

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

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The therapeutic control of tuberculosis has still to be accomplished. Of the specific therapeutic agents thus far proposed, tuberculin alone seems to exercise a favorable influence over certain forms of the disease. The results of sanatorium and climatic treatment, however, have amply demonstrated that there are in the body natural curative powers against this disease. While the mechanism of the natural cure has not as yet been fully worked out, it would nevertheless appear that phagocytic cells play an important part in it. It therefore becomes of interest to determine the extent of the action of the body phagocytes under controllable experimental conditions. The leucocytes are the phagocytic cells most readily obtained for experimental purposes.

Kling¹ has shown that leucocytes, injected simultaneously with tubercle bacilli, have the power of decreasing the pathogenic properties of these microorganisms. To Opie,² however, belongs the credit of a painstaking and systematic study of the effects of injected leucocytes upon a tuberculous lesion already developed. Opie inoculated the pleural cavity of dogs with tubercle bacilli, and found that leucocytes, brought into contact with the tuberculous tissue thus produced, made very striking alterations in it. The flat plaques of tuberculous tissue upon the pleura of the control ani-

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¹ Kling, Untersuchungen über die bakterientötenden Eigenschaften der weissen Blutkörperchen, *Ztschr. f. Immunitätsforsch.*, 1910, vii, 1.

² Opie, The Effect of Injected Leucocytes upon the Development of a Tuberculous Lesion, *Jour. Exper. Med.*, 1908, x, 419.

mals, were almost absent in the treated dogs. The pleura was sometimes returned even to the delicate translucent appearance observed in the normal animal. The masses of tuberculous tissue in the mediastinum and in the sub-pericardial membranes were also smaller after the injections, and there were fewer metastatic lesions in the extrathoracic organs.

The work embodied in this paper is part of the study of the experimental therapeutics of meningeal infections,³ which is being carried out at the Rockefeller Institute. The cerebrospinal membranes differ from the true serous membranes in that they are almost impervious to the blood proteids, and normally receive little or no protective substance from this source, and few, if any, leucocytes. The meningeal cavity is, therefore, always in an inferior condition of defense against infection. The method of lumbar puncture, however, provides a ready means of bringing healing substances into direct contact with pathological processes existing within these membranes, and has made possible a study of the local therapeutics of diseases of this surface. The experiments were made on dogs and consisted, first, in producing a tuberculous infection of the meninges, and, second, in attempting to control the lesion by the local injection of dog leucocytes.

METHOD.

Inoculation of the subdural cavity in the dog is attended with some difficulty. The spinal cord of this animal extends throughout the entire spinal canal, making the introduction of a needle, as for lumbar puncture, impracticable. Injury of the cord follows such introduction, and the resulting paralyses and other secondary effects rule out this method of procedure. A trephine opening in the cranium was therefore substituted.⁴

In devising the method, it was borne in mind, not only that inoculation of tubercle bacilli was to be provided for by this means, but also that provision must be made for the subsequent injection of

³ Flexner, *The Biological Basis of Specific Therapy*, *Boston Med. and Surg. Jour.*, 1911, clxv, 709.

⁴ All the operative procedures were carried out under morphin-ether anesthesia.

leucocytes. This was achieved by dividing the operation into two stages. In the first stage, the trephine opening was made, the button of bone removed was replaced by a disk of paraffin, the wound closed by cat-gut sutures, and allowed to heal under an anti-septic dressing. As soon as healing was complete, the actual inoculation was made, by passing a long, slender, blunt hypodermic needle through the skin, muscle, and paraffin disk, into and through the frontal lobe of the brain to the base of the skull. Leakage into the subcutaneous tissue was prevented by pressure on the paraffin on withdrawing the needle.

Preliminary experiments with suspensions of Berlin blue showed that a fluid so injected distributes itself quite uniformly over the base of the skull, extending a quarter, even a third of the way down the spinal meninges.

