A CASE OF SUSPECTED RABIES WITH ISOLATION OF BACILLUS DIPHTHERIÆ FROM THE CENTRAL NERVOUS SYSTEM.

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CLINICAL REPORT.

BY GEORGE DOUGLAS HEAD, M. D.

On the morning of September 28, 1897, Mrs. L. R. presented herself for the surgical treatment of a bite of the left cheek. She stated that about 4 o'clock that morning she was awakened by a noise in the chicken coop, and hurrying on her clothing went out to investigate. As she pushed open the door of the coop an animal sprang out upon her, biting her on the left cheek. The wound bled profusely and to stop the hæmorrhage she applied some liniment and a bandage.

When she presented herself for treatment, five hours after the accident, there were two sharp, deep punctures on the left cheek, one-half an inch apart, sinking deeply into the underlying tissue, from which on pressure a bloody grumous fluid exuded. The wound was scrubbed with soap and water, washed out with peroxide of hydrogen and packed with gauze. Two days following, the same treatment was repeated. At the third dressing, as the wound seemed clean, it was allowed to granulate over and the patient was dismissed.

Nothing more was heard of the patient until December 3, two months and five days following the bite. On that day the patient again came complaining of severe pain and numbness over the left cheek in the region of the previous bite. The office notes were as follows:

Woman, 32 years, well nourished, married, two children, one miscarriage, mother and father and one sister living; mother well; father has ill health, of which the cause was not ascertained, previous history uneventful. Had neuralgia of right side of face some years ago. No other ailments. No history of hysteria or other neuroses.

The pain of which the patient complains is localized on the left cheek in the region of the scar of the previous bite. It commenced

two days ago as a mild aching and numbness in that region and has been gradually growing more severe. There is little swelling and only slight tenderness on pressure over the area of pain. There is complaint of some difficulty in opening the mouth owing to pain in the temporo-maxillary articulation. Patient complains of loss of sleep. Temperature 98.8° F. Pulse 100.

Diagnosis: facial neuralgia. Treatment: phenacetine and salicylates.

Dec. 4. Patient at office. Tenderness most marked over the site of the bite. Pain paroxysmal in character, running along the ramus of lower jaw, up the side of head, in front and back of left ear, and back along the left sternocleidomastoid muscle. As patient complains of difficulty in swallowing I give her a glass of water to drink. She raises the glass to her lips, gives a short, jerky inspiration, and swallows the liquid. Suspecting rabies, I call Dr. Hunter in consultation.

Patient carefully examined. No throat lesions. No decayed teeth. No ear trouble. Tenderness sharply localized over the area supplied by middle branch of fifth nerve. Temp. 99.4°. Diagnosis: neuralgia of middle branch of fifth nerve or beginning rabies. Treatment, morphine.

December 5. Fifth day of disease. Called at 4 a. m. Patient has spent a bad night with little sleep. Excruciating pain over same area as described. Mouth opened with more difficulty. Can swallow saliva, but if she attempts to drink water, tea or milk she takes a number of short, quick, catchy inspirations, her eyes start from her head, she looks wildly about, then swallows the fluid with a gulp. The swallowing seems a great effort and tires her. Temperature 99°. Pulse 108. Heart, lungs, and abdomen normal. No enlarged spleen. Bowels constipated. Urine, acid, 1024. No albumin, or sugar. Urea 2.5 per cent. No casts, blood or leucocytes. Diagnosis: suspected rabies.

December 5, 4 p. m. Patient resting easier. Laryngeal and respiratory spasms more pronounced. Now even the taking of a glass of fluid into her hand causes a faint spasm. As she puts the liquid to her lips her hand will shake violently, the catchy inspirations will begin, and it seems almost impossible for her to swallow. She can swallow solids, though with some difficulty. With the exception of the laryngeal spasms and the pain, she seems perfectly normal. Sits up in a chair, talks with her friends, and is undisturbed mentally. Temperature 99.2°. Pulse 110, irregular and rapid. Respirations normal.

December 6, 8 a. m. Drs. Hunter and Sweeney in consultation. Sleepless night. Has taken little nourishment and no fluids. Complains much of thirst. Bowels moved. Temperature 100°. Pulse 110.

Respiratory and laryngeal spasms on taking fluids. Applications of snow to face or arm causes the spasms. Pupils react to light and accommodation. Reflexes feebly present. Knee-jerk absent in right leg, present in left leg. No ankle-clonus. No areas of hyperæsthesia. On left tonsil white exudate which is removed with swab, supposed to be due to a strong solution of sulphuric acid used by her mother for swabbing out the throat. Heart, lungs, and abdomen normal. Treatment: morphine; fluid enemata.

December 6, 4 p. m. No change. Slept some after the morphia. Laryngeal and respiratory spasms present on taking liquids; also, in less pronounced degree, on taking solids. Complains bitterly of thirst but can scarcely be induced to attempt to drink. Temperature 101° . Pulse 108. Respiration normal. Cocainized pharynx and gave one pint of milk by stomach-tube.

December 6, 12 p. m. Pain in face not complained of. Laryngeal and respiratory spasms manifested as before. Temperature 101° . Pulse 110. Gave one pint of milk by stomach-tube; also morphia.

December 7, 9 a. m. Dr. Jones in consultation. Patient seems brighter and more cheerful. Slept some during the night. Can swallow oysters and crackers moistened in water. On offering her some milk to drink she pleads not to be asked to try but finally takes a swallow only to exhibit the same laryngeal and respiratory spasms in a more pronounced degree. Now as she attempts to take fluid her eyes bulge, she clutches a support to sustain herself, her whole body seems in a violent tremble, and as the fluid touches her lips there comes a series of quick respirations, followed by a convulsive cough which blows the milk out of her mouth over herself and the bed clothes. Temperature 100.5° . Pulse 120. Bowels moved. Gave one pint of milk by stomach-tube; also bromides.

December 7, 5 p. m. Temperature 101.5° . Pulse 120. Tongue coated white, pharynx normal. Eyes bright. Mind clear. No sleep. Treatment, morphia. Milk and water by stomach-tube.

December 7, 12 p. m. Patient seems languid and tired. Wants to sleep but cannot. Face flushed, eyes bright. Laryngeal and respiratory spasms easily produced. Temperature 102° . Pulse 130. No rose spots. No tympanites. No enlarged spleen. Heart and lungs normal. Give eggs and one pint of milk by stomach-tube, also morphia to induce sleep.

December 8, 8 a. m. Dr. Hunter in consultation. Patient passed a good night. Laryngeal and respiratory spasms present. Knee-jerk

absent. Pupils react to light and accommodation. No ankle-clonus. Superficial reflexes normal. No rose spots. No enlarged spleen, nor tympanites. Temperature 103.2°. Pulse 130. Take blood for Widal test. Give 2 eggs, milk and whiskey by stomach-tube. No morphia.

December 8, 4 p. m. Patient complains of pain in upper abdomen, extending around the body. Stomach distended with gas. Temperature 102.8°. Pulse 120. Blood examination: leucocytes, 6000; red corpuscles, 5,500,000. Hæmoglobin 90 per cent. Widal test (State Board of Health Laboratory) gives a positive typhoid reaction (see p. 456). Give 2 eggs, milk and whiskey by stomach-tube.

December 8, 8 p. m. Patient seems better. Complains of pain in limbs and back. Mind clear. Laryngeal and respiratory spasms present. Temperature 102.5°. Pulse 120. Tongue coated white. No morphia.

