

Effect of Radon-Containing Ointment on Cutaneous Lymph Flow and Minute Vessels of Skin.

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It had been the clinical experience of one of us (E.P.P.) that "Alphatron" Radon Ointment[†] was of value in the treatment of ulcers resulting from previous excessive irradiation. The study here reported is an attempt to explain this effect. For clarity of presentation, the evidence will be presented in the following order: (1) Experiments suggesting that decreased lymphatic absorption, and presumably therefore some degree of lymphatic block, is present following the subsidence of the acute stage of post-irradiation reaction. (2) Experiments indicating that there is hyperemia of the active type in man and in animals following the use of Radon ointment. (3) Experiments showing in man and in normal animals there is increased lymphatic flow in the cutaneous area treated with Radon ointment. (4) Experiments in animals indicating that the active hyperemia and increased cutaneous lymphatic flow in animals is not associated with increased permeability of the capillaries to the colloidal dye Congo red, whereas such increase in permeability does occur following excessive irradiation by x-rays. (5) Result of the application of Radon ointment to cutaneous areas in men in whom local lymphatic block was presumed to have occurred following the injection of penicillin.

The normal human subjects were men between the ages of 22 and 41; the abnormal subjects, who received the penicillin, were men between the ages of 30 and 50 who were being treated for subacute bacterial endocarditis. The animals used were white rats, of either sex, some normal and others,

where stated, having been previously treated with roentgen irradiation totaling 2385 r, given in a single dose. Radon ointment in all cases contained 500 electrostatic units per cubic centimeter, was applied in a film approximately 1 mm thick, covered with oil silk fastened down at the edges by adhesive tape, and left in place for 4 hours when it was removed by gentle wiping with cotton. Control areas were treated similarly but using anhydrous lanolin instead of Radon. Studies were made 24 hours after the application of the Radon ointment, unless otherwise expressly stated.

1. Lymphatic absorption following x-ray irradiation: Lymphatic flow in the skin may be measured either by (a) injecting a colored colloidal dye as patent blue¹ into the skin and measuring the speed and extent of streamer formation, or (b) measuring the amount of time required for complete absorption of a standard amount of dye so injected. The first method, measuring streamers, is well adapted to show increased lymphatic flow, but normal flow is so slow that decreased flow cannot be so demonstrated; the second method is better adapted to demonstrating diminished flow.

Sixteen rats were used that 3 months previously had received roentgen therapy to one leg. Patent blue, 0.04 cc in amount, was injected intracutaneously into the dorsum of both feet, one irradiated, and the other a control. After 24 hours, the dye had completely disappeared from both feet in 6 rats, while in the other 10 it was present on the irradiated but not on the control side.

2. Hyperemia following application of Radon ointment: The antecubital area of the forearm was examined under the capillary

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† Obtained from the Canadian Radium and Uranium Corporation, 630 Fifth Ave., New York City, which also provided funds to defray part of the expense of the study.

¹ McMaster, P. D., *J. Exp. Med.*, 1937, **65**, 347.

microscope² before and 24 hours after the application of Radon ointment, and compared with the other arm prepared as a control. The appearance of the control arm was the same on both days. The treated arm showed an increase in capillary loops of approximately 100% in 7 subjects, along with some increase in subpapillary venous plexus in 5 of the subjects. The color was bright red, not cyanotic, but otherwise it could not be told whether the hyperemia was active or passive. However, the dorsum of the foot of 6 rats, similarly treated and with the opposite leg as a control, indicated quite clearly that the hyperemia was active. In this area, the rat does not open and close cutaneous vessels as does man in the forearm, but usually only about 30% of the vessels show flow. This was true of the control leg on both days and of the treated leg before treatment, but after treatment about 90% of the vessels showed flow, a result very comparable to that obtained 4 or 5 days after lumbar sympathectomy.

3. Lymphatic flow following application of Radon ointment: Radon ointment was applied in the usual way for 4 hours to the antecubital area of one arm in 8 normal men, and 12 hours later 0.04 cc of patent blue was injected intracutaneously into the treated area and into a corresponding area of the control arm. Streamer formation was followed at 5 minute intervals for 15 minutes, and found to be significantly increased on the treated side in 6 cases and not significantly different in 2 cases. Disappearance time was measured in 5 cases, and ranged from 8 to 10 hours, averaging 9 hours, on the treated side, and 24 to 48 hours, averaging 36 hours, on the control side.

Under sodium phenobarbital anesthesia lasting about 24 hours, 14 rats were treated with Radon ointment to one foot using the same dosage and time factors as for the human subjects. However, immediately after the Radon and control dressings were removed, both feet were injected intracutaneously with an equal amount of patent blue. Twenty hours later it was noted that dye

was present in the control foot but not in the treated foot of 7 animals, and had cleared entirely from both feet in the remaining 7 animals. In no case was dye present in the treated leg after the control leg had cleared.

