TETANUS.

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THE occurrence of muscular spasm following a wound was recognized by the physicians of antiquity, and Aretæus emphasized the gravity of the prognosis in such cases. The comparative rarity of the disease, however, prevented it from attracting wide attention, and extended observations do not appear to have been made until the opportunity was afforded by the wars of the eighteenth century, with their high number of casualties, which included many cases of tetanus. As will be seen later, even provided that the terrain is suitable, it is only war that affords any considerable number of wounds favourable for the implantation of tetanus, though the employment of fireworks on a large scale, as in the fourth of July celebrations in the United States of America, has led to very similar conditions and has been productive of many cases.

To-day, knowledge with regard to the pathogenesis of tetanus is fairly complete, and consequently great advances in therapy, and especially in prophylactic measures, have been made. These all date from the commencement of the bacteriological epoch in the last quarter of the nineteenth century, and are directly due to the employment of animal experiment in the elucidation of disease. Thus, two Italian observers, Carle and Rattone, undeterred by the previous failures of other workers, succeeded in 1884 in producing tetanic symptoms in rabbits, which they inoculated with an emulsion of tissue from the neighbourhood of an acne pustule in a case of tetanus, further, they transmitted the disease by transference from one animal to another. Nicolaier, in 1885, showed that inoculation of mice and other animals with samples of earth caused tetanic symptoms, and he recognized the probable causal agent in a slender bacillus which he grew in mixed culture with other organisms on blood-serum. In 1889, Kitasato obtained pure cultures of Nicolaier's organism under anaerobic conditions, he grew mixed cultures, and then heated these at 80° C. for one hour, by which means the other organisms were killed, the tetanus bacilli, which form resistant spores, survived the heating, and happening to be the only sporing organisms present, they were then obtained as a pure growth when cultures were subsequently incubated in an atmosphere of hydrogen. It was found that although the tetanus bacillus is comparatively uncharacteristic in the unspored stage (a Gram-staining filamentous organism of variable length, which possesses flagella and in the living state is motile), a highly distinctive appearance is presented when spores are fully developed. Each bacillus then possesses one large, perfectly terminal spore, practically spherical in outline, so that the descriptive term 'drumstick bacillus' is extremely appropriate. It must

be remembered, however, that tetanus bacilli cannot be recognized with certainty in pathological material, such as the secretions from wounds, by microscopical examination alone, this point will be referred to in detail later.

As regards the manner in which the disease-effects are produced, Knud Faber, in 1889, produced tetanic symptoms by the injection of bacterium-free filtrates of impure fluid cultures of *B. tetani* mixed with other organisms, he showed that, as in the case of diphtheria toxin, the filtrates were deprived of their toxic power by heating at 65° C. Kitasato then completed the proof by demonstrating the presence of toxin in sterile filtrates from pure cultures of *B. tetani*.

Under suitable conditions *B. tetani*, when growing in fluid medium, produces one of the most potent soluble poisons known, for a wide variety of animals, thus, the horse, which is exceedingly susceptible, but probably not more than man, can be killed by a subcutaneous injection of 0·003 c.c. of concentrated toxin. In 1890, Behring and Kitasato showed that when an animal was injected with repeated doses of tetanus toxin in non-lethal amounts, it gradually acquired marked tolerance to the poison, in addition, the blood-serum of such an actively immunized animal neutralized tetanus toxin when mixed with it *in vitro*. The mixture of many times the fatal dose of tetanus toxin with a suitable amount of such 'antitoxic' immune serum proved quite harmless when injected into a susceptible animal. This observation constitutes the basis of serum therapy.

In what follows, no attempt is made to deal equally fully with tetanus in all its aspects, but special attention is devoted to those observations, both clinical and experimental, which have a direct bearing on the prophylaxis and treatment of the disease, every effort has been made to avoid omission of any important fact, especially in the light of recent experience, but only representative works have been quoted from the literature.

PATHOGENESIS.

Source of Infection.—Tetanus bacilli or their spores are so widely distributed on the earth's surface as to be almost ubiquitous, but they are especially numerous in the dust of roads, in gardens and highly cultivated arable land, and in horse and cattle manure. They have been repeatedly demonstrated in the intestinal contents of horses and cattle, and even of men, especially those working in stables or with horses. Noble found that out of four horses in one stable, which were examined over a period of six months, one showed the presence of *B. tetani* in cultures made from 0·1 gram of faeces during the first three months, the results thereafter, even with 1 gram of faeces, being negative, a second horse gave positive results with 1 gram in three tests during the first fourteen days, but not again, the other two remained negative throughout. This author therefore concludes that certain horses afford a more favourable habitat for the tetanus bacillus than others, and that in suitable animals multiplication of the organism must occur in the intestine, in order to explain the continued excretion of bacilli, hence these animals are tetanus 'carriers.' Whether intestinal multiplication is the whole explanation of the prevalence of tetanus bacilli is not clear, but it is

certain that the presence of these organisms in horses is variable, since Lukas obtained 16 positive results among 17 horses, while McLeod, Soga, and Roger in this country (unpublished) obtained only 1 positive out of upwards of 30 horses (half of these animals were at a knackery, the others were farm horses working in the neighbourhood of London). Römer found tetanus antitoxin constantly present in the blood-serum of cattle which were over two years old, and as the presence of *B. tetani* in the intestinal contents has been proved by the inoculation of mice with faecal cultures, he considers that adult cattle are, as a rule, the subjects of latent infection with *B. tetani*.

Manure and highly manured soil are therefore pre-eminently the sources of infection with B. tetani (and also of the other potentially pathogenic anaerobes, the malignant oedema bacillus, *B. Welchii*, etc.), but whether the organism flourishes exclusively, or even mainly, inside or outside the animal body, is not yet settled. Owing to the resistant character of the spores, infected material may under ordinary conditions continue to be a source of danger for an almost indefinite period except when exposed to direct sunlight.

A wide variety of objects contaminated with tetanus spores have acted as the vehicles of infection, such as splinters of wood, portions of clothing and other foreign bodies, felt used to pad a plaster jacket (Kirmisson), plant fibre—pengawar djambi—employed as a dressing for wounds (Jacobsthal), vaccines, diphtheria antitoxin, and possibly catgut and vaccine lymph. Contaminated material employed to dress the stump of the umbilical cord appears to explain the heavy incidence of tetanus neonatorum in certain parts of the world, especially in the tropics. With reference to the production of tetanus by antidiphtheritic serum, it is to be observed that tetanus toxin may be present in the blood of horses some days before they manifest symptoms, hence harmful effects may follow the administration of a serum which is culturally sterile. Nowadays, precautions are taken to determine that antisera are free from tetanus spores or toxin by injecting a large amount—10 c.c.—into several guinea-pigs, and also by cultural tests, but, of course, it is possible that the serum may become infected in the course of bottling, after it has been tested.

It is of practical importance to know what procedures will sterilize articles infected with tetanus spores. Thus, Kitasato found steam at atmospheric pressure (i.e., 97·5°–98° C.) killed these spores in five minutes (this contrasts with the spores of malignant oedema, which survive heating for an hour under similar conditions), 5 per cent carbolic acid required fifteen hours to kill, 5 per cent carbolic acid + 0·5 per cent hydrochloric acid required two hours, 1–1000 corrosive sublimate required three hours, 1–1000 corrosive sublimate + 0·5 per cent hydrochloric acid required half an hour.

Conditions of Infection.—The tetanus bacillus and its products are essential for the occurrence of the disease, the characteristic symptoms being due to absorption of the specific soluble toxins which it produces. *B. tetani*, however, is probably quite incapable of obtaining a foothold in the living tissues by its own unaided action. The circumstances which enable it to proliferate in the body under natural conditions are, as a rule, those which promote sepsis,—that is to say, a wound contaminated with pyogenic organisms. Further favouring conditions are present where the wound is deep,

penetrating—so as not to drain spontaneously—and where foreign matter of any kind is embedded in the tissues. The presence of much necrotic tissue seems to aid the growth of *B. tetani*, accordingly, lacerated contused wounds are specially liable to be affected, these conditions, of course, also tend to produce sepsis, and so they exert a double action in favouring the onset of tetanus.

Various workers have shown that living tetanus spores, freed from toxin by heating at 80° C., produce no harmful effect when injected subcutaneously into animals, they settle at the site of injection, but some are transported throughout the body, and remain alive for a considerable time in various organs, e.g., the liver. Vaillard and Vincent found that such detoxicated tetanus spores, when injected subcutaneously, were actively taken up by phagocytes, hence it is probable that these cells are responsible for the transport of the spores. The spores remain alive, and under favourable conditions are capable of germinating, and by giving rise to toxin may produce fatal tetanus. In guinea-pigs, subsequent injection of living staphylococci into the site at which the spores were introduced, at any time up to one month later, regularly lights up tetanus (Francis). Tarozi caused tetanus to develop in a rabbit six weeks after the intravenous injection of heated spores, by producing simple fracture of the femur. Semple found viable, virulent tetanus spores at the site of inoculation, six months after subcutaneous injection into guinea-pigs.

Quinine has the same effect as have staphylococci in lighting up a latent tetanus infection,* and in this way Semple explained cases of tetanus in men following subcutaneous injections of this drug made presumably with aseptic precautions. He advanced the hypothesis that spores which had by some means entered the tissues at a previous date were enabled to proliferate at the site of the injection of quinine owing to the resulting tissue damage.