December 9, 8 a. m. Patient complains of pain in abdomen which is relieved when bowels move. Laryngeal and respiratory spasms still severe. Lungs and heart normal. No rose spots. No tympanites. No enlarged spleen. Urine 1024, acid, a trace of albumin, no sugar, urea 3 per cent. No casts, leucocytes or blood corpuscles. Temperature 102.2°. Pulse 120. Ate six oysters and took a few swallows of milk.

December 9, 8 p. m. Patient slept some during the day. Laryngeal and respiratory spasms not so pronounced. Temperature 103.2°. Pulse 120. Knee-jerk absent. No ankle-clonus. Sensation normal. Grasp of right and left hand unimpaired. Pupils react to light and accommodation. Patient drinks a cup of milk. No morphia.

December 10, 8 a. m. Patient delirious with rational intervals. No sleep during the night. Had hallucinations of being drowned, of the house on fire, of persecution. Occasionally patient had a general tremor of the whole body. Temperature 102°. Pulse 110, irregular. Had eaten some bread and milk.

December 10, 8 p. m. Patient extremely nervous. Fingers twitch. Casts furtive glances this way and that. Low, incoherent muttering. Temperature 101°. Pulse 130. Passes urine, and bowels move. Has taken milk and broth.

December 11, 3 a. m. Called in haste. Patient in violent delirium. Had gotten out of bed, knocked down nurse, and run out of doors. Patient tries to tear her clothing, bites at those about her. Has to be held by force. Quieted by one-half grain of morphia. Temperature 103°. Pulse 120. Urine, 1022, large amount of albumin. No sugar. Urea 4 per cent., a few pus cells, with a few hyaline casts and cylindroids.

December 11, 8 a. m. Dr. Hunter in consultation. Patient perfectly rational. Recognizes those about her. Talks sensibly, but there are occasional twitchings about the eyes. Quick glances this way and that. Takes little nourishment. Temperature 102.2°. Pulse 110. Calomel administered to move the bowels.

December 11, 8 p. m. Patient seems worse. Muttering delirium. Constant twitching of muscles of whole body. Eyes bright, face red and injected. Tongue flabby with pasty coat. Passes urine and bowels move. Temperature 104°. Pulse 110. Treatment: morphia ($\frac{1}{2}$ gr.), whiskey and strychnia.

December 12, 10 a. m. Patient does not recognize me. Seems more quiet, inclined to lie still. Has taken three or four cups of water and some milk. She drinks now without the occurrence of spasms. Severe attacks of trembling of whole body or of one limb, then of another. Urine 1028. Large amount of albumin, a few hyaline casts. Temperature 102.2°. Pulse 140. Treatment: whiskey and strychnia.

December 12, 8 p. m. Dr. Sweeney in consultation. Patient worse. Has hoarse cough, rapid respirations. There is an increased flow of saliva from mouth. Muttering delirium; picking at bedclothes; violent shaking of limbs. Heart, normal. Lungs: fine, mucous rales at bases. Abdomen normal. Take blood for Widal test.

December 13, 8 p. m. Thirteenth day of disease. Patient has had no sleep, speech thick and incoherent. Can be aroused but if left alone lies in a semi-conscious state. Temperature 102°. Pulse 130. Blood examination: red corpuscles, 5,120,000; leucocytes, 14,500.

Widal test (State Board of Health Laboratory) gives a positive typhoid reaction (p. 457). Notwithstanding this report there is little clinical evidence of typhoid fever. The leucocyte count in the second week of uncomplicated typhoid would not be increased. Typhoid fever is therefore not the probable diagnosis.

The laryngeal and respiratory spasms are again produced by the swallowing of solids or liquids or the putting of one's hands on the body or wiping away mucus from the mouth.

December 13, 8 p. m. Patient is unconscious. Has taken no nourishment. Short, sharp, muscular contractions sweep over the arms and legs. Temperature 102°. Pulse 140. Respirations feeble and superficial. There is an occasional mucous rattle in the trachea. It does not seem possible that the patient can long survive.

December 14. Patient died at 5 a. m. in an unconscious state, the respirations being previously rapid and superficial and the heart's action irregular.

Autopsy, 16 hours after death, the body having been kept at low temperature. A complete examination was not permitted.

Body well nourished. Rigor mortis pronounced. Scar on left cheek. Patchy livores mortis over posterior surface of body.

Dura adherent along superior longitudinal sinus. No other adhesions. Slight hyperæmia of dura, which is not thickened. Pia somewhat hyperæmic, free from exudate.

An incision was made with sterilized scalpels into left lateral ventricle and 1 cc. of clear serum was removed in a sterilized pipette, the end of the pipette being immediately sealed in a flame.

Cerebral convolutions well developed and normal. No exudate or effusion. No adhesions. No areas of softening. Vessels somewhat injected. Punctate oozing on section of brain. Meningeal vessels normal. No tubercles, emboli, thrombi, or adhesions. Cerebellum normal. Basal ganglia normal.

The third ventricle was opened with a sterile knife, and by means of a sterile pipette some bloody fluid was withdrawn, the ends of the pipette being at once sealed in a flame.

The medulla and pons Varolii were removed with sterilized instruments and placed in a sterilized flask.

A complete post-mortem examination not being allowed, the abdominal cavity was opened by a median incision only 10 cm. long. There were no adhesions or increase of fluid in the peritoneal cavity. The peritoneum and the intestines, both small and large, showed no abnormality. Parts of the cæcum and ileum were removed. Peyer's patches and the solitary follicles were not affected.

The right kidney was somewhat enlarged and congested. Capsule stripped off with ease. Dark red areas were scattered over the cortex. The left kidney presented the same appearance. Pieces of kidney removed and placed in 95 per cent alcohol. Liver appeared normal. Spleen was not enlarged. All tissues and fluids taken at the autopsy were kept on ice until taken to the State Board of Health Laboratory 13 hours later.

PATHOLOGICAL AND BACTERIOLOGICAL REPORT.

By LOUIS BLANCHARD WILSON, M. D.

(From the Bacteriological Laboratory of the Minnesota State Board of Health.)

The connection of the laboratory with the case of which the clinical history has been given by Dr. Head, began with the receipt, on Dec. 8, 1897, of a specimen of blood for examination for the serum reaction for typhoid fever. The specimen was dried on a visiting card, and con-

sequently only a colorimetric estimate of the dilution employed could be made. The reaction was present in a dilution of 1:25 so estimated. On Dec. 13, another specimen collected, weighed and accurately diluted after the author's method,* was found to give a good reaction in a dilution of 1:25, partial in 1:50, and a trace in 1:100.† In consequence of these two reactions special care was taken in the bacteriological examination of the specimens secured at the autopsy.

On December 15, at 9 a. m., there were received at the laboratory: one sealed bulb, containing about 1 cc. of fluid from the left lateral ventricle of the brain; a similar bulb, with about 0.5 cc. of fluid from the third ventricle; the lower two-thirds of the medulla, with about 1 cm. of the cervical cord, in a sterile flask plugged with cotton wool and about 2 ccm. of the pons, which had been placed in 4 per cent formaldehyde solution at the autopsy; and a portion of one kidney wrapped in gauze. Later there was also received another portion of the kidney, which had been placed in 80 per cent alcohol at the time of the autopsy.

