CONCERNING A SERUM-THERAPY FOR EXPERIMENTAL INFECTION WITH DIPLOCOCCUS INTRACELLULARIS.

By SIMON FLEXNER, M.D.

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

The high mortality of epidemic meningitis and the deplorable deformities caused by it demand that incessant effort be made to discover therapeutic measures which may mitigate the consequences of the disease. The epidemic through which the city of New York has recently passed, and the almost co-incident Silesian epidemic, have been scientifically fruitful in establishing more firmly the belief in the spread of the disease through immediate or mediate contact with the sick, and in tracing the common occurrence of *Diplococcus intracellularis* in the nasal and pharyngeal secretions of the sick, and the exceptional occurrence of the micro-organism in these secretions in the well who have been in contact with the sick. This mode of spread of the disease through directly and indirectly infected persons must come to exercise an important influence on the hygienic measures which will be enforced hereafter to limit the dissemination of the disease.[1]

[1] Goodwin and Sholly: The frequent occurrence of meningococci in the nasal cavities of meningitis patients and of those in direct contact with them. *Journal of Infectious Diseases,* 1906, Supplement No. 2, p. 21.

Flatten: Die übertragbarre Genickstarre im Regierungsbezirk Oppeln, im Jahre 1905 und ihre Bekämpfung. *Klinisches Jahrbuch,* 1906, xv, 211.

Schneider: Idem im Regierungsbezirk Breslau, *ibid.,* p. 300.

Rieger: Idem im Kreise Brieg, *ibid.,* p. 321.

Schmidt: Idem im Regierungsbezirk Leignitz, *ibid.,* p. 341.

Flügge: Die im hygienischen Institut der königl. Universität Breslau während der Genickstarre-epidemie im Jahre 1905 ausgefuhrten Untersuchungen, *ibid.,* p. 353.

v. Lingelsheim: Die bacteriolog. Arbeiten der kgl. hyg. Station zu Beuthen, etc., *ibid.,* p. 373.

Göppert: Zur Kenntnis der Meningitis cerebro-spinalis-Epidemica mit besonderer Berücksichtigung des Kindersalters, *ibid.,* p. 313.

A less certain advance has been made in the therapeutics of epidemic meningitis. The one therapeutic measure growing out of the study of the epidemics in America and Germany which offers any hope is an antiserum for the diplococcus. It is true that the experience of the past is not favorable to the hope of achieving remarkable success by the employment of antibacterial immune sera. All indications point to the pathological effects of the diplococcus as being caused by endotoxic constituents; and thus far, according to many investigators, these endotoxins have failed to yield, by methods of immunization, active antisera which have proved valuable in the treatment of infectious diseases. Opinion is, however, considerably divided on this subject;[2] and in the absence of more certain methods of reaching the desired goal tests of antisera for the diplococcus are certainly justified. These tests can in the preliminary stages be carried out on certain animals, since the course of infection in them with the diplococcus is now fairly well understood.[3]

The main question which would seem to be involved in the search for an active antiserum against meningitis is whether the quantity of antibody which can be produced will suffice to neutralize such a quantity of the poison of the diplococcus as to influence the result of the infection. In fact, the problem may not be so simple, or, indeed, so hopeless, as this proposition indicates. It is, of course, important that neutralization of the poison should if possible be secured, but the effect of the restraint of growth and multiplication of the diplococcus may, at some periods of the disease, be of greater significance than the neutralization of free endotoxin. Fortunately, many agents, some of them quite indifferent, are able to affect the power of multiplication in the body of the diplococcus. It has been shown, indeed, that serum in the fresh state and after

Meyer: Bericht über rhinolog. Beobachtung bei der Genickstarre-epidemie, 1905, *ibid.*, p. 427.

Westenhoeffer: Pathologisch-anatomische Ergebnisse der oberschlesischen Genickstarre-epidemie von 1905, *ibid.*, p. 447.

Jehle: Entstehung der Genickstarre-epidemie, Wien. klin. Woch., 1905, xix, 25.

[2] Besredka, *Annales l'Institut Pasteur,* 1906, xx, 4.

[3] Attention is directed to the two previous papers of this series published in this number of the Journal.

heating to 60° C., preserves the power to destroy in test-tubes large numbers of the diplococcus, and sterile fluid inflammatory exudates possess this power in even greater degree. An antiserum, therefore, even though it contain relatively small amounts of antibodies, as indicated by neutralization experiments, may be effective beyond this calculated value by restraining the multiplication of the diplococci, possibly by reducing outright their number, and by supporting the power of resistance normally at the disposal of the body.

