STUDIES IN EXPERIMENTAL SYPHILIS.

VII. REINOCULATION OF TREATED AND UNTREATED SYPHILITIC RABBITS WITH HETEROLOGOUS STRAINS OF TREPONEMA PALLIDUM. *

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Previous communications in this series have dealt with the results of experiments in which both treated and untreated syphilitic rabbits were inoculated a second time with homologous strains of Treponema pallidum. The results of those experiments are in close accord with the findings of other investigators in the same field,1 and the evidence thus far assembled, both by others and by ourselves, indicates that in rabbits during the course of an experimental infection with syphilis there is built up in time a specific state of resistance against the infecting organism to the extent that subsequent inoculations with homologous strains of the latter are not followed by the customary phenomena of the disease. It is possible to prevent the development of this state of resistance by suitable treatment early in the course of the disease (before the 45th day), but if treatment be postponed until the resistant state is fairly well established (after the 90th day), as was first shown by Kolle, the rabbit apparently remains refractory, for months at least, to a second inoculation with the homologous strain of treponemata, that is to say, refractory in the sense that reinoculation is not followed by any manifest phenomena of disease. It has been established in experiments already reported in this series (1) that this resistant

* Aided by a grant from the Ella Sachs Plotz Foundation for the Advancement of Scientific Investigation.
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1 The present status of our knowledge of this subject has been reviewed recently by one of us in another periodical (18), and need not therefore be presented here in detail.
state against homologous strains of the organism is not absolute but may be broken down in part by resort to procedures which favor the inciting agent.

The purpose of the present communication is to record the results of a series of experiments in which the reaction of treated and untreated syphilitic rabbits toward second inoculations with heterologous strains of *Treponema pallidum* was observed. Since these studies were first undertaken in the latter part of 1924, there have appeared in the literature reports of experiments conducted along similar lines with results, in general, similar to those obtained by ourselves. There are also available in the older literature accounts of experiments of like character in which a few animals were studied. These will be briefly summarised at this point, attention being directed principally to those experiments in which the second inoculation was carried out 90 days or more after the first, or in which treatment was begun after a like interval had elapsed following the first inoculation.

**HISTORICAL.**

Zinsser, Hopkins and McBurney (2) inoculated 12 untreated syphilitic rabbits with heterologous strains of *Treponema pallidum*, 106 to 389 days after the first inoculation, and obtained 3 positive results. Pearce and Brown (3) were apparently able to produce a second infection in an untreated syphilitic rabbit by the inoculation of a more virulent strain of treponemata 55 days after the first inoculation, at a time when the disease phenomena produced by the latter were subsiding. Reiter (4) obtained 4 successful second infections in 9 reinoculations of rabbits with heterologous strains carried out 133 to 602 days after the first inoculation, and Kolle (5) in a series of cross-inoculation experiments with 51 rabbits obtained 23 successful second infections with heterologous strains. Adachi (6) successfully infected 7 syphilitic rabbits a second time by inoculating with heterologous strains late in the course of the disease, while reinoculations with homologous strains gave uniformly negative results. Nothaas (7) has recently reported 3 successful reinoculations with heterologous strains in a series of 16 syphilitic rabbits, the interval between inoculations ranging from 14 to 42 weeks. Manteufel and Worms (8) carried out a series of cross-inoculations in which 7 rabbits infected with the Nichols strain and untreated were subsequently inoculated with another strain of *T. pallidum*. Of the 7, 2 were successfully infected a second time.

The behavior of treated syphilitic rabbits toward a second inoculation with heterologous strains of treponemata has received scarcely any attention thus far. Reiter (4) treated 3 syphilitic rabbits 70, 91 and 182 days, respectively, after
inoculation and subsequently reinoculated them with heterologous strains but failed to obtain any evidence of infection. Manteufel and Worms (8) reinoculated one treated rabbit with an heterologous strain of *T. pallidum* with a negative result.

**EXPERIMENTAL.**

The observations to be reported in this communication deal with a series of reinoculations in 68 rabbits, most of which were treated with arsphenamine prior to the introduction of the second infection.

