

Adaptation of Virus of Epidemic Keratoconjunctivitis to Development in Extra-Embryonic Fluids of Chick Embryo.

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Recent discussion^{1,2,3} of the size and nature of influenza virus has been based upon the properties of strains which have been adapted to the extra-embryonic fluids of the chick embryo.

This note is presented in the belief that it is important for other viruses to be adapted to a similar technic so as to be available for similar study.

The strain of epidemic keratoconjunctivitis virus⁴ used in this investigation, was isolated by tissue culture and established in mouse brain by Major M. Sanders, MC, AUS. The original material received from Major Sanders was given 2 mouse brain passages, and approximately 10% suspensions of whole macerated brain from the last passage were inoculated into the chorio-allantoic fluid of 8-day chick embryos. The embryos died in 5 or 6 days, the chorio-allantoic and amniotic fluids were collected, tested for sterility, and pooled.

After 2 more embryo-fluid passages, the

fluid from the third passage was reinoculated into young mice. The mouse brain material was again passed three times in embryo fluids and returned to mouse brain.

This last mouse passage material has been maintained in 12 consecutive embryo-fluid passages, with increase in virulence so that the present material is lethal in chick embryos in 0.001 ml quantities, killing them in 3 or 4 days. The material still produces a fatal encephalitis in mice.

Human convalescent and hyperimmune rabbit sera, furnished us by Major Sanders, will neutralize the embryo-fluid passage strain so that it will not cause the death of chick embryos. (Table I.)

The ease of handling this virus in extra-embryonic fluids, and the success of the neutralization tests, suggest that this method might be of value in serological diagnostic work. Investigations along this line are now in progress.

TABLE I.
Neutralization of Keratoconjunctivitis Virus in Chick Embryos.

Tube	*Virus, cc	Antiserum	cc	Diluent, cc	8-day inoculated embryos	Embryos dead in 8 days
1	0.1	Human convalescent	0.3	4.6	12	0
2	0.1	Hyperimmune rabbit	0.3	4.6	12	1
3	0.1	Normal sheep serum	0.3	4.6	12	12
4	0.1	—		4.9	12	11

* Chorio-allantoic fluid—10th passage.

¹ Chambers, L. A., *et al.*, *J. Exp. Med.*, 1943, **77**, 265.

² Taylor, A. R., *et al.*, *J. Immunol.*, 1943, **47**, 261.

³ Stanley, W. M., *J. Exp. Med.*, 1944, **79**, 267.

⁴ Sanders, M., and Alexander, R. C., *J. Exp. Med.*, 1943, **77**, 71.