The fact that under ordinary conditions of artificial culture *B. tetani* is a strict anaerobe, seems to have obscured somewhat the view of the pathology of the disease, the idea being prevalent that conditions strictly comparable with an anaerobic culture apparatus require to be arranged in the tissues before *B. tetani* will flourish. This has been dispelled by recent work, since even in artificial cultures the organism will grow in bouillon if the minced pieces of flesh are left in the broth, although no growth is likely to occur if a clear filtered specimen of the same broth be employed, as Tarozi and Theobald Smith first demonstrated, solid tissue particles promote growth. Again, mixed infection with staphylococci favours the proliferation of *B. tetani* in the tissues, though not *in vitro*, and a number of other organisms exert a similar favouring action. Whether other anaerobes, like Welch's bacillus and the bacillus of malignant œdema, specially assist *B. tetani* in the tissues is not clear, these organisms are usually present in wounds contaminated with 'rich' soil, and, as has been seen, their habitat is the same as that of *B. tetani*. As the present war has shown, shrapnel and grenade

* It is interesting to note that the favouring agencies vary to some extent with the species of animal investigated, thus staphylococci activate latent tetanus infection in mice, while quinine fails to do so.

wounds afford the combination of conditions specially favourable for tetanus, but a variety of other lesions have led to the disease, such as burns and frost-bite. Although the importance of marked sepsis in the causation of tetanus can scarcely be exaggerated, it must be remembered, on the other hand, that numerous instances have been recorded in which the site of infection was an otherwise trivial wound, which had healed perfectly before the onset of tetanic symptoms, sometimes, but by no means invariably, foreign bodies have been found in the cicatrices. Thus it comes about that there are no clinical features of a wound pathognomonic of tetanus infection. Tetanus has also been observed to follow injuries unaccompanied by solution of continuity of the integuments, e.g., in cases of simple fracture, hæmatoma, etc. (see Natonek). Accordingly, the presence of aseptic necrotic tissue or trauma, apart from mixed infection, has been considered to afford conditions suitable for the germination of tetanus bacilli, as in the classical work of Kitasato, who produced tetanus in monkeys by introducing subcutaneously splinters of wood impregnated with tetanus spores, it appears, however, that in most experiments of this kind other organisms also are found at the site of inoculation.

The site of wounds which give rise to tetanus is most commonly on the limbs, first on the legs, then on the arms, the rarity of tetanus complicating wounds of the thorax was noted in the American Civil War. Other paths of entry of the tetanus bacillus apart from wounds have been proved, such as the tonsils and carious teeth, and in one case *B. tetani* was shown by animal inoculation to be present in an abscess of the lung in a young man who died of tetanus when convalescent from pneumonia, also the genital tract affords entrance to the bacillus in women who develop tetanus shortly after parturition. Although, as Ransom showed in guinea-pigs, the healthy alimentary tract has little tendency to absorb tetanus toxin, even when administered in such large amounts that it can be detected in the fæces, it is probable that intestinal lesions, caused by organisms or otherwise, may afford a foothold to *B. tetani*, the complication of typhoid fever with tetanus observed in the wars at the beginning of the nineteenth century is probably to be explained in this way. There remains, however, a certain proportion of 'idiopathic' or 'rheumatic' cases, said to be most frequent in children and of relatively good prognosis ('pseudotetanus' of children—Pexa), in which no site of entry of the organism can be determined. In some of these the disease has supervened after exposure to cold or wet, so suggesting that depression of vitality has enabled the bacillus to proliferate and form toxin (in this connection it is noteworthy that Larrey concluded on clinical grounds that exposure to cold and wet favoured the outbreak of tetanus after war wounds), but in others no favouring circumstance could be held responsible for the attack. Whether auto-infection in the sense of absorption of spores from the alimentary canal ever takes place has not been determined, but there appears to be no theoretical ground against this path of entrance of the organism. Spores absorbed in this way might then lie latent in the tissues until infection was lighted up by suitable local conditions.

The question occasionally arises as to whether a wound has been infected with tetanus subsequent to the time of infliction, especially when the incubation period is long. Noetzi's experiments suggest that when once granulation

tissue has covered the raw surface, infection with tetanus bacilli is extremely unlikely to occur. Pocketed subcutaneous wounds were made in sheep, and were kept open by packing with gauze without aseptic precautions, when the surface was covered with granulation tissue, in the secretion from which staphylococci were present. a virulent fluid culture of *B. tetani* was poured into the wound, which was then packed with gauze soaked in the culture. All four animals treated in this way remained alive and well. Thus, granulation tissue, in correspondence with its protective properties in general, seems to afford an almost insuperable barrier to the subsequent entrance of *B. tetani* or its toxins. Conversely, in the course of the present war Nivière has found that operative procedure on a wound was followed in a number of cases by tetanic symptoms in twelve to forty-eight hours, thus suggesting that the onset of the disease had been determined or accelerated by the disturbance of the tissues.

The occurrence of tetanus following surgical procedures—most frequently gynæcological operations—has raised a number of points which have not been satisfactorily settled. E. Koch drew attention to imperfectly sterilized catgut as a source of infection, and he produced tetanus in mice by inoculation with portions of ligature which were found surrounded by pus in a case dying from tetanus after abdominal hysterectomy for impacted fibroids. This work served the useful purpose of emphasizing the necessity for securing sterile gut, and the difficulties attendant thereon; it is well known that organisms may survive in gut which has been boiled for half an hour in absolute alcohol. It is probable, however, that a variety of causes are responsible for post-operative tetanus.

It is obvious that the *geographical distribution* of the disease should correspond with the wide distribution of the specific organism. Where the bacillus is abundant, wounds are, of course, most liable to contamination, on the other hand, where the nature of the soil is such that *B. tetani* is scanty, similar wounds will yield a lower proportion of cases, the relative frequency of tetanus at the beginning of the present war in France and Belgium, and the comparative rarity of the disease in the South African war, are illustrations in point.

As regards *sex incidence*, males are more commonly affected, apart even from war wounds, a fact explicable on the basis of occupation and pursuits. As a rule the disease is sporadic in occurrence, but of course a common source of infection may lead to the outbreak of a series of cases. There is no definite evidence as to whether individuals exhibit marked differences in *susceptibility* to the implantation of tetanus in contaminated wounds. But Dehne and Hamburger, and Aschoff and Robertson, failed to find any notable amount of antitoxin in the blood of normal individuals, thus indicating that natural immunity to the toxin is not common or well marked in the human subject.

TETANUS IN THE HUMAN SUBJECT.

In the first place, the disease will be dealt with in the form unmodified by treatment with specific antitoxin. Alterations in the clinical picture due to the administration of serum will be referred to later.

Incubation Period.—Although the transport of tetanus spores throughout the body has been proved to occur under certain conditions, this does not invalidate the view that in most cases the symptoms of tetanus are due to toxin produced by bacilli restricted to a particular site, usually a septic lesion. *B. tetani* has been recovered from the lymph glands draining the infected wound, also from the blood, lungs, and spleen in human cases (Reinhardt and Assim), but there is no evidence that a general septicæmic process occurs ordinarily. The symptoms of tetanus appear after a latent stage following infection. According to Rose's collected statistics, symptoms begin in the second week after the infliction of the wound in 46 per cent of the cases; and in the recent analysis of 180 cases treated in home military hospitals (Bruce), over 50 per cent began in the same period, more cases occurring on the tenth day than on any other (it is not stated whether any of these cases had received prophylactic doses of antitoxin), 75 per cent of the cases manifested symptoms within the first fortnight. While this is the average, remarkable variations in the incubation period are met with, ranging from one or two days to a month or even longer. Where the incubation period is unusually long, the possibility that infection has occurred subsequent to the infliction of the wound must be considered, but as will appear clearly later, a variety of factors determine the duration of the latent stage.

Symptoms.—The usual symptoms of a definitely declared attack of tetanus in the human subject are too well known to require more than brief mention, they correspond to the experimental type termed 'descending' tetanus (see p. 24). *Increased tonus of the voluntary muscles is the characteristic phenomenon.* This usually commences in the masseters ('lockjaw'), where it is often first observed on waking in the morning after a good night's sleep, by passing the index finger in between the cheek and the teeth, which are held closed, the tense anterior margin of the masseters can be very well made out. The muscles of the neck are involved practically at the same time. Then the recti abdominis, and later other groups, are affected symmetrically on both sides of the body. Finally, opisthotonus is established, this position being probably due to the relative strengths of the various groups of muscles affected. Pleurosthotonus and emprosthotonus are very exceptional. Associated exaggerated action of the unaffected facial muscles on attempting to antagonize the spastic muscles, as in the effort to protrude the tongue, causes 'risus sardonicus.' After the condition has lasted several days, exacerbations of the tonic spasm, i.e., tonic convulsions, usually tend to supervene suddenly, without warning, and not as a reflex response, these seizures vary widely in intensity, frequency, and duration (minutes to hours). Again, reflex spasms very frequently follow external stimuli of various kinds, such as handling the patient, a draught of air, bright light, noise, or in fact any sensory stimulus, or a particular act—for example, swallowing may alone excite the condition (tetanus hydrophobicus). The forearms and hands tend to remain unaffected. The senses and consciousness remain normal as a rule. The spasm of the muscles causes cramp-like sensations of varying severity. Pyrexia may or may not be present, and fever may be accounted for by mixed infection. Profuse sweats are a frequent accompaniment of the exacerbations of spasm. The violence occasionally manifested by the muscular spasms may

be appreciated from cases recorded by Anders and Morgan in which the spinous processes of the vertebræ were fractured or the teeth crushed down to the roots.* In a small proportion of cases the spasm is at first confined to the muscles of the injured limb, *partial or local tetanus* (see Courtellemont), this corresponds to the sequence of events in animals which have been experimentally inoculated—'ascending tetanus.' A degree of this phenomenon is seen when in narcosis the injured limb remains stiff although the other members are relaxed. On recovery, spasm may disappear last from the injured limb. A distribution of spasm limited to the two upper or two lower limbs has also been described. Sleeplessness is the rule after the tonic spasm has set in, and in cases which progress rapidly to a fatal termination sleep cannot be produced by any means. When sleep occurs—as the result usually of drugs—the spasm relaxes more or less completely, and commonly does not again become declared until several minutes after waking. Rose appears to have been the first to emphasize the occurrence of facial paralysis in tetanus following wounds in the distribution area of the facial nerve. Green vision has been recorded. Symptoms affecting the other systems are in the nature of accidental complications. Thus, in the period of spasm, retention of urine and constipation are common; in the convulsions, evacuations tend to occur. Respiratory and cardiac affections are dealt with below †

Causes of Death.—The most frequent causes of death are asphyxia, exhaustion, and cardiac failure. When an exacerbation of muscular spasm affects the respiratory muscles, especially the diaphragm or glottis, death from asphyxia often occurs, hence cases in which spasm of the glottis or the diaphragm supervenes early are relatively unfavourable, such an attack may, however, cause death at a late date, when diminution in the general spasm indicates convalescence. Œdema of the lungs and pneumonia are liable to set in, and are very dangerous complications. As a result, apparently, of increased peripheral resistance during the spasm, the heart may come to a standstill either suddenly or gradually. Marked elevation of the pulse-rate is an unfavourable sign. Exhaustion may supervene rapidly, thus, an apparent improvement, in the form of diminution of the trismus, which is often accompanied by otherwise inexplicable hyperpyrexia‡, may be the herald of impending death. According to Rose, spasmodic flexion of the arms is a terminal phenomenon. Again, when convalescence appears to be established, patients whose nutrition and general condition have suffered severely may die quite suddenly. No doubt the diminished intake of food and fluid, owing to the trismus, account in part for the exhaustion and wasting, but doubtless the action of the toxin is also directly responsible.

