AN INVESTIGATION OF THE ETIOLOGY OF MUMPS*

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PLATES 1 TO 3

The earlier investigations concerning the etiology of mumps dealt with the cultivation of microorganisms by ordinary bacteriological methods. Cocci were isolated and cultivated from the aspirated fluid of the swollen glands, from the blood, and from the saliva; but attempts to reproduce the disease in experimental animals by inoculation of these bacteria into the parotid glands always met with negative results.

Granata in 1908 was the first to report attempts to reproduce the disease in animals by introducing into the glands material from the patient (1). He was able thus to produce in rabbits a rise in temperature of 3 days duration by injecting intravenously a bacterially sterile filtrate of saliva of patients suffering from mumps. He was also able to produce swelling of the parotid glands of rabbits by the direct inoculation of the gland with the filtrate. Granata therefore suggested that the etiological agent of epidemic parotitis is a filterable virus.

Nicolle and Conseil in 1913 produced swelling of the parotid in one of three monkeys by injecting directly into the parenchyma of the gland material aspirated from the parotids of children suffering with the malady. The other monkeys developed only a fever of 4 to 7 days duration and a mononuclear leucocytosis after an incubation period of 16 to 30 days (2).

Gordon in 1914 used a bacteria-free filtrate of saliva from patients with mumps for intracerebral inoculation of ten monkeys, five *Macacus rhesus* and five *cynomolgus*. Four of these animals, three of which were *Macacus cynomolgus*, died after having developed meningeal symptoms on the 4th day. They were found to have had a sterile lymphocytic meningitis, degenerative changes in the cortical neurones and anterior horn cells. Unsuccessful attempts were made to transfer the infection from one monkey to another by the use of filtrates of emulsions of brain and spinal cord. One monkey, inoculated intraperitoneally and intravenously, became ill after an incubation period of 7 days and showed swelling of the parotid glands (3).

Wollstein in 1916, using cats as the experimental animal, having observed that they gave more promising results than rabbits and monkeys, inoculated the parotids and testicles with filtered saliva which induced, after an incubation period of 5 to 8 days, a parotitis characterized by congestion, interstitial edema, mononuclear interstitial infiltration, and an orchitis characterized by degeneration of the epithelium with interference with spermatogenesis ("spermatorrhexis"), and an inconstant swelling and multiplication of the interstitial cells with cellular invasion between the tubules. She used a bacterially sterile filtrate of saliva of patients ill not longer than 3 days with the disease. By incubating, at 37°C. for 2 hours, a mixture of an emulsion of a gland removed at the height of infection, with immune serum obtained from a cat which had recovered from the infection, she was able to decrease the reaction of the cat to the inoculation. The virus increased in virulence through the fourth generation but rapidly decreased after the sixth transfer; and in only one instance were the effects observed at the eighth transfer. The virus remained virulent after 4 months of storage in 50 per cent glycerine (4). In 1921 Wollstein reported the production of an aseptic meningitis in cats by the intracerebral inoculation of bacterially sterile filtrate of saliva from patients ill with epidemic parotitis (5).

Kermorgant in 1925 reported that he had been successful in anaerobic cultivation of a specific spirochete in association with a small Gram-negative bacillus from the sediment of washings of the buccal cavity of patients ill with mumps. The culture medium used was composed of rabbit serum, 4 parts; extract of the red blood corpuscles of the horse, 1 part; and physiological saline, 5 parts. The spirochete was observed to break up into small granules, in which state it was possible to filter it; but it could not be cultivated from the filtrate unless the original small Gram-negative bacillus were added to it. He was able to produce in the *Macacus sinicus* a parotitis identical with that produced by inoculation of the saliva from the patients, by injecting the cultured spirochetes into the lumen of Stensen's duct or directly into the parenchyma of the gland. He was unable, however, to transmit the disease from monkey to monkey, but reported the production of an orchitis in rabbits which was transmissible in series (6).

A review of the literature shows that the causative agent of mumps has been at various times and by different investigators attributed to bacteria, to a filterable virus, and lately to a spirochete. One gains no conviction however, from a study of the experiments reported up to the present time, that anyone has unquestionably succeeded in inducing mumps experimentally, or has demonstrated the true etiological agent of this disease. There has been a varied choice of experimental animals, of methods of inoculation, and of the character of the supposedly infectious material.

In planning an experimental investigation of mumps we have been

guided by the following assumptions: that monkeys would more likely be a susceptible host than lower animals; that the active agent should come in direct contact with the parenchymal cells of the parotid gland, preferably attended by some injury; and that the causative agent is in the saliva at least in the first stages of the disease.

In order to bring about the most favorable conditions for the experiment it was thought to be advisable to introduce a considerable amount of fresh untreated saliva directly into the parotid glands through the duct of Stensen.

This procedure would of course introduce along with the undiluted hypothetical causative agent a variety of microorganisms, but our experience had indicated that *M. rhesus* has a strong natural resistance to many microorganisms both saprophytic and pathogenic derived from human sources. There would be by this method also an associated injury to presumably susceptible cells. It was expected, since the incubation period of mumps appears to be at least several days, that in the meantime the non-specific microorganisms would disappear. If they should persist we hoped it would be possible to separate the true cause from contaminants by passage in series or by filtration.

By the application of this method of procedure we have been able to obtain from the saliva in a proportion of cases of mumps, in the early stage of the disease, three strains of a filterable virus, free of demonstrable microorganisms even in the first monkey generation, which is uniformly pathogenic for *rhesus* monkeys and induces an acute non-suppurative parotitis which we judge to be mumps. One strain of the virus has been passed in series through seven generations in *M. rhesus* monkeys and has increased in its virulence. We think that the experimental disease can be reproduced at will and indefinitely in series.

Material and Method Used for Inoculation

The original material used for inoculation was fresh saliva obtained by having the patients, with the clinical history, symptoms, and signs of epidemic parotitis, rinse their mouths thoroughly with sterile physiological saline. After disposing of the washings they expectorated for about 2 hours into sterile, wide mouth, glass bottles. During the collection of the saliva the patient was asked to suck the sides of the cheeks in an attempt to obtain as much saliva from the parotid glands as possible.

