Effect of Protein Depletion upon Susceptibility of Rats to Total Body Irradiation. (17478)

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Although there have been many studies of the effects of penetrating radiation on normal animals of various species, there has been little investigation of the effects of radiation upon abnormal animals. Some components of diet, particularly the vitamin B series[1] and some of the amino acids,[2] have been reported to alter the response to radiation but the broader problems of protein, fat, or carbohydrate nutrition have not, to our knowledge, been investigated to any great extent. Since widespread tissue necrosis is a prominent feature of radiation damage, it appeared that investigation of the effect of protein, particularly a deficiency of protein, on the response to radiant energy might prove instructive. Therefore a series of experiments, of which this is the first report, was undertaken to try to evaluate the role of protein in radiation damage.

Procedure. This experiment was planned to determine the effect of moderately severe depletion of the protein reserves on the susceptibility to radiation. Since young adult rats showing 25% reduction in weight while on a protein-poor diet are known to have but little reserve protein,[3] this type of animal was selected for this study. There is considerable confusion regarding the effect of age and weight on susceptibility to radiation. For this reason two types of control animals were used, one group having the same weight as the test animals, the other being the same age. A total of 141 rats was almost equally divided into the 3 groups in this experiment. Protein-depletion, as it is produced by the method developed by Cannon and his group, consists of placing the rats on a low protein diet that is adequate in calories, vitamins, and minerals. The diets and methods used in obtaining protein-depleted rats have been described elsewhere by Cannon[4] and Wissler.[5] In this experiment male albino rats of the Sprague-Dawley strain were used. Rats with an initial weight of about 200 g were placed on a diet (3E) containing 1.9% protein. The animals were kept in large, wire-bottom cages with 6 rats to a cage. The rats were continued on the depletion diet until they had lost 25% of their starting weight. Such a weight loss occurred in about 10 weeks. The animals were then given total body irradiation.

Total body irradiation of all of the animals was accomplished by x-ray. The radiation was done at 200 kv and 15 ma with 0.5 mm copper and 3 mm bakelite filters. The half-value layer of the radiation was 1.2 mm of copper. The target to animals distance was 71 cm and the dose rate in all cases was 17-18 r per minute. The dosage rate was measured before and after each exposure with a Victoreen electrometer. The animals were placed in individual perforated aluminum cages and radiated in a horizontal beam. The cages were regrouped and turned several times during the exposure to give the animals uniform distribution in the beam.

The two groups of control rats were maintained on a diet (3C) identical with the protein-deficient ration except that it contained 22% protein supplied by casein. The age control rats were placed on this diet after they had reached a starting weight of about 200 grams. The animals were continued on this program until they had been

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1 Martin, C. L., Moursand, W. H., *Radiology*, 1938, **30**, 277.

2 Patt, H. M., Tyree, E. B., Straube, R. L., Smith, D. E., *Science*, 1949, **110**, 213.

3 Benditt, E. P., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 243.

4 Cannon, P. R., Wissler, R. W., Woolridge, R. L., and Benditt, E. P., *Ann. Surg.*, 1944, **120**, 514.

5 Wissler, R. W., Woolridge, R. L., Steffee, H. S., and Cannon, P. R., *J. Immun.*, 1946, **52**, 267.

on the 22% protein diet for a period of time equal to the period of depletion for the protein-depleted rats. At time of radiation these animals were 20 weeks old and weighed about 280 g. For the second control group, rats with an initial weight of about 100 g were fed the 22% protein ration at a rate of 15 g per rat per day until they had reached a weight of approximately 150 g. These rats were then about 8 weeks old and were given total body irradiation. Three groups of these rats were radiated before they had reached a weight of 150 g. The results show that this did not lessen their value as control animals.

During the 30-day observation period following radiation all of the animals were continued on the same diets they had been receiving prior to radiation. All of the rats were weighed at 2-day intervals through this observation period.

These experiments were performed over a 9-months period. For the most part corresponding groups of depleted and age control animals were radiated at the same time and these groups were under observation at the same time with similar animal colony conditions. It was not feasible to radiate weight control animals at the same time as the other groups. Nevertheless groups of all 3 types of rats were scattered through the 9-months period in an attempt to keep the conditions in each series as nearly comparable as possible.

