A COMPARATIVE STUDY OF THE DIPLOCOCCI OCCURRING IN EPIDEMIC CEREBRO-SPINAL MENINGITIS AND POSTERIOR BASIC MENINGITIS.[1]

By MARTHA WOLLSTEIN, M.D.

(*From the Laboratories of the Rockefeller Institute for Medical Research, New York.*)

PLATE XXVI.

As long ago as 1898, Still (1) concluded from a study of eight cases of simple, posterior basilar meningitis occurring in infants, that the disease is a sporadic form of epidemic cerebro-spinal meningitis, and that the Gram negative diplococcus found in the meningeal exudate of both forms is identical, such slight differences as greater vitality and lack of virulence being due to natural variation. This view was generally accepted, and the observations made on cases which occurred in this country confirmed Still's findings without adding anything important to our knowledge of the subject. According to the clinical view that posterior basilar meningitis, when not of syphilitic origin, is the chronic stage of an acute cerebro-spinal meningitis (Holt) (2), there seemed no reason to doubt the identity of the causative microörganism in the two affections.

During the past two years, since the recent epidemic of cerebro-spinal meningitis appeared in the British Isles, several observers have claimed that posterior basilar meningitis is a disease due to a specific microörganism definitely and uniformly differing from the meningococcus causing the ordinary epidemic disease, the difference consisting in a total lack of agglutination and opsonin reactions of the diplococcus from cases of posterior basic meningitis with the serum from an epidemic case, and vice versa. Thus in 1907, Houston (3), after studying diplococci isolated from two cases of posterior basilar meningitis at the Great Ormond Street Hospital for Sick Children, which reacted with the blood from these

[1] Received for publication April 1, 1909.

children but not with that from epidemic cases in Belfast, while cocci isolated from the Belfast cases failed to react with the blood from the London cases, concluded that there are two races of meningococci differing in agglutination and opsonic reactions. Houston studied both reactions in the same slide, prepared according to Wright's technique for the study of opsonins, thus using a dilution of one to three. At the onset of meningitis the opsonic index for the meningococcus is low, but it rises gradually. Agglutinins appear about the sixth day, and after that time the two reactions may be studied together.

At the meeting of the British Medical Association held at Sheffield in July, 1908, Houston and Rankin (4) reiterated the view that "Still's disease is due to a meningococcus which has much the same cultural characteristics as the coccus of epidemic cerebro-spinal fever, but differs from it entirely in its opsonic and agglutinating properties." Eve and Clements (5) expressed the same view even more forcibly, stating that the sporadic organism shows little or no tendency to agglutinate or phagocyte in the patient's own blood or in the serum from an epidemic case and will not agglutinate in the Flexner-Jobling antimeningitis serum. Ker (6) was enabled to pick out three cases of post-basic meningitis by means of the agglutination reactions, but one of these cases was very favorably influenced by the Flexner-Jobling anti-serum.

Dr. Flexner having obtained, through the kindness of Dr. Houston, three strains of diplococci from cases of posterior basilar meningitis, kindly gave me the privilege of studying them in comparison with a number of strains from epidemic meningitis cases isolated before and after this study was undertaken. In all, seventeen cultures of diplococci from cases of meningitis were at my disposal. Their histories were as follows:

Two were isolated from typical cases of epidemic meningitis, grown in the laboratory for one and two years.

Two were isolated from typical cases of epidemic meningitis, grown in the laboratory for three months.

Seven were isolated from typical cases of epidemic meningitis, during the course of this work.

One was isolated from the circulating blood of a patient with indefinite meningeal symptoms but without acute meningitis.

Three were obtained from Dr. Houston and regarded as posterior basilar strains.

Two were isolated from typical cases of epidemic meningitis, but were not meningococci.

These cultures were divided into three groups for purposes of study as follows: old, proved strains, recent strains and posterior basilar strains. The two which proved not to be meningococci will be described later.

Morphology.—All were biscuit-shaped diplococci lying in single pairs or in larger groups, and never forming chains. They were not motile.

To Gram's stain they reacted negatively in smears from cerebrospinal fluid, cultures, and in the sections of organs of inoculated animals.