Coverslip preparations. Four coverslip preparations each were made from the fluid from the left ventricle, from that from the third ventricle, from the substance of the medulla and from that of the kidney. They were stained with methylene-blue and eosine. Those from the lateral ventricle showed very few cocci, and two or three bacilli rather unevenly stained. In those from the medulla were found a few isolated cocci. No bacteria were found in any of the others.

Sections.—Kidney. In a portion of kidney placed in alcohol at the autopsy, many of the glomeruli were apparently normal. A few showed more or less complete hyaline degeneration and some cloudy swelling, with disappearance of the nuclei of the epithelium of the convoluted tubules. No bacteria could with certainty be distinguished.

Nerve tissues. Portions of tissue from the pons, medulla and cord were fixed and hardened in 96 per cent alcohol, embedded in celloidin and in paraffine, and sections 3, 5, 10 and 20 microns in thickness cut therefrom. These were stained by Nissl's method, by Weigert-Gram,

* Wesbrook and Wilson; *Philadelphia Med. Journ.*, 1898, i, 549.

† These reactions are perhaps explicable on either of two hypotheses: (a) a typhoid infection existed, and other pathological evidence of it was not obtained, owing to the incomplete autopsy permitted; (b) though no history of a previous typhoid fever was elicited, the exclusion of a previous typhoid infection would seem to be almost impossible, since the patient had been for years a resident of a city in which for a decade typhoid fever has been endemic, and from the public water-supply of which the typhoid bacillus was isolated a year ago (See Wilson and Wesbrook, *British Med. Journ.*, 1897, ii, 1774.)

and by Löffler's methylene-blue alone. The walls of some of the vessels were thickened. Where this occurred, the vessels were filled with blood, and the walls infiltrated with leucocytes. In some instances the perivascular spaces were completely filled with leucocytes. No accurate determination of the condition of the nerve fibres could be made by the methods employed. With Nissl's stain, many of the cells of the grey matter of each of the regions studied appeared to be perfectly normal. A large number of these cells, however, showed chromatolysis in varying degrees, from simple irregularity and subdivision of the Nissl granules to the almost complete disappearance of the chromatin. In most instances the periphery of the cell-body was least affected. Where chromatolysis was at all marked, the nucleus and nucleolus were in most cases eccentrically placed. In a few instances, the nucleus was distorted in shape; in others enlarged, irregularly crescentic nucleoli were present. Apparent complete absence of processes from some of the most affected cells was noted.

The foregoing changes were common to pons, medulla and cord. In addition, in the medulla and cord—which had been 13 hours longer out of preserving fluid—were found occasional cells, in which the nucleoli, while not enlarged, were partially divided, the nuclei stained light blue throughout and the cell-body was filled with well-stained, finely divided granules, the otherwise even distribution of which was broken up by “vacuoles.” The writer, however, agrees with Ewing* and others in considering these cadaveric rather than vital changes.

In the sections of the pons and medulla stained by Weigert-Gram and by Löffler's methylene-blue, several groups of bacilli were found. These in size, shape and staining characteristics could not be distinguished from *Bacillus diphtheriae*, and, in view of the cultural findings to be described later, in all probability were such. The groups consisted of two to five individuals each and were located within the bodies of the larger nerve cells. They were not observed in the vessels or perivascular spaces.

Though many observers, among others Löffler,† Kolisko and Paltauf,‡ Strelitz,§ Johnston,|| Abbott and Ghiskey,¶ Flexner,** Frosch,††

* Ewing, J., *N. Y. Med. Record*, 1898, liii, 513.

† Löffler, *Mittheil. a. d. k. Gesundheitsamte*, 1884, ii, 421.

‡ Kolisko and Paltauf, *Wien. kl. Wochenschr.*, 1889, ii, 147.

§ Strelitz, *Arch. f. Kinderheilk.*, 1891, xiii, 468.

|| Johnston, *Montreal Med. Journ.*, 1891, xx, 161.

¶ Abbott and Ghiskey, *Bulletin of the Johns Hopkins Hospital*, 1893, iv, 29.

** Flexner, *Ibid*, iv, 32.

†† Frosch, *Zeitschr. f. Hyg.*, 1893, xiii, 49.

Booker,* Canon,† Kutscher,‡. Wright§ and Stokes,|| Belfanti,¶ Kanthack and Stephens,** and Flexner and Anderson,†† (the last two papers contain good reviews of the previous literature) have demonstrated the presence of *B. diphtheriæ* in the internal organs of patients dead of clinical diphtheria, or similarly in animals dead from experimental inoculation, yet, so far as I am aware, Frosch alone has hitherto shown their presence within the central nervous system.

Cultures. Tubes of plain bouillon, glycerine agar and glycerine serum were sown direct from the bulb of ventricular fluid, from the substance of the central portion of the medulla, and from the kidney. The latter was then wrapped in 1:1000 bichloride gauze, incubated for 24 hours and second cultures were made.

Of four tubes of solid media inoculated from the right ventricle one—serum—developed 3 colonies of a bacillus indistinguishable in cover-slip preparations from *B. diphtheriæ*—and shown later to be such—and one colony of a diplococcus. Another—agar—developed one colony of a diplococcus. The broth cultures gave both *B. diphtheriæ* and diplococci. Four tubes from the third ventricle gave two to ten colonies each (broth abundant growth), about two-thirds of which were *B. diphtheriæ* and the remainder streptococci and diplococci.

Though the sowings from the medullary substance were abundant only two of seven tubes showed growth. One of these—agar—gave one colony of diplococci and the other—broth—many *B. diphtheriæ* and diplococci.

From the kidney a few colonies of a large white staphylococcus and an abundant growth of *B. coli communis* developed.

The diphtheria-like bacillus was isolated and carefully compared with specimens of known virulent diphtheria bacilli. Parallel cultures were made on Löffler's and glycerine serum, on plain glycerine and litmus-lactose agar, on plain and glucose gelatine, on potato, in plain glycerine, glucose and sugar-free bouillon, and in plain and litmus milk. The cultures were compared as to rate and character of growth, reaction of the media and appearance of stained preparations. In no particular did the organism under investigation differ materially from those with

* Booker, *Arch. of Pediatrics*, 1893, x, 642.

† Canon, *Deutsche med. Wochenschr.*, 1893, p. 1038.

‡ Kutscher, *Zeitschr. f. Hyg.*, 1894, xviii, 167.

§ Wright, *Boston Med. and Surg. Journ.*, 1894, cxxxi, 329; 357.

|| Wright and Stokes, *Ibid*, 1895, cxxxii, 271; 293; 330. Stokes, *ibid*, 1895, cxxxiii, 581.

¶ Belfanti, *Lo Sperimentale, Sez. biol.*, 1895, xlix, 278.

** Kanthack and Stephens, *Journ. of Path. and Bact.*, 1896-7, iv, 45.

†† Flexner and Anderson, *Bulletin of the Johns Hopkins Hospital*, 1898, ix, 72.

which it was compared. In the earlier cultures—on glycerine serum—it produced involution forms (large clubbed and “ghost” forms) somewhat more quickly than the controls, but this property was lost by the time the tenth generation was reached.

Animal inoculations with cultures. In order to determine the virulence and identity of the bacillus, guinea-pigs were inoculated, with the results shown in Table I.

