Experimental rabbit syphilis

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Syphilis is an infectious disease and its aetiological agent Treponema pallidum (Tp) is subject to the same biological laws as other organisms, which evolve in five stages when cultured in vitro:

I—Incubation
II—Exponential growth
III—Stationary phase
IV—Retrogression
V—Latency (in which persistent organisms can sometimes be demonstrated)

T. pallidum cannot be cultivated in vitro, but inoculation into the testicles of the rabbit permits the organism to be cultivated in vivo, and similar stages can be seen to develop (Fig. 1) (Monod, 1959).

**I. Incubation**

1. **Appearance of the lesions**
   There is no lesion as in the eclipse phase of virus infections.

2. **Duration**
   This is extremely variable; it is generally 6 days, but may be up to 3 months, depending on the following factors:
   
   (a) **Method of inoculation**
   See Chesney and Schipper (1950)

   (b) **Strains used for inoculation** (Collart, 1964).
   e.g. 1 million Tp of Nichols strain (rabbit) 6 to 8 days.
   1 million Tp of Gand strain (rabbit) 39 days.

   (c) **Origin of strain**
   A strain of Tp has difficulty in adapting itself to a host for which it was not previously specific. Thus, after fifteen transfers by biopsy of human syphilitic lesions to rabbits, we have observed only nine positive results: Tp appeared in only six cases during the first 3 months and in only one during the second 3 months. In the two others latent syphilis resulted and was revealed only by a positive TPI test after the 6th month (Collart, Dunoyer, and Dunoyer, 1968).

   (d) **Age of inoculum**
   Organisms taken from the rabbit during the latent phase of syphilis show a longer incubation period.

   (e) **Age of the animal**
   In young animals the incubation period is shorter than in older ones.

   (f) **Ambient temperature**
   Hollander and Turner (1954) showed that, after inoculation of the skin at temperatures above 20°C., the lesions were very slight and had a long period of incubation (72 days); below this temperature the lesions were larger and incubation was shorter (about 20 days). Longhin, Popescu, and Volosceanu (1957) found that, at 5°C. or less, generalized lesions may occur.

**FIG. 1 Stages in the evolution of an organism during culture or development in vivo**

In this paper we propose to consider the following factors at each stage:

1. Appearance of the lesions
2. Duration of lesions
3. Characteristics of the parasite
4. Infectivity of the animal's organs
5. Pathological findings
6. Serology (TPI and FTA tests)
7. Correlation of these findings with treatment with penicillin

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(g) Size of the inoculum (Wiggall and Chesney, 1950)
With the less virulent Truffi and Hoffmann strains, Levaditi and Levaditi (1941) and Gastinel and Pulvenis (1934) found that an inoculum of less than 6,000 Tp caused only latent syphilis. Magnuson, Eagle, and Fleischman (1948) and Collart (1964) have shown that, with the Nichols strain, there is practically no threshold and one or two treponemes are sufficient to produce orchitis.

(h) Previous treatment of the donor
A subcurative dose of penicillin reduces virulence (Eagle, Magnuson, and Fleischman, 1947), whereas cortisone increases virulence and the multiplication rate of the infective organism (Collart, Poggi, Dunoyer, and Dunoyer, 1968).

(3) The parasite
Tp are present for only 1 or 2 days at the inoculation site and cannot be found later during the eclipse phase (Gastinel, Vaisman, and Dunoyer, 1961).

(4) Infectivity of the organs of the host animal

(a) Testicles
Extremely infective although no Tp are visible on microscopy.

(b) Popliteal lymph nodes
According to Tani, Ōgiuti, Hutaki, and Ōya (1935) these nodes contain Tp 5 minutes after the inoculation, but it is often very difficult to prove this so early.

(c) Other organs
Tp can be found in the cerebrospinal fluid (CSF) as early as 18 hrs after inoculation (Fig. 2).