Biology.—On sheep's-serum-agar slants the growth was profuse and typical: greyish-white, smooth, moist showing a slightly metallic sheen on drying, and when moist of a tenacious, mucoid consistency on removal with the needle. On human ascitic fluid agar and on glucose agar, the growth was abundant. On plain agar it was very scant, but readily visible. In plain neutral bouillon a slight turbidity appeared within twenty-four hours and a small, flaky precipitate formed in another day; no pellicle formed. The reaction of the bouillon became alkaline. In serum broth the precipitate was more profuse; the reaction also became alkaline. Litmus milk remained unchanged.

The old, the recent and the posterior basilar strains showed no appreciable differences in any of these culture media. It was not possible to tell one strain or group from another. The two strains designated as not meningococcus were very different. They were also Gram negative cocci occurring in pairs, but they were slightly larger and less strictly biscuit-shaped. They grew profusely on plain agar, caused a marked clouding of serum-glucose agar (the albumin precipitation of Libman) and a diffuse cloudiness of plain and serum bouillon with the formation of a thin pellicle and a thick precipitate. Milk remained fluid. In serum-litmus water containing dextrose, coagulation occurred within twenty-four hours; also in galactose. The other sugars remained unchanged. The facts

that they were Gram negative diplococci and had failed to ferment lactose, mannite, saccharose, raffinose and levulose were their only points in common with true meningococci. The latter showed, in their reactions to sugars in the presence of serum-litmus water, the first points of differences encountered among the various strains; but again they were not race but individual variations. Dextrose, for instance, was fermented by all but the oldest strain, which had lost the power it possessed in this direction eighteen months earlier. The next oldest strain fermented neither dextrose nor maltose, though it had produced acid in both a year before. All the other strains, thirteen in number, fermented maltose. One recent strain, three months old, failed to ferment dextrose at any time. The culture isolated from the blood during life formed acid rapidly in both maltose and dextrose, coagulating the former after three weeks, the latter not at all. The three posterior basilar strains fermented maltose and dextrose, one coagulating maltose only, one producing a sufficient amount of acid in both varieties of sugar to cause coagulation, and the third leaving both sugar media fluid. Arkwright (7) mentions the fact that four strains of meningococcus studied by him fermented no sugars, and Wilson (8) observed the same phenomenon with one strain. The duration of laboratory cultivation of these cultures is not stated. Arkwright gives it as his opinion that the sporadic strains diverge more than the epidemic ones do, as regards the sugar reactions. With this view our observations do not agree. Galactose, lactose, mannite, saccharose, raffinose and levulose were not affected by any one of these fifteen strains of meningococci.

Autolysis was apparent to a marked degree in stained cover slips from all these meningococcus cultures more than twenty hours old, but it was not possible to pick out any one strain as being definitely more or less resistant than another. Suspension of the fifteen cultures in salt solution kept at 4° C. showed no differences in the amount of autolysis present at the end of one, two and three days. The same was true of a series of suspensions kept at 37° C. Nor did the viability of the several strains differ appreciably on solid or in fluid media. One of the older strains invariably outlived all three of the posterior basic strains when kept in the thermostat (37° C.)

on sheep's-serum-agar with or without calcium carbonate. Twenty-four hours after having been heated in a salt solution suspension for thirty minutes at 65° C. the posterior basic cocci were as well preserved as were the older and the recent strains. Evidently the autolytic ferment (9) was the same in all; at least the effects were indistinguishable.

Agglutination.—It is much to be regretted that only one human serum was obtainable for agglutination tests. The early administration of the Flexner-Jobling antimeningitis serum made all other cases under observation valueless for this purpose. The single specimen of human serum came from a seven months' old infant on the second day of an attack of epidemic meningitis, before the injection of the anti-serum. As was to be expected, it gave only negative agglutination reactions with its own strain of meningococci and with the older strains under observation.

