

THE EFFECTS OF SUBDURAL INJECTIONS OF LEUCOCYTES ON THE DEVELOPMENT AND COURSE OF EXPERIMENTAL TUBERCULOUS MENINGITIS.

SECOND PAPER.\*

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In the first paper<sup>1</sup> it was shown that the injection of virulent tubercle bacilli into the basal meninges of dogs causes a tuberculous meningitis characterized by a latent period of from five to thirty days, depending upon the dosage and virulence of the injected organism, followed by a period of increasing paralysis and incoordination, ending almost invariably in death. It was further shown that the injection of living canine leucocytes into the menigeal cavities of these dogs, during the latent period of the disease, causes a delay in the development of the paralytic symptoms and a prolongation of the life of the treated animals.

This delay was most marked in dogs inoculated with small doses of the less virulent strains of tubercle bacilli. A number of the treated dogs so inoculated had developed no paralytic symptoms by the time the first paper went to press (five months), while the untreated control dogs had all developed paralytic symptoms after an incubation period of about four weeks, from which half of the control dogs had already died. The clinical history of these dogs is reviewed in figure 1.

This result was sufficiently encouraging to warrant a more extensive study of the effects of leucocytic injections, particularly a study of the effects of injections under conditions applicable to human medicine. It evidently would be quite difficult with human beings

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<sup>1</sup> Manwaring, W. H., *Jour. Exper. Med.*, 1912, xv, 1.

*Effects of Subdural Injections of Leucocytes.*

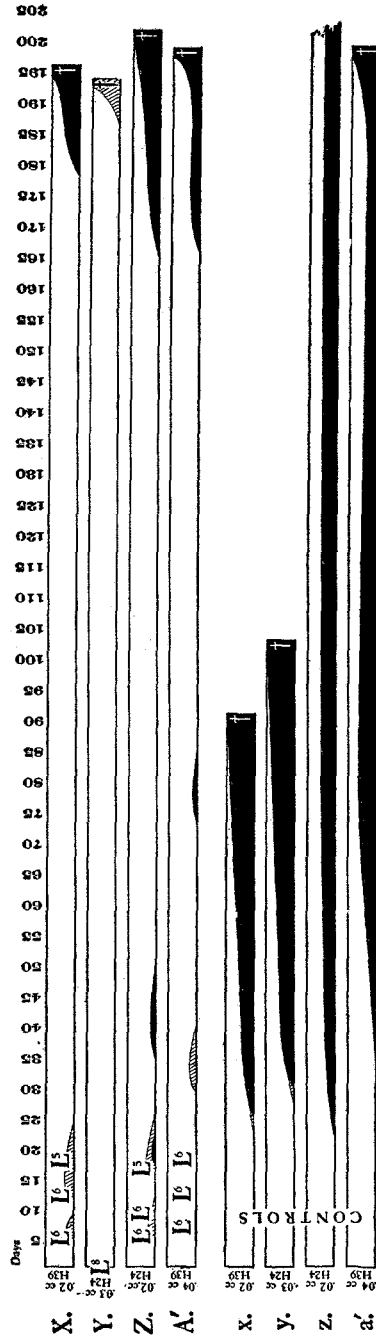


FIG. 1. EFFECTS OF SUBDURAL INJECTIONS OF HOMOLOGOUS LEUCOCYTES IN DOGS. Four dogs (X, Y, Z, A'), and controls (x, y, z, a'), were inoculated by the cranial-trephine method with small doses of tubercle bacilli, and given simultaneous or subsequent subdural injections with dog leucocytes (L). The dark areas show the development and the changes in the severity of the ataxic and paralytic symptoms. For further explanation of the figures see first paper.

