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AN EXPERIMENTAL STUDY OF THE POSSIBILITY OF TRANSMITTING SYPHILIS BY A CORNEAL GRAFT*†

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The purpose of this experiment is to determine whether or not syphilis can be transmitted from one animal to another by means of a corneal graft. From the point of view of the clinician performing a corneal graft operation, the pertinent question is: "Can syphilis be transferred from donor to host if the donor's eye is free from all evidence of past or present inflammation even though the donor has an undetected early or latent syphilis?" This question was investigated experimentally.

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LITERATURE

It has been abundantly demonstrated that syphilis can be transmitted by blood transfusion during certain early stages of the disease. Uhlenhuth and Mulzer,[1] and Frühwald[2] showed that the blood from patients in the primary stage of syphilis transmitted the disease in 84.2% of cases tested. In secondary syphilis, they found the transmission incidence to be 75%. Brown and Pearce[3] have shown that the blood of a syphilitic rabbit becomes so infectious between the seventh and fourteenth day following inoculation that 5 cc. will transmit the disease. They concluded that the syphilitic virus is present in the blood during the acute or active stage of the infection and with the disappearance of active lesions the blood loses its infectiousness. Eberson and Engman[4] tested blood from 73 individuals with latent or chronic syphilis by injection into rabbits' testes. The results were negative.

The blood of patients in the incubative (sero-negative) phase of syphilis is apparently infectious. Morgan[5] reported 16 cases, Salkind[6] 39 cases, Klauder and Butterworth[7] 35 cases and Eichenlaub and Stolar[8] 41 cases. In these patients with early incubative syphilis they found the blood to be infectious. On the other hand, according to Morgan, there is not a single instance reported of indubitable transmission of syphilis by transfusion in which the disease was transmitted by blood from a donor with well-established latent or chronic syphilis (uninfluenced by pregnancy). McNamara,[9] in an emergency, was forced to transfuse nonsyphilitic patients with blood from chronic syphilitics. No evidence of infection was found from 4 to 5 months after transfusion. McCluskie[10] states that in his experience, infection during the tertiary stage does not occur even though the Wassermann reaction of the blood is positive.

Despite these rather conclusive statements, Moore[11] emphasizes that it is not possible to set any definite time limit on the infectiousness of blood since cases have been reported where symptoms have occurred from donors infected

at least 7 years. He states that in blood transfusion, the best practical method of avoiding syphilitic infection is the rejection of all possible donors with syphilis. Stokes[12] also vigorously emphasized that the clinical principle of avoiding transfusion infection is not to use blood or serum, spinal fluid or tissues of persons known to have had syphilis without adequate sterilization. Turner and Diseker's[13] report indicates that the risk of transfusion syphilis is minimized by the use of blood banks. They showed that in isolated rabbit blood or tissue extract, treponemes apparently do not survive longer than 72 hours. They conclude that under conditions obtaining in blood banks, the treponeme undergoes complete deterioration in citrated whole blood during the usual storage period. There is a risk in the transmission of syphilis by transfusion with freshly stored blood but blood stored for 4 days or longer will no longer transmit the disease. It is interesting to note that their experiments were performed with fresh tissue extract which contained obviously motile treponemes from which transfusion syphilis would be expected to occur.

While it thus appears well established that in latent syphilis, the possibility of transmission by blood transfusion is minimal, this apparently is not true with tissue extracts. Eberson and Engman[4] isolated treponemes in 5 instances from latent syphilitics—3 times from inguinal glands and twice from the semen, the tissue extracts producing typical syphilitic lesions when injected into a rabbit's testis from which it could be recovered and propagated indefinitely. This investigation seems to demonstrate that those persons giving a history of an old syphilitic infection may harbor active treponemes in the tissue for years although the Wassermann reaction may be negative or only slightly positive with cholesterinized antigens.