It will be seen from the experiments recorded in Table I that the bacillus under investigation possessed an ordinary degree of virulence, that the autopsy findings were the same as those in death from known diphtheria bacilli, that in every instance the organism was recovered in pure culture and unchanged morphologically or culturally from the seat of inoculation (though from no other part of the body) and that diphtheria antitoxine invariably protected against otherwise fatal doses of the organism.

For experimental purposes a toxine was prepared by growing the bacillus—tenth generation from the original source—for 23 days in sugar-free bouillon in the incubator, and filtering through a Chamberland filter. Its toxicity was then tested with the results shown in Table II, p. 462.

Rabbit No. 1, weight 2860 grammes, whose serum had been shown by inoculation tests to be not antidiphtheritic, was then immunized by increasing doses of the toxine prepared as described above, and finally by inoculation with a living culture of the bacillus. The rabbit was then bled and its serum used as noted in the experiments given in Table III, p. 463.

Inoculations to determine the presence of the virus of rabies. About 0.5 ccm. from the central portion of the medulla of Mrs. R., received as noted above, was removed in a sterile manner and with sterile 0.67 per cent sodium chloride solution made into as thick an emulsion as could be passed through an ordinary hypodermic needle. With this emulsion the following inoculations were made:

Rabbit No. 2. Weight 1120 grammes. Inoculated December 15, 1897, 11 a. m., in left subdural space with 0.2 ccm. of emulsion above mentioned.

Appeared perfectly well until 9 a. m. Jan. 4, 1898, 20 days after inoculation, at which time it showed disinclination to move, and when made to hop exhibited weakness in posterior extremities with slight incoördination. Temperature 35.7° C. 11.30 to 12 m. incoördination became much more marked. Animal very restless, moving about constantly and grinding its teeth frequently. During afternoon animal

TABLE I.
GUINEA-PIG INOCULATIONS WITH BACILLUS X.

No.	Weight. Gms.	Subcutaneous inoculation in right groin.	Protected (?) by subcutaneous injection in left groin of	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at the autopsy: examined after 24 and 48 hours in the incubator.
1	520	0.5 cc. 40-hr. plain bouillon culture of Bacillus X.	Not protected.	4 days.	Intense œdema at seat of inoculation. Liver slightly enlarged. Meninges slightly congested.	Seat of inoculation = Bacillus X, pure. Heart's blood = sterile. Spleen = sterile. Ventricles and sub- stance of brain and spinal cord } = sterile.
2	450	Same dose and cul- ture as with No. 1.	Not protected.	4 days.	Intense œdema at seat of inoculation. General œdema over whole ventral portion of body.	Same as in No. 1.
3	390	"	60 units, N. Y. City B'd of Health diph- theria antitoxine.	40 days.	Small area of extravasation at seat of inoculation. Animal much emaciated.	All cultures sterile.
4	380	"	30 units of the same.	32 days.	Animal much emaciated.	All cultures sterile.
5	430	0.43 cc. 40-hr. sugar- free bouillon cul- ture of Bacillus X.	Not protected.	2 days.	Intense congestion and œdema at seat of inoculation.	Same as in No. 1.
6	410	0.41 cc. of same cul- ture as in No. 5.	Not protected.	2½ days.	Spleen enlarged, congested. Same as No. 5, and, in addition, kid- neys much congested.	Same as in No. 1.
7	250	0.25 cc. of same cul- ture as in No. 5.	Not protected.	2 days.	Same as No. 5, and, in addition, liver much congested.	Same as in No. 1.
8	410	0.41 cc. of same cul- ture as in No. 5.	50 units, Parke, Davis & Co.'s antiphther- itic serum. (Mixed with bacteria imme- diately before their injection into right groin.)	38 days.	Area of slight infiltration at seat of inoculation. Three abscesses in liver. Subcapsular abscess on surface of right kidney. Spleen pale.	Seat of inoculation, heart's blood, spleen and brain sub- stance = sterile. Liver abscess, } Kidney abscess, } { Short, thick bacilli, not diphtheria.
9	160	0.16 cc. 70-hr. plain bouillon culture of Bacillus X.	Not protected.	2 days.	Much œdema at seat of inoculation. Kidneys pale. Spleen much congested.	Same as in No. 1.
10	600	0.6 cc. of same cul- ture as in No. 9.	Not protected.	3 days.	Same as in No. 9, and, in addition, ad- renals and liver congested.	Same as in No. 1.
11	275	0.275 cc. of same cul- ture as in No. 9.	150 units, P. D. & Co's antiphtheritic serum.	14 days.	Lungs fill thoracic cavity, in state of congestion. Kidneys slightly softened.	Heart's blood = staphylococci. Seat of inoculation, spleen and brain substance = sterile.
12	250	0.5 cc. of same cul- ture as in No. 9.	150 units of same.	18 days.	As in No. 11.	As in No. 11.

* These 2 guinea-pigs were from a lot of 50 received but a few days before, and all the others of which died of pneumonia (?) in periods of from 1 to 3 weeks after their receipt in the laboratory.

TABLE II.
GUINEA-PIG INOCULATIONS WITH TOXINE FROM BACILLUS X.

No.	Weight.	Subcutaneous inoculation in right groin.	Protected (?) by	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at autopsy and examined after 24 and 48 hours in the incubator.
13	300	0.15 cc. filtered toxin — prepared by growing Bacillus X 23 days in the incubator in sugar-free bouillon.	Not protected.	3½ days.	Much œdema at seat of inoculation. Spleen enlarged and congested. Liver enlarged and congested.	Seat of inoculation, Spleen, Heart's blood, Ventricles and substance of brain and spinal cord, } = sterile.
14	310	0.31 cc. of same material as in No. 13.	Not protected. 0.3 cc. serum of Rabbit No. 1. (Normal — afterwards immunized with toxine noted in G. P. 13.) Serum mixed with toxine immediately before inoculation.	1½ days.	Same as in No. 13.	Same as in No. 13.
15	330	0.15 cc. of same material as in No. 13.		3½ days.	Same as in No. 13.	Same as in No. 13.

TABLE III.
GUINEA-PIGS PROTECTED WITH ANTITOXIC SERUM PREPARED WITH BACILLUS X.

No.	Weight. Grms.	Subcutaneous inoculation in right groin.	Protected (?) by subcutaneous injection of	Period from inoculation till death.	Positive gross findings at autopsy.	Glycerine serum and bouillon cultures made at the autopsy; examined after 24 and 48 hours in the incubator.
16	400	0.25 cc. 31-day sugar-free broth culture of known B. diphtheriæ (series De C.).	Not protected.	1½ days.	Intense oedema and congestion at seat of inoculation. Spleen congested.	Seat of inoculation = B. diphtheriæ, pure. Heart's blood, Spleen, Ventricles and substance of brain = sterile.
17	370	Same dose and culture as with No. 16.	0.5 cc. serum from Rabbit No. 1. (Immunized with toxin from Bacillus X.) Mixed with bacteria immediately before inoculation.	36 days.	Slight area infiltrated at seat of inoculation. Entire lungs in red hepatization.	Seat of inoculation = sterile. Heart's blood = staphylococci. Spleen = staphylococci. Brain and cord = sterile.
18	810	0.31 cc. 70-hr. plain bouillon culture of B. diphtheriæ (series De C.).	Not protected.	1½ days.	Considerable oedema and congestion at seat of inoculation. Kidneys congested. Liver and spleen pale.	Same as in No. 16.
19	140	0.28 cc. same culture as with No. 18.	0.3 cc. same serum as with No. 17.	26 days.	Right lung oedematous.	Same as in No. 17, except spleen sterile.
20	150	0.15 cc. 70-hr. plain bouillon culture of known B. diphtheriæ (series W. W.).	Not protected.	2½ days.	Intense congestion and oedema at seat of inoculation. Kidneys and adrenals congested. Spleen enlarged. Liver pale, mottled.	Same as in No. 16.
21	115	0.12 cc. same culture as with No. 20.	0.5 cc. same serum as with No. 17.	Still alive after 96 days.		

remained quiet. Jan. 6, 1898, 9 a. m., rabbit found lying on side, stretched out, head thrown backwards, all power of motion in posterior extremities apparently gone. Temperature 35.5° C. 1 p. m., condition unchanged. Sniffed at food which was placed near nose but did not attempt to eat. (Culture from throat taken at 5 p. m., Jan. 4, showed staphylococci, streptococci and short slender bacilli, unidentified.) Jan. 6, 8 p. m., 22 days after inoculation, rabbit was found dead and in rigor mortis.

