TPI TEST IN UNTREATED SYPHILIS*

A SEROLOGICAL RE-EXAMINATION OF 50 PATIENTS BELONGING TO "THE OSLO STUDY OF UNTREATED SYPHILIS"

BY

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The Boeck-Bruusgaard series of cases of untreated syphilis consists of approximately 2,000 patients with primary and/or secondary syphilis, hospitalized in the Dermatological Department, University Hospital, Oslo, in the 20 years between 1891 and 1910. Prof. Caesar Boeck, who was head of the Dermatological Department in those years, had very little belief in the effect upon early syphilis of the various antisyphilitic drugs which were then available; consequently drugs were very rarely given to patients with early syphilis in his department and this policy was continued until Salvarsan was introduced in 1910.

Mercury was administered only in exceptional cases, and potassium iodide to some patients whose clinical course was presumed to indicate its use, but in general, the patients with early syphilis were left untreated. They were, however, thoroughly examined and systematic records were kept, so that an outstanding series of patients with untreated early syphilis was created. This material was re-examined clinically by Bruusgaard (1929); and later by Danbolt, Clark, and Gjestland (1954), Gjestland (1955), and Clark and Danbolt (1955). These publications, especially Gjestland's great monograph published in 1955, have furnished extremely valuable information on the clinical course of untreated syphilis. This "Oslo Study of Untreated Syphilis" was reviewed by Harrison (1956), but the present investigation is serological, its chief aim being to procure new information on the sensitivity of the TPI test in very old syphilis, untreated in the early stages.

Validity of the Original Diagnoses

Having scrutinized the original records, we consider the diagnoses of primary and/or secondary syphilis to be correct in 48 of the fifty patients. The group includes no case of congenital syphilis. In one patient (Case 1) the symptoms and signs recorded appear to us insufficient for the diagnosis of secondary syphilis, but we have not removed the patient from the group under study, since she stayed in hospital for more than 2 months, and was thus submitted to prolonged clinical observation. In another patient (Case 11) an insoluble identity problem exists, but this does not appear to justify his exclusion from the group.

Specific Treatment in the Primary and Secondary Stages

Six patients had been given potassium iodide in amounts ranging from 100 to 775 g. during their stay in the University Hospital, and a further fourteen had received potassium iodide in very small or unknown amounts. Four of these fourteen patients had mercury treatment as well; the amounts are unknown but are probably small and inadequate. Two patients were given mercury treatment by the Oslo City Health Department soon after their discharge from the University Hospital.

We may conclude, therefore, that our group comprises fifty patients with acquired syphilis; the
majority received no specific treatment whatever during the early stages of the disease, and most of the remainder were given treatment which could not have had any significant influence upon the subsequent course of the disease.

Later Specific Treatment
Six patients were later given adequate treatment with arsphenamines, bismuth, or penicillin, or combinations of these drugs; this treatment was given from 7 to 47 years following infection. Three other patients received inadequate treatment, and a further eight received treatment to which no curative effect can be attributed, judging from the nature and amounts of the drugs administered.

Clinical Course
47 of the fifty patients were examined clinically by Gjestland in the years 1949 to 1951: two suffered at that time from active late syphilis (one with tabes dorsalis, and the other with benign tertiary syphilis); seven who showed no clinical signs or symptoms of active late syphilis when examined in 1949–1951 had previously developed manifestations of benign tertiary syphilis; 38 patients had no clinical evidence of syphilis, nor had they experienced any manifestations of the disease since their discharge from the University Hospital at the end of the secondary stage.

Age at Time of Infection and at Time of TPI Examination (Table I)
Nine patients had contracted syphilis in childhood and the remaining 41 as adults. More than 50 years had elapsed between infection and the present serological examination, a remarkably long interval.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Max.</th>
<th>Average</th>
<th>Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>At infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 in childhood</td>
<td>10</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>41 as adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At TPI Examination</td>
<td>98</td>
<td>75</td>
<td>54</td>
</tr>
<tr>
<td>Interval between Infection and TPI Examination</td>
<td>68</td>
<td>57</td>
<td>50</td>
</tr>
</tbody>
</table>

Sex Distribution
The group comprised 38 women and twelve men. This proportion agrees with the sex distribution in the whole series examined by Gjestland (68.2 per cent. females and 31.8 per cent. males). As mentioned by Gjestland (1955), this unequal distribution may be partly accounted for by the fact that the capacity of the female ward in Boeck's department was twice that of the male ward.

