useful method for the detection of lymphogranuloma venereum infection particularly in individuals who have not been tested with Frei antigen, as well as for immunological studies in infections of humans and animals with this etiological agent.

Summary. In individuals with lymphogranuloma venereum, the serum has been found to fix complement regularly in the presence of antigens containing the virus in high concentration. Such fixation was observed only once with sera taken from supposedly uninfected individuals but was obtained frequently in syphilitic sera showing markedly positive Wassermann reactions.

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Persistence of St. Louis Encephalitis Virus in the Brains of Chicks.*

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St. Louis encephalitis virus has been cultivated *in vitro* on minced chick embryonic tissue[1] as well as in the yolk[2] and on the chorio-allantoic membrane[1, 3-5] of chick embryos. On chorio-allantoic membranes, serial passage through 68[3] and more than 100[6] transfers has been possible. Brains of the corresponding embryos have been found to contain slightly more virus than the membranes, virus being also present in the livers and spleens.[3, 5] Virus has been demonstrated in the brains of chicks allowed to hatch[1] though only slight or no microscopic changes were demonstrable.[1, 3] It has been observed that embryos may survive until the time of hatching,[3] or die within a few days after inoculation.[1, 6]

Harrison and Moore[1] have reported that young chicks (4 to 6

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

2 Stimpert, F., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 483.

3 Smith, M. G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 191.

4 Smith, M. G., and Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 323.

5 Schultz, E. W., Williams, G. F., and Hetherington, A., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 799.

6 Schultz, E. W., *et al.*, unpublished work.

days old) were found somewhat susceptible. After inoculation with mouse brain virus, 3 out of 6 showed clinical (paralysis) or microscopic evidence of infection, or both, and virus was recovered from the brains of 3 out of 4 of the chicks tested for virus.

The present report deals with the susceptibility of young chicks to

TABLE I.
Results Obtained on Inoculating Suspensions of Chick Brains into Mice.

Days after inoculation chicks were killed	Dilution of chick brain employed	Death of mice in days		
2	1:10	6	6	7
	1:100	6	6	6
2	1:10	6	6	*
	1:100	6	8	—
	1:1000	8	10	—
	1:10,000	—	—	—
4	1:10	5	7	*
	1:100	5	5	12
4	1:10	5	6	—
	1:100	6	—	—
	1:1000	—	—	—
6	1:10	4	7	11
	1:100	7	8	—
6	1:10	8	10	*
	1:100	6	8	8
	1:1000	—	—	—
8	1:10	6	—	—
	1:100	—	—	—
10	1:10	5	8	8
	1:100	8	—	—
10	1:10	6	—	—
	1:100	—	—	—
12	1:10	7	8	9
	1:100	11	16	*
	1:1000	—	—	—
12	1:10	—	—	—
17	1:10	10	—	—
20	1:10	8	10	—
21		—	—	—
31	1:10	—	—	—
42		—	—	—
56		—	—	—

Six survivors were discarded.
— Survived.
* Accidental death.

mouse brain passage virus. White leghorn, barred rock and Rhode Island red chicks were tested for susceptibility. The presence of virus in brains, livers or spleens of the chicks was determined by inoculating 3 mice each intracerebrally with 0.03 cc of a suspension (usually 10%) of material. Surviving test mice were observed for 21 days. All virus suspensions were also tested for bacterial sterility.

Webster's No. 3 strain of the virus was used. It regularly killed mice when inoculated intracerebrally in a dilution of 10^{-5}.

Of forty 2-day-old leghorn chicks which were inoculated subdurally with 0.03 cc of a 10% suspension of mouse brain virus, none showed any sign of infection. The object of the investigation then was to determine whether or not the brains harbored active virus. The chicks were killed 2 at a time at intervals of several days over a period of 56 days. The brains of the 2 chicks were pooled, ground in a mortar and 3 mice were inoculated with each dilution. In some cases one brain only was ground, while the other was sectioned for microscopic examination. The results of the tests for virus are given in Table 1.

Histological examination of the brains of 11 chicks, not tested for virus, but killed on the 2nd to 20th day after inoculation showed only slight, and inconstant changes, consisting at most of small, perivascular, mononuclear infiltrations. The livers, spleens, and kidneys of these animals in all cases appeared normal. No changes were observed in the brains and other tissues from chicks killed more than 20 days after inoculation.

