The apparent infrequency of infection, as compared with exposure, has led to the belief that the infectivity of Treponema pallidum is much less than that of the great majority of pathogenic organisms, and that infection is not apt to occur unless virulent spirochetes are brought in intimate contact with some skin or mucous surface where there is an abrasion or other lesion. This conception of the infectivity of Treponema pallidum is supported, to some extent, by experimental observations, and in principle, is doubtless correct. Still, the great mass of evidence bearing on these points is based on the occurrence or non-occurrence of outspoken manifestations of syphilis and very little is known as to what actually happens among those who are equally exposed but, in the absence of obvious lesions, may never consult the syphilologist. There are, however, many patients in whom a syphilitic infection of obscure origin is eventually detected. Some of these give a history that indicates the previous occurrence of some manifestation of syphilis, while in others the infection appears to have pursued an asymptomatic course for a long or indefinite period of time.

The group of persons in whom syphilis tends to pursue a mild or asymptomatic course is of especial importance from the standpoint of the transmission of the disease, but they are also important in that they show that infections may occur which do not pursue the classical course. At the present time, it is impossible to say how frequent such infections are, or to what they may be attributed.
The resistance of the patient and the virulence of the organism certainly play a part in the occurrence of these conditions, but there is some evidence to show that the circumstances under which the infection is contracted may be of equal importance.

In man, it is a well established fact that infection may take place without the production of a characteristic primary lesion (1), and such cases are frequently observed in rabbits inoculated in the skin. Moreover, Reasoner (2) has shown that with rabbits, the presence of a gross lesion of the genital mucosa is not essential to successful inoculation; that in occasional instances mere contact of Treponema pallidum is sufficient to lead to infection, as indicated by the development of a chancre. What happened in other instances could not be determined on account of the lack of suitable diagnostic criteria.

In like manner, experience has shown that many factors may affect the results of experimental inoculations. This fact is clearly brought out in a recent paper by Chesney (3) who studied the influence of such individual factors as sex, age, and site of inoculation, in animals where all other conditions were constant. In fact, it is apparent from these experiments, that the circumstances under which a syphilitic infection is contracted form one of the major conditions that determine the general course of the disease.

In this connection, we wish to report the results of experiments that were carried out with three strains of Treponema pallidum to determine whether infection could be produced in any considerable proportion of animals by bringing these organisms in contact with apparently normal mucous membranes, and how such infections compared with those produced by mechanical inoculations of the same organism. The experiments were carried out in the spring of 1921. A preliminary report was published at that time (4) and reference has been made to the experiments on several occasions, but hitherto they have not been reported in full.

EXPERIMENTAL.

Methods and Material.—The results of the application of Treponema pallidum to normal mucous membranes were studied in three groups of male rabbits, using the penile mucosa and the conjunctiva as sites of inoculation. The animals were carefully examined prior to in-
oculation in order to make sure that there was no visible lesion of the mucosa in particular, and that there were no lesions in other parts of the body that might give rise to confusion. In like manner, the inguinal and popliteal nodes were examined in order to exclude animals with preexisting abnormalities.

Inoculations were made with testicular emulsions using a dose of 0.05 cc. which was carefully instilled into the conjunctival sac or the sheath. In the first instance, the lower lid of one eye was retracted to form a pouch for the reception of the emulsion, while with genital inoculations the sheath was drawn forward in a similar manner. In each case, manipulations were confined to the skin and the parts were held in position for about 30 seconds after the emulsion had been introduced. Most of the fluid escaped when the parts were released, leaving only a thin film covering the surface of the membranes.

Experiment 1.—In the first experiment, six animals were inoculated in the right eye and on the sheath with the Nichols strain of Treponema pallidum. The emulsion used contained three to six spirochetes to the microscopic field (Table I, Group A). The experiment was controlled by six other animals inoculated intracutaneously on the sheath with 0.05 cc. of the same emulsion (Table I, Group B).

