result. (6) After a single large dose of dye the reticulo-endothelial cells of liver and spleen are rapidly filled with dye droplets. These disappear or diminish substantially after several days. (7) In addition to tumor and reticulo-endothelial systems of liver and spleen, the kidneys take up considerable quantities of dye, as this is the chief channel of excretion. Accumulation in the kidney might prove to be a complicating factor in the use of radioactive materials, but in the case of induced activity, as with lithium by slow neutrons, appropriate shielding during irradiation should overcome this difficulty. (8) Small repeated doses of dye do not localize in greater quantities than does the same amount given at a single injection. Furthermore, the reticulo-endothelial system is blocked by small repeated doses. (9) X-ray treatment does not appreciably alter the amount of dye localizing in tumors, as determined by histological examination. (10) There is a marked difference in behavior, as regards tumor localization, between the colloidal dyes used and colloidal carbon. (11) Brief preliminary report is made on the localizing properties of some heretofore untested dyes.

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Immunological Reaction of the Hamster to Inoculations with the Virus of St. Louis Encephalitis.*

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The immunological response of mice to inoculation with St. Louis encephalitis virus has been rather extensively studied by Hodes and Webster.[1]

These authors[1] observed that mice inoculated subcutaneously with the virus failed to develop clinical evidences of encephalitis, but quickly developed immunity against subsequent intracerebral inoculation. This immunity, however, was not accompanied by the appearance of virus-neutralizing antibodies in the peripheral blood. Such antibodies were noted some 8 weeks after subcutaneous inocu-

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1 Hodes, H. L., and Webster, L. T., *J. Exp. Med.*, 1938, **68**, 265.

lation at which time, however, immunity to intracranial inoculation was lost.

We have shown[2] that the Syrian hamster *(Mesocricetus auratus)* is susceptible to inoculation with the virus of St. Louis encephalitis. This observation has been confirmed by Lennette.[3] Since the hamster is considerably larger than the mouse, it is possible to secure blood specimens repeatedly from the same animal and so follow the appearance of virus-neutralizing antibodies. The time of appearance of such antibodies is of clinical importance since it may indicate how rapidly virus neutralization tests may be expected to become positive during the course of human infection.

Virus neutralization tests were carried out according to the technic previously described by Broun and Ruskin,[4] which is similar to that of Webster and Fite.[5]

With each series of tests a non-immune serum was used as a negative control and a known immune serum as a positive control. The highest virus dilution in which two-thirds of the animals died in the non-immune serum control was considered as one lethal dose. This usually was in the 1/1,000,000 dilution. A serum which protects two-thirds of the animals against this quantity of virus, but not against greater quantities would be considered to have a titer of one minimal lethal dose protective unit. If a given serum protected two-thirds of the animals in all dilutions including the 1/5,000 virus dilution such a serum would have a titer of 200 minimal lethal dose protective units. No dilutions lower than 1/5,000 were used in the present study. Hence, 200 protective units is not necessarily the maximal titer of the sera studied. This degree of protection is, however, comparable with the more potent human convalescent sera which we have collected from patients who have recovered from St. Louis encephalitis.

Prior to inoculation, the serum of all hamsters so far studied contains no protective antibodies against St. Louis encephalitis virus.

Subcutaneous inoculation of 2 cc of 1/10 dilution of encephalitic mouse brain in no instance has been followed by the appearance of evidence of encephalitis. Several animals in this series died as a result of the cardiac puncture used to secure the blood specimens.

[2] Broun, G. O., Muether, R. O., Mezera, R. A., and LeGier, M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 601.

[3] Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 178.

[4] Broun, G. O., Ruskin, J., *J. Mo. State Med. Assn.*, 1936, **33**, 19.

[5] Webster, L. T., and Fite, G. L., *Science*, 1933, **78**, 463.

