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Local Skin Reactivity in Rabbits to an Extract of Ascaris Lumbricoides.

JUI-WU MU. (Introduced by C. N. Frazier.)

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

Since Shwartzman[1] reported that a local skin reaction characterized by pronounced hemorrhagic necrosis could be induced by intravenous administration of the culture filtrate of *B. typhosus* 24 hours after intracutaneous injection of the same filtrate, many workers have confirmed his observation. It has been noted,[2] however, that this local skin reactivity cannot be obtained by intravenous injections of various non-bacterial substances following intradermal preparation with the same material. The present communication deals with the reproduction of this phenomenon with the extract of *Ascaris lumbricoides.*

The extract of *Ascaris lumbricoides* was prepared by putting 4 gm. of the whole ascaris worm in a mixture of one part of Coca's solution[3] and 3 parts of normal saline for 2 weeks, during which time the mixture was shaken from time to time. After the extraction, the mixture was filtered through one layer of ordinary filter paper, and 0.25 cc. of concentrated phenol was added to 100 cc. of the filtrate. Before the extraction, the ascaris worm was washed in running water, immersed in 10% formalin solution for 10 minutes, and then again washed in running water, following which it was dried at room temperature and ground into a fine powder.

Altogether 45 adult male albino rabbits were used, 20 of them being employed as test animals and 25 as controls. Twenty-four hours prior to each experiment, the skin over the flank was shaved and the hair was brushed with only ordinary soap. The test rabbits were injected intradermally with 0.1 cc. of ascaris extract and 24 hours later treated intravenously with 3 cc. of the same extract per kilogram of body weight. The control rabbits were divided into 3 groups. The first group, 10 animals, were injected intradermally with 0.1 cc. of ascaris extract and 24 hours later treated intravenously with 3 cc. of a mixture of one part of Coca's solution and 3 parts of normal saline with 0.25% of phenol per kilogram of

[1] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 560.

[2] Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.

[3] Coca, A. F., and Milford, E. L., *J. Immunol.*, 1925, **10**, 555.

body weight. In the second group, 10 animals were injected with 0.1 cc. of ascaris extract and 24 hours later nothing was given intravenously. In the third group, 5 animals were injected intradermally with 0.1 cc. of a mixture of one part of Coca's solution and 3 parts of normal saline with 0.25% of phenol, and 24 hours later nothing was administered intravenously. All the intradermal injections were given with a sharp tuberculin needle and a tuberculin syringe calibrated to 0.01 cc., and were made equally deep in the epidermis. In the case of test animals and the first group of the control animals, the sites of intradermal injections were examined 24 hours after the intradermal preparation and 4 hours after the intravenous injections. In the case of the second and third groups of the control animals, the sites of intradermal injections were examined 24 hours and 28 hours after the intradermal injections had been given. The skin of the sites of intradermal injections was removed from the test and control animals 28 hours after the preparatory intradermal inoculations for histological study.

Results. Twenty-four hours after the preliminary intradermal injections of ascaris extract, erythematous papules appeared in all test animals and those of the first and second groups of the control animals. The size of this primary reaction varied from 2 to 5 cm. in the test animals, 4 hours after the intravenous injections, the erythematous skin became extremely dark blue and swollen in the center with a deep red zone at the periphery in 8 of them and moderately so in 7 of them. The remaining 5 animals showed no detectable reaction. The discolored skin resembled closely a severe bruise. The intensity and size of the discoloration was not related to the intensity and size of the erythema produced by the preparatory intracutaneous inoculations. Microscopically, the sections of the skin exhibiting frank hemorrhage showed very marked exudation, massive infiltration of polymorphonuclear neutrophil leucocytes, pronounced *endovascular* necrosis, partial obliteration of blood vessels by thrombosis and hyalinization, extensive hemorrhage in the subcutaneous tissues and rupture of blood vessels. Sections of the skin of the negatively reacting rabbits revealed mild edema with slight migration of polymorphonuclear neutrophil leucocytes, and moderate dilatation and congestion of blood vessels. No signs of endovascular necrosis, rupture of blood vessels or hemorrhage were found. Regarding the control animals, no gross alteration was observed in the erythematous papules 4 hours after the intravenous injections in the first group. In the second group, the erythematous papules remained unchanged in appearance, when they were exam-

ined 28 hours after the preparatory intradermal injections. In the third group, no reaction whatever was noticed both 24 hours and 28 hours after the intradermal injections. Microscopically, the sections of the skin removed from the first and second groups of control animals showed about the same changes as those of the negatively reacting test animals. In the sections of the skin of the third group of control animals, there were no abnormal findings except for a mild edema in the subcutaneous tissues.

Summary. Intradermal injection of rabbits with an extract of *Ascaris lumbricoides,* followed 24 hours later by intravenous administration of the same extract, produced hemorrhagic necrosis which grossly and microscopically conformed with that described by Shwartzman.

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Development of Female Characteristics in Adult Male Rabbits Following Prolonged Administration of Estrogenic Substance.

C. N. FRAZIER AND J. W. MU.

From the Division of Dermatology and Syphilology, Department of Medicine, Peiping Union Medical College.

Estrogenic substance was prepared by extracting acidulated human pregnancy urine with butyl alcohol in a continuous extractor as described by Veler, Thayer, and Doisy.[1] The alcohol was removed by distillation, using a vacuum pump, and the residue dissolved in ethyl ether. The ether extract was added to olive oil, and the ether evaporated. The resulting olive oil solution was then assayed for its estrogenic content by the technique of Coward and Burn.[2] The quantity required to produce estrus in 50% of 20 ovariectomized, sexually mature, albino rats constituted the rat unit used in the following experiment.

Twenty-four male albino rabbits of known parentage, 16-17 months of age, were employed. Eight of these were injected subcutaneously once a day, 6 days a week, with from 20-60 rat units of estrogenic substance in olive oil. Injections were continued in 6 animals for 250 days or more. Eight rabbits were injected in like

[1] Veler, C. D., Thayer, S., and Doisy, E. A., *J. Biol. Chem.*, 1930, **87**, 357.

[2] Coward, K. H., and Burn, J. H., *J. Physiol.*, 1927, **63**, 270.