**Rabbits.**—All the commoner breeds of rabbits were represented in the experiments, the grays and browns predominating.

**Mode of Inoculation.**—First inoculations were invariably made into the testis and in nearly every instance subsequent inoculations were made in the opposite testis. In a few instances second inoculations were made intracutaneously into the sheath of the penis. Because of the occurrence of metastatic orchitis in many of the animals, it happened that frequently the second inoculation was made in a testis which had previously been the site of a syphilitic process, while in the others the second inoculation was made into an apparently normal testis as judged by clinical examination. In some instances the animals were reinoculated first with the homologous strain, later with an heterologous strain of treponemata.

**Strains.**—Six strains of *Treponema pallidum* were used in this study. These included two which have long been adapted to the rabbit, namely the Nichols strain and the Truffi strain (for the latter of which we are indebted to Professor Kolle). The remaining 4 strains were isolated by us from syphilitic patients in the Johns Hopkins Hospital. Of these latter, 1, designated "A," was isolated from the spinal fluid of a patient with secondary syphilis, but without clinical or serological evidence of involvement of the central nervous system (9); 1, designated "C," was isolated from the spinal fluid of a patient with syphilitic meningitis of the neuro recurrence type (10), a third, designated "F," was isolated from the synovial fluid of a patient with syphilitic arthritis; and the fourth, Strain "H," was obtained from a lymph node of a patient with arthritis (11). All of these 4 strains were isolated in the years 1923 and 1924.

**Treatment.**—Where the animals were treated, the mode of treatment was invariably the same and consisted in the administration of 6 doses of arsphenamine, 10 mg. per kg., administered at weekly intervals. The time at which treatment was begun varied somewhat but was always late in the course of the infection, that is to say, after the 90th day. This time interval was selected because of the now well established fact that when treatment is postponed until this interval has elapsed a second inoculation with homologous strains of *Treponema pallidum* does not, except in rare instances, lead to the development of a syphilitic lesion at the site of inoculation.

**Wassermann Reaction.**—In many of the experiments the behavior of the Wassermann reaction was followed at regular intervals. The technic employed
has already been described elsewhere (12). Further experience with the method has confirmed our previous impression that it can be successfully employed in the study of experimental syphilis in the rabbit. The only modification which we have introduced since the publication of our previous communication on the test, and have employed throughout in these experiments, has been the use of 0.05 cc. of the serum instead of 0.1 cc. as originally employed. All other quantities of reagents have been the same. We have gained the impression that with the use of the smaller amount of serum a number of anticomplementary reactions have been eliminated.

Criteria of Reinfection.—In all the experiments cited in the literature, the criterion of successful production of a second infection has been the development, after an appropriate incubation period, of a syphiloma at the site of inoculation in which the presence of Treponema pallidum could be demonstrated. As was suggested several years ago by Brown and Pearce (13), however, second infections might be produced without the occurrence of any visible syphilitic phenomena at the site of reinoculation, and in previous papers in this series (14, 1) evidence has been presented which strongly indicates that treated syphilitic rabbits can react in such a manner to a second inoculation with homologous strains of the organism. Data of a similar nature have also been obtained by Voegflin and his associates (15). Recently Kolle and Schlossberger (16) reported experiments which indicate that the same state of affairs may obtain when heterologous strains are employed for the second inoculation. The recognition of such asymptomatic reinfections is made possible through the study of the infectivity of lymph nodes or internal organs of the reinoculated animal. The utilisation of this method of study necessarily entails the expenditure of a large number of animals and in the present study it was deemed advisable to forego this procedure since the object was not to obtain absolute figures as to the susceptibility of syphilitic rabbits to infection with heterologous strains, but to contrast the effect of reinoculation with the latter as opposed to reinoculation with homologous strains. For that reason we adopted as a criterion of successful second infection in this series of experiments, the development of a characteristic syphilitic lesion at the site of inoculation with the demonstration of treponemata therein. It is freely admitted that such a criterion will probably fall short of telling the whole story, and that the number of successful second infections in our experiments may have been, and almost certainly was, greater than the figures would indicate, but the expense attendant upon ascertaining the actual number of asymptomatic reinfections, involving as it does the identification of the strain isolated after reinoculation, did not seem to be warranted in view of the fact that only relative and not absolute data were desired.