The control dogs of this group all developed paralytic symptoms after an incubation period of from 25 to 35 days. The treated dogs were practically free from paralytic symptoms till the middle of the sixth month. One of the treated dogs (Y) died of distemper during the seventh month, and showed at autopsy no tuberculous lesion. The other treated dogs died toward the end of the seventh month, and showed at autopsy typical subdural tuberculosis.

to use homologous leucocytes as a therapeutic agent. Leucocytic therapy would be easy, however, if it were found possible to use leucocytes other than those of human origin. It was therefore desirable, as an initial step, to determine whether or not the same delay can be brought about in the development of the paralytic symptoms in inoculated dogs by the use of foreign leucocytes, and, if so, whether or not a similar delay can be produced by foreign leucocytes in animals whose susceptibility to tuberculosis more nearly approaches that of human beings. In the present paper an attempt has been made to answer these two questions.

Considerable work has already been done on the possible therapeutic uses of foreign leucocytes. Of particular interest in the present connection are the studies of Pettersson and his co-workers, who have tested the prophylactic and curative effects of subcutaneous and intraperitoneal injections of foreign leucocytes in a number of experimental infections, including experimental tuberculosis in the guinea pig.<sup>2</sup> Equally suggestive is the work of Hiss and his colleagues, who have studied the effects of injecting foreign leucocytic extracts.<sup>3</sup>

#### EXPERIMENTS ON DOGS.

*Toxicity of Foreign Leucocytes.*—The use of foreign leucocytes in meningeal infections in dogs is limited by their great toxicity when injected into the subdural spaces of these animals. Rabbit leucocytes,<sup>4</sup> injected in doses of from 0.7 to one cubic centimeter, apparently always cause death.<sup>5</sup> The dogs recover normally from the etherizations necessary for the injections, and show immediately after recovery no unusual symptoms. About two hours later there is beginning incoördination, rigidity, and respiratory distress, increasing to collapse by the fourth hour, and death by the sixth hour. An occasional dog survives till the twenty-fourth hour.

<sup>2</sup> Kling, C., *Ztschr. f. Immunitätsforsch., Orig.*, 1910, vii, 1.

<sup>3</sup> Hiss, P. H., Jr., and Zinsser, H., *Jour. Med. Research*, 1908, xiv, 321; 1909, xv, 245.

<sup>4</sup> For the method used in obtaining aseptic rabbit and horse leucocytes, see Manwaring, W. H., *Jour. Exper. Med.*, 1912, xvi, 250, 253.

<sup>5</sup> The inoculations and treatments of the dogs were made under morphin-ether anesthesia.

The cause of death in these injected dogs has not yet been fully determined. Rabbit leucocytes, killed by repeated freezing and thawing, cause no serious symptoms. The same is true of an aqueous extract of rabbit leucocytes. Autopsies on dogs dying after the injection of rabbit leucocytes show a hemorrhagic condition of the subdural tissues, suggesting the action of a hemorrhagin.

Horse leucocytes are less toxic for dogs. From one to three cubic centimeters can be injected into the cerebral meninges with comparatively few toxic symptoms. Approximately a quarter of the dogs so injected die within a few hours after the injection; but this mortality is not greater than the mortality previously noted after the injection of dog leucocytes. Horse leucocytes were therefore selected as the therapeutic agent to be tested with dogs.

The use of horse leucocytes, however, is limited by their increased toxicity on a second injection, or when injected into meninges the seat of a pathological lesion. The second injection of horse leucocytes into normal meninges, from five to seven days after the first injection, is apparently invariably fatal. A fatal result apparently always follows the injection of horse leucocytes from seven to ten days after the inoculation of the meninges with tubercle bacilli.

This limits the study of the effects of local injections of horse leucocytes in meningeal tuberculosis in dogs to a determination of the effects of a single injection of the leucocytes made simultaneously with the initial inoculation with tubercle bacilli.

*Effects of Simultaneous Inoculation with Horse Leucocytes and Tubercle Bacilli.*—The injection of from one to three cubic centimeters of horse leucocytes into the basal meninges of dogs, simultaneously with the inoculation of the meninges with minimal doses of tubercle bacilli, is apparently without effect upon the development and course of the subsequent tuberculous meningitis in the majority of the treated animals (figure 2). There is, however, in a certain number of the treated animals, a slight prolongation of the latent period, and in one of them (E) the subsequent paralytic symptoms have been unusually mild.