The first patient from whom saliva was thus obtained was a medical student who was admitted to Vanderbilt University Hospital about 12 hours after the onset of slight pain and slight swelling of the gland. He gave a history of contact with a case of epidemic parotitis 4 weeks previously while spending the Christmas holidays at his home in Pennsylvania. The saliva was collected over a period of 2 hours at which time the patient's left gland was quite swollen, and rapidly increasing in size. His temperature was 103°F. The saliva was immediately used for experimental inoculation after the completion of its collection.

About 2 months later an epidemic of parotitis appeared in the Tennessee Industrial School located at Nashville. Saliva was collected individually from six of the patients in the same manner as that described above. This material was placed in an electric refrigerator for a period of about 2 hours after its collection before being injected into monkeys.

Material from a case in southeast Alabama, where there had been an epidemic for 3 months, was sent to us at about the same time the epidemic appeared at the Industrial School. The saliva from Alabama was collected in the same manner as that of the first case. The bottle was tightly stoppered, sealed with paraffin, chilled by packing in ice, and mailed to us. The saliva was received the following day and was used immediately.

Altogether fresh saliva from six cases of mumps has been used for the inoculation of monkeys. Four of these specimens induced the specific parotitis, and two of them induced no recognizable clinical disease. These two specimens were introduced into the same monkey, one in each parotid. This monkey was not killed. Of the four positive specimens of saliva two were obtained within the first 24 hours after the first noticeable swelling of the parotids of the respective patients; one was obtained on the 2nd day; the other, from Alabama, not later than the 2nd day. Of the two negative specimens one was obtained on the 3rd day of the disease, the other possibly on the 3rd day, although we are not certain as to the exact time.

These results indicate that saliva taken within the first 24 hours after the onset of mumps is more likely to be infectious for monkeys than that recovered at later periods.

The sides of the face of the monkeys were shaved closely so that the changes that the glands might undergo could be observed easily. The animal was anesthetized with ether. The orifice of Stensen's duct was exposed by sticking a cotton-covered forefinger between the lips and down the inside of the cheek, then retracting the cheek by holding it between the finger and thumb. The duct was cannulated by inserting a size 22 Luer needle, which had had the point cut away to the shaft producing a blunt end to prevent the penetration of the walls of the duct. It was

found that the procedure of cannulation and injection was more easily performed if a 10 cc. Luer syringe with needle attached and containing the material to be injected were used. The orifice of the duct lies rather deeply and the longer syringe affords a better handle. The usual volume injected into each duct was 2 cc. It is necessary to anesthetize the animal in order to relax the muscles of the cheek sufficiently to permit a free injection. As the injection proceeds under moderate pressure the parotid may be seen and felt as it enlarges, so that one has no doubt about the entrance of the material.

The temperature and the total differential and leucocyte counts were taken before inoculation. The temperature was taken twice daily thereafter; and the total and differential leucocyte counts were made once daily from the day of inoculation until the animal was either killed or completely recovered. In several experiments the temperature and the leucocyte counts were made for a few days before the injection.

Results of Inoculation of Saliva

(a) *Normal Saliva.*—Saliva was collected from two normal individuals by the same method used to collect the saliva from the patients suffering from epidemic parotitis. One of the normal individuals gave no history of mumps, while the other had had the disease during early childhood.

The parotid glands of each of two *Macacus rhesus* monkeys were immediately inoculated by the previously described method of cannulation of Stensen's duct. Each gland of each monkey received 2 cc. of the whole saliva from each of the two individuals. This was the amount of saliva from patients with mumps which was injected into the ducts of each monkey in the reciprocal experiments.

These two monkeys, like those receiving the saliva of patients ill with parotitis, developed an immediate enlargement of the glands which increased in size for 12 to 24 hours and then returned to normal within 72 to 96 hours after the inoculation. They were observed daily for 3 weeks in the same manner as those which received the saliva supposedly containing the virus of epidemic parotitis. Their glands did not show any evidence of enlargement or tenderness after their return to normal. Their temperature showed no secondary elevation and they did not develop a leucopenia.

Ordinarily the parotid glands of a *rhesus* monkey are not at all palpable or barely so; consequently by palpation one can determine quite readily any moderate enlargement.

(b) *Saliva of Patients with Epidemic Parotitis.*—Saliva from Case 1 of mumps was diluted with an equal volume of 0.9 per cent NaCl solution and filtered through a Berkefeld N candle. The filtrate was bacteriologically sterile. 3 cc. of clear fil-

trate were injected into each parotid gland of a monkey through Stensen's duct. After a preliminary swelling which lasted about 48 hours, the glands returned to normal, and remained so. The unfiltered saliva from this patient was very active when injected into monkeys, consequently we believe that the virus in the saliva did not pass the candle, or was so diluted thereby that it was rendered innocuous. Therefore in all other experiments in which saliva from cases of mumps was used, whole fresh saliva was injected. Bilateral injections were made in nearly all instances.

The inoculation of the glands was followed immediately by a definite enlargement due to the volume of the injected fluid. There was a gradual enlargement for 12 to 24 hours, but this was followed by a rapid return to normal in from 48 to 96 hours following the injection. The immediate reaction and the return to normal was similar to that following the injection of normal saliva. The leucocyte count usually rose during the first 24 hours, in some of the cases the number was doubled. This rise was followed by a progressive fall in leucocytes until a definite leucopenic stage was reached which averaged about 4 days after the inoculation. With the development of the leucopenia there was a true and relative monocytosis, a relative lymphocytosis, a marked fall in the polymorphonuclear percentage and total count. In many instances there was a slight rise in temperature on the 3rd to the 5th day following inoculation. This rise in temperature was only of 1 to 2 days duration and the return to normal was usually paralleled by a slight rise in the leucocyte count which was only 1 day in duration in the majority of the cases. (Figs. 1 and 2.)

