A FURTHER STUDY OF EXPERIMENTAL PAROTITIS.

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PLATE 29.

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In a previous paper¹ it was shown that cats injected into the parotid gland and testicle with a bacterial sterile filtrate of the salivary secretion of children in the active stage of parotitis can be made to develop a pathological condition having several points of resemblance to that present in mumps in human beings.

The presence of acute cases of parotitis in military camps near New York City provided the opportunity to repeat the experiments with material from adult cases.²

EXPERIMENTAL.

Saliva from Patients.

In order to repeat the work done 2 years ago, mouth washings in normal saline solution were obtained from soldiers suffering from acute parotitis for 1 to 12 days. The washings were filtered through a Berkefeld candle N and the filtrate, which was sterile by ordinary aerobic and anaerobic culture methods, was inoculated into the parotid glands and testes of healthy, half grown cats.

There are several precautions to be observed in inoculating parotitis material into cats. The animals must be only half grown; old males do not react, even if they are well inoculated through the very tough

¹ Wollstein, M., *J. Exp. Med.*, 1916, xxiii, 353.

² I am greatly indebted to Captain Meader for placing material at Camp Mills at my disposal, and to Captain Monaghan and Lieutenants Cowdrey and Braiton for many courtesies at the Army Hospital at Secaucus, New Jersey. Material was also obtained at Camp Jackson, Columbia, South Carolina, through the kindness of Lieutenant Colonel Roberts.

connective tissue over the parotids on the side of the face. The most marked reactions, as expressed by severe symptoms and lesions, develop when the material is inoculated within a few hours after it is obtained from the patient; that is, as soon as it can be filtered. With proper care of the filters and the glassware, contaminations do not occur; and while the filtrates must always be tested for sterility, neither time nor animals are wasted by immediate inoculation if the technique is right.

Attempts to probe Steno's duct and obtain saliva directly from the swollen parotid were unsuccessful. The opening is normally small; during an attack of parotitis the mucous membrane is red and swollen, thus reducing the size of the orifice and making it even more difficult to introduce a cannula.

Filtrates of mouth washings obtained from patients ill 1 to 3 days produced characteristic symptoms and lesions in the inoculated cats, consisting of a rise in temperature, leukocytosis with an absolute increase in mononuclear cells, and tenderness and swelling of the injected glands with definite histological changes. All the symptoms appear after an incubation period of 5 to 8 days and endure about 8 days, when they decline.

PROTOCOL I.

Cat A.—Inoculated Nov. 23, 1917, with sterile filtrate made from the combined saliva of three soldiers who had been ill 36 hours. 2 cc. injected into the right parotid.

Date.	Temperature.	Leukocytes.	Differential counts and remarks.
1917	°C.		
Nov. 23	38.9	23,000	Polynuclears 60; large mononuclears 18; small mononuclears 17; eosinophils 5.
" 24	40.0	25,000	No signs.
" 26	40.3	27,000	Polynuclears 70; large mononuclears 17; small mononuclears 11; eosinophils 2.
" 27	40.5	32,000	Cat is very quiet; ill.
" 30	40.8	33,000	Polynuclears 75; large mononuclears 16; small mononuclears 19; eosinophils 0.
Dec. 3	39.6	30,000	Less ill.
" 5	39.1	35,000	Polynuclears 63; large mononuclears 12; small mononuclears 19; eosinophils 6.
" 8	38.8	38,000	Polynuclears 71; large mononuclears 16; small mononuclears 9; eosinophils 4.
" 11	38.7	40,000	Polynuclears 71; large mononuclears 13; small mononuclears 11; eosinophils 5.
" 15	39.0	35,000	Chloroformed.

Right parotid fully twice as large as the left; the adjacent lymph nodes were swollen. The parotid was edematous, pink, and granular on section contrasting with the smaller, paler, and smoother left parotid. Cultures gave no growth. Microscopically the right parotid showed edema, some degree of mononuclear cellular infiltration around the ducts, and a less degree about the blood vessels.

Material was obtained from soldiers at Camp Jackson, Columbia, South Carolina, very early in the course of a parotitis attack. It was filtered in the usual way and inoculated into two cats. Both animals reacted well. Fig. 1 illustrates the parotid, submaxillary gland, and adjacent lymph nodes from one of the cats, removed after death on the 17th day.

On the 6th day of the disease the filtered saliva produced less marked symptoms, while inoculation of material obtained from a patient 9 days or longer after onset of the mumps attack was apparently without effect. The following protocol illustrates the latter point.

PROTOCOL II.

Cat B.—Inoculated Feb. 13, 1918, with sterile filtrate made from the saliva of a soldier at Secaucus, New Jersey; onset of mumps 10 days before. 2 cc. injected into right parotid.

