oxygen uptake at first are proportional. However, with time there is a disproportional decrease in the oxygen consumed at the different cell concentrations.

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Use of Guinea Pig Eye in Study of Intraocular Infections Produced by Mumps Virus.* (19255)

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The injection of mumps virus into the guinea pig eye has been found to produce corneal opacity and result in the appearance of complement-fixing antibodies in the convalescent sera(1). The experiments in this paper deal with the use of the guinea pig eye in (a) the study of the neutralization of mumps virus by specific immune serum and pooled sera from normal mumps susceptible children. Further investigations were performed using the intraocular injection of mumps virus to demonstrate (b) the rise, persistence and decline of complement fixing antibodies in sera, (c) the persistence of mumps virus in ocular tissue, and (d) the corneal reaction and the subsequent appearance of complement fixing antibodies in the convalescent sera after the intraocular injection of 3 recently isolated strains of mumps virus.

Materials and methods. The mumps virus used in experiments I-III was Ender's embryo-adapted strain employed in the earlier study(1); it had undergone 54 serial passages in chick embryos. In the allantoic sac of chick embryos, the virus had a titre (ID_{50}) of $10^{8.3}$ 50% infective doses per ml. In the fourth experiment, 3 recently isolated strains of mumps virus were employed for intraocular inoculation. The JWE_2 virus was recovered from emulsified parotid tissue obtained from a child who had parotitis and meningoencephalitis at the time of his death(2), and had undergone 2 embryo passages. The $WTFE_6$ was recovered from aspirated testicular fluid obtained from a patient with orchitis, and the $135E_8$ from saliva obtained from a patient with parotitis(3). Each of these strains had undergone 6 and 8 embryo passages, respectively. The materials from which these 3 viruses were recovered were injected into the amniotic cavity of 7-day-old chick embryos. The titres of the viruses were not determined. The sera employed in the neutralization tests in guinea pig eyes were from 2 sources. The immune sera were obtained from the authors (V.S.B. and G.R.L.) 18 days after the intramuscular injection of 1 ml of mumps vaccine.† The complement fixing

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TABLE I. The Use of the Guinea Pig Eye as an Indicator for the Neutralization of Mumps Virus by Immune and Non-Immune Human Sera.*

Material injected	Guinea pig No.	Dilution of virus	No. of reacting corneas	No. of eyes with persisting corneal opacity (days) 1 2 3 4 5 6 7 10 11 12 13 14 15 16	Results of CF tests† Initial sera	Conv. sera
Pooled sera from mumps susceptible children (MSC) + virus	504-506	10^{-3}	4	4 2 0 0 1 1 1 1 1 1 1 1 0 0	<5	<5 <5 <5
	507-509	10^{-2}	1	1 0 0 0 0 0 0 0 0 0 0 0 0 0	<5	40 20 <5
	510-512	10^{-1}	2	2 2 0 0 0 0 0 0 0 0 0 0 0 0	<5	80 40 <5
	513-515	1:2	2	2 1 0 0 0 0 0 0 0 0 0 0 0 0	<5	40 80 40
Virus‡	528-530	10^{-3}	2	2 2 1 0 0 0 0 0 0 0 0 0 0 0	<5	80 80 160
	531-533	10^{-2}	6	6 6 4 4 0 0 0 0 0 0 0 2 2 0 0	<5	160 320 1280
	534-536	10^{-1}	5	4 4 5 5 5 5 5 5 1 1 4 3 2 1	<5	640 320 160
	537-539	1:2	6	4 6 6 6 3 3 4 4 3 3 3 3 3 3	<5	1280 80 160
Immune sera + virus§	516-525	10^{-3} 10^{-2} 10^{-1}	0		<5	<5
	526	1:2			<5	<5
	527	”			<5	5
Immune sera + normal allantoic fluid	540-542	—	0		<5	<5
Pooled sera from MSC + normal allantoic fluid	543-545	—	0		<5	<5

* Guinea pigs in groups of 3 injected in both eyes with each sample of sera and dilution of virus.

† Normal allantoic fluid controls were negative in each instance of lowest serum dilution tested (1:5). Titres expressed as the denominator of the dilution.

‡ Convalescent serum titres are individually expressed (except No. 540-545) and correspond to the respective guinea pig number.

antibody titre of each of these sera prior to pooling for the neutralization tests was found to be 1:20. Serum was obtained from 3 children who gave a negative history of mumps. The ages of these children were 2.5 to 5 years. Each serum in a final dilution of 1 in 8 was tested against 1000 ID_{50} mumps virus. Each serum-virus mixture was injected into 8 chick embryos. The results showed that the sera prevented the growth of mumps virus in 1/8, 2/8, and 1/8 chick embryos. In each instance the protective titre of serum was less than a dilution of 1 in 8, and the tests were considered negative(4). When the unheated sera were employed in the neutralization tests, the 3 samples were pooled and mixed with the desired dilutions of virus. Each sample of sera was stored in a glass vial at —20°C until used.

