

THE CURRENT STATUS OF *TREPONEMA CUNICULI**†

REVIEW OF THE LITERATURE

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Experimental ocular and neurosyphilis has been investigated in this laboratory during the past three years in three species:

- (1) Rabbits.
- (2) Owl monkeys (*Aotus trivirgatus*).
- (3) Squirrel monkeys (*Saimiri sciurea*).

Rabbits were selected as a control to the primate experiments because they were used in the great majority of previous studies, and the obvious advantage of having a large available literature pertaining to experimental syphilis in the rabbit. The disadvantage of this species, however, is the occurrence of an endemic spirochaetal disease in the rabbit—benign venereal spirochaetosis—due to *Treponema cuniculi*.

The paucity of reports in English concerning *T. cuniculi* made detailed information concerning rabbit spirochaetosis difficult to obtain. No papers on this subject have appeared in the literature for nearly two decades. Furthermore, the older and foreign literature was found to bristle with differences of opinion concerning major aspects of the disease, such as the incidence, incubation period, morphology of the organism, infectiousness for other species, serological responses, ocular involvement, and the like. These factors prompted this review of the literature in order to determine the current status of *T. cuniculi* as related to experimental syphilis research.

History

The first mention of spontaneous rabbit spirochaetosis in the literature was made by Halford

Ross (1912) in a footnote in the *British Medical Journal*:

“Similar parasites to those found in syphilis have been found in the blood of rabbits. These rabbits exhibit chancres, buboes, ulcers of the genitalia, mouth, anus, etc., and some have white patches of the liver like the infected guinea-pigs. The rabbit parasites, which are very numerous in the sores, possess phases indistinguishable from the human parasites”.

Bayon (1913) reported on “A New Species of *Treponema* found in the Genital Sores of a Rabbit”. In the serum expressed from a penile lesion found in a rabbit he found spirochaetes by dark-field examination. He succeeded in infecting another rabbit with this material, and found that the inoculated lesion developed in 25 days. Bayon described the new organism as 1.5 times as long as the diameter of a rabbit erythrocyte, having 5 to 8 curves, being slightly oval on transverse section, and having tapered ends without terminal flagella. Transversely dividing forms were described. The movements on dark-field were noted to be slow and rotatory without lashing. He called the attention of workers on experimental rabbit syphilis to the existence of this new species of *Treponema*. Review of Bayon’s paper reveals, however, that the only evidence for considering this to be a “new species” of *Treponema* was the fact that the organism had been found in a rabbit rather than in man.

Ross (1914a), in a second and more definitive paper on rabbit spirochaetosis, again commented upon the occurrence of lesions in the livers of these rabbits and called these “tumours resembling gummata”. Ross stated that he was trying to infect monkeys with the organism, and also noted that in America reactive blood tests had been reported to occur in some sera from normal rabbits.

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Ross's statement about blood reactions referred to the work of Kolmer and Casselman (1913), who found positive complement-fixation reactions when testing sera from normal rabbits with various lipoidal extracts. Thus, using alcohol, acetone, and other solvents to prepare extracts of syphilitic liver, they found positive complement-fixation reactions to occur in 27.5 to 56.8 per cent. of normal rabbit sera. They mentioned that there was no evidence that this was related to coccidiosis, and stated that the cause of this phenomenon was as yet unexplained. Kolmer's observation was particularly interesting since it was made prior to definitive reports on *T. cuniculi*.

Ross (1914b) reported the successful transfer of rabbit spirochaetes to two rhesus monkeys:

"The transfer of the rabbit intracellular parasites to monkeys has now been accomplished. In January last, Dr Penfold, of the Lister Institute, inoculated two rhesus monkeys with an extract of the spleen of a naturally-infected syphilitic rabbit. At first, penile vaccination was performed, but this failed to infect. Then inoculation in the testicle was tried. No visible local lesions occurred, but in April both monkeys began to lose their hair. On May 18, one monkey sickened as of a septicaemia and died. *Post mortem*, no local lesions could be discovered, but the heart's blood was found to be teeming with intracellular parasites, showing the developmental spirochaete forms, as well as the free amoeboid bodies similar to those pictured in the *British Medical Journal* on December 14, 1912, as occurring in human syphilis. No free spirochaetes were found in the blood... The transfer of these parasites to monkeys would appear to fill the last gap in the train of evidence in favour of their being the causative agent of rabbit syphilis and seems to show conclusively that these intracellular parasites are developmental forms of the spirochaetes; for by it Koch's postulates are fulfilled and proof is thus gained that intracellular parasites which develop into spirochaetes are the cause of this disease, which resembles both clinically and pathologically human syphilis."

It would appear fair to state that Ross's observation must be taken *sub judice* at this juncture. The facts were that two rhesus monkeys inoculated in the testis with extract of spleen from a rabbit with spirochaetosis showed no local lesions, had no blood tests reported, were found to have no focal lesions at autopsy, and had gross autopsy findings suggestive of a parasite of the heart rather than a treponematosis. We have observed in this laboratory monkeys who at autopsy after spontaneous deaths were found to have a large bolus of worms in the heart, and which were seen

histologically to seed by embolic basis to the vessels of the brain and various viscera. Such cases sound quite similar to the findings in Ross's monkeys. Furthermore, the intracellular forms of parasites presented in his first paper show an awareness of the morphology of treponemes at a much less developed state than is accepted to-day.

