Electrophoretic Patterns from X-irradiated Blood Serums. (29369)

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Muntz *et al*(1) reported that electrophoretic analyses of serums from dogs given 350 r of ionizing rays revealed diminished albumin levels beginning 7 days after irradiation until the dogs died. Three to 5 days before death, there was a striking increase in the alpha and beta globulins. Dogs treated with sublethal doses of X-rays (200-250 r) showed only slight changes in their plasma protein patterns beginning 14 days after irradiation until they had recovered. Later studies(2,3, 4) further established that whole body irradiation of rabbits, monkeys, and mice results in definite protein shifts. Kepp and Michel(5) called attention to increases in the alpha and beta globulins when human serum proteins were separated electrophoretically after *in vitro* exposure of the serum to 10^5 r.

In this report the comparative changes in electrophoretic distribution of proteins in serums from rats, rabbits, and man after *in vitro* exposure to large doses of ionizing rays, and in sera from rabbits and man after *in vivo* exposure to lethal and sublethal doses of ionizing rays are described.

Materials and methods. Rat, rabbit, and human sera were obtained as follows: Six-month Sprague-Dawley rats, fed on Rockland Lab Diet, were exsanguinated by cardiac puncture under nembutal anesthesia. Four-to-six month New Zealand albino rabbits fed Aubrey rabbit pellet diet were bled from the marginal ear vein. Normal human males were bled by venipuncture. After allowing these various blood samples to clot at 5°C, the sera were collected by centrifuging and decanting. Human plasmas were from oxalated blood collected from normal males. The human sera (but not the human plasmas) were heated at 56°C for 30 minutes for use in other serological tests. All samples were stored at −15°C and were thawed when needed.

Total protein determinations and paper electrophoresis were generally carried out on the samples within 4 hours after exposure to radiation. At times, however, the sera were maintained frozen for as long as 2 weeks after irradiation before being tested. The electrophoretic and total protein results were similar when the same serum was tested immediately after exposure to X-rays or after being maintained frozen for an interval of 2 weeks subsequent to irradiation. Except for changes in total protein, human sera showed no differences in the electrophoretic distribution of proteins after heating at 56°C for 30 minutes.

Complete details of the paper electrophoresis method may be found in (6).

Total protein determinations were made by the biuret method(7). The standard was crystalline bovine albumin prepared by Armour.

One ml samples from 8 different pools of rat sera (4-6 rats per pool) were exposed to X-rays. The sera were contained in round open glass planchets (1.5 × 2 cm) and each sample was removed after exposure to the desired dose. Sera from 11 individual rabbits and from 8 humans were treated similarly.

X-rays were delivered by a Maxitron unit operated at 250 kvp, 30 ma, 4.7 mm Be inherent filtration. Dose rate measurements were made using chemical dosimetry methods under identical experimental conditions. A dose rate of 16,900 r/min was determined by measuring spectrophotometrically the reduction of ceric sulfate solution(8).

For comparative purposes 4 rabbits were irradiated individually in revolving Lucite boxes (12″ × 5″ × 5″) with 750 r to 1000 r delivered by a 1000 Curie Co source. The half value layer for this radiation was 12 mm of lead. The dose rate was measured with a 100 r Victoreen chamber inserted into a paraffin phantom within the Lucite box: 52 r/min were given at a source to a mid-line distance of 51.0 cm. In addition, X-ray doses of 2000 r to 5000 r were given to 18-

TABLE I. Results of 11 Rabbit Sera (A) Separated into Protein Fractions by Electrophoresis After 6 *in vitro* Doses of X Rays, Along with Total Protein Values, and (B) Treated by Analyses of Variance. Two groups of control rabbit sera (11 in each group) were not X rayed.