Several strains of tubercle bacilli were thus inoculated:

(1) Bovine. Originally obtained from Dr. M. P. Ravenel. Extremely virulent.

(2) Human (H39). Obtained from Dr. E. L. Trudeau. Virulent.

(3) Human (H24). Obtained from Dr. Theobald Smith. Slightly less virulent.

(4) Human (H38). Stock culture, Rockefeller Institute. Avirulent for dogs. Subdural injections of this culture at no time caused symptoms of meningitis. The bacilli gradually decreased in number and, while still present in considerable numbers as late as the fortieth day after injection, had disappeared by the sixtieth day.

The inoculation material was prepared by filtering emulsions of these cultures through closely packed absorbent cotton, and diluting the resulting filtrates to the desired turbidity. Bacterial emulsions so filtered are practically free from clumps. As a turbidity standard, a $\frac{1}{20}$ per cent. lecithin suspension was used, prepared by diluting a 1 per cent. alcoholic solution of lecithin with nineteen volumes of physiological saline. The volume of fluid usually injected was two cubic centimeters of the bacterial suspension, followed by one cubic centimeter of physiological saline to wash out the needle, a separate syringe barrel being used for the salt solution.

EXPERIMENTAL TUBERCULOUS MENINGITIS.

The injection of virulent tubercle bacilli into the subdural space of dogs by the above technique, produces a disease characterized by a latent or incubation period of from five to thirty days, followed by a period of increasing paralysis and incoördination that terminates, almost invariably, in death. The clinical picture, during the paralytic stage, varies in different animals, depending on the groups of muscles most involved, and on whether the paralyzes are of the flaccid or of the spastic type. In order to record these paralyzes, the following symbols have been adopted:

□ = Apparently normal; no paralysis, no incoördination.

◻ = Beginning paralysis, beginning incoördination. Such an animal may show a slight spastic condition of the posterior extremities, or a tendency to stumble on running. A slight arching of the back and a beginning tenseness of all the trunk muscles are characteristic of this period.

◻ = Well-marked paralyzes and incoördinations. Such an animal may have all its extremities either in a flaccid or a spastic condition. Its movements may be so incoördinate that the animal falls to its side; the neck and back may be held rigidly and the animal may cry out on being handled.

◻ = Extreme paralysis, collapse. The animal in this stage is unable to stand. It usually lies on its side, refuses food, and rapidly emaciates. The back and neck are usually held rigidly.

◻ = Death.

The following symbols have been adopted to represent symptoms unassociated with paralyzes and incoördinations:

◻ = Quieter than normally. The animal refuses to play, spends most of its time curled up in a corner of the cage, and may object slightly to being handled.

◻ = Very quiet, weak, objects to being handled.

◻ = Profound stupor, collapse. ◻ = Death.

A typical course of the disease as represented by these symbols is shown in figure 1.

For the purpose of the present paper, the slight daily variations in the paralysis are of little moment. To obtain a less detailed



FIG. 1. TYPICAL COURSE OF TUBERCULOUS MENINGITIS. A fox-terrier, weighing 8 kilos, trephined 16 days previously. The wound healed by first intention. 0.1 c.c. of a suspension of bovine tubercle bacilli in 2 c.c. of physiological saline was injected, followed by 1 c.c. of salt solution to wash out the needle. The animal showed an incubation period of 11 days, mild constitutional symptoms beginning on the 12th day, definite paralysis on the 15th day, increasing to collapse on the 25th day; death on the 28th day. Autopsy showed a tuberculous process involving the entire meningeal cavity; abdominal and thoracic viscera negative.

picture, the above symbols have been combined,



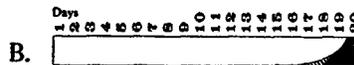
and the daily variation eliminated by changing the profile to a smooth curve.



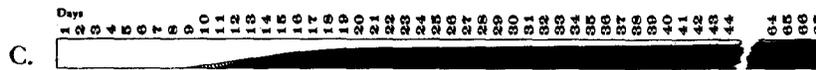
This method of representation has been adopted in the subsequent parts of the paper.

