or two points of interest which deserve comment.

Perhaps the most striking aspect of the experiments is the enormous number of viable organisms found necessary to establish a fatal infection. That the reaction was a true infection seems established by tissue sections from dead animals, which showed characteristic, developing, granulomatous areas containing numerous giant cells congested with the yeast cells of *H. capsulatum*. Furthermore, the injection of heat-killed organisms in maximal number was without significant pathological effect. Our results are in accord with Campbell and Saslaw(1), who used an inoculum which was about equivalent to ours, in terms of the number of organisms per gram of body weight necessary to produce death in the infected animal. (It may be noted, however, that Campbell and Saslaw used dosage levels based on the absolute numbers of yeast cells, stating that "it (is) impracticable to attempt to determine the actual number of viable cells.") Howell *et al.*(2) used intracerebral inoculation, and took into consideration the number of viable organisms in the inoculum. Interestingly enough, it may be calculated from their data that the intracerebral route of administration requires only 1/200 the number of viable yeast cells of *H. capsulatum* as does the intravenous route. It seems unlikely that a difference of this magnitude could be attributable to a difference in virulence of the strains employed; probably it is a reflection of the difference in the capacity of the host's defense mechanisms to cope with invasion by these different routes. This point is further illustrated by the ineffectiveness of intraperitoneal injection in our tests, and in those of Campbell and Saslaw, who found it necessary to add gastric mucin in 5% concentration to the yeast cell suspension in order to obtain infection by the intraperitoneal route.

From the standpoint of using the infected rat for *in vivo* studies of fungicidal agents, it is perhaps unfortunate that a change from 0% to 100% mortality should occur with only a ten-fold increase in the number of viable organisms injected. This fact, together with the inevitable uncertainty regarding the percentage of viable organisms in an inoculum, makes it essential to employ more than one dosage level of viable organisms, in order to achieve good approximation to any particular desired percentage mortality in the control animals.

Summary. The yeast cell phase of *Histoplasma capsulatum*, when injected intravenously in suitable numbers, has been shown to yield a consistently reproducible fatal infection in the rat. The necessity for standardizing the inoculum on the basis of the number of viable organisms, rather than the absolute number of organisms, is emphasized.

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Experimental Intraocular Infection of Guinea Pigs with Mumps Virus.*† (18133)

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The virus of mumps is known to infect

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the human being(1), the monkey(2), and the chick embryo(3) with the production of complement fixing antibodies in man and monkeys. Previously, the guinea pig has been found to produce no complement fixing antibodies after intraocular injection of filtrate from infected parotid gland of the monkey(4).

This paper presents experiments on (a) the susceptibility to mumps virus of the ocular tissues of the guinea pig; (b) the recovery of mumps virus from the ocular tissue of guinea pigs after five successive passages; (c) the production of high complement fixing antibody titers after intraocular injection of mumps virus, and (d) the irregular production of complement fixing antibody after extraocular inoculation of mumps virus.

Material and methods. An embryo-adapted strain of mumps virus which had undergone 52 passages in embryos was employed in these experiments.‖ The virus pool was prepared by inoculating 0.1 ml of infected allantoic fluid into the allantoic sac of seven-day-old chick embryos. After 5 days of incubation at 35°C, the allantoic fluid was harvested, sealed in small pyrex tubes, and stored in a dry ice cabinet until used. In the allantoic sac of chick embryos this material had an average titer (ID_{50}) of $10^{9.3}$ 50% infective doses per ml. Allantoic fluid collected from uninoculated 13-day embryos was employed as control for the intraocular injections. The guinea pigs employed in these experiments were young adult, albino males. From each guinea pig two samples of serum were collected. The first was taken immediately before mumps virus injection and the second 18 days later. Aqueous humor (0.02-0.05 ml) was withdrawn from the anterior chamber of the guinea pig eyes with a tuberculin syringe, fitted with a 27 gauge needle. The needle was introduced through the sclera about 1 mm from the limbus. The aqueous humor of the left eye was replaced with 0.05 ml of virus-infected allantoic fluid, and that of the right eye was replaced with 0.05 ml of normal allantoic fluid. Beef heart-infusion broth (pH 7.4) was used as the diluent.

