Hæmagglutination and Hæmagglutination-Inhibition with African Horse-Sickness Virus

COMPLEMENT-fixation and mouse neutralization tests are the two techniques so far employed in serological work with African horse-sickness virus. It was thought worth while to study the hæmagglutinating properties of this agent with the view of obtaining yet another tool for epidemiological (epizootiological) studies of this dreaded horse disease.

The three strains studied were an Indian strain recently isolated from the lung of a dead horse, a Pakistan strain and an attenuated type 6 strain from South Africa. The latter two were kindly supplied by Dr. Alexander of the Onderstepoort Laboratory in South Africa. The Indian and the Pakistan strains had undergone approximately 20 and the type 6 strain probably 100 or more mouse brain passages.

The basic techniques for preparing antigens, sera and erythrocytes for hæmagglutination-inhibition test were those described by Clarke and Casals[1]. Antigens prepared from infected suckling mouse brains were: (1) acetone–ether extract; (2) sucrose–acetone extract; (3) sucrose–acetone extract treated with protamine sulphate; (4) crude aqueous alkaline extract; (5) the same treated with protamine sulphate. Control antigens were prepared from the brains of normal healthy mice of the same age. Erythrocytes collected from roosters, geese, sheep, guinea pigs, horse, mule and donkey were tested. The cell concentrations used ranged from 0·4 per cent to 1 per cent. The cells were suspended in 'adjusting diluents', which when mixed with an equal volume of 'serum–virus diluent' gave a *p*H range from 6·4 to 7·0. The hæmagglutination test was performed in tubes with 0·5 ml. of antigen and 0·5 ml. of required cell suspension. One set was incubated at 37° C., one at room temperature (25° C.) and one at 4° C.

Initial experiments revealed that this virus showed hæmagglutination with cells from the equines alone. As the most satisfactory pattern was obtained with horse cells, these were used for all further work. Only the two antigens (Nos. 3 and 5) treated with protamine sulphate showed hæmagglutination. Of the two, sucrose–acetone extract treated with protamine sulphate gave better results than crude extract treated with protamine sulphate. Control antigens prepared from normal mouse brains in the same manner did not show any hæmagglutination. The best results were obtained with 0·5 per cent horse red blood cells, using *p*H 6·4 and incubation at 37° C. The test was read within $1\frac{3}{4}$–2 hr., when the cell controls had settled. The three strains gave similar hæmagglutination titres ranging from 1 : 32 to 1 : 128.

Hæmagglutination-inhibition work is still in a preliminary stage. Removal of non-specific inhibitors from sera was attempted by treatment with kaolin, acetone extraction and inactivation at 56° C. for 30 min. Antigen and serial two-fold dilutions of serum were mixed in 0·25 ml. volumes and incubated either at 37° C. for 1 hr. or overnight at 4° C. After incubation, 0·5 ml. of cells was added. Of the three methods of treating sera, acetone extraction appeared to be the best. From the limited trials it seemed that kaolin might reduce the level of specific antibodies. Overnight incubation at 4° C. produced better results than those observed with incubation at 37° C. for 1 hr.

Table 1

Serum No.	CF titres (reciprocal of initial serum diluted with 4 units of antigen Indian strain)	Log neutralization index*	HI titres (reciprocal of initial serum diluted with 4 units of antigen Indian strain)
603360–3	32	3·1 to ⩽ 3·5	40–160
603366–5	8	3·0 to ⩽ 3·1	20–80
603381–4	32	2·5 to ⩽ 3·7	160–640
603390–3	16	⩽ 3·5	80–320
603661–2	8	2·2 to ⩽ 4·0	20–80
603665–2	32	2·7 to ⩽ 4·0	40–160

* Adult mouse IC test with several Indian strains, using constant serum-varying virus dilutions.
CF, Complement-fixation; HI, hæmagglutination-inhibition.

A group of horse sera collected during a recent epidemic in India were tested for hæmagglutination-inhibition. Six sera that did not show detectable complement-fixing and neutralizing antibodies were also negative for hæmagglutination-inhibiting antibodies. Table 1 compares the complement-fixation, neutralization and hæmagglutination-inhibition results of the remaining six sera. The hæmagglutination-inhibition pattern, while clearly indicating inhibition, is not yet entirely satisfactory. The end-point is not as sharply defined as in tests with other viruses but is spread over several tubes. For this reason the hæmagglutination-inhibition titres have been presented as ranging over several tubes.

McIntosh[2] has reported that the complement-fixation test is less type-specific than the neutralization test and that no antigenic differentiation between the heterotypic strains was possible. Work is in progress to determine if the hæmagglutination-inhibition test is type-specific.

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[1] Clarke, D. H., and Casals, J., *Amer. J. Trop. Med. Hyg.*, **7**, 561 (1958).
[2] McIntosh, B. M., *Onderstepoort J. Vet. Res.*, **27**, 165 (1956).

GENETICS

Developmental Barriers in a Selection Experiment

THE present experiments describe the effect of selection on expression of the *ocelli-less* mutant, sex-linked recessive, affecting the ocelli and three pairs of macrochætes on top of the head in *Drosophila subobscura*.

In the foundation population, homozygous for the *ocelli-less* mutant, a large number of flies lacked all 3 ocelli and all 6 bristles; in the rest, varying combinations of bristles and ocelli were present. The ocelli in some of the flies were found to be slightly displaced from their normal positions, and in certain cases their sizes were also affected. In a few flies the bristles were found to be repeated; instead of one normal bristle on one side, additional bristles were found to lie close to the normal bristle. A positive correlation ($r = 0{\cdot}46$) was found between the presence of bristles and of ocelli. Therefore, in measuring the degree of expression, a unit score was given for the presence of each of the 6 bristles and 3