FIG. 2 Smear of CSF from a rabbit inoculated 18 hrs earlier

Poooled CSF from four rabbits inoculated with the Nichols strain 7, 9, 9, 11 days earlier respectively produced orchitis in the rabbits precisely 26 days later (Fig. 3).

FIG. 3 Smear of serous fluid obtained from acute orchitis 26 days after inoculation with 1·5 ml. pooled CSF from four rabbits infected 7 to 11 days earlier

(5) Pathological findings
No marked lesion seen histologically (Fig. 4).

FIG. 4 Skin lesion biopsy taken 5 days after inoculation at the point of injection on the flank. There are no treponemes and the skin is practically normal, but round cell infiltration is present.
(6) SEROLOGY
TPI test negative.

(7) PENICILLIN TREATMENT (Pechère, Franceschini, and Collart, 1971)
After administration the concentration of penicillin in the lymph nodes of a normal rabbit is about one-seventh that of the blood level, and four times higher than that in the testicles.

The following shows the average in ten animals:

<table>
<thead>
<tr>
<th>Protein Type</th>
<th>Average U/ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>3.4</td>
</tr>
<tr>
<td>Normal testicle</td>
<td>0.13</td>
</tr>
<tr>
<td>Lymph node</td>
<td>0.50</td>
</tr>
</tbody>
</table>

II. Exponential Growth

(1) APPEARANCE OF THE LESIONS
Swelling of the testicles is beginning.

(2) DURATION
This is variable, depending on the factors mentioned above (i.e. the strain, its origin, the age and the size of the inoculum, the ambient temperature, and previous treatment of the donor animal).

For example, with a well-adapted Nichols strain ($10^6$ Tp), the general rule is 3 to 4 days, but variations have been observed from 5 to 36 days as follows:

<table>
<thead>
<tr>
<th>No. of treponemes</th>
<th>Strain</th>
<th>Pre-treatment with cortisone</th>
<th>Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100,000</td>
<td>Nichols</td>
<td>Yes</td>
<td>5.6</td>
</tr>
<tr>
<td>100,000</td>
<td>Nichols</td>
<td>No</td>
<td>14.5</td>
</tr>
<tr>
<td>100,000</td>
<td>Gand</td>
<td>No</td>
<td>38.4</td>
</tr>
<tr>
<td>10,000</td>
<td>Nichols</td>
<td>Yes</td>
<td>12.8</td>
</tr>
<tr>
<td>10,000</td>
<td>Nichols</td>
<td>No</td>
<td>26.3</td>
</tr>
<tr>
<td>10,000</td>
<td>Gand</td>
<td>No</td>
<td>36.3</td>
</tr>
</tbody>
</table>

(3) THE PARASITE
The treponeme is often small and thin and shows reduced motility when swelling of the testis is beginning. During this accelerated growth stage, Tp divides every 30 to 33 hours, according to Magnuson and others (1948) and Cumberland and Turner (1949), who say that 'under favourable conditions' the division rate apparently depends on the activity and degree of virulence of the strain and other factors such as temperature (cf. Longhin and others, 1957). According to Rosahn and Rowe (1950) and Willcox and Guthe (1966), division takes place in the mouse every 25 days.

Logarithmic increase takes place only during this stage, in accordance with the generally accepted biological law. During this period, we can sometimes, but very rarely, see Tp under or in a cell (Fig. 5a, b, c) and boring through the head of a spermatozoon (Fig. 6, overleaf). This observation confirms the finding of Ovčinnikov and Delektorskij (1969, 1970, 1971) using the electron microscope.

(4) INFECTIVITY OF ORGANS
(a) Testicles
Very high.

(b) Lymph nodes
These are more extensively invaded, and transfer of infection is possible, but not constant; Tp are less difficult to detect on smears than at the earlier stage (Bessemans and Potter, 1934).

(5) PATHOLOGICAL FINDINGS
The infiltrate is now much more obvious and is located around the vessels of the superficial layers of the epidermis (Figs 7 and 8, overleaf).

