	4.1	
	0.016	17.5	4.4	
	0.012	14.5	3.3	
2 days after 1000 r	0.013	15.5	3.7	Mortality 100 per cent 4th to 6th day
	0.028	15.5	4.7	
	0.010	14.5	3.1	
	0.015	14.5	3.6	
	0.010	16.0	3.4	
2 days after 1200 r	0.017	14.5	3.7	Mortality 100 per cent 4th and 5th day
	0.041	14.0	4.9	
	0.013	13.5	3.2	
	0.037	10.0	3.3	
	0.014	14.5	3.5	
	0.011	15.0	3.3	
Mean values from 800 to 1200 r.....	0.017	15	3.7	

fered with the recovery of normal phagocytic function after a blocking dose of carbon (Table III). The effect was observed with both 16 or 48 mg. doses of carbon.

Effect of X-Irradiation on Stimulation of Phagocytic Function by Zymosan.— Previous experiments have shown that intravenous injections of zymosan into

mice cause considerable stimulation of the phagocytic activity of the RES (27) because of proliferation of the phagocytic cells. Control mice and irradiated mice (600 r, 5 days previously) were each injected with 1 mg. zymosan

TABLE III

Effect of Whole Body X-Irradiation (850 r) on the Recovery of the Phagocytic Activity of the RES in the Rat, following Blockade with 16 or 48 Mg. of Carbon per 100 Gm. Body Weight, Given 24 Hours after Radiation

Phagocytic indices K and α for the test dose of 8 mg. carbon per 100 gm., 4 days after the blocking dose.

	X-irradiated			Control		
	K	$\frac{W}{WLS}$	α	K	$\frac{W}{WLS}$	α
A. Blocking dose 16 mg. carbon per 100 gm.						
	0.006	21.3	3.8	0.026	18.5	5.5
	0.016	24.0	6.0	0.019	24.0	6.4
	0.0045	24.6	4.0	0.018	28.0	7.3
	0.007	31.0	5.9	0.018	21.0	5.5
	0.008	19.7	3.9	0.019	28.0	7.4
	0.030	20.5	6.4			
	0.012	19.0	4.4			
	0.008	20.3	4.0			
	0.007	23.0	4.4			
Mean values.....	0.011	22.6	4.8	0.020	23.9	6.4
B. Blocking dose 48 mg. per 100 Gm.						
	0.009	14.6	3.0	0.012	20.6	4.7
	0.006	19.6	3.5	0.012	19.8	4.5
	0.006	18.2	3.3	0.007	22.4	4.3
	0.006	18.6	3.3	0.007	20.0	3.8
	0.006	18.8	3.4	0.012	21.0	4.8
	0.008	20.2	4.0	0.013	23.0	5.4
	0.010	21.0	4.5			
	0.007	23.0	4.4			
Mean values.....	0.007	19.2	3.7	0.0105	21.1	4.6

intravenously (Standard Brands lot No. 7B-340) and 48 hours later the phagocytic activity of the RES was investigated using 16 mg. of carbon per 100 gm. The values of K, $\frac{W}{WLS}$, and α were calculated in each case. The data presented in Table IV establish that x-irradiation of mice with 600 r prevents the RES

from responding to an injection of zymosan with a stimulated phagocytic activity.

In normal mice, zymosan produces as much as a 10-fold increase in the clearance of carbon from the blood by the macrophages of the liver and spleen. It has been found to be due to the proliferation of the macrophages in these organs under the effect of zymosan (30). X-irradiation prevents this phenomenon through its inhibitory effect on the proliferation of the RE elements.

Effect of X-Irradiation on the Clearance of P^{32}-Labelled E. coli from the Blood.— Previous experiments with P^{32}-labelled *E. coli* (strain 0.111B4) have shown

TABLE IV

Effect of Whole Body X-Irradiation (600 r) on the Stimulating Action of Zymosan on the Phagocytic Activity of the Reticulo-Endothelial System in Mice, as Measured by the Phagocytic Indices K and α for the Dose of 16 Mg. Carbon per 100 Gm. 1 Week after X-Radiation