Controls.—The virulence of the strain used for reinoculation was, of course, always tested by simultaneous inoculation of a series of normal rabbits with equal amounts of the same virus emulsion. In no instance did the control rabbits fail to show characteristic syphilitic lesions. A series of 13 animals was twice reinoculated with homologous strains of treponemata in order to ascertain
whether or not the resistance to the homologous strain persisted for the duration of the experiments.

Results in Untreated Rabbits.

Reinoculations with an heterologous strain of Treponema pallidum were carried out in 11 untreated syphilitic rabbits, the second inoculation in each instance being performed with the Nichols strain. The interval between inoculations varied from 93 to 170 days. In 5 of the animals the second inoculation was made intracutaneously on the sheath of the penis. In 4 of the remaining 6 animals, in which the second inoculation was made into the testis, that particular organ had previously been the site of a syphilitic lesion. The results of the experiment are shown in Table I.

Table I shows that of the 11 untreated syphilitic rabbits reinoculated with an heterologous strain of Treponema pallidum 93 to 170 days after the first inoculation, 7, or 63 per cent, reacted with the formation of a syphilitic lesion at the site of inoculation. In 9 of the animals the strains used for the first inoculation had been isolated from human cases of syphilis at a more recent date than had the strain used

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Strain of 1st inoculation</th>
<th>Strain of 2nd inoculation</th>
<th>Interval between inoculations</th>
<th>Mode of 2nd inoculation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Nichols</td>
<td>107</td>
<td>Testicular</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>C</td>
<td>&quot;</td>
<td>93</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>&quot;</td>
<td>93</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>&quot;</td>
<td>93</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>&quot;</td>
<td>107</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>&quot;</td>
<td>170</td>
<td>Intracutaneous</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>&quot;</td>
<td>142</td>
<td>&quot;</td>
<td>+</td>
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<td>8</td>
<td>F</td>
<td>&quot;</td>
<td>107</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>H</td>
<td>&quot;</td>
<td>153</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Truffi</td>
<td>&quot;</td>
<td>121</td>
<td>&quot;</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>&quot;</td>
<td>121</td>
<td>Testicular</td>
<td>-</td>
</tr>
</tbody>
</table>

Total positive............................................................ 7
" negative............................................................. 4
### TABLE II.
Reinoculations of Treated Syphilitic Rabbits with Homologous and Heterologous Strains of *T. pallidum*.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Strain of 1st inoculation</th>
<th>Treatment days after inoculation</th>
<th>2nd inoculation</th>
<th>3rd inoculation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Strain</td>
<td>Day of disease</td>
</tr>
<tr>
<td>12</td>
<td>A</td>
<td>139</td>
<td>A</td>
<td>282</td>
</tr>
<tr>
<td>13</td>
<td>A</td>
<td>139</td>
<td>A</td>
<td>282</td>
</tr>
<tr>
<td>14</td>
<td>A</td>
<td>170</td>
<td>A</td>
<td>267</td>
</tr>
<tr>
<td>15</td>
<td>A</td>
<td>198</td>
<td>A</td>
<td>295</td>
</tr>
<tr>
<td>16</td>
<td>A</td>
<td>170</td>
<td>A</td>
<td>267</td>
</tr>
<tr>
<td>17</td>
<td>C</td>
<td>188</td>
<td>C</td>
<td>271</td>
</tr>
<tr>
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<td>C</td>
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<td>238</td>
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<tr>
<td>19</td>
<td>C</td>
<td>115</td>
<td>C</td>
<td>184</td>
</tr>
<tr>
<td>20</td>
<td>C</td>
<td>206</td>
<td>C</td>
<td>275</td>
</tr>
<tr>
<td>21</td>
<td>C</td>
<td>169</td>
<td>C</td>
<td>238</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>213</td>
<td>F</td>
<td>326</td>
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<td>23</td>
<td>F</td>
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<td>F</td>
<td>230</td>
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<td>24</td>
<td>F</td>
<td>157</td>
<td>F</td>
<td>240</td>
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<tr>
<td>25</td>
<td>F</td>
<td>124</td>
<td>F</td>
<td>207</td>
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<tr>
<td>26</td>
<td>F</td>
<td>124</td>
<td>F</td>
<td>207</td>
</tr>
<tr>
<td>27</td>
<td>F</td>
<td>157</td>
<td>F</td>
<td>240</td>
</tr>
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<td>28</td>
<td>F</td>
<td>208</td>
<td>F</td>
<td>291</td>
</tr>
<tr>
<td>29</td>
<td>H</td>
<td>210</td>
<td>H</td>
<td>344</td>
</tr>
<tr>
<td>30</td>
<td>H</td>
<td>141</td>
<td>H</td>
<td>275</td>
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<td>31</td>
<td>H</td>
<td>265</td>
<td>H</td>
<td>348</td>
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<td>32</td>
<td>H</td>
<td>210</td>
<td>H</td>
<td>293</td>
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<td>33</td>
<td>H</td>
<td>137</td>
<td>H</td>
<td>240</td>
</tr>
<tr>
<td>34</td>
<td>H</td>
<td>120</td>
<td>H</td>
<td>203</td>
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<td>H</td>
<td>120</td>
<td>H</td>
<td>203</td>
</tr>
<tr>
<td>36</td>
<td>Nichols</td>
<td>91</td>
<td>Nichols</td>
<td>160</td>
</tr>
<tr>
<td>37</td>
<td>&quot;</td>
<td>91</td>
<td>Nichols</td>
<td>160</td>
</tr>
<tr>
<td>38</td>
<td>&quot;</td>
<td>116</td>
<td>&quot;</td>
<td>185</td>
</tr>
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<td>39</td>
<td>&quot;</td>
<td>91</td>
<td>&quot;</td>
<td>160</td>
</tr>
<tr>
<td>40</td>
<td>&quot;</td>
<td>91</td>
<td>&quot;</td>
<td>160</td>
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<tr>
<td>41</td>
<td>&quot;</td>
<td>116</td>
<td>&quot;</td>
<td>185</td>
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<td>42</td>
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</tr>
<tr>
<td>45</td>
<td>&quot;</td>
<td>91</td>
<td>&quot;</td>
<td>160</td>
</tr>
<tr>
<td>46</td>
<td>&quot;</td>
<td>91</td>
<td>&quot;</td>
<td>160</td>
</tr>
</tbody>
</table>