Series	X ray dose in r	Albumin	Globulins α_1	Globulins α_2	Globulins β	Globulins γ	Total protein in g/100 ml serum
			A. Mean % values				
9	$10^{6.07}$	54.5	8.1	6.8	14.0*	16.5	7.1
10	$10^{6.10}$	50.4*	9.9*	8.0	14.4*	17.3	7.6
11	$10^{6.18}$	51.8*	9.4*	9.4*	15.3*	14.1*	7.1
12	$10^{6.30}$	47.1*	11.3*	10.9*	17.2*	13.6*	6.8
13	$10^{6.40}$	45.5*	11.3*	11.9*	18.7*	12.6*	7.2
14	$10^{6.48}$	43.0*	13.5*	11.7*	20.8*	11.1*	7.7
C15	—	57.9	6.9	6.3	11.2	17.8	7.7
C16	—	56.9	6.8	6.4	10.9	19.1	7.1
			B. Analyses of variance				
Source	D.F.	F ratio‡					
Dose levels	7	20.5† *321*	12.3† *60*	11.0† *62*	12.5† *132*	6.1 *89*	.6 *1*
Subjects	10	20.0† *313*	3.5† *17*	4.6† *26*	9.0† *95*	9.5† *139*	15.7† *31*
Interaction	70	*16*	*5*	*6*	*11*	*15*	*2*

* and † indicate .05 and .01 probability levels, respectively. D.F. = degrees of freedom.
‡ Mean square values are shown in italics.

week rabbits restrained as for the Co^{60} source. The machine was operated at 250 kvp, 30 ma, 4.7 mm Be inherent filtration with a 0.5 mm Cu filter added. Dose rates were measured as for the Co^{60} source and 90 r/min were given at a mid-line distance of 60 cm. At various times thereafter the animals were bled and the sera were separated electrophoretically.

Serum was obtained from one human subject who was accidentally exposed to a massive supralethal dose of radioactivity(9). Fig. 1F shows the electrophoretic separation of this serum which was collected 30 hours after exposure.

Statistical method. Rat, rabbit, and human sera and human plasmas were irradiated *in vitro* at 6 doses from 0.51×10^6 to 3.04×10^6 r. These doses consisted of $10^{6.07}$, $10^{6.1}$, $10^{6.18}$, $10^{6.30}$, $10^{6.40}$ and $10^{6.48}$ r, respectively. After irradiation the sera or plasmas were separated electrophoretically into albumin, alpha$_1$, alpha$_2$, beta and gamma globulin fractions as shown in Fig. 1. Because the rat sera were indistinctly separated at the higher dose levels, it was necessary to use combined percentages for the alpha$_1$ and alpha$_2$ components, and the beta and gamma globulins. Percentage distribution of proteins in irradiated and unirradiated serums were examined by an analysis of variance. This procedure determined whether the relative concentration of any given protein fraction was significantly changed by irradiation. When significant changes were apparent, the test of "critical differences"(10) was applied to determine at which dose level they occurred. For this study, the interest was in differences between each of the control groups means and the means of the irradiated groups. When the mean of any irradiated group differed by more than "d" from both of the control group means, then the mean of the irradiated group was marked by an asterisk to indicate significance.

Fig. 1 shows the results for the rabbit sera in complete form and indicates the method of data treatment for the other species of sera studied.

In vitro *irradiation.* Significant changes in the electrophoretic distribution of proteins in rabbit sera occurred in the albumin fraction and in all of the globulin fractions (Table I). The significant "critical differences" shown in Table IA further indicate that the beta globulin was altered by expo-