These effects, however, are very slight when compared with the remarkable prolongation of the latent period previously observed

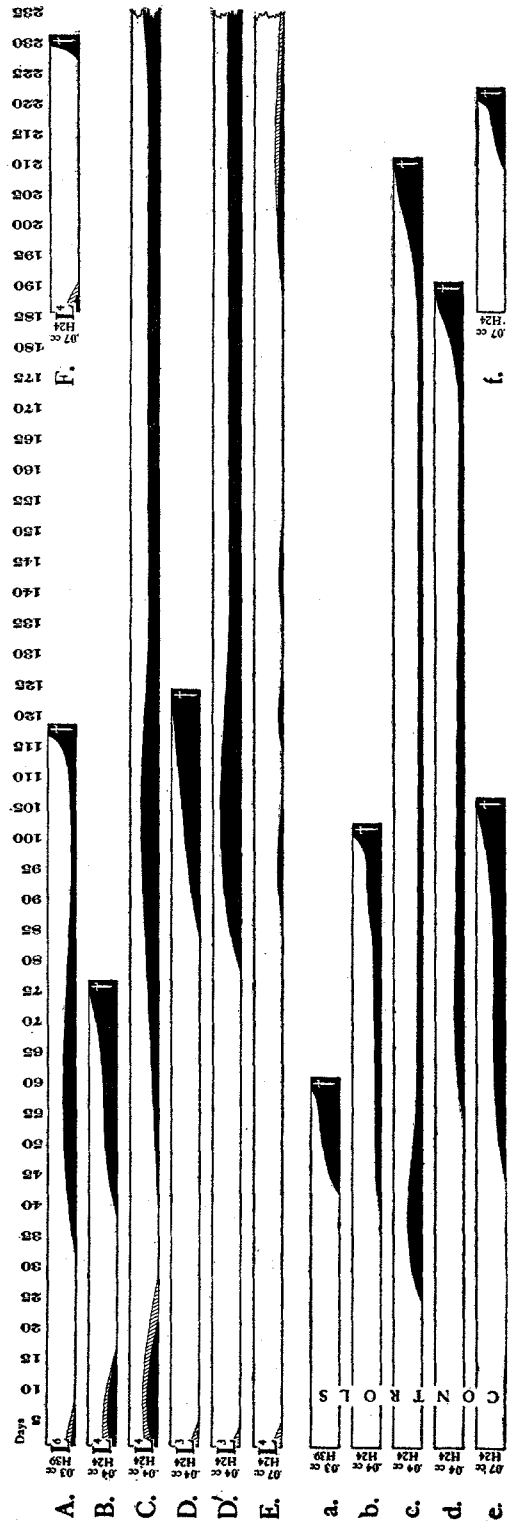


FIG. 2. SIMULTANEOUS INJECTIONS OF HORSE LEUCOCYTES AND TUBERCLE BACILLI IN DOGS. Seven dogs (A, B, C, D, D', E, F), with controls (a, b, c, d, e, f), were inoculated with small doses of tubercle bacilli, and given simultaneous injections of horse leucocytes (L). The cranial-trephine method was used in this group.

Three of the treated dogs (A, B, C) showed marked toxic and paralytic effects immediately after the injections, the symptoms lasting for from one to three weeks. These dogs all developed paralytic symptoms after an incubation period practically the same as that in the untreated controls (a, b, c). The other treated dogs (D, D', E, F) showed fewer immediate symptoms and all gave a distinct prolongation of the latent period. In one of the dogs (E) the subsequent paralytic symptoms have been unusually mild.

after the injection of dog leucocytes (figure 1). The average length of the latent period in the untreated control dogs of this group was thirty-eight days, that of the treated dogs, fifty-seven days, giving an average prolongation of but nineteen days, as compared with the five or six months' prolongation obtained with dog leucocytes.