The conditions are made theoretically less discouraging, perhaps, because the main pathological lesions are limited to the cavity of the cerebro-spinal axis. They can, therefore, be brought directly under the influence of the antisera by injecting the latter into the spinal canal. A large advantage is gained by this circumstance. It is, on the other hand, discouraging to reflect that in monkeys infected with the diplococcus, severe cortical lesions already exist at the end of ten or twelve hours. The question arises whether these deeper lesions tend to appear as early in the human infections. In respect to this question it should be stated that observation is against the occurrence of any such development of the diplococcus in the early stages of the human disease as is represented by the prodigious number of diplococci required to be injected into monkeys to produce the rapidly lethal effect with which the cortical lesions are associated. It is worthy of note that the more slowly developed lesions in the monkey remain more superficial, agreeing in this respect with the more common lesions present in fatal cases of the human infection. Hence, some encouragement may be taken from the power of the antiserum to influence favorably the course of meningitis in the monkey, although it has been injected as late as six hours after the inoculation.

If we are at all permitted to apply test-tube experiments to what may happen in the body, it would not be remarkable if the normal serum of animals, and perhaps of human beings, proved to be beneficial to a degree when brought into direct relation with the focus of development of the diplococcus. At first sight, judging from test-tube experiments, it would appear as if the exudate, called out by the inflammation, should suffice to destroy the diplococci; this manifestly does not happen in many cases. Indeed, it

is found that incubation outside the body will even increase the number of diplococci in the inflammatory fluid withdrawn from the spinal canal. It is safe to assume, therefore, that the exudate withdrawn has been exhausted of its power to destroy the diplococcus. It is quite possible that the introduction of fresh serum, of the same species of animal, may be helpful by bringing quickly into contact with the diplococci a quantity of actively destructive serum. The results of some of my experiments show that normal serum reduces appreciably the toxic effect of given doses of the diplococcus.

In experiments upon the monkey there is a definite low limit, beyond which it is not safe to go, for injection of fluids into the spinal canal. The species which I studied contain a small amount only of free spinal fluid. If one attempts to inject several cubic centimeters of fluid, symptoms of pressure may develop. In this respect the monkey is far less satisfactory to treat by intraspinal injections than are human beings.

I am far from having any conviction that cerebro-spinal meningitis in man can be influenced favorably by injections of immune sera into the spinal canal, or elsewhere in the body. The experiments to be described merely show that guinea-pigs and monkeys, in which the conditions of infection can be controlled, can be saved from the otherwise fatal effects of the diplococcus by the use of antisera, and to a less extent by the use of normal sera and other fluids. A preliminary note on this subject has already been made.[4] The protocols show that the experiments on immunity were begun during the spring of 1905. While the work was in progress two papers on the same subject appeared in Germany.[5] The use of monkeys for testing the antisera by direct injections into the infected and inflamed cerebro-spinal canal has not been made by the other investigators whose experimental studies were confined to guinea-pigs. Jochmann injected an antiserum prepared in the horse into the spinal canal of several human subjects of epidemic meningitis. The number of cases was too few to permit any conclusion of the value of the injections; but they showed that the injection of

[4] *Jour. of Amer. Med. Assoc.*, 1906, xlvii, 560.

[5] Kolle and Wassermann, *Deutsch. Med. Wochenschrift*, 1906, xxxii, 16. Jochmann, *ibid.*, p. 20.

horse's serum into the inflamed canal is not attended with special danger.

My first experiments on guinea-pigs were made with goat's sera. A female goat had been injected twice with cultures from several sources (12) of the diplococcus within a period of two weeks. The injections were made subcutaneously and gave rise to tumefaction which soon disappeared. After the second injection the goat aborted. The first bleeding was made two weeks after the second injection. As the table shows the serum at this time had little or no immunizing power. The experiment was designed to test the effect of an injection (1) previous to the injection of the diplococci, and (2) after the inoculation of the diplococci. The serum in the first instance was injected at 5 P. M. the day before, and in the second instance two hours after the inoculation with the diplococcus. All the injections, except bouillon in one pig, were intraperitoneal. The emulsion of the diplococcus was injected at 11 A. M., November 29, 1905.

Series No.	Weight in Grams	Protective Substance Injected.	Result.
144	189	"Immune" serum 0.03 c.c. 5 P.M. Nov. 28	Died 9 A.M. Nov. 30.
145	189	" " 0.04 c.c. " " "	Died 8 A.M. Dec. 2.
146	182	" " 0.05 c.c. " " "	Survived.
147	197	"Normal" " 0.05 c.c. " " "	Survived. Lost weight.
148	197	" " 0.10 c.c. " " "	" " "
149	220	Bouillon intraper. 1.0 c.c. " " "	" Weight 12/11 182 grams.
150	199	" subcut. 1.0 c.c. " " "	Died morning, Dec. 3.
151	190	"Immune" serum 1.0 c.c. 1 P.M. Nov. 29	Survived. Lost weight.
152	185	" " 2.0 c.c. " " "	" " "
153	192	"Normal" " 1.0 c.c. " " "	Died 5 P.M. Nov. 30.
154	190	" " 2.0 c.c. " " "	Died in night, Nov. 29.
155	190	Nothing: control	Died morning, Dec. 1.
156	210	Idem.	Died in night, Nov. 29.

