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from "Radiation Bio-Effects" Report by Hodge, DM (ed) p. 98 (1965)

D. EFFECT OF X-RAY RADIATION AND MICROWAVE RADIATION IN VITRO AND IN VIVO ON HUMAN AND RAT GAMMA GLOBULINS

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Pregnant rats were X irradiated on day 13 of gestation with a dose of 180 rads and killed 24 hours later. Serum was separated from each rat. Gamma globulins were precipitated with 18%, 14% and 12% sodium sulphate. The same procedure was repeated with non-irradiated control rats.

The serum of the offspring of X irradiated pregnant rats was individually collected and gamma globulins were precipitated with 18%, 14%, and 12% sodium sulphate. Gamma globulins were also prepared from rats after exposure to microwave irradiation for 9 minutes and 15 minutes. High-titre antisera were prepared in rabbits against human gamma globulin (Cohn Fraction II) and normal rat gamma globulins using Freund's complete adjuvant.

The various gamma globulins of rats exposed to X and microwave radiation will be tested for the changes in their antigenic structures by using acrylamide gel electrophoresis, Ouchterlony's gel diffusion technique, immunoelectrophoresis as well as the other chemical methods to study properties after denaturation such as release of sulphydryl groups, increase in optical levo rotation, and precipitation with the homologous antisera.

Human gamma globulin (Cohn Fraction II) was fractionated on Sephadex-G-200 using 0.01 M phosphate buffer pH 7.6 containing 0.15 M NaCl. The first peak of IgM gamma globulin was separated and recycled on the same column using the same buffer. A single peak of IgM was obtained. Similarly the peak containing the IgG human gamma globulin was fractionated on DEAE cellulose column using 0.0175 M phosphate buffer pH 6.3. A single peak of IgG was obtained. The results showed that human gamma globulin (Cohn Fraction II) from Pentex Corporation contains 91.9% IgG and 8.1% IgM.

Optical rotation of human gamma globulin in H_2O and 0.9% NaCl was determined at specific wavelengths (365 mμ, 405 mμ, 436 mμ, 546 mμ, and 578 mμ). Lambert-Beer plots for human gamma globulin (Cohn Fraction II) from Pentex Corporation were determined for four wavelengths, 260 mμ, 278 mμ (peak), 280 mμ and 290 mμ. The spectra for human gamma globulin at a concentration of 0.1 mg/ml in 0.15 M NaCl were determined for wavelength range 200 mμ-310 mμ. The nature of aggregation of human gamma globulin (Cohn Fraction II) for native form and heat denatured form in 0.9% NaCl solution were determined with modified Coulter Counter apparatu

The method for the quantitative determination of sulphydryl groups using 5,5'-dithiobis (2-nitrobenzoic acid) was standardized for human gamma globulins. Pure samples of IgG and IgM human gamma globulins will be irradiated with X-rays and microwaves in vitro. The configurational

changes occurring in IgG and IgM after X-ray irradiation and microwave irradiation will be studied by determining the four parameters of optical rotation:

1) (α) specific rotation
2) a_o, constant for helical and intrinsic residue contributions
3) b_o, constant characteristics of helix and
4) λ_c, optical rotatory dispersion constant.

The changes in the functional properties of IgG and IgM human gamma globulins such as precipitation with the homologous antiserum, and complement fixation will be studied. The mechanism of denaturation of IgG and IgM human gamma globulins after exposure to X-ray radiation and microwave radiation will be studied by separating the aggregates on Sephadex G-200, determining their sedimentation constants, and their amino acid composition. These studies should help in explaining the following question: Can radiation cause human auto-immune diseases and if so, how?