Effect of Intestinal Tract Irradiation on Serum Proteins of the Rat.* (24926)

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Several authors have reported that protein composition of rat serum is altered after whole-body x-irradiation(1,2,3). When extensive intestinal tract irradiation studies were initiated in this laboratory(4), it was felt that a study of effects of various types and methods of intestinal tract irradiation on serum proteins might shed light on the mechanism of radiation damage to intestinal tract. This report describes results obtained by paper electrophoretic fractionation of proteins in sera of animals at various intervals following x-irradiation of whole body, or intestinal tract, both *in situ* and exteriorized. Results are also included from animals that received beta irradiation of the GI tract from administration of Y^{91} by stomach tube, and from animals maintained on starvation diet to simulate decreased food consumption of irradiated animals.

Methods. Animals used were adult female Sprague-Dawley rats weighing 200 to 240 g. Twelve animals were exposed to 600 r of whole-body x-irradiation. The radiation factors were 250 kvp, 30 ma, HVL 1.15 mm Cu, TSD 96 cm and dose rate was about 35 r/min as measured in air with Victoreen ionization thimbles. Seventy-four animals were exposed to x-irradiation of gastrointestinal tract, which was irradiated either *in situ*, or surgically exteriorized, while remainder of body was protected by lead shielding. Intestines of rats, under Nembutal anesthesia (30 mg/kg), were exteriorized through midline incision by gentle manipulation and exposed to radiation on cardboard plaque. The remainder of body was incased in one-fourth inch thick, cylindrical, half shell-type lead shield(4). The intestine was moistened with Ringer's solution and maintained at body temperature throughout exposure. Asepsis was practiced in all surgical and exposure manipulations. After irradiation the GI tract was replaced, the abdominal wall was sutured and skin closure was made with skin clips. Rats exposed to abdominal irradiation were placed on their side while under anesthesia. Shields for these exposures were constructed of one-fourth inch lead with aperture approximately 3 x 5 cm (4). Region receiving direct irradiation extended anteriorally about 3 cm from greater trochanter and posterior to rib cage. The vertebral column, spleen, kidneys and almost the entire liver were shielded from direct radiation. Surgical and/or normal control animals were run in parallel with irradiated animals. Radiation factors were as above. Nineteen animals received 3 cm of Y^{91} by stomach tube as yttrium chloride at pH 3. Control rats were administered an equal volume of pH 3 hydrochloric acid. Starvation control animals were deprived of food for 3 days, then maintained on 50% of normal diet for additional 6 days. Blood samples were obtained from tail vein for serial determinations and by cardiac puncture, when animals were to be sacrificed. Electrophoretic characterization of serum samples was done by the Durrum method (5) using Veronal buffer at pH 8.6 and 0.1 ionic strength. Protein composition of serum was determined by scanning dyed filter paper strips in Spinco Analytrol Photometer-Computer.

Results. In all groups of irradiated animals per cent albumin in sera showed largest and most consistent changes following irradiation. This consistency was probably due to better precision of interpreting the "Analytrol" trace for per cent albumin than for other serum protein fractions. To present dose dependency of composition of serum proteins as clearly as possible, the percent albumin in irradiated animal sera was divided by average of per cent albumin in control animal sera fractionated in the same electrophoresis "run." This quotient, expressed as A/A_c, tends to minimize day to day variations in electrophoresis procedures. It is recognized that this electrophoretically separated "albumin" fraction

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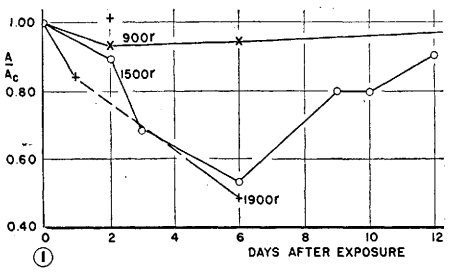

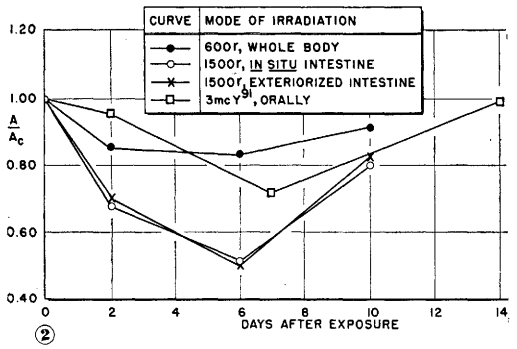

FIG. 1. Effect of *in situ* intestinal x-irradiation on A/A_c value in rat serum.

FIG. 2. Effects of various modes of irradiation on A/A_c value in rat serum.

may have a complex composition(6,7).

Fig. 1 shows variation with time and dose of average A/A_c values of sera from rats whose intestinal tracts were irradiated *in situ*. Each point is the average of results from 3 to 9 animals. The reason for the apparent discrepancy in results from one- and 2-day animals that received 1,900 r is not known. Fig. 2 compares effects of different modes of irradiation of comparable acute lethality on the A/A_c values obtained at different times after exposure.

