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THERMAL STABILITY OF AFRICAN HORSE SICKNESS VIRUS (*)

(Brief Report)

By

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With 2 Figures

In 1964, we described (1, 2) a method for the preparation of culture-adapted polyvalent African Horse Sickness (AHS) live vaccine in monolayers of two cell lines of monkey kidney (MS) and baby hamster kidney (BHK 21). This vaccine has already been used on a large scale in some North African States where outbreaks of AHS were reported. It is the purpose of this paper to report the study conducted on the resistance of fluid or lyophilized AHS vaccine to various temperatures in order to ensure the validity of the vaccine used in tropical areas.

Monovalent vaccine was prepared in MS cells with type 9, strain S2 (Shiraz) as described previously. Thin-walled, 5 ml. ampoules containing 1 ml. of this vaccine were sealed and placed in a refrigerator at 4° C or in water baths at 25° and 36° C. At selected intervals, ampoules were removed and their contents chilled and assayed. Plaque technique was used according to our previous report (3). Plaques, however, were stained on the fifth day with a solution of tetrazolium salt as suggested by *Cooper* (4).

The tissue culture infective dose (TCID50/ml.) of these vaccines was tested in MS cells. For each dilution of vaccine two MS tissue culture tubes were used. All vaccine titers are expressed as the reciprocal of the dilution of vaccine which showed cytopathic effect in one MS tube inoculated.

Results

Monovalent Vaccine

Results of the comparative titration of AHS virus type 9, strain S2 at 4°, 25° and 36° C are shown in Fig. 1. While no significant change appeared in titer after forty days of keeping virus suspension at 4° C, there was, however, a fall of

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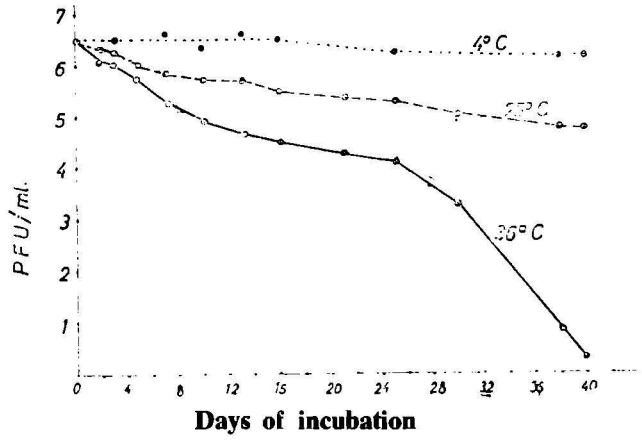

Fig. 1. Inactivation of African horse sickness virus at various temperatures.

Fig. 2. Plaques in monkey kidney cell line produced by AHSV, 5 days after seeding.

two logs in titer for virus kept at 25° C and a nearly complete inactivation for virus maintained at 36° C for the same period of time. Another point of interest is the stability of plaque size during thermal inactivation. Suspension of virus kept for twenty-four days at 36° C showed minute plaques of less than 1 mm. and large plaques of 2—5 mm. (Fig. 2). This is in agreement with our previous finding for all 8 types of AHS viruses freshly harvested from MS tissue culture.

Polyvalent Vaccine

The rate of decline in tissue culture titer of five batches of lyophilized vaccine at 4° C are summarized in Table 1. The initial titers were nearly preserved for over nine months, and the fall of titer was not more than two logs after eighteen months of storage. Two batches of polyvalent fluid vaccine were also stored at +4° C. After six months of storage, these vaccines lost 50% of their original titer. The lyophilization of the vaccines prolongs the admissible time of storage and a period of 12 to 18 months of storage appears acceptable for production purposes.

Table 1. Effect of Storage at 4 °C on the Activity of AHS Lyophilized Live Tissue Culture Vaccine

Vaccine No.	Titer (log TCID 50 ml.) after months of storage						
	1	3	6	9	12	15	18
1	6.5	—	5.5	—	5.0	—	5.0
2	7.0	—	—	6.0	—	5.5	—
3	6.5	6.0	6.0	—	5.5	5.0	5.0
4	6.0	—	5.5	5.5	—	5.0	—
5	6.5	6.0	—	5.5	5.0	—	5.0

References

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3. *Mirchamsy, H.,* and *H. Taslimi:* Canad. J. comp. Med. **30,** 47—51 (1966).
4. *Cooper, P. D.:* Virology **7,** 469—470 (1959).