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Change of sex under these conditions is merely the premature realization of the animal's definitive genetic characteristics.

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PROTECTIVE VACCINATION OF HORSES WITH MODIFIED EQUINE ENCEPH- ALOMYELITIS VIRUS

By serial passage through pigeons a strain of equine encephalomyelitis virus of the eastern type has been so changed that it promises to be of value as a vaccine. The pigeons were inoculated by the intracerebral route, under ether anesthesia, and the brain tissue for passage secured from birds that had just died or were killed when moribund. The virus has been carried through 100 passages, but most of the work to be reported was done with brains from the 40th and 49th serial passages.

In order to secure more material than is provided by the pigeon brain, a young lamb was inoculated intracerebrally with brain from the 40th pigeon passage and another lamb was likewise inoculated with brain from the 49th passage. Both animals promptly developed encephalomyelitis and died. Their brains were preserved in sterile 50 per cent. glycerin and suspensions were made as needed for the experiments. As little as 1 cc of a 10^{-3} dilution of a 10 per cent. suspension of the brain of either lamb injected subcutaneously into guinea pigs would immunize against from 10,000 to 100,000 infective doses of the unmodified virus injected either subcutaneously or intracerebrally. Of 117 guinea pigs inoculated with the 10 per cent. brain suspension, 8, or 7 per cent., died with symptoms of encephalitis and all but 15 of the remainder were immune. The majority of those that were not immune were tested by intracerebral injection of large amounts of virus. Had they been tested by the subcutaneous route they would probably have lived.

Although the modified virus usually fails to produce disease when injected subcutaneously, if it is brought directly into contact with the central nervous system an encephalomyelitis results. Its activity following intracerebral injection is, however, about 100 times less than that of the unmodified virus. Intracerebral passage of the modified virus through a horse, calf, sheep, rabbit, and serially through five guinea pigs has not restored the lost property of invasion of the central nervous system following subcutaneous injection.

Under controlled laboratory conditions 11 horses have been inoculated subcutaneously with suspensions of the lamb brains mentioned above. The majority of the animals were given 10 cc of a 10 per cent. suspension. Not one horse developed a temperature nor

could virus be demonstrated in blood drawn at various intervals after the injection. With the assistance of Dr. J. H. McNeil, state veterinarian for New Jersey, 67 horses were each given subcutaneous injections of 5 cc of the 10 per cent. lamb brain suspension. The inoculations were made in a region where there were many cases of encephalomyelitis, and two of the inoculated animals developed the disease. The virus present in the one brain secured was highly virulent for guinea pigs and was evidently not the strain injected. The other 65 horses showed no reaction to the virus, except that many of those tested as well as all those inoculated at the laboratory developed neutralizing antibodies.

Testing the immunity of horses is a difficult problem because the only certain method of producing disease in these animals is by the intracerebral injection of virus and only a horse with a very high degree of immunity can withstand such an inoculation. Four out of nine vaccinated animals tested by this method showed no temperature reaction or other sign of infection. The other five animals, after an incubation period that was from one to two days longer than that in the controls, developed the disease and died. Two other vaccinated horses inoculated intravenously with virus showed no evidence of disease, but since only one of two controls was infected the results are not conclusive.

In spite of the fact that more than half of our vaccinated horses died from a test intracerebral inoculation, we believe that vaccination with the modified virus will protect against the natural disease. This belief is based on the results of the experiments with guinea pigs and on the facts that vaccinated horses developed neutralizing antibodies and that four horses became so highly immunized that they resisted the intracerebral injection of active virus.

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