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ENCLOSURE 21

Alterations of immune mechanisms in mammals following exposure to ionizing radiation have been described (1). It has been reported that in vitro exposure of pooled human serum gamma globulin (HSGG) to massive doses of ionizing radiation results in the production of molecules electrophoretically and immunologically distinct from the original protein (2, 3). Therefore antibodies stimulated by radiation altered HSGG (anti-XI-HSGG) were used to detect specific antigens in the serum from seven patients who received total body irradiation for malignancy.

MATERIALS AND METHODS

Selection of Subjects. Sera from seven patients (Table 1) were studied. The patients were in relatively good nutritional status with a stable hemogram. Each subject served as his own control. Serum was collected during a pre-irradiation control period of two weeks and at several times post-irradiation. Although pre-irradiation samples were known, post-irradiation sera were coded and were identified as to the time interval post irradiation only after completion of all analyses.

Radiation. The gamma radiation was delivered by a Co-60 Teletherapy unit with the beam directed horizontally and with the patient midline at 282 cm from the source. The beam area for the 50 per cent isodose curve at the patient

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midline distance was a square approximately 70 cm x 70 cm. The patients were exposed bilaterally in a sitting position with legs raised and head tilted forward. Patients received total-midline air doses of 149 to 238 roentgens (absorbed doses of 100 - 150 rads) given at an air dose rate of 5 roentgens/minute. A detailed description of the dosimetry and radiation conditions pertaining to subjects included in this study may be found in (4).

Blood serum collection. Blood was obtained by venipuncture and allowed to clot. The serum was separated by centrifugation, decanted and stored at -5° C.

Preparation of immune serum. Female and castrated male goats under prolonged immunization with X-irradiated HSGG were used for anti-XI-HSGG production. Complete details for the preparation of XI-HSGG antigen and immunization schedule and blood collection may be found in an earlier report (2).

Absorption of immune serum. Pooled anti-XI-HSGG from three goats was incubated with 50 μg of HSGG protein N/ml of antiserum for 2 hours at 38° C and overnight at 5° C. The antiserum was separated from the precipitate by repeated centrifugation until only negligible precipitation resulted on subsequent additions of HSGG. Sera were preserved in 0.01% Merthiolate and frozen until used. All analyses were performed with the same anti-XI-HSGG. Fifty ml of this antiserum was further absorbed with 1.0 ml of a patient's pre-irradiation serum before precipitin tests on that patient's serum were performed. Therefore base line values differed between subjects so quantitative comparisons between patients were not made.

Quantitative precipitin tests. Complete details of the quantitative immunochemical techniques may be found in Kabat and Mayer (5). In the titrations each sample of patient serum was used as antigen and was diluted serially to 1 - 64 with phosphate buffer 0.15 M, pH 7.6. One ml of absorbed anti-XI-HSGG was added to 1.0 ml of this diluted serum and incubated at 38° C for 2 hours and overnight at 5° C. Antigen-antibody precipitates were analyzed for protein N by the Lowry modification of the Folin-Ciocalteau procedure (6). Control antigen-antiserum precipitate N values were subtracted from the total antigen-antibody precipitate N. The highest precipitate N value obtained from a sample was used in the plotted data.

RESULTS

Figure 1 illustrates the changes in N precipitated from anti-XI-HSGG by the sera of six human patients before and after a single exposure to total body irradiation. The amount of precipitate N rose immediately following irradiation and fell to the pre-irradiation level in about five days. Within thirty days post-irradiation there was a decrease in precipitate N which was followed by a second rise. Two subjects, #037 and #027, showed the decrease but were not followed beyond thirty days.