Animals inoculated in this manner show prompt and intense development of humoral antibodies. A serum antibody titer capable of neutralizing 200 lethal doses of virus developed in 4 out of 7 animals as early as 48 hours after inoculation and in 3 other animals this level was reached by the third day. None of the animals which were inoculated subcutaneously with virus failed to show at least some protective power in all serum specimens collected after the first 48 hours. Once the high titer of 200 neutralizing doses is reached, it persists in most animals at this level for periods as long as 24 weeks.

All animals reinoculated intracerebrally as long as 24 weeks after subcutaneous inoculation have survived as much as 10,000 lethal doses. Humoral antibodies in a titer of 200 lethal dose protective units were present at the time of this reinoculation in each animal.

Twelve animals were studied after intracerebral inoculation of .06 cc of 1/10 to a 1/1,000,000 dilution of encephalitic mouse brain. All except 2 resistant animals succumbed to the infection within 4 or 5 days. Three of these animals, bled when moribund, show no neutralizing antibodies in their serum. Some of the other animals as early as the fourth day after inoculation developed antibodies of such titer that the serum will protect against from 10 to 200 lethal doses. Even the latter titer does not prevent the fatal termination of the infection.

Two resistant animals were observed which did not succumb to the intracerebral inoculation. One of these animals developed antibodies capable of neutralizing only 10 lethal doses on the eighth day after inoculation. Later this animal showed protection against 100 lethal doses on the sixteenth day after inoculation and against 200 lethal doses on the twenty-first and thirtieth days after inoculation. The animal then died as a result of the cardiac puncture used in securing the blood specimen. In this case the titer of protective antibodies appeared to develop very gradually. A second resistant animal whose serum was not examined until 12 days after inoculation showed at that time 200 protective units. He has maintained this level, with the exception of a temporary drop during the third week for over 12 weeks.

When inoculated intranasally with .06 cc of 1/10 to a 1/1,000 dilution of encephalitic mouse brain 7 out of a group of 9 hamsters succumbed to the infection between the fourth and seventh days. Three animals showed no protective antibodies when bled immediately prior to death as late as the sixth day after inoculation. Other animals from 3 to 7 days after inoculation developed antibodies capable of neutralizing from 10 to 200 lethal doses of virus

and still succumbed to the infection. Two resistant animals survived even though a serum titer of only one and 10 protective units was developed within the first week after inoculation. By the fourth week these animals had developed a titer of 200 protective units and it was maintained for at least 9 weeks at this level with only occasional fluctuations.

We have so far studied only 2 animals after feeding the virus orally. Blood withdrawn from one of these 8 days after the feedings began showed a serum titer of only one protective unit. The animal died as a result of the cardiac puncture. The brain contained no demonstrable virus. The second animal showed a serum titer of 10 protective units on the twelfth and again on the nineteenth day after the feedings began. This animal still survives in good health. It would, therefore, appear that oral feeding can excite some immunological response in these animals although we have not yet seen a high titer of neutralizing antibodies so produced.

By all routes of inoculation all animals which survived as long as 7 days show at least some neutralizing power in their blood serum. In no instance has protective power been completely lost in animals surviving the first week during periods of observation as long as 14 weeks. One animal inoculated intranasally showed 10 protective units 3 days after inoculation but on the fifth day the serum failed to show protective power. The animal died on that day.

Summary. Humoral antibodies against the virus of St. Louis encephalitis can be demonstrated in the sera of hamsters inoculated by subcutaneous, intracerebral, intranasal and oral routes provided the animals survive the infection 7 days or more.

After subcutaneous inoculation, antibodies may appear in the serum within 48 hours after inoculation. They persist at high titer for many weeks. When inoculated intranasally or intracerebrally, some animals fail to develop antibodies prior to death and others which develop a high protective titer still succumb to the infection. Animals dying as long as 6 days after intracerebral or intranasal inoculation may fail to show neutralizing antibodies. No animal inoculated subcutaneously has failed to show humoral antibodies after the second day. The presence of humoral antibodies does not insure immunity against the disease.