The above changes were in turn followed by a palpable and visible enlargement and a tenderness of the glands, a rise in rectal temperature, and an edema of the soft tissues over the gland by the 6th to the 8th day after inoculation. If the animal were not killed the edema and enlargement of the glands disappeared after 2 to 4 days. The rise in temperature usually was only 1 to 3 days in duration.

The animals were usually killed the day of or the day following the appearance of edema in order to obtain their parotids for study and for transmission of the virus. They were killed, during ether anesthesia, by exsanguination. The chest was shaved closely, cleansed with alcohol, dried with ether, and then painted with 7 per cent iodine. The heart was located and pierced with a needle, gauge 18, length 3½ inches, attached to a 20 or 30 cc. syringe. As much blood as possible was removed in this manner, and if it were impossible to withdraw sufficient blood to cause death the femoral arteries were dissected and sectioned. The animal after death was placed on one side and the upward cheek shaved, cleaned with alcohol, dried with ether, and painted with 7 per cent iodine. The remainder of the body was covered with lysol-soaked towels. A V-shaped incision was made in the skin over the gland. The point of the V was about 1 cm. from the corner of the lips with the forked ends extending to the neck over the angle of the jaw and the other superiorly to the ear. Care was taken not to include the cheek pouch in the incision. The skin and the fascia were then dissected and held retracted by sterile hemostats. A small piece of the gland was immediately removed, with the ex-

posed gland *in situ*, for bacteriological studies.   Aseptic technique was continued
as the gland was dissected from its bed and placed in a sterile Petri dish.

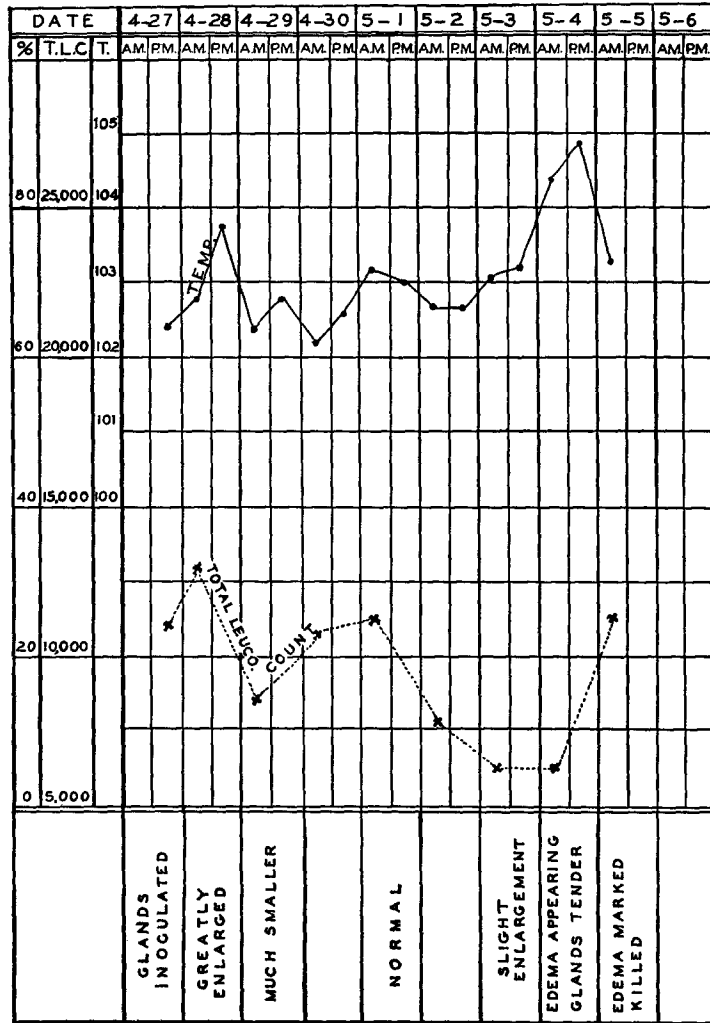CHART 1.   Temperature and leucocyte count curves of Monkey 23.   It received
into each gland 2 cc. of ground emulsion of the left parotid from Monkey 31.

The other gland was removed in the same way.   Each of the glands was weighed
in a sterile Petri dish and sectioned.   Pieces for histological study were fixed in
Zenker's solution, 80 per cent alcohol, 10 per cent neutral formalin, and formol-

Zenker's solution. Pieces of the supposedly sterile gland were placed in small bottles containing sterile 50 per cent neutral glycerine. The remainder of the gland was placed in small, sterile, rubber-stoppered bottles. These were placed in an electric refrigerator very near the freezing unit, where the temperature was at about the freezing point.

By the use of the methods just described three strains of the virus we are dealing with were isolated from the saliva of patients from three states of the Union. Bacteriological examination of the parotids inoculated with mumps saliva containing two of these strains, showed no microorganisms; one gland infected with another strain contained a staphylococcus which apparently played no part in the lesion, as determined histologically.

Transmission of Virus from Monkey to Monkey

The material used for passage of the virus through seven generations in monkeys up to date was prepared by taking small pieces of the gland, previously shown to be bacterially sterile, weighing them, and grinding them in a sterile mortar. About 1 gm. of the ground material was then taken up in 10 parts of 0.9 per cent NaCl solution. The heavy material was allowed to settle to the bottom of the tube by allowing the emulsion to sit overnight in the ice box. The emulsion was tested for sterility and if no growth became apparent in the media by the following day, 2 cc. of the turbid emulsion at the top of the tube were injected into each gland of a monkey through Stensen's duct by the same method used in the inoculation of the saliva. (Fig. 3.)

It was found that if the infected glands were allowed to remain in the ice box 2 to 7 days before being ground they were more easily macerated and the emulsion was more uniform in its results, than if the emulsion were made and used immediately after removal of the glands.

As shown in Table I there has been very little or no change in the incubation period, in the temperature curves, and in leucocyte counts in the seven generations of virus; but the most pronounced general reactions of the monkey have been observed in this series. A heightened reaction was noticed in the last three generations when it was observed that at times the animals would be drowsy and listless, and at other times easily excitable and very nervous. The tenderness of the glands was more marked and there was a slight increase in the duration of the edema of the subcutaneous tissues and swelling of the parotids.