Results. The results of this experiment are shown in Table I. Here it is apparent that the mortality in the protein-depleted animals was considerably greater at any given dosage than in the two control groups. Most of the deaths in the protein-depleted groups of rats occurred below 625 r while there were few deaths in the control groups below this dose.

In order to plot definite dosage-mortality relationships, the data were treated in the manner developed by Bliss.[6,7] The regression lines derived from this procedure are shown in Fig. 1. This treatment of the data demonstrates clearly that the protein-depleted rats were considerably more susceptible to total-body irradiation than were either animals of the same age or of the same weight when fed adequate protein in their diet.

TABLE I.
Mortality in Rats Following Total Body Irradiation.

Dose	No. animals	Avg wt, g	Died
	Depleted rats		
300r	4	158	1
450	6	160	0
525	6	162	1
525	3	154	2
575	6	146	2
575	3	155	3
600	6	156	6
625	3	150	2
625	3	161	3
750	6	157	5
	Age controls		
525r	3	286	0
575	6	293	0
625	3	304	0
650	3	297	1
675	9	268	6
700	3	299	1
725	9	269	6
750	3	299	2
775	9	269	8
800	2	291	2
	Wt controls		
500r	5	155	0
600	5	153	2
600	10	128	1
650	10	128	3
700	5	156	4
700	10	128	4

The equation for the regression line for the protein-depleted animals is: $Y = 5.3460 + 8.9179\ (X - 2.7463)$ where Y equals the probit value of the mortality and X is the logarithm of the observed dose. The standard deviation for this line is .1121. The regression line of the animals of the same age is: $Y = 5.1545 + 21.0772\ (X - 2.8476)$ with a standard deviation of .0474. Rats of the same weight give: $Y = 4.7268 + 15.0372\ (X - 2.8135)$ and a standard deviation of .0665. The fiducial ranges, or confidence limits of the dosage, with a probability of .05 for the regression lines at the lethal dose for 50% of the animals in 30 days (LD 50/30) were calculated according to Bliss[8]

[6] Bliss, C. I., *Ann. Applied Biol.*, 1935, **22**, 134.
[7] *Ibid.*, 1935, **22**, 307.
[8] Bliss, C. I., *Quart. J. and Yearbook Pharmacy*, 1938, **11**, 192.

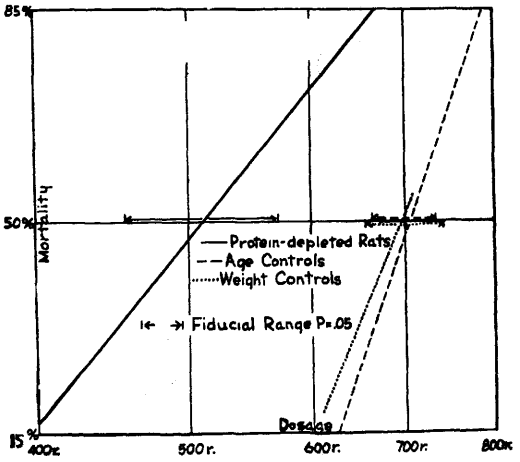

FIG. 1.
Thirty-day dosage-mortality regression lines in rats following total body irradiation.

and are indicated by corresponding arrows in Fig. 1. As can be seen, there is a wide zone separating the limits of variation of the protein-depleted animals from the limits of the controls.

In this experiment the weights of the protein-depleted radiated rats that survived the 30-day observation period closely paralleled the weight loss in non-radiated depleted animals except for some additional weight loss in the period immediately following radiation. Both the age control and the weight control rats also showed weight loss in the first few days following radiation when diet consumption was poor. This loss was quickly recovered when these animals regained their appetite and they continued to gain weight through the remainder of the observation period. It is important to note that a period of 100 days on the protein-deficient diet, as was the case with these depleted animals, causes no mortality in non-radiated rats. Rats have been kept on a protein-deficient diet for as long as 6 months in this laboratory with little spontaneous mortality, providing care is taken to prevent any infectious diseases of rats. Thus it is not likely that any of the increased mortality observed in the protein-depleted rats was due to spontaneous mortality.