Mortality.—All observers agree that tetanus following wounds is an extremely fatal disease, but figures collected from large groups of statistics

* Superficial and deep reflexes are greatly increased, and Goldscheider (*Berl. klin. Woch.*, No. 10, 1915) has frequently observed the Babinski phenomenon (extension of the great toe on stroking the foot sole), either bilateral or confined to the wounded limb.

† Davies has noticed a tendency to improvement, followed by exacerbation of the symptoms, about the third or fourth day, which may be quite a feature of the disease.

‡ The temperature has frequently been observed to continue to rise for about forty minutes post mortem.

show marked variations. In general, when the incubation period is less than ten days, the mortality is greater than in cases in which the disease appears later. Permin has investigated with great thoroughness the statistics of the mortality in patients not treated with serum. Six observers who gathered their cases from the literature, found the mortality to range from 17·4 to 50·8 per cent, the figure being under 30 in three series, all Italian. Permin points out that such figures are likely to be fallacious, on account of the fact that there is a tendency to publish cases which recover or which present unusual features, and to suppress those running the ordinary fatal course. On the other hand, taking the figures of eleven observers in hospital cases, the mortality ranged from 78 to 89·3 per cent in nine: while the two others, both Italian, gave the mortality as 44 and 84·9 per cent. Permin's own series of 124 cases taken from the records of the larger hospitals in Denmark (and not including tetanus neonatorum), along with Faber's Danish series of 75 cases (each series by itself giving practically the same result), making a total of 199 cases, gave the following figures —

Incubation of 10 days or less,	94 cases,	deaths = 94·7 per cent
„ of over 10 days	57 „	„ = 70·2 „
„ unknown	48 „	„ = 58·3 „

Thus, the average mortality was 79 per cent. This corresponds well with the general average of hospital figures already quoted. Only the Italians seem to have a much milder type of disease. Rose's collected hospital statistics of cases following wounds, from his own wards, from Great Britain, and in the American Civil War—in all, 716—yielded a mortality of 88 per cent, the extremes being 84·2 and 89·8 per cent.

Duration of the Disease.—Four-fifths of the fatal cases die within a week of the onset of symptoms, and over 90 per cent are dead within fourteen days (Rose). Death after the case has lasted a month is a rarity. In cases which ultimately recover, the spasm may persist for many months, the masseters being the last muscles to become free. In a case of Rose's, the last exacerbation was observed on the fifty-eighth day of the disease, and trismus was still noticeable on the seventy-fifth day. In another case the exacerbations occurred over a period of five months. Persisting stiffness of the joints of the lower limbs may require treatment by passive movement, as after fracture, it is probable also that impairment of muscular movement results from cicatrices following tearing of the muscle fibres.

Prognosis.—As has been seen under *Causes of Death*, the prognosis in this disease is always extremely doubtful. Those cases in which the spasm sets in after a short incubation period—under ten days—or in which successive groups of muscles become involved in rapid succession (which usually happens when the incubation period is short), are the least favourable. The occurrence of spasm in the diaphragm (pain in the hypogastrium and base of the chest) and glottis (stridor) is in itself a bad omen. But exhaustion, pneumonia, or cardiac failure may supervene unexpectedly at any period. According to Hill's observations, the most important point in prognosis is the time during which the patient has already survived the disease. If the case has lasted less than ten days, the chances of death are greater than of recovery, after ten days of illness the chances of recovery progressively increase.

Hyperpyrexia is a bad sign, and a temperature over 104° F on or before the fifth day, practically always means death.

Premontory Symptoms.—A number of observers have drawn attention to early or 'premonitory' symptoms. Where they are recognized, specific serum treatment can be begun without loss of time, the vital importance of avoiding delay in the administration of antitoxin will be discussed later. Where patients are already under observation, the occurrence of premonitory symptoms can, of course, be watched for. A number of observers have recorded the occurrence of pain in the wounded limb, which was taken to be 'rheumatic' in character, or of twitching or contracture in the muscles in the affected part, with or without marked pain. A temperamental change, such as the development of quarrelsomeness, has also been noted. Evler, who himself acquired tetanus, gives an extended list of early symptoms, as follows —

A day or so after the infection there may be general restlessness, changing suddenly to a desire to rest.

Sleeplessness, with distressing dreams and, it may be, nightly delirium. Difficulty in micturition, due to spasm of the sphincter vesicæ, which may last from a few minutes to half an hour.

Temporary giddiness, violent headache, excessive yawning.

The facial appearance changes and the patient looks anxious, though there is no risus sardonicus yet.

There may be trembling of the tongue, which is put out to one side.

There is often a profuse sweating, and darting pains in various parts may occur.

The patient may have a feeling of chilliness, and there may be some swelling, without redness locally, of the injured member, and throbbing of its arteries, notwithstanding that the limb is raised.

Slight jerking may follow pressure on the flexor tendons, and these muscles may be noticed to be in a condition of increased irritability

If symptoms such as these are present, with a history of possible infection with the tetanus bacillus, Evler considers that the administration of serum is justified. The later symptoms include —

Increased flow of saliva.

Reflex cramps of œsophagus.

Ocular symptoms, e.g., nystagmus, strabismus.

Ear trouble, and spasmodic cough.

Tremors and clonic spasms, which are not painful, and which may not attract attention.

Pain persisting after muscular contraction induced by effort.

With regard to these early symptoms, MacConkey says, "I have carefully read through a considerable number of cases of tetanus described in the literature, and I have found that practically all the symptoms mentioned by Evler have been noticed as being present early in the disease. All have not been observed in the same patient, but usually the one or two present have been sufficiently prominent to force themselves upon the notice of the physician, and, if he had read Evler's paper, to remind him that such a disease as tetanus might be before him." On the other hand, a criticism which has been passed on these observations is that, on reading them, all would feel that

they had those symptoms themselves (Embleton). But when there is the possibility that one may be dealing with so grave a disease as tetanus, it is surely advisable to be on the watch for symptoms such as Evler has described, the significance of which is, of course, unknown to the ordinary patient.

Diagnosis.—*Bacteriological.*—The occurrence of 'drumstick,' spored bacilli in the discharge from a wound is highly suggestive, but not conclusive, since other organisms practically identical in appearance with *B. tetani* are met with, and *B. tetani* in the non-spored condition is not characteristic. A much more certain procedure than microscopic examination is the production of tetanus in mice, either by the direct subcutaneous injection of secretion from the wound or by filtrates of anaerobic fluid cultures, the latter may give a positive result where direct inoculation fails to produce tetanus (Dean and Mouat), but results are obtained too slowly to be of use for treatment. The cerebrospinal fluid shows no abnormality in the albumin or cell-content, and no coagulum formation (Stintzing, Plaut, Rehn and Schottmüller). points of importance in diagnosis. The demonstration of toxin in the patient's blood or cerebrospinal fluid when several cubic centimetres are injected subcutaneously into mice, may be successful early in the course of the disease. According to several observers, failure to obtain fluid on entering the subarachnoid space is frequent in cases of tetanus; others again find the pressure of the fluid increased. There is no characteristic leucocytosis.

Clinical Differential Diagnosis.—*Masseter spasm* occurs in a variety of local lesions—dental abscess, abscess of the temporomaxillary joint, angina, parotitis, irritation associated with the eruption of wisdom teeth—which must be excluded should trismus be the only sign. *Spasm of the neck muscles* occurs in meningitis and cerebral abscess, but trismus is absent in these conditions, and the general features are unlikely to cause confusion with tetanus, in meningitis, too, the cerebrospinal fluid is abnormal. In *tetany*, the marked spasm of the hand muscles, leading to the 'obstetric position' of the fingers, is usually a prominent feature, further, the spasm comes and goes, which is not the case in tetanus. *Strychnine poisoning* is characterized by the presence of reflex spasms from the outset, with intervals of relaxation during which, in contrast to tetanus, the masseters and neck muscles also relax completely.

EXPERIMENTAL PATHOLOGY AND SERUM THERAPY.

As has been noted, the muscles first affected by spasm in man are usually the masseters, wherever the site of the infected wound may be, thence the affection spreads to other muscular groups—*descending* tetanus. This is the case likewise in the horse and other large animals. In smaller animals, on the other hand, when inoculated with tetanus toxin or bacilli, either subcutaneously or intramuscularly, the spasm begins, as a rule, in the muscles of the inoculated limb, and spreads later on to the rest of the body—*ascending* tetanus, with a small dose of toxin, spasm may remain confined to the inoculated limb.

The action of tetanus toxin has formed the subject of a great amount of experimental investigation, the principal contributors being Marie and Morax, Meyer and Ransom, and Permin. The practical as well as the theoretical

importance of this work can scarcely be overrated, since upon a clear understanding of the pathology of the disease, as elucidated by the action of toxin and antitoxin under experimental conditions, depends the successful application of antiserum therapy. The following summary of the principal facts and the theoretical considerations to which they have given rise takes no account of the historical development of the subject, as the present comparatively complete state of knowledge depends on the verification and amplification of one observer's work by others.

The local muscular spasm of 'ascending' tetanus is due to the action of the toxin on the related segment of the spinal cord. The toxin reaches the cord by passing up the muscular nerves. This has been demonstrated by the following experiments taken in conjunction —

1. Taking advantage of the fact that tetanus toxin when injected into a muscle soon reaches the nerve supplying the muscle, and when in the nerve is practically insusceptible to the neutralizing action of antitoxin contained in the blood and lymph, a rabbit is passively immunized by an intravenous injection of antitoxin, which soon reaches the lymph, and then receives a relatively small dose of tetanus toxin into the muscles of the hind leg, the result is that *local tetanus develops in the inoculated limb and nowhere else (too large a dose of toxin will spread in the cord—see p. 27).* The same result follows if antitoxin is injected into the thigh muscles and toxin is introduced into the sciatic nerve at the same spot. But if in such a passively immunized animal the sciatic nerve be first cut below the gluteus maximus, and then toxin be injected into the peripheral end, no tetanus occurs. Subcutaneous injection of toxin produces no effect in a passively immunized animal.