The above case, selected as the typical course of the disease, is characteristic of the majority of cases. About a quarter of the animals, however, do not conform to this type. For the sake of presenting these atypical cases, they are here divided into four groups. In these groups are included only uncomplicated cases of tuberculous meningitis, animals developing distemper and other intercurrent infections being carefully excluded from these records.

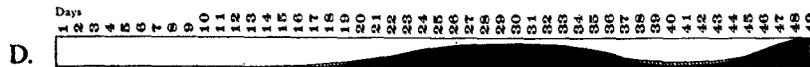
The first of these atypical cases is characterized by a slightly prolonged latent period, followed by sudden death, with or without slight preliminary paralytic symptoms. The animals are usually quieter than normally for a day or two before death. Death is usually preceded by convulsions and coma.



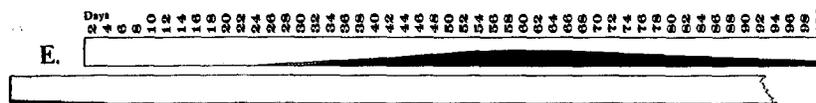
The second atypical group is characterized by an unusually prolonged paralytic stage. Animals of this type usually show the flaccid, rather than the spastic type of paralysis. The head and neck muscles are usually but little involved, thus interfering only slightly with the movements necessary for taking food.



The third group is characterized by a remission or actual disappearance of the paralytic symptoms, followed by their reappearance and death.



In the fourth group are included the spontaneous recoveries from the disease.



On account of the existence of these atypical cases, care must be exercised in drawing conclusions from clinical data, since parallel animals, inoculated with equal doses, may give quite different clinical pictures (figure 2).

To reduce these variations to a minimum, care was exercised in

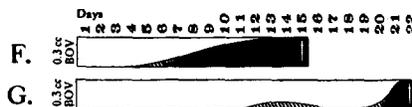


FIG. 2. CLINICAL VARIATIONS. Two fox-terriers inoculated with equal doses (0.3 c.c.) of bovine tubercle bacilli. In the first animal (F), definite paralysis began on the 6th day, progressed to collapse on the 11th day; death on the 15th day. The second animal (G) showed no paralytic symptoms till the 20th day. Collapse on the 21st day; death on the 22d.

choosing animals for parallel inoculations. The animals selected were as nearly as possible of the same weight, the same breed, the same age, and in the same condition of nutrition. For most of the experiments, large, well nourished fox-terriers and bull-terriers were selected. Collies, spaniels, Irish terriers, and other long-haired dogs were avoided, as they were found irregular in their reactions and very susceptible to intercurrent infections. Young dogs were also avoided for the same reason. By exercising this care, it was found possible to produce approximately the same clinical picture in parallel inoculations (figure 3).

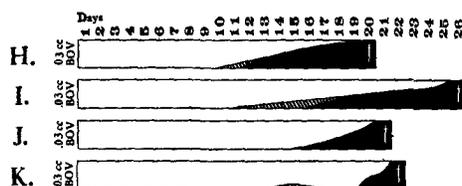


FIG. 3. PARALLEL INOCULATIONS. Four carefully selected adult fox-terriers, inoculated with equal doses of tubercle bacilli, showing comparatively slight variations in the clinical picture.

The clinical manifestations varied with the virulence of the culture injected (figure 4). With the very virulent bovine culture, the incubation period was short, the development of the paralytic symptoms rapid, and the outcome invariably fatal. With the less virulent human cultures, the incubation period was prolonged, the paralytic symptoms developed slowly, and an occasional animal recovered spontaneously.

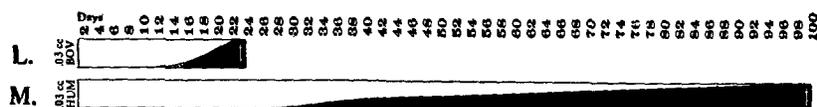


FIG. 4. RELATION TO VIRULENCE. Parallel inoculations with equal doses of bovine (L) and human (M) tubercle bacilli.