The Flexner-Jobling antimeningitis serum remained the only available means for making agglutination tests with these cultures, normal horse serum being used as controls. A recent non-carbolized specimen was obtained. With it, the three posterior basilar strains agglutinated in dilutions of one to twenty only, but five recent epidemic strains did not agglutinate even in so low a dilution. On the other hand, the two oldest strains used for the inoculation of the horses from the first were agglutinated by the serum in dilution as high as one to two hundred, as was the diplococcus isolated from the cerebro-spinal fluid from the case of the infant referred to above, the fluid having been withdrawn within thirty-six hours of the onset of the meningitis and before any serum had been injected. Two facts are brought out by these results: (1) that prolonged artificial cultivation was not the determining factor, and (2) that agglutination reactions with the Flexner-Jobling anti-serum do not serve to differentiate epidemic from posterior basilar strains of meningococci. That all strains of meningococci are not agglutinated in the same dilutions of a serum has been noted by Bruns and Hohn (10). The two non-meningococcus cultures were not agglutinated by this serum in dilutions of one to five, nor was the Staphylococcus aureus.

Phagocytosis.—The final test which the English observers applied to the differentiation of the two races of meningococci was that of

phagocytosis. In this, as in the agglutination tests, I was handicapped by the lack of human serum from patients and convalescents. The only specimen obtainable (vide supra) was taken on the second day of the disease, when its opsonic content was low. The antimeningitis horse serum remained as in the agglutination tests the only available means of study until the inoculation of monkeys was begun. I am aware, therefore, that these tests are not comparable with those upon which Houston based his conclusions. Nevertheless, they are interesting and suggestive. The results with the monkey's serum are given with the protocols of the cases.

Two parallel sets of experiments were made. In one Wright's technique for the study of opsonins, but with diluted serum according to the method of Klien (11), was used; in the other the technique devised by Neufeld and Hüne (12), also with diluted serum, and applied by Neufeld (13) to the standardization of antimeningitis serum was employed. It is based on the observation that immune serum favors phagocytosis because it contains bacteriotropic antibodies which are thermo-stable. The serum was used in dilutions as high as one to five thousand, and the results with both methods were fairly the same. Human leucocytes were used with the Wright technique, those from the peritoneal cavity of a guinea-pig for the Neufeld method. The diplococcal suspensions required were thick. The most striking result of both methods was the variability in the degree of phagocytosis displayed by the same corpuscles to different strains of meningococcus. A table on page 585 gives the results of the first series, made with the Neufeld method.

By the Neufeld method no actual counts are made, but the amount of phagocytosis is estimated and compared in the series of prepared slides, a strain of diplococcus which phagocytes readily, but not spontaneously, being used as a comparison control throughout all the experiments. The opsonic strength of the serum is measured by the dilution at which specific phagocytosis exceeds that which is spontaneous and present in the salt solution control. In Wright's technique two sets of tubes were prepared, one being incubated one and one-half hours, as in the Neufeld method, the other, fifteen minutes only. In the slides prepared from these tubes the engulfed cocci were counted. The results were similar to those given in the

One and One-half Hours Incubation.

	Strain.	Dilutions of anti-meningitis serum.						Salt sol. control.
		1–100	1–200	1–500	1–1000	1–2000	1–5000	
Old	720	+++	+++	++	+	+	±	±
	MSS.	+++	++	+	+	±	—	+
Recent	St. Luke's	+++	+++	+++	++	+	±	±
	Lebanon	+++	+++	++	+	+	±	±
	A. S.	+++	+++	++	+	+	±	±
	Simons	++	+	+	—	—	—	±
	Ward	++	+	+	—	—	—	±
	Fischer	++	+	—	—	—	—	±
	Johnston	++	+	—	—	—	—	±
	Price	++	+	—	—	—	—	±
	T. C.	+	+	—	—	—	—	±
	Silverman	+	+	—	—	—	—	±
Post-Basic	Moseley	++	+	+	—	—	—	±
	Cooper	++	+	—	—	—	—	±
	Windsor	++	+	—	—	—	—	±

above table, showing that one of the recent strains was most susceptible to phagocytosis, two other recent strains and an old strain but slightly less so, while one of the posterior basilar strains was as resistant as two recent cultures, and less so than the other two post-basic strains, which were comparable with five recent strains in susceptibility to phagocytosis in immune serum. Expressed in another way, the opsonic power of this serum was present in higher dilutions for some strains of meningococci than for others, exactly as the agglutinins were present in higher dilutions for some strains than for others. Definite group distinctions are not deducible from these results.