The rate of ascent of toxin in the muscular nerve may be judged from the following experiment —A series of rabbits are immunized intravenously with antitoxin, and then several times the lethal dose of toxin is injected into the leg muscles, at varying times thereafter in different animals, the sciatic nerve on the same side is cut just below the gluteus. It is found that if an interval of five hours or more is allowed to elapse between the injection of the toxin and the neurotomy, local tetanus develops.

If the posterior nerve roots only of one leg are cut, and then later on, after the animal has been passively immunized intravenously, toxin is injected into the muscle of the neurotomized leg, no tetanus follows, a similar injection on the sound side causes spasm as usual. But the injection of toxin into intact posterior nerve roots does not produce tetanic spasm. This is of importance in connection with Sherrington's work (see below).

2. If antitoxin is injected into the sciatic nerve and then toxin is immediately injected into the corresponding limb, tetanus fails to occur in that limb. The intraspinal injection of antitoxin also prevents ascending tetanus.

3. The spasm, when recent, disappears during general narcosis, also on the injection of cocaine intraspinally. On the other hand, after spasm has existed for some time (over thirty hours), changes occur in the affected muscles, for even section of the motor nerve does not then entirely abolish the spasm.

4. Direct injection of toxin into the spinal cord causes tetanus starting, after a very short incubation period, in the muscles innervated from the site of inoculation, but there are also symptoms of pain, restricted to the part innervated from the site of injection (see below, *tetanus dolorosus*). The extremely short incubation period after direct injection of toxin into the substance of the cord—three to five hours in cats, as compared with twenty-eight to thirty hours after subcutaneous or intravenous injection—leads Meyer and Ransom to attribute the ordinary incubation period in great part to the time taken by the toxin in ascending the peripheral nerves.

The Nature of the Nervous Lesion in Tetanus.—It has been supposed that the anterior cornual cells of the cord are irritated by the toxin, thus giving rise to increased muscular tonus. These cells are said to exhibit a definite lesion, viz., swelling of the Nissl bodies without disturbance of their arrangement (Flatau and Goldscheider), and recently Manouelian has described other characteristic changes in the motor cells of the cord, pons, and medulla, how far these lesions are the cause of the muscular spasm remains uncertain, however. But it is important to note that there are, in addition, degenerative changes widespread throughout the ganglion cells of the central nervous system, including the cerebral cortex (Foulerton and Campbell Thomson).

According to Sherrington's investigations on animals poisoned by tetanus toxin, there exists a *disturbance of reciprocal innervation*. Thus, stimulation of that area of the cerebral cortex which in a healthy animal causes opening of the mouth—due to contraction of the depressors of the jaw, accompanied by relaxation of the elevators—in a tetanic animal leads to trismus, since the normal inhibition of the closing muscles, which form the more powerful group, is now converted into a stimulation of them. The condition greatly resembles that of 'decerebrate rigidity' in an animal whose cerebral hemispheres have been removed. Clinically, the patient with tetanus exhibits the same phenomena, since the effort to open the mouth, instead of inhibiting the closing muscles, leads to excitation of them, so that his mouth closes all the tighter. The site of the lesion would appear to be in the neighbourhood of the connection between the upper and the lower motor neurons.

The general muscular spasm of 'descending' tetanus depends on the diffused action on the nervous system of toxin widely disseminated by the blood and lymph. Even in small animals, when the injection of toxin is made into a non-muscular region, e.g., subcutaneously near the knee-joint (Zupnik), general descending tetanus results. Whether the toxin passes to the central nervous system directly from the blood stream, or whether it can reach the nerve centres only by way of the muscular nerves after being taken up from the lymph by the nerve-endings, is a point which has not been satisfactorily determined. After toxin has been injected intravenously, it is possible, by introducing anti-toxin into one sciatic nerve, to prevent the occurrence of spasm in this leg, this result may be due not to the antitoxin blocking the ascent of toxin in the nerve from the periphery, but to its having passed up to the spinal centres and exercised its neutralizing action in the latter situation.

Those who uphold exclusively the neural route have sought to explain the regular commencement of tetanus in the masseters on the ground of the shortness of the nerve, or of its having a greater affinity for the toxin than other nerves. It is not the case, however, that the sequence of onset of spasm in particular muscles depends entirely on the length of their nerves, since in cats spasm begins in certain muscles of the fore-limbs, the nerves of which are among the longest (Meyer and Ransom). Another view is that the centres of the earliest affected muscles have a special 'predilection' for the toxin. A probable explanation of the early appearance of trismus is that the weak action of the antagonizing muscles renders the spasm of the masseters

obvious (Zupnik), this is in harmony also with the fact that trismus may exist as the only symptom for days before the involvement of other muscles is apparent.

While the muscular nerves act as excellent and rapid conductors, they do not bind the toxin as the central nervous system, especially the grey matter, does (Wassermann and Takaki). The precise mode of conduction in the nerve has not been decided. Marie and Morax, Meyer and Ransom, and others, consider that the axis cylinder is the conductor. In support of their view, Meyer and Ransom quote an experiment in which a rabbit, very highly immunized to tetanus toxin, died of tetanus as the result of the injection of toxin into the sciatic nerve, its blood—and consequently also the lymph—at the time of death containing more antitoxin in two drops than sufficed to neutralize all the toxin injected. But Field (quoted by Park), and Aschoff and Robertson, hold that the toxin ascends in the neural lymphatics. Experiments showing that toxin injected into a muscle fails to pass into its nerve when the nerve has been cut high up a considerable time previously, do not really support the former view, while the fact that toxin which has reached the nerve is insusceptible to neutralization by the circulating antibodies in an immunized animal, can probably be explained by the comparatively slight access of lymph which is possible in that situation, the consequence is that when toxin has once entered the nerve, its action becomes concentrated exclusively on the nervous system. This appears also from the fact that a very much smaller dose of toxin will cause fatal tetanus when introduced intraneurally than when injected into the blood stream or subcutaneously.

When the toxin reaches the cord, it does not remain localized, but spreads, thus, if section of the cord is performed in the upper lumbar region, and afterwards toxin is injected into the sciatic nerve, tetanus remains localized in the hind limbs, whereas if the cord is intact, the anterior part of the body becomes spastic also.

A number of other interesting facts have also been elicited, as, for example, the observation of Meyer and Ransom that an injection of toxin into the posterior nerve-roots between the ganglion and the spinal cord does not produce muscular contraction, but causes severe pain, which recurs in spasms (tetanus dolorosus), the pain is also produced reflexly by slight stimulation of the skin supplied by the affected nerve, as by blowing on it. Again, the injection of an otherwise fatal dose of tetanus toxin into the infra-orbital nerve of a dog caused very little effect of any kind—there being in one animal spasm of the ear on the side of the injection, and in another spasm of the masseter. The fact that tetanus dolorosus never occurs clinically, or after intravenous or intraneural injections of toxin, is held by Meyer and Ransom as a proof that, under natural conditions of infection, the toxin reaches the cord only by the axons of the motor nerves, not by lymphatics; the posterior-root ganglion cells are considered to oppose an insuperable barrier to the toxin reaching the cord by sensory nerves. Loewi and Meyer have observed that the injection of a sublethal dose of tetanus toxin into a nerve renders an animal hypersensitive to a subcutaneous dose of the toxin administered some days later, this may have a bearing on the pathology of relapses.

The spontaneous convulsive seizures are probably of cerebral origin.—The evidence in favour of this conclusion is as follows —

1. Clinically, in many instances the seizures are not associated with any obvious peripheral stimuli.

2. Roux and Borrel observed that intracerebral injection of tetanus toxin caused epileptiform convulsions, but there remained the possibility that this might be due to the mechanical irritation of the injection. Permin accordingly produced what was in effect an intracerebral injection without this possible fallacy, by introducing the toxin into the vitreous humour in rabbits previously passively immunized by an intravenous injection of antitoxin. The result was the development of recurring universal spontaneous convulsions, with practically no tonic spasm (as has been seen, the latter is produced by toxin acting on the spinal cord).

3. Confirmatory observations made by Permin were (a) The occurrence of convulsive spasms in the leg of a rabbit which, by means of an intraneural injection of antitoxin, was prevented from sharing the general tonic spasm produced by an intravenous administration of toxin, the non-spastic leg participated equally with the others in the convulsions, (b) In such an animal, the removal of all brain substance from below the corpora quadrigemina upwards abolished the convulsions, whereas, when the animal recovered from the effects of the ether under which the operation had been performed, the general tonic spasm remained marked.

4. As has been noted, marked lesions of the cells of the cerebral cortex have been found (Foulerton and Campbell Thomson).

Of course, in the naturally occurring disease, both the brain and the spinal cord are exposed to the action of the toxin.

There are a number of further points regarding the behaviour of toxin and antitoxin, and their interaction in the tissues, which have a direct bearing on the prophylaxis and treatment of the disease. Tetanus toxin reaches the blood stream when there is a local infection, this has been proved both in animals and in man, and it has been found that the amount of toxin present in the blood is usually at a maximum before and at the commencement of the tetanic symptoms (McClintock and Hutchings). Toxin also has been demonstrated in the cerebrospinal fluid in human cases (Permin, Stintzing), positive results here are obviously more important than negative. When a fixed amount of tetanus toxin is introduced directly into the blood stream, it disappears rapidly from the circulation, this no doubt being due partly to its passing into the lymph, but also to its combining with certain tissues, especially the central nervous system. Even after the toxin has been removed from the blood, it is, up to a certain point, still capable of being neutralized by a subsequent injection of antitoxin—that is to say, the antitoxin exerts a 'curative' effect, but with the lapse of time it becomes more difficult to produce cure. This is proved by the experiments of Dönitz, who injected a lethal quantity of tetanus toxin intravenously into rabbits, thereafter he injected antitoxin intravenously at increasing intervals, and determined the amount necessary to save life at different times. It was found that when the interval between the injection of toxin and antitoxin was an hour, over ten times as much antitoxin was required as sufficed if the interval was only four minutes.