Arzt and Kerl (1914) studied a total of 853 rabbits in an attempt to determine the incidence of venereal spirochaetosis. They found few broods entirely free from this infection. They noted that 26.9 per cent. of their adult rabbits had flat, scaly, papular or ulcerative anogenital lesions, and that these lesions contained numerous spirochaetes morphologically indistinguishable from *T. pallidum*. They pointed out that rabbit genital lesions were familiar to breeders who used compound solution of creosol as therapy in such instances. They successfully inoculated uninfected animals from the spontaneous lesions and found genital lesions with spirochaetes present in the transfer animals at 27 days. These authors did not find intratesticular inoculations successful (contrary to the later literature, to be cited). No further reports appeared until after the end of the first world war.

The year 1920 was a vintage year for the literature of rabbit spirochaetosis, however. Jacobsthal (1920) proposed the first name for the organism. He suggested that the disease be called "paralues cuniculi" and the infective organism *Spirochaeta paralues cuniculi*. He found the morphology of the organism essentially the same as that of *T. pallidum*, and stated that the average number of turns was ten to twelve. He found both testicular and corneal inoculation to be unsuccessful. Jacobsthal did transmit the disease by light scarification on the vulva with infective material, and infected three of four female rabbits by this method, with the lesions developing in 3½ to 4 weeks. Jacobsthal was unable to transmit the infection to mice and guinea-pigs. He believed the infection could be distinguished from true syphilis by the incubation period and by difference in the pathological picture. Arzt and Kerl (1920) disagreed with Jacobsthal by concluding that neither the inoculation period nor the histological changes warranted differentiation of venereal spirochaetosis from experimental syphilis in the rabbit.

Schereschewsky (1920) described the genital lesions in spirochaetosis as papular or ulcerative, and containing numerous spirochaetes identical with *T. pallidum*. The incubation period was said to be like that of syphilis, 14 to 30 days. This author noted exacerbations of fading skin lesions

after mechanical irritation. In a few rabbits, the liver and spleen were enlarged, but no spirochaetes were seen. Apes showed no lesions 25 days after inoculation with the rabbit spirochaetes.

Outstanding in the literature of experimental syphilis in rabbits were the classical studies from the United States of Brown and Pearce (1920). A review of their papers reveals, however, that they did not consider *T. cuniculi* in the differential diagnosis of lesions affecting the eyelids and conjunctiva in rabbits. Indeed, in their many reports, only the following statement was found concerning rabbit spirochaetosis:

"Arzt and Kerl have described a similar condition in rabbits due to a spirochaete infection which is capable of transmission from one animal to another. It is claimed that the lesions produced by this organism bear some resemblance to syphilitic lesions. *We have never encountered infections of this type.*"

The name "*Treponema cuniculi*" was first suggested for this organism by Noguchi (1921), and in 1922 he published the first observations of the occurrence of spontaneous rabbit spirochaetosis in American rabbits. He found five out of twenty rabbits examined in June, 1921, to show genital lesions. Of twenty rabbits obtained in Pennsylvania in November, 1921, he found six females to have similar papulosquamous, chronic, frequently ulcerating lesions, characterized by the presence of spirochaetes morphologically resembling *T. pallidum*. The incidence of infection in American rabbits studied by Noguchi was thus between 10 and 30 per cent. He noted that the spiral organism in rabbits closely resembled *T. pallidum*, that the disease was transmissible to normal rabbits, and that the incubation period varied in the first passages from 20 to 88 days, but that one strain subsequently produced a lesion in 20 days on the second passage and in 5 days on the third. No typical orchitis or keratitis was noted in his animals. Eight rhesus monkeys inoculated on the genitalia showed no lesions during a 4-month period of observation. The Wassermann reaction was found to be negative in five rabbits with spontaneous lesions and in eighteen rabbits experimentally infected, but the period of observation was not stated. Noguchi found salvarsan as effective against *T. cuniculi* as against experimental lesions due to *T. pallidum* in the rabbit.

Two dramatic studies appeared in 1921. Lersey, Dosquet, and Kuczynski (1921) reported a case of typical syphilis in a man simultaneously with its occurrence in a male rabbit which he owned. The spirochaetes were identical from each case. These

authors held that the idea of a specific rabbit syphilis due to a spirochaete distinct and different from that of human syphilis was to be regarded with great caution, and believed that transmission of genuine *T. pallidum* to the rabbit had not been excluded.

In the *pièce de résistance* of all experiments with *T. cuniculi*, Levaditi, Marie, and Nicolau (1921) and Levaditi, Marie, and Isaïcu (1921) reported that Levaditi and Nicolau had inoculated themselves with a rich emulsion of *T. cuniculi*, and had also inoculated a monkey and a rabbit at the same time. The rabbit later developed the disease. The human lesions and also those of the monkey healed within 5 days. The Wassermann reaction was negative and remained negative after the inoculation, and all remained perfectly healthy. It was concluded that *T. cuniculi* was non-pathogenic for man and monkeys (Sazerac and Levaditi, 1922). One would certainly like to have the opportunity to study the sera of these volunteers with the TPI and FTA-ABS tests! In an attempt to gain a follow-up to this experiment, we wrote to Dr Pierre Collart, at the Alfred Fournier Institute, in Paris, France. Dr Collart kindly replied that Professors Levaditi and Marie had both died some 15 years ago, but that Dr Nicolau was still alive (over 45 years later!) and living in Bucharest, Roumania. We attempted to contact Dr Nicolau and to obtain a serum specimen from him, but received no reply to our letter.

