14034

Placental Transmission of Immunity to St. Louis Encephalitis Virus Inoculated Intraperitoneally in Mice.*

MARGARET G. SMITH. (Introduced by R. A. Moore.)

From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.

The resistance of young mice born of mothers vaccinated with the active virus of St. Louis encephalitis was tested to determine whether any transference of immunity for this virus occurred *in utero* in mice, and also to determine what the relation between intracerebral and intraperitoneal resistance might be in young immune animals. Since the mouse placenta is of the hemochorial type,[1] the transfer of humoral antibody from the immunized mother might be expected to occur.[2] The blood of adult mice immunized by a single subcutaneous inoculation of the active virus of St. Louis encephalitis contains demonstrable neutralizing antibody as early as 1 week following vaccination, although the degree of resistance which develops to the intracerebral inoculation of the virus is not great under these circumstances.[3]

It is only in young mice that the relation between specific intracerebral and intraperitoneal immunity to the virus of St. Louis encephalitis might be demonstrated, since after 2 weeks of age mice develop an increasing natural resistance to the intraperitoneal inoculation of the virus. Adult mice can be infected by the intraperitoneal route only when extremely large doses of virus (10 million minimal intracerebral lethal doses) are given. On the other hand, young and adult mice are equally susceptible to the virus of St. Louis encephalitis when inoculated intracerebrally.[4] With the strain of the virus used in these experiments the highest infective dilution of mouse brain emulsion in broth when inoculated intracerebrally is approximately 10^{-8} for both young and adult mice.

In each of 2 experiments a group of 50 female Swiss mice was immunized by 2 subcutaneous inoculations of active St. Louis encephalitis virus given at weekly intervals. Each group of mice was 2 months of age when the first subcutaneous inoculation of virus was made. The inoculation consisted of .5 cc of a 10^{-3} dilution in broth of an infected mouse brain emulsion. The vaccinated mice were used for mating 3 weeks following the last subcutaneous inoculation. This period was allowed to elapse to lessen the possibility of active virus being transferred to the young *in utero*. Webster[5] was unable to demonstrate the virus of St. Louis encephalitis in the blood of mice later than 5 days following a subcutaneous inoculation, although virus was regularly demonstrated in the spleen for 4 days following the inoculation, and on rare occasions over periods as long as 30 days.

In one experiment the resistance to intraperitoneal and intracerebral inoculation of active virus of 32 young mice, 7 to 9 days of age, born of immune mothers, and in another experiment the resistance of 34 young mice, 13 and 14 days of age, born of immune mothers, was compared with that of control groups of mice of the same age (Table I). Both the control mice and those born of immunized mothers included male and female animals. Each group consisted of 6 or more litters.

In both experiments the young mice of immunized mothers succumbed to a dilution of 10^{-7} of the infective material inoculated intracerebrally in .03 cc amounts, a susceptibility approximately the same as that shown by the control animals. They were

* Sponsored by the National Foundation for Infantile Paralysis, Inc.

1 Grosser, Otto, Munchen, 1927, J. F. Bergmann.

2 Mossman, H. W., *Am. J. Anat.*, 1926, **37**, 433.

3 Smith, M. G., to be published.

4 O'Leary, J. L., Smith, M. G., and Reames, H. R., *J. Exp. Med.*, 1942, **75**, 233.

5 Webster, L. T., and Clow, A. D., *J. Exp. Med.*, 1936, **63**, 433.

TABLE I.
Resistance of Young Mice Born of Immune Mothers and of Control Young Mice to Virus Inoculated Intraperitoneally and Intracerebrally.

Route of inoculation	Dilution of virus	Mice born of immune mothers, 13 day old	Control mice, 14 day old	Mice born of immune mothers, 7-9 day old	Control mice, 7-9 day old
I.P.	10^{-1}	2/6	—	5/5	—
	10^{-2}	1/4	—	5/5	—
	10^{-3}	0/4	4/4	1/5	3/3
	10^{-4}	0/4	4/4	0/5	4/4
	10^{-5}	—	4/4	0/5	4/4
	10^{-6}	—	4/4	—	1/3
Infective dilution	(50% end point)	$<10^{-1}$	$>10^{-6}$	$10^{-2.6}$	$10^{-5.8}$
		14 day old	14 day old	7-9 day old	7-9 day old
I.C.	10^{-4}	4/4	—	—	—
	10^{-5}	4/4	—	—	—
	10^{-6}	4/4	4/4	3/3	5/5
	10^{-7}	4/4	4/4	4/4	4/4
	10^{-8}	—	3/4	—	1/4
Infective dilution	(50% end point)	$>10^{-7}$	$>10^{-8}$	$>10^{-7}$	$10^{-7.7}$

Numerator—No. of deaths.
Denominator—Total No. of mice injected.

not tested with higher dilutions. In each experiment the control mice succumbed to dilutions of approximately 10^{-8} of the infective material ($10^{-7.7}$ and 10^{-8} respectively) (Table I).