The preceding experiment is of interest in showing the irregular action of the serum injections, and especially, as bringing out the fact that such an indifferent substance as bouillon can, if it is injected in advance of the diplococcus, impart power of successful resistance to the guinea-pig. It developed subsequently that the bouillon need not be injected into the peritoneal cavity to achieve this effect; one cubic centimeter injected subcutaneously in pigs of 225 to 250 gram weight, the day before inoculation, frequently

saves the animals. Using the goat serum of low protective value, the fact was determined that the protecting power of the serum has a definite limit (about 0.05 c.c. for pigs of 250 grams weight) under the most favorable conditions, namely, intraperitoneal injection the day before the infection. By using larger quantities (up to 1.0 c.c.) of serum simultaneously with the injection of the diplococcus the pigs can also be saved; subcutaneous injections of the serum (1.0 c.c.) in advance are effective. The smallest number of successful results is obtained in pigs in which the serum injections follow, after one or more hours, the infection. Several experiments were carried out in order to determine the fate of the diplococcus in the peritoneal cavity in the "protected" and "unprotected" animals. The plan was to withdraw fluid, after a suitable interval, by means of capillary tubes. The results were not wholly uniform, but in the main showed more rapid disappearance of the

Guinea-pigs weighing 230 to 260 grams, received 0.1 c.c. "immune" goat's serum intraperitoneally and subcutaneously, 1.0 c.c. bouillon intraperitoneally and subcutaneously, followed the next day by an emulsion of the diplococcus injected into the peritoneal cavity.

Series No.	How Injected.	Examination During Life.	Autopsy Findings in Peritoneum.
119	Goat's serum intraperitoneal; emulsion cocci at 11:30 A.M.	4 P.M. Many leucocytes containing a few diplococci; no extracellular diplococci.	Survived.
120	Goat's serum subcutaneous; emulsion cocci at 11:30 A.M.	4 P.M. Enormous number of diplococci; very few leucocytes overlaid with diplococci.	Died during the night. 2 c.c. fluid exudate; more pus present than usual; many leucocytes including diplococci.
121	Bouillon intraperitoneal; emulsion cocci at 11:30 A.M.	4 P.M. Diplococci more numerous than in 120; no leucocytes.	Died during the night. 3 c.c. fluid exudate; no leucocytes; many diplococci.
122	Bouillon subcutaneous; emulsion cocci at 11:30 A.M.	4 P.M. Many leucocytes; some intracellular diplococci; very few extracellular ones.	Survived.
125	Control; emulsion cocci at 11:30 A.M.	4 P.M. Enormous number of diplococci; almost no leucocytes.	Died during the night. Usual p. m. appearances; very large number of diplococci free; almost no leucocytes. The omentum contains some leucocytes including cocci.

diplococcus from the peritoneum of the treated as compared with the untreated pigs. Exudation of cells was more abundant in the former animals.

The injections of the goat were carried on subsequently, during and after lactation, until the end of February, 1906. Blood was withdrawn from time to time and the serum tested for its protective and therapeutic value. The results were never uniform, but the general indication was that the protective properties were increased measurably. The animal fell ill on March 15, 1906, and was bled to death. The serum obtained from this bleeding was used for many subsequent experiments. It was found by simultaneous injection to protect small pigs (190 to 200 grams) against a twelve hour fatal dose of the diplococcus in quantities varying from 0.5 to 0.01 c.c., but not regularly. On the whole, as the next experiment given in detail will tend to show, the serum had acquired protective and therapeutic properties.

An emulsion of the diplococcus was prepared of which 0.5 c.c. caused death in the control pigs in about eighteen hours. Three series of pigs were tested; the first received the serum and emulsion simultaneously, the second the serum two hours, and the third the serum four hours after the emulsion. One tenth c.c. of serum was injected in each pig. The emulsion was given intraperitoneally.

Series No.	Serum Injected.	Result.
102	Intraperitoneal; simultaneously.	Survived.
103	Subcutaneous; "	Died in 4 hours.
105	Subcutaneous; after 2 hours.	" 16 "
106	Intraperitoneal; " 2 "	Survived.
107	Subcutaneous; " 4 "	Died in 56 hours.
108	Intraperitoneal; " 4 "	Survived.

I shall supplement this table by another in which the goat serum is compared with an immune serum made in monkeys (vide infra). This experiment has, in this place, a two-fold value in showing that certain of the series of tests proceed in a regular manner, and the two immune sera have about equal protective value for guinea-pigs. The emulsions of the diplococcus killed six control pigs in from nine to fifteen hours. The injections were simultaneous.

