isms and the contours of their surfaces; shadowing also makes evident their flagellar and other extra-cellular processes. Illustrative shadowed electron micrographs are given of subtilis-like and typhoid-like organisms and of several forms of cocci.

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Cultivation of Viruses of the Psittacosis Group in the Allantoic Cavity of Chick Embryos.*

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Many of the viruses of the psittacosis group have been cultivated successfully in fertile hens' eggs, the yolk sac being most satisfactory for this purpose. Using this particularly susceptible tissue, preparation of large amounts of virus has been possible.[1] Such preparations cannot be regarded as ideal for some purposes, however, such as attempts at purification of the virus[2] or for production of antigens, because of the large amount of admixed tissue debris and yolk material, removable only with difficulty. It occurred to us that the allantois, being embryologically similar to the yolk sac, might also show special susceptibility to the viruses of this group. The allantoic cavity has been extremely useful in cultivation of influenza virus,[3] and the advantages in the case of the latter virus might be expected to hold for others as well.

After our first attempts to adapt mouse pneumonitis virus, one of the agents of this group, to the allantoic cavity, the publication of Williams[4] appeared, in which he reports successful cultivation of psittacosis virus in the allantoic cavity and in greater amounts than obtained in the yolk sac. We have been able to confirm his finding and have extended the study to other viruses of the group. The present preliminary report records the successful cultivation of a strain of psittacosis, human pneumonitis (SF), and meningopneumonitis (F-97) virus in the allantoic cavity. The few attempts made so far to adapt mouse pneumonitis virus to the allantoic cavity have not been successful. Williams reported failure to cultivate lymphogranuloma venereum virus in this tissue.

The strains of virus used in the present experiments have been employed in previous work in this laboratory and their sources have been recorded.[2] The first inoculations into allantoic cavities were made with emulsions of infected mouse tissue or yolk sac, and subsequent passages were made with 0.25 cc quantities of undiluted allantoic fluid. The preparations of human pneumonitis and feline pneumonitis virus, designed for the first allantoic injections, proved to be contaminated with bacteria, a circumstance overcome by adding sulfadiazine to the contaminated emulsions in the amounts of 68 to 75 mg per egg. Before use the eggs were incubated at 38 to 39°C for 7 or 8 days and at 37°C for 2 days. After the inoculations were made, incubation was continued at 35° C. When movements of the embryo became feeble, indicating impending death, usually on the 3rd, 4th or 5th day, the eggs were chilled at 4° C for a few hours and the allantoic fluids then withdrawn. Five to 6 cc per

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1 Grace, A. W., Rake, G., and Shaffer, M. F., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 259.