On the other hand, in each of the 2 experiments, when inoculated intraperitoneally rather than intracerebrally, the young of immunized mothers were resistant to considerable amounts of virus as compared with control young mice inoculated in the same manner. The two groups of mice born of immunized mothers succumbed to dilutions of 10^{-1} and $10^{-2.6}$ respectively, while the control groups succumbed to dilutions of 10^{-6} and of $10^{-5.7}$ respectively (Table I), inoculated in .25 cc amounts.

Morgan[6] has obtained results similar to ours in young mice actively immunized during the first 2 weeks of life by vaccination with the formol-treated virus of Eastern equine encephalomyelitis. In her experiment mice tested between 10 and 15 days of age were fully susceptible to the virus of Eastern equine encephalomyelitis given by the intracerebral route although they resisted large doses of virus by the intraperitoneal route. The lack of resistance to virus inoculated intracerebrally was attributed to the fact that the amount of humoral antibody present was too small to reach the central nervous system in an effective concentration. If the difference in the protection afforded when the virus is inoculated intraperitoneally rather than intracerebrally in both our experiments and in those of Morgan is dependent upon a hematoencephalic barrier which does not allow the passage of antibody into the brain, this barrier must be well developed in mice as early as 7 to 9 days of age. However Stern and Peyrot[7] have demonstrated that the hematoencephalic barrier of the mouse, as judged by the passage of sodium ferrocyanide into the brain and the spinal fluid, is not fully developed until 12 to 15 days of age. They point out that the full development of this barrier is coincident with the opening of the eyes in several species of animals.

Olitsky and Harford[8] have demonstrated a difference in the protection against Eastern equine encephalomyelitis when the inoculations are made by different routes. This phenomenon was elicited by the injection of immune serum-virus mixtures by the intraperitoneal and intracerebral routes. They found the injection of young animals by the

[6] Morgan, I. M., *J. Exp. Med.*, 1941, **74**, 115.

[7] Stern, L., and Peyrot, R., *Compt. Rend. Soc. de Biol.*, 1927, **96**, 1124.

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

intraperitoneal route to be a more sensitive test than injection by the intracerebral route for the recognition of protective antibody in the serum-virus mixture. This "selective protection" is another possible explanation for the difference in the resistance shown by both actively and passively immunized young mice to virus inoculated intraperitoneally as compared to that inoculated intracerebrally.

Conclusions. Placental transmission of specific immunity to the virus of St. Louis encephalitis has been demonstrated. Mice between 7 and 9 days of age and 13 to 14 days of age born of mothers immunized by the subcutaneous inoculation of active virus show no increased resistance to the virus of St. Louis encephalitis inoculated intracerebrally but are more resistant than control mice of the same age to virus inoculated intraperitoneally.

14035

Action of Certain Drugs on the Sea-Star, *Asterias forbesii.*

WM. A. HIESTAND.

From the Laboratory of Animal Physiology, Purdue University, and the Marine Biological Laboratory, Woods Hole, Mass.

Moore[1,2,3] has shown that strychnine causes reversal of reciprocal inhibition in the sea-star resulting in dorsiflexion of the rays. My own observations bear out his contentions, although it should be stated that lateral flexure of the rays occurs as well as dorsiflexure. The tube feet are rendered useless by immersion of the sea-star in sea water containing 1:10,000 parts of strychnine sulfate and when placed dorsal side down strychninized sea-stars are unable to right themselves due to the loss of reciprocal inhibition as well as loss of adhesion of the distal cups of the tube feet. The animals suffer no permanent effects of the strychnine even after remaining immersed for as long as 36 hr in .01% strychnine sulfate solution for they will recover normality when placed in running sea water for a few hours. Sea-stars placed in weak solutions of atropine sulfate (.01%) promptly show signs of irritability. The tube feet become greatly extended and the rays become curled in all directions though chiefly dorsally. If one observes sea-stars under the influence of atropine one soon realizes that no particular posture is characteristic of this drug. Recovery is complete after the animals are returned to fresh sea water.

Caffeine produces two types of effects, (1) an early period of excitement with extension of the tube feet and (2) a later effect of paresis of the tube feet musculature in which state the feet are slowly retracted and remain entirely motionless in rigor. Recovery is complete in animals previously immersed in solutions of caffeine when washed with running salt water. If however, sea-stars paralyzed in rigor with caffeine are placed in a solution of .02% picrotoxin the rigor is promptly abolished with the tube feet becoming extended and very active. Thus picrotoxin abolishes (reverses) caffeine rigor.

Sea-stars placed in a .02% solution of picrotoxin show prompt extension of the tube feet with intense waving motion. No dorsiflexion results but locomotion is greatly accelerated, becoming many times faster than normal. When inverted the animal promptly rights itself indicating that nervous coordination has not been diminished. Recovery from picrotoxin is complete if the animals are placed in running sea water. If picrotoxinized sea-stars are placed in a solution of .02% caffeine the motion of the tube feet

[1] Moore, A. R., *J. Pharmacol. Exp. Ther.*, 1916, **9**, 167.

[2] Moore, A. R., *J. Gen. Physiol.*, 1918, **1**, 97.

[3] Moore, A. R., *J. Gen. Physiol.*, 1920, **2**, 201.