The various samples of horse leucocytes used in these injections differed in their toxic effects. While one sample caused stupor and even a paralysis, lasting for from one to three weeks, a second sample, obtained under apparently identical conditions, caused only a slight quietness of the injected animal, which disappeared within a few days. It was noted that the prolongation of the latent period was produced only by the less toxic samples of the leucocytes.

The autopsy findings in the dogs dying after these inoculations and treatments are similar to those described in the previous paper. No constant differences have yet been found between the anatomical pictures in the treated and untreated dogs.

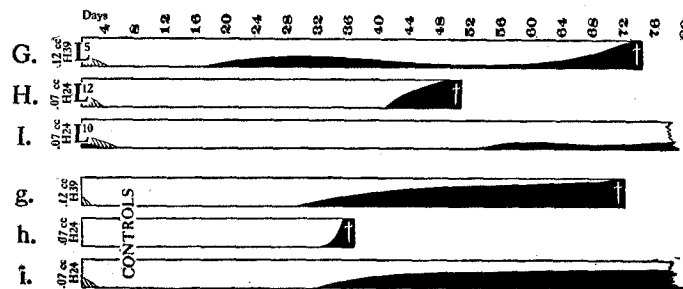


FIG. 3. SIMULTANEOUS INJECTIONS OF HORSE LEUCOCYTES AND TUBERCLE BACILLI IN DOGS. Three dogs (G, H, I), with controls (g, h, i), were inoculated and treated by the method of lumbar puncture. One of the treated dogs (I) has shown a slight prolongation of the latent period, and exceptionally mild subsequent paralytic symptoms.

Following an observation with monkeys, described in the latter part of this paper, an attempt was made to vary the technique of the inoculations by injecting the bacilli and leucocytes into the spinal meninges rather than into the basal meninges of the brain. Spinal inoculation can be accomplished in dogs by a modification of the ordinary method of lumbar puncture. The fore quarters of the

dog are elevated till its back is nearly vertical, and a long, slender, hypodermic needle is then passed obliquely through the lumbar enlargement of the cord, till the escape of cerebrospinal fluid shows that the needle has passed into the subdural space. In the subsequent clinical picture allowance has to be made for the occasional paralysis resulting from the necessary injury to the cord substance.

A small group of dogs in which the inoculations were made by the method of lumbar puncture is shown in figure 3. While the average length of the latent period is the same in the treated and untreated dogs of this group, one of the treated animals (I) has shown a slight prolongation of the latent period and a distinct lessening of the severity of the subsequent paralytic symptoms. This result, however, is no more striking than the result obtained above, by the routine method of cranial inoculation.

The results from the study of these two groups of dogs, however, were considered sufficiently encouraging to warrant an extension of the problem to other animals in which the study was not so greatly limited by the toxicity of the injected leucocytes. The work was, therefore, repeated on monkeys.

#### EXPERIMENTS ON MONKEYS.

*Toxicity of Foreign Leucocytes.*—Foreign leucocytes are apparently much less toxic for monkeys. This possibly depends upon the relatively greater capacity of the subdural space in monkeys, which tends to prevent any great or sudden increase in cerebrospinal pressure as a result of a local inflammatory reaction.

Rabbit leucocytes can be injected into the subdural space of monkeys in doses as high as one cubic centimeter, without the production of fatal symptoms. This injection can be made either into the spinal meninges by the ordinary method of lumbar puncture, or into the cerebral meninges by the cranial-trephine method previously described for dogs. The monkeys are usually somewhat quieter than normal for a day or two after the injection, and there are occasionally cranial tenderness and transient paralyses; but there is not the collapse and respiratory distress observed in dogs. Horse leucocytes are apparently even less toxic, and can be injected in doses of two cubic centimeters.

