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Effect of Properdin on Whole Body Irradiated Mice and Rats.* (24492)

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The parenteral administration of properdin of heterologous origin into rats and mice has been reported to protect significantly against $LD_{100}/30$ day and $LD_{100}/6$ day doses of whole body x-irradiation(1,2). Degree of protection was reported to be dependent on dose, route, and time of administration of properdin. Ross(2) observed that mice exposed to 600 r and treated with 50 units of partially purified bovine properdin administered intravenously showed a 4-fold greater 30 day survival rate than the untreated controls. However, mice treated with properdin on the second, third, fourth, seventh, or tenth postirradiation days showed similar or accelerated death rates as compared to the controls. In order to further assess the role of properdin in protection of animals against the effects of whole body x-irradiation, experiments were conducted to determine the value of properdin as a therapeutic agent.

Materials and methods. Adult albino mice and adult albino rats (Sprague-Dawley) were randomly arranged into groups of varying numbers according to the design of each experiment. Dosage of x-rays administered for each experiment is given in the section on results. The x-irradiation was administered from above the animals by means of a Westinghouse Quadrocondex x-ray machine. The technical factors were: 250 KVP; 15 ma, 1 mm aluminum and 0.5 mm copper filters in addition to inherent filtration of 2.5 mm aluminimum and 0.25 mm copper; size of field, whole-body; target distance, 16 inches. The mice were irradiated in groups of 10 in a cylindrical container having a diameter of 18.5 cm and a height of 3 cm. The rats were irradiated in groups of 4 in a cylindrical container having a diameter of 23 cm and a height of 5.5 cm. Average dose rate as measured with a Victoreen r meter was approximately 90r per minute when recorded in the center of a lifelike wax phantom. Variation in radiation dose did not exceed 2r per minute when measured in any position in the container. Properdin solutions were prepared from fresh bovine serum according to the zymosan adsorption technic of Pillemer *et al.*(3) and also by a modified Cohn ethanol fractionation procedure. In addition, purified human properdin was used in the experiments.† The titers of the partially purified properdin (PPP) solutions and the human properdin (HP) were determined by the zymosan assay technic described by Pillemer *et al.*(3).‡ Route and time of administration of the properdin solutions is given in each experimental design.

Results. In the first experiment, 5 randomly selected groups of mice were exposed to a single 550 r total body dose of x-irradiation. Animals of the first group of 11 mice were in-

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‡ Original RP and R3 assay reagents were kindly supplied by the late Dr. Louis Pillemer and Mr. Earl W. Todd of the Inst. of Pathology, Western Reserve Univ., Cleveland, O. Portions of reagents were used to standardize subsequent assay reagents prepared in this laboratory.

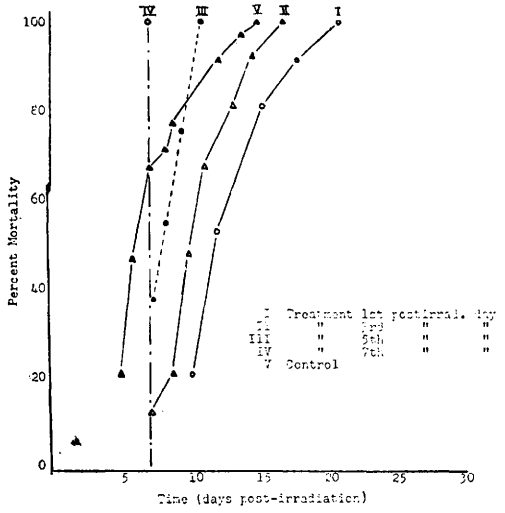

FIG. 1. Mortality of adult albino mice treated intrav. with 50 units of partially purified bovine properdin (PPP) following 550 r total body x-irradiation.

jected intravenously with 50 units of PPP on the first postirradiation day. The next 3 groups of 11 mice each were treated with 50 units of intravenously administered PPP on the third, fifth and seventh postirradiation days, respectively. The fifth group of 40 mice was used as untreated controls. The mortality curves following this irradiation and treatment procedure are shown in Fig. 1. It can be seen that the intravenous injection of PPP did not afford protection to the x-irradiated mice regardless of time of administration. However, there appeared to be a trend toward delayed mortality if treatment was initiated early following total body x-irradiation.