**FIG. 5 A treponeme on or in a cell**
FIG 6  A treponeme perforating the head of a spermatozoan

FIG 7  Biopsy on 10th day after inoculation of flank. Infiltration is seen in vicinity of blood vessels

FIG 8  Section showing orchitis 9 days after inoculation. Infiltration has not yet appeared. Spaces between tubules are normal

(6) SEROLOGY
TPI test consistently negative.
FTA test begins to become positive.

(7) PENICILLIN TREATMENT
The concentration in the testicles and lymph nodes begins to rise in comparison with the level in the blood.

III. STATIONARY PHASE
(1) APPEARANCE OF THE LESIONS
Acute orchitis. The testicle is enlarged, turgid, and of characteristic firm consistency.

(2) DURATION
Variable, depending on the strain. With the Nichols strain it is 6 to 7 days.

(3) THE PARASITE
The treponeme is always very motile; it is of classical appearance with regular spirals (Fig. 9) and about 10μ in length. The number of parasites in the medium seems to be stable and does not increase.
FIG. 9 Treponemes of Nichols strain removed from a rabbit with an acute orchitis

(4) Infectivity of Organs
(a) Testicles
Particularly infective, with very virulent organisms.

(b) Lymph nodes
Infective. Inoculation of material from one of these nodes into the scrotum of a new animal leads to infection which is seen after an incubation period of approximately 18 days. Positive results are obtained in 95 per cent. of transfers.

(c) Treponemes are disseminated throughout the body of the host via the blood stream, but above all via the lymphatic vessels.

(5) Pathological Findings
After the 12th day, sections of the testicles show vascular proliferation with an increased number of inflammatory cells (Fig. 10). Plasma cells begin to appear within the lumen of the seminal canal (Franceschini, 1970).

(6) Serology
TPI still negative. FTA shows a marked rise in titre.

(7) Penicillin Treatment (Pechère and others, 1971)
With an average blood level of 2.74 U/ml., the concentration in the testicles will be 0.85 U/ml., and that in the lymph nodes 0.73 U/ml.

FIG. 10 Section from orchitis of 13 day's duration. Intercanalicular spaces are thickened and infiltrated

IV. Regression
(1) Appearance of the Lesions
In France we use the term 'syphiloma' for this lesion in contrast to the term 'acute orchitis' for that of the early period.

The testicle is now very firm and hard. A nodular lesion appears deep within it or in the tunica vaginalis. In 95 per cent. of cases ulceration develops. The lesion usually appears about 15 to 20 days after the inoculation, sometimes much later (44 days). At this stage, the signs of diffuse acute orchitis have disappeared completely. Papules appear on the skin at the site of inoculation; these soon ulcerate and then become covered with a crust.

(2) Duration
Extremely variable. Even with the use of a uniform emulsion, so that the same number of treponemes was inoculated into fifty rabbits, the duration of the syphilomas varied from 15 to 195 days (Collart, Poggi, Dunoyer, and Dunoyer, 1966).

(3) The parasite (Jacquet and Sézary, 1907; Collart, 1964)
The organisms are much less numerous than in
diffuse orchitis. They are generally less motile and longer, and may present some anomalies of morphology (Fig. 11).

FIG. 11 Smear from a syphiloma 56 days after inoculation. There are some atypical straight forms as well as typical treponemes. (From Collart, 1970, p. 1266, Fig. 1)

(4) INFECTIVITY OF THE ORGANS
The Tp are disseminated throughout the body of the rabbit, but are not necessarily evenly distributed (Bessemans and van Haelst, 1933). According to Chesney (1927), the serial transfer of emulsions from various organs of untreated infected rabbits to fresh rabbits gave positive infectivity test results as follows:

<table>
<thead>
<tr>
<th>Material</th>
<th>Percent. infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardium and brain</td>
<td>0</td>
</tr>
<tr>
<td>Blood from heart, spleen, bone-marrow</td>
<td>25</td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
</tr>
<tr>
<td>Testicle</td>
<td>62</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>75</td>
</tr>
</tbody>
</table>