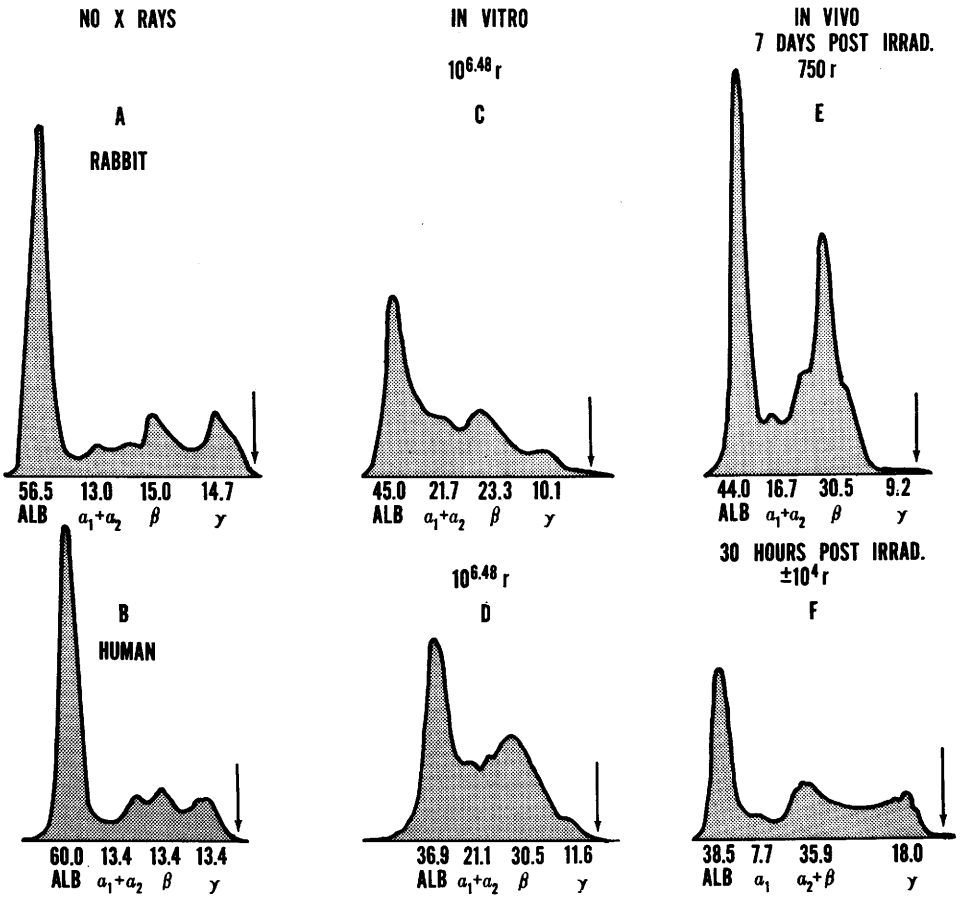

FIG. 1. Per cent total protein of albumin and various globulins after electrophoretic separation of non-irradiated and irradiated rabbit and human sera. Arrows indicate starting point of electrophorized sera. Note that, as compared to non-irradiated sera (A and B), sera irradiated *in vitro* with $<10^6$ r (C and D) or from subjects given total body irradiation (E and F) in general possessed a lower proportion of gamma globulin and albumin and a higher proportion of alpha and beta globulins.

sure to $10^{6.07}$ r, that the albumin fraction and the alpha$_1$ globulin were altered by exposure to $10^{6.1}$ and that the alpha$_2$ and gamma globulins were not altered until exposed to $10^{6.18}$ r.

Compared to the rat sera, the rabbit sera were differently affected. As irradiation was increased, the beta and gamma globulins were significantly altered sooner than the albumin fraction. The alpha$_1$ and alpha$_2$ fractions could not be compared as they were not separated for the rat sera. The total protein remained unchanged for both kinds of sera.

Rat blood sera showed significant changes in the albumin fraction and in the combined alpha globulins early in the irradiation series, at $10^{6.1}$ and $10^{6.07}$ r, respectively, whereas a significant change for the combined beta and gamma globulins did not appear until much later, *i.e.*, at $10^{6.48}$ r. No significant changes occurred in total protein values between dose levels, although there was an initial significant difference between the rat sera before they were irradiated.

Although there were differences in levels of the albumin and alpha$_2$ components, as well as in total protein values between individual samples of human blood plasmas and sera before irradiation, the mean values indicated that increases in irradiation affected all fractions of the human sera. In addition,

the changes resembled those shown for the rabbit sera for the albumin fraction and the $alpha_1$ and $alpha_2$ globulins, but appeared after a higher irradiation ($10^{6.3}$ r) for the beta and gamma globulins.

The human plasmas and the human sera showed the first X-ray change at the $10^{6.1}$ level in the $alpha_1$ globulin and at the $10^{6.3}$ level in the gamma globulin. The plasma samples were affected later than the serum samples in the albumin and $alpha_2$ globulin fractions and sooner for the beta globulin.

Rabbit irradiation. The albumin and gamma globulin concentrations decreased with a corresponding increase in the alpha and beta components following irradiation of rabbits. Such changes, after a sublethal dose of 750 r occurred in 2 days in one rabbit, in 3 days in 2 rabbits and in 7 days in a fourth rabbit. This last pattern is shown in Fig. 1E. Similar changes occurred earlier in the sera of rabbits given total body irradiation with 1000 r, *i.e.,* at one day after irradiation. In general, the 4 animals irradiated with 2000 or 5000 r showed changes similar to Fig. 1E when tested at 20 hours.