In applying these results to the treatment of human cases, it is necessary to realize precisely what the conditions are when symptoms have appeared (1) Toxin has reached the central nervous system, and a certain amount, though probably only a portion, of the toxin has had time to enter into firm combination, and has injured the nerve cells, (2) Toxin is circulating in the blood and lymph as well as in the nerves throughout the body, (3) Toxin is probably still being produced at the site of the wound.

Experimental Serum Therapy.—Now, as regards the means at disposal for combating these conditions, toxin in the blood and lymph can be neutralized by antitoxin injected either subcutaneously, intravenously, or intramuscularly. In order that toxin diffused throughout the body may be neutralized, a similar diffusion of the antitoxin must be attained, the subcutaneous injection of antiserum, however, produces this result only slowly, about two days being required before the antitoxin content of the blood reaches a maximum (as Henderson Smith showed for antidiphtheritic serum, and Dehne and Hamburger for tetanus antitoxin). Hence, in order to ensure rapid neutralization of the circulating toxin, antitoxin should be administered intravenously, it has been found experimentally that half an hour after an intravenous injection of antitoxin, both blood and lymph are rendered atoxic (Ransom).

When we come to consider the conditions which are present in the nervous system, there is more difficulty. The central nervous system is not, like the rest of the tissues, bathed by lymph, which in its composition is closely related to blood plasma, the cerebrospinal fluid which permeates the nervous tissue is peculiar in being extremely poor in protein, and it is well established that antibodies and drugs do not pass readily into it from the blood stream (see Dixon and Halliburton). Hence, however rich the blood may be in antitoxin, whether introduced or as the result of active immunization, the concentration of antibodies will be low in the fluid surrounding the nerve cells. This explains the fact that a highly immunized animal is susceptible to a comparatively small dose of tetanus toxin introduced directly into the nervous tissue, since it must be remembered that the protective action of antibodies is due to their being present in the surrounding fluid and so neutralizing toxin before it can combine with susceptible cells. In order to bring antitoxin into the closest possible contact with the nervous tissue, various workers have recommended the intraspinal injection of antiserum, and Park and Nicoll have shown experimentally in the clearest fashion the efficacy of this form of treatment. They injected twice the fatal dose of tetanus toxin into the hind leg in guinea-pigs, and seventeen to twenty-four hours later introduced antitoxin by various routes into different animals, viz., subcutaneously, into the general circulation (intracardiac), and intraspinally. The results were as follows —

6	animals	were	treated	subcutaneously	—none	survived.
15	„	„		intracardially	—2	survived.
16	„	„		intraspinally	—13	survived.

Further, the dose of antitoxin which was efficacious when administered intraspinally was much smaller than that injected into the blood stream. In view of these results it is difficult to comprehend how any theoretical considera-

tion should be allowed to stand in the way of the intraspinal treatment of tetanus. It has been objected that antiserum does not enter the nerve cells, but experiments have shown that toxin which has been bound by tissue cells can be removed by contact with a solution containing antitoxin (Besredka, Noon, Graff and Menschikoff). Injection of antiserum into the lateral ventricles has been advised, but does not commend itself on account of the danger of damaging the cerebral tissue, even if this be obviated (Ballance), the method appears unsuited for frequently repeated injections. Again, to inject antitoxin into all the nerves in a case of general tetanus is obviously impossible even if it were desirable, and as Park and Nicoll point out, intraspinal injection probably achieves the same result. The necessity for commencing antitoxin treatment at the earliest possible moment has already been emphasized, the following experiment demonstrates quantitatively the vital importance of attention to this point. MacConkey determined the amount of antitoxin sufficient to save the life of a guinea-pig when injected under the skin *simultaneously, but at another site*, with a dose of toxin just sufficient to kill an untreated animal, he then found that (1) one-tenth of this amount of serum sufficed to neutralize 100 fatal doses of toxin when toxin and antitoxin were *mixed together in vitro prior to injection*, and (2) two thousand times as much serum was required to save the life of the animal when injected *twenty-four hours after the toxin*, i.e., at a time when symptoms of tetanus had already appeared. In other words, the amount of antitoxin required to cure the animal was two million times as great as the amount which, mixed in the test tube, would render the same dose of toxin inert.

PROPHYLACTIC TREATMENT, ESPECIALLY THE USE OF ANTISERUM, AND MODIFICATIONS OF THE DISEASE DUE TO SUCH TREATMENT.

Laboratory experiment, as well as veterinary practice, have demonstrated the high value of antitoxic serum—derived from horses immunized by injections of tetanus toxin—for the prevention of tetanus. Thus, Vaillard quotes the result of the preventive inoculation of 13,124 animals during the years 1898–1906 in the practice of eight veterinary surgeons, with no cases of tetanus, while in two other veterinary practices during the same period, 139 cases occurred in untreated animals. Mohler and Eichhorn have determined that 500 U.S.A. units is a sufficient prophylactic dose for a horse, even when infection has occurred four days prior to the injection of antitoxin. In the United States the value of antitoxin in preventing tetanus as a consequence of ‘fourth of July’ injuries has also been clearly apparent, thus, the number of deaths from tetanus fell from 417 in 1903, to 150 in 1909, and 3 in 1914, a reduction “far out of proportion to the reduction in the number of accidents from which tetanus might have resulted” (*Jour Amer Med. Assoc.*, No. 26, 1915).

With regard to the prophylactic use of antitoxic serum, the following points require consideration (1) *Dosage and route of introduction of the serum*, (2) *Duration of immunity thus conferred*.

As regards *dosage and route of introduction of antitoxin*, there is a consensus

of opinion among observers in different countries that for the great majority of wounds 500 to 1000 U.S.A. units* represents a sufficient prophylactic dose when injected subcutaneously, preferably as soon as possible after the infliction of the wound, or at least within twenty-four hours afterwards. Results obtained during the present war have in this respect confirmed previous experience (see MacConkey's summary of the literature). In the British Official *Memorandum on the Treatment of Wounds in War* (July, 1915), it is stated that there is no evidence that 500 U.S.A. units is an insufficient dose if given promptly. But the broad principle may well be followed, that "as the amount of soiling of the wound and contusion of the tissues increases, so should the amount of antitoxin be increased" (MacConkey).

As to the route of introduction, it is recommended by some that the injection be made in the vicinity of the wound, so that the toxin may be neutralized before it can diffuse, but in any case, the result of a subcutaneous injection is to render the blood and lymph antitoxic, so that toxin is neutralized before it can reach the nervous system. After a subcutaneous injection the antitoxin content of the blood reaches a maximum in forty-eight hours. This is sufficiently rapid to prevent the outbreak of tetanus in most cases, but occasionally, whether as the result of the introduction of very large numbers of tetanus spores or of some special favouring condition, symptoms of tetanus may appear in spite of the prophylactic injection. Thus, Wolf records the case of a grenade wound which tore the tendo Achillis from its attachment and splintered the os calcis, and in which the early injection of 20 German units failed to prevent the appearance of trismus thirty hours after the infliction of the wound, the patient received further antitoxin and drug treatment, and recovered. In view of the extremely short incubation period, it is probably safe to say that without the use of antiserum this case would certainly have died. Intravenous administration causes the rapid diffusion of antitoxin throughout the body fluids, accordingly this method would probably prevent a proportion of cases which occur after subcutaneous treatment, and the same dose or even less would suffice. In a number of instances (see Montais, Pozzi) the occurrence of tetanus commencing in the wounded limb, and either spreading to the rest of the body or remaining localized, has been observed in cases where antitoxin was administered during the incubation period, but too late to prevent altogether the outbreak of tetanus, e.g., two to four days after the infliction of the wound. Thus, the effect is similar to that where an animal is passively immunized and toxin is injected intramuscularly or intraneurally. When the leg is affected in such cases, the first evidence of tetanus is that the foot becomes fixed in the position of equinus, the spasm being aggravated by any local stimulus or by attempts at voluntary movement, later, the whole limb is rigid, and any stimulus causes painful exacerbation of the spasm (*partial* or *local* tetanus).

* Forty U.S.A. units are equivalent to 1 German unit, also known as 1 Behring unit or 1 A.E. The U.S.A. unit of tetanus antitoxin is ten times the least quantity of serum which is required to preserve the life of a 350-gram guinea-pig for ninety-six hours when mixed with the official test-quantity of standard toxin (100 minimum lethal doses) and injected subcutaneously. Commercial sera frequently contain 1500 U.S.A. units in a volume of about 10 c.c. and consist of 'concentrated' antitoxin, i.e., a considerable amount of the albumin has been removed by fractional precipitation of the serum.

The local application to wounds of dried antitoxin in the form of a dusting powder has been recommended, and, as the result of experiments, Robertson suggests that a more efficient procedure is to apply a dressing impregnated with antiserum, of course this should not replace the injection of antitoxin.

Duration of Immunity.—It must be remembered that the passive immunity conferred by an injection of antiserum derived from another species is of relatively short duration, and it has been found both in the human subject and in animals, that the immunity will have fallen to a comparatively low level after the end of a week following the injection of antitoxin, as shown by the content of the serum in antitoxin (Dehne and Hamburger, Levin). On the other hand, clinical estimates of the duration of protection afforded by a dose of antitoxin based on the date of development of the disease afterwards, yield extremely divergent results (see MacConkey), this is readily understood when one remembers how many variable factors are concerned, such as the extent of the wound, the number and possibly the virulence of the tetanus bacilli introduced, the local conditions favouring multiplication of the bacilli and absorption of the toxin, etc.

It might be thought that in order to maintain immunity for a long period it would merely be necessary to administer more serum at the beginning. But this is not the case, the antitoxin disappears largely in virtue of its association with the foreign protein of the horse-serum, which is disposed of by the tissues, and there is no basis for supposing that double the amount will persist in circulation for double the time. Further, the amount of antitoxin which reaches the blood after a subcutaneous injection varies greatly in different persons who have received the same dose, thus indicating that there is also a marked individual factor in the destruction of antitoxin. Repetition of the subcutaneous dose of antiserum is strongly indicated at weekly intervals so long as the wound is not definitely in a healing stage, and especially if necrotic tissue be still present.

