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The Immunization of Mules with Formalysed Horsesickness Virus. II.

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Introduction.

In 1932 du Toit and Neitz reported upon the application of the formalysed spleen virus method to the immunization of mules. They showed that a concentration of one part of formaldehyde to 3,000 parts of a 20 per cent. spleen emulsion could not be injected into mules in a dose of 30 c.c. with safety. The majority of the mules which survive this single injection are solidly immune, but on receiving an intravenous injection of 5 c.c. of O virus as an immunity test a small proportion may develop horsesickness and die.

In a second experiment upon a total of 40 mules the single immunizing dose was replaced by two injections at an interval of 14 days, the first injection consisting of virus inactivated by one part of formaldehyde to either 2,000 or 2,500 parts of spleen emulsion. The second injection one part of formaldehyde to 3,000 parts of emulsion. As a result of the immunizing injections no deaths occurred, in fact no clinical symptoms developed, but on applying an immunity test by injecting O virus intravenously two animals developed typical horsesickness and died.

This mortality of only 5 per cent. was considered quite satisfactory, more especially when it is considered that the immunity test was exceedingly severe, in fact far more severe than would be encountered under field conditions. It was therefore decided to repeat the experiment, and at the same time to introduce our modification namely, for one group of mules, to emulsify the infective spleen material in Tyrode solution instead of saline. Also the second injection was made one part of formaldehyde to 3,500 parts of emulsion.

Object of the Experiment (S. 4718 and 4719).

To verify the previous results obtained with the subcutaneous injection of formalysed spleen material and to ascertain whether Tyrode solution has any advantage over 0·85 per cent. saline, as a vehicle for the virus.

Method.

The technique of preparation of the vaccine did not differ from that employed in previous experiments (du Toit and Neitz, 1932). The Tyrode solution was made up according to the formula given by Fisher namely, NaCl 3 gm., KCl 0·20 gm., $CaCl_2$ 0·20 gm., $MgCl_2$ 0·10 gm., NaH/PO_4 0·05 gm., $NaHCO_3$ 1·0 gm., Glucose 1 gm., H_2O add 1,000 c.c.

Sterilization was performed by filtration through a Seitz filter under positive pressure. Spleens from horses 20286 and 20313 destroyed *in extremis* on the 4th and 5th days following the intravenous injection of 5 c.c. of O virus were used for the preparation of the vaccine. Approximately half of each spleen was used for the saline and the Tyrode emulsions; before injection the two respective portions were mixed.

The vaccine was given subcutaneously in 30 c.c. doses at an interval of 21 days. Twenty-one days after the second injection 5 c.c. of O virus was given intravenously as an immunity test.

The majority of the mules were quite unbroken and all were too wild to permit the taking of daily temperatures. The reaction to any injection therefore could only be gauged by careful daily observations on the general habitus of the animals. The results are indicated in Tables I and II.

Results.

(a) *Saline emulsion.*—Of eleven mules injected one developed typical Dunkop Horsesickness to which it succumbed on the 18th day, the reaction being due to the injection of the 1 : 2,000 formaldehyde material; one mule showed diarrhoea on the 14th day; the remainder showed no apparent departures from normal health. On applying the immunity test the ten survivors proved to be solidly immune, no clinical symptoms of Horsesickness being observed at any time.

(b) *Tyrode Emulsion.*—Of ten mules injected one developed typical Dunkop Horsesickness and died on the 9th day after the 1 : 2,000 injection; one developed a mild but typical Dikkop attack from the 15th day but made an uneventful recovery. The remainder showed no clinical symptoms. On immunity test the nine survivors were solidly immune to O virus.

Conclusions.

No significant difference could be ascertained in favour of either saline or Tyrode solution as a fluid for emulsifying infective spleens. In both groups one animal died, but no importance can be attached to the fact that in the Tyrode group an additional animal developed clinical Horsesickness since individual susceptibility must be considered an important factor amongst small groups of only 10 animals.

2. It is confirmed that spleen virus attenuated by a concentration of one part of formaldehyde to 2,000 parts of infective spleen emulsion cannot be injected subcutaneously into mules with safety. Probably a concentration of 1 : 1,500 would be adequate.

3. The 1 : 2,000 formaldehyde concentration produces an immunity sufficient to protect against the 1 : 3,500 concentration. Mules which survive the 1 : 3,500 injection are solidly immune to fully virulent O virus.

4. The degree of immunity set up by 1/3,500 formaldehyde concentration is greater than that set up by 1 : 3,000 formalysed material as will readily be seen by a comparison with the results in the previous report.

5. The general result in previous work is confirmed namely that formalization of infective spleen material is capable of attenuating the virus so that it will become relatively avirulent but antigenic. The method as developed so far is not free from danger as it can be expected that 5 per cent. of animals will develop Horsesickness and die.

REFERENCES.

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TABLE I (SALINE EMULSION).

Experiment (*S.* 4718).

	D.O.B. No. of Mules.	Concentration of Formaldehyde in spleen emulsion.		Interval in days between injections.	Result of Immunization.	Date of injection of O virus.	IMMUNITY TEST.	
		1/2,000 M.	1/3,500 M.				Interval in days since last injection.	Result.
1....	20397	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
2....	20398	22.6.33	13.7.32	21	14 days after injection of 1/2,000 mule developed diarrhoea	3.8.32	21	,, ,, ,,
3....	20399	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	,, ,, ,,
4....	20400	22.6.32	—	—	Showed typical clinical Dunkop Horsesickness reaction and died on the 18th day after 1/2000 injection	—	—	—
5....	20401	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
6....	20402	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
7....	20403	22.6.33	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
8....	20404	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
9....	20405	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
10....	20406	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
11....	17897	18.8.32	8.9.32	21	,, ,, ,,	6.10.32	28	,, ,, ,,

TABLE II (TYRODE EMULSION).

Experiment (*S.* 4719).

	D.O.B. No. of Mule.	Concentration of Formaldehyde in Spleen Emulsion.		Interval in days between injections.	Result of immunization.	Date of injection of O virus.	IMMUNITY TEST.	
		1·2,000 S.	1/3500 S.				Interval in days since last injection.	Result.
1....	20377	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
2....	20378	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
3....	20379	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
4....	20380	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
5....	20381	22.6.32	13.2.33	21	15 days after the injection of 1/2,000 mule showed typical clinical symptoms of Horsesickness and recovered	3.8.32	21	,, ,, ,,
6....	20382	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	,, ,, ,,
7....	20383	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,
8....	20384	22.6.32	—	—	Showed typical clinical Dunkop Horsesickness reaction and died on the ninth day after injection of 1/2,000	—	—	—
9....	20385	22.6.32	13.7.32	21	No clinical symptoms noticed	3.8.32	21	No clinical symptoms observed. Immune to Horsesickness.
10....	20386	22.6.32	13.7.32	21	,, ,, ,,	3.8.32	21	,, ,, ,,