Neutralization tests were carried out by injecting mixtures of serum and increasing dilutions of virus into the anterior chamber of the eye. The serum-virus mixtures were incubated for one hour at room temperature prior

to injection. The methods employed for intraocular injection of materials, the use of antibiotics in the inoculum, and the means of determining the levels of complement fixing antibodies in the sera of guinea pigs have been described(1). The normal allantoic fluid employed as a control for eye injection and complement fixation tests was collected from 13-day-old chick embryos. In these experiments convalescent blood was obtained from the guinea pigs on the 16th to 18th day after the injection of virus into the eye.

Experimental. Exp. I. Thirty-six guinea pigs were divided into 12 groups of 3 guinea pigs each. Mumps virus was injected into both eyes of the guinea pigs of 4 of the 12 groups. One group was injected with virus dilutions 10^{-3}, one with 10^{-2}, one with 10^{-1}, and one with 1:2, while the remaining 8 groups were injected with either pooled sera from normal mumps susceptible children or specific immune sera mixed in similar final dilutions of virus. Immune sera and sera from normal mumps susceptible children were each mixed with an equal volume of normal allantoic fluid, and each mixture was injected into both eyes of 3 guinea pigs. The volume of each mixture injected into each eye was 0.03 ml. The results are shown in Table I.

The specific immune serum neutralized the virus and completely prevented the corneal reaction in the 24 guinea pig eyes. Fifteen of the 24 eyes injected with pooled serum from normal mumps susceptible children and virus developed no corneal reaction. The corneal opacity in the 9 reacting eyes was mild and lasted only 24-48 hours. The corneal reaction in the eye of one of the latter animals (No. 506) reoccurred 3 days later.

Of the 24 eyes injected with the various dilutions of virus, 5 failed to develop the corneal reaction. Four of these 5 eyes were injected with virus dilution 10^{-3}. The corneal opacity that developed in 19 of the 24 eyes persisted from 48 hours to 16 days. The corneal reaction in 2 of the 3 guinea pigs injected with virus dilution 10^{-2} persisted for 96 hours and in the third guinea pig (No. 533) for 48 hours. Guinea pig No. 533 developed a second mild corneal reaction and severe iritis 11 days after the initial corneal opacity. Four guinea pigs (534, 535, 536, 539) injected with virus dilution 10^{-1} and 1:2 developed similar recurrent corneal reactions 5 to 11 days after the injection of virus into the eye.

Of the 12 guinea pigs injected with pooled sera from mumps susceptible children, mixed with virus, 5 developed no complement fixing antibodies. The remaining 7 guinea pigs developed relatively low complement fixing antibody titres ranging from 1:20-1:80. Of the 12 guinea pigs injected with specific immune serum and various dilutions of virus, 11 showed no complement fixing antibody in sera diluted 1:5, the lowest dilution tested. The 12th guinea pig (No. 527) injected with virus dilution 1:2 developed a complement fixing antibody titre of 1:5. The 12 guinea pigs injected with the various dilutions of mumps virus without serum developed complement fixing antibody titres that ranged from 1:80-1:1280.

The 6 guinea pigs injected with normal allantoic fluid mixed as a 1:2 dilution with pooled sera from mumps susceptible children or immune sera demonstrated no corneal opacity and no complement fixing antibody in a dilution of 1:5, the lowest dilution tested.

Exp. II. The rise, persistence and decline of complement fixing antibody against the mumps virus was studied in 4 guinea pigs. Each animal was injected in the left eye with 0.05 ml of mumps virus. The right eye was not injected. Sera were collected prior to virus injection and every 4 days thereafter for 28 days. Eight samples of sera were obtained from each animal.

Fig. 1 demonstrates the absence of complement fixing antibody in the sera of the 4

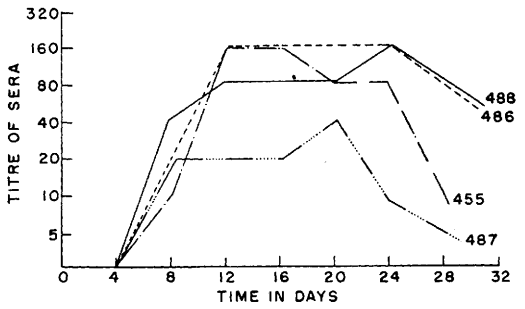

FIG. 1. Rise and decline of complement fixing antibodies against the mumps virus in the convalescent sera of 4 guinea pigs.

TABLE II. Production of Corneal Opacity and Complement Fixing Antibodies in Guinea Pigs as a Result of Intraocular Injection of Recently Isolated Strains of Mumps Virus.

