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Usefulness of Organ Emulsions of Infected Animals in Diagnosis of Lymphogranuloma Inguinale.

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To make available a larger supply of L. I.* antigen for clinical and laboratory investigations than could be obtained by the method of Frei,[1] and completely to eliminate the possibility of an occasional false positive intradermal reaction with the use of human material for antigen, we have produced experimental meningo-encephalitis in white mice and monkeys with the virus of lymphogranuloma inguinale, after the method of Hellerström[2] and of Levaditi[3] and his coworkers. The diluted sterilized brain emulsions of both white mice and monkeys (marmosets) have consistently given intradermal reactions, comparable to those obtained with antigens prepared from acute L.I. inguinal buboes after the method of Frei.

The original source of the virus is pus from an acute inguinal bubo in a patient with a positive Frei reaction. The excised lymph nodes must present the characteristic histologic changes of L.I. The lymph gland emulsion may be used in the event that little or no free pus is available. With the finely divided sterile suspension of this material in physiological saline, white mice and monkeys are inoculated intracerebrally. For mice, .01-.02 cc. are used, and for monkeys, 0.2-0.3 cc. The animals employed are the white mouse and the common marmoset (*Hapale penicillata*), which are highly susceptible to infection with the virus of lymphogranuloma inguinale. The mice are killed at the end of one week, their brains being pooled for convenience, but the monkeys are permitted to succumb to diffuse meningo-encephalitis (1 to 2 weeks) or are killed when signs of paralysis develop. Microscopic brain sections show a more or less intense meningeal reaction. Bacteriologic cultures must be consistently negative.

For the preparation of antigen, we employ a 20% sterile brain emulsion in physiological saline. The brain emulsion is heated at 60° C. for 2 hours and at the same temperature for one hour the

*L.I. used in text as abbrebiation for lymphogranuloma inguinale.

1 Frei, W., *Klin. Woch.*, 1925, **4**, 2148.

2 Hellerström and Wassén, *Verh. 8th Intern. Kongr. Dermat. u. Syph.*, Kopenhagen, Aug., 1930.

3 Levaditi *et al.*, *Ann. Inst. Pasteur*, 1932, **48**, 27.

following day. For intradermal tests 0.1 cc. of this antigen is employed and the reaction is read after 48 hours as with the Frei test.

The brain of a single marmoset weighing approximately 6.0 gm. will provide 30 cc. of antigen, sufficient for 300 intradermal reactions. Similarly, a mouse brain weighing approximately 0.3 gm. will furnish 1.5 cc. of antigen, sufficient for 15 reactions. We prefer to employ the monkey brain antigen because it is prepared somewhat more conveniently and the cost is not appreciably greater.

Monkey brain antigens prepared in the manner described above have been tested in 350 patients with uniformly satisfactory results. Mouse brain antigens, employed for approximately 150 intradermal tests, have yielded equally satisfactory reactions. The latter checked consistently with those obtained by the method of Frei. In a consecutive series of 450 intradermal reactions obtained with the use of monkey or mouse brain antigens, prepared according to our method, no more than 32 reactions were equivocal, an incidence of 7%. In the negative reactions the response to the animal brain tissue itself was so insignificant that it was often difficult to detect the site of injection. In patients with active or old healed lesions of lymphogranuloma inguinale, the intracutaneous response was an unmistakable tender area of induration and swelling with a red areola varying in size from 1 to 3 cm.

Emulsions of the abdominal viscera of white mice, prepared one week after intracerebral inoculation with L.I. virus, also possess some antigenic potency. Thus, splenic tissue, injected intradermally as antigen in patients with L.I., will give positive reactions, which are not as strong, however, as those obtained with brain emulsion. Similarly, kidney emulsion will give weakly positive or equivocal reactions, while liver emulsion fails to produce any intracutaneous response. For the diagnostic intracutaneous test for L.I. we prefer to use brain emulsions exclusively.

Conclusion.—Brain emulsions of white mice and of monkeys (*Hapale penicillata*) infected with the virus of L.I. provide a large supply of a uniformly potent antigen for the diagnostic intracutaneous test. There is no danger of contamination with pathogenic organisms, particularly of the spore-bearing type, as there may be when pus from spontaneous buboes in humans is employed directly for the preparation of antigen. Finally, the false positive reactions obtained occasionally with the Frei test are completely eliminated by this method.