Warthin, Buffington, and Wanstrom (1923) reported a study of eighteen rabbits infected with *T. cuniculi* and presented a significant review article. They stated that the lesion was superficial, papillomatous, or condylomatous in nature, limited to the mucous membranes or skin, appeared to spread by contiguity or autoinoculation, and was transmissible by inoculation, contact, and coitus. They found no evidence of systemic infection, no lesions containing spirochaetes in any internal organs, and negative blood Wassermann reactions. They proposed that *T. cuniculi* could be differentiated from *T. pallidum* by morphology in silver-agar cover glass smears and by the pathology of the lesions. They stated, however:

"Nevertheless, it is disconcerting to find that there is a spirochete infection of rabbits, apparently widespread throughout the world, caused by a spirochete that resembles pallida sufficiently closely to make possible the occurrence of mistaken identifications of the one for the other, in the case of any worker who is not acquainted with the two organisms and their differential diagnosis. It is unfortunately true that the great mass of experimental work on the transmission of human syphilis

to the rabbit has been carried out in such ignorance of the spontaneous rabbit disease, and the value of that work now becomes legitimately doubtful. It is extremely likely that mistakes have been made. Now that the knowledge of this primary rabbit spirochetosis has been obtained, the possibility of future error should disappear with intimate acquaintance with the cuniculi organism. It will, however, be necessary to repeat much of the work in this light, particularly that concerned with the production of rabbit syphilis from parietic brains and human semen, and the production of re-infection and superinfection, immunity and cure. Too many important deductions have been made from rabbit experimental work to admit of any doubt being allowed to remain as to their accuracy."

Adams, Cappell, and McCluskie (1928) stated that 20 to 40 per cent. of the wild rabbits in England were infected with *T. cuniculi*, according to reputable observers. They noted that Klarenbeek (1921) had observed spirochaetosis in Holland, and that he had found intra-ocular injections sometimes successful. Adams and others (1928) found spirochaetes in fourteen of 228 animals that were specifically examined by dark-field. They found no spirochaetes in sections of internal organs and noted that female rabbits were more readily infected than males, and that the incubation period varied from a fortnight up to 2 months. They also stated:

"Apparently the disease confers little or no general or local immunity, as rabbits in which the lesions have disappeared and in which no spirochaetes have been found for a long period can be readily infected."

They pointed out that rabbit dealers used blue ointment (ungentum hydrargyri) as a specific therapy, and confirmed that it cleared the lesions within 7 to 10 days. They concluded that "the infection may constitute an important source of fallacy in work on human syphilis in the rabbit where superficial inoculation is practised". Klarenbeek (1922) had also stated that "the rabbit is not a perfectly reliable test animal for experimental syphilis".

Another important paper on *T. cuniculi* was that of the Russian workers Fried and Orlov (1932). They also stated that the morphological identity of *T. pallidum* and *T. cuniculi* cast doubt on the results of the study of experimental syphilis in the rabbit. They quoted Artz and Kerl, Klarenbeek, Klarendon, and Simon and others, who believed that the existence of a spontaneous spirochaetosis in the rabbit made the animal altogether useless for the study of experimental syphilis. However, Fried and Orlov (citing

Jacobsthal, Kolle, Mulzer, and Uhlenhuth) stated that the majority of investigators believed that, in spite of the morphological similarities of the strains, the contrast of diseases they caused was rather sharp and that the margin of error in their discrimination was practically nil.

Fried and Orlov (1932) had witnessed two epidemics of rabbit spirochaetosis in Moscow in 1927-8, which enabled them to make an interesting observation regarding the age of the animals and the incidence of the disease.

Early in the autumn of 1926, the managers of a large rabbit farm discovered anogenital lesions in about 100 rabbits. They were not isolated, and by February 1927, 640 (16.1 per cent.) of 5,000 rabbits were ill with spontaneous spirochaetosis. Energetic treatment with bismuth and arsphenamine was undertaken and the epidemic was apparently controlled. However 8 months later, when the number of animals in the farm had reached 12,000, a new epidemic was discovered. At that time, 704 (19.3 per cent.) of 3,650 rabbits were found to have the spontaneous disease. All animals were susceptible. It was noted in 54.9 per cent. of females aged one year, and in 34.9 per cent. of males of the same age. Age was found to be most important in spirochaetosis. Thus, of 2,893 animals one year old or above which had mated, 1,304 (46.4 per cent.) were infected. However, of 5,800 rabbits less than 8 months old most of which had not yet mated, only forty (0.7 per cent.) were diseased.

They considered this strong evidence that extragenital transmission of the disease should be considered non-essential. They considered the organisms to be morphologically identical, and as producing infections with nearly the same incubation period (2½-3½ weeks in syphilis versus 3-4 weeks in spirochaetosis). Since the incubation period has been reported in experimental syphilis to vary from 7 days to 10 months, it is obvious that this difference in incubation period was of no help in differentiating the diseases.

Fried and Orlov pointed out that experimental syphilitic lesions were usually associated with regional adenopathy, but that this was seen in only 9.3 per cent. of rabbits with spirochaetosis. They found the inguinal nodes to be usually smaller and less firm than in syphilis, and stated that they "apparently contain no organisms". They quoted Arzt and Kerl and Frei as the only authors who had reported two positive results. It should be stated at this point that it is now well-recognized that lymph nodes which are morphologically normal and which contain no organisms on silver stains are frequently infectious on passive transfer experiments.

Fried and Orlov further stated:

"Contrary to what one observes in experimental syphilis, *T. cuniculi* causes a parenchymatous keratitis in very rare instances. Orchitis is seen even more rarely, for none of our rabbits showed these lesions . . . Unlike experimental syphilis, spontaneous spirochaetosis becomes generalized apparently in very rare instances; only a few observers were able to demonstrate *S. cuniculi* in the blood and also in the lymph nodes (Warthin and others; Arzt and Kerl; Frei). Attempts to produce the disease by inoculating normal animals with liver, spleen, and bone marrow of sick rabbits have invariably given negative studies."