* Lesion produced was a small syphilitic nodule.
for the second inoculation, and in general could be regarded as less virulent strains than the latter. There was no significant difference in percentages of successful reinoculations obtained by intratesticular inoculation as contrasted with intracutaneous inoculation.

**Results in Treated Rabbits.**

For the purpose of presentation, the experiments with treated rabbits have been arranged in two groups, first (Group 1) those in which the animals were reinoculated with the homologous strain and then with an heterologous strain, and those (Group 2) in which only one reinoculation and that with the heterologous strain was carried out.

**TABLE III.**

Possible and Actual Results of Reinoculations with Homologous and Heterologous Strains of *T. pallidum*.

<table>
<thead>
<tr>
<th>Category</th>
<th>1st reinoculation</th>
<th>2nd reinoculation</th>
<th>No. of rabbits encountered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain</td>
<td>Result</td>
<td>Strain</td>
</tr>
<tr>
<td>A</td>
<td>Homologous</td>
<td>+</td>
<td>Homologous</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>-</td>
<td>Heterologous</td>
</tr>
<tr>
<td>F</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>G</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Group 1. In this group there were observations upon 35 animals including 13 controls which received two successive reinoculations with the homologous strain. The results are shown in Table II. From Table II it will be seen that of 35 rabbits originally inoculated with various strains of *T. pallidum* and treated from 91 to 265 days after inoculation all but 2 gave negative results when inoculated a second time with the homologous strain. Of the 33 animals which were thus shown to be refractory to their own strain, 13 were inoculated a third time with the same strain as controls and again proved refractory, while the remaining 20 which were inoculated with heterologous
strains, gave a positive result in 12 instances, or 59 per cent. The two animals (Nos. 24 and 25) which proved to be susceptible to a second inoculation with their own strain were subsequently inoculated with an heterologous strain and proved to be refractory to the latter, rather to our surprise. We have no satisfactory explanation for this result.

The foregoing results may perhaps be better appreciated if one considers all the theoretically possible combinations and contrasts them with the results actually obtained. If syphilitic rabbits are twice reinoculated, first with the homologous strain and then with either the same or another strain, it is apparent that there are 8 possible results to such an experiment. In Table III these are listed together with the number of rabbits encountered in each category.

### Table IV.

Reinoculation of Treated Syphilitic Rabbits with Heterologous Strains.