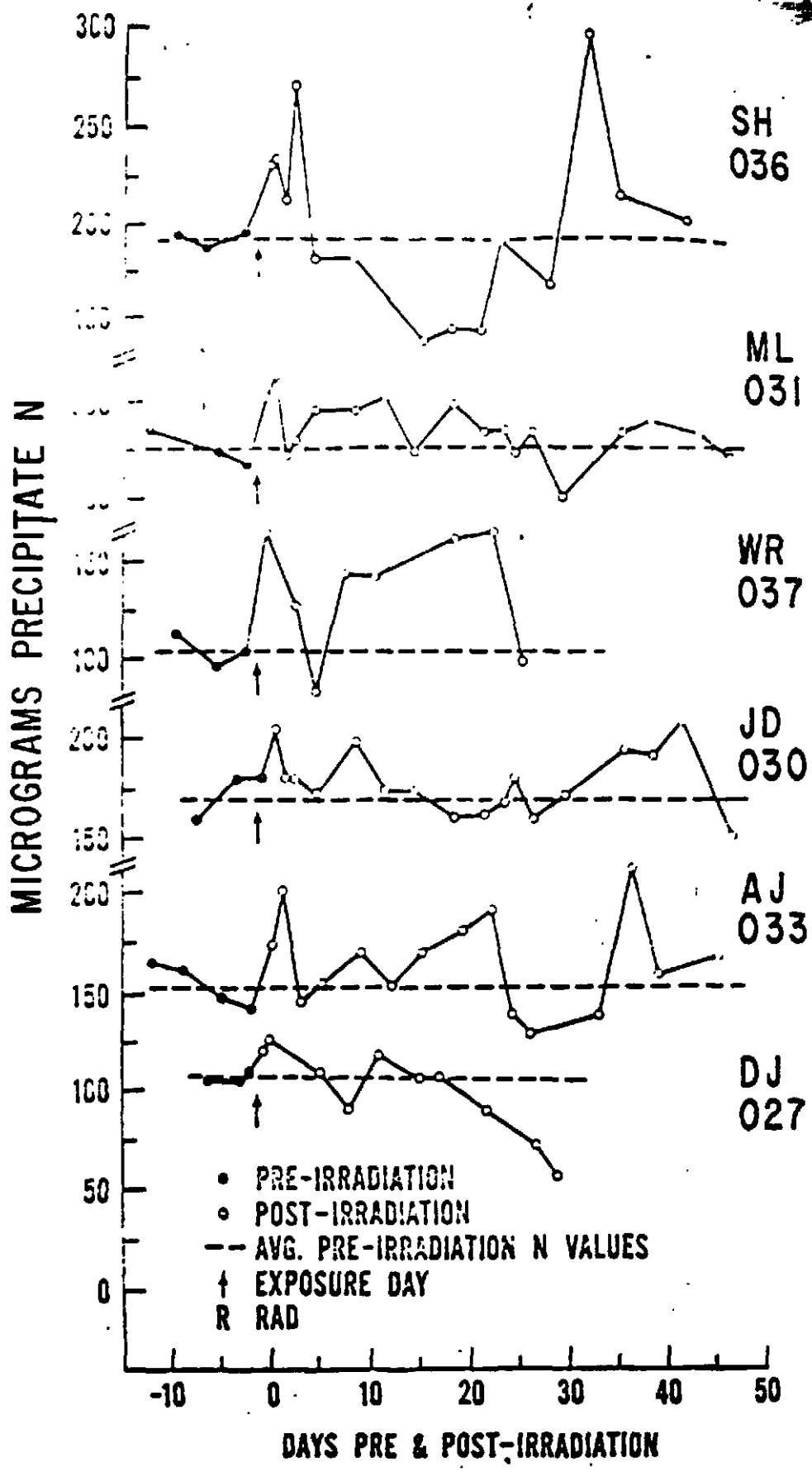
Figure 2 shows the results from one patient who received a total dose of 150 rads delivered in fractions of 50 rads on day 0, day 2 and day 10. The precipitate N rose steadily, peaked on the twentieth day post-irradiation, and then declined slowly, approaching the base line thirty days after treatment.