Experiment 2.—The second experiment was also carried out with six animals, inoculated in this case with the Zinsser-Hopkins strain of pallidum, using an emulsion containing twelve to fifteen spirochetes to the microscopic field. Three of the animals were inoculated in the right eye (Table II, Group A) and three on the sheath (Table II, Group B). There were again six controls, but in this instance the control animals were inoculated in both testicles with 0.2 cc. of the emulsion (Table II, Group C). This was done as a means of establishing a basis for comparison with large series of animals in which this method of inoculation had been used.

Experiment 3.—The third experiment was carried out merely for the purpose of determining whether organisms of low virulence were capable of gaining entrance to the body through normal mucous membranes. Three animals were inoculated in the right eye and sheath with a recently isolated strain of pallidum. The emulsion contained twelve to fifteen spirochetes to the microscopic field. No controls were inoculated.

Results.

The results of the experiments cited above may be considered from two standpoints: First, the occurrence of infection, and second,
the character and course of the disease as compared with that produced by various methods of inoculation involving a mechanical introduction of spirochetes into the body.

Occurrence of Infection.—The criteria used for determining infection were: (1) the occurrence of a local lesion in which spirochetes could be demonstrated; (2) enlargement and induration of drainage lymph nodes; (3) general lymphadenitis; (4) the occurrence of metastatic lesions in other parts of the body, especially the scrotum and testicles. The data covering these points in Experiments 1 and 2 are tabulated in Tables I and II, respectively.

By reference to Table I, it will be seen that all of the animals in the first experiment developed a local lesion at one or the other of the two sites of inoculation. In only one instance, however, was there a lesion at both sites (Figs. 1 and 2), while two other animals showed suggestive conditions about the eye with definite lesions of the penile mucosa.

All of the animals also showed definite enlargement and induration of the inguinal lymph nodes (Fig. 3) as well as a general lymphadenitis.

Metastatic lesions occurred in the scrotum (Fig. 3) of all animals and definite lesions were detected in the testicles of four of the six animals.

The time of occurrence of the several groups of lesions is especially interesting. The incubation period of those at the site of inoculation varied between 34 and 108 days, with an average of 54 days in the case of the eye, and 59 days for the sheath, which is much longer than that of primary lesions in control animals. A definite inguinal adenitis was recognized within a few days after inoculation, while enlargement and induration of the popliteal lymph nodes occurred within 3 to 7 weeks. In several instances scrotal lesions were recognized before any abnormality could be detected at the site of inoculation.

It was possible, therefore, to establish the occurrence of an infection in all of the animals of this group on the basis of a lesion at one or the other of the two sites of inoculation, as well as by the occurrence of a local and general lymphadenitis, and metastatic lesions in the testicles or scrotum. It is important to note, however, that in several instances the first lesions to appear were not at the site of
inoculation, and further, that during the prescribed period of ob-
servation, none of the animals inoculated in this manner developed
generalized lesions other than those of the scrotum and testicles.

The results of the second experiment (Table II) were much the
same as those of the first. The one difference to be noted is in the
case of Rabbit 1, Group B. This animal developed no lesion at
the site of inoculation, but infection was first established by the
development of a small cutaneous papule in the scrotum, and later
by the occurrence of other lesions in the testicles and scrotum, as
well as by enlargement and induration of the inguinal and popliteal
nodes.

The third experiment was carried out with a recently isolated
strain of *pallidum* whose virulence was comparatively low, and here
the results were less obvious than in the two preceding experiments.
Only one of the three rabbits developed clearly defined syphilitic
lesions at the sites of inoculation. The two others showed no lesion
about the eyes but both developed minute and ill defined areas of
redness and apparent infiltration in the region of the urinary meatus,
which may or may not have been syphilitic. One of these animals
showed a papular lesion in the scrotum, and a second papule in the
skin near the anus, while the other developed a definite metastatic
orchitis, and all three animals showed a moderate but definite en-
largement and induration of the inguinal lymph nodes (Fig. 4).

It was possible, therefore, to demonstrate infection in these animals
as in the others, although only one of them developed a characteristic
lesion at the site of inoculation.