In all groups of radiated animals, as is the case in most radiation mortality studies, death usually occurred before the fifteenth post-irradiation day. Autopsies were done on most of the rats that died and no differences were noted between the lesions in the control and depleted rats. Most of the animals that died in the first few days following radiation showed widespread interstitial hemorrhages. Some of the rats that died later showed pneumonia. Blood cultures were done on 12 of the protein-depleted rats that died after exposure of less than 650r. Two organisms were found consistently in the 10 animals that died before the fifteenth post-irradiation day. One organism was a gram-negative rod (probably *Bacillus dysenteriae*) and the other a micrococcus. The 2 depleted animals that died after the fifteenth day both had sterile blood cultures.

Discussion. The present experiment shows that the protein-depleted rat is materially more susceptible to the physical injury of penetrating radiation than is the animal on an adequate protein diet. The LD 50/30 for the protein-depleted rat is approximately 520r while the LD 50/30 for the control rats in this series proved to be in the region of 700r. Thus at the LD 50/30 there is a great difference in the dosage between depleted and control rats. This increased susceptibility of the protein-depleted rat becomes even more apparent when one realizes that the dose of total body irradiation that killed 50% of the protein-depleted rats killed less than two per cent of the control rats. The slope of the regression line for the protein-depleted animals indicates that these animals are proportionately somewhat more susceptible to lower doses of radiation than the controls.

The fiducial range for each of the regression lines, as shown in Fig. 1, shows a wide zone separating these ranges at the LD 50/30. Calculation of the differences at the LD 50/30 indicates that the P value for the data at this point is somewhat less than .001. Therefore the observed increase in mortality from radiation in the protein-depleted rat is highly significant.

Since there is confusion about the influence of weight and age on radiation suscepti-

bility, control rats for both of these factors were used. Quastler[9] found that in mice, with doses in the range of the LD 50, the survival time was proportional to body weight and age. Hagen and Sacher[10] and Hagen and co-workers[11] reported that in a series of mortality studies on rabbits the animals weighing more than 2 kg were less sensitive to x-ray than rabbits weighing less than 2 kg. Naiman[12] recently reported that older, and therefore heavier, rats could sustain larger doses of radiation than small rats. In contrast to these conclusions Hagen and Simmons[13] studied a series of 638 rats of the same strain as that used in this experiment and concluded that "within the range tested (median weight 210 g) any age or weight effect on radioresistance of rats must be small." Boche and Bishop in a study of 240 rats of the Wistar strain concluded that age and sex did not cause significant differences in radiation mortality.[14] The coincidence of the two control regression lines in this experiment and the corresponding fiducial ranges indicate that age and weight in the adult rat, in the range of 150 to 280 g, have little effect on susceptibility to radiation under the conditions of this study.

The LD 50/30 of about 700r for the 2 groups of control rats in this experiment is in close agreement with the LD 50/30 being obtained at present for similar animals in another laboratory.[15] This figure is in some disagreement with a LD 50/30 of 600r as reported by Prosser[16] but is in only moderate disagreement with a LD 50/30 of 640 ± 5r for a Wistar strain of rats as recently reported by Clark and Uncapher.[17]

The finding of increased susceptibility to radiation damage in protein-depletion coincides with findings of increased susceptibility in protein-depleted rats to other types of injury. The studies of Cannon,[4,18] Wissler[5,19] and Benditt[3] have demonstrated that antibody production is seriously impaired by severe and prolonged protein-depletion. Taliaferro and associates[20] recently demonstrated a decreased immunity to trichinosis in the protein-depleted rat. A delay in wound healing was noted by Kobak and associates[21] in protein-depletion. The leukocytic response of the bone marrow, particularly to repeated stimulation, is also markedly impaired by depletion of protein reserves.[22]

Protein-depletion, in addition to causing some decrease in plasma proteins and hemoglobin level,[23] also causes a decrease in production of several of the enzymes. Tobin and co-workers[24] have shown a reduction in hyaluronidase and antihyaluronidase. Benditt and associates[25] have demonstrated that cytochrome oxidase and succinoxidase are decreased in liver homogenates in protein-depletion. Kidney phosphatase is also reduced while the total liver phosphatase remains constant during the depletion of the protein reserves. It is known that radiation also inhibits several of the enzymes, particularly the sulfhydryl-containing enzymes, as shown by the recent work of Barron and his group.[26]

