pensions as was observed on the addition of distilled water alone. On the other hand, ethyl alcohol dissolved in phosphate-Ringer solution had little or no lethal effect on thymic and testicular cells even in high concentrations (2 molar). A 3-molar solution had a marked cytocidal action in fifteen minutes at 37.5°C.

From these experiments, it seems that ethyl alcohol exerts little if any osmotic tension on the cells. This would indicate that the cell wall was readily permeable to the reagent. In spite of its apparent capacity to penetrate into the cell, ethyl alcohol has only a slight cytocidal action.

Similar experiments with urea showed that this reagent also failed to exert an osmotic pressure on the cell. Evidently the cellular wall was also permeable to this compound. Urea in 1 molar solution had no appreciable effect on the number of viable thymic cells or polymorphonuclear leukocytes and did not cause the hemolysis of erythrocytes but did cause a marked decrease in the number of viable testicular cells (from 220 to 7 cells per millimicroliter). Evidently urea had a marked toxic effect on testicular cells but not on the other cells studied.

Summary. By means of the method of unstained cell counts both distilled water and urea were found to be more toxic to testicular cells than to lymphocytes and polymorphonuclear leukocytes. Ethyl alcohol was not cytocidal even in relatively high concentrations. The cell membrane of viable thymic, testicular and other cells appeared readily permeable to urea and ethyl alcohol but not to sodium chloride or sucrose. The addition of distilled water or freezing and thawing produced a characteristic gelatinous precipitate only in suspensions of thymic cells but not in suspensions of splenic or testicular cells.

14808

Effect of Penicillin on Certain Viruses.

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In view of the wide antibiotic activity of penicillin, it is of interest to learn whether it has any effect on the growth of viruses. The present paper deals with experiments designed to gain information on this subject.

Certain representative viruses were used as follows: Vaccinia BH,[1] a strain of vaccine virus derived originally from the New York Board of Health Strain and maintained for several years by rabbit testicular passage; Vaccinia CV II,[1] derived from BH by serial passage in Rivers-Li culture medium now of apparently stable virulence; St. Louis Encephalitis, Hubbard strain, obtained from Dr. Margaret E. Smith, and maintained by serial intracerebral mouse passage; Psittacosis 6 BC, obtained through the courtesy of Dr. J. E. Smadel, maintained by serial yolk sac passages; Meningo-pneumonitis virus, M.P., obtained from Dr. T. Francis, Jr., and maintained by mouse lung and chick embryo yolk sac passage; Equine Encephalomyelitis virus, the Eastern strain, secured through the courtesy of Dr. J. E. Beard, was used in its third chick embryo passage.

Penicillin used was prepared by the Reichel and Abbott Laboratories and was part of a supply allocated to the University Hospitals of Cleveland by the Committee on Therapeutics and Other Agents of the National Research Council. Assays were made by the Oxford cup method using spores of *Bacillus subtilis* for inoculating the solid medium as suggested by Foster and Woodruff.[2] It was found that

[1] Parker, R. F., Bronson, L. H., and Green, R. H., *J. Exp. Med.*, 1941, **74**, 263.

[2] Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1944, **47**, 43.

a much greater yield of spores could be obtained under conditions of partial desiccation of the culture than in the liquid medium originally proposed. The culture was accordingly seeded on the surface of nutrient agar, and incubated for 7 days. It was then harvested and pasteurized and the spore suspension stored at 4°C. If assays were not carried out immediately, the sample was stored at −15°C. Appropriate controls were always included.

Two general methods were used for testing the effect of penicillin on virus growth: (1) cultivation in Rivers-Li culture medium[3] consisting of chick embryonic tissue suspended in Tyrode solution, in a total volume of 10 ml; and (2) cultivation in the intact chick embryo. When the first method was employed, a measured quantity of virus was added to the culture medium, titration of an aliquot portion being carried out as soon as possible after setting up the culture. After 5 days' incubation 0.1 ml of the culture was transferred to fresh medium, giving a dilution for each passage of 10^{-2}. The culture after incubation was titrated for virus and a penicillin assay was made. When the intact chick embryo was used, inoculation was made into the yolk sac[4] or onto the chorio-allantoic membrane.[5] Death of the embryo or development of lesions on the membrane was taken as the criterion of virus growth, appropriate bacteriologic controls being included.