	Control mice						X-irradiated					
	Untreated			Zymosan 1 mg i.v. 48 hrs. previously			Untreated			Zymosan 1 mg i.v. 48 hrs. previously		
	K	$\frac{W}{WLS}$	α	K	$\frac{W}{WLS}$	α	K	$\frac{W}{WLS}$	α	K	$\frac{W}{WLS}$	α
	0.020	12.8	3.5	0.050	13.3	4.9	0.024	15.1	4.3	0.015	19.4	4.8
	0.016	15.5	3.9	0.039	13.9	4.6	0.013	16.9	4.0	0.005	14.4	2.5
	0.047	11.0	4.0	0.074	12.5	5.2	0.032	14.3	4.5	0.024	14.5	4.2
	0.038	12.3	4.1	0.172	14.2	7.9	0.049	14.0	5.1	0.022	15.1	4.2
				0.144	12.9	6.7				0.017	16.2	4.2
										0.014	15.4	3.7
										0.015	15.7	3.9
Mean values....	0.030	12.8	3.9	0.096	13.3	5.8	0.030	15.1	4.5	0.016	15.8	3.9

that these microorganisms, whether alive or heat-killed are removed from the blood of mice according to an exponential function of the time, until concentrations of about 10 per cent are reached, when the curve begins to change. As in experiments with carbon particles, the rate of clearance can be expressed as the slope of the clearance curve, K. The bacteria are phagocytized principally by the macrophages of the liver and spleen. The clearance of P^{32}-labelled *E. coli* in mice is slow and remarkably constant in control animals in the dosage used; it is independent of the number of bacteria injected in this dose range. The limiting factor is the level of opsonizing antibody in the blood, which controls the maximal efficiency with which these bacteria are taken up by the phagocytic system of the liver and spleen.

E. coli 0111 B_4 from Difco Laboratories was grown in the following medium:

Na_3 citrate	$3H_2O$	0.1 gm.
Mg SO_4	$7H_2O$	0.02 gm.
Glucose		0.4 gm.
Casamino acids		2.0 gm.
P^{32}-phosphate		1 mc.

Distilled water to make a total of 200 ml.

The *E. coli* was killed by heating at 60° for 20 minutes, washed, and suspended in saline. Heparin (0.1 ml., 100 U.S.P. units) was injected intravenously and 10 minutes later 2×10^8 *E. coli* per 20 gm. body weight were injected intravenously into 13 controls and 5 irradiated mice (600 r 8 days previously). The same doses of labelled bacteria were injected into controls and irradiated animals which had been previously immunized. Immunization was carried out by intraperitoneal injection of 3×10^8 cells, 4 days after radiation. Testing was done 8 days after immunization.

In order to determine whether or not the phagocytic mechanism in irradiated animals would remain unaffected when sufficient levels of opsonizing antibody was present, 2 irradiated mice were injected with 0.2 ml. of serum from immunized control mice before receiving the P^{32}-labelled *E. coli*.

The rate of clearance of P^{32}-labelled *E. coli* from the blood was measured as previously by drawing 0.05 ml. of blood with a calibrated glass pipette from the retroorbital venous plexus (29). The blood was then spread on circles of filter paper cemented on glass slides and the radioactivity measured with a Geiger-Müller counter keeping the geometry constant. When the level of radioactivity in the blood had reached about 10 per cent of the injected amount, the animals were sacrificed, the organs were digested in 10 per cent NaOH in a water bath and the radioactivity recovered in each organ was measured using aliquot samples.

The results of these experiments are presented in Fig. 1 and Tables V and VI.

The ability of the RES to clear *E. coli* from the blood was not significantly altered by 600 r of x-irradiation. Nearly the same rates of clearance were observed in control and irradiated mice, reflecting the same basic level of opsonizing antibodies and an intact clearance mechanism. However the total amount of radioactivity recovered in the spleen of irradiated animals is much smaller than in the spleen of control animals (Table VI). This can be explained by the differences in size of the organs. The spleen of the control mice had an average weight of 178 mg./20 gm., while the spleen of the x-irradiated mice weighed 56 mg./20 gm.

As expected the irradiated mice responded poorly to immunization. In control immunized mice, well opsonized *E. coli* are very rapidly cleared from the blood and removed almost exclusively by the liver (because of the greater capacity of this organ to clear very efficiently phagocytized material due to its greater blood supply). Irradiated immunized mice were not able to clear the bacteria as rapidly as the controls. The data indicate that this difference was due to a deficiency in antibody response and not to a deficiency of the phagocytic mechanism since passive transfer of serum from immunized controls increases significantly the rate of clearance of *E. coli* in irradiated mice.