The clinical manifestations also varied with the dosage (figure 5). The latent period was perceptibly prolonged on decreasing

the dose, while with the smaller doses the paralytic symptoms developed slowly and there was a greater number of spontaneous recoveries.

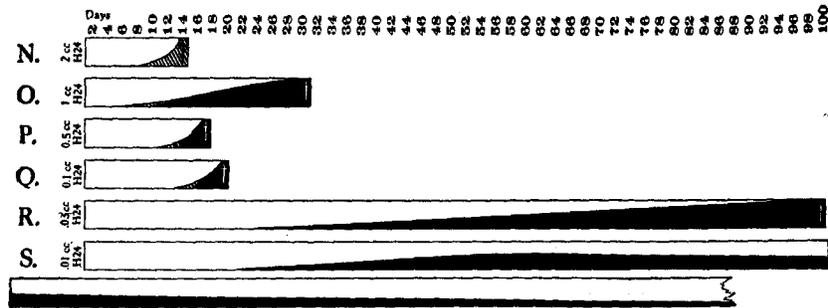


FIG. 5. RELATION TO DOSAGE. Parallel inoculations with decreasing doses of the same organism.

The local anatomical changes in the disease varied with the virulence and dosage of the organism injected. There was always more or less thickening of the meninges, with adhesions to adjacent structures. The subdural space usually showed an active tuberculous process, varying from an accumulation of fluid, fibrin, and semi-necrotic cells in the more acute cases, to well organized masses of diffuse tuberculous tissue in the more chronic cases. Smears made from the subdural space showed tubercle bacilli. There was usually a considerable accumulation of round cells about the cerebral and spinal capillaries, with occasional areas of necrosis.

The other organs of the body usually showed no characteristic change. Miliary tubercles were found in the livers of about a quarter of the dogs injected with the bovine culture, more rarely in the lungs and other organs. Such nodules, however, were never found in dogs injected with the human strains.

This does not necessarily mean that the bacilli of the human type did not occasionally escape from the meningeal cavity into the general circulation, but it indicates presumably only that the virulence of the human cultures was too small for the few that did escape to set up the disease in the extrameningeal tissues. This view is strengthened by experiments, in which small doses of the bovine

and human cultures were injected intravenously. With the bovine culture, a generalized miliary tuberculosis was always produced by this means. With the human cultures, such nodules were never formed.

Dogs recovering spontaneously from the paralytic symptoms showed, a few months later, simply a slight opacity of the meninges, with a few old adhesions. No tubercle bacilli were then found.

EFFECTS OF LEUCOCYTIC INJECTIONS.

The leucocytes used in treating the infected animals were obtained by the method perfected by Opie.⁵ Five days before the proposed treatments, one cubic centimeter of turpentine was injected into the pleural cavity of a normal dog, and three days later a second injection of a similar amount was given. The resulting rapidly formed serous effusion was withdrawn and discarded on the fourth day, following which withdrawal a pleural exudate very rich in leucocytes was usually formed. This was used on the fifth day.

To prevent clumping, this exudate was drawn into about a third of its expected volume of 1.5 per cent. sodium citrate. The exudate was then filtered through gauze, freed from plasma by centrifugation, washed with salt solution, refiltered if necessary to remove lumps, and made up into a 50 per cent. suspension in physiological saline.

A microscopic examination of suspensions so prepared showed them to consist of mononuclear and polymorphonuclear leucocytes, mixed with a few red cells. Bacteria were never seen. Routine sterility tests, by transferring a sample to nutrient agar, gave uniformly no growth. About a third of the leucocytes stained immediately on the addition of very dilute methylene blue, from which it was concluded that these cells were probably necrotic, although still preserving their normal microscopic appearance.