Date.	Temperature.	Leukocytes.	Differential counts and remarks.
1918	°C.		
Feb. 13	38.9	14,000	Polynuclears 56; large mononuclears 14; small mononuclears 20; eosinophils 0.
" 14	38.7	18,000	No symptoms.
" 16	38.6	12,000	Polynuclears 72; large mononuclears 12; small mononuclears 15; eosinophils 1.
" 18	38.8	13,000	Polynuclears 75; large mononuclears 6; small mononuclears 19; eosinophils 0.
" 20	38.7	17,000	No symptoms.
" 23	39.0	20,000	Polynuclears 73; large mononuclears 8; small mononuclears 19; eosinophils 0.
" 26	39.4	22,000	Polynuclears 75; large mononuclears 11; small mononuclears 14; eosinophils 0.
" 28	39.0	23,000	Chloroformed.

The right parotid was not larger than the left, and the adjacent lymph nodes were not swollen. Microscopic examination showed no lesion in either parotid.

It will be noted that in this animal the mononuclear leukocytes were actually decreased, and the polynuclear leukocytes slightly increased, a result which had been previously noted after the injection of saliva not containing the virus of parotitis.

Cats inoculated with material from soldiers on the 9th, 11th, and 12th days of an attack of parotitis gave results similar to those described in Protocol II; that is, the temperature range was only 0.5°C., the leukocytic count was not even doubled, the mononuclear cells were decreased instead of increased, the polynuclear cells were slightly increased, and at autopsy the parotid gland showed neither macroscopic nor microscopic changes. From this we may conclude that the period of infectivity of the mouth secretions, as far as this test is capable of indicating, is comparatively short, and covers about 1 week, corresponding to the swelling of the parotid. A fresh swelling appearing in the opposite parotid gland would, of course, prolong the infectious period for a given patient.

Saliva from Cats.

The saliva from inoculated cats was employed for further injection. By anesthetizing the animals large amounts of ropy saliva are readily obtained, and at the height of the symptoms, at the end of the 2nd week after inoculation, the saliva thus obtained was found to incite symptoms and lesions in other and normal cats. The salivary secretion from normal cats obtained in the same manner and injected gave only negative results.

Following the inoculation of infected materials not only was the parotid gland affected, but the submaxillary, the sublingual, and the adjacent lymph nodes also. The extent of the swelling of the salivary glands was greater in this series of experiments than in the first series reported.¹ Moreover, the lymph nodes on the uninoculated side were observed to be swollen and moist, as though the infectious agent present in the saliva had been carried to them, presumably by lymphatics, leading to reaction on the uninoculated side. On microscopic examination the swollen lymph nodes showed necrosis of the center of several lymph nodules, while the sinuses were distended and the lining cells swollen. In this connection it may be remarked that swelling of cervical lymph nodes was a marked condition in many of the soldiers ill with mumps.

As regards the lesions in the parotid glands and testes, it may be stated that they agreed with the descriptions given in the previous paper¹ and briefly are as follows: In the parotid there are edema of the interlobular connective tissue and mononuclear cell infiltration about the ducts, and occasionally about the blood vessels as well. Similar lesions have been described in the pathology of cases of human mumps. In the testis there is principally an epithelial degeneration resulting in diminished spermatogenesis.

Blood.

The marked constitutional symptoms observed in many patients with mumps suggested an investigation of the blood at the height of the attack.

Films were stained with Wright's and with Giemsa's stain. Cultures were made in the usual way and by the anaerobic (tissue ascitic

broth) method of Noguchi.³ No parasites were detected under the microscope or cultures obtained. Blood was taken from a vein at the elbow in seven patients ill 2 to 3 days who were suffering from marked constitutional symptoms. The blood was defibrinated and had been proved sterile in glucose broth and on agar plates; it was inoculated into normal cats. The protocol of such an experiment is given, together with its control.

PROTOCOL III.

Cat C.—Inoculated Mar. 13, 1918, with defibrinated blood taken from the arm vein of a patient ill 3 days. 2 cc. injected each into right testis and right parotid.

Date.	Temperature.	Leukocytes.	Differential counts and remarks.
1918	°C.		
Mar. 13	39.0	13,000	Polynuclears 67; large mononuclears 28; small mononuclears 5; eosinophils 0.
" 14	39.2	34,000	Polynuclears 80; large mononuclears 9; small mononuclears 11; eosinophils 0.
" 15	40.4	47,000	No signs.
" 18	39.2	35,000	Polynuclears 74; large mononuclears 20; small mononuclears 5; eosinophils 0. Right testis tender.
" 22	39.3	40,000	Testis tender.
" 26	39.5	43,000	Polynuclears 84; large mononuclears 4; small mononuclears 11; eosinophils 1.
" 28	38.8	40,000	Seems quiet and ill. Polynuclears 82; large mononuclears 6; small mononuclears 12.
" 30	39.0	38,000	Chloroformed.

Right parotid, submaxillary, and lymph nodes are swollen and pink and larger than the left (Fig. 2). Right testis also swollen. Microscopically the right parotid gland showed marked edema and swelling of the epithelial cells in some acini. The lymph nodes showed distention of the sinuses and swelling of the lining cells. The right testis showed a degree of characteristic epithelial degeneration.