In another experiment, the first group of 10 mice each received 50 units of HP intravenously on first postirradiation day following a single exposure to 550 r of total body x-irradiation. A similar number of mice received the same treatment on third postirradiation day and a third group of 10 mice each received 50 units of HP intravenously on fifth postirradiation day. A fourth group of 33 mice were used as untreated controls. The mortality ratios are shown in Table I. It can be seen that postirradiation treatment with 50 units of HP administered intravenously did not yield protection to the whole body x-irradiated mice regardless of time of administration.

TABLE 1. Mortality of Adult Albino Mice Treated Intravenously with 50 Units of Purified Human Properdin (HP) following 550 r Total Body X-irradiation.

Treatment	Treatment day (PID)*	No. of mice	Final mortality %	Day
HP	1	10	100	12
"	3	10	"	9
"	5	10	"	9
Control		33	"	15

* PID, postirradiation day.

It is difficult to obtain properdin with high activity, that is, high unitage/unit volume. To increase host systemic concentration of properdin, it was suggested that intraperitoneal injection of large doses of properdin solution would enable the host to absorb more properdin than could be administered by intravenous route. This increased level of properdin might then be effective in protecting against the effects of whole body x-irradiation. To test this hypothesis, mice were subjected to 600 r of whole body x-irradiation and randomly separated into 5 groups. The first group of 20 mice received 200 units of PPP intraperitoneally on the third post-irradiation day; the second and third groups of mice (20 mice/group) each received 200 units of PPP injected intraperitoneally on fifth and seventh postirradiation days respectively. The fourth group of 20 mice received a total of 600 units of PPP administered intraperitoneally at a dosage of 200 units/day given on third, fourth and fifth postirradiation days. The 25 mice of fifth group were held as controls. The mortality curves of this irradiation and treatment experiment are shown in Fig. 2. The results were that properdin was not effective as a therapeutic agent against x-irradiation even in concentrations as high as 600 units when given by intraperitoneal route, regardless of time of administration.

Various investigators have reported that mouse serum is deficient in complement components(4-6). The lack of bactericidal activity of mouse serum reported by Marcus, *et al.*(7) could be explained as a result of deficient complement components. Since the properdin system has been reported by Pille-

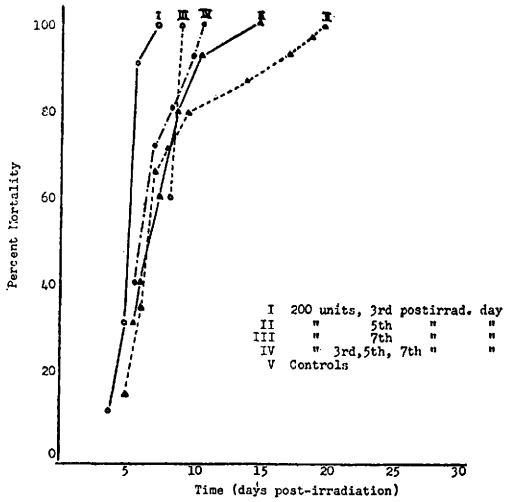

FIG. 2. Mortality of adult albino mice treated intraper. with partially purified bovine properdin (PPP) following 600 r total body x-irradiation.

mer *et al.*,(8) to require the 4 components of complement and Mg^{++} for its activation, failure of properdin to protect mice against effects of whole body x-irradiation might be explained on this basis. An experiment was carried out to determine if the properdin system could be activated *in vivo* in the mouse by concomitant injection of guinea pig complement with properdin. Groups of mice were subjected to 500 r of total body x-irradiation and treated with 200 units of PPP and 33 units of guinea pig complement (GPC'). The mixture was administered intraperitoneally rather than intravenously since a total volume of 1.1 ml was injected. The results are shown in Table II. The x-irradiated mice were not protected even though all components of complement were present. Mortality rates of treated mice were, in all cases, accelerated as compared to mortality rates of the control group. GPC' was not toxic in the concentrations employed.