There is considerable evidence that treponemes are present in the eyes of congenital syphilitics in the first stages of the disease even though the eyes are free of clinical inflammation. Yoshida,[14] Rumbaur[15] and Friedenwald[16] made histologic

examinations of the eyes of syphilitic fetuses and stillborn syphilitic infants and found minor changes throughout the uveal tracts in a large percentage of such eyes. Treponemes in the noninflamed corneas of such eyes have been demonstrated by Stock,[17] Bab,[18] Waetzold,[19] Schlimpert[20] and von Hippel.[21]

It is, however, not fully agreed that living treponemes are present in the corneas of patients with active interstitial keratitis. In favor of this assumption are the reports of von Hippel[21] who in 1908 demonstrated by Levaditi's stain that treponemes were present in the cornea of a stillborn syphilitic fetus; of Igersheimer[22] who removed a section of the cornea of a 14-year-old child with active interstitial keratitis and demonstrated treponemes in the stained section; and of Clausen[23] who also found treponemes in the cornea of a 4½-month-old infant, the child later dying of a generalized syphilitic infection. However, the Levaditi technique may be misleading and modern syphilologists feel that other proof is necessary to establish the actual presence of the treponeme. Further in favor of treponemes being in the cornea is the fact that it has been clearly shown by transmission experiments that they are invariably present in the corneas of experimental syphilitic rabbits with spontaneous interstitial keratitis. There is, however, some clinical evidence which has been interpreted as indicating treponemes are not present in the corneas of patients with interstitial keratitis. If such corneas did contain treponemes, one might reasonably expect them to be more responsive to antisyphilitic therapy than they usually are. As Duke-Elder[24] states, one would not expect the well-known refractoriness to treatment if the disease were due to the actual presence of the treponemes. Although it may undoubtedly be difficult for arsenical preparations to reach an avascular structure, such as the cornea, nevertheless, that arsphenamine does reach the cornea is shown by the experiments of Woods and deSchweinitz[25] who demonstrated the immediate beneficial effect from a

single injection of arsphenamine in the interstitial keratitis due to trypanosome infection. The trypanosome equiperdum is, however, infinitely more sensitive to arsenicals than are treponemes. Interstitial keratitis develops only rarely in acquired syphilis. The Cooperative Clinical Group reported only 0.3% incidence of interstitial keratitis in 3,244 patients with early acquired syphilis. It is well known that interstitial keratitis occurs rarely in early congenital syphilis where treponemes are widely disseminated throughout the body, but does occur in late congenital syphilis when patients may be receiving strenuous antisyphilitic treatment and when the other syphilitic lesions are progressing favorably. It seems, therefore, that proof as to the actual presence of treponemes in the corneas of patients with interstitial keratitis is not conclusive.

Summing this up, it is quite clear that the blood and tissue extracts of individuals or experimental animals in the early stages of acquired or experimental syphilis are definitely infectious. It has been shown that treponemes are present in the eyes of congenital syphilitics in the early stages of the disease. Woods and Chesney[26] believe they lie dormant in the cornea for years, later producing symptoms on fluctuations of immunity. The existing evidence, however, indicates that the blood of individuals with latent syphilis is at best only occasionally infectious, especially blood isolated from the body for 3 or 4 days. It is not established, however, that the tissues are equally noninfectious.

As concerns corneal transplantation, the essential question is: Can syphilis be produced in the recipient by the transplantation of a cornea from a syphilitic donor, where the cornea shows no evidence of any old or active inflammation? This question is an immensely practical one, especially in this day of Corneal Banks and the increasing number of corneal transplant operations.

This question was investigated experimentally in the following manner:

Experiment 1. Using 10 rabbits, each with latent syphilis but without a keratitis, corneal grafts were performed, the clear corneas from these rabbits serving as donors.* In none of the donor rabbits was there any clinical evidence of ocular syphilis and during the course of the systemic syphilis, ocular syphilis had not been observed. In 6 rabbits, the graft remained transparent and in 4 rabbits the graft became either nebulous or completely opaque. In no host rabbit was there the slightest clinical evidence of syphilitic keratitis. After 3 months, the host rabbits were sacrificed. Under rigid aseptic technique, the following procedure was carried out on each rabbit:

The whole cornea containing the graft was removed in its entirety, washed with saline, sliced in several sections and placed in a mortar to which was added 1 cc. sand and 2 cc. 80% normal saline. The corneal tissue was then thoroughly macerated and either centrifuged or allowed to stand for 10 minutes; 1 cc. of the supernatent emulsion was then drawn into a tuberculin syringe and ¼ to ½ cc. injected subconjunctivally into the right eye and the remainder into one testis of a normal male rabbit. These rabbits were observed at periodic intervals and during a period of 8 months no rabbit developed keratitis or orchitis.