Autopsy 9 p. m., Jan. 6. Marked injection of vessels of pia; minute extradural plastic exudate at seat of inoculation; mouth and trachea clear of mucus; vessels of trachea not congested; small light-colored patch on inferior border of median lobe of liver; bladder much distended and its vessels injected; other organs normal.

Cultures made from pia, fourth ventricle, and heart's blood, all remained sterile. Those from substance of medulla gave abundant pure growth of a bacillus with the same morphological and cultural characteristics as that obtained from the original source and identified as the diphtheria bacillus.

Rabbit No. 3. Weight 1270 grammes. Inoculated at same time (Dec. 15), in the same manner, and with the same substance and dose as Rabbit No. 2.

Jan. 5, 9 p. m., 21 days after inoculation, animal showed first symptoms. History similar to that of Rabbit No. 2, except that at no time was any excitement present, and that much flowing of saliva was noted throughout the second day after the appearance of symptoms. Death Jan. 6 at 11 p. m., 22 days after inoculation.

Autopsy findings parallel with those of No. 2, except that the throat was filled with mucus and the trachea was slightly injected in upper portion. The liver was apparently normal. Cultures from surface of brain at seat of inoculation and heart's blood remained sterile. Those from substance of medulla developed pure growth of *B. diphtheriæ*.

Though the *Bacillus diphtheriæ* was recovered in pure culture from the central nervous system of Rabbits Nos. 2 and 3, yet the clinical history and gross autopsy findings in the animals were so like those observed in rabbits inoculated subdurally with the virus of known rabies that it was impossible to decide whether death had been caused by the virus of rabies, by the diphtheria bacillus, or by a combined infection with the two. Hence the following inoculations were made from the substance of the medulla of Rabbit No. 2.

Rabbit No. 4. Weight 1390 grammes. Inoculated 2 p. m., Jan. 6, 1898, in left subdural space with 0.2 ccm. of thick emulsion of medulla

of Rabbit No. 2 in 0.67 per cent. NaCl. solution. (A small quantity of the emulsion escaped from the wound in the dura on the withdrawal of the needle.)

Rabbit remained apparently well up to and including 4 p. m., Feb. 1, 1898. Found dead and in rigor mortis 9 a. m., Feb. 2, 27 days after inoculation.

At *autopsy* animal very much emaciated. Pia slightly congested. Trachea normal. Liver congested, not markedly enlarged. Gall bladder much distended. Urinary bladder distended. All other gross findings negative. Cultures from surface of pia, heart's blood and gall bladder all remained sterile after 7 days in the incubator. Those from the substance of the medulla developed a few colonies of *B. diphtheriæ*.

Rabbit No. 5. Weight 1030 grammes. Inoculated Jan. 6, 2 p. m., in left subdural space with 0.2 ccm. of the same emulsion as that used in inoculating Rabbit No. 4 (*i. e.* medulla in physiological salt solution).

Jan. 23, 10 a. m., 17 days after inoculation, the rabbit showed disinclination to move; when compelled to hop exhibited slight posterior incoördination. Temperature $38^{\circ}.5$, 5 p. m. Symptoms grew rapidly worse during afternoon—animal lying on side with posterior limbs completely paralyzed. Found dead Jan. 24 at 9 a. m., 18 days after inoculation.

Autopsy. Body not emaciated. Meninges very intensely congested. Trachea slightly congested. Heart and adjacent vessels engorged with blood. Liver very dark, congested, enlarged and friable. Spleen pale. Right kidney slightly congested. Other gross findings negative.

Serum and broth cultures from the meninges and substance of medulla gave a few colonies of *B. diphtheriæ*. Similar cultures from the ventricles of the brain, the heart's blood, and the spleen, remained sterile after 5 days in the incubator.

Rabbit No. 6. Weight 1210 grammes. Inoculated Jan. 6, 1898, 2 p. m., in left subdural space with 0.2 ccm. of emulsion prepared by rubbing up an unmeasured portion of the medulla of Rabbit No. 2 with P., D. & Co.'s diphtheria antitoxine (date of Oct. 9, 1897, 2000 units to bulb). The emulsion was as thick as could be passed through the needle of a hypodermic syringe.

This rabbit has shown no symptoms up to the present time, 130 days after the inoculation.

Rabbit No. 7. Weight 970 grammes. Was inoculated Jan. 6, 1898, in the left subdural space with 0.25 cc. of the same emulsion as that used in inoculating Rabbits Nos. 4 and 5. Immediately preceding this inoculation the animal had been given subcutaneously in the right

groin 1 cc. (about 500 units) of antidiphtheritic serum (P., D. & Co.'s issue of Oct. 9, 1897). This rabbit has exhibited no symptoms up to the present time, 130 days after the inoculation.

The extreme difficulty of procuring rabbits at the time of death of Nos. 4 and 5, coupled with the strong belief that Nos. 6 and 7 would eventually die of rabies, was deemed sufficient reason for not carrying forward the series from the medullæ of those already dead.

Effects of association of the virus of rabies with diphtheria antitoxine and B. diphtheriae.—The experiments on Rabbits Nos. 6 and 7 indicate that the virus of rabies was absent from the material used to inoculate these animals and presumably also from the medulla of the patient, but before this inference could be justifiably drawn it was necessary first to determine what influence diphtheria antitoxine may have upon the virus of rabies or upon a combination of this virus with the diphtheria bacillus.

In numerous experiments made in this laboratory large doses of antidiphtheritic serum have been administered subcutaneously to rabbits just prior to their inoculation with the fixed virus of rabies, and in other instances with the virus of street rabies, without any appreciable effect either on the time of onset or the character of the symptoms. Equally without effect upon the manifestations of rabies was the mixture of the rabic virus with diphtheria antitoxine before inoculation of the animal.

The experiments recorded in Table IV (p. 467) were made to determine the effect, if any, on the incubation period, symptoms, etc., of inoculation of rabic virus simultaneously with diphtheria antitoxine and diphtheria bacilli.