³ Noguchi, H., *J. Exp. Med.*, 1911, xiv, 99.

PROTOCOL IV.

Cat D.—Inoculated Mar. 19, 1918, with sterile defibrinated blood from a healthy adult man. 2 cc. injected each into the right parotid and right testis.

Date.	Temperature.	Leukocytes.	Differential counts and remarks.
1918	°C.		
Mar. 19	39.1	19,000	Polynuclears 60; large mononuclears 22; small mononuclears 18; eosinophils 0.
" 20	38.9	26,000	Polynuclears 72; large mononuclears 24; small mononuclears 4; eosinophils 0.
" 23	39.2	20,000	Animal well.
" 25	39.5	20,000	Polynuclears 72; large mononuclears 16; small mononuclears 12; eosinophils 0.
" 28	39.0	25,000	No signs.
Apr. 1	38.9	25,000	Polynuclears 65; large mononuclears 10; small mononuclears 25; eosinophils 0.
" 4	39.1	23,000	Chloroformed.

Both parotid glands of equal size; slight subcapsular erythrocytic discoloration in the right. No difference in the two testes.

An examination of Protocol III shows that while the temperature range was small it was somewhat greater than in Protocol IV; the blood count, on the other hand, showed a polynuclear leukocytosis in both animals, but while the mononuclear cells were diminished in the control cat they were actually increased throughout in the animal receiving the blood from the patient ill of parotitis. Identical results were obtained with the blood of four other patients which had been pooled before inoculation. The blood taken from a case with slight symptoms on the 5th day of illness gave no result on injection into a cat.

The conclusion drawn from this experiment is to the effect that the blood of parotitis patients, especially those suffering from severe constitutional symptoms, is infective for cats. This conclusion is in keeping with the clinical course and metastatic complications of certain cases. It may be remarked that one of the four patients whose blood was pooled suffered from orchitis. Meningitis has not infrequently been described, especially in the French literature, as complicating mumps. In one suspicious case among our series of patients, the spinal fluid was clear, contained no lymphocytes, and was without effect when inoculated into cats.

Recurrent Parotitis.

An opportunity was afforded of studying a case of so called recurrent mumps. The patient, a soldier, had a first attack in November, 1916. He came under my observation in March when he was suffering either from another attack or merely from an exacerbation, as the swelling had persisted somewhat since November. Cases of parotitis which recur after 3 or 4 weeks are not uncommon, and Jochmann⁴ states that the swelling may endure for months or even for a year. In this patient the right parotid remained enlarged longer than the left; both had returned to about normal size in 6 months. Three sets of inoculations were made with the salivary secretion: one on March 13, one on May 1, and the last on May 15. The first two gave positive, the last a negative result. As regards the last it may be stated that the swelling of the parotid had disappeared soon after May 1. Fig. 3 shows the condition found in the glands after the second inoculation.

SUMMARY.

A new series of inoculations into cats of the filtered sterile salivary secretions derived from cases of parotitis has been described. They confirm the observations made in 1915-1916 and extend them to include the epidemic parotitis occurring in our military forces.

Incidentally confirmatory evidence of the filterable nature of the causative agent of mumps has been obtained.

It has been determined that the saliva of man and of inoculated cats, and the inoculated glands of the latter animals, contain the filterable, infective agent.

The lesions present in the inoculated organs conform to those described in our first publication. In addition, the lymph glands adjacent to the salivary glands on the uninoculated side were sometimes found to be swollen and to exhibit microscopic lesions. Probably the involvement resulted from salivary and lymphatic infection.

The "virus" of parotitis was detected most readily in the saliva during the first 3 days of the disease, less easily on the 6th day, and not

⁴ Jochmann, G., *Lehrbuch der Infektionskrankheiten für Aerzte und Studierende*, Berlin, 1914.

at all on the 9th day. It was detected also in the blood of patients showing marked constitutional symptoms, and in the saliva of a case of recurrent mumps at the periods of enlargement of the parotid glands, but not 2 weeks after the swelling had subsided. It was not detected in the cerebrospinal fluid.

EXPLANATION OF PLATE 29.

FIG. 1. Parotid (*P*) and submaxillary (*SM*) glands with adjacent lymph nodes (*L*), showing swelling of the inoculated right side. Cat killed after 17 days.

FIG. 2. Parotid (*P*), submaxillary (*SM*), sublingual glands, and adjacent lymph nodes (*L*), showing swelling of the right side inoculated with blood from a patient ill 3 days. Cat killed after 18 days.

FIG. 3. Parotid (*P*), submaxillary (*SM*), sublingual (*SL*) glands, and adjacent lymph nodes (*L*), showing swelling of the right side inoculated with saliva filtrate from a recurrent case (second recurrence). Cat killed after 15 days.

FIG. 1.

FIG. 2.

FIG. 3.

(Wollstein: Experimental parotitis.)