One of these recently infected rabbits, having been used for fourteen passages from different organs, gave only one positive response, although it had never been treated before (Chesney and Kemp, 1925a, b). However, we have found atypical Tp in brain, and the inoculation of a rabbit with material from this organ gave a positive result (Fig. 12). We have also found Tp in aqueous humour (Fig. 13) (Smith, Singer, Moore, and Yobs, 1965; Smith, Singer, Reynolds, Moore, Yobs, and Clark, 1965; Wells and Smith, 1967; Smith, 1969) and in CSF (Fig. 14).

FIG. 12 Smear from brain of Rabbit 74. The treponemes are atypical but the transfer of a fragment of this brain to a new rabbit produced infection.

FIG. 13 Treponeme in a smear of aqueous humour from Rabbit 61, infected 81 days earlier but showing no eye lesion.

FIG. 14 Treponeme in a smear of CSF from Rabbit 21, infected 108 days earlier.
(5) PATHOLOGICAL FINDINGS (Franceschini, 1970)

Three stages may be observed:

(i) The blood vessels are obstructed with inflammatory cells, the intercanalicular septa are thickened and infiltrated with round cells, lymphocytes, and plasma cells, and some eosinophilic polymorphonuclear leucocytes. The seminal canals are completely obstructed.

(ii) 20 to 25th day. The structure of the testicle is much altered, the lumina of the seminal canals cannot be discerned; all blood vessels are obstructed with infiltrates, some being closed by endothelial thickening; the septa are thickened; the lymphoplasmocytic infiltrates, containing large numbers of eosinophils are plainly seen.

(iii) Ulceration results from vascular thrombosis in portions of nodules adhering to the scrotum. The lymphoplasmocytic and eosinophilic infiltration reaches its peak deep to the ulceration and spreads on each side of it, predominantly around the hair follicles. The infiltrated areas are separated by clear zones containing only a few round cells but much mucin (Figs 15 and 16).

The tunica vaginalis is markedly thickened and fibrotic and is infiltrated with inflammatory cells.

The testicle is now a gumma, and no seminal canals can be recognized. With the electron microscope Tp appear to be encapsulated by a granular substance which we know to be mucopolysaccharide in nature (Fig. 16).

(6) SEROLOGY

The quantitative TPI and FTA tests are at their maximum values and maintain this level for some 18 to 24 months, although the titres differ from animal to animal, and are not related to the number of organisms inoculated initially.

Immobilizing antibody seems to have no action on the Tp in vivo, otherwise the lesions would be of shorter duration in cases with high antibody
TABLE I  
Titres of TPI and duration of lesion (days)

<table>
<thead>
<tr>
<th>TPI titre</th>
<th>Duration of lesion (days)</th>
<th>TPI titre</th>
<th>Duration of lesion (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>135</td>
<td>2,500</td>
<td>45</td>
</tr>
<tr>
<td>800</td>
<td>75</td>
<td>2,500</td>
<td>15</td>
</tr>
<tr>
<td>900</td>
<td>105</td>
<td>2,500</td>
<td>45</td>
</tr>
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<td>1,000</td>
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<td>0</td>
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<tr>
<td>1,000</td>
<td>75</td>
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</tr>
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</tr>
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</tr>
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<td>135</td>
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<td>45</td>
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<td>2,500</td>
<td>75</td>
</tr>
<tr>
<td>1,500</td>
<td>45</td>
<td>2,500</td>
<td>165</td>
</tr>
<tr>
<td>1,500</td>
<td>90</td>
<td>2,500</td>
<td>60</td>
</tr>
<tr>
<td>1,800</td>
<td>105</td>
<td>3,000</td>
<td>165</td>
</tr>
<tr>
<td>1,800</td>
<td>15</td>
<td>3,000</td>
<td>75</td>
</tr>
<tr>
<td>1,600</td>
<td>30</td>
<td>3,000</td>
<td>90</td>
</tr>
<tr>
<td>2,000</td>
<td>0</td>
<td>3,500</td>
<td>90</td>
</tr>
<tr>
<td>2,000</td>
<td>195</td>
<td>4,000</td>
<td>105</td>
</tr>
<tr>
<td>2,000</td>
<td>30</td>
<td>4,000</td>
<td>105</td>
</tr>
<tr>
<td>2,000</td>
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<td>15</td>
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<tr>
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<td>5,000</td>
<td>60</td>
</tr>
<tr>
<td>2,000</td>
<td>60</td>
<td>5,000</td>
<td>30</td>
</tr>
<tr>
<td>2,000</td>
<td>180</td>
<td>5,000</td>
<td>30</td>
</tr>
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<td>2,500</td>
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<td>5,000</td>
<td>60</td>
</tr>
<tr>
<td>2,500</td>
<td>105</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From Poggi (1965)