It will be observed from Table IV that Rabbits Nos. P85, P87, 268, 270, were used merely as controls, the first two being inoculated in the ordinary manner with the fixed virus of rabies from two different animals, and the last two with the virus of street rabies, second generation from a rabid dog. In Nos. P86 and P88 the addition of diphtheria antitoxine and virulent *B. diphtheriae* to the fixed virus some time before inoculation did not appear to alter in any way either the incubation period, the train of symptoms, or the autopsy findings. Rabbits Nos. P109, P111, P113, P115, P117 and P121 of Table V may also be cited in this series. The diphtheria bacilli had been in contact with the living nerve tissue and rabic virus for from 2 to 15 days in the material with which these latter animals were inoculated, yet the rabic virus appeared unchanged thereby.

In Rabbits Nos. 269 and 271, in the inoculation of which to the rabic

TABLE IV.

INOCULATION OF RABBITS WITH RABIC VIRUS COMBINED WITH DIPHtheria BACILLI
AND DIPHtheria ANTITOXINE.

No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoculation till death.	Autopsy.
P. 85 (control)	1460	In left subdural space with 0.2 cc. of emulsion of 0.2 cc. of medulla of rabbit dead of inoculation with the fixed virus of rabies mixed with 0.5 cc. of plain broth.	7	Short period of excitement followed by stupor; posterior incoördination; ascending paralysis; retraction of head; escape of much saliva from mouth; death in coma.	13	Meninges and trachea slightly congested. Bladder distended. Serum and broth cultures from meninges and heart's blood sterile after 48 hours incubation.
P. 86	1520	Same as No. P. 85 except 0.5 cc. of P., D. & Co's diphtheria antitoxine (500 units) used instead of broth. Emulsion inoculated with 1 loopful of a 40-hr. broth culture of <i>B. diphtheriae</i> ½ hour before inoculation into rabbit.	7	Same as No. P. 85.	13	Same as No. P. 85.
P. 87 (control)	3050	Same as No. P. 85 except medulla of another rabbit (dead of fixed virus of rabies) used.	7	Same as No. P. 85.	12	Same as No. P. 85.
P. 88	2380	Same as No. P. 87 except 0.5 cc (500 units) P. D. & Co's diphtheria antitoxine used instead of plain broth. Emulsion inoculated 1 hr. before use with 1 loopful of a serum culture of a virulent <i>B. diphtheriae</i> .	7	Same as No. P. 85.	11	Same as No. P. 85.
268 (control)	1300	In left subdural space with 0.2 cc. of emulsion of 0.2 cc. of medulla of rabbit No. 266 (original from street rabies, Case 33) made up with 0.5 cc. of plain broth.	13	Excitement followed by stupor; posterior ascending paralysis; retraction of head; much saliva escaping from mouth; death in coma.	15	Slight meningitis; trachea congested; bladder distended. Cultures from seat of inoculation and heart's blood sterile after 48 hrs. in incubator.

TABLE IV—*continued.*

No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoculation till death.	Autopsy.
269	1420	In same manner, dose and medulla as with No. 268, but emulsion made by using 0.2 cc. of medulla to 0.5 cc. of P. D. & Co's diphtheria antitoxine (500 units). The emulsion was inoculated $\frac{1}{2}$ hr. before use with 1 loopful of 40-hour broth culture of virulent <i>B. diphtheriae</i> .	14	Slight excitement of animal noticed on day following inoculation which subsided within 18 hrs. No further symptoms till the 14th day, when began the usual course as observed in rabbits suffering from rabies.	16	Same as in No. 268, and in addition liver slightly congested.
270 (control)	2050	Same in every respect as No. 268, except that the medulla of rabbit No. 267 (mate to No. 266, original from street rabies, Case 33) was used.	19	Same as No. 268.	21	Same as No. 268.
271	2000	Same as No. 269 except same medulla as used with No. 270 and emulsion inoculated with 1 loopful of 24-hr. serum culture of virulent <i>B. diphtheriae</i> $\frac{1}{4}$ hr. before use.	20	Three days after inoculation rabbit excited, showed some posterior incoördination; 24 hours later less excited, incoördination very slight; ate food freely. 72 hours later animal apparently perfectly well. No further symptoms until the 20th day after inoculation when began the usual course as in No. 268.	24	Same as No. 268, except small hæmorrhagic area—subdural—at site of inoculation.

virus had been added diphtheria antitoxine and virulent diphtheria bacilli, symptoms of slight cortical irritation were present on the first and third days respectively after trephining. These symptoms rapidly disappeared and the animals remained well until the expiration of the ordinary incubation period of street rabies when they exhibited the same train of symptoms as their controls, and after death gave the same autopsy findings.

The foregoing experiments demonstrate that the medulla of Rabbit No. 2 (dead 22 days after inoculation with an emulsion of the medulla of Mrs. R.) could not have contained the rabic virus, for if this virus had been present it would have manifested itself in Rabbits Nos. 6 and 7 which were inoculated with the medulla of Rabbit No. 2 combined with diphtheria antitoxine.

The question may, however, still be raised whether a combined infection with rabies and diphtheria may not have existed in the patient and that only the virus of diphtheria was transmitted to the rabbits inoculated with her medulla. As the ultimate findings could not be anticipated, the opportunity of determining the protective influence of diphtheria antitoxine upon the animals inoculated directly with the medulla of the patient was unfortunately lost.

Inasmuch as Roux * found that the medulla and cord of an animal inoculated 4 days before with fixed virus—that is, 3 days before the appearance of symptoms—were capable of producing rabies when inoculated into other animals, it seems most improbable that the virus of rabies should not, if it were originally present in the medulla of the patient, have been demonstrable in the medullæ of the rabbits inoculated with this medulla and dying 22 days after the inoculation.

In order to shed further light upon the question of the possibility of a mixed infection in the case of Mrs. R., the experiments recorded in Table V were undertaken to determine:

(a) How soon after subdural inoculation with the virus of rabies the medulla of a rabbit may contain sufficient virus to produce symptoms of rabies in other animals inoculated therefrom.

(b) The effect, if any, on the incubation period, course of symptoms, etc., in rabbits of inoculation with the medullæ of rabbits killed, during the incubation period of rabies, by subdural inoculation of *B. diphtheriæ*.

It will be noted from Table V that the medullæ of rabbits Nos. P103

* *Annales de l'Institut Pasteur*, 1889, iii, 77 and 1888, ii, 24.