Antitoxin does not appear to be effective in leading to the destruction of tetanus bacilli in a wound, on the contrary, in persons immunized with antitoxin, septic wounds may continue to harbour living virulent tetanus bacilli for many weeks. Antitoxin treatment, therefore, has created many wound carriers of tetanus, who on this account may be a source of danger to others as well as to themselves, whether in the absence of prophylactic treatment with antitoxin many individuals may receive *B. tetani* into the tissues at the time of injury and continue to harbour the organism in a septic wound without developing symptoms of tetanus (as in a case recorded by Dudgeon, Gardner, and Bawtree) is not clear, but there can be little doubt that the employment of antitoxin has greatly multiplied such carriers. The danger which a tetanus carrier himself incurs is due to the fact that his infection does not appear, as a rule, to excite his own tissues to develop any considerable degree of active immunity (Löwy). Hence, if the process of healing of the wound has not led to the elimination of *B. tetani* or the formation of an efficient granulation-tissue barrier against absorption, the effects of the toxin will become manifest when the passive immunity has expired, there is also always the possibility that increased proliferation of the bacilli, accompanied by increased toxin formation, may occur, or suitable conditions for toxin absorption may develop,

and so lead to fatal tetanus. These considerations probably afford a full explanation in many cases of the long incubation period of the disease observed in patients who have received prophylactic injections of serum (e.g., two months in a case recorded by Don, and five months in a case of Teutschlaender's),* and also of spontaneous recurrences (Brandt).

The outbreak of the disease at a late stage is specially liable to occur when further operative procedure is undertaken on an infected wound, since the solution of the granulation-tissue barrier permits the spread of infection and the absorption of toxin. Bérard and Lumière have drawn attention to several distinctive features which may be presented by such cases of *delayed tetanus*, thus, the onset may be insidious, the tonic spasm, which may be localized in the wounded limb, progresses very gradually, and trismus and stiffness of the neck may remain slight for days or even weeks; permanent contracture of the abdominal muscles is, however, said to be strikingly constant. Convulsive seizures are absent, or appear late and are slight. In spite of the gradual onset and slow evolution of the disease, however, the prognosis in such cases is grave, death occurring from asphyxia owing to the contracture of the respiratory muscles. It is, therefore, an excellent rule to administer a prophylactic dose of antiserum subcutaneously twenty-four to forty-eight hours before operating on a patient with an infected wound, or if an emergency operation is required, antitoxin should be administered intramuscularly five or six hours earlier, or an intravenous injection may be given immediately beforehand. Recrudescence of septic infection also may afford conditions suitable for the occurrence of tetanus in wound carriers. In a case recorded by Huppel, a severe shrapnel wound of the left thigh, with splintering of the bone, was cleaned out five days after infliction, and at the same time a dose of antitetanus serum was given, severe tetanus occurred three weeks later, but recovery took place. Almost four months after the original injury, erysipelas developed from a small area of the wound which alone had remained unhealed, tetanus appeared four days later, and led to death.

The administration of antiserum after the infliction of a wound does not affect in any way the details of local treatment. It is superfluous to go into this matter here, beyond saying that whatever method of wound treatment is best in allaying or preventing sepsis will also prove most efficient in obviating conditions suitable for the development of tetanus. It will probably be found that a distinct value attaches to the use of those 'disinfectants' which can be combined with the 'physiological' method of treatment, and which are highly antiseptic and at the same time comparatively harmless to the living tissues, such as the organic chloramides (see Dakin, Cohen, and Kenyon), and also substances like brilliant green (see Leitch, and Browning and Gilmour). Such benefit as accrues from treatment with peroxide of hydrogen or similar reagents can scarcely be attributed to the inhibitory action which oxygen exerts on anaerobes in artificial cultures.

* In this case a grenade splinter passed through the thorax and lodged in the deep muscles of the back. Five months later fatal tetanus developed and led to death within two days. Tetanus bacilli were recovered from the tissue encapsulating the splinter, but not from a chronic empyema due to the perforation of the thorax.

With regard to the *indications for the use of antitetanic serum*, all wounds contaminated with 'rich' soil, or manure, or into which foreign bodies so contaminated have penetrated, should be regarded as highly suspicious, and the presence of much contused or necrotic tissue should be held to aggravate such suspicion. The presence in a wound of other anaerobic organisms, *B. œdematis maligni* and *B. Welchii*, etc., which have the same habitat as *B. tetani*, also indicates the likelihood that the latter is present. As has been already seen, antitoxin should be administered at the earliest possible moment, or at least within twenty-four hours. Later on, larger amounts of serum will be required, exactly how much it is difficult to say, but it may be advisable to give up to 10,000 or 15,000 U.S.A. units. The following case (Teutschlaender) is instructive as demonstrating efficacious treatment where the presence of living virulent tetanus bacilli was proved. The case was one of wound of the foot-sole due to a fragment of grenade. On the sixth day bacilli resembling *B. tetani* were found in the discharge from the wound, and 20 German units of antitoxin were given subcutaneously. Late on the eighth day transient painless contractures occurred in the sole of the wounded foot. Accordingly next day 100 units of antitoxin were injected intravenously, mice inoculated on the eighth day with secretion from the wound developed tetanus. No further symptoms of tetanus occurred, but a guinea-pig inoculated with secretion from the wound on the twenty-fifth day developed tetanus after the long incubation period of fifteen days, two mice inoculated at the same time remained unaffected.

The chances of grave anaphylactic symptoms following repeated subcutaneous injections of serum are fortunately not serious—this matter will be treated later. The further question arises, however, as to whether a case which has already received one or more injections of antiserum is thereby rendered refractory to subsequent passive immunization, in other words, whether, if a patient is to be treated again with antitoxin, the fact that he has received a previous injection impairs in any way the specific efficiency of the later dose of serum. This point has been investigated for diphtheria antitoxin by Park, Famulener and Banzhaf in the human subject, under conditions strictly comparable with those likely to arise in practice, and they conclude that *the same quantity of antitoxin is indicated whether the case has or has not received a previous injection of horse serum or globulins*. There is every reason for believing that their results apply equally to tetanus antitoxin, and this conclusion is borne out by an observation of Dehne and Hamburger on the behaviour of tetanus antitoxin in a man who had been repeatedly injected with horse serum.

The effect of the extensive prophylactic administration of antitoxin since the earlier days of the present war has had the result of practically abolishing tetanus (see MacConkey). It is true also that the organization for the treatment of wounds has been greatly perfected, and that for this and other reasons the diminution in sepsis might be held to account for the diminished incidence of tetanus, but regarding the extraordinary reduction after 'fourth of July' injuries in the United States in the past thirteen years, no corresponding revolution in the treatment of wounds can be discovered. Again, evidence such as that afforded by Wolf is extremely conclusive, and could no doubt be

readily duplicated, this observer deals with the results of a German field hospital which had been stationed several miles behind the trenches at a point on the Western front, where little change of position occurred during the whole period of observation. Prior to December, 1914, antitoxin treatment was not a regular procedure, and the cases of tetanus amounted to 1·4 per cent of the wounded. During the subsequent seven months, for the greater part of which the conditions as regards cold and wet were strictly comparable with the foregoing period, the incidence of tetanus was only 0·16 per cent; but during this latter time all cases of wounds due to grenades received antitoxin, and also those due to shrapnel where there was evidence of contamination, rifle bullet wounds were not so treated.

The use of ultra-violet light has been recommended for the purpose of killing off tetanus bacilli and spores in wounds and cicatrices, but the absorption which these rays suffer in passing through an extremely thin layer of tissue or body fluids is so great (Russ), that little practical result can be expected from the procedure.

THE TREATMENT OF DECLARED TETANUS.

It is clear from the pathology of the disease that here one is dealing with an extremely difficult therapeutic problem, since the outbreak of symptoms indicates that toxin has already combined with the central nervous system, and has exerted injurious effects. In cases in which the incubation period is short and in which symptoms develop rapidly, the damage may be irreparable before the patient comes under observation. On the other hand, a considerable proportion of cases are not of this fulminant type, and in these it is only rational to believe that adequate treatment may turn the scale in favour of recovery. The modes of death and the study of the action of the toxin afford indications for treatment. Uncombined toxin in the body fluids must be neutralized, toxin must, as far as possible, be removed from combination with the nervous tissue by the contact action of concentrated antitoxin, and purely mechanical causes of death, such as asphyxia and starvation due to the muscular spasm, must be obviated. At the same time it is to be remembered that symptomatic remedies which produce muscular relaxation can exert only a limited beneficial effect, they are themselves for the most part poisons, super-added to that of the bacterial toxin, whose action is not limited to irritating motor neurons. The *tetanus sine tetano* experimentally observed by Dönitz indicates that tetanus toxin produces a general exhausting effect which is ordinarily concealed by the prominent motor symptoms of the acute stage, but which becomes marked when the patient survives for a time.

Where spasm is already present, the subcutaneous injection of serum entails such delay in its action as may render the remedy useless, the rate of absorption is much too slow. Hence, the intravenous route is indicated, by this means free toxin in the tissue fluids will be rapidly neutralized, so that further toxin cannot reach the nervous system. This is probably the main value of moderate intravenous doses of antitoxin, but it is difficult to believe that the intimate proximity of an abundance of antitoxin in the capillary blood-vessels will be altogether without neutralizing effect on the

toxin in the central nervous system, and this is supported by von Graff's experiments. As has been especially demonstrated by Park and Nicoll, however, intraspinal injections of serum are the most effective in antagonizing the action of the toxin on the nervous tissue, and a much smaller dose achieves this result by the intraspinal route than by the intravenous. Accordingly, both an intravenous and an intraspinal dose of antitoxin should be given without delay. Where for any reason an intravenous injection cannot be given, the serum should be administered intramuscularly.

With regard to *dosage*, it is certain that much more antitoxin will be required than before the outbreak of symptoms, and even then the result will be less assured, hence the wastefulness of delay till the disease is declared, in cases where there is strong presumptive evidence that infection has occurred. Where the intravenous route alone is employed, a moderate amount of antiserum will neutralize all the toxin which is likely to be in circulation, but probably very large doses are required to influence toxin already present in the nervous tissues. Some observers record the administration of enormous amounts of antitoxin intravenously, thus, Torres has recommended 120 c.c. every six hours. This serves at least to show how much foreign protein can be tolerated, but the expense is almost prohibitive. There is, however, strong experimental evidence against administering a small dose of serum every few hours, the *concentration* attained by the antitoxin is important, hence one large dose of serum should be given. As regards the intraspinal administration of serum, antitoxin rapidly passes out of the cerebrospinal fluid into the blood (Ransom), although the reverse does not hold, hence, to maintain an adequate concentration in the subarachnoid space, repeated injections are required.