Discussion. The data presented here are in agreement with the well established concept that shifts in electrophoretic patterns of blood serum follow lethal and sublethal doses of ionizing radiation delivered *in vivo*. The observations illustrated in Fig. 1 are particularly interesting. They show that, other than differences in magnitude, the patterns produced by sera after *in vivo* irradiation parallel those produced by sera after *in vitro* irradiation. In both categories there is a marked decrease in the albumin and gamma globulin concentrations with a compensatory increase in the alpha and beta components. It is likely that alteration of proteins originally in the albumin and gamma globulin region results in these molecules becoming electrophoretically indistinguishable from the alpha and beta components. It is significant that sublethal doses are sufficient to do this *in vivo* and that at least $10^{6.07}$ r are required to produce similar electrophoretic shifts by *in vitro* irradiation. The *in vitro* changes, by massive doses, are immediate and of sufficient magnitude to be readily discernible by electrophoretic methods.

One human subject exposed to a massive dose(9) showed protein shifts within 30 hours after exposure (Fig. 1F). It is likely that in humans, as well as rabbits, the time at which protein shifts appear is dependent on dose, since rabbits given massive irradiation demonstrated protein shifts earlier than those given sublethal doses. These observations indicate that perhaps a disruption of certain functions governing protein synthesis may be a predisposing factor. Immediate alterations in the molecular structure of serum proteins probably occur as a result of direct action during *in vivo* irradiation. However, the amount of protein denatured is not sufficient to be detected by electrophoresis. Recently, Luzzio(6) showed the antigenic specificity of radiation altered proteins and the potentialities of detecting such proteins *in vivo* and *in vitro* with specific sera.

It was found that at least $10^{6.07}$ r were necessary to affect a protein shift in any of the sera tested. $Alpha_1$ globulin was the most uniformly affected. It was significantly altered by $10^{6.1}$ and by all larger doses. The albumin fraction was altered for all 3 groups at the $10^{6.1}$ or $10^{6.18}$ r levels while the gamma globulin was altered for all 3 groups at higher doses, *i.e.,* at $10^{6.18}$ or $10^{6.3}$ r. The $alpha_2$ and beta globulins showed the greatest differences among the 3 kinds of sera. Thus, the beta globulin was altered at the lowest dose for rabbit serum, at the third dose for human plasma and at the fourth dose for human serum, whereas the $alpha_2$ globulin was affected at the third dose for both sera, but only at the fifth dose for human plasma.

Table II shows the consistant decrease in albumin fractions and in gamma globulins and the consistent increase in alpha and beta globulins which occurred in all cases with increased doses of X-radiation. Moreover, the qualitative changes in the various sera were comparable, although as noted previously there were marked quantitative differences between sera irradiated at the same dose levels. The observations summarized in Table II also show that, after a specific dose level,

TABLE II. % Change* from Control Values in Protein Fractions Separated by Electrophoresis of Rat and Rabbit Sera, and Human Sera and Plasmas Treated *in vitro* with 6 Doses of X Rays.

X ray dose in r	Albumin				Globulins													
					Alpha$_1$				Alpha$_2$				Beta				Gamma	
	Rat	Rabbit	Human serum	Human plasma	Rat†	Rabbit	Human serum	Human plasma	Rat†	Rabbit	Human serum	Human plasma	Rat‡	Rabbit	Human serum	Human plasma	Rat‡	Rabbit	Human serum	Human plasma
$10^{6.07}$	*19*	*6*	*5*	*4*	32	18	12	18		9	1	17	8	25	10	13		*7*	*2*	*5*
$10^{6.10}$	*22*	*13*	*7*	*7*	46	43	41	56		29	1	34	4	28	18	20		*3*	*5*	*13*
$10^{6.18}$	*37*	*10*	*16*	*11*	79	37	60	83		51	20	36	5	36	21	35		*21*	*17*	*16*
$10^{6.30}$	*42*	*19*	*18*	*16*	88	64	64	124		74	24	46	7	54	37	48		*24*	*18*	*21*
$10^{6.40}$	*48*	*21*	*24*	*23*	122	64	69	139		91	49	85	5	67	38	63		*29*	*33*	*26*
$10^{6.48}$	*50*	*26*	*31*	*26*	144	96	95	159		87	50	90	14	86	52	97		*38*	*31*	*38*

* Italic type = negative change; Roman type = positive change.
† Alpha$_1$ and alpha$_2$ globulins together in alpha$_1$ globulin category.
‡ Beta and gamma globulins together in beta globulin category.

decreases in albumin and in gamma globulin for all sera are accompanied by corresponding increases in alpha and beta proteins. The degree of shift in each fraction is apparently a function of dose since the magnitude of the change increases as the X-ray dose is increased. Furthermore, species is a determining factor, as indicated by the greater drop in albumin in the sera of the rat than in the sera of other species after exposure to the same dose of X-radiation (Table II).

