support growth of the virus was investigated in roller tube cultures prepared with fragments of the organ, using the 7-2-1 mixture as nutrient fluid. The tissues suspended in the fluid (taken as a 10^0 dilution) were titrated in mice at 3-, 6- and 9-day intervals. The results are given in Table II.

The virus grew well in all tissues tested except brain, heart and skeletal muscle. In brain tissue the virus apparently survived but did not increase. In skeletal muscle and heart the virus survived but in repeated trials only irregular and small increases in virus content were found. The virus appeared to increase more slowly in yolk sac tissue than in other tissues.

Summary. During a period of 30 days' continuous cultivation of the Melbourne strain of influenza A virus in roller tubes, the respective titers of virus in the chick embryo tissue and fluid components after initial increases remained almost constant, provided the fluid contained serum and embryonic extract. When Tyrode solution or physiological saline was substituted, the amount of virus declined gradually after one week. The virus grew well in cultures of various organs from 14-day chick embryos except in the case of brain, heart and skeletal muscle.

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Resistance of Chicks to Infection with Influenza A Virus.

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St. Louis encephalitis virus may be recovered from chicks allowed to hatch after inoculation of the egg[1, 2] but attempts to infect young chicks have resulted only in survival of virus for a limited period without signs of infection.[2, 3] That the body temperature of chicks may be a factor in such resistance to infection has been suggested.[2] Higher temperatures decreased the intensity of reaction of mice inoculated with St. Louis encephalitis virus and of guinea pigs in-

1 Harrison, R. W., and Moore, E., *Am. J. Path.*, 1937, **13**, 361.

2 Sulkin, S. E., Harford, C. G., and Bronfenbrenner, J., *J. Inf. Dis.*, 1940, **67**, 252.

3 Pearson, H. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 413.

fected with endemic typhus.[4, 5] Incubation of eggs at temperatures below 39.5°C favors the growth of some viruses.[6] Thus in Burnet's hands influenza A virus proved lethal or produced lesions in eggs kept at 36°C, whereas neither embryonic death nor lesions occurred at 39°C. Nelson also reported that chick embryos at 28°C reacted less to variola and vaccinia viruses than at 37°C.[7] Furthermore, the multiplication of St. Louis encephalitis virus[8] and lymphogranuloma inguinale viruses[9] in tissue culture has been found to be greater at room temperature than at 37°C. In addition to alteration in body temperature, humoral factors in resistance associated with age have been noted[10] in connection with the reaction of chickens to vaccinia virus. In this paper experiments are described which were undertaken to elucidate the resistance of the young chick to influenza A virus.

The Melbourne strain of influenza A virus passed only in eggs or chick embryo tissue cultures for more than 300 transfers was used. A dilution of 10^{-4} to 10^{-5} of embryo suspension killed all mice after intranasal (i.n.) inoculation of 0.05 cc. The eggs used were from Leghorns except for an occasional one from the Barred Rock variety.

A number of 15-day embryos were inoculated[6] on the chorio-allantoic membranes each with 0.05 cc of 1:500 virus and incubated at 37°C Three were killed after 3 days and the pooled lungs, spleens and intestines were titrated in mice. Enough virus was present in a dilution of 10^{-3} to kill mice and to produce pulmonary lesions in these animals in a dilution of 10^{-5}. Most of the remaining eggs hatched on the 5th day (12 of 39 died but were found to be without definite gross pathology). Three of the chicks were killed immediately after hatching and their pooled organs were tested in mice as described above. No virus was recovered. Additional groups of 3 or 4 chicks were killed at 7, 9, 15 and 19 days after the original inoculation and the lungs and spleens were tested for virus. None was found. The individuals in the 9-, 15- and 19-day groups were bled before death and the respective pools of sera were tested for the

[4] Lillie, R. D., Dyer, R. E., Armstrong, C., and Pasternack, J. G., *Pub. Health Rep.*, 1937, **52**, 1805.

[5] Castaneda, M. R., *J. Immunol.*, 1937, **33**, 101.

[6] Burnet, F. M., *Med. Res. Council, Spec. Rep. Series*, London, 1936, No. 220.

[7] Nelson, J. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 110.

[8] Molloy, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 563.