The scheme of treatment which is advocated by Park and Nicoll appears to rest on a thoroughly rational basis. It is as follows. Inject, as soon as possible, 3000 to 5000 U.S.A. units intraspinally after lumbar puncture, diluting the serum with sterile normal saline and introducing a volume of 10 to 15 c.c. according to age and the amount of cerebrospinal fluid withdrawn, the dilution aids thorough diffusion, but where there has been a 'dry tap,' inject only 3 to 5 c.c. Allow the fluid to run in slowly, by gravity if possible, while the patient is anæsthetized. Give at the same time 10,000 to 15,000 units intravenously. A similar amount injected subcutaneously three or four days later will maintain the tissue fluids rich in antitoxin for the next five days. The intraspinal injections should be repeated at intervals of twenty-four hours. It has been recommended that for several hours after the intraspinal injection the patient should be kept with the head of the bed lower than the foot, to aid the upward diffusion of the antitoxin, Doyen keeps his patients in this position, at an angle of 45° for ten hours following each injection. The immediate effect of a dose of serum may be a temporary exacerbation of the symptoms, followed later by improvement.

Certain harmful effects occasionally follow the administration of serum, especially by the intravenous or intraspinal routes. These may be grouped under the following headings (1) Acquired hypersensitiveness (anaphylaxis), which is due to a reaction between the tissues and body fluids of the host and foreign protein in the injected serum, this reaction occurs only as the result

of repeated injection of serum derived from the same foreign species on each occasion, and is altogether independent of the antitoxin present in the serum, (2) *Primary hypersensitiveness*, and possibly (3) *Effects due to chemical preservatives in the serum*.

1. *Anaphylaxis* is, as a general rule, established ten days after an injection of serum, hence, if a patient has received a previous injection of horse serum, whether as diphtheria or tetanus antitoxin, ten days or more previously, he should not receive a further massive dose of serum intravenously or intraspinally without previous preparation to induce anti-anaphylaxis (see below). Neglect of this precaution has led to serious results, and might cause death.*

2. *Primary hypersensitiveness* leads to symptoms identical with anaphylactic shock, but here the effects follow the first injection. This condition is exceptional, but serious results tend to follow intravenous or intraspinal injections of serum in such individuals;† thus, if circumstances permit, it is a good rule to take measures to induce anti-anaphylaxis in all cases before administering serum by these routes. The following *method of rapidly inducing anti-anaphylaxis* is based on the work of Besredka and of Friedberger 5 c.c. of the serum are diluted with 50 c.c. of normal saline. Of this mixture 1 c.c. is injected intravenously, and is followed at intervals by further amounts—3 c.c. after four minutes, 10 c.c. two minutes later, 25 c.c. after another two minutes. This fractional introduction of serum can be best effected with the ordinary apparatus for giving intravenous injections by gravitation, the tube connecting the reservoir to the needle being pinched in the intervals. After a further interval of ten to fifteen minutes, the full therapeutic dose of serum may be administered intravenously or intraspinally. By this means the dangers of hypersusceptibility may be reduced to a minimum. Of course, in view of the gravity of tetanus, no consideration of any kind should interfere with the prompt administration of antiserum. Park recommends that the intraspinal injection of serum should be carried out according to Sophian's method, whereby the serum is allowed to run in slowly by gravitation, 30 c.c. requiring over ten minutes to enter. According to Sophian, the blood-pressure should be observed during the whole operation, and a fall of 20 mm. pressure in an adult indicates that the safe limit of quantity has been reached, British observers, however, do not appear to regard this method of control as a valuable one (*Proc. Roy. Soc. Med.*, Therapeut. and Pharmacol. Section, Oct. 19, 1915). Therapeutic measures which reduce the dangerous effects of anaphylaxis are ether anaesthesia and injections of atropine sulphate.

3. *The effect of preservatives in the serum* (0·3 per cent trikresol being that commonly employed) has been held by some to account for injurious effects following intraspinal injection. Flexner, in discussing the question, held that increased intracerebral pressure and anaphylaxis explained the accidents,

* Collapse due to anaphylactic shock following intravenous or intraspinal injections usually appears within a few minutes, and tends to pass off rapidly, the symptoms include convulsive movements, dyspnoea and cyanosis, soft and rapid pulse, low blood-pressure, pains in head and limbs, feeling of impending death, vomiting, and diarrhoea, coma may supervene, and in cases terminating fatally, death may not occur for some hours.

† A history of asthmatic attacks following exposure to emanations from horses should always be inquired for, as this indicates the idiosyncrasy.

since death has followed intraspinal injections of serum free from preservatives (from the Pasteur Institute). The question has been investigated by Worth Hale, Voegtlin, and lately most extensively by Auer, the results obtained vary according to the animal studied and the anæsthetic employed, but the general conclusion clearly follows that trikresol contributes to the toxic action of serum introduced intraspinally. As the respiratory centre is mainly affected, Meltzer's insufflation apparatus should be kept in readiness, and if ill effects follow the introduction of serum, a portion should also be withdrawn at once by gravitation.

Additional treatment comprises thorough cleansing of the wound, adequate special nursing and feeding,* avoidance of external stimuli, such as bright light, draughts, noise, etc., and the use of drugs which abolish the muscular spasm. The drugs used include practically all the sedatives and narcotics—chloral hydrate by mouth, chloretone in oil per rectum, morphine hypodermically, and ether inhalation (chloroform appears to be less suitable), being especially employed. The dosage, of course, is regulated so as just to secure and to maintain relaxation of the muscles.†

Recently, as a result of Meltzer and Auer's experimental work, magnesium sulphate has been much used to relax the spasm, and has been widely if not universally approved. The following account of its employment embodies the procedures of Meltzer, and of Kocher, a warm advocate of the method. The drug may be administered (1) Subcutaneously. (2) Intramuscularly, (3) Intraspinally, or (4) Intravenously.

1. *Subcutaneous injection* suffices in milder cases, the dose is 1·2 to 2 c.c. (for a young infant 0·8 c.c.) of a sterile 25 per cent solution of $MgSO_4$ crystals (not anhydrous) per kilo of body weight, administered after morphine or under light ether anæsthesia. This represents a quarter of the maximum safe dose, and may be repeated four times in the twenty-four hours for a number of days. The effect is gradual.

2. *By the Intramuscular route* the dose is two-thirds of the subcutaneous, and should be injected into the thigh muscles, the site should then be massaged well. The effect is very potent when ether is used in combination, the patient is etherized before the injection, and a moderate anæsthesia is maintained for twenty minutes afterwards. The action sets in more rapidly, but is more transient, than with subcutaneous injections, on account of the local reaction, repetition is inadvisable.

3. *The Intraspinal method.*—When the case is very severe, with involvement of diaphragm and larynx—a condition most resistant to other treatment—this method produces rapid and prolonged relaxation, which sets in in half an hour and lasts for twenty-four hours. The dose is 1 c.c. of a 25 per cent solution per 10 kilos of body weight, introduced under ether anæsthesia. The patient should be kept horizontal, with the head alone

* Embleton has found constantly aceto-acetic acid in the urine in cases of tetanus, however slight the spasms, and has suggested that it pointed to the possibility of death being due to acidosis, but of course the common cause of acetonuria, viz., relative carbohydrate starvation, must first of all be excluded.

† Average doses are chloral hydrate 75 to 150 gr., or morphia 1½ gr. daily, chloretone 30 gr. every eight to sixteen hours.

raised, for some hours, if the thoracic muscles do not relax, the head should be lowered, and it may be necessary to incline the whole body with the feet upward. Deep narcosis results, which may last for twenty-four hours. After the symptoms have been controlled by an intraspinal injection, the attempt should be made to maintain relaxation by subcutaneous administrations.

4. *Intravenous administration* produces the most rapid action, Straub employs a 2·5 per cent (i.e. isotonic) solution, from 50 to 150 c.c. of which should be injected in two minutes. Spasm relaxes in from one to five minutes, but the effect passes off in half an hour. On account of the effect of intravenous injections on the heart, Meltzer urges caution in the use of this method, and recommends injecting a 6 per cent solution at the rate of from 2 to 3 c.c. per minute, slowing or shallowness of respiration is an indication for immediate discontinuance of the injection, if this does not cause prompt improvement, inject slowly through the venous cannula 'a small amount' of 2·5 per cent calcium chloride (see below).

An objectionable effect which may follow the administration of MgSO_4 is paralysis of respiration, which can be counteracted at once, except in the case of intraspinal administration, by calcium chloride (30 to 40 c.c. of a 2 per cent solution intramuscularly, or 500 to 600 c.c. of a 0·2 per cent solution along with 0·6 per cent NaCl intravenously), then, of course, the spasm may also recur. An injection of 1·5 mgm of physostigmine may also be employed, but is probably less safe. Especially in the case of intraspinal administration, arrangements should always be made for insufflation of air or oxygen by Meltzer's apparatus. Kocher states, however, that with the doses mentioned the occurrence of respiratory failure is very rare, and he has not seen a fatality from this cause. Depression of the circulatory function, as shown by softness or intermittency of the pulse, is especially likely to follow intravenous administration, and is a sign for discontinuance; and if the administration is stopped when ill effects commence, they tend soon to pass off. While the necessary precautions have been gone into in detail, it must not be concluded that the method is dangerous, in fact, few remedies are so well under control provided that the administrator knows what preparations are required. Obviously, cases treated in this way must be kept under close observation, and in the use of intraspinal injections the attention must be unremitting. The remarkable property which calcium chloride possesses of immediately awakening an animal narcotized with magnesium sulphate (Meltzer and Auer) suggests that the alteration produced in the nervous tissues is, within limits, a perfectly reversible one, this is a rare and most valuable feature in any drug, and warrants a wider trial of the method. The subcutaneous injection of concentrated MgSO_4 is painful at the time, but the pain is said to disappear soon, whereas a weaker solution (5 to 10 per cent), though painless at first, causes pain later on (Stadler). Zuelzer employs magnesium glycerophosphate, which he states has very little effect on the blood-pressure, safe doses are of a 25 per cent solution, 10 c.c. intravenously or 5 c.c. intraspinally, intramuscularly, scarcely any pain is produced.