However, there are striking inconsistencies between the data given in Fried and Orlov's paper and their conclusions. Concerning the cerebrospinal fluid, they concluded: "Likewise the cerebrospinal fluid in this disease shows no cytologic or chemical changes." However, in the text of their paper, they studied the spinal fluid in 47 rabbits by cisternal puncture. This revealed in "eighteen, or 41 per cent., of this number . . . an abnormal condition such as an increase in the amount of albumin and a pleocytosis with cells numbering from ten to about 200 per cubic millimetre". Furthermore, they noted abnormal spinal fluids in 12 per cent. of normal rabbits, and in other rabbits after treatment with arsphenamine. They further stated:

"Pathological lesions in the brains of normal animals akin to those seen in spontaneous spirochaetosis were described by Iliin, Muradova, Saveneiev, Pette, Olivier (of San Francisco), and others. Professor Finkelstein of the State Venereologic Institute has found similar lesions in experimental syphilis . . . It is interesting that a pathologic study of normal rabbits that had received arsphenamine revealed lesions in the central nervous system analogous to those in rabbits with spontaneous spirochaetosis."

They did not elucidate further on these lesions but stated:

"The changes found in the cerebrospinal fluid of rabbits in spontaneous spirochaetosis do not correspond either with the duration of the process or with its gravity. This and the fact that in no instance were spirochetes found in cerebral foci make us believe that the changes in the fluid are not specific responses to spirochaetosis."

Fried and Orlov stated in their conclusions that "the Wassermann and the Sachs-Georgi reactions are, as a rule, negative in spontaneous spirochaetosis". Yet in the text of their paper they found that, of 393 sera from rabbits with spirochaetosis, a positive Wassermann was found in 50 per cent. of their cases! Their "control

syphilitic rabbits yielded positive Wassermann reactions varying between 70-80 per cent. while a percentage of positive normal serums was negligible". Certainly it is important to compare their data with their conclusions!

Fried and Orlov could be further criticized about their statement on visceral lesions in this disease because of the limited nature of their study in this respect. They stated:

"To ascertain whether the visceral lesions contain specific organisms we examined four rabbits with widespread cutaneous lesions. In only one did we find a miliary granuloma in the cortical layer of the suprarenal gland. A thorough search for the presence of spirochetes in this nodule gave negative results. This and the fact observed by us that granulomas of the viscera and the brain may be observed in non-spirochetal disease in rabbits make us believe that this growth was most likely not of spirochetal origin. The question, then of *Spirocheta cuniculi* causing lesions in the viscera ought to be answered at the present in the negative."

Thus, Fried and Orlov's statement that "no visceral lesions occur" was from a study of only four rabbits, one of which had an adrenal granuloma in which no spirochaetes were seen.

Pertinent studies on lymph node involvement in the rabbit, as well as the transmission of *T. cuniculi* to other species, were reported in the years 1937 to 1939 by Bessemans and his collaborators. Their major studies on ocular involvement with *T. cuniculi* are discussed below in more detail. They noted that, of eighteen rabbits inoculated once or more with *T. cuniculi* (by scarification of the lids, retrobulbar injection, or testicular injection), five were apparently uninfected. They wondered if rabbits might harbour inapparent infection with *T. cuniculi*. As a consequence, they removed lymph nodes from these apparently uninfected rabbits, found that they all were normal in appearance, and transferred these to new rabbits. They was able to obtain positive results by such transfers. They similarly infected five guinea-pigs with *T. cuniculi* by genital scarification or by injection into the scrotum or abdominal skin. No specific clinical manifestations were noted in these guinea-pigs. After intervals of 1 week to 3 months, they removed the inguinal nodes and transferred these by intratesticular injections to three rabbits. They succeeded in obtaining positive dark-field recoveries of organisms by this method from two of the guinea-pigs. These authors similarly infected white mice with *T. cuniculi* and again recovered the organism by testicular transfer of lymph nodes to new rabbits and by recovery of treponemes by dark ground examination. One

rabbit which received a transfer node remained clinically in good health, but transfer of its popliteal nodes subsequently revealed motile treponemes in another animal. These workers also successfully proved the presence of latent cuniculi infection in a female common hamster. They concluded that they had established for the first time not only clinically inapparent spirochaetosis in the rabbit, but also specific infectiousness of the lymphatic system, and positive infection of the guinea-pig, white mouse, rat, and common hamster with this organism. In a later paper, they showed that the rabbit not only harboured *T. cuniculi* in the testes, but that the organism maintained its virulence for long periods of time in inguinal, axillary, popliteal, and retrocaecal mesenteric lymph nodes. They recovered the organisms from inguinal and axillary nodes of white mice. They were not able to prove infection of the common dormouse, small dormouse, squirrel, or spermophile.

McLeod and Turner (1946a, b) gave final corroboration to the subject of lymph node involvement in rabbits with *T. cuniculi* infection by finding positive results on five of six occasions when they transferred popliteal lymph nodes from *T. cuniculi*-infected rabbits to normal rabbits by intratesticular inoculation. Indeed, Turner, McLeod, and Updyke (1947) later found positive lymph nodes from rhesus monkeys infected cutaneously with *T. cuniculi*.