In Exp. II and III the aqueous humor from the anterior chamber of the injected eyes was not cultured for bacteria. Emulsified ocular tissue from Exp. I was cultured on blood agar plates at 37°C for 48 hours. These cultures showed no bacteria under aerobic or anaerobic conditions. Certain specimens that had been frozen (some contained penicillin and streptomycin while others did not) were cultured for pleuropneumonia organisms with negative results. The pleuropneumonia culture technic included the use of aerobic and anaerobic methods with media containing horse serum and had proved adequate for the isolation of such organisms from rats, mice, and human prostatic secretion.¶ Eyes that have been infected with bacteria are easily recognized by macroscopic inspection. In these experiments bacterial infections were rare, but to minimize the possibility of such infections both penicillin and streptomycin were employed, each in a concentration of 2.5 μg per ml(5). Kolmer's(6) method was employed for the determination of the levels in the sera of complement-fixing antibody against the mumps virus. The sera were preserved at −70°C or at 4°C so that all of the complement-fixation tests for each experiment could be performed simultaneously. Each serum was tested with normal allantoic fluid and complement for the detection of complement-fixing antibody against non-viral embryo material. The sera were inactivated by heating twice at 60°C for 20

1. Johnson, C. D., and Goodpasture, E. W., *Am. J. Hyg.*, 1935, v21, 46.

2. Johnson, C. D., and Goodpasture, E. W., *J. Exp. Med.*, 1934, v59, 1.

3. Habel, K., *Publ. Health Rep.*, Wash., 1946, v60, 201.

4. Enders, J. F., Kane, L. W., Cohen, S., and Levans, J. H., *J. Exp. Med.*, 1945, v81, 93.

‖ Originally supplied to one of us by Dr. J. F. Enders.

¶ Pleuropeneumonia cultures were performed by Dr. John C. Nunemaker.

5. Leymaster, G. R., and Ward, T. G., *J. Immunol.*, 1949, v61, 95.

6. Kolmer, J. A., and Boerner, F., *Approved Laboratory Technic*, 4th Ed., Appleton Century, 1945.

minutes to reduce anti-complementary effects. Titers were expressed as the final dilution of serum prior to adding the sensitized erythrocytes. End points were recorded as the highest dilution of serum which gave 3 or 4 + fixation of complement. For chicken red blood cell agglutination-inhibition tests, a modification of Salk's method for influenza was employed(7). Agglutination-inhibition tests for identification of the virus recovered from guinea pig ocular tissue were performed, using sera from both acute and convalescent human cases and from guinea pigs experimentally infected with mumps virus. The antigens used were (1) allantoic fluid infected with stock virus of the "Enders" strain, and (2) allantoic fluid infected with "recovered virus" from ocular tissues of guinea pigs.

Exp. I—Serial passage of mumps virus in guinea pig ocular tissue, employing a 10% pooled suspension of retina, uvea, cornea and lens for inoculum, has been performed. The passage suspensions were ground in a mortar with a pestle and centrifuged at 2000 r.p.m. for 5 minutes. The supernatant was passed serially every 48 hours until 7 passages were achieved. Three to 8 guinea pigs were employed in each passage. Convalescent sera collected from the 2 guinea pigs of passage I (injected with undiluted stock virus) and 1 of 3 guinea pigs of passage II showed complement-fixing antibodies in titers of 1:640, 1:1280 and 1:20, respectively, against the stock virus. The convalescent sera obtained from the guinea pigs in passages III, IV, V, VI and those injected with Seitz filtrate from which virus was recovered in embryonated eggs, failed to show complement-fixing antibodies against the stock virus.

Six guinea pigs were injected with suspensions from passage VI. Of the 6 animals, 2 were injected with pooled suspensions of corneas and 4 with pooled suspensions of retina, uvea and lens. The sclerae were discarded. The convalescent sera from 2 of the 4 guinea pigs in passage VII, injected with ocular tissue other than cornea, showed complement-fixing antibody titers of 1:160 and 1:320 against the stock virus, indicating infection with the adapted guinea pig virus. Pooled Seitz filtrate from ocular tissues of passage V with either VI or VII was injected into both eyes of 7 guinea pigs and into 8 seven-day-old embryonated eggs, according to the technic previously described(5).

On the 5th day after inoculation of the above filtrate, the pooled allantoic fluid collected from 7 of the 8 embryos caused hemagglutination of chicken red blood cells at a titer of 1024. The identity of the recovered virus subsequent to a second egg passage was established in 3 ways. First, comparative agglutination-inhibition tests were performed, employing pools of stock and recovered virus as antigens against acute and convalescent sera from human patients with mumps and from experimentally infected guinea pigs.