2 Hilleman, M. R., *J. Infect. Dis.*, 1945, **76**, 96.

3 Burnet, F. M., *Aust. J. Exp. Biol. and Med. Sci.*, 1941, **19**, 291.

4 Williams, S. E., *Aust. J. Exp. Biol. and Med. Sci.*, 1944, **22**, 205.

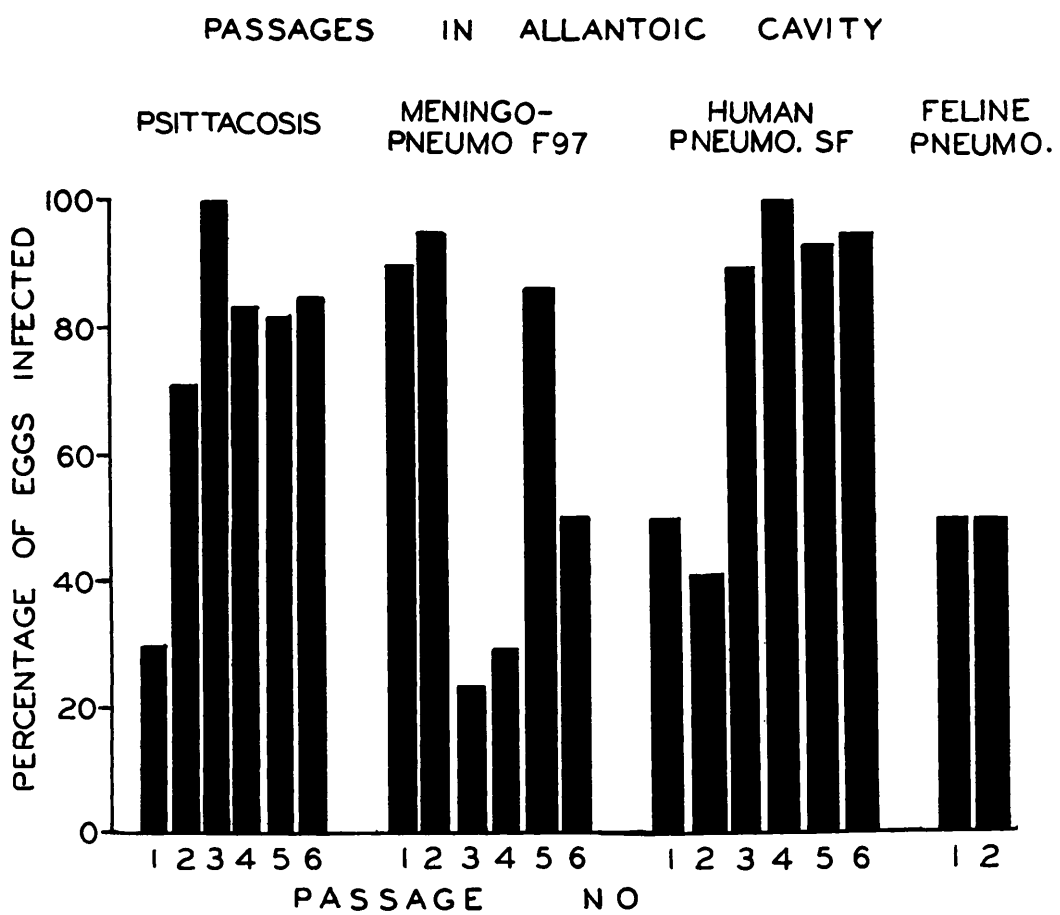

CHART 1.

Passages of Virus in the Allantoic Cavity of Fertile Eggs.

Each passage consisted of 6 to 60 eggs and infection was determined by stained smears of allantoic fluid.

TABLE I.

Titration of Allantoic Fluids by Mouse Inoculation.

Virus	Egg passage No.	Route of inoculation* of mice	Results in mice: Representative mean infectivity scores† 10⁻³	10⁻⁴	10⁻⁵	LD_{50}
Psittacosis	3	intracerebral				$10^{-8.4}$
"	6	"				$10^{-9.2}$
Meningopneumonitis	3	intranasal	4.7	4.7	2.0	$10^{-4.4}$
"	6	"	5.0	4.3	2.3	$10^{-4.5}$

* Each inoculum consisted of a pool of 10 or more allantoic fluids which contained large amounts of elementary bodies as revealed by stained smears.

† Computed according to the method of Horsfall.[5]

egg were obtained. From 6 to 60 eggs were used for each passage and the determination whether an egg was infected was made by the presence or absence of elementary bodies in stained (Macchiavello) smears of the allantoic fluid. Chart 1 indicates the percentage of infected eggs at each passage and suggests that satisfactory adaptation to the allantoic

[5] Horsfall, F. L., *J. Exp. Med.*, 1939, **70**, 209.

cavity had occurred by the 3rd passage in the case of psittacosis and human pneumonitis virus. The reason for the irregular results with meningopneumonitis virus is unknown. In the 2 passages with feline pneumonitis virus completed to date only one half the eggs became infected, results which are similar to those of 2 of the other agents.

Bacteria-free allantoic fluids of a single passage were pooled in groups of 10 or more, only those fluids being chosen in which stained smears had revealed large numbers of elementary bodies. Several of these pools have been titrated by mouse inoculation, the results of which are given in Table I. The titers indicate concentrations of virus in these preparations which compare favorably with preparations from other sources. For example, an emulsion of yolk sac tissue infected with meningopneumonitis virus, when titered in mouse lung, gave infectivity scores[5] of the same range as those indicated in Table I for allantoic fluid, 5.0, 4.3 and 2.6 for the 10^{-3}, 10^{-4} and 10^{-5} dilutions respectively.[6] The LD_{50} value of the meningopneumonitis virus† from yolk sac was $10^{-4.3}$; the comparable values for infected allantoic fluids were $10^{-4.4}$ and $10^{-4.5}$ (Table I). Our strain of psittacosis virus, when propagated in mouse brain, gave an LD_{50} in mice[7] of $10^{-4.5}$. The comparable figure for allantoic fluid (*e.g.*, LD_{50} equals $10^{-8.4}$) indicates approximately a 10,000-fold difference in favor of the allantoic fluid. This figure confirms closely that reported by Williams.[4]

Judging by the numbers of elementary bodies in stained smears, large amounts of virus are present also in the allantoic fluids infected with human pneumonitis but only small amounts were present in the first 2 passages of feline pneumonitis virus Quantitative determinations by mouse titration have not yet been completed in the case of these 2 latter viruses.

Preliminary attempts at concentration of psittacosis elementary bodies from allantoic fluid indicate that this is easily accomplished by centrifugation, with little difficulty arising from the presence of tissue debris.

These studies suggest that growth in the allantoic cavity of fertile eggs may offer certain advantages over previous methods of cultivation, in the case of some members of the psittacosis group of viruses. The infected allantoic fluid represents a ready-prepared suspension of virus in high concentration, available in large amounts, with relatively little extraneous matter present.

[6] We are indebted to Elizabeth St. John for these figures from unpublished work.

† A personal communication from Dr. Thomas Francis, Jr., states that the allantoic route of inoculation has been used for some time in his laboratory for propagation of meningopneumonitis virus. The allantoic fluid, rich in elementary bodies, proved satisfactory as antigen in the complement fixation test, and the infective titer was as good or better than that of similar quantities of mouse lung.

[7] Calculations made by method of Reed, L. J., and Muench, H., *Am. J. Hygiene*, 1938, **27**, 493.