Clinical Description

Rabbits

The clinical disease caused in rabbits by *T. cuniculi* has been noted by most investigators, and most of the descriptions are in substantial agreement as to the appearance of the lesions. Fried and Orlov (1932) stated that the disease involved the external genitalia in most instances, often accompanied by perineal lesions. There was early hyperaemia and oedema of the prepuce or labia majora. With time the oedema increased in intensity and the inflamed areas appeared as bluish-red spots at the edges of the prepuce or labia majora where small haemorrhagic spots were found, removal of which led to bleeding ulcers. The anal lesions were similar. No induration was seen. It should be emphasized that most authors have repeatedly emphasized the fact that lesions due to *T. cuniculi* show little or no induration as a point of differentiation from lesions in the rabbit due to *T. pallidum*. Naturally occurring *T. cuniculi* lesions are commonly seen about the prepuce, vagina, anus, or scrotum, and less frequently on the nose, eyelids, lips, and paws. In the description of McLeod and Turner (1946a), the disease was

characterized by slightly elevated scaly patches or eroded sores with a brown crust which bled easily upon slight scarification. The clinical picture was said to be quite similar in general appearance to experimental syphilis. Lesions of the prepuce, which were also scaly patches varying from 0.5 cm. to extensive crusted lesions, extended to the anus. Again no induration was found. With intratesticular infection, lesions developed in 6 to 60 days, but in less than 21 days in most of the animals, and lesions varied from an almost imperceptible full feeling to the testis becoming enlarged and firm throughout. The initial reaction subsided within a few weeks and left little or no reaction, to slight atrophy. A different picture was observed after multiple animal passages, however; for example, *T. cuniculi* passed through six to eight rabbits yielded a small erythematous papule on the back or flat scaly areas 3–5 mm. in size. When the strain was passed through more than eight animals, a marked change in the size and characteristics of the lesions was noted. Metastatic lesions increased. Some of these rabbits developed large, raised, indurated nodular lesions with ulcerated surfaces which were indistinguishable from experimental syphilis. A still larger number developed cutaneous lesions said to be indistinguishable from yaws. Metastatic lesions were found mainly in the genital area. McLeod and Turner (1946a) emphasized the occurrence of granular lesions in the tunica albuginea of inoculated testes as characteristic of *T. cuniculi*. They noted occasionally that such inoculated testes, although normal in character, showed spirochaetes. Generalized skin lesions developed frequently before or in the absence of preputial or scrotal lesions. Mucocutaneous lesions were frequent near the nose and mouth, and usually bordered on the skin or immediately adjacent to it. These were erythematous papular lesions, which developed into hypertrophic areas with thin crusts. Occasionally, deep ulcerative lesions with crusted tops were seen on the nose. Flat, pale scaly patches were seen near the eyes in some rabbits, which developed rapidly. Hypertrophic round crusted lesions, which showed bleeding granulomatous surfaces on removal of the crusts, were most frequently seen at the inner canthus. At times the posterior canthus was involved. Some rabbits developed lesions at the anterior canthus which then spread around the lids to encircle the eye. No keratitis was noted in McLeod and Turner's series. Other lesions were noted on face, neck, ears, paws, and back. The individual lesions of prepuce, testes, scrotum, and skin were found to abound in treponemes.

The varying incidence of cuniculi infection noted by various authors is of interest. Thus, the high figures previously cited by Noguchi (1921), Arzt and Kerl (1920), Fried and Orlov (1932), should be compared with the statement of McLeod and Turner that, of approximately 1,800 rabbits of mixed breeds purchased in the open market which had passed through their laboratories, only six had, upon careful examination, shown evidence of natural spirochaetal infection. Each of these six animals showed genital lesions from which *T. cuniculi* was recovered and successfully propagated in two or more generations of rabbits.

McLeod and Turner (1946b), in another important paper, conducted a controlled experiment to compare *T. pallidum* with *T. cuniculi* (and also *T. pertenuae*). They used a highly inbred stock of Dutch Belt rabbits which had been maintained for a number of years, and in which naturally occurring *T. cuniculi* infection had never been observed. The rabbits were divided into four groups of twenty animals each. One group was not infected, a second was inoculated with *T. pallidum*, a third with *T. pertenuae*, and the fourth with *T. cuniculi*. All injections were given into both testes of each rabbit, and all were inoculated on the same day. It should be noted that throughout the course of the 6 months of observation of this experiment, the uninoculated controls remained free from any lesion suggesting a spirochaetal disease. Even though the animals were housed in the same room (each of the rabbits was kept in an individual cage), not the slightest indication of cross-infection was noted. In examining the animals, these authors stated that it was their custom to examine the control rabbits first, but other than this no unusual precaution was taken to prevent transfer of infection from one of the infected groups to the control animals. They suggested that accidental cross-infection of treponemal diseases in the laboratory therefore does not readily occur.

The findings of McLeod and Turner (1946a, b) are of great interest, and both of these papers

should be carefully read in the original by those interested in this subject. The incubation period with *T. pallidum* varied between 13 to 21 days in all but one rabbit and averaged 19 days. The incubation period with *T. cuniculi* was from 13 to 28 days and averaged 21 days. The serological responses of the three infected groups to the Eagle test were essentially the same. Their findings are reproduced in the Table.

With regard to the serological responses in these rabbits, McLeod and Turner (1964a), noting that many previous investigators had found serological tests for syphilis to be negative during the course of experimental venereal spirochaetosis in the rabbit, pointed out that Bessemans and Asaert (1935) had found a higher percentage of positive sera in rabbits with *T. cuniculi* infection than in normal rabbits, although they had questioned whether the finding could be attributed to the presence of a spirochaetal infection. McLeod and Turner (1964b) noted that, in general, the clinical course of experimental syphilis, yaws, and *T. cuniculi* infection was similar. They stated:

“While the lesions observed in each disease exhibited certain similarities, they were, on the whole, sufficiently distinctive to permit easy differentiation of one experimental disease from the others.”