Since the time of incubation or time to death of the embryo would for most of the viruses be measured in days, it was necessary to gain information on the rate of deterioration of the penicillin. Accordingly several flasks of the culture medium were set up and penicillin was added, but no virus. They were incubated at 37°C. A sample was removed each day from one of the flasks, stored at −15°C, and finally all samples were collected and titrated by the cup method together with controls. Data obtained in such an experiment are presented in Fig. 1. The deterioration of the substance is seen to be regular over the whole period of observation. A number of assays were made on cultures seeded with virus, and the results of 8 of these are included in the figure. Similar data were obtained on the stability of penicillin when injected into the chick embryo, and are presented in Fig. 2. In these experiments penicillin was injected into the yolk sac

Fig. 1.

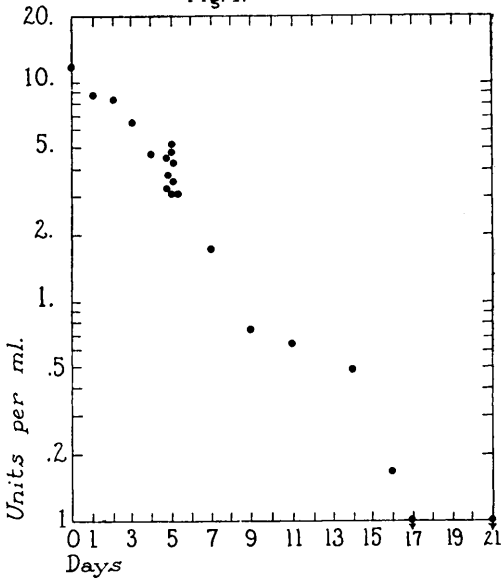
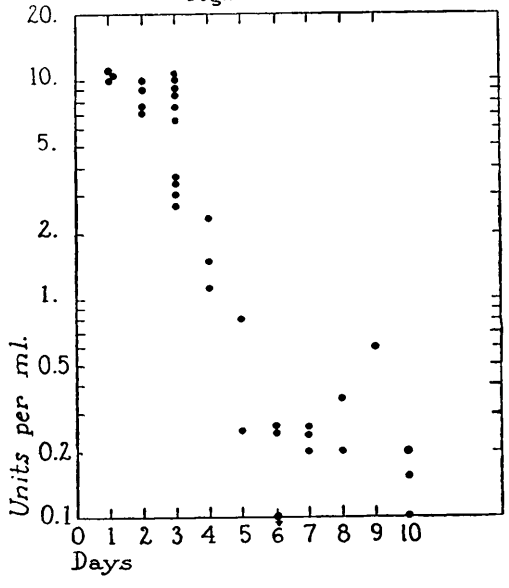


Fig. 2.



[3] Li, C. P., and Rivers, T. M., *J. Exp. Med.*, 1930, **52**, 465.

[4] Cox, H. R., *U. S. Pub. Health Rep.*, 1938, **53**, 2241.

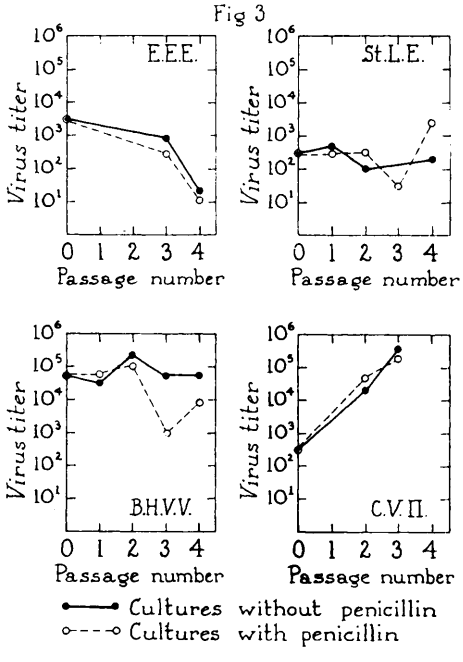
[5] Barret, F. M., *J. Path. and Bact.*, 1933, **37**, 107; Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

TABLE I.
Virus-Penicillin Experiments with Intact Chick Embryos.

Days after injection of virus and penicillin	M.P.V.*		M.P.V.†		6 B.C.‡		6 B.C.§	
	Controls	Penicillin	Controls	Penicillin	Controls	Penicillin	Controls	Penicillin
0	0	0	0	0	0	0	0	0
1	2‖	0	2	1	1	0	0	0
2	1	1	2	1	1	0	0	1
3	0	0	0	2	1	0	0	0
4	0	0	0	1	2	0	0	2
5	0	1	0	0	8	4	1	0
6	0	0	2	1	13	0	14	4
7	3	0	7	1	1	2	3	1
8	11	0	4	1	1	1	0	3
9	0	1	2	1	0	3	0	2
10	2	0	1	1	1	9	0	2
11	0	2		0		1	0	1
12	0	1		4		7	0	4
13	0	0		5			0	4
Survivors at 14th day	1	24	0	11	0	2	2	5

* Meningo-pneumonitis virus. $10^{3.2}$ infectious units injected per egg.
Penicillin: 200 U per egg injected initially + 200 U on 4th day.
† Meningo-pneumonitis virus. $10^{3.2}$ infectious units injected per egg.
Penicillin: 50 U per egg injected initially + 50 U on 3rd day.
‡ Psittacosis virus. $10^{2.5}$ infectious units injected per egg.
Penicillin: 500 U per egg initially.
§ Psittacosis virus. $10^{2.5}$ infectious units injected per egg.
Penicillin: 666 U per egg initially + 600 U on 3rd day.
‖ Figures indicate number of embryos dying on indicated day.