Results as presented in Table I show that the sera had comparable agglutination-inhibition titers against each virus. Employing the two viruses, convalescent human and guinea pig sera and complement fixation tests, comparable levels of complement fixing antibodies were demonstrated, indicating the serological relationship of the recovered virus to stock virus. The recovered virus was inoculated into the anterior chamber of the eyes of 5 guinea pigs. The inoculated eyes developed a mild corneal reaction. The convalescent sera from these guinea pigs with stock virus fixed complement and resulted in titers of 1:40-1:160. Complement fixation tests, for the detection of antibody formation in guinea

TABLE I. Comparative Agglutination-Inhibition Titers Produced by Human and Guinea Pig Sera Against Stock and Recovered Virus.

Source of sera	Sera phase	Recovered virus	Stock virus
Human (LL)	Acute	32	32
	Conv.	2048	2048
Human (NW)	Acute	128	128
	Conv.	2048	2048
Guinea pig #390	Acute	<2	<2
	Conv.	1024	512
Guinea pig #373	Acute	16	16
	Conv.	2048	1024

7. Robbins, F. C., Kilham, L., Levens, J. H., and Enders, J. F., *J. Immunol.*, 1949, v61, 235.

TABLE II. Production of Complement-Fixing Antibodies and Corneal Opacity as a Result of Intraocular Injection of Mumps Virus.

Guinea pig No.*	Eye inj.	Dilution of inoculum†	Persistence of corneal opacity			Results of CF tests‡	
			24 hr	72 hr	18 days	Initial sera	Conv. sera
199	Rt.	A,10^{-3}	0	0	0	<10	80
	Lt.	V,10^{-3}	+	±	0		
200	Rt.	A,10^{-3}	0	0	0	<10	<10
	Lt.	V,10^{-3}	0	0	0		
201	Rt.	A,10^{-2}	0	0	0	<10	80
	Lt.	V,10^{-2}	0	±	0		
202	Rt.	A,10^{-2}	0	0	0	<10	320
	Lt.	V,10^{-2}	++	++++	++++		
203	Rt.	A,10^{-1}	0	0	0	<10	320
	Lt.	V,10^{-1}	++	++++	++++		
204	Rt.	A,10^{-1}	0	0	0	<10	320
	Lt.	V,10^{-1}	+++	++++	++++		
211	Rt.	A,10^{0}	0	0	0	<10	320
	Lt.	V,10^{0}	++	++	++++		
212	Rt.	A,10^{0}	0	0	0	<10	320
	Lt.	V,10^{0}	+++	++++	++++		

* Eyes of guinea pigs (189-198) injected with virus dilution 10^{-4} or higher, developed no corneal reaction while the initial and convalescent sera of these animals showed no complement-fixing antibodies in a dilution of 1:10, the lowest dilution tested.

† A = Dilution of 13-day normal allantoic fluid injected into right. V = Dilution of mumps virus-infected allantoic fluid injected into left eye.

‡ Normal allantoic fluid controls were negative in each instance of lowest serum dilution tested (1:10). Titers expressed as the denominator of the dilution.

pigs against non-viral material in allantoic fluid, proved to be negative in a dilution of 1:10, the lowest dilution of sera tested. This was found to be true also for the two experiments reported below.

Exp. II.—This experiment was performed to determine the relative amounts of injected stock mumps virus required to produce infection in the eye of the guinea pig as indicated by the corneal reaction and the subsequent appearance of complement-fixing antibodies in the convalescent sera of guinea pigs against the stock mumps virus. Mumps infected allantoic fluid, undiluted and in tenfold dilutions through 10^{-8}, was injected into the left eyes of eighteen guinea pigs, 2 animals being used for each dilution. The right eyes were injected in a similar manner with similarly diluted normal allantoic fluids. As may be seen in Table II, one of the two guinea pigs injected with virus dilution 10^{-3}, as well as one of those two injected with virus dilution 10^{-2}, and all of those receiving lower dilutions, developed complement fixing titers of 1:80-1:320. Guinea pigs injected with virus dilutions 10^{-4} or higher, and one guinea pig (No. 200) injected with virus dilution 10^{-3} developed no complement fixing antibodies against the mumps virus. Guinea pigs injected with virus dilution 10^{-2}, or lower, developed a severe corneal reaction with subsequent pannus that was still present in about the same degree 18 days later. The corneal reaction began to appear within 24 hours after the inoculation of material which contained relatively large amounts of virus. At 48-72 hours after injection the entire cornea was involved. When smaller amounts of virus were employed, the corneal reaction was transitory and the normal translucency returned to the cornea in 3 to 14 days. This has been observed previously in experiments not reported in this paper. A minimal corneal opacity that disappeared in 72 hours was seen in guinea pigs No. 201 (10^{-2} dilution) and No. 199 (10^{-3} dilution). These minimal corneal reactions were associated with complement-fixing antibody titers of 1:80. Four of the 8 left eyes injected with dilutions of virus beyond 10^{-4} developed a transitory corneal reaction which disappeared within 24 hours. No complement-fixing anti-