Comparing the results obtained in the specimens incubated fifteen minutes with those incubated one and a half hours, we find that phagocytosis occurs in the same serum dilutions in both, but the number of cocci taken up by the cells increases with the time of exposure allowed them. Thus:

Serum Dilutions.

	Wright's technique, 15′ incubation.							Wright's technique, 90′ incubation.						
Strains.	100	200	500	1,000	2,000	5,000	Salt sol.	100	200	500	1,000	2,000	5,000	Salt sol.
720	4.0	2.5	2.2	1.5	1.0	0.1	0.5	8.0	8.0	3.0	2.5	1.5	1.0	1.0
A.S.	1.9	1.5	1.2	1.0	0.8	0.2	0.2	4.0	3.2	2.5	1.5	1.2	0.3	0.4
Johnston	1.4	0.5	0.1	0.1	0.0	0.0	0.1	2.2	0.6	0.2	0.1	0.1	0.0	0.1
Moseley	2.0	1.4	0.6	0.3	0.2	0.1	0.2	2.9	1.7	1.0	0.4	0.2	0.2	0.5

When normal horse serum was used the control diplococcus ceased to be taken up by the leukocytes in a dilution greater than one in two hundred, while in the immune serum this point was not reached until a dilution of one to two thousand was employed. The phagocytic value of the immune serum for this diplococcus was, therefore, ten times the normal. The results with the other strains in normal horse serum were similar.[2]

The three posterior basilar cultures were inoculated into the spinal canal of monkeys and recovered from them by lumbar puncture. These cultures, after having been passed through an animal, did not agglutinate nor phagocyte to higher degrees than before.

The two non-meningococcus cultures showed no difference in the numbers of organisms taken up by human or guinea-pig leukocytes in the presence of salt solution as compared with antimeningitis horse serum, diluted or in full strength. The phagocytic index of the serum toward the two cultures was practically nil. It has not been possible to identify these two strains of cocci, obtained in pure culture from two different adult cases of apparently typical meningitis. Von Lingelsheim (14) encountered ten varieties of bacteria in his studies with material from patients suspected of having meningitis. These were meningococcus, Diplococcus crassus, staphylococcus, streptococcus, Streptococcus mucosus, Diplococcus mucosus, Diplococcus pharyngis flavus II, Micrococcus cinereus and Gram negative, plump bacilli. From the nine varieties of cocci enumerated, our strains can be differentiated without much difficulty. From Diplococcus intracellularis by its profuse growth on all media, lack of autolysis, fermentation of galactose, and its inability to affect maltose. The cocci under consideration are Gram negative, while Diplococcus crassus is still described by von Lingelsheim (14) as being Gram-doubtful; moreover, the latter ferments levulose, saccharose, lactose and maltose, in addition to dextrose and galactose. The Gram positive staphylococcus, streptococcus and Streptococcus mucosus need no further differentiation, and the morphology and

[2] Dr. Alice Taylor of London very kindly sent serum from two cases of posterior basilar meningitis. Unfortunately the quantity was very small, and the results of an opsonic test (Wright) with one of the posterior basilar strains was entirely negative when the serum reached us. There was not sufficient serum for study with other diplococci.

capsule of Diplococcus mucosus exclude it. Diplococcus pharyngis flavus II, as also I and III form a yellow pigment and ferment levulose instead of galactose. Micrococcus cinereus does not split any sugar, therein resembling Micrococcus catarrhalis, from which its smaller size and less profuse growth serve to differentiate it. That one or more of the diplococci described in the literature as found in air or water may be identical with these cocci found in two specimens of spinal fluid is possible, but the lack of data as to their staining power with Gram's method and their behavior toward the saccharids make it absolutely impossible to give an opinion on the subject. Half an agar culture twenty-four hours old inoculated into the peritoneal cavity of a medium-sized guinea-pig killed the animal in from twenty to thirty hours. At autopsy the point of inoculation was found to be hemorrhagic, the peritoneal cavity contained one or two centimeters of turbid fluid, the omentum was rolled up and hemorrhagic and the liver much congested. The edema about the pancreas, so characteristic of meningococcus infections in these animals, was absent. The pleura and lungs presented nothing abnormal. The cocci were recovered from the peritoneal exudate and from the heart's blood.