[9] Sanders, M., *J. Exp. Med.*, 1940, **71**, 113.

[10] Duran-Reynals, F., *Yale J. Biol. and Med.*, 1941, **13**, 693.

presence of neutralizing antibodies which were not demonstrated in any case.

When 18-day embryos similarly inoculated were killed after 3 days and the whole embryo (except head and limbs) was tested for virus, irregular results were obtained. Certain of them contained as much virus as was found in 10-day embryos, whereas in others none was revealed by the mouse test.

One-day-old chicks were inoculated, under ether anaesthetic, in the nares each with 0.1 cc of 10% chick embryo virus. Groups of 3 were killed at intervals and their pooled lungs and spleens were tested separately for virus. After 1 and 19 hours, sufficient virus was present in the lungs to kill all mice inoculated with a 10% suspension of those organs. No virus was detected in the lungs at 48 hours nor in the spleens at any of the 3 intervals of time. Two-day-old chicks were similarly inoculated except that the dose was reduced to 0.05 cc of chick embryo virus and the pooled lungs (of 3 or 4) were tested at intervals of 3, 7 and 14 days. No virus was recovered. The chicks killed at 7 and 14 days were bled prior to death and the respective pools of sera were examined for the presence of neutralizing antibodies with negative results. Moreover, antibodies were not found in pooled sera from normal 2-day-old chicks nor from adult chickens.

The ability of the lung tissue from chicks of various ages to support growth of the virus was tested by setting up roller tube tissue

TABLE I.

Titrations in Mice of Influenza A Virus from Roller Tube Cultures (Pooled Tissue and Fluid) of Chick Lung Maintained for Various Intervals of Time at 37°C.

Age of chick	Age of culture (days)	Dilution of culture		
		10^0	10^{-2}	10^{-4}
18 day embryo	3	D	D	+
	6	D	D	+
	9	D	D	—
	14	—	—	—
20 " "	3	D	D	D
	6	—	—	—
	9	+	+	—
	15	—	—	—
2 " chick	3	D	D	D
	6	—	—	—
	9	D	—	—

D = 50% or more of mice died within 10 days with typical lung lesions.
+ = All mice survived 10 days but had lung lesions at autopsy.
— = All mice survived 10 days but had no lung lesions at autopsy.

cultures of lung in which Tyrode's solution was employed as the nutrient fluid.[11] The results are given in Table 1.

It is evident that tissue removed from chick embryos just before the time of hatching and of 2-day-old chicks supported the growth of virus *in vitro* at 37°C. This finding suggests that no virus-inhibitory property of the tissue, therefore, developed at the time when a mechanism of resistance to infection had been shown to appear.

The body temperature (cloacal) of young chicks was found to be 40° to 41°C and that of adult chickens 41° to 41.6°C. The effect of such temperatures on the propagation of influenza virus in eggs and tissue cultures was studied. The virus was inoculated on the chorio-allantois of 15-day embryos and the eggs then incubated at 41°C. No virus was demonstrated in such embryos when they were tested after 2- and 4-day intervals. Tissue cultures of 15-day embryonic lung and of 2-day chick lung were then set up in roller tubes in the manner mentioned above except that incubation was carried out at 41°C and the tubes were kept stationary. No virus was found in these cultures after intervals of 2, 4 and 6 days despite the fact that the 15-day embryo lung tissue grew fairly well and the 2-day lung tissue grew better at 41° than at 37°C. These data suggest that the high body temperature which presumably becomes established at or about the time of hatching may be the limiting factor in the resistance of chicks to infection with influenza A virus.

Summary. Virus was not recovered after hatching from chicks infected as embryos with the Melbourne strain of influenza A virus. This virus survived and increased in cultures of 18- or 20-day embryo lung and lung tissue from 2-day-old chicks at 37°C, but not in cultures of embryo or chick lung at 41°C. Virus was not recovered from embryos inoculated by the chorio-allantoic route and kept at 41°C. Virus was demonstrated in the lungs of 1-day-old chicks at 19 but not at 48 hours after inoculation in the nares. No neutralizing antibodies for the virus were detected in sera of chicks following such inoculation nor in the sera of normal chicks or adult chickens.

[11] Pearson, H. E., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 140.