There are 2 possible reasons for failure of this experiment: (a) either the cornea of the rabbit with latent syphilis does not contain treponemes, or (b) the cornea from which the graft was taken contained treponemes in such small quantities that transmission of the infection was not possible. Perhaps the latter is more significant inasmuch as the average graft taken is not more than $^1/_7$ to $^1/_3$ of the entire corneal surface.

Experiment 2. Assuming that the clear corneas of syphilitic rabbits contain treponemes and that the reason for failure in Experiment 1 was the small number of treponemes in an average graft, it should be possible to transfer syphilis

* These rabbits were obtained from the laboratory of Dr. Alan M. Chesney.

by direct testicular or subconjunctival inoculation of a large amount of corneal emulsion. The following experiment was carried out to explore this point.

Ten rabbits with latent syphilis were used. In none had there been evidence of ocular syphilis in any form. In 5 rabbits, 1 entire cornea from each rabbit was used, and in the other 5 rabbits, both corneas from each rabbit were used. Using the same aseptic technique as in Experiment 1, an emulsion was made of each cornea or corneas and injected subconjunctivally and intratesticularly into normal rabbits. These rabbits were observed over a period of 9 months and in none was there any evidence whatsoever of keratitis or orchitis. It can be assumed from this experiment that treponemes are not present in the corneas of rabbits with latent syphilis.

Experiment 3. To determine whether or not it was possible to transmit systemic syphilis by transferring the corneas from rabbits with active syphilis, the following experiment was carried out.

Twenty rabbits were used. Ten rabbits (Group 1) had been inoculated with syphilis from the syphilitic liver of either an ape or monkey and at the time of the experiment had syphilitic orchitis confirmed by positive dark field examination. The other group of 10 rabbits (Group 2) was injected with the Nichols strain of *t. pallida*, this group developing orchitis within 10 days to 3 weeks. At the height of the orchitis, both entire corneas from each rabbit in the 2 groups were excised, emulsified, and injected subconjunctivally and intratesticularly in 20 normal rabbits. As in Experiments 1 and 2, the same aseptic technique was carried out. These rabbits were observed at periodic intervals and during a period of 8 months there was no evidence of either orchitis or ocular involvement.

This experiment shows that the asymptomatic, noninflamed cornea of a rabbit with active syphilis is noninfectious.

COMMENT

The Nichols strain of *t. pallida* is an extremely potent one and has been passed through rabbits hundreds of times and is presumably well adapted to its host. Using this strain, Magnuson, Eagle and Fleischman[27] have shown that 2 organisms inoculated intracutaneously, produced a dark-field positive lesion in 47% of the cases. This was increased to 71% with 20 organisms and 68% with 200 organisms and finally, 100% with 200,000 organisms. With intratesticular inoculation, 1 to 2 treponemes were shown to be regularly infectious.

It seems reasonable, therefore, that in view of the negative response in the host rabbits outlined in the above experiments that the nondiseased, apparently normal corneas of rabbits with latent or active syphilis are noninfectious and do not transmit syphilis when transferred to normal rabbits.

One might assume the following clinical application to this experiment: Should the eye from an individual with undiscovered or latent syphilis be used as a donor for corneal grafting, these experiments would indicate that it is highly unlikely syphilis would be transmitted.

SUMMARY

1. The corneas from 10 rabbits with latent syphilis were transplanted to the corneas of 10 nonsyphilitic rabbits without any evidence of the development of keratitis during a period of 3 months.

2. These 10 corneas were removed in their entirety, emulsified and injected into the subconjunctival tissue of 1 eye and to 1 testis of 10 normal rabbits. Over a period of 8 months there was no evidence of keratitis or orchitis.

3. The corneas of 10 rabbits with latent syphilis (1 cornea in 5 rabbits and both corneas in 5 rabbits) were emulsified and injected into the subconjunctival tissue of 1 eye and to 1 testis of 10 normal rabbits. During a period of 9 months, there was no sign of either keratitis or orchitis.

4. Both corneas of 20 rabbits with active clinical syphilis were emulsified and injected into the subconjunctival tissue of 1 eye and to 1 testis of 20 normal rabbits with completely negative results for syphilis after a period of 8 months.

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