EXPERIMENTAL MENINGITIS.

The strains of diplococcus from posterior basilar cases were inoculated into the spinal canal of monkeys, in order to test the pathogenicity of these organisms in relation to meningitis, it having been shown that Diplococcus intracellularis of epidemic cerebro-spinal meningitis causes the disease experimentally in these animals (15).

Experiment I: Strain Windsor.—Two twenty-four hours old sheep-serum-agar slant cultures were suspended in 2 c.c. normal salt solution and inoculated into the lumbar spinal canal of a healthy monkey (*Macacus rhesus*). One hour later the animal seemed ill; in three hours he lay quietly on the floor of his cage, then became restless and evidently very ill. Eighteen hours after inoculation lumbar puncture was performed and a drop of turbid fluid obtained. This showed in films many polymorphonuclear leucocytes, some closely packed with Gram negative diplococci which were swollen and stained poorly. A pure culture of Diplococcus intracellularis was grown from this fluid. The animal was still ill and very weak but improved throughout the next twenty-four hours.

Lumbar puncture on the second day withdrew 1 c.c. of turbid fluid, containing many leucocytes, many intracellular diplococci and more extracellular ones than on the first day. No growth resulted from this fluid. On the following day the fluid was almost clear, and no cocci were found in the films. On the fourth day the fluid was quite clear and the monkey seemed well, though weak. Recovery was complete. The phagocytic index of this animal's serum was examined on five days with the following results. (Wright's method, 1½ hours incubation.)

20 hours after inoculation.			2 days.		3 days.		4 days.		9 days.	
Dilution.	(A. S.)		W.	A. S.	W.	A. S.	W.	A. S.	W.	A. S.
	Strain Windsor.	Recent strain.								
100	6.0	3.4	3.0	2.6	1.6	1.7	1.3	1.0	2.3	2.0
200	4.8	3.2	2.5	2.2	1.0	0.9	0.8	0.6	1.8	—
500	3.2	3.1	—	2.0	—	—	—	—	1.0	0.9
1,000	2.8	3.0	2.4	1.5	1.0	—	—	—	0.8	0.6
2,000	2.5	1.5	1.8	1.2	0.8	1.5	—	—	—	—
Control	0.8	0.7	0.8	0.7	0.6	0.4	0.7	0.5	0.6	0.5

It becomes apparent from these results that a recent (epidemic) strain of Diplococcus intracellularis was phagocyted in as high dilutions of this animal's serum as was the strain of posterior basilar meningitis used for inoculation. The comparatively high phagocytic index one day after the injection, the short duration of the rise and the rapid fall after the second day are also of interest.

Experiment II: Strain Cooper.—(*a*) Three sheep-serum-agar slants, twenty-four hours old, were suspended in 1.5 c.c. salt solution and injected into lumbar region of spinal canal of a monkey. Within one hour animal seemed ill. Died in seven hours. Autopsy—No purulent exudate visible. Vessels of the pia mater intensely engorged over entire brain and cord. Ventricles not dilated. Brain substance of normal color and consistence. Pia-arachnoid over entire cord and medulla, looks turbid. *Films: Cerebral cortex.*—No leucocytes and no diplococci found. *Medulla*—Many leucocytes, in many of which cocci are contained; cocci also outside the cells; autolysis of cocci marked. *Lumbar and dorsal cord*—Enormous numbers of diplococci, both extra- and intracellular; very large numbers of lucocytes. *Choroid plexus*—Few diplococci within and without leucocytes. *Cultures*—No growth from heart's blood or spleen. Pure growths from medulla, lateral ventricles, and spinal cord. *Microscopic examination of sections*—The pia arachnoid of the spinal cord contains an exudate, in some places quite heavy, consisting of pus cells and fibrin. The exudate covers and surrounds the nerve roots and the intervertebral ganglia. The latter seem not to have been invaded. In a few places, probably corresponding with the lumbar puncture wounds, the dura is infiltrated with pus cells. The number of diplococci in the exudate is very large and they are contained chiefly in the leucocytes. The cerebral meninges also contain leucocytes, fibrin, serum in excess

and extravascular red corpuscles. The blood vessels are widely distended. In the depth of the sulci the cellular exudate is replaced by an inflammatory edema with fibrin formation. Over the cerebellum the leucocytic exudate is heavy; within the cortex the perivascular lymph sheaths contain an excess of polynuclear leucocytes.