**Character and Course of Disease.**—In comparing the disease pro-
duced in rabbits by entrance of spirochetes through apparently intact
mucous membranes with that produced by ordinary methods of
mechanical inoculation, the points to be given especial consideration
are: (1) the occurrence or non-occurrence of a so called primary
lesion, (2) the character of the primary lesion, (3) the occurrence of
generalized lesions, and (4) the general course and severity of the
disease.

Of twelve animals inoculated by applying a spirochete emulsion
to the genital mucosa, seven developed definite lesions at the site
of inoculation, but none of these presented the appearance of a typical
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Group A. Animals inoculated on mucous membranes.
Group B. Control animals inoculated intracutaneously on the sheath.

? indicates result or condition uncertain; -, negative; +, positive where used with reference to lymph nodes, elsewhere indicates also slight or mild affection; ++, moderate affection; ++++, well developed lesions or severe disease; +++++, marked lesions or very severe disease. B., bone; E., eye; M. M., mucous membranes; S., skin.
### TABLE II.

**Results of Experiment 2.**

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Group A. Conjunctival inoculations.
Group B. Sheath inoculations.
Group C. Controls.

? indicates result or condition uncertain; -, negative; +, positive where used with reference to lymph nodes, elsewhere indicates also slight or mild affection; + +, moderate affection; + ++, well developed lesions or severe disease; + ++++, marked lesions or very severe disease. B., bone; E., eye; M. M., mucous membranes; S., skin.
cutaneous chancre. The majority of them were situated in the region of the urinary meatus. In three instances the lesions measured as much as 6.0 to 8.0 mm. in their greatest diameter; they were clearly defined, well indurated, and showed some tendency to superficial necrosis and ulceration (Figs. 2, 5, and 6). The lesions in other animals were either extremely small, as in Fig. 7, or were rather ill defined processes of infiltration, and with the exception of the three animals in which the lesions were most characteristic, the conditions present might have been overlooked, had attention not been focussed on the genital mucosa, or they might have been mistaken for *cuniculi* lesions, had there been no other evidence of a syphilitic infection.

In like manner, from the same number of eye inoculations there were also seven instances in which definite lesions developed at the site of inoculation. In two animals the lesions were large, clearly defined granulomas. One of these involved the conjunctiva, episcleral tissues, and cornea and was associated with an erosion on the contiguous surface of the upper lid (Fig. 8), while the other was confined to the lower lid (Fig. 1). All other lesions were of the type of small indurated papules, most of which were on the margins of the lids, as in Fig. 9, suggesting that infection had taken place by entrance of spirochetes into the ducts of palpebral or sebaceous glands.

Of the three animals inoculated with the strain of low virulence, there was only one that developed a characteristic local affection, and even in this instance the lesions were very slight. This feature of the results serves to indicate that in experiments of this kind, the virulence of the organism used operates in precisely the same way as with other forms of inoculation.

Among the fifteen animals used in the three experiments, there were only eight in which a diagnosis of syphilitic infection probably could have been made on the basis of the lesions at the site of inoculation; three showed no definite lesions, and four showed slight lesions of indefinite character. Nevertheless, all of the animals presented other evidences of syphilitic infection. An inguinal adenitis was present in all of those inoculated on the genitalia, and nearly all of the animals showed a definite enlargement and induration of the popliteal lymph nodes. In addition, there were lesions in the testicles and scrotum.
The disease as a whole differed materially, however, from that in the control animals or from the disease ordinarily observed after mechanical inoculations of the testicles or skin. To begin with, the lesions at the site of inoculation were relatively small and ill defined; the incubation period was long, and in several instances definite lesions appeared at some remote point, such as the scrotum or testicles, before any lesion could be recognized at the site of inoculation or where no definite primary lesion occurred.

Moreover, the metastatic lesions in the testicles and scrotum were, like those at the site of inoculation, extremely slight. The scrotal lesions were all minute indurated papules (Fig. 3) or small plaques of infiltration, while those in the testicles assumed the form of small nodules or slight diffuse enlargements and infiltrations followed by atrophic changes. In only one instance (Table II, Group A, No. 3) was there an extensive orchitis and this did not develop until 7 months after inoculation.