Effect of X-Irradiation on the Metabolic Activity of the Kupffer Cells.—When heat-denatured proteins, trace-labelled with radioactive iodine (I^{131}) are in-

jected intravenously into mice, they are efficiently phagocytized by the RES principally by the Kupffer cells of the liver (22). The ingested protein is then broken down and iodide and diffusible products containing diiodotyrosine are

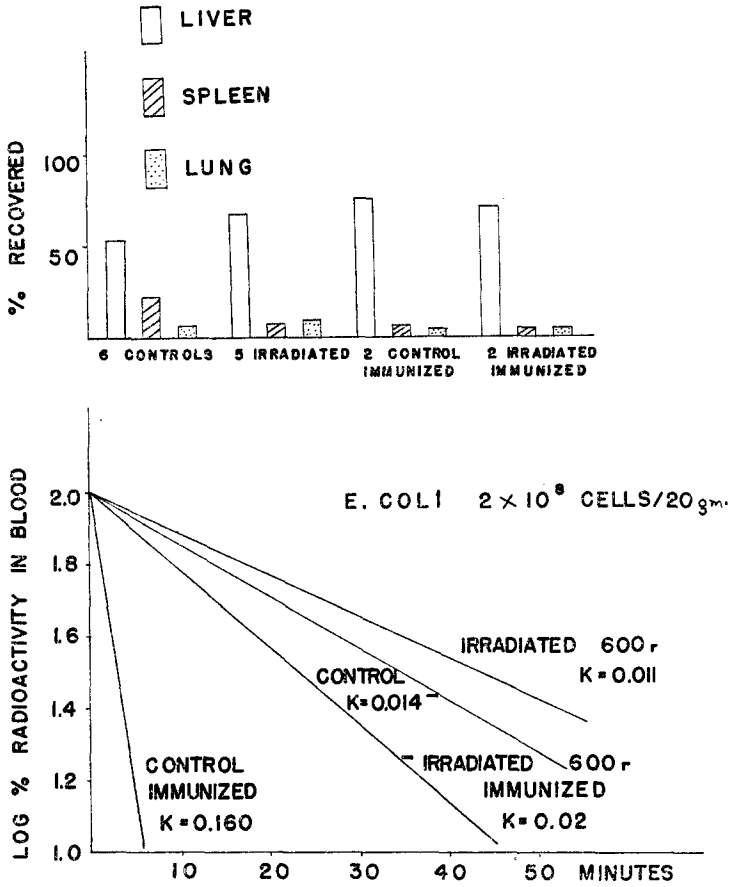

FIG. 1. Clearance from the blood and distribution in the various organs of P^{32}-labelled *E. coli* in normal mice, immunized normal mice, irradiated mice, and immunized irradiated mice.

eliminated from the liver. This method constitutes the basis for measuring an enzymatic function of the Kupffer cells, which is the result both of protein breakdown and deiodination. Using varying doses of a well characterized heat-denatured rabbit serum albumin containing about 6.4 per cent of iodine (C.A.[1] I^{131}). Biozzi *et al.* (25) have shown that the cellular enzymatic activity of the

[1] Complex albumin.

Kupffer cells obeys the laws of enzymatic kinetics. It is therefore possible to estimate accurately the amount of enzyme present in the cells.

In the experiments described here, the product used, C.A. I^{131}, was prepared from human serum albumin, heat-denatured according to the method described (22), and labelled with 3 per cent of iodine containing traces of I^{131}.

TABLE V

Effect of Whole Body X-Irradiation (600 r) on the Rate of Clearance of E. coli *from the Blood by the RES of Mice, as Measured by the Phagocytic Index K for the Dose of 2 × 10^8 Cells/20 Gm. Body Weight; 8 Days after Radiation*

Effect of previous immunization with 3 × 10^8 cells of *E. coli* 4 days after radiation and 8 days before test.

	Controls	X-irradiated	Control immunized	X-irradiated immunized	X-irradiated + 0.2 ml. serum from control immunized
K	13 animals 0.014±0.003	0.012 0.013 0.011 0.010 0.0105	0.124 0.160 0.146 0.210	0.014 0.054 0.011 0.018 0.011	0.065 0.074
Mean values.....	0.014	0.011	0.160	0.021	0.069

TABLE VI

Distribution of P^{32}-labelled E. coli—in the Organs of Control, X-Irradiated, Control Immunized and X-Irradiated Immunized Mice after Clearance of 2 × 10^8 Bacteria from the Blood

	6 control mice Per cent of injected dose	5 x-irradiated mice Per cent of injected dose	2 control immunized mice Per cent of injected dose	2 x-irradiated immunized mice Per cent of injected dose
Liver.....	54	68	76	72
Spleen.....	21	5	4	2
Lung.....	5	10	2	5
Kidney.....	1	2	1	1
Blood.....	15	14	10	10

The enzymatic capacity of the Kupffer cells, to break down the product was measured simultaneously in control animals and in irradiated mice having received 600 r 6 days previously. Control and irradiated mice were injected with 1 of 3 doses of C.A. I^{131} 0.1, 0.5, or 1.5 mg. per 20 gm. body weight.