They found the tissue reaction among the syphilitic animals to be more intense and more extensive than among the rabbits infected with *T. pertenuae* or *T. cuniculi*. They made the following important statement:

“On the basis of inadequate experimental evidence, *T. cuniculi* has been regarded as non-pathogenic for man. In view of the similarity in the disease picture produced in rabbits by *T. cuniculi* to that produced by *T. pallidum* and *T. pertenuae*, it seems wise to consider the non-pathogenicity of the former organism for man as not yet established.”

Other Species

T. cuniculi was assumed by some to be pathogenic for rabbits only. This statement was not

TABLE
HIGHEST SERUM TITRE OBSERVED AMONG RABBITS BELONGING TO THREE GROUPS EXPERIMENTALLY INFECTED

Experimental Disease	Number Tested	Number of Rabbits of each group whose serum reached titre					
		1:512	1:256	1:128	1:64	1:32	1:16 or lower
<i>T. pallidum</i>	19	2	1	4	10	2	0
<i>T. pertenuae</i>	20	0	1	5	6	4	4
<i>T. cuniculi</i>	20	0	4	1	4	3	8

true, however, as several experiments have documented. Bessemans and de Moor (1939) proved the occurrence of latent infection with *T. cuniculi* (as well as *T. pallidum*) in the white mouse, white rat, guinea-pig, hedgehog, and golden hamster. Large numbers of organisms were found in the lymph nodes of these animals. These findings were verified by further experiments noted by Turner and Hollander (1957). They inoculated 25 hamsters with *T. cuniculi*. Positive lymph nodes were found in eleven of the 23 animals examined, and one animal had crusted lesions. Furthermore, they noted that *T. cuniculi* was able to produce lesions on the back in 818 guinea-pigs, although scrotal lesions were not demonstrated. Although early authors did not appear to be able to infect the monkey with *T. cuniculi*, it should be noted that the period of observation was short in such instances. They inoculated thirteen monkeys with *T. cuniculi*, and found dark-field-positive lesions in two. It was of interest, however, to note the results of infectivity tests in these animals. Thus, infectivity tests of the lymph nodes were performed on twelve of the thirteen monkeys inoculated with *T. cuniculi*. Two of these were positive. One of these nodes was from an animal which had shown dark-field-positive lesions. The other monkey is equally, if not more, interesting, for in this instance a primate inoculated with *T. cuniculi* had shown no clinically evident lesions, and yet was proven to have the organism in the lymph nodes in infectious form. One half of the monkeys inoculated with *T. cuniculi* showed low titted Wassermann tests, and this did not corroborate with the infectivity tests. The authors noted that because *T. cuniculi* was shown to produce both local and general infection in monkeys, this also suggested that the organism might be infectious for man.

Histopathology

It is the consensus of the literature, summarized by Turner and Hollander (1957) that the histology of *T. cuniculi* infections is not sufficiently distinctive to permit ready differentiation of these lesions from those of syphilis.

Immunology

Khan, Nelson, and Turner (1951), using serum from rabbits infected with seven syphilis strains, yaws, and *T. cuniculi*, compared the responses to the TPI (*Treponema pallidum* immobilization) test. A single specimen from each rabbit infected with each strain was tested several times against homologous strains. The maximum variation found was less than a 3-fold dilution. In comparing the

largest variation of five sera (one was pooled Nichols strain sera), there was slightly more than a 2-fold dilution with the TPI tests. The authors felt that some antigenic differences were suggested by these studies, but that the evidence was still uncertain. It should be noted that Turner and Hollander state that *T. cuniculi*-infected rabbits definitely showed reactive blood tests to not only the blood reagin tests but also to the TPI test.

Ocular Involvement

Only two groups of investigators have been primarily interested in *T. cuniculi* and the eye—Klarenbeek (1921), and Bessemans and Van Canneyt (1939). However, numerous isolated comments about ocular involvement have been made in the general literature, and therefore the following brief chronological review is presented from the ophthalmological point of view. Jacobsthal (1920), who, it will be recalled, first named the organism, found corneal inoculation unsuccessful. Arzt and Kerl (1920) stated that brow inoculations were unsuccessful in the monkey.

Schereschewsky and Worms (1921) reported an interesting ocular phenomenon. A healthy female rabbit (No. 32) was placed in the same cage with a male rabbit known to have spirochaetosis, and following coitus rabbit 32 acquired genital lesions. One month after cohabitation these showed the typical spirochaetes; 2 days later, both eyes were scarified with a sterile knife, and then human syphilitic material from a dark-field-positive condylomatous lesion of a woman was applied to the left cornea of rabbit 32. This rabbit developed a specific parenchymatous keratitis in the left eye. 4 months later both eyes and genitalia showed no further change. The authors thus noted that having a primary infection with *T. cuniculi* did not prevent the development of a corneal lesion from inoculation with *T. pallidum* in this animal. They pointed out that this work was in agreement with that of Tomaczewski (cited by Truffi, 1913), who was able to infect animals by the corneal route after previous scrotal infection. It may be fair to state that, since re-infection can occur in syphilis and superinfection as well, the significance of these experiments between *T. cuniculi* and *T. pallidum* must be viewed with an open mind.

Klarenbeek (1921) produced the first experimental keratitis in rabbits with *T. cuniculi*. He inoculated small portions of infected perineal tissue into the anterior chamber of the eyes of rabbits. After 41 to 43 days a keratitis was obtained, and spirochaetes were seen in superficial scrapings from this cornea. He also made subcutaneous inoculations

with *T. cuniculi* in the upper lids. In another case, after an anterior chamber inoculation, the rabbit developed upper lid ulcers 2 months later. Noguchi (1922) commented on lid lesions in spontaneous rabbit spirochaetosis, but noted no keratitis in his animals.