TABLE I

Summary of the Transmission of the Virus in Series for Seven Generations

Monkey No.	Generation No.	Return to normal after inoculation	Beginning enlargement	Definite enlargement	Appearance of edema	Duration of edema	Leucocyte response to inoculation 1st day	Leucocyte response to inoculation 2nd day	Beginning of leucopenia stage	First rise after leucopenia	Temperature rise after leucopenia	Duration of rise	Temperature rise with symptoms	Duration of fever	Monocytosis	Lymphocytosis
		days	*days*	*days*	*days*	*days*			*days*	*days*	*days*	*days*	*days*	*days*		
5	1	4	6	6	8	Killed	Rise	Fall	4	6	?	?	8	Killed	True	Relative
6	2	4	5	5	6	Killed	None	None	4	None	2	1	6	Killed	True	Relative
11	3	4	6	7	7	Killed		Rise	3	4	3	?	6–8	Killed	True	Relative
16	4	3	5	6	6	Killed	Fall	Fall	3	3	3	1	5	2	True	Relative
17	4	4	5	6	7	3	Fall	Rise	6	None	3	1	5	2	True	Relative
21	5	2	4	5	6	3	Fall	Fall	3	5	2	2	7	Killed	True	Relative
9	5	2	3	5	6	Killed	Fall	Rise	2	3	2–3	2	5	Killed	True	Relative
40	6	3	5	6	7	Killed	Fall	Fall	5	5	3	1	6	Killed	—	—
42	7	3	6	8	9	Killed	Fall	Rise	4	5	5	1	8	Killed	—	—
Average..	—	3	5	6	7	3	Fall		3	5	3	1	5–8	2	True	Relative

The figures in the columns refer to the number of days after the inoculation of the glands with exception of the columns referring to the duration of rise in temperature and the duration of edema.

Gross and Microscopic Lesions of the Glands

When the specific experimental parotitis manifests itself by swelling of the glands the subcutaneous tissues and fascia surrounding them usually become quite edematous. The parotids are enlarged both in weight and volume by 2 to 4 times their normal size. They are easily palpable and can be picked up between the fingers; they are boggy and often contain shotty lymph nodes at the lower pole. There is a pitting edema of the overlying cheek. On exposing the glands they are found to be quite congested and through their capsule can be seen small hemorrhagic areas varying in size from pin-point up to 1 to 2 mm. in diameter. The glands as well as the surrounding tissues are very edematous, and there is a weeping of a serous exudate after removal. (Figs. 4 and 5.)

Histologic sections disclose marked destructive changes in the parenchyma of the glands. The parotid of the *rhesus* monkey is a serous gland, and the earliest stage of the lesion in the experimental parotitis is found in acinar cells. The essential lesions are focal in distribution. They are primarily a degeneration and necrosis of a single acinus or small groups of acini. The affected cells become swollen, lose their normal granulation, and become detached. The nuclei show the various changes indicative of necrosis. No nuclear inclusions have been observed, although there are often discrete though faintly staining masses in the cytoplasm in earliest stages. The exact nature of these structures has not yet been determined.

The disintegration of acinar cells takes place before there is a cellular exudate. As the cells die mononuclear phagocytes enter and take up the remains. Later, within the area of necrosis and in the interstitial tissue about many of the ducts, there is an infiltration by lymphocytes in which there are a few eosinophilic leucocytes and plasma cells. Polymorphonuclear neutrophilic leucocytes play no part in the inflammatory reaction. Accompanying the cellular injury there is a diffuse edema of the gland affecting the cells and the interstitial tissues generally. The parenchymal cells become edematous and often elevated from the basement membrane. In the interstitial tissue there is an abundant serofibrinous exudate, and in the neighborhood of the areas of acute parenchymal destruction petechial hemorrhages are

observed. No changes have thus far been found in the submaxillary glands. (Figs. 6 and 7.)

In the parotids of monkeys killed at the height of the disease there are focal areas of parenchymal necrosis of different ages, and we have interpreted this to mean that the changes begin 3 or 4 days after the inoculation, and spread through the gland until there is a sufficient general response to result in enlargement and edema.

Characteristics of the Virus

(a) *Bacteriology*.—The small portions that were removed from each gland *in situ* for cultural study were cut into pieces of about 2 to 3 mm. square. A piece was placed into each of a tube of plain infusion broth, blood broth, and anaerobic beef heart media. In practically every case the gland was found to be bacterially sterile. Noguchi's medium for leptospira was also used for several of the glands but no growth of microorganisms was obtained.

Dark-field examinations of emulsions of the fresh glands made immediately after removal, and also of the emulsion in the transfer of the virus from monkey to monkey, have shown no evidence of spirochetes. Smears, and sections of the glands stained by Levaditi's method for demonstrating spirochetes in tissues, have been entirely negative. Thus cultivation on artificial media and the staining characteristics of the virus still remain unsolved problems.

(b) *Filterability*.—Emulsions of the glands were prepared as for the passage of the virus from monkey to monkey. They were allowed to remain in the ice box for 24 hours, and were then centrifuged at a moderate but sufficient speed for the supernatant fluid to be clear but opalescent. This was decanted and to 10 cc. of the supernatant fluid 0.5 cc. of a 24 hour broth culture of *B. prodigiosus* was added to check the filter. The material was then slowly filtered through a Berkefeld V or N filter at 2 to 5 pounds pressure. Cultures of the filtrate were made and the filtrate stored in the ice box. The cultures being sterile, the filtrate was then inoculated into the glands of the monkeys in the usual volume and by the previously described method for the inoculation of saliva.

In every instance of five trials the filtrate proved to be virulent. The incubation period was from 1 to 4 days longer than for unfiltered material but this was the only change detected in the reaction to the filtered virus.

(c) *Resistance to Freezing*.—The saliva of Case 1, which induced parotitis following its initial injections, was kept in a stoppered bottle in the freezing unit of an electric refrigerator. The saliva was removed from the unit after 3 weeks and allowed to melt. Another monkey was inoculated with it in the same manner as the first. Its reaction was identical with that of the first monkey with the exception that the incubation period was shorter by 1 day.

