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Experiments on Maternal Transmission of Creutzfeldt-Jakob Disease in Guinea Pigs[1] (40425)

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Four diseases caused by unconventional viruses, Creutzfeldt-Jakob disease and kuru of man, scrapie of the sheep, and transmissible mink encephalopathy have been grouped together as subacute spongiform virus encephalopathies (1). Creutzfeldt-Jakob disease has been successfully transmitted in this laboratory to convenient laboratory animals, such as guinea pigs (2, 3), hamsters (4, 5), and mice (6). Using guinea pigs as an experimental model a study was undertaken to investigate whether maternal transmission of this disease occurs in this species.

Materials and methods. The Creutzfeldt-Jakob inoculum for maternal and paternal injection consisted of a 10% suspension of brain in physiological saline from one guinea pig that developed the disease during the second serial passage. The guinea pig had the characteristic histological features of Creutzfeldt-Jakob disease.

Nine female and 3 male young healthy guinea pigs, weighing 250–350 g, from the Yale stock of the Hartley strain, were inoculated on May 3, 1974 with 0.1 ml intracerebrally and 0.2 ml intraperitoneally of the 10% brain suspension. Three female and one male guinea pigs were kept together in three separate cages. The subsequent litters born to the inoculated parents were kept in the same cages with their mothers. Parents and offspring were inspected almost daily for clinical signs of the experimental disease.

Four female guinea pigs inoculated with 10% suspension of normal guinea pig brain with identical amounts and routes of inoculation served as controls to the infected parents.

Results. The three male guinea pigs performed successfully their pleasant task and all 9 females became impregnated and gave birth to litters at various times after inoculation (Table I). All parents developed clinical signs of the disease and they were killed when moribund; in all 12 guinea pigs the diagnosis of Creutzfeldt-Jakob disease was confirmed by histological examination. The paternal and maternal incubation times (Table I) varied between 193 and 224 days with an average of 211.79 ± 2.67 (SEM).

A total of 24 guinea pigs (litters I–IX) were born to the nine inoculated females (Table I). The birth of the offspring in various litters occurred between 42 days for litter I, to 209 days for litters VIII and IX, after maternal inoculation. Knowing the exact day of the maternal inoculation (5/3/74), the date of birth of the litters (Table I), and the gestation period in guinea pigs which is 59–67 days with an average of 65 days, one can calculate the gestational days during which the embryos were exposed to the maternal infection. Accordingly, litter I which was exposed for 42 gestational days to the maternal infection was conceived roughly 3 weeks prior to the maternal inoculation, and the only pig of litter II which was exposed for 56 gestational days to the maternal infection was conceived approximately 9 days prior to her inoculation. Litters III and IX which were born between 90 and 209 days after the maternal inoculation were exposed to the infection of their mothers for the whole period of gestation.

None of the 24 guinea pigs born to the infected mothers developed any clinical signs characteristic of experimental Creutzfeldt-Jakob disease. Some have lived as long as 1213 days (>3 years old). Six offspring guinea pigs were found dead without any clinical signs (Table I). In all these guinea pigs autopsy revealed changes consistent with death from causes unrelated to Creutzfeldt-Jakob disease, e.g. pneumonia. They were 758 days old (litters II), 340 and 435 days old (litter III), 179 and 735 days old (litters V and VI), and 258 days old (litters VII). Of the six guinea pigs found dead, in two guinea pigs autolysis prevented histological evaluation of the brain

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TABLE I. EXPERIMENTS WITH MATERNAL TRANSMISSION OF THE DISEASE.[a]

Litter	Animal	Date of birth	Days born after maternal inoculation	Gestational days exposed to maternal infection	Age of offspring when sacrificed or found dead	Spongiform enceph.
I.	1	6/14/74	42	42	350/s.	–
	2	6/14/74	42	42	1213/s.	–
II.	1	6/28/74	56	56	758/f.d.	a.
III.	1	8/1/74	90	65	340/f.d.	a.
	2	8/1/74	90	65	435/f.d.	–
	3	8/1/74	90	65	438/s.	–
	4	8/1/74	90	65	438/s.	–
IV.	1	8/30/74	119	65	409/s.	–
V. and VI.	1	9/18/74–9/20/74	139	65	179/s.	–
	2	9/18/74–9/20/74	139	65	179/f.d.	–
	3	9/18/74–9/20/74	139	65	735/f.d.	–
	4	9/18/74–9/20/74	139	65	745/s.	–
	5	9/18/74–9/20/74	139	65	1113/s.	–
	6	9/18/74–9/20/74	139	65	1113/s.	–
VII.	1	11/1/74	182	65	258/f.d.	–
	2	11/1/74	182	65	1034/s.	–
	3	11/1/74	182	65	1071/s.	–
VIII. and IX.	1	11/26/74–11/28/74	209	65	937/s.	–
	2	11/26/74–11/28/74	209	65	1044/s.	–
	3	11/26/74–11/28/74	209	65	1044/s.	–
	4	11/26/74–11/28/74	209	65	1044/s.	–
	5	11/26/74–11/28/74	209	65	1044/s.	–
	6	11/26/74–11/28/74	209	65	1044/s.	–
	7	11/26/74–11/28/74	209	65	1045/s.	–

