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EFFECT OF LYSOZYME IN MAINTENANCE MEDIUM FOR FL. CELLS INFECTED WITH PARA - INFLUENZA 3 VIRUS*

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The addition of egg white in a final concentration of 1 per cent to medium 199 that contained 15 per cent tryptose phosphate broth provided a favorable medium for the propagation of para-influenza 3 virus in FL cells (Annual Report 1960, p. 33). Experiments were continued to investigate the role of lysozyme in the effect of egg white.

Crystalline egg albumin, crystalline bovine albumin, or lysozyme (crystallised from egg white) in a final concentration of 0.1 per cent or egg white treated with charcoal (Norit-A) and Kaolin or untreated in a final concentration of 0.1 per cent were added to medium 199 with 15 per cent tryptose phosphate broth. The lytic activity of each medium was tested with a beef infusion broth culture of **Micrococcus lysodeikticus.** The egg white preparations and the lysozyme were highly active, the crystalline egg albumin showed markedly reduced enzyme activity and no lysis occured with bovine albumin. The effect of the various test substance on the growth of parainfluenza 3 virus was determined by infectivity titrations, based on cytopathic effects, in tubes of FL cell cultures, and by hemagglutination titrations of cell culture fluids. The highest virus titers were obtained with medium containing lysozyme alone or combined with crystalline egg albumin or with charcoal-treated egg white. The mean 50 per cent infective dose per ml in six comparative titrations was 2.4×10^7 for the control without added protein, 13.8×10^7 with lysozyme and 14.1×10^7 with both lysozyme and crystalline egg albumin, each in a final 0.1 per cent concentration. With the crystalline egg albumin alone virus titers were slightly lower than

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when lysozyme was also added. Treatment of egg white with charcoal apparently removed some virus inhibiting factor in three comparative titrations, the mean titers were 1.2×10^7 when medium with untreated egg white was used and 6.9×10^7 when the treated egg white was substituted. With crystalline bovine albumin in the medium, virus titers were essentially the same as when no protein was used, but when lysozyme was also added, the virus titer was definitely increased. The mean figures from three tests were control without added protein 1.4×10^7, bovine albumin 0.8×10^7 bovine albumin and lysozyme 4.6×10^7. Hemagglutination titers of fluids from infected FL cell cultures were usually low and differences were not as clear as with infectivity titrations, but there was some general agreement between the results of the two methods. In each of three tests of infected monkey kidney cell cultures, the fluids showed increased hemagglutination content if lysozyme was added to the medium.

This lysozyme in the concentration tested appears to be an important factor in the favorable effect of egg white in maintenance medium for FL cells infected with para-influenza 3 viruses. The whole egg white has a practical advantage, however, over pure lysozyme in that FL cells are maintained in better condition and for longer period in the presence of the egg white.