4. Permeability of capillaries to Congo red after the application of Radon ointment: Under sodium phenobarbital anesthesia, Radon ointment was applied to one foot of 8 normal rats, and immediately thereafter 0.3 cc of a 5% solution of the colloidal dye Congo red was injected into a jugular vein. The animals were observed at intervals for 24 hours. At no time was the treated leg any pinker than the control leg or the rest of the body. By contrast, Congo red was injected similarly into 7 animals that had received 2385 r of x-ray irradiation to one leg one month previously. Within 5 minutes the treated leg had become very red, as compared with the control leg and other parts of the body.

5. Four men were available in the medical ward who had received many injections of penicillin. One man in particular had received hundreds of injections over a period of 8 months, with so much resulting discomfort that he was about to refuse further therapy. It seemed likely that the local swollen areas represented areas of local lymphedema due to acute partial lymphatic block from colloidal and particulate matter in the penicillin. Improvement was striking in 12 hours. An arm that had been held rigidly because of a painful swelling near the scapula was freely moveable and the swelling was greatly decreased in size and not tender. Previous therapy, over a period of more than 3 months, with heat, baking, light et cetera had been completely ineffective in relieving the pain and swelling. In all, 12 applications of Radon ointment were given to 4 patients over areas where a painful swelling had appeared following an injection of penicillin. Six control applications were given using either the inactive ointment or Radon ointment from a tube at least 2 weeks old. In every case the control applications were recognized by the patient as being ineffective, and the treatments as effective.

Summary and Conclusions: Evidence is

² Griffith, J. Q., Jr., Roberts, E., and Corbit, H. O., *Am. Heart J.*, 1941, **21**, 47.

presented suggesting that the effect of Radon ointment applied to the skin of men and animals is: (1) To produce active hyperemia. (2) To increase lymph formation and lymph flow without appreciably increasing capillary permeability to colloids. (3) It is suggested that the therapeutic effect of Radon ointment may be apparent in these cases where lymph flow is diminished, per-

haps due to partial block. Evidence is offered suggesting that lymph flow is diminished in a limb that has, in the past, been exposed to excessive x-ray irradiation. Also, the result of treating acute lesions thought to represent partial lymphatic block following penicillin injections is described, with results that are suggestively good although the series is too small for absolute certainty.

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Effects of Glutathione and Thioglycollic Acid on Acid-Production by *Lactobacillus arabinosus* in Presence of Atabrine.

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The action of atabrine as a competitive inhibitor of certain enzyme systems has been investigated by Wright and Sabine¹ and Haas.² The influence of different types of diets upon the toxicity of atabrine for various laboratory animals has been studied by several groups of workers, and a recent article by Siegel, Mushett and Emerson³ reviews the contributions in this field.

Silverman and Evans⁴ reported on the effects of spermine, spermidine, and other polyamines on the growth inhibition of *Escherichia coli* and certain soil organisms by atabrine. These organisms are capable of growth on an inorganic salts-glucose medium and atabrine bacteriostasis is relieved by spermidine. On the other hand, they observed that atabrine bacteriostasis produced in soil organisms with complex nutrient requirements is not relieved by spermidine and related polyamines.

The purpose of this paper is to present data respecting the response of the *Lactobacillus arabinosus* (an organism with relatively

complex nutrient requirements) to atabrine with and without the addition of glutathione and thioglycollic acid to the medium.

Materials and Methods: A strain of *Lactobacillus arabinosus* 17-5 was used. This was supplied by Dr. Jerald G. Wooley of the Division of Physiology, National Institute of Health. The procedure for growth of stock cultures and preparation of culture for inoculation was that of the U. S. Pharmacopeia,⁵ using the technics employed by the Food and Drug Administration. The basal medium and stock solutions were that of the U. S. Pharmacopeia for nicotinic acid assays⁵ except that 0.25 g of bacto-dextrose, 0.20 g of sodium acetate and 0.5 μ g of nicotinic acid were added to each tube, i.e., 10 ml final volume.

A distilled water solution of C.P. atabrine dihydrochloride dihydrate (2.0×10^{-3} M) was added to the tubes in varying amounts in such a range that minimum and maximum bacteriostatic effects were demonstrated. Comparable tubes were set up which contained distilled water solutions of glutathione, Merck, or thioglycollic acid practical, Eastman, in a final concentration of 1.6×10^{-3} molar. In these experiments the pH of the medium was not altered by the addition of

¹ Wright, C. L., and Sabine, J. C., *J. Biol. Chem.*, 1944, **155**, 315.

² Haas, E., *J. Biol. Chem.*, 1944, **155**, 321.

³ Siegel, H., Mushett, C. W., and Emerson, G. A., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58**, 157.

⁴ Silverman, M., and Evans, E. A., *J. Biol. Chem.*, 1944, **154**, 521.

⁵ U. S. Pharm., XII First Bound Supplement, 1943.