(d) *Resistance to Drying*.—Pieces of the glands of the monkey receiving the fifth transfer were placed in a Petri dish immediately after removal. The Petri dish was packed in carbon dioxide ice and allowed to remain for 30 minutes. Com-

pletely frozen, it was transferred to a desiccator in which there was phosphorus pentoxide. The desiccator had been packed in ice and was connected to a vacuum pump. The pressure was then reduced to 2 mm. of mercury. The ice-packed

DATE			3–22		3–23		3–24		3–25		3–26		3–27		3–28		3–29		3–30		3–31		4–1		4–2	
%	T.L.C.	T.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.	AM	P.M.

TOTAL P.M.N.	6,786	11,762	4,922	5,341		1,740	1,680	1,219	3,525	3,108		5,580
TOTAL LYM.	4,563	4,251	5,457	5,123		3,712	3,840	3,604	3,525	4,704		6,200
TOTAL MONO.	351	327	321	436		348	480	477	450	588		670

Glands Inoculated · Normal · Slight Enlargement · Enlarging Tender · Edema Appearing · Edema Marked · Edema and Size Decreasing · Almost Normal
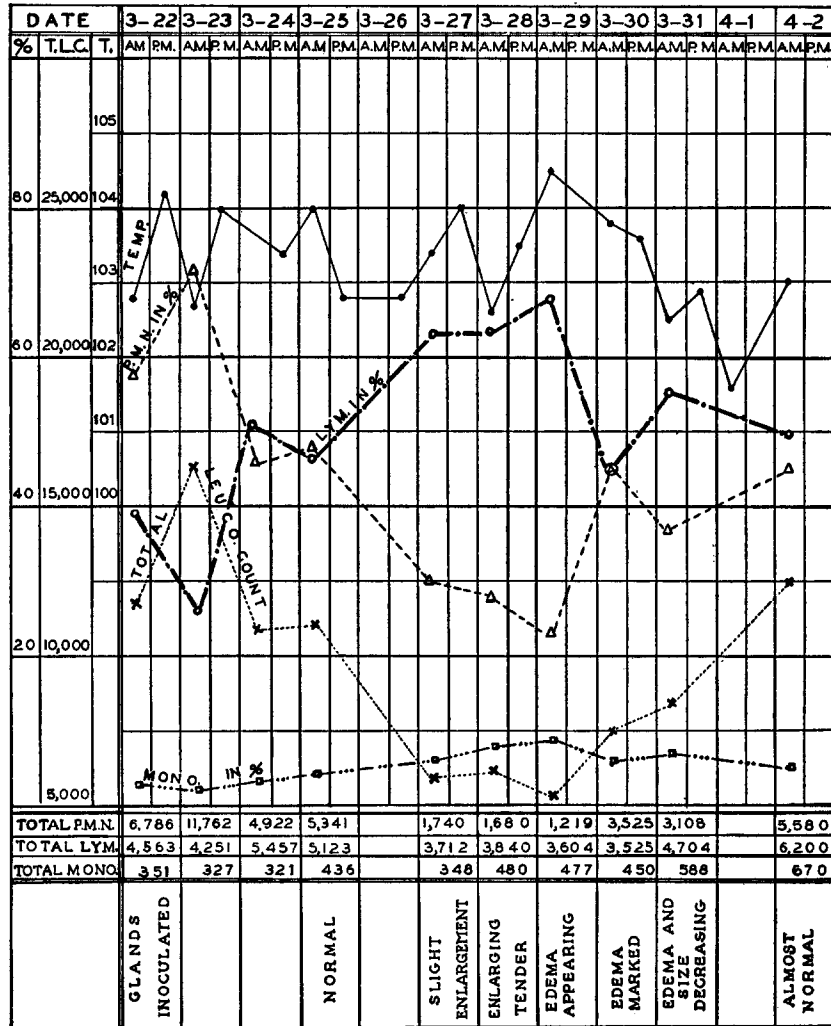
CHART 2. Temperature, total leucocyte count, and differential count curves of Monkey 20. The base numbers refer to the total polymorphonuclear neutrophils, lymphocytes, and monocytes. Each gland was inoculated through Stensen's duct with 2 cc. of a filtered emulsion of the gland of the fourth generation monkey.

desiccator was placed in the ice box and allowed to remain there for 4 days.   The dried pieces of the glands were removed and placed in small sterile ampules and sealed.

CHART 3. Temperature and total leucocyte count curves of Monkey 30, sixth generation.   It was inoculated by the routine method described in the text with bacterially sterile filtrate of the emulsion of Monkey 19 which had in turn been infected with the same inoculum as Monkey 20 (Chart 2).

TABLE II

Summary of the Findings of the Filtrate Experiments

Monkey No.	Return to normal after inoculation	Beginning enlargement	Definite enlargement	Appearance of edema	Duration of edema	Leucocyte response to inoculation 1st day	Leucocyte response to inoculation 2nd day	Beginning of leucopenia stage	First rise after leucopenia	Temperature rise after leucopenia	Duration of rise	Temperature rise with symptoms	Duration of fever	Monocytosis	Lymphocytosis
	days	*days*	*days*	*days*	*days*			*days*	*days*	*days*	*days*	*days*	*days*		
15	3	5	7	8	Killed	Fall	Rise	3	6	5	1	8	Killed	True	Relative
19	2	5	6	8	Killed	Fall	Fall	3	5	5	1	8	Killed	True	Relative
20	3	5	6	7	3	Rise	Fall	3–5	4	5	1	7	2	True	Relative
28	3	6	7	8	Killed	Rise	Level for 3	5–7	6	5	2	7	2	—	—
30	2	7	8	9	Killed	Fall	Fall	4	None	5	2	8	2	—	—
33	4	7	9	10	Killed	Rise	Fall	6	8	6	2	10	1	—	—
Average	3	6	7	8	3	Rise–fall	Fall	3–5	4–6	5	1–2	8	1–2	True	Relative

The figures in the columns refer to days after inoculation like those of Table I.