(*b*) Two sheep-serum-agar slants suspended in 1 c.c. of salt solution were inoculated into the lumbar region of the spinal canal. The animal became very sick within two hours and died in nineteen hours. *Autopsy*—In the cervical region of the spinal cord the pia-arachnoid was edematous and hemorrhagic. Over the rest of the brain and cord there was marked congestion, but no visible exudate. *Films and cultures* as in *Experiment* (*a*). *Microscopic examination of sections*—The exudate in the spinal meninges is less in quantity than in the preceding monkey and it is almost or entirely leucocytic in character. The exudate in the cerebral meninges is also less and consists of a mixture of polynuclear leucocytes and of red corpuscles. The number of diplococci in the leucocytes in the spinal exudate is large; in the cerebral, small.

(*c*) One solid culture, twenty-four hours old, was suspended in 1 c.c. of salt solution and injected into the lumbar region of the spinal cord of a monkey. The animal was weak and ill during the night. Lumbar puncture on the following morning withdrew very turbid fluid, showing many leucocytes crowded with diplococci; also some extracellular organisms. On the second morning 0.5 c.c. of turbid fluid was withdrawn, showing numerous leucocytes and large numbers of well staining diplococci. Cultures grew well and were pure. On the third day there was some improvement in the animal's condition. The fluid withdrawn by lumbar puncture was still turbid, containing leucocytes but very few diplococci. The following day no diplococci were found and on the fifth day the spinal fluid was clear. The monkey was then well. Examination of the blood showed that while before inoculation the meningococcus (strain Cooper) was not taken up by the leucocytes in dilutions exceeding 1 to 200, that on the first and second days after injection they were phagocyted in dilutions of 1 to 2,000, on the fourth day to 1 to 1,000, and on the seventh day not over 1 to 200. A recent strain (A.S.) again parallel with the one used for inoculation. In the slides prepared from the serum on the seventh day agglutination of the diplococci was apparent. In the earlier specimens it had been absent.

(*d*) Two-thirds of one serum-agar-culture was injected into the lumbar region of the spinal canal of a healthy monkey. No symptoms of illness developed, but on the day following the inoculation the spinal fluid was slightly turbid, containing leucocytes with intracellular diplococci in small numbers, and still fewer outside the cells. The cocci grew in pure cultures. The phagocytic index of the serum before the beginning of the experiment was nil in dilutions over 1 to 100. It was apparent in dilutions of 1 to 2,000 after twenty-two hours and had fallen to what it was before inoculation on the fifth day, the recent strain (A.S.) running a parallel curve with the one used for inoculation.

Experiment III: Strain Moseley.—One and a half sheep-serum-agar cultures, twenty-four hours old, were injected into the lumbar region of the spinal canal of a monkey. He became ill within two hours, and on the following

morning was very sick, lying on the floor in his cage. Two cubic centimeters of turbid fluid were withdrawn by lumbar puncture and in it many leucocytes containing well staining diplococci were found; a small number of autolyzed diplococci were also present. Cultures from this fluid grew well. On the following morning opisthotonos was marked; fibrillary twitchings and general convulsions were produced by disturbance. The pupils were irregularly contracted and reacted slowly. The animal was perfectly rigid unless disturbed by a touch or a loud noise, when a general convulsion came on. The cerebro-spinal fluid was very turbid, and contained many well-staining diplococci inside leucocytes and outside them as well. On the third morning the monkey was very quiet, lying on his side. No convulsions and no twitchings occurred. The eyes were less irregular. The fluid was still turbid, with many diplococci in the leucocytes. The condition changed but slightly during the following day; the animal made attempts to sit up but soon resumed the recumbent position. On the morning of the fifth day it died. The symptoms of the first days were very much like those of a human patient with meningitis and the clinical picture on the second day was most suggestive. The photograph illustrates the condition at this time.