The animals were sacrificed at varying times after the denatured labelled protein had disappeared from the blood; the radioactivity present in the liver was measured with a scintillation counter and compared with the amount injected. The amount of C.A. I^{131} broken down by the liver was labelled in micrograms of C.A. I^{131} metabolized in controls and irradiated

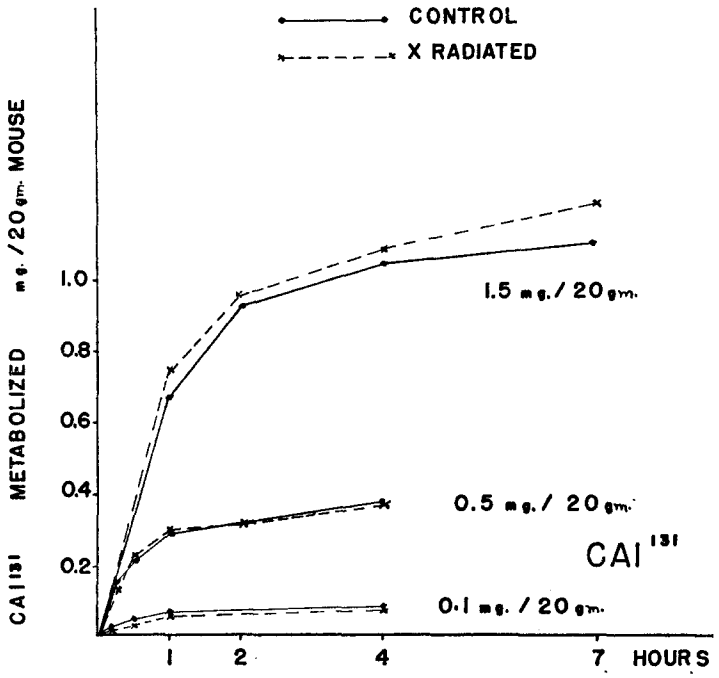

FIG. 2. Release of radioactivity from the liver Kupffer cells measuring enzymatic breakdown of heat denatured iodinated serum albumin (C.A. I^{131}) by cells in normal and irradiated mice (600 r 7 days previously).

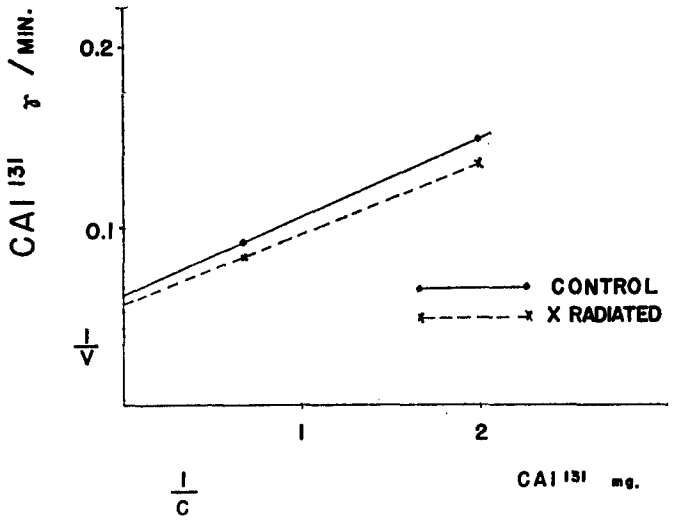

FIG. 3. Effect of x-irradiation, 600 r 7 days previously, on the speed of enzymatic breakdown of heat denatured iodinated serum albumin, C.A. I^{131}, by liver Kupffer cells.

mice. Then the maximum early speed of breakdown was related to the concentration of product in the cells by plotting $\frac{1}{V}$ against $\frac{1}{C}$. The intercept of the curves on the $\frac{1}{V}$ axis when $\frac{1}{C} = 0$, is directly related to the enzyme activity of the Kupffer cells.

The results of the experiments are presented in Figs. 2 and 3.

In Fig. 2, we see that for the various doses of C.A. I^{131} used ranging 0.1 to 1.5 mg./20 gm. body weight, the rate of release of radioactivity from the liver Kupffer cells was approximately the same in controls and irradiated mice. There was no evidence that exposure of mice to 600 r, 6 days earlier impaired the ability of the Kupffer cells to break down denatured protein enzymatically. From the data presented in Fig. 3 the maximum value of $\frac{1}{V}$ was calculated in both groups and found to be 16 to 17 μg. of protein per minute per 20 gm. mouse.

