

Stimulation of Local Antibody Formation in Cornea by Grenz Rays.

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In a previous communication¹ we presented evidence that, when a solution of egg-albumin is injected into the corneal stroma, specific precipitins are produced locally. By pooling the tissues from several rabbits and the use of a capillary-tube technic, it was possible to measure the precipitin-concentration in the small amounts of juice obtained by subjecting the tissues to high pressure in a hydraulic press. Following the intracorneal injection of antigen, precipitins could be detected in the corneal tissues earlier and in greater concentrations than in the serum, in the other tissues of the eye, or in the spleen or bone marrow. When the albumin was injected into only one cornea of each animal no precipitins could be obtained from the uninjected corneæ. The removal of the corneal epithelium and endothelium before the tissue-juices were expressed did not affect the precipitin-titer.

Since a number of different rays of the electromagnetic spectrum are known to exert an influence on the healing of tissues, and Grenz rays, in particular, have been used for a number of years in this institution² and in several European eye clinics for the treatment of various corneal conditions it seemed desirable to determine whether antibody-formation in the cornea could be influenced by these rays. Grenz rays* are electromagnetic waves of about 2 Ångström units occupying a position in the electromagnetic spectrum between ultraviolet (1300 Å) and the Röntgen ray (1/100-1/10,000Å). The waves of this length are not very penetrating and are absorbed by the superficial tissues. Bucky³ has calculated that half of the radiation is absorbed in 0.47 mm. of water; in 0.52 mm. of muscle or *cutis vera*; in 0.56 mm. of epidermis and in 0.77 mm. of subcu-

¹ Thompson, R., Gallardo, E., Jr., and Khorazo, D., *Am. J. Ophth.*, 1936, **19**, 852.

² Pfeiffer, R., unpublished work, in Institute of Ophthalmology.

* Grenz rays are sometimes called Bucky's rays; Bucky designed the machine for their generation and has done much to establish their value as a therapeutic agent. See Bucky.³

³ Bucky, G., *Grenz Ray Therapy*. 1926, Macmillan Co., N. Y.

taneous tissue. Approximately 60% of a 10 K.V.P. Grenz beam is able to penetrate 0.5 mm. of cutaneous tissue.

The methods used in the preparation of the purified egg-albumin solution; for the corneal injections; the expression of the tissue-juices, and the technic for the titration of the precipitins by the optimal-proportions method were as described in the previous paper.¹ Before the Grenz ray treatments the rabbits' eyes were locally anesthetized by a few drops of 0.5% pantocaine. The lids, and when necessary the nictitating membrane, were retracted and the whole cornea exposed, so far as possible, to the rays. Each treatment† lasted for one minute. Four hundred and eighty-five R. were delivered with the following factors: 10 Ma., 11 K.V.P. and 9 cm. distance. The number and spacing of treatments are indicated in each experiment.

Experiment 1. Eight rabbits were injected into both corneæ on 2 successive days with 0.05 cc. of egg-albumin solution (containing approximately 9 mg. of N per cc.). The corneæ of the right eyes were exposed to the Grenz rays for one minute on the day of the second injection and on the succeeding 3d, 6th, and 11th days. The left eyes of all animals were subjected to the same pantocaine drops and to the one-minute exposure to drying as the right eyes, the rays being omitted. Fifteen days after the first injection of antigen the animals were killed by bleeding and the tissue-juices and serums titrated for precipitin content.

Experiment 2 was similar to Experiment 1 except that 5 ray treatments were given and a 14-day interval elapsed between injections and killing.

Experiment 3. A group of 8 rabbits were given Grenz ray treatments of the right corneæ on alternate days for 8 days. On the 10th day 0.05 cc. of egg-albumin was injected into 2 places in both corneæ of all rabbits. A fifth treatment of the right corneæ was given the following day. The rabbits were killed 14 days after the injections.

The titrations of precipitin in these 3 experiments are shown in Table I.

The rayed corneæ consistently contained greater concentrations of precipitins than did the untreated corneæ. The difference was most marked in Experiment 3 in which the Grenz ray treatments were begun 8 days before the injection of antigen; the titer of the treated corneæ being approximately 12 times that of the untreated. The increased precipitin concentration in the corneæ treated by rays of such little penetrating power is additional evidence for the local

† We are indebted to Mrs. D. Albert, X-ray technician of the Institute, for her coöperation.

TABLE I.
Comparative precipitin-titers of treated and untreated corneæ.
(Optimal-proportions method.)