Source of virus*	Guinea pig No.	Eye†	Persistence of the corneal reaction 24 hr	48	72	8 days	16 days	CF results‡ Initial sera	CF results‡ Conv. sera
JWE_2	267	R	2+	2+	1+	0	0	<10	160
		L	±	0	0	0	0		
	268	R	4+	4+	4+	4+	4+	<10	40
		L	0	0	0	0	0		
	269	R	±	±	0	0	0	<10	160
		L	±	±	±	0	0		
$WTFE_6$	500	R	4+	4+	3+	3+	2+	< 5	160
		L	0	0	0	±	0		
	501	R	0	0	3+	3+	3+	< 5	80
		L	0	0	±	0	0		
$135E_8$	502	R	±	0	0	0	0	< 5	20
		L	0	0	0	0	0		
	503	R	0	0	0	0	0	< 5	80
		L	±	±	±	0	0		

* Numerical subscript of JWE = number of egg passages.
† Right eye, etc.
‡ Normal allantoic fluid controls were negative in each instance of lowest serum dilution tested (1:5 or 1:10). Titres expressed as denominator of dilution.

guinea pigs before the injection of virus and on the fourth day. On the eighth day significant levels of complement fixing antibodies were demonstrated. These levels persisted until the 20th to 24th day and then commenced to decline.

Exp. III. The next experiment demonstrates the persistence and levels of mumps virus in ocular tissue and the appearance of complement fixing antibodies in the convalescent sera of the guinea pigs after the removal of the virus injected eye. The left eyes of 42 guinea pigs were injected with 0.05 ml of undiluted mumps virus. After virus inoculation, both right and left eyes were removed at intervals of 2 hours to 8 days. There was a total of 15 intervals, the longest being 192 hours and the shortest 2 hours. The tests for virus were performed by injecting ground suspensions of ocular tissue (sclera was discarded) in increasing dilutions into the allantoic cavity of 7-day-old chick embryos. The virus diminished rapidly in the injected eyes, since at 2 and 4 hours the amounts of virus in the ocular tissue showed end points of 10^{-5} and 10^{-3}, respectively. The 10^{-3} level of virus persisted for 2 days. This level of virus then dropped to 10^{-2} and persisted for 2 days. During the next 4 days the end point was 10^{-1} or less. At 8 days, one of 8 eggs showed that ocular tissue still contained mumps virus. The right eyes were tested for virus. In each instance, the test was negative.

Of the 4 guinea pigs that had their eyes removed at 2 hours, 2 developed complement fixing antibody titres of 1:10 while no complement fixing antibody could be demonstrated in the sera of the other 2. In 36 guinea pigs the convalescent sera showed complement fixing antibody titres ranging from 1:10-1:80. These animals had their eyes removed at intervals of 4 hours to 8 days. One guinea pig that had its eyes removed 8 days after virus injection developed a complement fixing antibody titre of 1:320, and one with the eyes removed at 44 hours developed no complement fixing antibody.

Exp. IV. Three recently isolated strains of mumps virus were injected into both eyes of 7 guinea pigs. Control eyes injected with normal allantoic fluid were not included. Two animals were each injected with WTF_6 and $135E_8$ viruses, while 3 were injected with the JWE_2 virus. The volume of undiluted egg virus injected into each eye was 0.05 ml.

As may be seen in Table II, the 3 recently isolated viruses subsequent to 2, 6, and 8 passages in eggs produced an irregular corneal reaction in a majority of the eyes of the guinea pigs. The 7 guinea pigs developed

complement fixing antibody titres that ranged from 1:20-1:160.

Discussion. From the data presented in Table I, it is clear that specific immune serum antibody will neutralize the mumps virus and completely prevent the corneal reaction and the appearance of complement fixing antibodies in the convalescent sera of guinea pigs. When mumps virus is not completely neutralized in serum-virus mixtures, low titres of complement fixing antibodies may occasionally occur in the convalescent sera as was in the case of guinea pig No. 527.

The pooled sera from mumps susceptible children clearly showed an antiviral effect against the mumps virus. The duration of the corneal reaction in those guinea pigs injected with increasing dilutions of virus was 16 days. In contrast, the corneal reaction lasted only 72 hours in those guinea pigs injected with virus mixed with normal sera. All 12 of the guinea pigs injected with increasing dilutions of virus without sera developed complement fixing antibody titres that ranged from 1:80-1:1280. The antiviral effect of the pooled sera from mumps susceptible children appeared to prevent and modify the production of complement fixing antibodies, since 5 of the 12 guinea pigs failed to develop complement fixing antibodies while the remaining 7 developed titres ranging from 1:20-1:80.