Monkeys, also, can be safely reinjected with foreign leucocytes after an interval of from five to seven days, without the production of unusually severe symptoms. If reinjected sooner than that, as after an interval of but three days, the monkeys often die with the symptoms of collapse and respiratory distress described above. No unusually severe symptoms are observed in monkeys if the leucocytes are injected into meninges previously inoculated with tubercle bacilli.

*Inoculation of the Meningeal Cavity with Tubercle Bacilli.*—In monkeys, inoculation of the meningeal cavity can be readily made by the method of lumbar puncture. The tuberculous meningitis produced by such inoculations gives a clinical picture similar to that observed in dogs. Following the inoculation there is a latent or incubation period of several weeks, during which time no characteristic symptoms are observed. In the later stages of this period the animal may be somewhat quieter than normal and may show a greater tendency to chatter or cry out when disturbed. This latent period is followed by a period of increasing paralysis and incoördination, ending in collapse and death. The paralysis after lumbar inoculation invariably begins in the hind legs.

The beginning of the paralysis is usually less easily detected in monkeys than in dogs, due to their great activity. In monkeys, as in dogs, the disease is apparently invariably fatal.

The autopsies on monkeys dying as a result of lumbar inoculation usually show a layer of diffuse tuberculous tissue filling the subdural space, with more or less necrosis and infiltration of the superficial portions of the spinal cord and nerve trunks. The most important finding for our present purpose, however, is the almost constant presence of extradural tuberculosis in these animals. Extending in some cases the entire length of the spinal cord, but generally confined to a few centimeters above and below the site of inoculation, are numerous miliary and conglomerate tubercles, which fill and often entirely replace the extradural spinal tissue. These extradural tubercles are usually not adherent to the dura, and evidently arise from a leakage of the infectious agent into the extradural tissue spaces through the dural puncture made at the time of the inoculation.



Since these extradural lesions cannot be reached by a therapeutic agent injected into the meningeal cavity, and since their presence alone would be sufficient to cause paralytic and ataxic symptoms, the constant presence of extradural tuberculosis in monkeys inoculated by the method of lumbar puncture renders inoculation and treatment by this method impracticable in the present investigation. The later inoculations and treatments herein reported were therefore made through a permanent wax-trephine opening in the skull, as described for dogs in the preceding paper.

The clinical picture in monkeys inoculated by the cranial method<sup>6</sup> is similar to that after inoculation by lumbar puncture. The initial paralyzes are possibly less liable to be confined to the hind quarters, and occasionally death occurs even without the presence of a definite paralysis, there being only an increased quietness and stupor, with a slight spasticity of the neck muscles.

For our present purpose the most important autopsy finding in monkeys inoculated by the cranial method is the complete absence of extradural tuberculosis. The extradural tissues have been found to be free from tuberculosis in all monkeys inoculated by this method.

The distribution of the tuberculous lesion within the meningeal cavity, however, differs somewhat in these animals from the distribution after lumbar inoculation. Scattered over the surface of the brain are numerous tubercular foci, usually small, well marked tumor masses. The main lesion, however, in these monkeys, as in monkeys inoculated by lumbar puncture, is situated in and around the lower half of the spinal cord. Here the subdural space is usually completely filled with diffuse and conglomerate tuberculous tissue. It would appear as though most of the injected tubercle bacilli had gravitated to the lower levels of the cord, as a result of the constant upright position of the animals.

*Effects of Leucocytic Injections.*—In the earlier experiments a number of monkeys were inoculated and treated by the simpler method of lumbar puncture. Those of the earlier monkeys that received simultaneous or subsequent injections of horse leucocytes are shown in figure 4. All the treated monkeys of this group devel-

<sup>6</sup>The inoculations were made under ether anesthesia.

oped paralytic symptoms occasionally even after a shorter incubation period than the untreated controls. The average length of the incubation period in the treated monkeys of this group was shorter than that of the untreated controls, as was also the average duration of life.