The dried gland remained in the ampules for 7 weeks. An ampule was then opened and the contents emulsified for inoculation into a monkey. The emulsion was prepared much in the same way as that for the transfer of the virus in series, by grinding the dried material in a proportionate volume of 0.9 per cent saline solution. The animal developed the typical leucocyte response, rise in temperature, enlargement of the glands, and edema of the subcutaneous tissues in the usual sequence.

(e) *Resistance to Glycerination.*—Small pieces of the same gland that was frozen and dried were also placed at the same time in 50 per cent neutral glycerine. After being stored in the ice box at about 10°C. for 7 weeks an emulsion of the pieces was prepared. A monkey inoculated with the usual volume of the emulsion developed the typical clinical response previously mentioned after the usual incubation period, showing that there had been no loss in the virulence of the virus.

Immunity

(a) *Reinoculation Experiments.*—Two of the monkeys which were allowed to overcome their infection for clinical observations were again inoculated, 2 months after recovery. An emulsion of the glands of the monkey of the sixth transfer was prepared by the previously described method. Each parotid of the two monkeys previously ill with the malady received 2 cc. of the emulsion. A normal monkey was given 2 cc. of the emulsion into the left parotid as a control.

The two monkeys which had previously been inoculated and had developed swelling and tenderness of the parotids, a rise in temperature, and a leucopenia, failed to show any symptoms of infection following reinoculation. These two monkeys were observed for 3 weeks after the inoculation.

The normal monkey which received the emulsion in only the left gland developed a slight rise in temperature, a typical enlargement of the inoculated parotid on the 7th day after the inoculation, and 4 days after its initial return to normal size. Edema of the subcutaneous tissues appeared on the 8th day after inoculation, and the animal was killed on the 9th day after the temperature had reached its peak and had begun to recede. The gross and microscopic changes were characteristic of the experimental disease.

(b) *Attempt to Neutralize the Virus with Sera of Patients Recently Recovered from Mumps.*—An attempt was made to determine whether or not the sera from patients recently recovered from mumps would have an inhibiting effect upon the virus inoculated into monkeys. In pursuance of this object two sets of experiments were made.

In the first set the serum from an adult human donor who seemed to have a re-
liable history of never having had an attack of mumps was used as a control.
Sera from two patients who had recently recovered from mumps—about 2 months
previously—were used to test their possible antiviral effect. Four monkeys were
used. Into one gland of each monkey a mixture of equal parts of virus emulsion
and "normal" serum was injected. Into the opposite parotid of each a similar
mixture of virus and "immune" serum, from two recently recovered patients, was
injected at the same time.

The virus suspension was made by emulsifying an infected gland in 0.9 per cent
saline solution in a proportion of 1 to 10, just as the inoculum in other experiments
had been prepared. The mixtures of serum and virus were incubated at 37°C. for
2 hours, and were then injected into the parotids.

The results were quantitatively inconclusive as to the antiviral effect of the
immune serum. Three of the parotids receiving normal serum and virus became
swollen and edematous in due time; the fourth showed no recognizable change.
Of the four glands which received immune serum, one became swollen and edema-
tous 2 days later than the opposite, control side; one gland became slightly enlarged,
without facial edema; the remaining two showed no change. One of the monkeys,
however, showed no enlargement of either parotid.

In a second set of experiments an attempt was made to compare the effect of
immune serum upon the usual virus emulsion, and a sample of the same diluted to
half its strength. Each inoculation was made into a separate monkey. As a con-
trol the serum from a supposedly normal placental blood was used. Two pairs of
monkeys were inoculated. One monkey received a mixture of full strength virus
emulsion plus an equal quantity of normal serum; another received half strength
virus emulsion plus an equal volume of normal serum. In the second pair im-
mune serum and virus emulsion were prepared and inoculated in the same way.
The serum-virus mixtures were kept in a refrigerator just below the freezing point
for 24 hours before the inoculation. They were not incubated at 37°C.

Each of the two monkeys which received normal serum and virus developed in
due course swelling of the parotids and facial edema. The monkey which received
diluted virus plus immune serum remained normal, at no time showing a swelling
of the gland. The one which received strong virus and immune serum developed an
erythematous rash on the 14th day associated with a swelling of the parotids, and
this condition, not observed by us before, persisted for several days. The parotids
were distinctly enlarged but there remained the question in our minds whether or
not the skin eruption associated with edema was a manifestation of serum sickness
which involved the parotids in the inflammatory reaction as well.

While this problem requires much more extensive investigation, we
may say that none of the parotids inoculated with virus plus immune
serum presented so marked an enlargement as the corresponding con-
trol, and where both serum-virus mixtures were inoculated into the

same animal the enlargement of the immune side was delayed 2 to 3 days.   In two of the experiments there was no response from the glands receiving immune serum plus virus while the corresponding controls were positive in the usual manner.

We judge from these few preliminary experiments that there is some inhibiting effect of serum from patients recently recovered from mumps upon the virus with which we are working.

DISCUSSION

The infectious agent which we have obtained from the saliva of patients in the early stages of mumps presents characteristics of a filterable, cytotropic virus having a predilection for the parenchymal cells of the parotid glands of *rhesus* monkeys.   The virus is free of demonstrable microorganisms; it is filterable and resistant to drying and glycerination; it causes a lesion which is primarily a degeneration and necrosis of the parenchymal cells of the parotid; and it confers immunity to reinoculation.   There are no visible structures associated with it which we can interpret as microorganismal.   No spirochetes have been observed in smears, in dark-field preparations of fresh lesions, or in sections of tissue stained by Levaditi's method.

There is good evidence, we consider, that this virus is the cause of mumps.   We have not found a similar virus in saliva other than that of patients suffering from mumps, although we have not yet tested a large number of specimens.   The virus has been demonstrated in four out of six specimens of saliva from cases of mumps.