Bacelli's treatment consists in the subcutaneous injection of a 2 or 3 per cent watery solution of carbolic acid, commencing with the daily dose of 0·3 to 0·5 gram—the urine being observed in order to test the patient's tolerance—

and then rapidly increasing to 1 to 1·5 gram phenol daily, administered in repeated injections. A remarkable reduction in the mortality was reported by Baccelli (a collection of individual cases from the literature), which should render this the only method worthy of consideration, but a general confirmation is lacking.

Amputation of the inoculated part has been proved experimentally to serve no useful purpose in the treatment of tetanus (McLintock and Hutchings), and this coincides with the opinion of most clinical observers.

Various heroic measures have been practised as further aids in treatment, such as section of the phrenic nerves.

Coming now to the *statistical aspect*, what reduction in the mortality from tetanus has resulted from the use of antitoxin? Permin, who has collected what is probably the most satisfactory series of comparable statistics from Danish hospitals—not individual cases compiled from the literature—concludes that antitoxin therapy has an undoubted, although not a pronounced, effect in reducing the mortality. Thus he found that out of 189 cases (not including tetanus neonatorum) treated with serum, 42·3 per cent recovered, as compared with 21·1 per cent of recoveries where antitoxin was not used. The details are as follows, and the table of untreated cases is added for comparison —

INCUBATION	CASES NOT TREATED WITH SERUM		CASES TREATED WITH SERUM	
	Number of Cases	Percentage of Recoveries	Number of Cases	Percentage of Recoveries
10 days or less	94	5·3	92	27·2
Over 10 days	57	29·8	57	59·6
Unknown	48	41·7	40	52·5
Totals	199	21·1	189	42·3

It might be objected that the untreated cases belonged to an earlier period, and that the severity of the disease had altered spontaneously or as the result of other treatment, but 100 of the untreated cases occurred subsequent to the antitoxin epoch, and of these only 24 per cent recovered, they were distributed as follows —

INCUBATION	NUMBER OF CASES	PERCENTAGE OF RECOVERIES
10 days or less	47	6·4
Over 10 days	32	37·6
Unknown	21	42·9
Total	100	24

Accordingly, the beneficial effect of antitoxin treatment appears to be clearly established, although this view is by no means universal. A closer analysis of Permin's cases did not establish any advantage following a particular route of introduction of serum, or any other factor. In comment, however, it may be said that, so far, no satisfactory detailed statistics on

the antitoxin treatment of tetanus exist, and further, until recently, knowledge as to what is even presumptively the best procedure as to dosage and route of administration has been extremely deficient. Nicoll's recently published series of unselected cases treated by the combined intraspinal and intravenous method of Park and himself, yielded 16 recoveries out of 20 patients, 9 of the cases had an incubation period of ten days or less, and 6 of these recovered. Doyen, also, has reported 21 recoveries out of 24 cases treated by large intraspinal doses of serum, 13 of the cases had an incubation period of ten days or less, and 10 of these lived. Although these series are small, the recoveries seem much too numerous to be the result of chance, and afford the hope that an effective procedure has at length been evolved for the treatment of declared tetanus.

REFERENCES.

- ANDERS and MORGAN, *Jour Amer Med. Assoc.*, xlv, 314.
 ASCHOFF and ROBERTSON, *Med. Klin.*, 1915, Nos. 26, 27
 AUER, *Jour of Exper Med.*, 1915, xxi, 43.
 BACCELLI, *Berl. klin. Woch.*, 1911, 1021.
 BALLANCE, *Lancet*, 1914, i, 1525.
 BESREDKA, *XVIIth Internat. Med. Congr.*, Sect. IV, London, 1913, *Ann. de l'Inst. Pasteur*, 1903, 138.
 BÉRARD and LUMIERE, *Bull. de l'Acad. de Med.*, 1915, Aug. 31.
 BRANDT, *Zentr. f. inn. Med.*, 1915, 565.
 BROWNING and GILMOUR, *Jour of Path. and Bact.*, xviii, 144.
 BRUCE, *Brit. Med. Jour.*, 1915, ii, 593.
 COURTELLEMONT, *Paris Med.*, 1915, May 8.
 DAKIN, COHEN, and KENYON, *Brit. Med. Jour* 1916, Jan. 29.
 DAVIES, *Ibid.*, 1915, i, 140.
 DEAN and MOUAT, *Ibid.*, 1916, i, 77
 DEHNE and HAMBURGER, *Wien. klin. Woch.*, 1907, July 4.
 DIXON and HALLIBURTON, *Jour of Physiol.*, 1916, i, 198.
 DÖNITZ, *Deut. med. Woch.*, 1897, 428.
 DOYEN, *Compt. rend. Soc. de Biol.* 1914, 77, 504.
 EMBLETON, *Trans. Med. Soc. Lond.*, xxxviii.
 FOULERTON and CAMPBELL THOMSON, *Med.-Chir Trans.*, London, 1900, lxxxiii, 105.
 FRANCIS, *Hygienic Laboratory Bulletin No. 95, United States Public Health Service*, 1914.
 GRAFF, *Mitteil. a. d. Grenzgeb. d. Med. u. Chir.*, 1913, xxv, 145.
 GRAFF and MENSCHIKOFF, *Centr. f. Bakt.*, 1912, I, Orig., lxi, 226.
 HALLIBURTON and DIXON, *Jour of Physiol.*, 1916, i, 198.
 HILL, *Arch. Internal Med.*, 1911, viii, 747
 HUPPEL, *Münch. med. Woch.*, 1915, No. 30.
 JACOBSTHAL, *Münch. med. Woch.*, 1914, 2079.
 KIRMISSON, *Bull. de l'Acad. de Med.*, 1914, lxxi, 746.
 KITASATO, *Zeits. f. Hyg.*, 1890, vii, 1891, x, 1892, xii.
 KNUD FABER, *Berl. klin. Woch.*, 1890, No. 31.
 KOCH, *Deut. Zeits. f. Chir.*, 1898, xlviii, 417
 KOCHER, *Corresp.-Blatt f. schweiz. Aertze*, 1915, No. 40.
 LEITCH, *Brit. Med. Jour.*, 1916, Feb. 12.
 LEVIN, *Zeits. f. Immunitätsforsch.*, Orig., i, 3.
 LOEWI and MEYER, *Arch. f. exper Path. u. Pharmakol.*, 1908, Supplement. vol., 355.
 Löwy, *Wien. klin. Woch.*, 1915, 1288.
 MACCONKEY, *Brit. Med. Jour.*, 1914, ii, 609, *Ibid.*, 1915, ii, 849.
 MCCLINTOCK and HUTCHINGS, *Jour Infect. Dis.*, 1913, xiii, 309.
 MANOUELIAN, *Ann. de l'Inst. Pasteur*, xxix, 440.
 MARIE and MORAX, *Ibid.*, 1902-03.
 MELTZER, *Lancet*, 1915, i, 1330, *Jour Amer Med. Assoc.*, 1916, lxvi, 931.
 MEYER and RANSOM, *Arch. f. exper Path. u. Pharmakol.*, 1903, xlix, 369.
 MOHLER and EICHHORN, *Report 28, Bureau of Animal Industry*.

- MONTAIS, *Ann. de l'Inst. Pasteur*, 1915, xxix, 369.
 NATONEK, *Cent. f. d. Grenzgeb. d. Med. u. Chr.*, 1913, xvii, 196.
 NICOLL, *Jour Amer Med. Assoc.*, 1915, lxiv, 1982.
 NIVIERE, *Bull. de l'Acad. de Med.*, 1915, March 30.
 NOBLE, *Jour Infect. Dis.*, 1915, xvi, 132.
 NOON, *Jour Hyg.*, 1907, vii, 101.
 NOETZL, *Forts. d. Med.*, 1898, xvi, 201.
 PARK, FAMULENER, and BANZHAF, *Jour Infect. Dis.*, 1914, xiv, 350.
 PARK and NICOLL, *Jour Amer Med. Assoc.*, 1914, July 18.
 PERMIN, *Mitteil. a. d. Grenzgeb. d. Med. u. Chr.*, 1914, xxvii, 1.
 PEXA, *Wien. med. Woch.*, 1907, 1554 and 1603.
 PLAUT, REHN, and SCHOTTMULLER, *Leitfäden z. Untersuchung d. Zerebrospinalflüssigkeit*, Jena, 1913.
 POZZI, *Bull. de l'Acad. de Méd.*, 1915, Nov 9.
 RANSOM, *Berl. klin. Woch.*, 1901, 387, 373.
 REINHARDT and ASSIM, *Centr f. Bakt.*, 1909, I Orig., xlix, 583.
 RÖMER, *Zeits. f. Immunitätsforsch.*, 1909, Orig., i, 363.
 ROSE, *Der Starrkrampf beim Menschen*, Stuttgart, 1897.
 ROUX and BORREL, *Ann. de l'Inst. Pasteur*, 1898.
 RUSS, *Brit. Med. Jour.*, 1916, Jan. 22.
 SEMPLE, *Scientific Memoranda by Officers of the Medical and Sanitary Departments of the Government of India*, 1911, No. 43, new series.
 SHERRINGTON, *The Integrative Action of the Nervous System*, London, 1911.
 SMITH, *Jour Hyg.*, 1907, vii, 205.
 SOPHIAN, *Epidemic Cerebrospinal Meningitis*, London, 1913.
 STADLER, *Berl. klin. Woch.*, 1914, pp. 15, 109.
 STINTZING, *Centr f. d. Grenzgeb. d. Med. u. Chr.*, 1898, iii, 461.
 STRAUB, *Münch. med. Woch.*, 1915, No. 10.
 TAROZZI, *Centr f. Bakt.*, 1906, I Orig., xl, 305.
 TEUTSCHLAENDER, *Deuts. med. Woch.*, 1915, Nos. 20 and 49.
 TORRES, Abstract in *Jour Amer Med. Assoc.*, 1912, ii, 2014.
 VAILLARD, *Medicaments Microbiens*, Paris, 1912.
 VAILLARD and VINCENT, *Ann. de l'Inst. Pasteur*, 1891-92, 1904.
 WOLF, *Münch. med. Woch.*, 1915, No. 39.
 ZUELZER, *Berl. klin. Woch.*, 1915, No. 26.
 ZUPNIK, *Deut. med. Woch.*, 1900, 837.