body was demonstrated in these guinea pigs. The injection of the 16 right eyes with the various dilutions of normal allantoic fluid produced no corneal opacity.

Exp. III.—The comparative effectiveness of intraperitoneal, intraplantar space and intracardial virus injection for complement-fixing antibody production was studied. Three to five hundredths ml. of undiluted mumps virus-infected allantoic fluid was injected into the peritoneal cavity of 6 guinea pigs and into the plantar spaces of the hind foot of a similar number. Four animals were injected intracardially with 0.05 m. of the same fluid.

Of the 16 guinea pigs, one developed a complement-fixing antibody titer of 1:40, 5 of 1:20, 3 of 1:10, and 7 less than 1:10, the lowest dilution of sera tested.

Discussion. The characteristic corneal reaction that develops after the injection of relatively large amounts of mumps virus into the eyes is probably a result of the direct action of the virus itself on the endothelial cells of the inner surface of the cornea. The nature of this effect is being tested by experiments now under way. From the data shown in Table II, it is clear that relatively large amounts of embryo virus must be injected into the eye before the guinea pig can produce complement-fixing antibody. Subsequent to intraocular inoculation, 99% of the virus disappears from the eye within 48 hours, following which it grows or persists within the ocular tissues for at least 8 days(8).

The intraperitoneal, intraplantar space or intracardial inoculation of six to ten times the amount of mumps virus that is adequate for antibody production after intraocular injection has failed to produce complement-fixing antibodies, or resulted in low titers only. The explanation for this is probably related to failure of growth or limited growth of virus in extraocular guinea pig tissue.

The production of complement-fixing antibodies in the guina pigs of passage II may be in part the result of the transfer of "residual" virus from passage I, 48 hours after inoculation. The failure of complement-fixing antibodies to appear in the sera of guinea pigs of passages III, IV, V, VI, and of those injected with the Seitz filtrate pool against the stock virus is not known. In passage VII, 2 of 4 guinea pigs developed complement-fixing antibody titers of 1:160 and 1:320. This indicates that these guinea pigs developed a virus infection as a result of the ocular injection of passage VI. Mumps virus was recovered on two different occasions from a Seitz filtrate pool of passage V pooled with either passage VI or VII injected into 7-day-old embryonated eggs. It is unlikely that the virus recovered from the fifth passage was merely "residual" virus carried along from the first passage since 5 passages would produce about a 10,000 fold dilution of egg virus, which is beyond the range of infectivity for guinea pig ocular tissue as may be seen in Table II.

Additional studies have been planned to work out the inconsistencies which have occurred during the serial passage of virus in guinea pig eyes.

Summary and Conclusions. 1. Chick embryo-adapted mumps virus has been shown to infect the tissues of the guinea pig's eye after intraocular inoculation.

2. Mumps virus has been carried through 7 successive guinea pig passages by intraocular injection with the subsequent production of complement-fixing antibodies in the sera of guinea pigs in passages I, II and VII. A virus culturally and immunologically indistinguishable from the original virus has been recovered from ocular tissue of passages V, VI and VII by inoculation into chick embryos.

3. When doses of virus 6 to 10 times as large as those necessary to infect the anterior chamber were introduced into the plantar spaces, peritoneal cavity, or heart, antibody formation was low or absent as shown by titers of the complement fixing antibody in the convalescent sera.

4. The guinea pig appears to be a suitable animal for the study of mumps virus infections when inoculations are made into the anterior chamber of the eye.

8. Bolin, V. S., Bosma, J. F., and Newton, J. D., Unpublished data.

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