Summary. Rat, rabbit, and human blood sera, when analyzed by paper electrophoresis, showed marked changes after they had been irradiated *in vitro* at 6 dose levels from $10^{6.07}$ to $10^{6.48}$ r. In general, as the dose of X-radiation was increased, the percent of albumin and gamma globulin in the total protein decreased as alpha and beta globulins increased. It is suggested that perhaps albumin and gamma globulin molecules are so altered by irradiation that they become electrophoretically indistinguishable from alpha and beta proteins. Similar changes occurred 2 to 7 days after irradiation when rabbits were given doses of 750 r to 5000 r. Differences between types of sera as a result of *in vitro* irradiation were (1) less pronounced increases in the alpha$_2$ and beta globulins for the human serum than for the others, (2) a more marked increase in the alpha$_1$ globulin of human plasma than for the other sera tested, (3) a more marked decrease in the albumin fraction of the rat serum than of the other sera tested.

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Effect of Blood Volume Changes on Renin-Like Activity in Blood. (29370)

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Although renin secretion is said to be increased in shock(3,4,7,10), no exact data have been presented either on the degree or onset of the rise. It was assumed that, by its effect on the blood pressure, renin might counteract severe hypotension(10). On the other hand, despite various hypotheses(13, 14) the mechanism of renin release is still not defined. Employing isovolemic cross circulation in the rat, with a nephrectomized animal highly sensitive to renin as indicator, we found a very constant concentration of a renin-like substance in the blood of intact animals(6,11). This method is suitable to determine variations in the concentration of renin-like activity in blood under various experimental conditions. To obtain more precise information on a possible role of the renin-angiotensin system in the regulation of homeostasis we investigated the effects of changes in the intravascular volume on renin secretion in rats. The present paper reports on the influence of acute blood loss and of forced blood transfusion on the concentration of renin-like substance in blood as well as on the renin content of the kidneys.

Material and methods. Isovolemic cross circulation between the donor rat and the indicator animal, nephrectomized about 20 hours previously, was established through the femoral vessels according to a previously described technique(6,11).

a. *Hemorrhage experiments.* White male rats of average weight 260 g were anesthetized with urethane (1.4 g/kg body weight). Blood pressure was recorded in the left carotid artery by means of a mercury manometer. After connection of the femoral vessels with those of the nephrectomized indicator animal but before starting cross circulation, 5 ml of blood were taken from the femoral artery of the donor rat within a 5-minute period. Blood pressure fell rapidly to values between 20 and 30 mm Hg but increased gradually during 10 minutes up to 30 to 40 mm Hg. Ten to 15 minutes after bleeding, cross circulation was established for 10 minutes. Following disconnection of the animals, the blood pressure of the indicator rat was recorded for another 10 to 15 minutes in order to see whether it remained elevated as occurs in nephrectomized rats after injection of renin. At the end of the experiment the donor animals were killed by intravenous injection of air, the kidneys were removed, and their renin content was determined as described previously(2).

In another series of experiments the donor rats were nephrectomized 5 to 10 minutes before bleeding. From these animals also, 5 ml of blood were removed and then cross circulation installed. Since, under these conditions, blood pressure recovered more slowly, cross-circulation flow in the femoral artery was less than half (0.8 ml/min) of that in the non-nephrectomized rats (2.0 ml/min). Earlier experiments had demonstrated that such a reduction of flow rate does not influence the concentration of renin-like substance in blood(11). Altogether 25 to 30 minutes elapsed between nephrectomy and the start of cross circulation.

b. *Overtransfusion experiments.* White male rats of average weight 250 g received 3 infusions of 4 ml each of heparinized (0.3 ml of a 0.5% heparin solution) rat blood which immediately before had been drawn from the carotid artery of a male rat, anes-