At the autopsy, encephalitis of the right frontal lobe was found. This portion of the cerebrum was very bright red in color, raised above the level of the adjoining lobes, translucent in appearance, studded with punctate hemorrhages. The layer of gray matter was double the thickness of that in the opposite hemisphere. The remainder of the brain and cord showed a moderate congestion of the vessels of the pia mater. *Cultures* from the cerebrum, cerebellum and spinal cord showed no growth. The microscopic examination of the sections of the spinal cord and brain of this animal indicates that it had recovered almost completely from the meningeal infection and that it succumbed to the cerebral lesion. There are present small remains of the exudate in the meninges consisting of leucocytes and proliferated mononuclear cells.

The chief interest centers in the encephalitis which proved to be an infarction affecting a large part of the hemisphere. The tissue in the infarcted area is necrotic, the contained blood vessels are necrotic, and nuclear staining is generally absent. Around some of the blood vessels, now thrombosed, are collections of leucocytes also undergoing necrosis, and in various parts are punctiform hemorrhages and beginning calcium salt deposits. The membranes over the encephalitic focus are inflamed; they show an accumulation of leucocytes and pus cells. The larger branches of the veins are closed wholly or partially by thrombi containing fibrin and trans-

formed, fused, red corpuscles. In places a heavier exudate lies between the membranes and the encephalitic tissue of which the larger part is composed of proliferated pial cells of large size, which have taken up extravasated red corpuscles that are undergoing decolorization.

This case is of unusual interest because of its typical clinical picture, its prolonged course compared with the cases described by Dr. Flexner in previous experiments, and the exceedingly severe and unusual lesion found at autopsy. It should be added that hemorrhagic encephalitis as a complication of cerebro-spinal meningitis in human beings is of occasional occurrence (16).

CONCLUSIONS.

The study carried out and recorded in this paper did not lead to the finding of any reliable criteria of difference between strains of Diplococcus intracellularis obtained from typical cases of epidemic meningitis and several cultures obtained from cases of posterior basic meningitis.

The successful experiments made with monkeys show that the diplococcus obtained from cases of posterior basic meningitis is capable of setting up rapidly and acutely fatal forms of meningitis and in producing organic lesions of the cerebral tissues of great severity.

This study would, therefore, suggest that the antimeningitis serum should be as useful in cases of posterior basic meningitis so-called, as it has been in epidemic meningitis, especially if it were employed early in the disease.

BIBLIOGRAPHY.

1. Still, *Jour. of Path. and Bact.*, 1898, v, 147.
2. Holt, Diseases of Infancy and Childhood, 4th edition, New York, 1908.
3. Houston, *British Med. Jour.*, 1907, ii, 1414.
4. Houston and Rankin, *Idem*, 1908, ii, 1340.
5. Eve and Clements, *Idem*, 1908, ii, 912.
6. Ker, *Idem*, 1908, ii, 1340.
7. Arkwright, *Lancet*, 1908, ii, 475.
8. Wilson, *Lancet*, 1908, ii, 477.
9. Flexner, *Jour. of Exper. Med.*, 1907, ix, 105.
10. Bruns and Hohn, *Klin. Jahrbuch*, 1908, xviii, 285.

11. Klien, *Johns Hopkins Hospital Bull.*, 1907, xviii, 285.
12. Neufeld und Hüne, *Arb. a. d. k. Gsndhtsamte.*, 1907, xxv, 164.
13. Neufeld, *Med. Klin.*, 1908, iv, 1158.
14. von Lingelsheim, Arb. über die übertragbare Genickstarre in Preussen im Jahre 1905, Jena, 1906.
15. Flexner, *Jour. of Exper. Med.*, 1907, ix, 142.
16. Albrecht and Ghon, *Wien. klin. Woch.*, 1901, xiv, 988. Maschke, *Berl. klin. Woch.*, 1908, xlv, 1561.

EXPLANATION OF PLATE XXVI.