The next table given shows that the sera of the goat and the monkey were both capable of protecting guinea-pigs from fatal doses of the diplococcus by simultaneous injection, and the previous

Series No.	Weight in Grams.	Serum Injected.	Result.
106	190	0.1 c.c. goat, interperitoneal.	Survived.
107	188	Idem.	"
108	180	0.1 c.c. monkey, intraperitoneal.	"
109	185	Idem.	"
110	185	0.1 c.c. goat, subcutaneous.	"
112	175	Idem.	Died in 12 hours.
114	195	0.1 c.c. monkey, subcutaneous.	Survived.
117	178	Idem.	"
175	210	0.1 c.c. goat serum, intraperitoneal.	"
178	205	0.2 c.c. " " "	"
181	170	0.5 c.c. " " "	"
188	185	1.0 c.c. " " "	"
182	205	0.1 c.c. monkey serum, intraperitoneal.	"
183	182	0.2 c.c. " " "	"
186	175	0.5 c.c. " " "	"
187	172	1.0 c.c. " " "	Died in 30 hours.

one indicates that the infected pigs can with less uniformity be rescued at the expiration of two to four hours after inoculation. The striking results of the last table are, however, relative merely, since it is found that certain normal goat sera possess a power that it almost equal to that of the immune serum in protecting by simultaneous inoculation. On reweighing, four days later, the surviving pigs of the last tabulation a marked loss of weight was found to have taken place in those which received the sera subcutaneously and not in those receiving it into the peritoneum.

It would be extremely hazardous to express, on the basis of my experimental results, the relative value, as protective agents against the diplococcal infection in guinea-pigs, of normal and immune goat sera. I was restricted to the use of a female goat for the purpose of immunization, which was, unfortunately, during a part of the process, lactating. This fact could account for the low relative value of the serum. I did not, however, find the injection of normal goat serum following the inoculation of the diplococcus, so effective as the injection of the immune serum in this way. But here again, I was, I should state, limited in this study to three specimens of normal serum. On the whole, the immune serum saved more guinea-pigs than the normal serum. I am not, however, convinced that in the instances of preceding or simultaneous injection of the serum, in respect to the inoculation, any very great stress can be laid upon the inherent antitoxic, or even bactericidal power of the specific serum, since, in the former case, the exudate

caused to be poured in the peritoneal cavity has itself, in vitro, the power of suppressing large numbers of diplococci, and, in the latter, the sera possess this power only in less degree than the exudate. If, however, it can be shown that the immune sera are more effective as therapeutic agents than normal sera, and by simultaneous and preceding injections, also, then it will have to be conceded that they contain some useful elements absent from the normal serum or present in it in less amount.

In the course of these experiments the effort was made to produce an immune serum in the horse. For this purpose, recent cultures were injected by Dr. Jobling, first subcutaneously and later intravenously. The doses had to be carefully chosen on account of the high sensibility of the horse to the diplococcus. The subcutaneous injections produced local swellings which frequently softened and discharged externally. After a period, intravenous injections of autolysates were begun; but the alarming symptoms which followed almost immediately the injection of a few cubic centimeters of this fluid caused a return to the use of cultures and autolysates by the subcutaneous method. After about five months of intermittent injection the serum was collected and compared for protective value on small guinea-pigs. I employed as controls several samples of normal horse serum kindly supplied by Dr. Park of the Board of Health. I shall give a few typical experiments from which it would appear that on the whole the serum of the treated horse has greater protective value than the serum of normal horses.

Two strains of the diplococcus of moderate virulence were employed in the tests. The suspensions were made in the morning, control pigs being inoculated with them immediately. About four hours later, the rectal temperatures indicated the probably fatal doses. It was subsequently found that 0.1 c.c. of the suspensions caused death in less than twelve hours and represented, for susceptible pigs of about two hundred grams, two or three fatal doses. The inoculation of the pigs for the experiments on immunity was made in the afternoon of the same day. This procedure is rendered necessary by the rapid deterioration of the suspensions in salt solution and Ringer's fluid which sometimes takes place at

the refrigerator temperature. The deterioration is associated with loss of viability and not with striking morphological changes in the diplococci, from which fact I have been led to believe that multiplication for a period of the inoculated diplococci may be a necessary condition of the lethal dose.

Three control guinea-pigs were injected at 10 A. M. with 0.1, 0.2, and 0.3 c.c. of a suspension of "Mt. Sinai culture" of the diplococcus. The pig receiving the largest dose died at 4.30 P. M., at which time the temperatures of the other pigs were 34.6 and 34.8° C., respectively. A series of guinea-pigs which had been injected into the peritoneum at 4 o'clock the previous day with normal and immune horse serum, were now inoculated into the abdominal cavity with the suspension of the diplococcus. The two remaining control pigs died at 11.30 P. M. the same day. All the pigs weighed between 165 and 190 grams.

NORMAL HORSE SERUM.

Series Number	Quantity of Serum Injected 24 Hours Previously.	Quantity of Suspension Injected.	Result.
68	0.01 c.c.	0.2 c.c.	Died midnight.
69	0.02 "	" "	Died 3 A.M.
72	0.05 "	" "	Died midnight.
75	0.07 "	" "	Survived.
78	0.10 "	" "	Died midnight.
82	0.30 "	" "	Died 1 A.M.