The most notable features of the infection produced by this method of inoculation, however, were the absence of recognizable lesions in distant parts of the body other than those in the testicle and scrotum, and the extremely mild course of the disease throughout the prescribed period of observation.

The deviation from the usual course of events is most clearly brought out in the first experiment (Table I), where control animals were inoculated intracutaneously with the same dose of the emulsion as was used for the mucous membrane inoculations. In this instance, four of the six controls developed generalized lesions; in two instances there was an extensive involvement and the course of the disease was very severe. In fact, one animal was never able to overcome his infection and still showed extensive lesions at the time of death, 12 months after inoculation. There was another animal in which the disease was moderately severe, but the remaining three showed conditions that were comparable to the most pronounced infections in the experimental group.

In this connection, the fact should be pointed out that the results obtained in the control animals of this series showed that the virus used was highly active, and that other conditions that influence the course of syphilitic infections were especially favorable, as indicated by both the incidence and severity of generalized lesions.
A similar situation is brought out in the second experiment (Table II). Here, the purpose of the control was primarily that of a gauge of the activity of the virus and of the prevailing course of the disease, as indicated by a comparison with numerous experiments in which this method of inoculation had been used. There again, the results obtained in the control animals showed that the disease produced was more severe than usual. The incidence of generalized lesions was considerably increased and the involvement more extensive.

In this experiment, there were two possible cases of generalized disease among the animals inoculated on the mucous membranes, but in both the lesions were very slight. One animal developed a single papule on the sheath (autoinoculation?) while, some 7 months after inoculation, the other developed a diffuse infiltration on the penis, which was indistinguishable from a cuniculi lesion.

Nothing can be said as to the generalized disease in the third experiment since this strain rarely produced generalized lesions by any method of inoculation, and those that occurred were always relatively slight.

DISCUSSION AND CONCLUSIONS.

The experiments reported show that under favorable conditions Treponema pallidum is capable of gaining entrance to the body of a normal rabbit when brought in contact with apparently normal mucous membranes. It is further shown that no recognizable lesion may occur at the portal of entry, or else it may be so slight as to escape detection; in any case, the lesion produced is apt to be small and ill defined as compared with primary lesions in the skin or testicles. Moreover, these lesions develop slowly and may not appear until some time after the development of lesions in distant parts of the body. Finally, it is shown that where infection originates in the manner indicated, the clinical course of the disease is apt to be entirely different from that produced by the usual methods of inoculation. With possible rare exceptions, the disease is extremely mild and in many instances, the existence of an infection might be overlooked, unless the most careful observations are carried out.

The explanation of these conditions is somewhat uncertain, but in general, the course of events is in line with what is known to occur
when infection takes place under conditions that are favorable to the host or are unfavorable to the spirochetes. In the first place, the failure to develop a typical chancre and the inconspicuous nature of the lesion at the portal of entry, as well as the prolonged incubation period, may be accounted for by the absence of any localizing influence, such as exists with other methods of inoculation, or in instances where there is a superficial trauma which favors both entrance and localization of spirochetes.

In like manner, the atypical course of disease may be attributable to the entrance of only a few organisms, or to lowering of the vitality of the spirochetes before they finally reach points that are favorable to their growth and multiplication. At all events, infection develops slowly, and wherever spirochetes become localized and begin to multiply, they are almost immediately suppressed, and for several months at least, they are unable to make sufficient headway to produce more than the most insignificant lesions in those parts of the body where the most pronounced lesions usually occur.