[a] Paternal–Maternal: ic and ip Inoculation 5/3/74. Animal #/Paternal–Maternal Incubation in days: #1 = 193, #2 = 203, #3 = 207, #4 = 207, #5 = 208, #6 = 208, #7 = 213, #8 = 215, #9 = 217, #10 = 223, #11 = 223, #12 = 224. a. = autolysis, – = negative, f.d. = found dead, s. = sacrificed.

(litter II, guinea #1 and litter III, guinea pig #1). In the remaining four guinea pigs found dead and in the 18 guinea pigs sacrificed, none of the microscopic features characteristic for Creutzfeldt-Jakob disease, namely neuronal destruction, astrocytosis and spongy changes of the neuropil, were found.

None of the four control guinea pigs inoculated with normal guinea pig brain developed any clinical signs and any microscopic findings characteristic of Creutzfeldt-Jakob disease.

Discussion. The present experiments indicate that there is no maternal transmission of Creutzfeldt-Jakob disease in guinea pigs. The embryos of the inoculated mothers were for all practical purposes exposed to the maternal infection during the whole period of gestation. In this context note should be taken of the occurrence of viremia in guinea pigs intracerebrally inoculated with the Creutzfeldt-Jakob agent, as data from this laboratory have shown (7). Some of these periods of viremia coincide with the gestational period of the offspring studied here. There are several possible pathways which could have been operative in maternal transmission, such as via the maternal circulation or transplacental route, yet in the present experiments there is evidence of no vertical transmission of Creutzfeldt-Jakob disease from the infected mothers to the embryos.

Our present data also strongly suggest that in experimental Creutzfeldt-Jakob disease there is no lateral transmission of the infection, via lactational routes, from contagion, through direct contact, or via urine and feces. In the present experiments the offspring were housed, as stated, at all times in the same cages as the infected mothers. Some of the

progeny were kept alive and in good health up to senility. Published data in scrapie research indicate lateral transmission from sheep to sheep (8–10), from sheep to goats (11–13), and from mice to mice (14–16). No transmission of scrapie by suckling (lactational route) in sheep (17) and no scrapie agent in urine and feces has been demonstrated (14, 18).

No maternal and no lateral transmission of Creutzfeldt-Jakob disease has been observed in non-human primates (Carleton Gajdusek, personal communication). Furthermore, 300 children born to mothers with kuru disease and observed over several years showed no evidence of maternal transmission of this disease (19). There are however, several investigations on the maternal transmission of scrapie and the results of these studies are contradictory. Maternal transmission has been reported in both natural (8, 9) and experimental scrapie in sheep (17, 20). In subsequent studies it has been demonstrated that the scrapie agent is present in the placenta of scrapie affected sheep (21, 22). In experimentally infected goats, no transmission of scrapie has been found from the infected mothers to their offspring (14). Despite early reports that scrapie can be maternally transmitted to mice (23, 24), all subsequent studies claim the absence of maternal transmission in this species (25–27).

It has been reported that susceptibility to scrapie in mice infected by the intraperitoneal route depends upon the developmental maturation of the host; younger mice are less susceptible (28). These observations, were considered to possibly explain maternal transmission of scrapie in sheep, and its absence in mice, "because sheep are born with more mature lymphoreticular responses" which are absent in newborn mice (28). By applying age equivalence to available data on the first appearance of small lymphocytes, onset of transplantation immunity, γ M-globulin and antibody formation, the onset of immunocompetence occurs at the same stage of physiological development in all mammals (29). Accordingly, guinea pigs and sheep, unlike smaller rodents (mice and hamsters), become immunocompetent during gestation while smaller rodents develop a large portion of their immunological systems after birth. On this basis, guinea pigs should thus be expected, as sheep, to display maternal transmission of Creutzfeld-Jakob disease. Similarly goats with gestation period of 148–156 days by age equivalence (29) should also show maternal transmission of scrapie, if this developmental immunocompetence is a critical factor in such transmission. In fact neither guinea pigs nor goats do show evidence of maternal transmission of Creutzfeldt-Jakob disease and scrapie, respectively, and factors other than or additional to "lymphoreticular responses" may be of importance.