IMMUNE HORSE SERUM.

47	0.01 c.c.	0.2 c.c.	Died 3 A.M.
50	0.02 "	" "	Survived.
58	0.05 "	" "	Died 9 A.M.
59	0.07 "	" "	Survived.
61	0.10 "	" "	Died 3 A.M.
66	0.30 "	" "	Survived.

This result, while it is not remarkably good, does, on the whole indicate that the immune serum possesses greater power of protection than the normal serum. The condition of the experiment was fairly severe, since the dose of suspension represented about five fatal doses; but what should be especially noted is the varying sensitiveness of the inoculated pigs owing to which the tests give quite irregular results.

The next tabulation, which would seem to bring out the same fact, gives the result in a series of simultaneous inoculations. The

mixtures of suspension and serum were permitted to stand at the warm room temperature for half an hour before injection.[6]

NORMAL SERUM NO. 1.

Series Number.	Serum Injected.	Suspension Injected.	Result.
51	0.01 c.c.	0.1 c.c.	Died after 8 hours.
54	0.02 "	0.2 "	" " " "
46	0.10 "	0.5 "	Survived.
	NORMAL SERUM NO. 2.		
49	0.01 c.c.	0.1 c c.	Survived.
43	0.02 "	0.2 "	"
38	0.10 "	0.5 "	Died after 8 hours.
	IMMUNE SERUM.		
41	0.01 c.c.	0.1 c.c.	Survived.
37	0.01 "	0.1 "	"
34	0.02 "	0.2 "	"
44	0.02 "	0.2 "	"
42	0.10 "	0.5 "	"
39	0.10 "	0.5 "	"

Two other kinds of antisera were prepared and tested upon guinea-pigs. The first was made by injecting large rabbits with the peritoneal exudate of guinea-pigs which succumbed to intraperitoneal inoculation with the diplococcus. The exudates were toluolized, freed from cells, the toluol was removed by evaporation at a low temperature, and injected into rabbits. The antisera obtained from the rabbit exercised a degree of protection which can be expressed as follows: simultaneous injections of the diplococcus and the antiserum into the peritoneal cavity tend to give protection; separate simultaneous injections, diplococcus into the peritoneum and serum under the skin, are effective in proportion to the quantity injected. Small doses of the serum (0.1 c.c.) do not protect, but they delay the lethal effect; larger doses (0.5 c.c.) prevent the lethal effect. Dosage of rabbit serum proved to be important; too little failed to protect, and too much (0.5 c.c. into the peritoneal cavity) prejudices the result by reason of its own toxicity. The pigs which received the culture and this dose of serum died soon after the controls. The second was an homologous serum

[6] It is my intention to make a later report, when the immunization shall have gone farther, upon the effects of the serum of the immunized horse as regards its power of protection for guinea-pigs and for monkeys.

yielded by large guinea-pigs which were injected at intervals for several months with cultures of the diplococcus, the peritoneal exudate from other guinea-pigs, and the autolysate, which proved not to have greater protective value than normal guinea-pig's serum.

If the result of these attempts to produce an antiserum for *Diplococcus intracellularis,* which should be effective in the experimental infection with the diplococcus in the guinea-pig are reviewed, they cannot be held to be particularly promising. Under the severe conditions of the experiments, the most that can be said is that various agents—bouillon, normal sera, immune sera—can at times affect favorably the course of the experimental infection; the lead in respect to this influence being taken rather by the immune sera. It is to be recalled that in small guinea-pigs the experimental infection is rapidly fatal; that the prostration of the pigs develops very quickly, and the animals are often moribund in six to eight hours after inoculation. To influence very favorably and systematically a pathological process which progresses as rapidly as this, would be, perhaps, an achievement. As the experiments show, this can be done, although not wholly in this degree, by appropriate dosage of certain antisera.

The next experiments to be reported relate to monkeys inoculated with the diplococcus and treated with anti-diplococcus serum made in the monkey. Two large monkeys (*Macacus nemestrinus*) were immunized, for the production of an homologous serum, by injecting them subcutaneously with exudates from the peritoneal cavity of guinea-pigs succumbing to diplococcus infection, and with emulsions of the diplococcus. The injections were made at intervals for a period of nine months, after which the animals were bled to death, and the sera tested. Before giving the protocols of these experiments, the chief facts of a much earlier experiment to influence the course of meningitis in a monkey by means of the antiserum of the goat should be given.

