3.0 to 3.6 mg after the eleventh dose and 2.96 to 3.0 mg after the thirteenth dose.

The results were entirely negative as the compound in the dosage administered was without apparent effect upon the evolution and course of the vaccinal lesions.

Conclusions. 1. Sulfanilamide by oral administration in dosage of 0.1 g per kilo of weight, twice daily for 10 to 14 doses, had no appreciable effect upon the evolution and clinical course of experimental vaccinia of rabbits. 2. Under the circumstances it is unlikely that sulfanilamide has any effect upon the virus of smallpox, although its administration may be helpful in the treatment of smallpox and generalized vaccinia by reducing secondary staphylococcus or streptococcus infections of the vesicles.

13249

Cultivation of Influenza A Virus in Roller Tubes.

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The prolonged cultivation of vaccinia in roller tube cultures of chick embryonic tissue has been reported.[1] After an initial rise, individual cultures were observed to maintain a high titer of virus for as long as nine weeks. The present communication presents the results obtained in the cultivation of influenza A virus by means of the same method.

The Melbourne strain of influenza A virus which had been passed exclusively in eggs or in chick embryo tissue culture for more than 300 transfers was used. Introduced intranasally 0.05 cc of a suspension of infected embryo diluted 10^{-4} or 10^{-5} in infusion broth killed all mice. Cultures were inaugurated by mixing a suspension of virus with finely minced, 10-day chick embryo (head removed). About 0.6 cc of infected tissue was distributed over the layer of chicken plasma on the inner surface of the lower half of a roller tube. Nutrient fluid consisted of 1.6 cc of so-called 7-2-1 mixture (Simms' solution 7 parts, chick embryo extract 2 parts, chicken serum 1 part). This fluid was changed daily when 20 cc of sterile

[1] Feller, A. E., Enders, J. F., and Weller, T. H., *J. Exp. Med.*, 1940, **72**, 367.

TABLE I.
Titration in Mice of Virus in Tissue and Fluid Components of Roller Tube Cultures After Various Intervals of Incubation at 37°C.

	Age of culture in days							
	5		10		20		30	
Dilution	T	F	T	F	T	F	T	F
10^0	D	D	D	D	D	D	D	D
10^{-2}	D	+	D	+	D	+	D	+
10^{-3}	nd	+	nd	+	nd	+	nd	+
10^{-4}	+	nd	+	nd	+	nd	+	nd

T = tissue.
F = fluid.
D = 50% or more of mice died within 10 days with typical lung lesions.
+ = mice survived 10 days but had lung lesions at autopsy.
nd = not done.

air were blown into the tube. At intervals of 5 to 30 days the tissues and fluids from the cultures were tested separately for their content of virus. The tissue was ground in 1.6 cc of broth and the resulting suspension designated as a 10^0 dilution. Serial dilutions of this tissue suspension and of the nutrient fluid were then administered intranasally to mice. The titer of virus was taken as that dilution which in a volume of 0.05 cc killed 50% or more of the mice within 10 days after intranasal inoculation. The results of these titrations are given in Table I.

The respective titers of tissue and fluid were fairly constant for at least 30 days. Furthermore, the titer or persistence of the virus did not vary with the amount of virus in the inoculum.

In other experiments the composition of the nutrient fluid was altered. The effect of such alterations on the increase and maintenance of the virus was then determined. Tyrode solution instead of the 7-2-1 mixture was first used. The results were entirely comparable on the 5th day of cultivation to those obtained at this time with the more complex fluid. At 10 days, however, there was less virus present and much less at 15 days. After 20 days only enough virus remained in both undiluted fluid and tissue suspension to produce small lesions in mice. No virus was detected at 25 days. When the Tyrode solution was not changed and the culture left stoppered until tested (tissue plus fluid tested together), as much virus was present at 5 days as if the fluid had been changed daily; at 10 and 15 days the amount of virus had decreased and at 20 days was barely detectable; by 30 days it had disappeared. These findings are in agreement with those reported by others[2] on the survival of influenza virus in a closed culture.

[2] Magill, T. P., and Francis, T., Jr., *J. Exp. Med.*, 1936, **63**, 803.

When a 0.9% solution of sodium chloride was substituted for the 7-2-1 mixture, the results were as follows: at 3 days both tissue and fluid contained as much virus as the cultures in 7-2-1; at 6 days there was slightly less virus in the tissue and much less in the fluid; at 9 days virus was barely detected in the tissue but was not found in the fluid and none was demonstrated in either component at 13 days. It is apparent from these observations that for relatively short term experiments the fluid component of the roller tube culture may be composed of very simple constituents—a fact of practical value should the method be employed in metabolic studies on tissues infected with virus.

An attempt was made to determine which tissues of the chick embryo in culture permitted the multiplication of virus. Previously it was shown[3] that egg-adapted strains induced rapidly fatal, generalized infections of the embryo *in vivo* in contrast to certain strains partially adapted to the egg which did not involve the embryo after inoculation on the chorio-allantoic membrane. Inoculation of the latter into the amniotic sac, however, resulted in lung lesions and a high titer of virus in the lung and amniotic fluid. When virus was inoculated between the yolk sac and chorio-allantois,[4] it was recovered in high titer from the yolk sac and in even higher titer from the chorio-allantois and amnion and from the amniotic fluid.[5, 6]

The capacity of various organs from 14-day chick embryos to

TABLE II.
Titration in Mice of the Virus Content of Various Embryonic Organs of the Chick Cultured at 37°C.

Age of culture in days	3			6			9		
Dilution	10^0	10^{-2}	10^{-4}	10^0	10^{-2}	10^{-4}	10^0	10^{-2}	10^{-4}
Whole embryo	D	D	D	D	+	+	D	D	+
Chorio-allantois	D	D	D	D	+	+	D	D	—
Amnion	D	D	D	D	—	—	D	+	—
Yolk sac	—	—	—	D	+	—	D	D	—
Brain	+	—	—	+	—	—	+	—	—
Skin	D	D	+	D	D	+	D	D	+
Lung	D	D	D	D	D	+	D	D	+
Intestine	D	D	+	D	D	+	D	+	+
Heart	+	—	—	—	—	—	D	+	—
Muscle (leg)	—	—	—	+	—	—	—	—	—

Legend same as Table I, except — = mice survived 10 days but had no lung lesions at autopsy.

[3] Burnet, F. M., *Brit. J. Exp. Path.*, 1940, **21**, 147.

[4] Nigg, C., Crowley, J. H., and Wilson, D. E., *Science*, 1940, **91**, 603.

[5] Burnet, F. M., *Austral. J. Exp. Biol. and Med. Sc.*, 1940, **18**, 353.

[6] Henle, W., and Chambers, L. A., PROC. SOC. EXP. BIOL. AND MED., 1941. **46**, 713.

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.

13250

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.