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Strain of 1st inoculation</th>
<th>Strain of 2nd inoculation</th>
<th>Result Positive</th>
<th>Result Negative</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>Nichols</td>
<td>A</td>
<td>2</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>F</td>
<td>7</td>
<td>4</td>
<td>63</td>
</tr>
</tbody>
</table>

From consideration of Table III it is seen that of the 8 theoretically possible results 4 were actually encountered in the experiments. The tendency of syphilitic rabbits treated late in the course of the disease to react to a second inoculation with the homologous strain in such a manner as to exhibit no evidence of a lesion at the site of inoculation is clearly shown in the table, and is in striking contrast with their behavior when reinoculated with heterologous strains.

Group 2. In this group there were observations upon 22 rabbits all of which were inoculated at the same time with the Nichols strain, were treated on the 122nd day of the disease and subsequently reinoculated with either Strain A or Strain F on the 197th day after the original infection. The results are shown in Table IV.

Study of Table IV shows that of 11 syphilitic rabbits inoculated with the Nichols strain and treated 122 days after inoculation, only 2,
or 18 per cent, showed lesions at the site of inoculation when inoculated subsequently with Strain A, whereas of 11 similar rabbits inoculated subsequently with the F strain 7, or 63 per cent, reacted with the development of a characteristic syphilitic lesion at the site of inoculation. In short, infection with the Nichols strain under the conditions of the experiment appeared to protect a much higher percentage of animals against infection with Strain A than against infection with Strain F. This rather marked difference in the reaction of the "Nichols rabbits" to two different strains can scarcely be attributed to differences in the size of the inoculum, since, as a matter of fact, in the group inoculated with the A strain the inoculum was considerably richer in treponemata than in the group inoculated with Strain F, in which a much higher percentage of positive results was obtained.

Indeed there is no evidence at present that variations in the size of the inoculum influence the response of syphilitic rabbits to a second inoculation, although it is possible that they may.

A more plausible explanation of the difference in the behavior of the two heterologous strains would be the assumption that a closer biological relationship existed between the Nichols strain and Strain A than between the former and Strain F. The validity of such an assumption would be strengthened if it could be shown that animals infected with Strain A were protected to a greater extent against subsequent infection with the Nichols strain than were those infected with Strain F. Unfortunately our experiments do not clear up this point since they contained too few animals upon which to base conclusions. However since it may be of interest to determine to what extent they do throw

<table>
<thead>
<tr>
<th>No. of rabbits</th>
<th>Strain of 1st inoculation</th>
<th>Strain of 2nd inoculation</th>
<th>Result</th>
<th>Per cent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Nichols</td>
<td>A</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>Nichols</td>
<td>A</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Nichols</td>
<td>Nichols</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>Nichols</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
light upon this matter, we have assembled all the cross-inoculation experiments conducted with these two strains and the Nichols strain. They are shown in Table V.

As will be seen from Table V, the percentage of successful second infections obtained when rabbits infected with the Nichols strain were subsequently inoculated with Strain A was much less than when Strain F was used for reinoculation, whereas when animals infected with Strain A or Strain F were subsequently inoculated with the Nichols strain the percentage of successful second infections was the same. In other words, although the Nichols strain appeared to protect against infection with Strain A to a much greater extent than against Strain F, nevertheless infection with Strain A did not, in this limited number of observations, appear to protect against the Nichols strain to a greater extent that did infection with Strain F. Cross-protection was not complete, therefore, and although the number of animals studied was perhaps too small to warrant generalisations, the fact that it was not complete suggests that if a biologic relationship exists between Strain A and the Nichols strain it is at best one-sided under the conditions of the experiment.