December 2, 1905, each of two spider monkeys (*Atales ater*) was inoculated with one agar slant culture of the diplococcus. Fluid flowed from the needles before injection. 3 P. M., control sick; mate to this perhaps not quite so sick. Into the spinal canal of the latter 2 c.c. of goat's immune serum were injected. The immediate effect was alarming: animal relaxed, heart's action tumultuous,

respiration sighing. The symptoms passed off in 10 to 15 minutes. L. p. before injection of serum showed in each many diplococci; no leucocytes. 9 P. M., control in stupor from which he could be aroused; easily handled. Responded to introduction of needle for l. p., no fluid obtained; point of needle carried a small amount of exudate which showed many leucocytes and a small number of intracellular cocci. Rectal temperature 40° C. Serum treated, less stupid than control; could be handled alone. No fluid obtained by l. p. The small amount of exudate on needle showed leucocytes some of which contained diplococci. Rectal temperature 36° C. December 3, 6 A. M., both still very sick; 8 A. M., serum treated animal very much depressed; 10 A. M., serum treated animal dead; control brighter. The latter animal finally recovered. Autopsy on serum treated monkey. The spinal canal showed hæmorrhagic imbibition of the membranes of the lower third of the cord. The pia-arachnoid of the cord was infiltrated with gelatinous-œdematous exudate. Small hæmorrhages beset the pia-arachnoid of the cortex, and the meninges of the brain were infiltrated with an exudate similar to that of the spinal cord. The ventricles contained turbid fluid in small amount. Microscopical examination of sections of the brain and cord show the exudate to be moderate in amount, and to be thicker over the convexity than over the base of the brain. The hæmorrhages in the membranes of the spinal cord are large, and of the brain small. The most striking lesion is an acute endarteritis which effects all sized branches of the arteries of the brain and cord. Very few diplococci can be found.

The symptoms in this animal and the lesions found at autopsy were taken to indicate that goat serum cannot be injected with impunity into the inflamed spinal canal of monkeys.

Preliminary to the tests of the antisera prepared in the two monkeys, the lethal dose of the diplococcus had to be established. A recently isolated culture (Mt. Sinai 596), which had proven virulent for guinea-pigs, was chosen. Two control monkeys were inoculated (1) with 0.5 c.c., (2) with 1.0 c.c. of a suspension of the culture. Brief histories follow:

Control No. 1. *Macacus rhesus.* June 27, 1906, 11 A. M., given 0.5 c.c. suspension. 6 P. M., very sick; June 28, 9 A. M., brighter; 12 M., l. p. small amount of thin fluid obtained; animal very weak. June 29, 8 A. M., moribund; chloroformed at 1 P. M. Autopsy: The membranes of the cord and brain were pale; little visible exudate. C. s. show leucocytes in small numbers over cord and brain; and a few intracellular diplococci. Sections of the tissues confirm these findings; the inflammatory exudate is small in quantity; no marked lesions of the nervous tissue itself are to be seen.

Control No. 2. *Macacus rhesus.* June 28, 12 M., 1.0 c.c. of same emulsion injected (in refrigerator over night). 6 P. M., monkey sick; 9 P. M., very sick; June 29, 9 A. M., died. Autopsy: The meninges were injected and contained small hæmorrhages. The fluid in the pia of the cord was increased, and turbid. The meninges of the brain and the ventricles contained similar fluid in excess; the basal meninges the largest quantity. C. s. show leucocytes and

diplococci throughout the membranes. The spinal membranes contain the largest number of free diplococci. Cultures positive. Sections of the tissues show the lesions of an acute inflammation of the meninges and of the superficial portion of the cortex of the brain.

In making the serum tests, the larger dose of emulsion was always employed. Brief protocols of the experiments follow:

Experiment 1, July 3, 1906: Medium sized *Macacus rhesus* given at 11 A. M. 1.0 c.c. emulsion of diplococcus "596" together with 1.0 c.c. of monkey antiserum No. 1. Fluid flowed from the needle before injection. July 4, 9 A. M., monkey appeared normal; active; on perch. L. p. yielded several drops of faintly turbid fluid, which showed on cover-slips free cocci, and leucocytes, some of which contained diplococci in moderate numbers staining feebly. Cultures from the fluid negative. July 5, 11 A. M., monkey apparently well. Lumbar puncture gave a small amount of clear fluid, which contained neither leucocytes nor cocci. Cultures negative. January 10, 1907, the monkey remained well.

Experiment 2, July 5, 1906: Medium-sized *Macacus rhesus* given 1.0 c.c. suspension of culture "596" at 11:45 A. M. Fluid flowed from needle before injection. At 2 P. M., the monkey was sick. L. p. gave a small quantity of rather thick, opaque fluid. One cubic centimeter of immune serum (monkey No. 2) injected rapidly. Immediately at conclusion of injection pressure symptoms of an alarming character developed. The animal was prostrated for two hours, after which it slowly got better. At 6 P. M., it responded to disturbance. At 8 A. M., next day, the monkey was up and appeared well. It remained so subsequently (January 10, 1907). The cover-slips from the lumbar puncture showed many polymorphonuclear leucocytes and a small number of lymphocytes, many extracellular and a few intracellular diplococci.

