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PATHOGENESIS OF VACCINE STRAINS OF MEASLES VIRUS IN SUCKLING HAMSTERS (*)

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Summary. – Four strains of attenuated measles virus were isolated in Vero cells from vials of vaccines manufactured in different countries. Three out of four strains were originally derived from three different virulent measles viruses. While all these strains were harmless for suckling mice they were more or less pathogenic when inoculated intracerebrally into 1 – 2 days old hamsters. Limited passage history of these viruses and the growth curve of one strain showing a higher titre in baby hamsters are described.

During a routine control of the safety of a live attenuated measles vaccine it was found that while intracerebral inoculation of virus in monkeys, guinea pigs and adult and suckling mice performed according to the minimum requirements of W.H.O. (WHO Expert Committee 1966) revealed no signs of illness and showed no histological changes in brain tissue some suckling hamsters inoculated at the same time with the same material exhibited two weeks later signs of encephalitis and measles virus was recovered from their brains. As a result of this finding a second study was begun on four vaccine strains isolated from vials of live attenuated measles vaccine used in immunization of children since several years in different regions of the world. In this report these strains are called A, B, C and D.

The viruses were isolated in Vero cells grown in Melnick medium supplemented with 5% calf serum and had undergone 3 passages in this cell line before use. Litters of five to seven suckling hamsters 1–2 days old originally received from the Grifith farm, State Laboratories of Health, Albany (N.Y., U.S.A.), were injected intracerebrally with 0.02 ml of fluid from the 3rd passage of each virus strains. The titres of all viruses were adjusted to $10^{3 \cdot 5}$ TCID50/ml before use. Seven to 15 days after inoculation, stunded hamsters were observed in each litter. The animals were killed, their brains removed, pooled and stored frozen at—65° C until used. For use, the brains were thawed and homogenized in chilled glass grinders to make a 10% suspension in cold phosphate saline

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(PBS) pH 7.2, containing 2% foetal calf serum, 200 units penicillin and 200 ug kanamycin per ml. Suspensions were centrifuged at low speed at 4°C for 10 minutes. The clear supernatant was used as infectious material for further passage in suckling hamsters or for titration in Vero cells.

The signs of illness in baby hamsters were incoordination and inability to right themselves after being turned on their back. Death normally occurred 1 to 2 days after onset of sickness, sometimes the animals recovered from the disease. The susceptibility of the hamsters was dependent on the age of animal. While baby hamsters 1—2 days old died of infection in nearly 100% after inoculation of strain D at its 8th intracerebral passage, hamsters five days old or older failed to develop any sign of illness.

To study the growth curve in hamster brain 9 litters of 5—7 suckling hamsters were injected intracerebrally with 0.02 ml of a 10% suspension of infectious hamster brain at the 8th serial passage of strain D. At intervals hamsters of one litter were killed, their brains aseptically removed pooled and stored frozen at—65° C until used. For virus titration a 10% suspension of each harvest was prepared as mentioned above and 0.1 ml of its tenfold dilutions was inoculated into replicate cultures of Vero cells in tubes. After an adsorption period of 1 hour at 35° C 1 ml of maintenance medium similar to the growth medium but containing 2% calf serum was added. The cultures were incubated at 35° C for 5 days. At this time the cytopathic effect (CPE) of virus in Vero cells was recorded.

Table 1. Intracerebral passage of 4 attenuated measles vaccine strains in suckling hamsters

Strain	Passage No.	Infection ratio*	Incubation (days)	Titre in Vero cells log TCID50/ml
A	1	1/5	15	1.0
	4	2/6	12—14	1.7
B	1	1/4	15	1.5
	4	1/3	10—14	2.7
C	1	1/5	12	2.7
	4	1/3	10—14	2.3
D	1	2/7	9—12	2.7
	4	3/4	7—10	3.5
	5	4/4	6—8	5.0
	8	5/5	4—6	5.0

* Numerator: animals either dead of infection or killed when moribund; denominator: total No. of animals inoculated.

The passage history and results of titration of brains suspensions in Vero cells are summarized in Table 1. Only 4 passages of each strain in suckling hamster were done. This experiment was repeated but no signs of illness were noticed in baby hamsters inoculated with strain A and virus was not isolated from these hamsters; 1 of 5 baby hamsters inoculated for the second time with strain B became sick after 17 days and measles virus was recovered. Regarding strains C and D the results of the second experiment were nearly similar to the first one. Since the latter strains were originally derived from the same virus we selected strain D for some further intracerebral passages in suckling hamsters.

The central nervous system disorders in this animal were more evident after 8 passages when the incubation period also was reduced to 4 days. A section 2 *u* thick of brain fragment from a baby hamster at the 9th passage killed 7 days after inoculation was stained with haematoxylin and eosin. The main characteristic feature of disease was a degenerative process along with formation of Warthin-Finkeldy type giant cells. The CPE characteristic of measles infection was seen in Vero cells by the second day for all strains passed in hamster brain except for strain A showing a CPE after 8 days. No CPE was observed when each of the 4 strains was mixed before seeding with appropriate dilution of known monkey antimeasles serum. Results concerning the growth curve (Fig. 1) showed an eclipse phase not exceeding 24 hours followed by

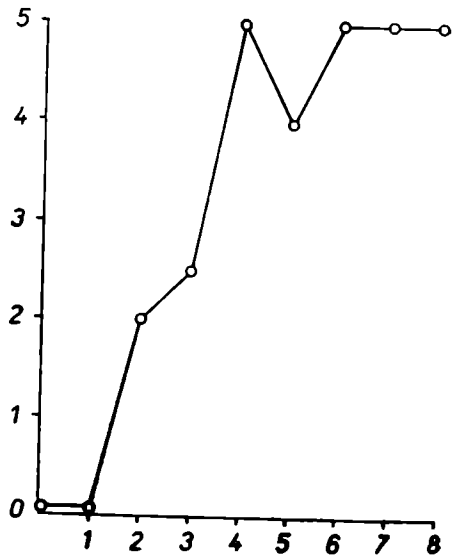

Fig. 1.

Growth curve of attenuated measles virus (strain D) in baby hamster brain
Abscissa: days after inoculation; ordinate: log TCID50 per ml hamster brain suspension

formation of new progeny of virus. Maximal titre was reached on the 4th day when encephalitis signs were also recorded.

The present data support the finding of other workers who adapted virulent (Burnstein *et al.*, 1958, 1964) or neurotropic measles virus isolated from subacute sclerosing panencephalitis cases (Byington *et al.*, 1970) to hamster brain. To our knowledge the attenuated measles vaccine strains have not so far been adapted to hamster brain. Recently however Fred Rapp (pers. comm.) adapted a vaccine strain intracerebrally in suckling hamsters. It is worth mentioning that the dissimilarity of the clinical and pathogenic characteristics of the hamster infection from those observed in children showing encephalitis after natural infection would seem to make the analogy between the infection of hamster and human rather difficult but the ease of neuro-adaptability of attenuated vaccine strains in the hamster system and frequent incidence of convulsion in children immunized with some vaccine strains (Anonymous, 1969*a*, *b*) is of particular interest. In assessing the significance of the adaptability of various measles vaccine strains to suckling hamster brain it is too premature to discredit any of the existing measles vaccine strains but one might more reasonably conclude that the hamster model may be useful for a better approach to the problem of post-vaccinal nervous system disorders.

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