One other possible explanation of the results recorded in Table V should be considered, namely, whether relative differences in virulence of Strains A and F would account for the differences in percentage of successful reinoculations obtained with these strains. It was suggested by Pearce and Brown (3) that relative differences in virulence of strains of *Treponema pallidum* might explain superinfection or reinfection with heterologous strains, and Nothhaas (7) has recently taken the same ground. If this is the correct and sole explanation for the differences in behavior of the two strains, A and F, when inoculated in rabbits previously infected with the Nichols strain, it follows that Strain F is more virulent than Strain A, and it may be said that our experience with these two strains, extending over a period of 3 years or more, tends to confirm that conclusion. On the other hand the behavior of the rabbits first infected with Strain A or Strain F and subsequently inoculated with the Nichols strain does not support that conclusion, since the percentage of successful reinoculations with the latter strain was the same in each group. However, since the number of animals in these groups was rather small we prefer to suspend judgment in
the matter until more data have been accumulated. It is possible, of course, that biologic relationship between strains of *Treponema pallidum* and relative differences in their virulence may each be a factor in determining the outcome of reinoculation experiments in which heterologous strains are employed, and in that case it may be very difficult if not impossible to ascribe to each factor its relative importance. However it is clearly shown by these reinoculation experiments that rabbits infected with a strain of *T. pallidum* which has been adapted to that species for a period of 12 years may, in a high percentage of instances, be infected a second time with other strains which have been recovered recently from the human body and have not had opportunity over so long a period for adaptation to the rabbit.

As has already been stated, the criterion of production of a successful second infection in these experiments has been the development of a characteristic lesion at the site of inoculation coupled with the demonstration of treponemata in the lesion. Using this criterion and taking into consideration all the experiments, the number of successful reinoculations obtained with heterologous strains amounted to 28 in 56, or 50 per cent, whereas the total number of successful reinoculations with homologous strains was 2 in 35, or 5.4 per cent. That this criterion is inadequate in that it would fail to disclose an asymptomatic reinfection ("stumme" infection of Kolle) has been admitted. If such asymptomatic reinfections occurred in the experiments they could not have been recognised since, for reasons already stated, we were unable to determine the infectivity of the lymph nodes and internal organs of animals in which no lesions developed following reinoculation with heterologous strains. However the behavior of the Wassermann reaction in a number of the rabbits which, upon clinical grounds, were regarded as not having been successfully reinfected, led us to suspect that a number of these animals had in reality been reinfected with heterologous strains, even though no lesions developed at the site of reinoculation. In order to show the grounds for this suspicion we have thought it wise to record the behavior of the Wassermann reaction in a number of treated animals reinoculated with heterologous strains, where the test was performed repeatedly upon the serum of the same animal over a period of weeks following reinoculation. These data are shown in Table VI.
TABLE VI.

Behavior of Wassermann Reaction in Treated Syphilitic Rabbits Reinoculated with Heterologous Strains of *T. pallidum*.

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Wassermann reaction Result of reinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days after reinoculation</td>
</tr>
<tr>
<td>36</td>
<td>0  A  0  Ac  Ac  Ac  D</td>
</tr>
<tr>
<td>37</td>
<td>0  0  0  0  0  0  0</td>
</tr>
<tr>
<td>38</td>
<td>0  0  0  4  4  4  4</td>
</tr>
<tr>
<td>39</td>
<td>0  0  0  Ac  4  4  4  4</td>
</tr>
<tr>
<td>40</td>
<td>0  0  0  4  3  1  1  1</td>
</tr>
<tr>
<td>41</td>
<td>0  0  4  4  4  4  D  +</td>
</tr>
<tr>
<td>42</td>
<td>0  0  4  3  4  4  4  0</td>
</tr>
<tr>
<td>43</td>
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</tr>
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<td>46</td>
<td>0  0  0  4  4  4  4  4</td>
</tr>
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<td>47</td>
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<tr>
<td>48</td>
<td>0  0  1  0  1  0  0  0</td>
</tr>
<tr>
<td>49</td>
<td>0  0  0  0  0  0  0  D</td>
</tr>
<tr>
<td>50</td>
<td>0  0  0  0  0  0  3  0</td>
</tr>
<tr>
<td>51</td>
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</tr>
<tr>
<td>58</td>
<td>0  0  1  2  4  4  4  D</td>
</tr>
<tr>
<td>59</td>
<td>0  0  Ac  Ac  Ac  Ac  Ac  D</td>
</tr>
<tr>
<td>60</td>
<td>0  0  Ac  Ac  Ac  4  4  4</td>
</tr>
<tr>
<td>61</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>62</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>63</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>64</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>65</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>66</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>67</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
<tr>
<td>68</td>
<td>0  0  Ac  Ac  Ac  4  4  D</td>
</tr>
</tbody>
</table>