The normal pooled human sera employed in the neutralization tests in guinea pig eyes was obtained from children who were considered to be non-immune to mumps virus infections. When serum-virus mixtures were injected into chick embryo, no neutralizing antibody was demonstrated in a sera dilution of 1 in 8. Numerous tests for mumps neutralizing antibody in human sera have shown that the single serum dilution of 1 in 8 seems to distinguish between the immune and non-immune. However, it is possible that the neutralization test in chick embryos is not sufficiently sensitive to demonstrate borderline cases of immunity in children (4).

The results in Table I demonstrate that normal allantoic fluid when mixed with either pooled normal sera or specific human sera and injected into guinea pig eyes plays no part in the corneal reaction or in the appearance of complement fixing antibodies in the convalescent sera.

Within 24 hours after the injection of large amounts of mumps virus into the eye, the corneal reaction occurs. The results in Table I show that mild corneal reactions were observed. The duration of these reactions was 48 hours to 12 days. During the interval of 10 days, they disappeared and reappeared several days later and persisted for variable lengths of time. This recurrent corneal reaction was observed in the eyes of guinea pigs 533, 534, 535, 536, and 539. In the case of guinea pig 533, the corneal reaction persisted for 48 hours and disappeared. Then on the 13th day a severe iritis appeared simultaneously with the second recurrent corneal reaction. The recovery of mumps virus in other experiments 8 days after intraocular injection indicated that the virus was in the ocular tissue at this time. The virus may be in part responsible for the unexplained iritis and the mild recurrent corneal reactions that occurred later in the course of infection.

No complement fixing antibodies were demonstrated in the sera of the guinea pigs before or on the fourth day after the injection of virus into the eye. However, on the 8th day significant amounts of antibody were present. It is possible that neutralizing antibody is in some way related to the final disappearance of virus in the ocular tissue. After the initial precipitous drop in titre, which was a 10,000-fold decrease in virus during the first few hours, the virus continued to diminish slowly in the ocular tissue after the fourth day. In general, the earlier the virus infected eye is removed after virus injection the lower the complement fixing antibody titre in the convalescent sera.

Recently isolated strains of mumps virus when injected into the guinea pig eye produce the corneal reaction and result in the production of complement fixing antibody titres that are comparable to those produced by Ender's strain of virus. The corneal reaction to the 3 viruses appears to be more irregular and less severe than the reaction produced by Ender's virus.

Conclusions. (1) When a mixture of mumps virus with specific immune serum was

injected into the eyes of guinea pigs, the serum completely prevented the corneal reaction and the production of complement fixing antibodies. Pooled sera collected from normal mumps susceptible children, when mixed with mumps virus and injected into the eyes of guinea pigs irregularly prevented the corneal reaction and the production of complement fixing antibodies. (2) Subsequent to the injection of mumps virus into the eye of the guinea pig, complement fixing antibodies appear in the convalescent sera between the 4th and 8th day, persist at significant levels until the 20th to 24th days, and then decline. (3) Mumps virus persists or grows in guinea pig eyes for at least 8 days after inoculation. (4) Chick embryo adapted strains of mumps virus recently recovered from parotid tissue, saliva and testicular fluid when injected into guinea pig eyes produce the corneal reaction, and complement fixing antibodies were demonstrated in the convalescent sera.

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Influence of Temperature upon Radiation Sensitivity of Thermophilic and Mesophilic Bacteria. (19256)

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Thermobiosis has been the subject of considerable speculation, but of little experimentation. Gaughran(1) indicated that there was no fundamental difference in the effect of temperature on the respiratory systems of stenothermophilic and mesophilic bacteria. He postulated that the thermophilic organism may produce a substance to protect its proteins against heat. Allen(2) postulated that growth at thermophilic temperatures was possible because of an increased rate of synthesis of cellular proteins. She pointed out the relative ease with which thermophilic strains may be obtained from mesophilic cultures, and suggested that a very simple genetic change must be involved. Militzer *et al.*(3-5), and Georgi *et al.*(6) have recently shown that a number of thermophilic enzymes are much more heat stable than are those of mesophiles. The influences upon radiation sensitivity of temperature and of oxygen tension during irradiation have been examined by several workers. Hollaender *et al.*(7) reported an increased X-ray sensitivity when *Escherichia coli* was irradiated in an aerobic atmosphere. The same was also observed at low temperatures (2°C), but only when oxygen was present. Wyss(8) reported that hydrogenase-producing organisms can resist radiation much more effectively in an atmosphere of hydrogen than of nitrogen or methane, and that an oxygen atmosphere markedly increased their sensitivity. The demonstration of heat-stable enzymatic proteins in thermophiles suggests the presence of heat-stable nuclear proteins. Since thermophilic strains may be readily selected from mesophilic cultures, it seems doubtful that an independent genic alteration would be necessary for rendering each specific protein heat-stable. It appears more reasonable to believe that the modification resulting in an increased heat stability is established in the non-specific protein molecule before its final specificity is determined, *i.e.*, early in its synthetic sequence.

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