Groups of 4 to 6 chicks, 2 days old, were then inoculated by various routes with 10% mouse brain virus. None of these showed any sign of infection. They were killed at intervals and the tissues pooled and tested for virus. No gross changes were noted in any of the tissues. Microscopic sections of brains were stained by Lentz A method, other tissues with hematoxylin and eosin. The observations on this group are given in Table II.

Ten leghorn chicks, 21 days old, were inoculated subdurally, each with 0.03 cc of 10% mouse brain virus. These showed no sign of infection over a period of one month.

Serial passage of virus was tried (using leghorns for the first 2 passages and barred rocks subsequently) in chicks 2 to 6 days old. Three chicks were inoculated subdurally each with 0.03 cc of 10% mouse brain virus. These were killed 3 to 4 days later. Their brains were ground together to make a 10% suspension and 0.03 cc were inoculated subdurally into each of 3 new chicks and intra-

TABLE II.
Results Obtained When Chicks Were Inoculated with a 10% Suspension of Mouse Brain Virus.

No. of chicks inoculated	5 Leghorns	4 Barred Rocks	5 Barred Rocks	6 R. I. Reds	6 R. I. Reds
Route and amount inoculated	0.3 cc subcutaneously	0.03 cc subdurally	0.3 cc intraperitoneally	0.03 cc subdurally	0.4 cc intraperitoneally
Days after inoculation chicks killed	30	28	28	21	21
Results on inoculating tissues into mice were negative.	3 brains	(a) 2 brains	3 brains	3 brains	3 brains
Kind of tissue (pooled)	3 (livers spleens)	3 (livers spleens)	3 (livers spleens)	(½ of each)	3 (livers spleens)
Microscopic examination of tissues of chicks which were not tested for virus	(b)	(b)	(c)	(d)	(e)

(a) One of 3 mice died

(b) No changes observed in brains, livers or spleens

(c) One brain had a small area of mononuclear cell infiltration in the choroid plexus; a second had a similar area but perivascular in distribution.

(d) Of 3 brains sectioned (in this case half of each brain was tested for virus), 2 showed several small foci of mononuclear cell infiltration, mostly in the cerebellum at the level of Purkinje cells.

(e) Brains not examined. Livers and spleens showed no changes.

TABLE III.

No. of the serial passage	1	2	3	4	5
Death of individual mice in days	5 5 5	6 6 7	8 8 10	19 — —	7 7 12
No. of the serial passage	6	7	8	9 10 days	9 25 days
Death of individual mice in days	4 9 14	9 10 *	7 7 7	7 8 —	— — —

— Survival
* Accidental death

cerebrally into 3 mice. None of the chicks showed any sign of infection. Six instead of 3 chicks were used in the 9th passage; 3 of these were killed 10 days and three 25 days after inoculation. The results of the mouse inoculation with material from each serial chick passage are given in Table III.

Microscopic sections of the 3 brains of chicks from the 9th passage sacrificed 10 days after inoculation showed several foci of mononuclear cell infiltration near the meninges, some diffuse mononuclear cell infiltration and perivascular infiltration in various areas and particularly in the cerebellum.

Serial passage in chicks 2 to 6 days old was also attempted, in which the initial inoculation consisted of 0.5 cc of a 10% suspension of mouse-brain-virus administered intraperitoneally to each of 3 chicks. Transfers were then made by grinding all the brains and spleens together to make a 10% suspension and inoculating each of 3 new chicks with 0.5 cc intraperitoneally and 3 mice each with 0.03 cc intracerebrally. None of the chicks showed any sign of infection. Virus was recovered from the first passage, but not from the subsequent 8 passages.

Summary. Two-day-old chicks, after inoculation with St. Louis encephalitis virus, subdurally or by other routes, failed to show any clinical evidence of infection. Nevertheless, the brains of chicks which had been inoculated subdurally proved infectious for mice in a dilution of 10^{-2} for at least 6 days and sometimes in a dilution of 10^{-1} up to 20 days. Histological sections of such brains showed only slight changes, consisting at most of small areas of perivascular infiltrations.

The virus was carried through nine serial passages at 3- to 4-day intervals in the brains of 2- to 6-day-old chicks. Chick brains of the ninth serial passage showed areas of focal, diffuse and perivascular infiltration.