0—negative Wassermann.
1—25 per cent fixation.
2—50 per cent fixation.
3—75 per cent fixation.
4—100 per cent fixation.
Ac—anticomplementary.
D—died or discarded.
A. M. Chesney, C. R. L. Halley, and J. E. Kemp 235

A consideration of Table VI shows that in 13 of 29 rabbits inoculated first with the Nichols strain, then treated and subsequently reinoculated with heterologous strains (Strain A or F) a characteristic syphilitic lesion developed at the site of reinoculation. In all but 1 of these 13 animals the Wassermann reaction, which before reinoculation had been negative, became completely positive about the time of appearance of the lesion associated with the second infection. In the 16 rabbits in which no lesion developed at the site of reinoculation the test, which at first was negative, became completely positive in 6 animals (Nos. 37, 55, 59, 61, 62 and 64), complete fixation being obtained upon at least 2 or more occasions. We are strongly inclined to the view that these animals were in reality successfully reinoculated, for in former experiments dealing with reinoculations with homologous strains (1) a close parallelism was found between infectivity of lymph nodes and the occurrence of a positive Wassermann reaction in rabbits in which no lesion developed at the site of reinoculation. Of the remaining 10 animals the serum of 1 (No. 51) gave 75 per cent fixation upon one occasion only and the sera of the other 9 gave repeated negative tests or at most only 25 per cent fixation, or were anticomplementary.

One more point may be considered, namely, what influence was exerted upon the occurrence of reinfections by the previous existence of syphilitic lesions in the area where the reinoculations were made. Zinsser (17) was of the opinion that reinoculation of a testis that had previously been the site of a syphilitic inflammatory process was less apt to yield a positive result than if the testis had not previously been involved. According to him a syphilitic lesion when it healed might leave the tissues in that area more highly resistant to second infection than other parts that had not been the seat of syphilitic inflammation. Our experiments do not bear out this conception since many successful reinoculations were obtained with heterologous strains in instances where testes were inoculated which had previously been extensively involved in the syphilitic reaction. In general, where successful second infections were obtained with heterologous strains, the character of the lesion produced was not greatly different from that of the controls. It is true, however, that in some of the animals the lesion associated with the second infection appeared earlier and was of the
abortive type, as if the preceding infection had conferred a slight resistance upon the animal, but in the majority it was fully comparable to that seen in the controls. In a few also the incubation period was prolonged by a few days but the difference in this respect was neither constant nor striking.

SUMMARY AND CONCLUSIONS.

Syphilitic rabbits, whether untreated or treated after the 90th day of infection, were found to be more refractory to subsequent inoculation with the homologous strain of *Treponema pallidum* than to inoculation with heterologous strains of the same organism, when clinical criteria alone were employed in judging the outcome of reinoculation. The incidence of second infection with homologous strains was 5.4 per cent, as against 50 per cent with heterologous strains. The resistance which develops in rabbits during the course of a syphilitic infection appears therefore to be strain-specific rather than species-specific. The protection afforded against homologous strains was found to persist for at least as long as 6 months after treatment was discontinued.

A given strain may afford a higher degree of protection against some strains than against others, but whether this is to be explained upon the basis of biologic relationship or of differences in virulence, or possibly as the result of both of these factors was not disclosed by the experiments. Rabbits infected with a strain (Nichols) which had been adapted to this species for over a decade could be infected with strains which had been recovered recently from the human body. The previous existence of a syphilitic lesion in the testis which was used as the site for reinoculation did not seem to exert any influence upon the incidence of successful second infections obtained with heterologous strains of *Treponema pallidum*. Sometimes the course of the second infection produced by inoculation with heterologous strains

2 In this respect it is of interest to call attention to some recent inoculation experiments upon untreated syphilitic human beings, carried out by Hashimoto (19). This observer noted a higher percentage of positive results when patients with untreated syphilis were inoculated with syphilitic virus from other patients than when inoculated with their own virus.
was less pronounced than that observed in the controls, but in most instances no significant alteration was observed.

In syphilitic rabbits treated late in the course of the disease and re-inoculated with heterologous strains of *Treponema pallidum* no lesion may develop at the site of reinoculation but nevertheless the Wassermann reaction may become positive and remain so for weeks thereafter. It is suggested that such animals may be examples of asymptomatic reinfection.

**BIBLIOGRAPHY.**