This experiment, however, is not thoroughly conclusive as to the negative value of the injection of horse leucocytes, since there was with each member of this group a loss from the meningeal cavity of

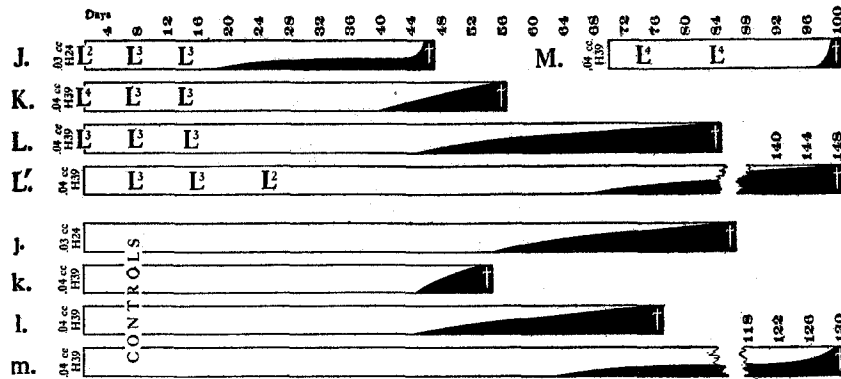


FIG. 4. EFFECTS OF HORSE LEUCOCYTES IN MONKEYS. Five monkeys (J, K, L, L', M), with controls (j, k, l, m), were inoculated by the method of lumbar puncture, and given simultaneous and subsequent injections of horse leucocytes. The number of cubic centimeters of a 30 per cent. suspension of leucocytes injected are shown by the small exponents.

Two of the treated monkeys (J, M) showed a distinct shortening of the latent period, and one monkey (L') a possible prolongation of that period. Autopsies showed extradural tuberculosis in all the members of this group.

part of the injected dose of tubercle bacilli, with the setting up of extradural tuberculosis.

A second group of monkeys, also inoculated by the method of lumbar puncture, was given simultaneous and subsequent injections with rabbit leucocytes (figure 5). In one of the treated monkeys of this group (O) there was a considerable prolongation of the latent period, and a second monkey (P), killed at the end of seven months, during which time it had shown no paralytic symptoms, was found on autopsy to be apparently free from tuberculosis. Whether this means an actual prevention of the development of tuberculosis

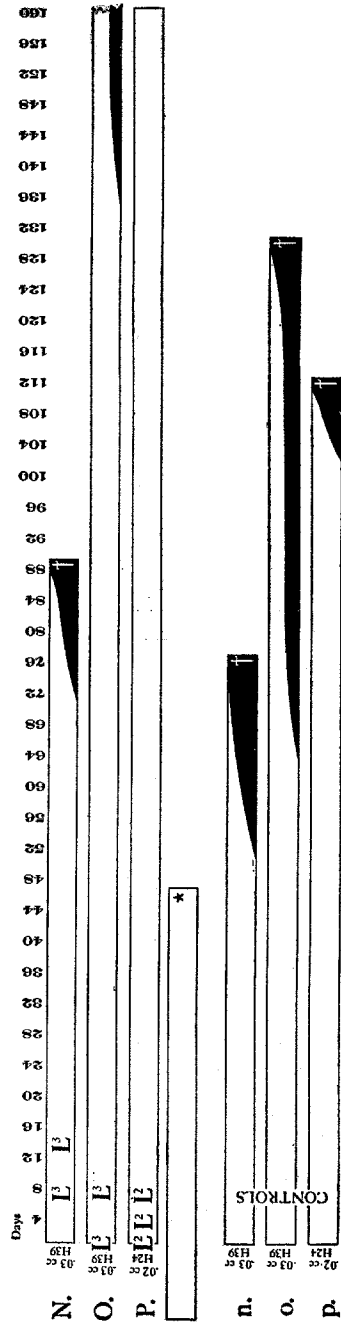


FIG. 5. EFFECTS OF RABBIT LEUCOCYTES IN MONKEYS. Three monkeys (N, O, P), with controls (n, o, p), were inoculated by the method of lumbar puncture, and given simultaneous or subsequent injections with rabbit leucocytes. Two of the treated monkeys (O, P) showed a marked prolongation of the latent period, and one of them (P), killed at the end of seven months (\*), was found to be free from tuberculosis. The interpretation of the result in this group is complicated by the presence of ex radural tuberculosis.

in this animal as a result of the injections of rabbit leucocytes, or merely that the monkey selected possessed an unusual resistance to tuberculosis, can be determined only by a repetition and extension of this work.