Methods

TPI Technique*
Proportion of test mixture: serum 0.05 ml., complement 0.20 ml., Treponema pallidum suspension 0.25 ml.
All sera were examined at least twice. First, they were tested qualitatively using 18 hrs' incubation, which is our routine technique. Sera giving TPI$_{18}$ < 20 were then re-examined qualitatively with 42 hrs' incubation, which is a more sensitive technique.
Sera giving TPI$_{18}$ = 100 were re-examined quantitatively to determine their TPI$_{50-18}$. Sera giving TPI$_{18}$ 20–99 per cent. S.I. were re-examined by the same technique.
With 42 hrs' incubation, only half of the complement dose (0.10 ml.) was added at the beginning, the remaining 0–10 ml. being added after 18 hrs' incubation.
A titration of the "WHO Reference Pool No. III for TPI Test" was carried out on each testing day. The TPI$_{50-18}$ was on the average 330 (varying between 180 and 520), which shows that the sensitivity of the test was satisfactory (W.H.O., 1958).

Standard Tests for Syphilis (STS) Technique
The sera were also examined with the following three standard tests for syphilis:

Bordet-Wassermann Complement-Fixation Test (WaR) (cardiolipin-lecithin-cholesterol antigen)
Meinicke Clarification Test II (MKR) (cardiolipin-lecithin-balsam of Tolu antigen)
VDRL Slide Flocculation Test (cardiolipin-lecithin-cholesterol antigen).

Positive sera were titrated in WaR and MKR.

Technique for WaR Titration.—First tube, serum dilution 1:5, further doubling dilutions for each successive tube. Each tube is given a titration score according to the degree of haemolysis (4 = complete inhibition of haemolysis, 0 = complete haemolysis).

Technique for MKR Titration.—First tube, serum undiluted, further doubling dilutions for each successive

* The following symbols are used:

TPI$_{18}$ = per cent. specific immobilization in qualitative TPI test with 18 hrs incubation.
TPI$_{42}$ = per cent. specific immobilization in qualitative TPI test with 42 hrs incubation.
TPI$_{50-18}$ = final serum dilution giving 50 per cent. specific immobilization in the quantitative TPI test with 18 hrs incubation.
TPI TEST IN UNTREATED SYphilis

Tube. Each tube is given a titration score according to the degree of clarification (4 = complete clarification, 0 = no clarification).

For both reactions the titre value is given as the sum of the titration scores; the titre values thus run roughly parallel to the logarithm of the reagent content in the serum.

The VDRL test is carried out as a qualitative test in undiluted serum only, and positive results reported as ± to +++ according to the size of the clumps.

Results

TPI Results

47 patients were TPI-positive, and three negative. Case 1, who had a doubtful clinical diagnosis, and Case 11, who had an identity problem, were both among the three negative reactors.

Table II shows the strength of the reactions. 26 sera immobilized all the treponemes after 18 hrs' incubation; the distribution of titres is given in Section A. The highest titre demonstrated was 1,700 and the lowest 20. Fourteen sera had titres as low as 100 or less.

**Table II**

RESULTS OF TPI TEST

For symbol definitions, see footnote, page 224.