TABLE 1

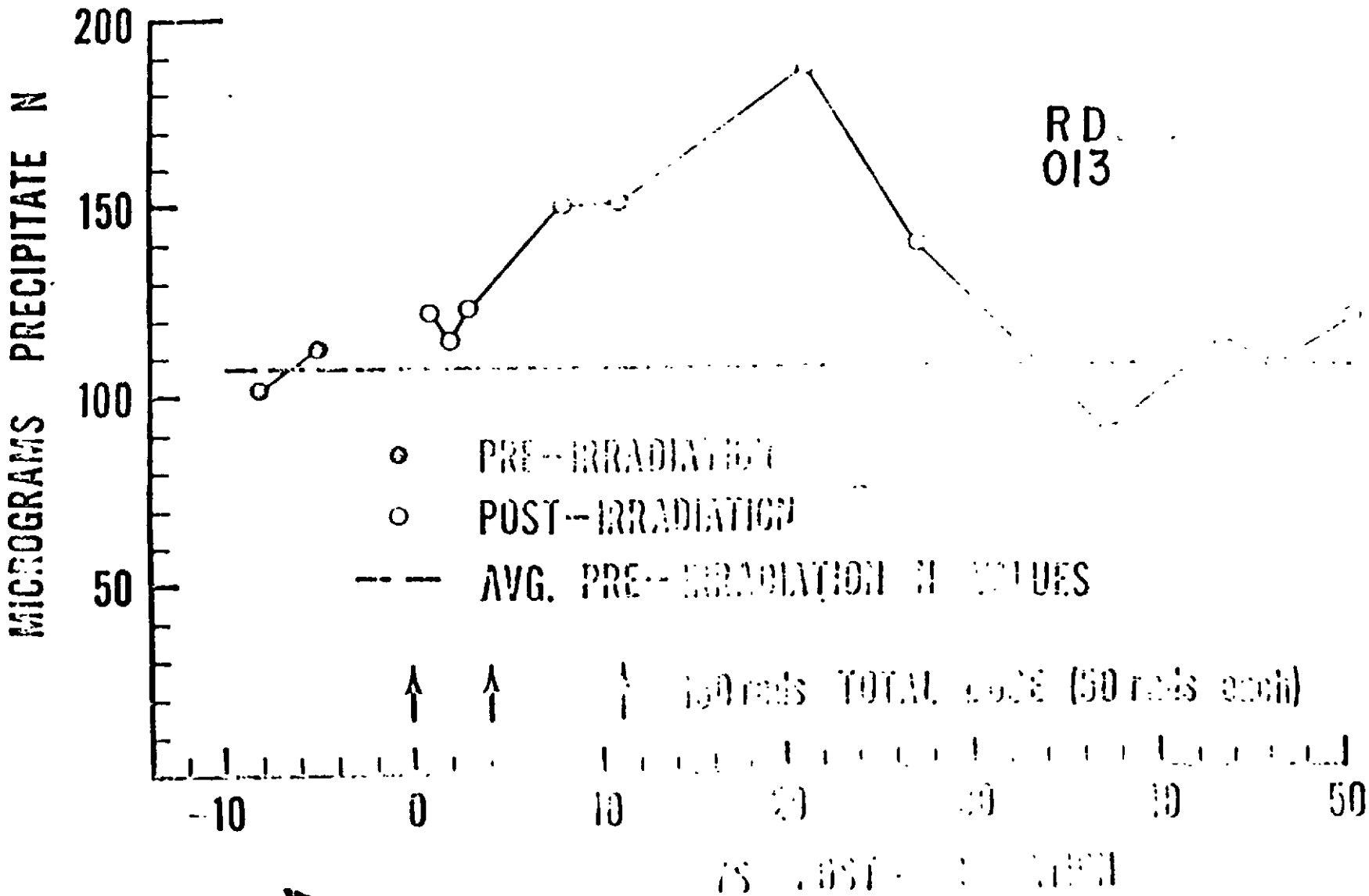
Patient	Study No.	Age	Sex	Race	Diagnosis	Dose	Previous Therapy
M.L.	031	81	F	N	Carcinoma of the left breast with metastases to the liver	100 Rad	Radiation therapy for fibroids 34 yrs. prior to present therapy
J.D.	030	54	M	N	Recurrent carcinoma of the stomach	100 Rad	A-8103 (piperazine derivative) 40 mgm/day (6 months prior)
S.H.	036	64	M	N	Carcinoma of the colon with metastases to the liver	100 Rad	None
W.R.	037	64	M	N	Carcinoma of the stomach	150 Rad	None
A.J.	033	64	M	N	Carcinoma of the colon with metastases to the liver	100 Rad	None
D.J.	027	17	M	W	Ewing's sarcoma	150 Rad	Four 450 r doses of Co^{60} sacrum (one year prior)
R.D.	013	49	M	N	Kaposi's sarcoma	50 Rad 50 Rad 50 Rad	None

Figure 1. Total N precipitated from anti-XI-HSGG by serum from six patients given total body irradiation in a single exposure.

Figure 2. Total N precipitated from anti-XI-HSGG by serum from patient given total body irradiation in three fractions of 50 rad each.


MICROGRAMS PRECIPITATE N
SH 036
ML 031
WR 037
JD 030
AJ 033
DJ 027
PRE-IRRADIATION
POST-IRRADIATION
AVG. PRE-IRRADIATION N VALUES
EXPOSURE DAY
R RAD
DAYS PRE & POST-IRRADIATION

The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.


MICROGRAMS PRECIPITATE N
200
150
100
50
RD
013
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AVG. PRE-IRRADIATION
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50

DISCUSSION

The data show that when sera from patients treated with a single dose of total-body irradiation is tested against anti-XI-HSGG by the quantitative precipitin method a biphasic increase in precipitate N occurs within fifty days. A comparison of the data in Figures 1 and 2 shows that a peak response and subsequent decline appears to be reached at a slower rate when the total body irradiation is fractionated.

The immediate rise in the biphasic curves which occur within five days suggests that irradiation directly modifies preformed protein so as to alter its specificity, that it causes increased release of preexistent molecules specific for anti-XI-HSGG into the serum, or that both factors may be operative. However, the subsequent decline in precipitate N, to below base line levels, preceding the second elevation suggests temporary inhibition of protein synthesis. High pre-irradiation control values, along with the observation that serum from non-irradiated humans free of neoplasia precipitated anti-XI-HSGG (7) is further evidence to support the above assumption. Furthermore, these high control values rule out neoplasia as the sole contributing element to the precipitate N base lines.

The biphasic appearance of C_x and C reactive protein (CRP) in rabbits, monkeys and man after irradiation shows certain similarities to the results reported here (8, 9, 10). However, in this study, CRP did not precipitate anti-XI-HSGG nor was there any correlation between the data in Figure 1 and parallel semiquantitative titrations for CRP.

The observations described above cannot be explained fully at this time. However, continuing efforts in this direction have provided some evidence which indicates that serum macroglobulins may be the specific protein involved. Experiments are currently underway to investigate this aspect and will be reported at a later date.

SUMMARY

In the quantitative precipitin reaction anti-XI-HSGG is precipitated by sera collected from seven patients at various times before and after treatment with total body radiation. The data are insufficient to determine whether the effects described are due to a direct effect on preformed proteins and/or an effect on the rate of synthesis of pre-existing protein. These studies may provide an approach to a better understanding of radiation-altered immunologic mechanisms in human beings.

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