DISCUSSION

The ability of the reticulo-endothelial system to clear particulate matter from the blood remains unaffected in rats and mice exposed to a dose of x-irradiation about 50 per cent lethal. When a higher dose of irradiation was used (lethal to 100 per cent), a significant, though small decrease in phagocytic activity was observed. These findings are in agreement with the observations of Taplin *et al.* (13) in rabbits using 1000 r and of Biozzi *et al.* (31) in mice in the same dose range 1000 to 1200 r. However such a massive dose of x-ray markedly affects many other biological systems, and the animals are in a very poor general condition, a fact which raises some doubt as to the significance of this effect on the reticulo-endothelial system. In general these current studies of phagocytosis of colloidal particles confirm the earlier histological observations describing the phagocytic elements of the reticulo-endothelial system as radioresistant (8, 9).

Whereas an LD_{50} dose of irradiation does not affect the phagocytic activity of the RES, it does inhibit the recovery of its normal phagocytic function after blockade with carbon. Exposure to x-rays also suppresses the increase in phagocytic activity observed after the injection of zymosan in mice (27). These two phenomena, the return of normal phagocytic activity after blockade, and the stimulation of phagocytic function by zymosan have been shown to depend upon the proliferation of macrophage elements of the reticulo-endothelial system (26, 27, 30). It is not surprising therefore that these processes are susceptible to ionizing radiation. The ability of the RES to respond to stimulation by cellular proliferation, is a fundamental protective mechanism against infectious agents (6, 7) and the loss of the defense reaction following irradiation may in part account for the greater susceptibility to infection of irradiated animals.

The ability of the RES to phagocytize *E. coli* was found to be unaffected by an LD_{50} dose of x-irradiation. These results are in agreement with the data reported by previous investigators who showed that the RES of irradiated animals is equally able to clear bacteria from the blood as effectively as normal animals (17, 18). The phagocytosis of P^{32} labelled *E. coli* was studied in mice, because the rate of clearance of these organisms from the blood depends in normal mice upon the level of antibody in the serum as its rate-limiting factor (23). We have observed that these bacteria are cleared from the blood at essentially the same rate in control and irradiated mice. The amount removed by the spleen of irradiated mice, however, is much smaller than that removed by control spleens. However the deficiency is largely made up by the Kupffer cells. These results can be explained on the basis of the difference in size of the irradiated and normal spleens. It must be stressed also that, as far as *E. coli* are concerned, the failure of irradiated animals to produce adequate amounts of antibody is reflected in the inability of x-irradiated, immunized mice to efficiently clear the blood of bacteria inadequately opsonized by antibody, in spite of adequate phagocytic function of the RES. When antibodies are passively transferred into these mice, the bacteria are then phagocytized as rapidly by the RES as in normal immunized mice. The impaired immunological response can also explain in part the inability of the RES to deal adequately with phagocytized organisms (18), especially in view of the observation by Rowley (24) that a heat-stable factor, presumed to be specific antibody, is required for a normal macrophage to be able to destroy phagocytized bacteria.

In the course of these studies the ability of the Kupffer cells to ingest and metabolize heat-denatured albumin was investigated in normal and irradiated mice by a technique which estimates the concentration of the intracellular enzymes involved. It is of interest to note that the results obtained in our control mice are almost identical with those originally reported by Biozzi *et al.* (25), confirming the accuracy of the technique. Since x-irradiation with an LD_{50} dose does not seem to affect the rate of enzymatic breakdown of denatured serum albumin by Kupffer cells, it can be assumed that x-rays do not affect in these cells, the level of enzymes involved in the process.

These findings may appear at variance with the results of Donaldson *et al.* (19) who reported that macrophages obtained from peritoneal exudates in irradiated animals failed to digest phagocytized chicken erythrocytes. However different phagocytic cells (Kupffer cells) and possibly a different set of enzymes systems were investigated in our study.

SUMMARY

The effect of moderately high doses of x-irradiation upon the reticulo-endothelial system of mice and rats was investigated.

A dosage of 600 r to mice and 850 r to rats did not interfere with the ability

of the RES to clear colloidal particles from the blood. However the 850 r x-ray dose to rats prevented recovery of normal phagocytic function after "blockade."

In mice, 600 r interfered with the ability of the RES to respond to the usual stimulating effect of zymosan.

The ability of the RES of mice which have received 600 r of x-rays to clear P^{32}-labelled *E. coli* from the blood was not significantly altered. These mice responded poorly to immunization, as demonstrated by the slow rate of clearance of the bacteria from the blood of immunized, irradiated individuals as compared with that of immunized controls. This reflected the lowered antibody production and not deficiency of phagocytic mechanism.

There was no evidence of a changed capacity of the Kupffer cells of mice which had received 600 r of x-rays to break down denatured protein enzymatically.

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