for neutralizing antibodies to E.E.E. virus by the intraäbdominal method in 14-day-old mice,[6] using sera of mice of the same ages as controls. The results are shown in Table I.

Thus, by the intracerebral route, the 2-day-old mice showed no immunity, the 10- and 15-day-old resisted 10 minimal cerebral lethal doses, the 30-day and 6-months, strikingly more, *viz.*, 100,000 and 10,000,000 doses, respectively. However, that the 2-day-old mice were not entirely without response to the formolized virus was shown by the development of resistance to 1,000 minimal lethal intraäbdominal doses. Repetition of these experiments yielded similar results.

Of the sera collected prior to immunity test, that of 2-day-old mice showed no neutralizing antibodies. The capacity to form neutralizing antibodies increased with age, from 10 up to 30 days, when a maximum of 100,000 doses neutralized was reached.

Summary. The ability of mice to be immunized by means of formolized virus of Eastern equine encephalomyelitis increases with age, as shown by the strikingly higher resistance of older immunized mice to the intracerebral injection of active virus, as well as by the amount of neutralizing antibodies developed.

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Histology of the Cutaneous Reaction in Guinea Pigs to Purified Brucella Protein.

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Recently, two of us[1] investigated cutaneous hypersensitiveness to Brucella among milkers and cattle handlers using purified Brucella protein (PBP) as antigen. In this paper we describe the histopathology of this test in normal and sensitized guinea pigs.

One group of guinea pigs was sensitized by the intraperitoneal injection, 3 weeks before the test, of 0.2 cc of a suspension of Brucella bacilli obtained by the emulsification of a 48-hour agar culture in 3

[6] Olitsky, P. K., and Harford, C. G., *J. Exp. Med.*, 1938, **68**, 173.

[1] Morales-Otero, P., and González, L. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 703.

cc of normal saline; these animals had all developed cutaneous hypersensitiveness by the time the tests were carried out. Five sensitized and 5 normal guinea pigs were injected intracutaneously in the shaven skin of the abdomen with 3 different dilutions each of PBP prepared as described previously.[2] The dilutions were so made that the injected volume (0.1 ml) of fluid contained 0.1, 0.01 and 0.005 mg of PBP, respectively. As a control, the same animals were injected with a suspension (corresponding to No. 2 McFarland nephelometer) of killed Brucella organisms.

A normal and a sensitized animal were killed at intervals of 6, 12, 24, 48, 72 and 96 hours. The sites of injection were excised so as to include all of the abdominal wall, fixed in Zenker's fluid, embedded in paraffin and stained with hematoxylin and eosin.

The unsensitized animals showed no response grossly; the sensitized ones reacted strongly with erythema and edema at the sites of inoculation, beginning at 6 hours, reaching a maximum at 48 and then regressing slowly.

Histologically, the unsensitized guinea pigs showed, at the site of injection, a slight mainly perivascular infiltration of the derma and subcutaneous fat with eosinophils and neutrophils. The reaction was already at its height 6 or 12 hours after injection and was accompanied by edema with the largest dose only. The outermost intermuscular plane always responded with a leukocytic infiltration predominantly eosinophilic. Beginning at 12 hours, monocytes and lymphocytes replaced the granulocytes, and some fibroblastic proliferation became evident deep in the derma, in the subcutaneous fat and in the outermost intermuscular plane. Qualitatively the histologic response in unsensitized animals was the same with dead bacilli as with PBP. With the latter, the reaction was always strongest with the highest dose, and the results with this dose were quite comparable in kind and degree with those evoked by dead bacilli.

The response provoked by dead bacilli and PBP in sensitized guinea pigs was characterized in all animals and with all doses by a very violent acute inflammatory reaction in the derma and subcutaneous layer at the site of inoculation, extending in some instances to the peritoneum. In the derma and subcutaneous tissue there was, with dead bacilli, very marked infiltration with neutrophils, mainly perivascularly, at 6 hours, but becoming almost massive at 72 hours, which marked the height of the process. Eosinophils were always

[2] Morales-Otero, P., and González, L. M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 703.

very scanty, while edema was very marked. Foci of liquefaction of the exudate without frank abscess formation were evident at this time. At 96 hours, although the reaction was still fairly marked, numerous large mononuclear cells and lymphocytes had made their appearance, and the number of neutrophils was much reduced. The response to Brucella proteins was like that to dead bacilli, qualitatively. In degree, the highest dose of 0.1 mg acted very similarly to dead bacilli. With both inocula there was a very sharp drop in the amount of edema between the 72nd and 96th hours. The tissues in the outermost intermuscular plane responded with edema to a similar degree as the corium and subcutaneous layer, but eosinophils always predominated in these tissues, and lymphocytes and monocytes appeared in numbers at least 24 hours before they did in the other layers. Subperitoneal changes were limited to fibroblastic proliferation without leukocytic infiltration.

Summary. Dead Brucella organisms and Brucella proteins injected intradermally in normal guinea pigs provoke an immediate but mild and evanescent inflammatory reaction with eosinophils and neutrophils, and without edema. In sensitized animals the local reaction is more violent, mainly neutrophilic and accompanied by marked edema. It rises to its height the 3rd day after injection and then rapidly regresses. Qualitatively, the changes provoked by Brucella protein are similar to those induced by dead bacilli.