Fig 3



of embryos of 5 days' incubation. The eggs were opened at intervals, the fluid mixed with a pipette and a sample withdrawn. Before assay, the fluid was centrifuged to remove red cells and debris which interfered with the diffusion of penicillin. Progressive desiccation of the embryo prevented an indefinite continuance of the assays. It is seen that as with the liquid cultures, penicillin disappeared slowly.

The results of the experiments with viruses cultivated in the chick-embryo medium are given in Fig. 3. The first 2 passages of BH Vaccinia and St. Louis Encephalitis were in medium containing 1 unit of penicillin per ml at the beginning of incubation. Later passages, and all passages of CV II Vaccinia and Equine Encephalomyelitis were in medium containing 10 units per ml initially. Samples taken from these flasks after 5 days' incubation contained from 3.0 to 5.2 units of penicillin per ml. It will be seen that the titration values for the control do not differ significantly from values for the "penicillin" cultures.

In one series of experiments, the 2 strains of vaccinia were inoculated on the chorio-allantois of 14-day embryos. A quantity of virus was used which gave 15 to 50 lesions on each membrane. In one series 500 units of penicillin were injected into the egg on the day before inoculation of virus, and in the

TABLE II.
Effect of Penicillin on Equine-Encephalomyelitis in Chick Embryos.

Dilution of virus	Control	Treated
10^{-3}	8/8	7/7
10^{-4}	18/18	14/15
10^{-5}	8/8	6/8

Numerator: number dying; denominator: number inoculated.
Titer of virus in previous experiment: 10^{-6}.
500 units penicillin injected into each egg just before virus inoculation.

other, 500 units of penicillin were mixed with the virus inoculum. In neither case could any difference between control and treated embryos be distinguished, either as to size or number of lesions when the membranes were examined at 48 hours.

The results of inoculation of chick embryos with psittacosis and with M.P. virus, and with penicillin plus these viruses, are given in Table I. It will be seen that with these 2 infections penicillin was effective in delaying, or with larger doses preventing death of the embryos within the period of observation. Not all embryos dying were tested for the presence of virus but enough passages were made to learn that in a fair proportion of the embryos dying late after penicillin treatment, no detectable virus was present.

In one series of experiments embryos were inoculated with equine encephalomyelitis virus, and the results are recorded in Table II. The penicillin was entirely without effect on the course of the infection.

It is evident from these experiments that penicillin in the doses used was without effect on the multiplication of the viruses of vaccinia, St. Louis encephalitis or equine encephalomyelitis, or on the course of the disease induced in chick embryos by vaccinia or equine encephalomyelitis. Penicillin did have a very definite effect on the course of the disease induced in chick embryos by the viruses of psittacosis and of meningo-pneumonitis. It is to be noted, however, that the doses used were large. To maintain a comparable blood concentration of the drug would require the injection into man of several million units of penicillin daily. The practical importance of this observation may, therefore, be questioned.

14809

Isolation of Influenza A Virus from Normal Human Contacts During an Epidemic of Influenza A.*

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Various investigators have reported the occurrence of sub-clinical infections during epidemics of influenza A as evidenced by significant increases in neutralizing or complement fixing antibodies in the sera of persons who were exposed but who did not become ill.[1,2,3] A review of the literature, however, has revealed no reports of the isolation of influenza virus from persons with subclinical infections following normal exposure to the disease.

In November, 1943, 171 typical clinical cases of influenza A occurred in a dormitory housing approximately 500 students at the

* This study was supported by funds from the International Health Division of the Rockefeller Foundation.

1 Francis, T., Jr., Magill, T. P., Rickard, E. R., and Beck, M. D., *Am. J. Pub. Health*, 1937, **27**, 1141.

2 Stuart-Harris, C. H., *et al.*, *Medical Research Council, Special Report Series*, No. 228, 1938.

3 Rickard, E. R., Lennette, E. H., and Horsfall, F. L., Jr., *Pub. Health Rep.*, 1940, **55**, 2146.