<table>
<thead>
<tr>
<th>TPI Result</th>
<th>Number of Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. TPI&lt;sub&gt;18&lt;/sub&gt; = 100%</td>
<td>26</td>
</tr>
<tr>
<td>1000 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 1000</td>
<td>3</td>
</tr>
<tr>
<td>500 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 500</td>
<td>4</td>
</tr>
<tr>
<td>100 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 100</td>
<td>5</td>
</tr>
<tr>
<td>30 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 20</td>
<td>10</td>
</tr>
<tr>
<td>30 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 20</td>
<td>4</td>
</tr>
<tr>
<td>B. 100 &gt; TPI&lt;sub&gt;18&lt;/sub&gt; &gt; 20%</td>
<td>16</td>
</tr>
<tr>
<td>C. TPI&lt;sub&gt;18&lt;/sub&gt; &lt; 20% and TPI&lt;sub&gt;12&lt;/sub&gt; &gt; 20%</td>
<td>5</td>
</tr>
<tr>
<td>Total TPI-Positive Sera</td>
<td>47</td>
</tr>
<tr>
<td>D. TPI&lt;sub&gt;18&lt;/sub&gt; &lt; 20% (TPI-Negative)</td>
<td>3</td>
</tr>
<tr>
<td>Total Sera Examined</td>
<td>50</td>
</tr>
</tbody>
</table>

Sixteen sera (Section B) caused only partial immobilization after 18 hrs' incubation; fifteen of them gave TPI<sub>18</sub> > 50 in at least one of the repeated examinations (and the remaining one gave a maximum of TPI<sub>18</sub> = 48).

Five sera (Section C) showed the weakest type of positive reaction, manifesting themselves with certainty in TPI<sub>18</sub> only. Two had TPI<sub>18</sub> < 20 in all the examinations performed, and the other three had TPI<sub>18</sub> < 20 in some examinations, and TPI<sub>18</sub> > 20 in others, up to 32. All these five sera had TPI<sub>18</sub> > 50 in at least one examination.

Obviously, the group as a whole is distinguished by a remarkably high frequency of weak and very weak positive TPI reactions.

STS Results

32 sera gave positive results in one or more of the standard tests employed (64 ± 6.8 per cent.) and the other eighteen were negative in all three tests. The following reaction patterns were seen among the 32 STS-positive sera:

<table>
<thead>
<tr>
<th>WaR</th>
<th>MKR</th>
<th>VDRL</th>
<th>No. of Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>18</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>19</td>
<td>31</td>
<td>31</td>
<td>32</td>
</tr>
</tbody>
</table>

The distribution of titres in WaR and MKR is given in Table III; as would be expected, the weakly positive reactions predominate.

**Table III**

RESULTS OF WASSERMANN (WaR) AND MEINICKE (MKR) REACTIONS

<table>
<thead>
<tr>
<th>Test</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>WaR</td>
<td>31</td>
</tr>
<tr>
<td>MKR</td>
<td>19</td>
</tr>
</tbody>
</table>

TPI Results compared with STS Results

Table IV (overleaf) compares the TPI with the MKR. The group is too small to warrant any detailed statistical analysis, but it can be shown that a positive correlation exists between the strength of the two reactions. The correlation can be demonstrated, for instance, by a higher frequency of the weakest positive TPI results among the MKR-negative sera: of sixteen MKR-negative sera, eleven (68·75 per cent.) had TPI<sub>18</sub> < 100, but of 31 MKR-positive sera, only ten (32·26 per cent.) had TPI<sub>18</sub> < 100. (The three sera which were negative in both TPI and MKR are not included in this calculation.) The difference between the two proportions (36·49 per cent.) is 2·55 times greater than its standard error, i.e. the difference would arise by chance only once in about eighty times. Furthermore, Table IV shows that, of the five patients
TABLE IV

COMPARISON BETWEEN RESULTS OF TPI TEST AND MKR REACTION
(For symbol definitions, see footnote, page 224)

<table>
<thead>
<tr>
<th>TPI Test</th>
<th>MKR Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (Negative)</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>TPI&lt;20%</td>
<td></td>
</tr>
<tr>
<td>TPI&gt;20%</td>
<td></td>
</tr>
<tr>
<td>TPI&lt;100%</td>
<td></td>
</tr>
<tr>
<td>30&gt;TPI&lt;100</td>
<td></td>
</tr>
<tr>
<td>100&gt;TPI&lt;100</td>
<td></td>
</tr>
<tr>
<td>500&gt;TPI&lt;100</td>
<td></td>
</tr>
<tr>
<td>1000&gt;TPI&lt;1000</td>
<td></td>
</tr>
<tr>
<td>TPI&gt;10000</td>
<td></td>
</tr>
<tr>
<td>Total No. of Cases</td>
<td></td>
</tr>
</tbody>
</table>

Table IV shows the comparison between TPI test and MKR reaction. The TPI test was performed, and the MKR titre was determined. The results are summarized in the table.