Before the next experiment with the serum was made, the culture "596" was again tested for virulence.

A medium-sized *Macacus rhesus* was given 1.0 c.c. emulsion at 10:30 A. M., July 11. 10 P. M., animal sick; on bottom of cage, July 12, 9 A. M., l. p., small quantity of a thin white exudate obtained. C. s. many leucocytes and few diplococci. Died 12:30 P. M. Lived about thirty-eight hours. The lesions found at autopsy were characteristic; exudate existed over the cord and brain, the base of the latter being chiefly affected. The cortical vessels were injected. C. s. showed cocci in the inflammatory exudate of the cord and base of the brain, and fewer in the exudate of the convex meninges and the ventricles. Sections of the brain and cord show marked inflammatory lesions of the usual character. Diplococci are abundant in the exudate.

Experiment 3, July 13, 1906: Moderately large *Macacus rhesus* given at 11:15 A. M. 1.0 c.c. emulsion of Coccus "596." Although no fluid was obtained, the canal was certainly entered; 4 P. M., animal sick; 5 P. M., depressed, but still sat up. L. p. gave a small quantity of turbid fluid, but during the operation the animal collapsed; 1.0 c.c. of immune serum from monkey No. 2 injected slowly. The monkey was watched until 10 P. M.; no progress of the disease. July 14, 10 A. M., animal appeared well. No future symptoms developed up to January 10, 1907. The fluid obtained by l. p. showed many leucocytes with an occasional intracellular coccus, and extracellular cocci.

Experiment 4, July 27, 1906: At 8:45 A. M., a medium-sized *Macacus rhesus* was given one full agar slant culture "610," 18 hours old, suspended in salt solution, into spinal canal Fluid obtained before injection. At 11 A. M., 5 c.c. of antiserum from monkey No. 2, were injected into the skin of the thigh. Before the serum injection the animal was sick; it lay half down on the bottom of the cage. No immediate effect followed from the injection, but the symptoms did not progress. The next day the animal appeared well. L. p. was unsuccessful after the injection until the next day, at which time clear fluid, containing neither cocci nor leucocytes, was obtained. The control for this monkey was a much smaller and weaker monkey of the same species, which succumbed in eight hours.

I do not regard this experiment as entirely free from doubt, but as I was unable to obtain more monkeys at this time, the experiment could not be repeated then.

The series of experiments was up to this point successful, and they indicated that an antiserum to the diplococcus could prevent the development of severe symptoms from following the injection of the cultures of the diplococcus into the spinal canal, and cause arrest of the symptoms which had already set in. This series of tests is, of course, incomplete without corresponding experiments with normal serum with which they may be compared. The latter will follow. But before citing them I wish to record a failure under conditions which, in view of the foregoing results, was wholly unexpected.

Experiment 5, July 20, 1906: Medium-sized *Macacus rhesus* given at 12 M. 1.0 c.c. of emulsion of Coccus "596"; 3 P. M. 1.0 c.c. of antiserum injected intraspinally. This monkey was sick when given the serum and the symptoms progressed fairly rapidly. At 11 P. M. the animal was much prostrated and sat in the cage with head depressed. It died about 7 A. M., July 21. The autopsy showed a very unusual amount of exudation in the membranes of the cord. Cover-slip preparations showed a purulent exudate with large numbers of diplococci, all within polymorphonuclear leucocytes. The exudate in the meninges of the brain and cord showed the diplococci in the same condition of complete phagocytosis, although the number of leucocytes and diplococci was smaller. The fluid withdrawn by lumbar puncture before the serum injection contained large numbers of diplococci and very few leucocytes; only an occasional leucocyte contained diplococci. Cultures made at the autopsy from the meninges of the cord, medulla and cortex, and from the lateral ventricle were positive; those from the heart and bone marrow of the femur were negative. Examination of sections of the brain and spinal cord bear out the macroscopic appearances. The exudate is remarkably thick over the entire nervous system, and is composed exclusively, or nearly so, of leucocytes. The lateral ventricle is shown to have been dilated and to contain pus cells. The brain tissue has escaped invasion with leucocytes, and the intracortical blood vessels are free from thrombi and do not show the perivascular infiltration with pus cells which is commonly present. The spinal cord at the level of the injection shows a superficial invasion with leuco-

cytes, but the higher levels do not show this condition. The dura is, in the former locality, infiltrated with pus cells.

The control for this experiment was a smaller *rhesus* monkey. The injection was made July 19, at 2 P. M., and as symptoms had failed to develop from the small dose given, a second injection of one third culture was made at 6 P. M. At 9 P. M., animal sick; lying down, but when disturbed rose and looked distrait. July 20, 7:30 A. M., lay on bottom of cage, but on being disturbed rose and displayed marked nystagmus; resumed recumbent position. Depression increased during the morning; 12 M., died. The autopsy showed a general thin exudate in the meninges, marked chiefly over the medulla. Cover-slips showed a remarkably large number of cocci which, while chiefly within leucocytes, were abundant outside. In no other experiment was so large a number of microorganisms seen. A suspicious circumstance was found in the appearance of short chain-like groups of cocci, 4 to 6 members long. The cocci were Gram-negative and in size like the diplococcus. Attempt at cultivation failed.