Bessemans and Van Canneyt (1929, 1932, 1938, 1939) were the most active workers in the field of ocular involvement with *T. cuniculi*. In 1929 they reported that they gave *T. cuniculi* by five different routes to rabbits: (1) into the anterior chamber, (2) scarification of lid border, (3) into the bulbar conjunctiva near the limbus, (4) into the upper layers of the cornea, and (5) in the nictitating membrane. They produced ulceropapulosquamous lid lesions in 85 per cent. of rabbits so inoculated and found the typical adherent brown crusts. They described three types of conjunctivitis: disseminated nodular, a tumour-like, and hyperplastic. They noted three types of keratitis: torpid, ulcerative, and tumourous. In 1932, they reported on corneal and iris lesions, and noted keratitis after anterior chamber inoculation. They stated that keratitis occurred in 9 per cent. of the animals after corneal scarification, but in 25 per cent. after anterior chamber inoculation. They also noted iritis in some of these animals. The organisms were not definitely identified *intra-ocularly* in their experiments to our knowledge.

Klarenbeek (1930) described keratitis as occurring 1 to 3 months after anterior chamber inoculations of *T. cuniculi* in rabbits. He stated that similar results had been obtained in 1921 and 1924. He noted definite intra-ocular manifestations (iritis, glaucoma, and the like) but attributed this to post-operative reaction. He noted generalized skin lesions in two rabbits after inoculations into the eye. He thought that he could differentiate *T. pallidum* and *T. cuniculi* by means of the ocular reactions obtained.

Fried and Orlov (1932) mentioned that keratitis was rare in *T. cuniculi* involvement, but it appeared that this was a statement from the literature and that it had not been seen in any of their rabbits. Burghraeve (1933), from Besseman's department, described three types of ocular lesions following injections with *T. cuniculi*: (1) lid lesions, (2) iridocyclitis, and (3) corneal granuloma. The iridocyclitis demonstrated in this paper may have followed corneal involvement, and there appeared to have been no definite demonstration of intra-ocular spirochaetes in this report. Bessemans, Van Haelst, and DeWilde (1935) mentioned that the blepharitis due to *T. cuniculi* was characteristic. Bessemans and Van Canneyt (1938) reported on

rare forms of keratitis with this organism. They noted one bilateral recurrent keratitis as the only such instance in forty cases of cuniculi keratitis, after 146 inoculations in 85 rabbits. In 1939, these authors reported on temperature studies and the eye. They thought that the cooler temperature of the external ocular segment was related to the propensity to external disease in rabbits after inoculations with either *T. cuniculi* or *T. pallidum*, and that the higher internal temperatures of the eye explained the lessened incidence of intra-ocular lesions with these organisms. McLeod and Turner (1946a, b) noted no keratitis in their cases of *T. cuniculi* infection in rabbits, but they did note keratitis in two of twenty rabbits infected with *T. pallidum*. Turner and Hollander (1957) reported on the previously-mentioned inoculations of monkeys with *T. cuniculi*. Organisms were usually given both by intracutaneous injections and eyebrow scarification. They found lesions similar to those seen in rabbits with adherent crusts, and such lesions on the backs of the monkey persisted for a long time. No generalized skin or mucous membrane lesions were noted, and there was no evidence that other organs were involved.

In summary, many authors have reported on rather characteristic forms of external ocular disease in rabbits infected with *T. cuniculi*: involvement of the lids in a typical blepharitis, and secondary involvement of conjunctiva, cornea, and nictitating membrane. Intra-ocular inoculations produced keratitis in several instances. The occurrence of synechiae, iridocyclitis, secondary glaucoma, and the like may have been due to the organism itself, or may have been secondary to the surgical trauma of inoculation. There appeared to be no good control series in which intra-ocular inoculations were made into the fellow eye but in which no *T. cuniculi* organisms were given. There is no question that external eye disease commonly occurs in rabbits infected with *T. cuniculi*, but the question of intra-ocular involvement awaits final clarification. It should be noted that visceral lesions were lacking in animals infected with *T. cuniculi*, but a similar situation prevails in rabbits infected with *T. pallidum* (Brown and Pearce, 1920). It must be stressed that many rabbits infected with *T. pallidum* have shown grossly normal liver, spleen, and lymph nodes; no spirochaetes could be demonstrated in these tissues with the silver stains, yet these organs have been subsequently proved to be infectious upon transfer to normal rabbits. Another difficulty with the older literature is the relative lack of photographs of the lesions, and hence the dependence upon

clinical descriptions which has often rendered differentiation of the lesions nearly impossible.