The hypothesis was proposed that mouse serum inactivates guinea pig complement *in vivo*. The failure of properdin to protect x-irradiated mice might be explained on this basis. It was reported that x-irradiation did not affect rabbit complement titers(9,10), and Ross(2) reported that complement component titers of the rat are not affected by x-irradiation. In other experiments, therefore, rats were used.

TABLE II. Mortality of Adult Albino Mice Treated Intraperitoneally with 200 Units of Partially Purified Bovine Properdin (PPP) and 33 Units of Guinea Pig Complement (GPC') following 550 r Total Body X-irradiation.

Treatment	Treatment day (PID)*	No. of mice	Final mortality %	Final mortality Day (PID)
PPP + GPC'	1	10	100	8
GPC'	1	5	"	14
PPP + GPC'	3	10	"	9
GPC'	3	5	80	30
PPP + GPC'	5	10	100	10
GPC'	5	5	"	9
PPP + GPC'	7	10	"	8
GPC'	7	5	"	10
Control		20	"	13

* PID, postirradiation day.

Groups of rats were subjected to 720 r of whole body x-irradiation and randomly divided into 7 groups. The first group of 15 rats received 200 units of PPP injected intraperitoneally on the third postirradiation day. The next two groups of 15 rats per group each received 200 units of PPP intraperitoneally on the fifth and seventh postirradiation days, respectively. The fourth group of 15 rats received a total of 1000 units of PPP intraperitoneally at a dosage rate of 200 units/day for 5 consecutive days starting with third postirradiation day. The fifth group of 15 rats received a total of 500 units of PPP administered intraperitoneally at 100 units/day for 5 consecutive days starting with third postirradiation day. The sixth group of 14 rats received a total of 250 units of PPP intraperitoneally injected at 50 units/day for 5 consecutive days commencing with third postirradiation day. The seventh group of 20 rats was untreated and held as controls. The results of this experiment are shown in Table III. It can be seen that properdin did not protect rats from the effects of whole body x-irradiation, even in doses as high as 1000 units when administered by intraperitoneal route. Mortality rates of groups receiving multiple injections were observed to be similar to or slightly accelerated compared to the control group and groups receiving a single treatment. It is also observed that a trend toward delayed mortality appears to occur if a single injection of properdin is given to rats early in treatment following whole body x-irradiation.

TABLE III. Mortality of Adult Albino Rats Treated Intraperitoneally with Partially Purified Bovine Properdin (PPP) following 720 r Total Body X-irradiation.

Treatment PPP (units)	Treatment day (PID)*	No. of rats	Final mortality %	Day
200	3	15	87	30
200	5	"	100	11
200	7	"	"	11
200	3, 4, 5, 6, 7	"	"	12
100	"	"	"	11
50	"	14	"	11
Control		20	"	15

* PID, postirradiation day.

Discussion. It has been reported by Ross (2) that properdin levels in rats and mice decrease to undetectable levels following whole body x-irradiation. Linder(11) reported that properdin levels of 500r x-irradiated rats falls to 52% of normal on third postirradiation day and reaches a minimum of 34% of normal by eighth postirradiation day. The properdin level begins to rise and reaches 60% of normal on thirteenth postirradiation day. We observed that properdin levels of 720r x-irradiated rats fell rapidly and reached undetectable levels between fifth and seventh postirradiation days. Furthermore, properdin levels of 500r x-irradiated mice rapidly decreased to 10% of normal levels by fifth postirradiation day and remained undetectable until all animals died on fifteenth postirradiation day.

If properdin has therapeutic value for whole body x-irradiated animals, it seems apparent that treatment should be given at a time when the host endogenous levels are at a minimum. In all our experiments, the time of administration of exogenous properdin was varied to include postirradiation time during which some endogenous properdin is still present (first postirradiation day) to when undetectable levels are found (fifth to seventh postirradiation days). It was found that properdin did not afford any protection to the whole body x-irradiated animals in doses as high as 600 units given intraperitoneally for mice and 1000 units by this route for rats. It was observed, however, that time of administration of exogenous properdin appears to be an important factor. Trends toward delayed mortality were observed to occur if treatment was initiated early following whole body x-irradiation. In all cases, treatment on the seventh postirradiation day accelerated the death rate considerably as compared to the controls. This observation was also reported by Ross (2).