The clinical disease induced in *M. rhesus* monkeys, whose parotids have been inoculated with the virus, is analogous to mumps in the human being.   The histology of the parotitis of human mumps is as yet undetermined, but the lesions of the experimental disease are quite comparable to those found in the specific orchitis of mumps, except that in the latter there is a considerable neutrophilic leucocytic response.   While our experiments attempting to neutralize the virus with serum of mumps-immune patients are inconclusive, they indicate so far as they go, that such sera have an inhibiting effect upon the virus.

It is well known that the saliva of a small proportion of people may harbor the virus of herpes simplex.   The possibility that we are deal-

ing with the herpetic virus, we think is ruled out by the fact that cutaneous, corneal, and intracerebral inoculations of rabbits with the experimental virus is innocuous so far as we have determined. There are likewise no nuclear inclusions in the experimental lesion. The insusceptibility of rabbits to the virus would also eliminate an accidental vaccinal contaminant. This is further obviated by the fact that monkeys immune to the experimental disease under consideration are susceptible to the vaccine virus. We are not aware of other known viruses with which the virus we have demonstrated may be confused.

Our judgment that we are dealing with a filterable cytotropic virus which is the true cause of mumps is based upon the above mentioned data. Further experiments are being conducted, however, to elucidate more clearly the characteristics of the virus.

CONCLUSIONS

1. From four out of six specimens of saliva from six cases of mumps in the early stages of the disease, a filterable cytotropic virus has been obtained which induces in *M. rhesus* monkeys, following inoculation of the parotid glands through Stensen's duct, an acute, non-suppurative parotitis analogous to mumps.

2. This virus has not been found in normal saliva, nor does it correspond to any known virus with which we are familiar.

3. The virus is free of demonstrable microorganisms including spirochetes.

4. It is judged that this virus is the causative agent of mumps.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. Monkey 31. Photograph taken on the 6th day following inoculation of saliva from two patients ill with epidemic parotitis. The left gland, the larger of the two, received 2 cc. of saliva from a patient during the 1st day of illness. The right gland, which is only moderately swollen, received 2 cc. of saliva from a patient during the 2nd day of illness.

FIG. 2. The left side of the monkey shown in Fig. 1. Note the pitting of the edematous tissues over the swollen gland which resulted from pressure.

PLATE 2

FIG. 3. Monkey 21. On the 7th day after inoculation and on the 2nd day of marked swelling. There was marked bilateral enlargement of the parotids and edema of overlying tissues. It received into each duct 2 cc. of the emulsion of the gland used for the fifth transfer.
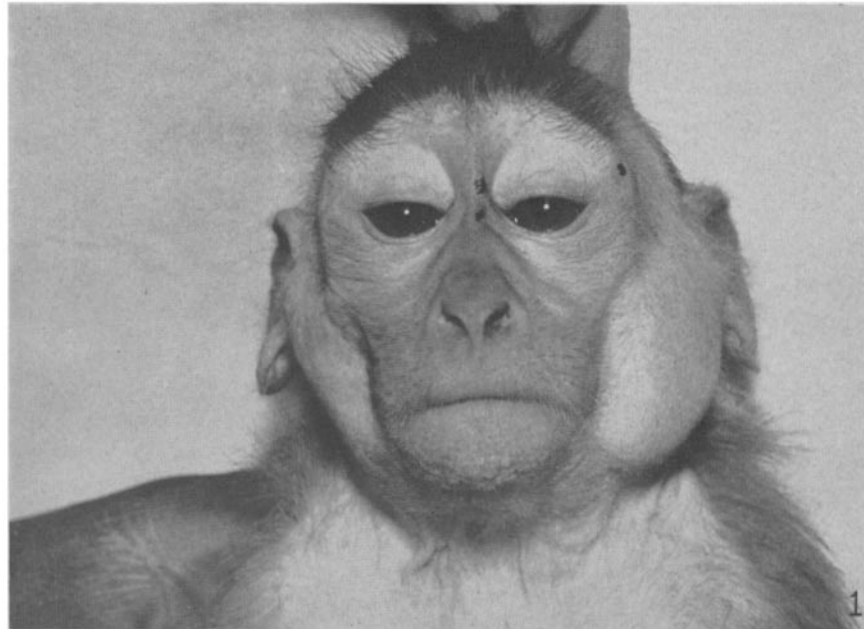
FIG. 4. One of the experimentally infected glands *in situ* showing the hemorrhagic areas and marked congestion as described in the text.