In making the subdural leucocytic injections, the same technique was employed as in the initial subdural inoculations with tubercle bacilli; a long, slender, blunt hypodermic needle was passed through

⁵ Opie, *loc. cit.*

the paraffin disk filling the permanent trephine opening, into and through the frontal lobe of the brain, and the leucocytes were forced into the basal meninges. The volume of suspension thus injected was usually about four cubic centimeters for fox-terriers, and six cubic centimeters for bull-terriers. Occasionally as much as ten cubic centimeters have been injected with apparently no ill effects.

The usual immediate effect of the injection was a temporary cessation of respiratory movements. As a routine, the animal was at once placed under artificial respiration, the tongue and epiglottis being retracted to insure a free respiratory passage. Spontaneous respiratory movements were usually resumed within two minutes, but were occasionally delayed for five or ten minutes, or even longer.

The later effects of the injections were less satisfactory. About a quarter of the animals died within a few hours. In a number of cases, death was attributable to edema of the lungs, or to unsuspected pneumonia from earlier etherizations; but many of the autopsies showed apparently no such accessory cause.

For several days after the injection, a number of the surviving animals were quiet, showed evidences of cranial tenderness, a few even showing disturbances in equilibrium, circulatory movements, and the like. These symptoms usually disappeared within two or three days, but occasionally persisted for a longer period. The majority of the animals, however, showed no symptoms the day after the injection.

The effect of the leucocytic injections varied with the dosage and virulence of the organism used. With large doses of tubercle bacilli the leucocytes were apparently without effect (figure 6).

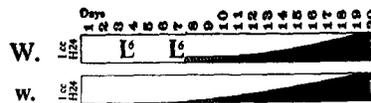


FIG. 6. EFFECT OF LEUCOCYTIC INJECTIONS. Composite picture of four treated dogs (W) and four untreated controls (w). In making these composites, the average day on which symptoms of a certain severity first made their appearance was taken as the day of its appearance in the composite animal. The number of cubic centimeters of leucocytic suspension injected is indicated by the small exponents; thus, $L^6 = 6$ c.c.

With small doses of the very virulent bacilli of the bovine type (figure 7), the injections produced a uniform prolongation of the latent period of the disease, but had a less evident influence upon the subsequent course of the paralytic symptoms. Possibly the paralytic symptoms were somewhat less acute in the treated dogs than in the untreated controls, but the number of experiments was too small to establish this beyond question. All animals of this series died as a result of the meningeal infection.

With small doses of the less virulent human cultures, the injections had an even greater influence (figure 8). The control animals, inoculated with these doses, invariably developed paralytic and ataxic symptoms, usually after an incubation period of about four weeks. The resulting paralyses usually increased slowly in severity, and in half the cases terminated fatally in about three months. In the other half, the paralyses were of a more chronic type, persisting to the present date (seven months). Half of the animals receiving the leucocytic injections have shown no paralytic symptoms up to the present day (seven months), while the others have developed paralyses only after very prolonged incubation periods, from which one animal of the series has thus far died.

The local histological changes produced by the leucocytic injections can be determined only by killing animals at various stages after such injections. This has not been done. What can be stated, however, is that when fatal paralyses develop in the treated animals, the autopsy usually shows approximately the same histological changes as in the untreated controls. In some of the treated dogs, however, the tuberculous lesion in the upper parts of the subdural space has apparently been arrested, or caused to undergo resolution by the leucocytic injections, while persisting or advancing to a chronic condition about the lower third of the spinal cord, to which situation the leucocytic suspension presumably did not penetrate.