These earlier observations with rabbit leucocytes, uncertain as their interpretation may be, were sufficiently suggestive to warrant a study of the effects of rabbit leucocytes when injected under conditions that eliminate the confusion from extradural tuberculosis, and rule out the possible experimental error from individual variations in resistance. A study of the effects of injections of rabbit leucocytes by the cranial method was, therefore, begun.

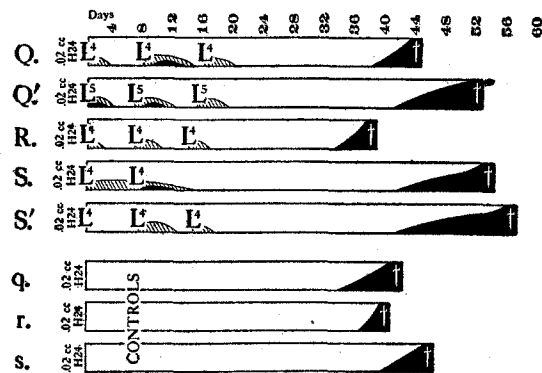


FIG. 6. EFFECTS OF RABBIT LEUCOCYTES IN MONKEYS. Five monkeys (Q, Q', R, S, S'), with controls (q, r, s), were inoculated and treated by the cranial-trephine method. There was practically no difference observed between the treated and untreated monkeys of this group. This negative result, however, is not necessarily final, due to the possibility of the monkeys of this group having been given an overdose of the infectious agent. All of the monkeys of this group were free from extradural lesions.

A group of monkeys, inoculated and treated by this method, is shown in figure 6. The five monkeys of this group receiving simultaneous and subsequent injections with rabbit leucocytes, all developed paralytic symptoms after practically the same incubation period as the three untreated controls. All the monkeys of this group died at practically the same time. The autopsies showed subdural tuberculosis in all the members of the group, the distribution of the lesions being practically the same in the treated and untreated animals.

In so far, however, as the dosage of the tubercle bacilli injected in this group was based upon the earlier experience with lumbar inoculations, in which there was a considerable loss of the infecting agents from the meningeal cavity, it is conceivable that all the monkeys of the group received an overdose of the infectious agent. It will be recalled that in the first paper the dogs receiving an overdose of tubercle bacilli showed no prolongation of the latent period on treatment with dog leucocytes. It was only when the dosage was reduced that a prolongation was observed. The probability, however, is against there having been such an overdose, since there was a long latent period with all the members of this group. The work with monkeys is being continued.

#### SUMMARY.

Rabbit leucocytes, injected into the basal meninges of dogs, in doses from 0.7 to one cubic centimeter apparently invariably cause death. Horse leucocytes, injected in the same amounts, cause death in about 25 per cent. of the dogs.

The injection of foreign leucocytes into the meninges of monkeys causes few if any symptoms.

The injection of from one to three cubic centimeters of horse leucocytes into the meningeal cavities of dogs, simultaneously with the inoculation of the meninges with tubercle bacilli, causes a slight delay in the development of the paralytic symptoms in about half the treated animals. This delay, however, is very slight when compared with the remarkable prolongation of the latent period previously observed after treatment with dog leucocytes.

The injection of foreign leucocytes into the meningeal cavities of monkeys has thus far given almost uniformly negative results. In one small group of monkeys, however, inoculated by the method of lumbar puncture, the injection of rabbit leucocytes has been associated with a prolongation of the latent period in one of the treated monkeys, and with a complete prevention of the subsequent tuberculosis in a second monkey.