Discussion. Maximum depression in albumin level was observed 6 days after x-irradiation (Fig. 1). Time of maximum depression and pattern of subsequent recovery are similar to the effect of intestinal irradiation on other blood elements(4). Amount of histological damage and time of maximum mortality caused by acute doses of radiation to the intestine suggest a relationship among these effects.

Although decreased albumin level was caused by exposure of abdominal region, the possibility remained that the liver may have absorbed sufficient "scatter radiation" to cause inhibition of serum albumin synthesis at this site. Therefore, serum protein pattern was determined in animals totally shielded except for an exteriorized portion of intestinal tract which was irradiated. The decrease in serum albumin was approximately the same, whether the gut was irradiated, exteriorized or *in situ* (Fig. 2). This effect must therefore be due either to direct action of radiation on the intestine, or to indirect action by some material produced in the gut during irradiation acting at site of serum protein synthesis.

Fig. 2 shows the effect on serum albumin of internal beta irradiation of the GI tract. The 3 mc dose of Y^{91} administered is of comparable acute lethality to 1,500 r of x-ray. Since yttrium is absorbed from the GI tract to a negligible extent, its effect must be attributed to irradiation of intestines and closely adjacent tissues. Albumin is decreased following oral administration of Y^{91}, but onset of this effect is delayed, and the magnitude decreased, as compared with x-radiation effects. This is probably related to the longer time required for intestine to absorb a beta-radiation dose comparable to the x-ray dose.

Exposure of rats to whole-body x-radiation of comparable acute lethality (600 r) caused a much smaller effect on serum albumin than did the 1,500 r dose to the intestinal tract. Maintaining animals for 3 days without food, then for 6 days on only 50% of their normal diet had no effect on A/A_c values measured.

Summary. The results show that irradiation of the gastrointestinal tract affects albumin content of rat serum in the same manner as does whole body irradiation. This effect has been attributed to irradiation of the liver during whole body exposure. Since only the intestinal tract was irradiated in these experiments, decreased albumin production caused by indirect action of some substance originating in the irradiated tissue is suggested. Another possibility is loss of albumin because of increased capillary permeability in the irradiated intestinal tract.

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Growth and S^{35} Uptake of Cotton Granulomas in Rats. (24927)

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The cotton pellet granuloma technic of Meier, *et al.*(1), has provided a new approach to studies of inflammatory potentials of animals under various physiological conditions(2-5). In addition it has provided the basis for development of several tests for anti-inflammatory materials(6-11). This is a study of long term growth of granulomas around cotton pellets and incorporation of labeled sulfate into the tissue in early stages of granuloma formation.

*Methods.** Male Sprague-Dawley rats averaging 175-200 g in weight were adrenalectomized 2 weeks after arrival and maintained on saline and Purina laboratory chow. The saline was supplemented with 0.5% glucose for 24 hours after adrenalectomy. In an experiment designed to study granuloma growth and histology, 4 cotton pellets averaging 6 mg in weight were implanted subcutaneously(11) into each rat the day following adrenalectomy, and the granulomas were removed at autopsy 2-100 days later. In a second experiment in which sulfate uptake in granuloma tissue was measured, each rat was implanted with a 6 mg pellet 12, 6, 4 and 2 days before autopsy. These animals were adrenalectomized on day before first pellet was implanted, and placement of pellets was varied to allow for possible position effects. Twenty-four hours before sacrifice all animals received 150 microcuries of S^{35} labeled sodium sulfate subcutaneously. In both experiments test compounds were administered subcutaneously as solutions or suspensions in oil beginning first day of implantation. At autopsy animals were sacrificed with ether, the granulomas removed, and those granulomas used for growth curve or S^{35} studies were trimmed of extraneous connective tissue. Granulomas used for histological purposes were fixed in Zenker's solution untrimmed. As control tissue for the S^{35} data normal connective tissue was removed from subcutaneous area of back according to method of Boas and Foley(12). Trimmed granulomas and normal connective tissue were dried to constant weight at 72°C. Granuloma weights were expressed as total granuloma weight/rat by subtracting original weight of the 4 cotton pellets. For radioactivity measurement individual granulomas and tissue samples were weighed and wet-ashed in perchloric acid. Aliquots of each tissue digest were mounted directly on lens paper discs supported by copper planchets and counted with end-window Geiger counter. In calculating percentage of administered S^{35} incorporated/g of tissue, corrections were made for self-absorption, radioactive decay, and variations in body weights of animals. Granulomas for histological examination were fixed in Zenker's solution 24 hours, then cut in half and washed in tap water. They were dehydrated with S-39 Technicon dehydrant, cleared with butyl acetate, infiltrated 3 or 4 days in paraffin and embedded. The paraffin blocks were trimmed to the tissue and soaked in detergent for several days to soften the cotton fibers for cutting. Chilled blocks

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