The photograph which was kindly made by Dr. Leaming shows the monkey of Experiment III in the condition of opisthotonos. The hair was cut away from the back of the neck in order to show the degree of curvature and retraction of the head.

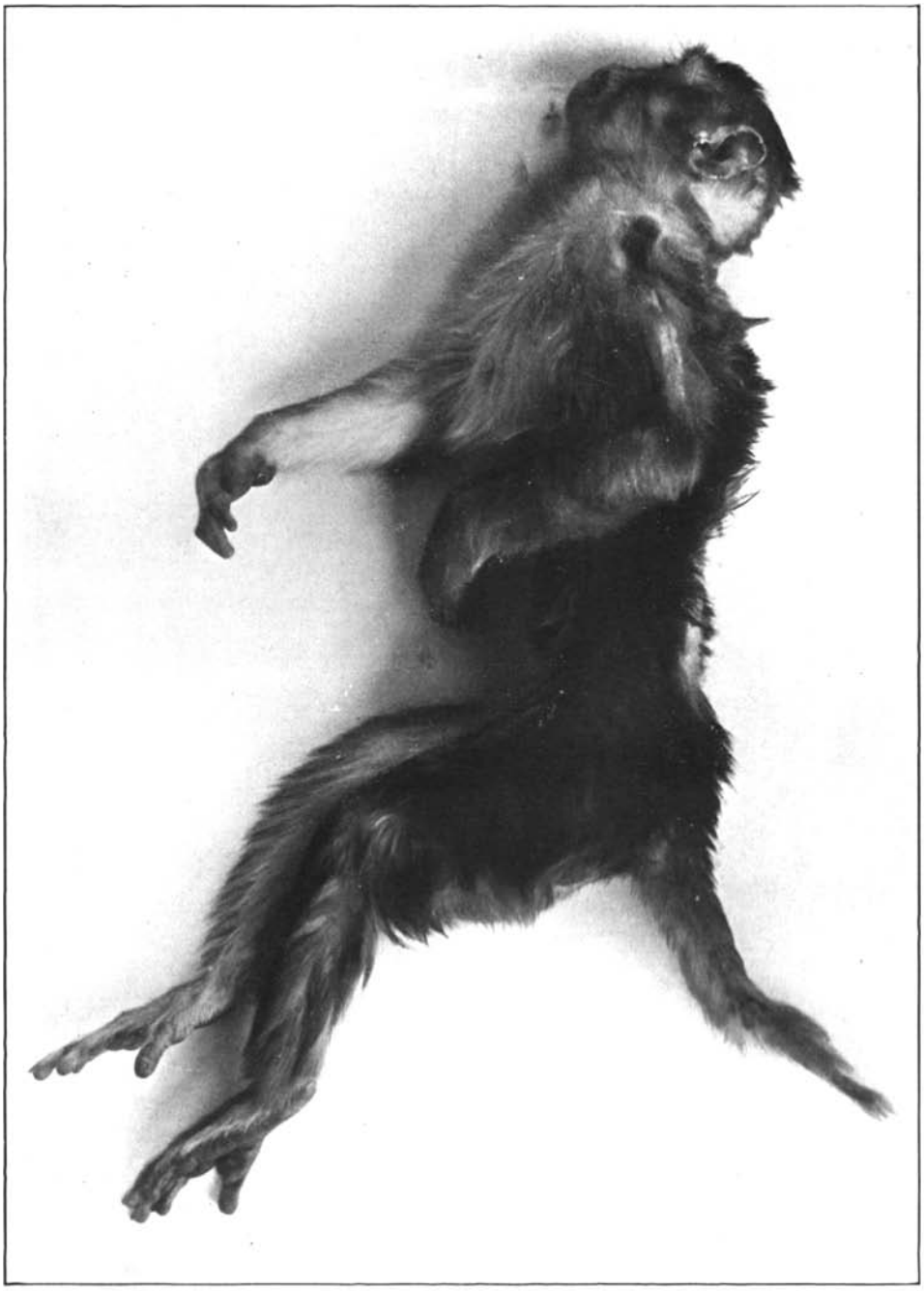

FIG. 1.