In summarizing these experiments, it may be said that by the employment of an homologous anti-diplococcus serum several monkeys were saved from death due to experimental infection with *Diplococcus intracellularis*. The conditions of the experiments were such that the inoculated monkeys could, by simultaneous injection of serum and culture be prevented from developing severe symptoms, although the diplococci persisted for a period in the spinal canal, and by separate injection of the culture, and six hours later of the serum, the already severely ill monkey could, apparently, be saved from certain death. The experiment in which the serum was used successfully by subcutaneous injection cannot be interpreted without suitable repetitions.

The tests with normal monkey serum to serve as controls for the above experiments were made so as to bring out two sets of facts. In the first place, the value of simultaneous injection of normal monkey serum and a quantity of culture which would cause death in the control animal within twenty-four hours, was studied. And in the next place, the value of the normal serum was studied in monkeys in which the dose of the culture was on the border line —that is, of such a size that certain monkeys survived and others succumbed after a greater period than twenty-four hours. As regards the second series of tests, it may be said that it appeared as if the injection of a mixture of the normal serum and the culture led, in certain cases, to the survival of the monkey after a period of illness which was sometimes severe. The first symptoms ap-

peared very soon—within one or two hours—and grew in intensity for five or six hours, after which they receded. I am, therefore, inclined to attribute to normal serum employed in this way, a certain definite protective value.

The results in the first class of experiments were different. I found that the normal serum not only failed to save the inoculated monkeys, but the injection of the mixture of culture and serum might even hasten the fatal outcome. I wish to speak with some reservations on this point, for I was greatly hampered in this entire series of tests by great difficulty in obtaining at the time a suitable number of monkeys for the experiments. The study must, indeed, be carried much further before a final answer can be given as to the availability of a serum therapy for this experimental diplococcal infection in monkeys. There follow two brief protocols relating to the use of normal serum with a certainly fatal dose of the diplococcus. Two animals of the same species—*Macacus rhesus*—of equal size were employed.

Control Monkey. At 12 o'clock noon, one cubic centimeter of a suspension of the diplococcus fatal to guinea-pigs was injected into the spinal canal. Fluid flowed from the needle before injection. At 5 P. M., the monkey was sick; 9 P. M., lay on bottom of cage, but could be roused. Next morning comatose; died at 2 P. M. Survived the inoculation 26 hours. The lesions found at autopsy were characteristic. Cover-slips showed that the diplococci had to a large extent disappeared. The cultures were negative.

Serumized Monkey. At 12 o'clock noon, one cubic centimeter of the same emulsion used in the previous experiment (in refrigerator over night) mixed with one cubic centimeter of normal monkey serum and placed at 37° C. for half an hour, was injected into the spinal canal. Fluid was obtained before the injection. No immediate effects were noted following the injection. The monkey was already sick at 3:30 P. M., the symptoms increasing with great rapidity. Death took place at 8 A. M. the next morning. Survived 22 hours. The autopsy showed vivid injection of the meningeal vessels, and many small hæmorrhages over the cortical convolutions. Cover-slips showed a rich emigration of leucocytes and almost total disappearance of the diplococci. The cultures remained sterile.

. . . . . .

I have no desire to attempt to apply, at this time, the results given here of the experiments with the various sera on guinea-pigs and monkeys to human beings the subjects of cerebro-spinal meningitis. The experimental results with the antisera were not sufficiently constant and striking to make this mode of treatment of human cases of cerebro-spinal meningitis of very hopeful au-

gury. On the other hand, it is not improbable that more active antisera, using appropriate means of immunization, may be produced. Possibly, such antisera may prove of value in the treatment by direct spinal inoculation, possibly even by intravenous or subcutaneous injection, in this hopeless disease. The evident disadvantages to which the human patient must always be subject, as compared with the animals used for experiments, arise from the difficulty often encountered of estimating exactly the duration of the disease, and applying the remedy at its most favorable stage. On the other hand, the exceptional cases only in man run so rapid a course, attended with such profound symptoms of intoxication, as are regularly seen in the inoculation disease in animals. The slower and more measured progression of the infection in human beings may, indeed, be a favorable circumstance, provided the treatment can be applied in the early stages and before too severe structural changes have taken place in the nervous system. The fact that normal serum exercises a certain degree of protection might possibly be taken advantage of in cases of human infection. It would, of course, be practicable to obtain normal human serum for such injections. This subject is one which, in view of the gravity of cerebro-spinal meningitis in man and the absence of any efficient therapeutic measure against it, would seem to deserve consideration.