FIG. 5. The gland of Monkey 42, the seventh generation, showing the marked difference in size of a normal, non-inoculated gland and a diseased gland of the same monkey. The diseased gland was 2½ times as heavy as the smaller normal gland which showed no evidence of being infected either in the gross or by histological study. Actual size.
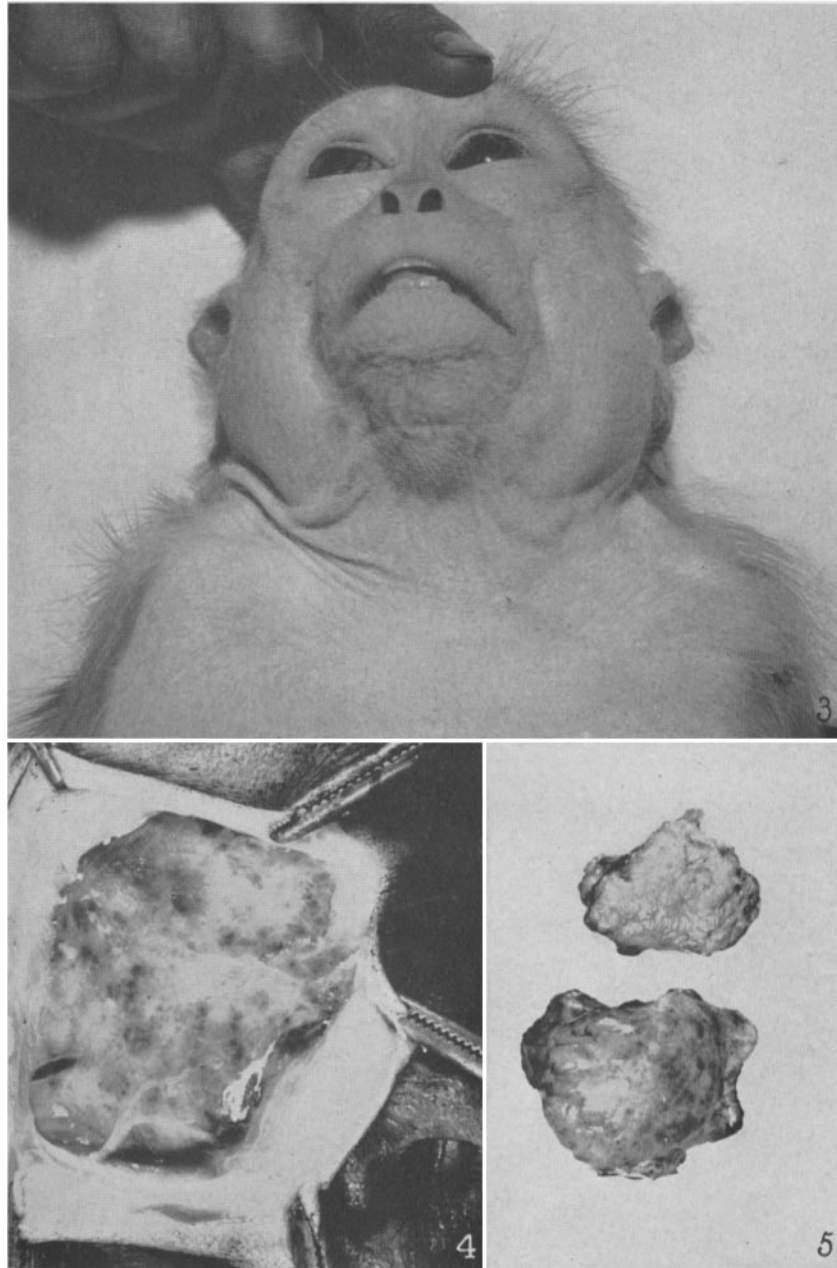
PLATE 3

FIG. 6. Photomicrograph of section of the diseased gland in Fig. 5 showing an infected lobule in which there are focal areas of necrosis with cellular infiltration. The interstitial edema is also apparent. × 80.

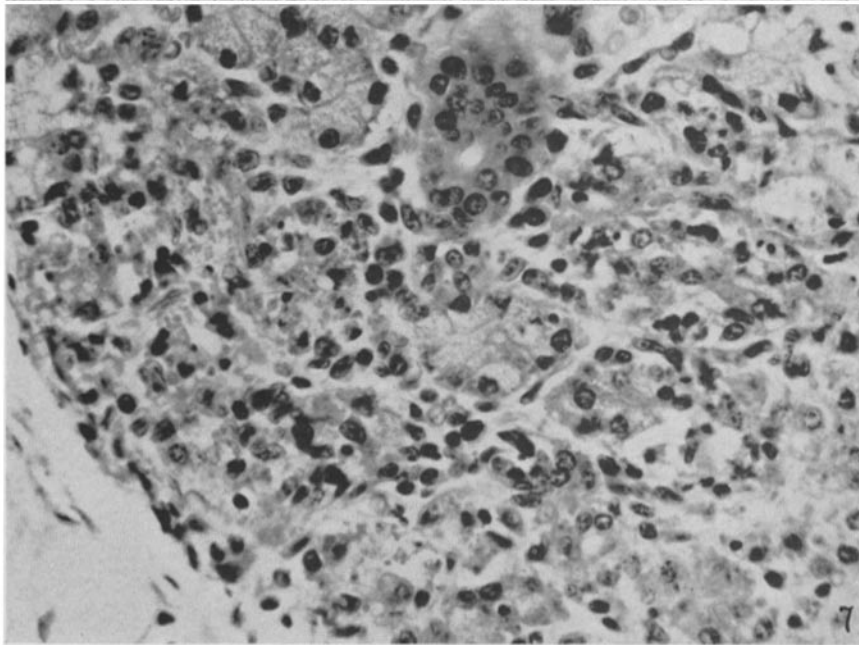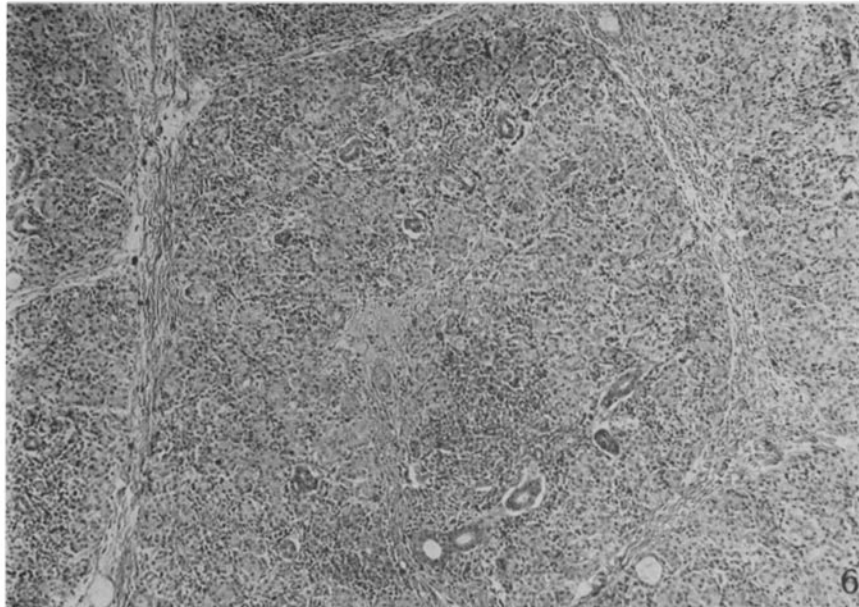FIG. 7. A focal area of necrosis in which there has been a moderate infiltration by large mononuclear phagocytic cells and lymphocytes. × 450.

(Johnson and Goodpasture: Etiology of mumps)

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