

oped typical infection characteristic of the respective organisms. Monkeys 3 through 9 likewise became infected after receiving infected blood from different sources maintained at the same temperature for 19 days. Of particular interest were Monkeys 2, 8, and 9, which became infected from 1 cc of blood in spite of the fact that the source monkeys had a maximum of less than one parasite per 10,000 red cells. The incubation periods observed in all of the monkeys were approximately the same as those obtained from comparable inoculations of fresh blood, thus indicating that there is relatively little loss of infectivity by freezing in the manner described. The data at hand indicate that the method as described for the preservation of malaria parasites of monkeys offers a more practical method than animal passage for maintaining strains or species. The possibilities whereby this method may facilitate the study of malaria are apparent.

Summary. A method of preserving monkey malaria parasites for as long as 70 days by rapid freezing, storage at -76°C , and rapid thawing without loss of viability is described.

10954 P

Relation of Age to Immune Response of Mice to Formolized Equine Encephalomyelitic Virus.

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This paper presents a study of the relation of age to the ability of animals to be immunized to a viral disease, as studied by injecting mice with non-infective formolized equine encephalomyelitic virus.¹ The immunity induced was measured by resistance to active virus and by development of neutralizing antibodies.

The capacity to form antibodies to non-infective antigens of non-viral nature is known to increase with age. This has been demonstrated with phenolized typhoid bacilli in guinea pigs and rats,² formolized typhoid bacilli, sheep red cells, horse serum and egg-albumin in rabbits,³ and formolized *Trypanosoma lewisi* in rats. In

¹ Cox, H. R., and Olitsky, P. K., *J. Exp. Med.*, 1936, **63**, 745; Olitsky, P. K., and Morgan, I. M., *J. Am. Vet. Med. Assn.*, 1939, **95**, 530.

² Kligler, I. J., and Olitzki, L., *Z. Hyg. u. Infektionskrankh.*, 1929, **110**, 459.

³ Freund, J., *J. Immunol.*, 1930, **18**, 315.

the last instance the older rats were shown to have developed also a greater resistance to infection.⁴

Age is known to be a factor in the susceptibility of mice to the virus of equine encephalomyelitis, for when virus is injected intraabdominally, young mice are highly susceptible, while old ones are almost entirely resistant. Using the intracerebral route, on the other hand, old and young are equally susceptible.⁵

The vaccine used for immunization¹ was a 10% suspension of brain of mice succumbed to infection with the virus of Eastern equine encephalomyelitis (E.E.E.), in 0.5% formalin. Before formolization the titer of the viral suspension was 10^9 , as tested intracerebrally in mice; after formolization, no active virus was detectable.

Albino mice of Rockefeller-Institute strain were of 5 age-groups, namely, 2-, 10-, 15-, 30- and 180- days, respectively, at the beginning of immunization. Each group consisted of about 20 mice to be injected, with 12 controls of the same age. The 2-day-old group was started with a larger number, since only a small proportion survive the procedure of immunization. 0.2 cc formolized virus was injected intraabdominally on the 1st, 3d, and 5th days of immunization. On the 12th day all mice were bled from the tail and the sera of each group pooled. On the 13th day, treated mice of all age-groups were tested for active immunity by injection of tenfold dilutions (4 mice for each dilution) of active E.E.E. virus. Controls were similarly injected to determine the titer of the virus for each age-group. An additional group of mice 2 days old at the beginning of immunization, as well as untreated controls, were injected by the intraabdominal route. The sera of the immunized mice were tested

TABLE I.
Active Immunity and Serum Neutralizing Antibodies in Mice Immunized with Formolized Equine Encephalomyelitic Virus.

Age of mice at beginning of immunization	No. of intracerebral doses resisted	No. of intraabdominal doses resisted	No. of doses neutralized by serum
2 days	0	1,000	0
10 "	10		>100
15 "	10		10,000
30 "	100,000		>100,000
6 months	10,000,000		100,000

>100 = at least 100 doses.

⁴ Culbertson, J. T., and Kessler, W. R., *Am. J. Hyg.*, 1939, **29**, 33, Sec. C. For a general review of the subject, see Baumgartner, L., *Yale J. Biol. and Med.*, 1934, **6**, 403.

⁵ Sabin, A. B., and Olitsky, P. K., *Proc. Soc. Exp. Biol. and Med.*, 1938, **38**, 597.

for neutralizing antibodies to E.E.E. virus by the intraabdominal 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 intraabdominal 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.

10955 P

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

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Recently, two of us¹ 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.