Unfortunately, the work had to be discontinued at this point and we were never able to complete the experiments that had been planned. But, the subsequent course of events may be predicted with a high degree of probability on the basis of the general principles of the interaction between Treponema pallidum and the animal host (5, 6). From what has been observed in many other experiments, it seems safe to assume that since no lesions of any consequence were produced during the first few months, the resistance or immunity developed was correspondingly slight and served mainly to curb the activity of the spirochetes in certain locations, but was not sufficient to suppress the infection or to prevent the occurrence of lesions in tissues that had not become involved. If this is true, as is the case with infections produced by other methods of inoculation, it would be expected that the infection would remain active longer than usual, and that from time to time new lesions would appear, and eventually the disease might assume relatively severe proportions with a tendency to the occurrence of lesions in parts that ordinarily would be protected by a vigorous reaction during the early stages of the infection. This did happen in the one animal of the series that was held beyond the allotted period of observation (Table II, Group A, No. 3).
The peculiar course of the infection observed in these animals appears to be closely analogous to the conditions that exist in those patients in whom syphilis pursues a mild or asymptomatic course during the early stages of the infection, and who eventually come under observation on account of some visceral affection or involvement of the central nervous system. As has been said, this course of events might be attributable, on the one hand, to an unusually high resistance on the part of individual patients, or to infection with an organism of low virulence, on the other. It is clear, however, that the same conditions may arise from still another cause; namely, the circumstances under which the infection is contracted. Just as the constitutional resistance of the patient or the virulence of the spirochete may affect the course of disease in accordance with well established principles of biological reaction, so also, such factors as the portal of entry, the mode of entrance, and the viability of the organism at the time of entrance into the body, may operate in such a manner as to increase or diminish the severity of the disease, or to change the clinical course of the disease in accordance with the same general principles of biological reaction.

It is believed, therefore, that the facts brought out in these experiments throw some light on the origin of obscure cases of syphilitic infection. Moreover, they are of equal importance in any consideration of conditions that are necessary for the production of an infection, or of factors that determine the clinical characteristics of the disease.

SUMMARY.

Experiments were carried out with three strains of Treponema pallidum to determine whether infection could be produced by applying an emulsion, rich in spirochetes, to normal mucous membranes of rabbits and whether an infection produced in this manner differed in any respect from one produced by other methods of inoculation.

It was found that a simple instillation of a spirochete emulsion into the conjunctival sac or the sheath was all that was necessary to obtain an infection. Still, the infection produced in this manner differed from that produced by intracutaneous or testicular inocula-
TREPONEMA PALLIDUM

tions in that it tended to pursue a mild or asymptomatic course and frequently without the development of a characteristic chancre.

It is pointed out that these experiments may have a bearing on problems pertaining to obscure and atypical cases of human syphilis as well as the more immediate question of the infectivity of Treponema pallidum.

BIBLIOGRAPHY.


EXPLANATION OF PLATES.

Figs. 1 to 4 are reproductions of photographs not retouched; Figs. 5 to 9 are reproductions of colored drawings made directly from the objects represented. All objects are represented at their natural size. Statements of time are from the date of inoculation.

PLATE 33.

**Fig. 1.** 84 days. Chancre of the lower lid. (Table I, Group A, No. 1.)

**Fig. 2.** 49 days. Chancre of penis. Same animal as Fig. 1.

**Fig. 3.** 54 days. Inguinal adenitis with multiple papular lesions in both scrota. Same animal as in Figs. 1 and 2.

**Fig. 4.** 10 days. Inguinal adenitis in an animal inoculated with a strain of low virulence. There was no lesion at the site of inoculation.

PLATE 34.

**Fig. 5.** 64 days. A later stage of the lesion in Fig. 2, showing a clearly defined nodule at one side of the meatus.

**Fig. 6.** 82 days. A meatal chancre. (Table II, Group B, No. 3.)

**Fig. 7.** 54 days. A minute but clearly defined chancre on the penis. (Table II, Group B, No. 2.)

**Fig. 8.** 64 days. Primary granuloma of the eye. (Table I, Group A, No. 3.)

**Fig. 9.** 67 days. Multiple papular lesions on the margins of the lids. (Table II, Group A, No. 3.)
(Brown and Pearl: Treponema pallidum.)
(Brown and Pearce: Treponema pallidum.)