Summary. No maternal transmission of Creutzfeldt-Jakob disease was observed in offspring born to guinea pigs at various times after intracerebral and intraperitoneal inoculation with the Creutzfeldt-Jakob agent. The offspring were exposed to the maternal infection during virtually the entire period of gestation. There was also no evidence of lateral transmission of the infection to the offspring. All inoculated parents died with histologically verified subacute spongiform virus encephalopathy.

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1. Gibbs, C. J. Jr., and Gajdusek, D. C., J. Clin. Pathol. **25** (Suppl. 6), 84 (1972).
2. Manuelidis, E. E., Science **190**, 571 (1975).
3. Manuelidis, E. E., Angelo, J. N., Kim, J. H., and Manuelidis, L., Proc. Nat. Acad. Sci. (U.S.A.) **73**, 223 (1976).
4. Manuelidis, E. E., Angelo, J. N., Gorgacz, E. J., and Manuelidis, L., Lancet **1**, 479 (1977).
5. Manuelidis, E. E., Gorgacz, E. J., and Manuelidis, L., Proc. Nat. Acad. Sci. (U.S.A.) **75**, 3432 (1978).
6. Manuelidis, E. E., Gorgacz, E. J., and Manuelidis, L., Nature (London) **271**, 778 (1978).
7. Manuelidis, E. E., Gorgacz, E. J., and Manuelidis, L., Science, **200**, 1069 (1978).
8. Dickinson, A. G., Young, G. B., Stamp, J. T., and Renwick, C. C., Heredity **20**, 485 (1965).
9. Dickinson, A. G., Young, G. B., Stamp, J. T., and Renwick, C. C. U.S. Department of Agriculture, ARS 91-53, p. 223 (1966).
10. Brotherston, J. C., Renwick, C. C., Stamp, J. T., Zlotnik, I., and Pattison, I. H., J. Comp. Pathol. **78**, 9 (1968).
11. Stamp, J. T., Vet. Rec., **74**, 357 (1962).
12. Stamp, J. T., Scrapie Seminar, U.S. Department of

Agriculture, ARS 91, p. 187 (1966).
13. Stamp, J. T., Brit. Med. Bul. **23,** 133 (1967).
14. Pattison, I. H., Vet. Rec. **76,** 333 (1964).
15. Dickinson, A. G., Mackay, J. M. K., and Zlotnik, I., J. Comp. Pathol. **74,** 250 (1964).
16. Morris, J. A., Gadjusek, D. C., and Gibbs, C. J. Jr., *in* "Slow, latent and temperate virus infections." (D. C. Gadjusek, C. J. Gibbs, Jr., and M. Alpers, eds.) NINDB Monograph No. 2., Washington, D.C., p. 273 (1965).
17. Dickinson, A. G., Young, G. B., and Renwick, C. C., Scrapie Seminar, U.S. Department of Agriculture, ARS 91-53, p. 244 (1966).
18. Pattison, I. H., and Millson, G. C., J. Comp. Pathol. **72,** 233 (1962).
19. Masters, C. L., Harris, J. O., Gajdusek, D. C., Gibbs, C. J., Bernoulli, C., and Asher, D. M., Ann. Neurol., in press.
20. Gordon, W. C., Scrapie, U.S. Department of Agriculture, ARS 91-22, p. 1 (1960).
21. Pattison, I. H., Hoare, M. N., Jebbett, J. N., and Watson, W. A., Vet. Rec., **90,** 465 (1972).
22. Pattison, I. H., Hoare, M. N., Jebbett, J. N., and Watson, W. A., Brit. Vet. J. **130,** 65 (1974).
23. Eklund, C. M., Hadlow, W. J., and Kennedy, R. C. Proc. Soc. Exp. Biol. Med. **112,** 974 (1965).
24. Gibbs, C. J. Jr., Gajdusek, D. C., and Morris, J. C., *in* "Slow, latent and temperate virus infections." (D. C. Gajdusek, C. J. Gibbs, Jr., and M. Alpers, eds.), NINDB Monograph No. 2., Washington, D.C., p. 195 (1965).
25. Dickinson, A. G., Lancet **1,** 1166, (1967).
26. Field, E. G., and Joyce, G., Nature (London) **226,** 971 (1970).
27. Clarke, M. C., and Haig, D. A., Brit. Vet. J., **127,** 32 (1971).
28. Outram, G. W., Dickinson, A. G., and Fraser, H., Nature (London) **241,** 536 (1973).
29. Solomon, J. B., *in* "Foetal and neonatal immunology" (Frontiers of Biology, A. Neuberger, and E. L. Tatum, eds.), p. 341, Elsevier Publishing Co., N.Y. (1971).

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