TABL

SERIAL INOCULATIONS TO DETERMINE THE PRESENCE OF THE RABIC VIRUS IN RABBITS IN

FIRST SET OF INOCULATIONS.							SECOND SET OF INOCULATIONS.		
No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days from original inoc. to onset of symptoms.	Symptoms.	No. of days from original inoculation till death.	Autopsy.	No. of rabbit.	Wt. in grammes.	Inoculation.
P102 (control)*	2020	In left subdural space with 0.2 cc. of emulsion of 0.4 cc. of medulla of rabbit No. P. 101 (dead from inoculation of fixed virus of rabies) rubbed up with 1 cc. of plain broth.	7	Short period of excitement followed by stupor; posterior incoördination; ascending paralysis; retraction of head; escape of much saliva from mouth; death in coma.	12	Slight congestion of meninges and trachea. Bladder distended. Serum and broth cultures from meninges and heart's blood remained sterile after 48 hours in incubator.			
P103	1900	Same as No. P. 102, except that in addition 4 days later animal was given through original opening in skull 0.2 cc. of emulsion of 48-hour broth with a 48-h. serum culture of B. diphtheriae.	5	Much excitement; posterior incoördination; ascending paralysis. (Excitement kept up longer than in rabies.) Death in coma.	7	Much congestion of meninges; no pus. Trachea congested. Serum and broth cultures from meninges showed B. diphtheriae pure. Similar cultures from heart's blood remained sterile after 48 hours in incubator.	P116	990	Same as No. P. 103, emulsion made of rabbit No. P. 103 up with plain broth.
P104	1960	Same as No. P. 103, except second inoculation given 3 days after first.	4	Same as No. P. 103.	5	Same as No. P. 103.	P117	960	Same as No. P. 116, emulsion made of 0.2 cc. of medulla of No. P. 103 with cc. of P. D. & Co. antitoxine.
							P112	930	Same as No. P. 103, medulla of rabbit used for inoculation.
							P113	880	Same as No. P. 112, 50 units P. D. & Co. antitoxine, making emulsion.
P105	2470	Same as No. P. 103, except second inoculation given 2 days after first.	3	Same as No. P. 103.	4	Same as No. P. 103.	P110	2200	Same as No. P. 105, emulsion made of No. P. 105.
							P111	1420	Same as No. P. 110, P. D. & Co.'s diphtherine used in making emulsion.
P106	2190	Same as No. P. 103, except second inoculation given 1 day after first.	2	Same as No. P. 103.	2	Same as No. P. 103.	P108	2200	Same as No. P. 103, sion made from No. 106.
							P109	1980	Same as No. P. 108, P. D. & Co.'s diphtherine used in making emulsion.
P107	2200	Same as No. P. 103, except 20-day broth culture of B. diphtheriae used instead of plain broth in making emulsion.	1	Same as No. P. 103.	5	Same as No. P. 103.	P114	940	Same as No. P. 103, sion made from No. P. 107.
							P115	900	Same as No. P. 114, P. D. & Co.'s diphtherine used in making emulsion.

* This animal forms one generation in the series used in this laboratory for the perpetuation of the fixed virus of r

E V.

OCULATED WITH THIS VIRUS AND SUBSEQUENTLY KILLED BY THE DIPHTHERIA BACILLUS.

SECOND SET OF INOCULATIONS.					THIRD SET OF INOCULATIONS.						
Inoc.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoc. till death.	Autopsy.	No. of rabbit.	Wt. in grammes.	Inoculation.	No. of days to onset of symptoms.	Symptoms.	No. of days from inoc. till death.	Autopsy.
102, but with from medulla P. 103 rubbed oth.	7	Same as No. P. 102, except shorter period from onset of symptoms till death. (This is almost always true with small rabbits.)	9	Same as No. P.102.							
1, except with by rubbing up alla of rabbit .50 units (0.5 Co.'s diphtheria	7	Same as No. P. 116.	9	Same as No. P.103.							
1, except with bit No. P. 104 ation.	7	Same as No. P. 116.	11	Same as No. P.103.							
2, except that Co.'s diphtheria were used in 1.	7	Same as No. P. 116.	10	Same as No. P.103.	P120	1220	Same as No. P. 116, except medulla of No. P. 113 used in making emulsion.	7	Same as No. P.116.	10	Same as No. P.102.
					P121	1420	Same as No. P. 120, except 50 uts. P. D. & Co.'s diphtheria antitoxine used in making emulsion.	7	Same as No. P.116.	10	Same as No. P.102.
2, except with from medulla	7	Same as No. P. 102.	14	Same as No. P.102.							
except 50 units diphtheria antitoxine emulsion.	7	Same as No. P. 116.	10	Same as No. P.102.							
2, except emulsion medulla of	7	Same as No. P. 102.	9	Same as No. P.102.							
except 50 units diphtheria antitoxine emulsion.	7	Same as No. P. 102.	15	Same as No. P.103.							
2, except emulsion medulla of No.	7	Same as No. P. 116.	9	Same as No. P.102.							
except 50 units diphtheria antitoxine emulsion.	7	Same as No. P. 116.	10	Same as No. P.102.							

abies, and is noted here incidentally as a control in the inoculation of Nos. 103-107.

to P107, inclusive, dying from diphtheria infection 7, 5, 4, 2, and 5 days respectively after subdural inoculation with the fixed virus of rabies were found to contain the virus of rabies apparently unchanged.

Rabbits Nos. 108 to 117, inclusive, comprising the second series of inoculations from the above showed the same incubation period, train of symptoms and post-mortem lesions as control rabbits inoculated in the ordinary manner with the fixed virus of rabies. Though from each of the emulsions used in inoculating these animals (Nos. P108-P117) *B. diphtheriæ* was grown in pure culture, yet the organism was recovered from the brains of but half of them after death. Its presence or absence seemed not to be determined by the use or non-use of diphtheria antitoxine in making up the emulsions, nor by the length of time elapsing from inoculation till death.

Nos. P120 and P121, the only animals inoculated in the third set, gave the incubation period, train of symptoms, and autopsy findings of controls inoculated in the ordinary manner. Diphtheria bacilli were not recovered from their brains after death though the organisms were present in small numbers in the emulsion from which they were inoculated.

In the light of the experiments recorded in Table V it does not seem possible to doubt that if the virus of rabies had really been present in the medulla of the patient, it would have been transmitted in demonstrable form to the medullæ of the rabbits inoculated therewith, but, as has already been shown, such transmission did not occur, and the inference is, therefore, warrantable that the rabic virus was absent from the patient's medulla.

Subdural inoculations of B. diphtheriæ.—Some experiments have been made in this laboratory and others are now in progress relating to the production of acute and delayed meningitis in rabbits by the subdural inoculation of pure cultures of *B. diphtheriæ*. The results of these, with report of tissue changes, are reserved until more extended observations have been made. One series, however, may be given here. *B. diphtheriæ*—nine generations removed from the original source—was grown 23 days in sugar-free bouillon and the following inoculations made:

Rabbit	8,	weight	2170 g.,	received	0.05 cc. in left subdural space.
"	9	"	2080 g	"	0.1 cc. " " "
"	10	"	2000 g.	"	0.2 cc. " " "
"	11	"	1680 g.	"	0.3 cc. " " "
"	12	"	1800 g.	"	0.4 cc. subcutaneously in right groin.

Nos. 9, 10 and 11 died within 24 hours. At the autopsies was found acute meningitis, and *B. diphtheriæ* was recovered from the point of inoculation. No. 12 died on the 4th day.

No. 8 remained somewhat stupid for two days and then became apparently normal until the 8th day, when it showed great excitement if irritated, whirling about rapidly and rushing around the room in a frenzied manner, this attack lasting about one minute. These phenomena could, with gradually decreasing readiness, be produced by stimulation during a period of 3 days, after which they subsided altogether. The rabbit then remained in apparent good health until the 23d day after inoculation, when its posterior extremities were noticed to be weak and slightly incoördinated. On the following day they were completely paralyzed. The paralysis rapidly ascended. 48 hours after the beginning of incoördination the rabbit was lying on its side with limbs extended, head retracted, and breathing spasmodically. At no time during its final sickness was there any excitement observed nor any marked escape of saliva from the mouth. It may be mentioned, however, that the symptoms were difficult to distinguish from those exhibited by two rabbits dying from inoculation with the fixed virus of rabies and one dying from "street rabies"—second generation from a rabid wolf—which were under observation at the time in the same room with Rabbit No. 8. The animal died on the 27th day after inoculation.

Autopsy. General condition of rabbit good. Meninges not congested. Portion of left cerebrum about 1 cm. in superficial diameter and extending, at seat of inoculation, down to ventricle, was markedly softened. Membranes of cord slightly congested. Liver deeply congested. Urinary bladder much distended.

