

NOTE ON THE PRESERVATION OF STOCK STRAINS OF
TREPONEMA PALLIDUM AND ON THE DEMON-
STRATION OF INFECTION IN RABBITS.

BY WADE H. BROWN, M.D., AND LOUISE PEARCE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, March 2, 1921.)

The maintenance of stock strains of *Treponema pallidum* for teaching or experimental purposes has been both time-consuming and expensive, due to the necessity for constant watchfulness and frequent transfer from animal to animal in order to guard against loss of strains, but no way has been found to overcome these difficulties, and in spite of the greatest care, valuable strains are frequently reported as lost.

In like manner, the usefulness of *pallidum* infections in rabbits for experimental purposes has been subject to serious limitations on account of the lack of reliable means for determining end-results as regards infection when no demonstrable lesions were present. This has been especially true in chemotherapeutic investigations in which the method for determining a cure, and the only one available, was more or less prolonged clinical observation. It has been assumed that healing of existing lesions without recurrence within a short period of time (1 to 3 months) constituted evidence of cure and the results of all experiments thus far reported have been based upon the use of criteria of this kind.

The supposed necessity for frequent transfers, the presumed loss of stock strains of *Treponema pallidum*, and the supposition that freedom from lesions constituted evidence of cure were all based upon the belief that *pallidum* infections in the rabbit are self-limiting—that with the healing of the lesions the infection also became extinct. Numerous isolated observations by ourselves and others have cast considerable doubt upon the validity of these earlier views, but until quite recently, no systematic experiments were carried out for the

purpose of determining the exact nature of the infection in the rabbit as regards dissemination of organisms, the duration of the infection, and the possibilities of recovering the virus from animals after all manifestations of disease had disappeared.

During the past 2 years, a large series of experiments has been carried out in an attempt to obtain definite information upon these points. One of the methods employed was that of test inoculations of normal animals with material from superficial lymph nodes of infected animals. Parts of this work have been reported in connection with studies on the dissemination of spirochetes (1, 2) and the demonstration of spirochetes in the lymph nodes during latent periods of infection (3).

The details of these experiments need not be repeated; it will suffice to state that test inoculations have been made from inguinal or popliteal nodes of 51 rabbits with positive results in all instances. The material studied included four classes of animals: (1) animals with developing or active infections of from 48 hours to 2½ years duration; (2) animals with latent infection in which no lesions had been present for from 3 months to 2 years with a period of infection ranging from 7 months to 4 years and 3 months; (3) drug-treated animals in which no lesions had recurred during a period of 3 months observation; (4) animals used for serial passage of *Treponema pallidum* from lymph node to testicle over a period of about 14 months. The majority of the tests were carried out during the first 3 months of the infection.

Before it could be concluded that the organisms recovered were localized in the tissues, it was necessary to exclude the blood as a possible source. During early stages of the infection, this could be done only upon the basis of relative infectivity. Later, however, blood inoculations were uniformly negative while the lymph nodes gave positive results.

The experiments cited showed that with old strains of *Treponema pallidum*, generalization and localization of the organisms in lymphoid tissues are a constant phenomenon of the infection; they also indicated that the infection is permanent and that the treponema can be recovered at any time by inoculation of material from superficial lymph nodes of infected animals. It thus appears that while rabbits acquire a high degree of protection against the toxic effects of *Treponema*

pallidum, they are no more capable of terminating the infection than is man.

Whether the same conditions hold true for recently isolated strains is not entirely certain, but since the latter exhibit the same tendencies to lymphoid involvement as the older strains, it is not unlikely that they too are capable of surviving in the rabbit for an indefinite period of time.

Once it has been shown that there is a constant and permanent localization of *Treponema pallidum* in the lymphoid tissues of infected animals, there are many applications of these facts which are quite obvious, and the possibility of utilizing them in connection with the preservation of stock strains and as a means of determining the presence of infection in experimental animals appeared to be of sufficient importance to warrant a special note upon this subject.

The method proposed for the preservation of stock strains of *Treponema pallidum*, when not in active use, is merely to keep a sufficient number of infected animals to guard against loss of the strain by their death. Serial transfers may be dispensed with. When it is desired to recover the organism for teaching or experimental purposes, a popliteal node may be excised with aseptic precautions, minced, and ground in a mortar; an emulsion is then prepared by the addition of about 1.5 cc. of sterile normal salt solution with further grinding. The resulting fluid is aspirated into a syringe fitted with a No. 22 gauge needle and about 0.5 cc. of the emulsion is injected into a testicle of one or more normal rabbits. In order to allow ample time for the development of a testicular infection, the inoculation should be made 6 to 8 weeks before the organism is needed.

Exactly the same method is applicable to the demonstration of infection in experimental animals. The circumstances should determine in each case when test inoculations are to be undertaken. In chemotherapeutic experiments, for example, it would appear to be advisable to follow the old system of clinical observation for at least 1 to 2 months before resorting to test inoculation; otherwise an infection which had been almost extinguished might not be given a sufficient opportunity to reestablish itself.

The essential requirements of the method are extremely simple: One is advised against complicating the technique by the use of

foreign substances to aid in the grinding of material; filtration is unnecessary; the injection of large amounts of fluid tends to produce inflammatory reactions in the testicles which may obscure subsequent lesions, and the use of strong antiseptics, either in the removal of lymph nodes or in making inoculations, is contraindicated.

Eberson and Engman (4) have used a similar method with success in demonstrating infection in the lymph nodes of human subjects with latent syphilis, thus establishing another analogy between the human and animal infection.

Positive results from testicular inoculation are usually not difficult to determine. It should be noted, however, that atrophy of the testicle may occur instead of the usual granulomatous enlargement and that in exceptional instances, infection may be recognized by the development of an adenopathy when no lesions can be detected at the site of inoculation.

By the use of the method described, a great saving in time and expense may be accomplished, and infection can be determined with comparative ease and with much greater certainty than was hitherto possible. However, until the delicacy of the method has been subjected to further test, negative results are still to be accepted with reserve.

SUMMARY.

Experiments carried out on rabbits infected with *Treponema pallidum* showed that there was a constant invasion and localization of the organisms in the superficial lymph nodes, that the infection persisted indefinitely, and that organisms could be recovered at any time from such nodes as the popliteals. Based upon these observations, a method is proposed for the preservation or recovery of stock strains of *Treponema pallidum* and for the demonstration of infection in rabbits.

BIBLIOGRAPHY.

1. Pearce, L., and Brown, W. H., *Proc. Soc. Exp. Biol. and Med.*, 1920, xvii, 164.
2. Brown, W. H., and Pearce, L., *Arch. Dermatol. and Syphilol.*, 1920, ii, 470.
3. Brown, W. H., and Pearce, L., *Am. J. Syphilis*, 1921, v, 1.
4. Eberson, F., and Engman, M. F., *J. Am. Med. Assn.*, 1921, lxxvi, 160.