It has been difficult to obtain properdin solutions of high activity/unit volume. Consequently, intravenous injections of properdin of high activity could not be completed without introducing large fluid volumes. The choice of intraperitoneal route of injection was made on the assumption that properdin could be absorbed by the animal in doses greater than could be administered intravenously without causing physiological stress. It is of interest to note that in the bactericidal tests conducted with the sera of x-irradiated rats, no bactericidal activity was measurable when the properdin levels of the animals were undetectable. Perhaps failure of properdin to protect the animals against bacteremia that follows whole body x-irradiation might be explained on the asumption that sufficient levels of systemic properdin were not obtained. However, *in vitro* studies by Wardlaw and Pillemer(12,13) show that bactericidal activity of the properdin system occurs with a system containing as little as 0.01 units of properdin. It is recognized that extrapolation of *in vitro* observations to *in vivo* predictions must be guarded. They also reported that concentrations of properdin above 10 units was inhibitory in the *in vitro* bactericidal process. Possibly a minimum of properdin would give maximal *in vivo* protection. In an initial experiment in this laboratory, use of properdin of low activity (1 unit/ml) yielded 60% 30 day mortality among 500r x-irradiated mice compared to 100% mortality of the controls in 15 days. However, attempts to confirm this observation have been uniformly negative.

Summary. Under conditions employed postirradiation treatment of mice and rats with partially purified bovine properdin and purified human properdin administered intraperitoneally or intravenously did not afford protection against the effects of whole body x-irradiation. There appeared to be a rela-

tionship between delayed mortality and time of injection.

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Enzymatic Determination of Sorbitol in Animal Tissues.* (24493)

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Chemical methods for determination of sorbitol and related polyols depend upon degradation of these substances by ferricyanide(1, 2) or periodate(3,4). These procedures suffer from the disadvantage of extreme lack of specificity, since a wide variety of sugars other than acyclic polyols react with these reagents. Although interference by substances such as glucose can be minimized by pretreatment with fermenting yeast(3) or by examining the reactions under carefully controlled conditions (4), application of these methods to determination of polyols in tissue extracts is not altogether satisfactory. Purification of a soluble liver polyol dehydrogenase(5) which catalyzes the reversible oxidation of certain 5, 6 and 7 carbon polyols to ketoses(6,7) made possible the present method for enzymatic estimation of sorbitol. As little as 1 μg of sorbitol can be measured by rapid and simple spectrophotometric procedures, not subject to interference by glucose or other naturally occurring sugars.

Methods and materials. Spectrophotometric measurements were made with Beckman model D.U. spectrophotometer using cells of 1 cm light path. Most sugars and polyols used were obtained from Pfanstiehl Laboratories, Waukegan, Ill., and were of the highest grade of purity. L-Iditol(8) was donated by Dr. Jean Sice. DPN and TPN were more than 80% pure when assayed enzymatically (9,10). DPNH was prepared by either chemical(11) or enzymatic(12) reduction of DPN. Ketoses were estimated by the methods of Dische and Borenfreund(13) or Roe (14). Protein was determined spectrophotometrically(15). Molar extinction coefficient of pure DPNH at 340 μ was assumed to be 6220.

Results. Purification of soluble polyol dehydrogenase of liver. Assay. Activity of the enzyme at each stage of purification was determined in a reaction mixture containing 0.033 M sodium pyrophosphate buffer pH 9.1, 0.0006 M DPN and 0.033 M sorbitol. Total volume was 1.5 cc. The reaction was initiated by addition of enzyme in total volume of 0.1 cc or less. Absorbency at 340 μ was measured at 30 second intervals at 25°. Reaction velocities were determined from zero order portions of plots of change in absorbency *vs.* time. One unit of activity was defined as equal

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