(7) PENICILLIN TREATMENT

With a blood level of 0.45 U/ml., the testicular concentration was 0.16 U/ml.

With an average blood level of 2.55 U/ml., the average lymph node concentration was 0.53 U/ml.

V. LATENCY

(1) APPEARANCE OF THE LESIONS

In most cases no more lesions develop but the testicle becomes slightly atrophied.

Rarely, some cutaneous peripheral papular lesions containing a few Tp appear. These are more common in cases pre-treated with cortisone (McLeod and Magnuson, 1956). Sometimes necrotic bone lesions develop (Brown and Pearce, 1920; Brown, Pearce, and Witherbee, 1921).

(2) DURATION

The situation remains the same throughout the life of the animal.

FIG. 17  Correlation between TPI titre and duration of lesions in fifty rabbits inoculated 9 months earlier

titres. A comparison of serological titres with the duration of the lesions in fifty rabbits inoculated 9 months earlier shows the lack of correlation between them (Table I and Fig. 17). Immobilizing antibodies have no apparent influence on the duration of the syphiloma; it therefore seems impossible that they should have a treponemicidal effect in vivo after the parasites have penetrated deep into the tissues.

FIG. 18  Smear of syphiloma 112 days after inoculation with Gand strain. The Tp is atypical, long and straightened

FIG. 19  Smear of aqueous humour of Rabbit 12, with spontaneous keratitis that developed 480 days after intratesticular inoculation with Gand strain
view of the difficulty of producing the disease by means of lymph node transfers, we are forced to conclude that Tp persists in those tissues in the commensal state, as other organisms do according to the laws of biology.

(5) PATHOLOGICAL FINDINGS
The tunica vaginalis remains fibrotic, but the infiltration with inflammatory cells has disappeared.
Fibrous trabeculae encircle the seminal canals, some of which are atrophied.

(6) SEROLOGY
The level of immobilizing antibodies (Fig. 21, opposite) decreases spontaneously, but never reaches the negative zone.

(7) PENICILLIN TREATMENT
With an average blood level of 6.3 U/ml., the average lymph node concentration was 1.9 U/ml., and the average testicular concentration 0.55 U/ml.

Effects of treatment
The influence of treatment and of the duration of the infection upon the results are shown in Table II.
When treatment is given soon after the infection the results for the control and treated rabbits are very different, but if treatment is given late in the course of the disease the differences are small (Collart, 1964, 1970; Yobs, Clark, Mothershed, Bullard, and Artley, 1968; Nicolau, Badanoiu, Nicolau, and Gavirlescu, 1969).