Experiment		Quantity* of Precipitate in Tubes Containing Various Concentrations of Egg-albumin (mg. of nitrogen per cc.)											
		.08	.07	.06	.05	.04	.03	.02	.01	.005	.0025	.001	Saline
1	Treated Corneæ	0	0	0	1	1	4	6	8	8	4	2	0
	Untreated Corneæ	0	0	0	0	0	0	0	2	4	4	2	0
	Pooled Serums†	0	0	0	0	0	0	0	0	0	0	0	0
2	Treated Corneæ	4	4	8	8	8	12	16	16	12	8	4	0
	Untreated Corneæ	trace	2	2	2	2	4	8	8	8	8	1	0
	Pooled Serums†	0	0	0	0	0	0	0	0	0	0	0	0
3	Treated Corneæ	7	8	12	10	10	9	8	8	8	2	2	0
	Untreated Corneæ	trace	trace	1	1	1	1	2	2	4	2	trace	0
	Pooled Serums	0	0	0	0	0	0	0	0	0	0	0	0

* Each 0.25 mm. of column of precipitate considered as 1 unit.

† When the serums were tested individually 2 serums in both experiments 1 and 2 showed minute traces of precipitins.

Experiment 3 was done a year later than the others. The tube had not been calibrated before this series, so that the R units may have varied somewhat.

production of the precipitins since the corneal tissue must necessarily be the only tissue affected. In an attempt to determine whether the ray treatment accelerated precipitin formation 2 experiments were done in which the intervals between injection of antigen and killing were respectively 7 and 10 days, in contrast to the 14-day intervals of the previous experiments. Five Grenz ray treatments were given following the injections of antigen. In both experiments antigen was still present, both in the treated and untreated corneæ, and no precipitins were shown. We have not yet determined whether treatment before injection of antigen, shown in Experiment 3 to be most effective, would produce an accelerated formation of antibodies.

We have not found any previous work dealing with the effect of Grenz rays on antibody-formation or of any type of electromagnetic wave upon *local* antibody-formation. General exposure to ultraviolet rays has been reported as stimulating the production of agglutinins,^{4, 5} and of hemolysins.⁶ The effect of general exposure to

⁴ Hansen, T., *Compt. Rend. Soc. Biol.*, 1921, **85**, 1199.

⁵ Bassemans, A., and Seldelachts, A., *Compt. Rend. Soc. Biol.*, 1929, **100**, 1055.

⁶ Omar, W., *J. Egyptian Med. Assn.*, 1935, **18**, 168.

Röntgen rays has repeatedly been described as a diminution of antibody-production due to injury of the bone marrow, spleen or lymphatic tissues.⁷⁻¹⁰

Summary. The local formation of precipitins following the injection of purified egg-albumin into the rabbit's cornea was increased by exposure of the cornea to the dosages of Grenz ray used in these experiments. The effect was most marked when the exposures were made for several days prior to injection of antigen.

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Factors Influencing Sedimentation Rate of Erythrocytes.

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There is increasing use of the sedimentation rate of erythrocytes as a test in clinical medicine, but there is equal difficulty in correct interpretation of the results obtained due to the many factors that influence it. Much work has been done to elucidate the value of this test in different clinical conditions, especially in infections.

Fahareus¹ has reviewed the literature and studied many of the factors that influence the sedimentation velocity of erythrocytes, especially the amount of fibrinogen and globulin in the plasma. Many other publications add new evidence. Westergren^{2, 3} states that a definite positive correlation exists between the plasma proteins and the sedimentation of erythrocytes. Zarday and Farkas⁴ have modified blood by the addition of normal fibrinogen and globulin and found that the sedimentation rate was increased in proportion to the amounts added. When they added albumin the rate was decreased. Coburn and Kapp⁵ arrived at similar conclusions and added that

⁷ Benjamin, E., v. Sluka, E., *Wien. Klin. Wchnschrift*, 1908, **21**, 311.

⁸ Löwen, 1909, quoted by Hektoen.⁹

⁹ Hektoen, L., *J. Inf. Dis.*, 1915, **17**, 415.

¹⁰ Murphy, J. B., and Sturm, E., *J. Exp. Med.*, 1925, **41**, 245.

¹ Fahareus, R., *Physiol. Rev.*, 1929, **9**, 255.

² Westergren, A., Theorell, H., and Widström, G., *Z. f. d. g. exp. Med.*, 1931, **75**, 668.

³ Westergren, A., Juhlin-Dannfelt, C., and Schnell, R., *Acta Med. Scand.*, 1932, **77**, 469.

⁴ Zarday, I., and Farkas, G., *Z. f. d. g. exp. Med.*, 1931, **78**, 367.

⁵ Coburn, A. F., and Kapp, E. M., *J. Clin. Invest.*, 1936, **15**, 715.