The study of the therapeutic control of tuberculous meningitis is made difficult in the dog by the necessity of making the injection and treatment through a trephine opening. Danger exists of producing fatal pressure effects, and the frequent etherizations expose the animal to intercurrent respiratory infections. Moreover, the

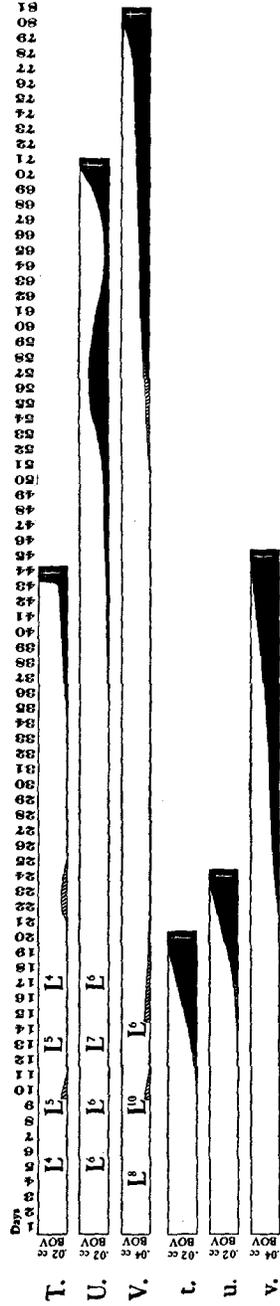


FIG. 7. EFFECT OF LEUCOCYTTIC INJECTIONS. Three animals (T, U, V), with controls (t, u, v), inoculated with small doses of the bovine culture.

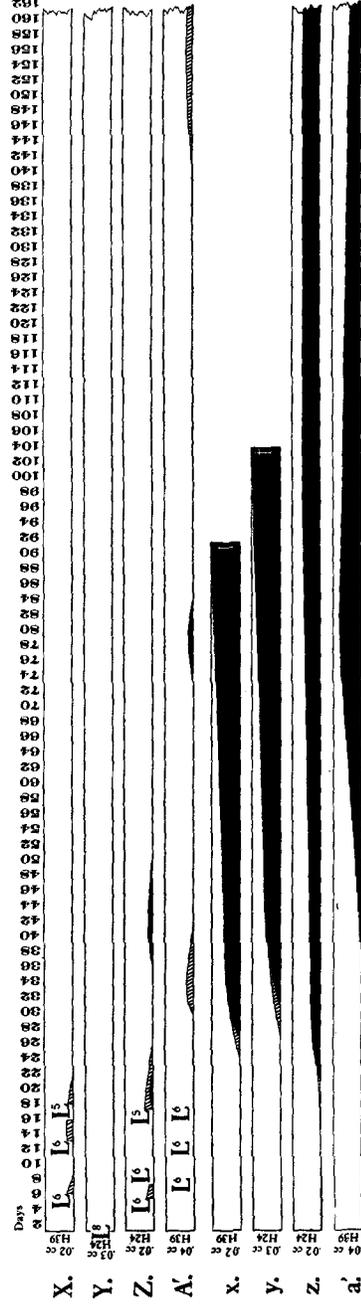


FIG. 8. EFFECT OF LEUCOCYTTIC INJECTIONS. Comparison as in figure 7, but with animals injected with small doses of the less virulent bacilli of the human type.

medicament introduced into the cranial cavity penetrates with difficulty to the caudal levels of the spinal cord, leaving that part of the cord practically untreated. It is intended therefore to pursue this investigation upon animals in which the inoculations and injections can be made with less traumatic injury and under conditions more nearly approaching those occurring in spontaneous tuberculous meningitis in human beings.

The later history of the animals now under observation will be reported in a subsequent paper.

SUMMARY.

Subdural inoculation of tubercle bacilli of established virulence for guinea pigs and rabbits, produces in the dog a tuberculous meningitis, followed by paralysis and death. When suspensions of canine leucocytes are injected subdurally, following such inoculations there occurs uniformly a delay in the development of the paralysis and a prolongation of the life of the treated animal. In dogs inoculated with small doses of tubercle bacilli of low virulence, the development of paralysis has been prevented by this means for periods of seven months (up to the present date), while the untreated animals injected with the same cultures have all developed paralytic symptoms within a period of about four weeks.