Typical results in rabbits treated with penicillin 2 years after the original infection are shown in Figs 22

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Stage of infection related to results of treatment in experimental syphilis in the rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage of syphilis</td>
<td>Early (treated 1 mth after inoculation)</td>
</tr>
<tr>
<td>Lesions</td>
<td>Controls (not treated)</td>
</tr>
<tr>
<td>Tp detectable in lesions</td>
<td>Orchitis or syphiloma</td>
</tr>
<tr>
<td>TPI test</td>
<td>+</td>
</tr>
<tr>
<td>Transfer of lymph node material to new rabbits</td>
<td>100% Lesion + Tp + TPI +</td>
</tr>
<tr>
<td>Cortisone reactivation</td>
<td>Not done</td>
</tr>
</tbody>
</table>

*According to Dr. Yobs 20 per cent. of rabbits are still positive.
†Benzyl penicillin G

From Collart (1970), p. 1289, Table IV.
and 23, and the further results of giving cortisone in Figs 24 and 25 (page 399).

Table III (Collart, Borel, and Durel, 1962a, b) shows that in man the difference between penicillin levels in serum and cerebrospinal fluid is similar to that found in the rabbit.

It may be concluded that we are dealing with a balance between, on the one hand, the host, which has been sensitized by the infection which existed before therapy started and has thus acquired a power of resistance, and, on the other hand, the parasite, vegetating in the body in the commensal state. But this does not exclude the possibility that, under certain biological conditions unknown to us, these organisms may recover all or a part of their virulence and become pathogenic once again, at least for the host which harbours them.

The latent phase may appear clinically as a 'cure', since many patients do not and will not show any further manifestation of disease throughout their lifetime, but this does not correspond to a state of 'bacteriological sterilization'.

**Summary**

The authors present a survey of experimental syphilis in rabbits based on their work at the Institut Alfred Fournier. The following stages in the evolution of the infection are considered: incubation, exponential growth, stationary phase, retrogression, and latency. At each stage the following features are considered: the appearance and duration of the lesions; the characteristics of the treponeme—
lesions, or reactivity in the TPI test. On the other hand, in some cases of infections of 2 years' duration or longer, treponemes could still be found in the tissues after penicillin treatment, and lymph node transfer showed continued infectivity in 5 per cent. of cases; treatment with cortisone could reactivate some infections with production of clinical lesions. The authors conclude that, in the period before treatment, the host acquires the power of resistance so that the parasites vegetate in a commensal state, but they do not exclude the possibility that in certain unknown biological conditions organisms can recover their virulence: the latent phase does not correspond to 'bacteriological sterilization'.

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LEVADITI, C., and LEVADITI, J. (1941) C.R. Soc. Biol., 135, 316
Les auteurs présentent une étude générale de la syphilis expérimentale chez le lapin d'après leurs travaux à l'Institut Alfred Fournier. Les différentes étapes de l'évolution de l'infection sont envisagées de la manière suivante : incubation, croissance exponentielle, phase stationnaire, rétrocision et latence. A chaque étape, différents faits sont considérés : apparition et durée des lésions ; caractéristiques des tréponèmes – particulièrement dans le changement du nombre, de la distribution et de la morphologie ; infectosité pour les tissus animaux ; changements histo-pathologiques ; comportement des épreuves TPI et FTA ; effet du traitement par la pénicilline et par la cortisonne sur les éléments précédents.

Le traitement par la pénicilline d'infections de moins de 3 mois de durée entraîne la guérison des lésions avec disparition des tréponèmes et le transfert de ganglions lymphatiques à des lapins neufs ne permet pas d'obtenir de lésion ou une positivité du TPI. D'un autre côté, dans quelques cas d'infection ayant duré deux ans ou plus, les tréponèmes purent être constatés dans les tissus après le traitement pénicilliné et le transfert des ganglions lymphatiques fut positif dans 5 pour cent. des cas ; le traitement par la cortisonne peut réactiver certaines infections et produire une lésion clinique. Les auteurs concluent que, dans la période qui précède le traitement, l'hôte acquiert une possibilité de résistance telle que le parasite subsiste à l'état commensal ; mais ceci n'exclue pas la possibilité que, dans certaines conditions biologiques inconnues, lesorganismes ne puissent retrouver leur virulence : la phase de latence ne correspond pas à la 'stérilisation bactériologique'.
Experimental rabbit syphilis.

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