Cultures. From the softened area of the cerebrum no growth was obtained; from the medulla a few colonies on serum of *B. diphtheriæ*; from the heart's blood a few colonies of staphylococci.

SUMMARY OF PATHOLOGICAL REPORT.

1. The bacillus isolated from the central nervous system of Mrs. R. was *Bacillus diphtheriæ*, and doubtless such was the identity of the bacillus discovered in the nerve cells of the pons and medulla.
2. The histological lesions, as far as they were observed in the central nervous system and kidney, were the same as those observed after death from ordinary clinical and experimental diphtheria.
3. The subdural inoculations in rabbits of portions of the medulla

of Mrs. R. produced symptoms simulating rabies in their time of onset and general character. The gross post-mortem findings in the animals, resembled in their negative character those of rabies, but *B. diphtheriæ* was isolated culturally from the central nervous system. Synchronous inoculation of diphtheria antitoxine protected rabbits against otherwise fatal doses of the emulsions of the medullæ of the first series of rabbits.

4. The mixture of diphtheria antitoxine, or of diphtheria antitoxine and diphtheria bacilli, with the virus of rabies is without influence upon the manifestations of rabies.

5. The medullæ of rabbits inoculated with the fixed virus of rabies and killed during the period of incubation, 2 to 7 days after the original inoculation, by subdural injection of cultures of the diphtheria bacillus, contain the virus of rabies in a form capable of transmitting rabies to rabbits inoculated in series with such medullæ.

6. The subdural inoculation of a small dose of pure culture of *B. diphtheriæ*, isolated from another source, produced symptoms which, in their time of onset and subsequent history, resembled in some degree those of rabies in rabbits.

I desire to express sincere thanks to Professor F. F. Wesbrook, under whose direction the above study has been conducted, to Dr. O. McDaniel and to Dr. E. Bates Block for their suggestions and criticism.

REVIEW AND CONCLUSIONS.

The history and the clinical symptoms in the case of Mrs. R. pointed toward the diagnosis of rabies. The well-authenticated history of a bite on the cheek by an unknown animal, the two months' incubation period, the onset with extreme pain and numbness in the region of the scar, the development of the characteristic laryngeal and respiratory spasms on attempting to take liquids, the spasms at first being slight, later most pronounced, and toward the close feeble or absent, the insomnia, the absence of fever in the beginning, which later in the disease became pronounced, the rapid pulse at all stages, the attacks of violent delirium interspersed with periods of calm and complete rationality, the absence of all symptoms pointing toward any other simu-

lating disease, and the fatal termination, all serve to make an almost complete picture of rabies.

One feature of the case not in harmony with the diagnosis of rabies is the long period over which the disease continued prior to the fatal termination. As is seen from the history, the course of the illness, dating from the onset with pain, sleeplessness and mental depression, lasted 14 days. If time is estimated from the onset of the first laryngeal spasm, ten days elapsed before the fatal outcome. On the other hand, most authorities place the death limit in rabies at five to eight days.

A second clinical feature manifested in this case which does not appear to correspond to the usual symptom-complex in rabies is the presence of a large amount of albumin and of casts in the urine. Most of the clinical writers make no mention of such a complication in rabies. Roger,* however, speaks of the occurrence of dysuria, albuminuria and glycosuria in rabies, and he cites Samson and Chippindale as having noted hæmoglobinuria and the presence of casts.

The gross pathological findings likewise seemed to confirm the clinical diagnosis, since the post-mortem examination revealed no lesions aside from mild cerebral congestion. But the value of such confirmation is slight, in view of the fact that no characteristic gross lesions of rabies have hitherto been determined by any observer, and a confirmation based on negative pathological conditions is of doubtful value. The microscopical changes found in the nervous centres, although definite, are not of decisive diagnostic significance.

An unexpected and important discovery was the finding of the Klebs-Löffler bacillus in the ventricular fluids and in the tissues of the medulla oblongata of the patient. The presence of the bacillus in microscopical sections confirmed the cultural results, and especially to be emphasized is the demonstration of characteristic diphtheria bacilli within nerve cells. The determination of the identity of the bacillus with the genuine *Bacillus diphtheriæ* was so complete that there can be no room for doubt upon this point. The bacillus was of

* Roger, Article "Rage" in Charcot, Bouehard and Brissaud's *Traité de Médecine*, T. i, p. 608. Paris, 1891.

ordinary virulence and produced toxine and led to the generation of antitoxine identical with diphtheria toxine and antitoxine.

Not less suggestive of rabies than the clinical history of the patient were the results of subdural inoculations of rabbits with emulsions prepared from the medulla of the patient. There occurred the long period of incubation (20 and 21 days) followed by phenomena similar to those in experimental rabies of rabbits, and the rabbits inoculated subdurally with the medullæ of the first rabbits behaved in a similar manner.

But here again the *Bacillus diphtheriæ*, which was present in the medulla of the patient, was demonstrated with similar distribution in the rabbits' medullæ.

Of decisive significance in the interpretation of the case is the demonstration that the mixture of the emulsion of the medulla of the first series of rabbits with diphtheria antitoxine, as well as the injection of antitoxine followed by inoculation of the medulla, prevented the appearance of any abnormal symptoms after subdural inoculation, whereas in control experiments diphtheria antitoxine manifested no appreciably protective influence as regards either the fixed virus of rabies or the virus of street rabies. It is, therefore, justifiable to infer that the virus of rabies was not present in the medullæ of the rabbits inoculated directly with the medulla of the patient, for otherwise it should have been capable of demonstration after the neutralization of the diphtheria toxine by antidiphtheritic serum. Although the opportunity to mix diphtheria antitoxine with the medulla of the patient was lost, the experiments recorded in Table V justify the conclusion that if the virus of rabies had been present in combination with the diphtheria bacillus in this medulla it would have been transmitted in demonstrable form to the medullæ of the rabbits inoculated therewith and dying 22 days afterward. As such transmission is excluded by the results of the inoculation of Rabbits Nos. 6 and 7, the absence of the virus of rabies from the patient's medulla must be regarded as established.

We are forced, therefore, to the conclusion that the case reported in this paper was one of infection of the central nervous system with

the diphtheria bacillus and not one of rabies, and this conclusion is confirmed by the experimental reproduction of a similar localization with a prolonged period of incubation by the subdural inoculation of a rabbit with 0.05 cc. of a 23 days' bouillon culture of the diphtheria bacillus (p. 472).

The portal of entry of the bacillus was not determined. There was no positive evidence of diphtheria in the throat, although the existence of the diphtheria bacillus in this situation was not excluded. The possibility that the patient was inoculated with the diphtheria bacillus through the wound and that it traveled along the nerves to the nervous centres, in the manner of the rabid virus, may be suggested, but of this there was no proof.

The close simulation of rabies both clinically and experimentally (a simulation all the more remarkable on account of the previous history of a bite on the cheek, followed by the usual period of incubation of rabies) by cerebral infection with the diphtheria bacillus is certainly most curious and interesting. We have abundant evidence that the cerebral localization of various infectious agents, such as those of tuberculosis, of tetanus, etc., may be attended by morbid phenomena very unlike those of the ordinary localizations of the same agents elsewhere in the body, and our case demonstrates that the same may be true for the diphtheria bacillus.