9 Quastler, H., *Am. J. Roentgen.*, 1945, **54**, 457.

10 Hagen, C., and Sacher, G., MDDC—1252 (CH-3754), 1946.

11 Hagen, C., Jacobson, L. O., Murray, R., and Lear, P., MDDC-999 (CH-2147), 1944.

12 Naiman, D. N., *Am. J. Roentgen.*, 1949, **61**, 95.

13 Hagen, C., and Simmons, E., MDDC-1210 (CH-3815), 1945.

14 Boche, R. G., and Bishop, F. W., MDDC-250, 1947.

15 Patt, H. M., personal communication.

16 Prosser, C. L., *Radiology*, 1947, **49**, 299.

17 Clark, W. G., and Uncapher, R. P., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 214.

18 Cannon, P. R., Chase, W. E., and Wissler, R. W., *J. Immun.*, 1943, **47**, 133.

19 Wissler, R. W., *J. Infect. Dis.*, 1947, **80**, 264.

20 Taliaferro, W. H., Woolridge, R. L., and Benditt, E. P., *Science*, 1949, **109**, 443.

21 Kobak, M. W., Benditt, E. P., Wissler, R. W., and Steffee, H. S., *Surg. Gyn. Ob.*, 1947, **85**, 751.

22 Asirvadham, M., *J. Infect. Dis.*, 1948, **83**, 87.

23 Benditt, E. P., Straube, R. L., and Humphreys, E. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 189.

24 Tobin, J. R., Bergenstahl, D., and Steffee, H. S., *Arch. Biochem.*, 1948, **16**, 373.

25 Benditt, E. P., Steffee, H. S., Hill, T., and Johnston, T. C., *Fed. Proc.*, 1949, **8**, 350.

26 Barron, E. S. G., Dickman, S., Muntz, J. A., and Singer, S. P., *J. Gen. Physiol.*, 1949, **32**, 537.

The manner in which protein-depletion increases susceptibility to radiation is not known. Several methods by which radiation may cause death have been postulated and protein-depletion affects most of these mechanisms. Death following radiation may be due to infection subsequent to the break-down of the normal immunological processes. It is well known that protein-depletion interferes with immunological responses and this in combination with the effects of radiation on the epithelial barriers, particularly the intestinal epithelium, may account for the increased susceptibility. Also it may be more difficult for the depleted animals to recover from the leukopenia of radiation so that the phagocytic barrier against infection is lowered. There is much evidence that the degree and duration of leukopenia following radiation have great influence on mortality.[27]

Since it is known that protein-depletion and radiation both interfere with enzyme production or action, it may be that there is an additive effect that interferes with enzyme activity to account for the increased radiation susceptibility.

It may be that death is due to anemia following bone marrow damage. That this mechanism accounts for the increased susceptibility does not seem likely for protein-depletion *per se* does not cause a marked lowering in the erythrocyte or hemoglobin levels. However it may interfere with the recovery of the bone marrow and with the production of both erythrocytes and leukocytes.

It seems possible that the increased susceptibility to radiation observed in these protein-depleted animals might be due to their poor immunological and leukocytic responses or to their decreased production of some essential enzyme. This problem is being investigated further.

Conclusions. 1. Protein-depletion in young adult male rats, as produced by a weight loss of 25% on a protein-poor diet, materially increases susceptibility to total body irradiation. 2. Age or weight in normally nourished adult rats, in the 150 to 280 g weight range, have little effect on susceptibility to radiation.

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[27] Brues, A. M., and Rietz, L., ANL-4227, **183**, 1948.

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An Autohemolytic Agent in Fetal Liver Extracts.* (17479)

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In the course of studies dealing with enzymatic activity during fetal and extrauterine development it was noted that extracts of fetal liver lysed the red cells of the same species. It was of interest to determine the nature of this phenomenon and to compare the activity of adult and fetal extracts.

Methods. Thirty experiments were performed, mostly on pregnant guinea pigs and their fetuses. A few experiments were made with pregnant rats or newborn rats and their mothers. The pregnant animals were killed by decapitation and the maternal blood was collected in oxalated tubes. The fetuses were then quickly removed and decapitated and

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