Case 14, a woman born in 1891, had early syphilis when 18 years old in 1909, and no further clinical signs or symptoms of syphilis. In 1951, the WaR and MKR were both positive in blood.

Serological Findings, 1960–1961
WaR: positive titre 3, MKR: positive titre 4, VDRL: ++.

Case 9, a man born in 1880, had early syphilis when 22 years old in 1902, and no further clinical signs or symptoms of syphilis. In 1950, the WaR and MKR were both negative in blood.

Serological Findings, 1961
WaR: positive titre 5, MKR: positive titre 5, VDRL: +++.

TPI Results Compared with Sex, Age, and Clinical Course

The two patients who suffered from active late syphilis in 1949–1951 had TPI<18 = 640 and 170 respectively.

No correlations were found between the TPI result and sex, age at infection, age at TPI examination, interval between infection and TPI examination, and occurrence of benign tertiary manifestations before 1950. It must be stressed, however, that our small group of patients is not very suitable for examination regarding such correlations; our negative findings in this respect do not exclude the possibility that one or more of the correlations mentioned might have been demonstrable in a larger series.

Discussion

Our present knowledge about the sensitivity of the TPI test in untreated late syphilis, symptomatic and latent, may be summarized as follows:

1. In syphilis which is not treated until after the secondary stage, the TPI will remain positive for several decades in nearly all patients, and in many of them throughout life.

2. In patients who eventually become TPI-negative the TPI has nevertheless remained positive for a much longer time than the STS.

This knowledge is based upon the work of several investigators—Nielsen and Reyn (1956) have selected from the literature altogether 197 cases of untreated
early and late latent syphilis, 195 of whom were TPI-positive and only two TPI-negative; of 107 cases of untreated late symptomatic syphilis, 106 were TPI-positive and one TPI-negative.

Our small group under study represents a natural selection of survivors from the original Boeck-Bruusgaard series of about 2,000 patients, and this fact probably explains why our group mainly comprises latent cases. Our serological results must be seen in close association with the distinctive character of the group: very long-standing syphilis, untreated in the early stages, and now latent in nearly every case.

Of our fifty patients, 47 were TPI-positive more than 50 years after the original infection, which confirms the lifelong reactivity of the TPI test in cases of untreated syphilis. But to this conclusion must be attached an important reservation; many patients reacted so weakly to the TPI test that an only slightly less sensitive TPI technique would have given a negative result. This has been proved by examining all the sera under study by the TPI test in another laboratory. There they were also tested by the fluorescent treponemal antibody test (FTA) in order to gather information about the sensitivity of the FTA test. The results of this comparative study are to be published separately (Eng, Nielsen, and Wereide, in preparation).

47 patients gave positive TPI tests, but only 32 gave positive STS. This overall result confirms the opinion that in cases of late syphilis the TPI is much more sensitive than the STS. This fact and the very high specificity of the TPI test form the basis of the use of the TPI test to distinguish between specific and non-specific positive standard tests. It is of great interest, therefore, that, out of fifty patients with late syphilis, as many as three appear to be exceptions (Cases 3, 9, and 14). These three patients gave positive STS, but the TPI tests were negative when performed by the usual routine technique, and the small amounts of immobilizing antibodies contained in their sera could be demonstrated with certainty only by 42-hr incubations. It must, however, be considered whether the positive STS demonstrated in these three patients were really caused by their old syphilitic infection, or were due to a non-specific formation of reagin. As the case histories show, two of them (Cases 3 and 9) were WaR- and MKR-negative when examined in 1949–1951. On the other hand, the WaR and MKR examinations in 1949–1951 were carried out in another laboratory with antigens made up from crude heart extracts, which were considerably less sensitive than the cardiolipin antigens in present use. It is quite possible, therefore, that in 1949–1951 as well as in 1960–1961 these three patients had specific reagins in the blood in small amounts which were not then detectable, but can now be readily demonstrated. Furthermore, the STS-patterns of the three sera oppose the idea of non-specific reagin formation in two respects:

(1) The patterns are of a type frequently seen in patients with late syphilis;

(2) The patterns are not distinguishable from those of the remaining sera under study, but fit smoothly into the scheme of the 32 STS-positive sera.

We consider it highly improbable, therefore, that a non-specific reagin formation alone accounts for the positive STS in the three patients under discussion. If their blood samples had been sent to our laboratory for routine serological examination, and the history of syphilis had been unknown to us, we should have regarded the positive STS as being very probably non-specific. It is obvious, therefore, that important practical lessons can be learnt from these cases:

(1) The sensitivity of the TPI technique should be kept as high as possible;

(2) The diagnosis of "false positive STS" should only be made in consultation between the serologist and the clinician.

As for the former point, the sensitivity of the TPI technique in our laboratory is kept at a high level by means of a high complement concentration (40 per cent.), and moreover, a number of the routine sera which give TPI18 < 20 are now re-tested with 42 hrs' incubation. In principle, the procedure of forcing sensitivity up to a very high level involves the danger that small amounts of immobilizing antibodies from the rabbits which are probably often absorbed on the spirochaetes may become demonstrable in the tests. In our experience this danger can be reduced by giving the rabbits cortisone daily throughout the incubation period (for technical details, see WHO, 1960).

It may be concluded that the sensitivity of the TPI test is considerably greater than, but not absolutely superior to, that of the STS. The present investigation appears to provide new information which modifies the general concept that in late syphilis the TPI test outdoes the STS in sensitivity. These results are, of course, enhanced by the high sensitivity of the modern cardiolipin-lecithin antigens. In Norway, as in many other countries, the majority of syphilitic patients are old, asymptomatic cases, and much
laboratory work consists in demonstrating and evaluating small and very small amounts of antibodies. Thus the serologist will occasionally encounter as in the present series, a few cases of late syphilis with positive STS and a negative TPI test, a phenomenon previously observed by other authors (see Eng, 1959). It is reasonable to assume, however, that most cases of this kind are weak STS-reactors; it is probably right to say that, in patients with positive STS and negative TPI, in whom fresh infection can be excluded and in whom there are no other signs or symptoms of syphilis, the validity of the diagnosis "false positive STS" is greater, the higher the STS titres.

Our series is characterized by an unusual predominance of weakly positive TPI tests, several of them only just demonstrable, probably because of the very long time which had elapsed since the patients were first infected. Another possible explanation is that antibody level deteriorates in old people with the general failure of protein synthesis, but this could have played only a minor rôle in our series, since no correlation could be demonstrated between the TPI test and the patient's age.

Although we examined only one blood sample from each patient, our series provides, in an indirect way, information about the course of the TPI titre in untreated latent syphilis. Nearly all the patients were TPI-positive, but most of them showed only weak reactions, and these two facts lead us to the following conclusions:

1. The immobilizing antibodies in the blood of patients with untreated latent syphilis decrease when—and because—the disease becomes old, but only in exceptional cases do they disappear completely.

2. In cases of very long standing, the antibody content has stabilized itself at a low level, often so low that only a very sensitive TPI technique will detect it.

3. At this stage very few patients still have high TPI titres.

The present results confirm the fact that, in late syphilis, a persistent positive TPI test does not mean that the disease is active or in need of additional treatment. Furthermore, a weakly positive TPI test may persist even if the disease is definitely cured. In 38 of our patients no clinical manifestations of syphilis could be traced since the secondary symptoms ended more than 50 years before. A negative TPI reaction in late syphilis is generally regarded as good evidence of definite cure, and a weakly positive reaction to a sensitive TPI test technique also appears to be not incompatible with a definite cure.