Summary

Benign venereal spirochaetosis is an endemic disease of rabbits due to a spirochaete, *Treponema cuniculi*, which is morphologically indistinguishable from *Treponema pallidum*. The disease primarily involves the genitalia and is transmitted by sexual contact. Lesions also often occur around the face, eye, ear, nose, and extremities. The organism has proved to be infectious not only for rabbits, but also for mice, rats, guinea-pigs, hamsters, hedgehogs, and monkeys. The disease cannot be differentiated serologically or histologically from experimental syphilis in the rabbit. Lesions respond to medications effective against *T. pallidum*. Bessemans reported the occurrence of latent (subclinical) infections in rabbits inoculated with *T. cuniculi*, and this is an extremely important point when using this species for research on syphilis. The incidence of the disease has varied widely, but it has been reported in 10 to 50 per cent. of rabbits examined from all over the world, and similar figures have been noted by different techniques (presence of genital lesions, dark-field surveys, and serological testing). The incubation period for rabbits infected with *T. pallidum* averages 19 days, and with *T. cuniculi* 21 days; and as a considerable spread occurs, it is evident that incubation period is not a valid criterion for differentiating these organisms. The only means by which differentiation of *T. cuniculi* from *T. pallidum* can be made at this time is by the clinical appearance of the lesions produced in rabbits, and it would appear that this point is still open to a reasonable question. Serial passage of *T. cuniculi* through six to eight or more rabbits causes lesions often indistinguishable from those seen in rabbits infected with *T. pallidum*. It would appear that this change is related to *rapidity* of passage rather than to the *number* of such passages, for it is obvious that, when a rabbit purchased from a breeder is found to have venereal spirochaetosis, the number of passages of the *T. cuniculi* organisms that this rabbit harbours is totally unknown and may reach an exceedingly high figure.

T. cuniculi has been said to be non-pathogenic for man, but the evidence for this is not sufficiently strong to conclude that this point has been finally resolved. It is evident that, although the rabbit has been the animal classically employed for research on syphilis, such work with this species must be considered *sub judice* at this moment. Important questions that need further study include:

- (1) The incidence of reactive reagin tests in healthy rabbit populations in various parts of the world.
- (2) A careful study of such reactors for genital lesions and dark-field analysis of those found.
- (3) An attempt to determine an objective biological point of differentiation between the organisms, such as electron microscopy, histochemistry, immunology, and the like.
- (4) An attempt to corroborate Bessemans' finding of subclinical *T. cuniculi* infection in rabbits.
- (5) Search for other suitable species for research on syphilis.

A repeat study involving infection of human volunteers with *T. cuniculi*, done on larger numbers of persons and with modern laboratory and serological techniques, would appear to be the only means of final clarification of this problem.

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Le statut actuel du *Treponema cuniculi*: Une revue de la littérature

RÉSUMÉ

La spirochétose vénérienne bénigne est une maladie endémique des lapins causée par un spirochète, le *Treponema cuniculi*, qui est morphologiquement indiscernable du *Treponema pallidum*. La maladie attaque essentiellement les parties génitales et est transmise par les rapports sexuels. Les lésions se trouvent souvent aussi sur la figure, les yeux, les oreilles, le nez et les extrémités. Le germe a prouvé qu'il était infectieux non seulement aux lapins mais aussi aux souris, rats, cobayes, hamsters, hérissons et aux singes. La maladie ne peut être distinguée sérologiquement ou histologiquement de la syphilis expérimentale chez le lapin. Les lésions répondent aux médicaments qui sont efficaces contre le *T. pallidum*. Bessemans et ses collaborateurs ont rapporté la présence des infections latentes (sous-cliniques) chez le lapin inoculé le *T. cuniculi*, et ceci est un point extrêmement important quand on se sert de cet animal pour les recherches sur la syphilis. L'incidence de la maladie a varié beaucoup mais il a été rapporté chez 10 à 50 pour cent des lapins examinés à travers le monde, et des chiffres semblables ont été notés par des techniques différentes (la présence des lésions génitales, les relevés faits par éclairage sur fond noir et les tests sérologiques). La période d'incubation chez le lapin infecté par le *T. pallidum* est en moyenne de 19 jours, et par le

T. cuniculi de 21 jours; et comme la période d'incubation varie beaucoup il est évident que celle-ci n'est pas un critère valide pour différencier ces deux germes. Le seul moyen de distinguer le *T. cuniculi* du *T. pallidum* à ce stage est par l'apparition clinique des lésions produites chez le lapin, et il semblerait que ce point attend toujours une réponse. Le passage en série du *T. cuniculi* à travers six à huit lapins ou plus cause des lésions qui sont souvent indiscernables de celles vues chez les lapins infectés par le *T. pallidum*. Il semblerait que ce changement est en relation à la rapidité du passage plutôt qu'au nombre de tels passages, car il est évident que si un lapin acheté d'un éleveur est atteint de spirochétose vénérienne le nombre de passages du *T. cuniculi* que ce lapin a hébergé est complètement inconnu et peut atteindre un chiffre très élevé.

Il a été dit que le *T. cuniculi* n'est pas pathogène à l'homme, mais la preuve de cette assertion n'est pas suffisamment forte pour conclure que cette question a été finalement résolue. Il est évident, malgré que le lapin ait été l'animal classique employé pour les recherches sur la syphilis, qu'un tel travail avec cet animal doit être considéré comme *sub judice* aujourd'hui. D'importantes questions qui demandent des études additionnelles comprennent:

- (1) L'incidence des tests positifs d'anticorps chez les lapins sains de diverses régions du monde.
- (2) L'étude exacte des lapins positifs y compris la recherche chez eux des lésions génitales et l'examen par éclairage sur fond noir.
- (3) La tentative de trouver un moyen biologique comme le microscope électronique, l'histochimie, l'immunologie ou d'autres moyens similaires afin de différencier ces deux germes.
- (4) La tentative de corroborer la constatation de Bessemans quant à l'infection sous-clinique par le *T. cuniculi* chez le lapin.
- (5) La recherche parmi d'autres animaux de ceux convenables d'aider les travaux au sujet de la syphilis.

Une reprise de l'étude comprenant l'infection par le *T. cuniculi* d'un grand nombre de volontaires humains aidée des techniques modernes de laboratoire y compris celles de la sérologie semblerait être le seul moyen d'éclaircir définitivement ce problème.