The last conclusion to be drawn from our results is that Prof. Caesar Boeck and his co-workers, who had no modern laboratory methods available to them, had nevertheless a thorough mastery of the clinical diagnosis of syphilis in its varying clinical manifestations. Today, the diagnosis of syphilis rests primarily upon the serologist and his intimate knowledge of the reactivity of the serological tests under varying technical conditions.

**Summary**

*Treponema pallidum* immobilization (TPI) tests were performed on blood samples from fifty survivors of the "Oslo Study of Untreated Syphilis". The series was characterized by the following features:

1. The diagnosis of syphilis was, with only two possible exceptions, indubitable.

2. The patients had been left untreated during the primary and secondary stages, and most of them had received no specific treatment at any time.

3. Most were latent cases.

4. More than 50 years had elapsed since the primary infection.

Table II shows that 47 of the fifty patients were TPI-positive, a result which confirms the reputed life-long reactivity of the TPI test in untreated syphilis. But there was an unusual predominance of weakly positive TPI reactions, several of them only just demonstrable. These findings appear to justify the following conclusions:

The amount of immobilizing antibodies in the blood of patients with untreated latent syphilis decreases with—and because of—the passage of time, but only in exceptional cases do they disappear completely.

After a very long interval the antibody content in most patients stabilizes itself at a low level, often so low that it can be detected only by a most sensitive TPI technique.

Very few patients still have high TPI titres at this stage.

In three patients showing positive standard tests for syphilis (STS) the TPI reactions were negative with 18 hrs' incubation, but could be demonstrated by 42 hrs' incubation. The practical consequences of this finding, primarily with regard to the diagnostic application of the TPI test, are thoroughly discussed.

The present studies suggest that a positive, or at least a weakly positive, TPI reaction may persist even if the patient is definitely cured.
The authors would like to express their sincerest thanks to Dr. H. Aage Nielsen, Head of the Treponematoses Department, State Serum Institute, Copenhagen, and Director of the WHO Serological Reference Centre, who has made many valuable suggestions.

REFERENCES

Immobilisation des treponemes dans la syphilis non traitée

RÉSUMÉ

Le sérum de 50 survivants de l'étude de Oslo fut soumis au test d'immobilisation des treponèmes (T.P.I.).

On nota les caractères suivants:
(1) Le diagnostic de la syphilis était incontestable, sauf dans deux cas.
(2) Les sujets n'avaient reçu aucun traitement aux étares précoces et secondaires, et la plupart rien du tout.
(3) La maladie était latente dans la plupart des cas.
(4) Plus de 50 ans s'étaient écoulés depuis la première infection.

On voit sur la Table II que 47 des 50 séums étaient positifs, ce qui corrobore que la réactivité du test TPI dure pour toute la vie chez les sujets non-traités. Il y avait néanmoins un nombre inattendu de réactions faibles, quelques-unes à peine visibles.

Ces résultats suggèrent les conclusions suivantes:
Les anticorps immobilisants dans le sang d'un sujet atteint de syphilis latente qui n'a reçu aucun traitement diminuent avec le temps, mais ne disparaissent complètement que dans les cas exceptionnels.
Après un long intervalle les anticorps se maintiennent à un niveau bas chez la plupart des sujets, souvent si bas que seules les méthodes les plus sensibles permettent d'en décéler la présence.
A cette étape très peu de malades présentent un titre élevé de T.P.I.
Chez trois malades présentant des S.T.S. positifs, les réactions T.P.I. étaient négatives après 18 heures d'incubation mais devinrent positives aprés 42 heures. On discute à fond les conséquences pratiques de ce résultat, surtout à l'égard de l'emploi du test T.P.I. dans le diagnostic.
Ces études indiquent qu'une réaction T.P.I. positive ou au moins faible peut persister même quand le malade est définitivement guéri.
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