STUDIES IN EXPERIMENTAL SYPHILIS.

IV. THE SURVIVAL OF TREPONEMA PALLIDUM IN THE INTERNAL ORGANS OF TREATED AND UNTREATED RABBITS.

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In the foregoing paper of this series (1) it was shown that when syphilitic rabbits are treated with arsphenamine their reaction toward a second inoculation of treponemata will depend upon the time at which treatment was instituted. If arsphenamine is administered early in the course of the infection the animals are almost always susceptible to a second infection, whereas, if treatment is postponed until a period when the primary manifestations have subsided, such animals are almost always refractory to a second infection. These results are in entire agreement with the work of Kolle (2) and Frei (3). It was further shown that in the same treated animals the lymph nodes are rendered incapable of transmitting the syphilitic infection to normal rabbits no matter at what time the treatment is begun.

The bearing of these facts upon the question of the biologic cure of experimental syphilis in the rabbit was discussed and it was pointed out that under the conditions of the experiments the two criteria of cure, namely the reinoculation test and the lymph node test, could not both be valid, since they gave absolutely opposite results in the same animal. In the discussion it was pointed out that the abolition of lymph node infection by arsphenamine administration did not necessarily mean biologic cure, since it was conceivable that animals so treated might continue to harbor the organisms in the internal organs, although the lymph nodes had been rendered incapable of transmitting the infection to normal rabbits.

It seemed of paramount importance to determine whether or not syphilitic lymph node infection in the rabbit is an index of the presence
of syphilitic infection in more remote parts of the body both in treated and in untreated rabbits. The present communication deals with our attempts to answer that question.

HISTORICAL.

The relationship of lymph node infection to infection of the internal organs in syphilitic animals has not, so far as we are aware, been the subject of extensive study by workers in this field. Neisser (4), working with apes, demonstrated that the spleen and bone marrow were infectious in slightly over half of his animals, in one instance as late as 283 days after inoculation, and in half of the animals in which one or the other of these organs was infectious, the blood was also infectious. In one animal in which the lymph nodes and testicles were transferred to normal animals 70 days after inoculation, the result was positive. Uhlenhuth and Mulzer (5), working with syphilitic rabbits, showed that the inguinal lymph nodes harbor the infection and that the blood, the liver, and a mixture of spleen and bone marrow may be infectious but are not uniformly so. Pearce and Brown (6) found that the lymph nodes in distant parts of the rabbit's body regularly harbor the microorganism. They succeeded in recovering the virus from popliteal lymph nodes of rabbits as late as 51 months after inoculation. Eberson (7) recovered treponemata from the testes of rabbits as late as 4½ months after inoculation. Plaut and Mulzer (8) have recently succeeded in recovering treponemata from the brain of a rabbit inoculated intrathecally with what they regard as a neurotropic strain of Treponema pallidum. The organisms were recovered by inoculating normal rabbits with brain substance removed 59 days after the original inoculation. No parallel inoculations with blood were reported by them, a fact which raises some question as to whether or not the organisms were really in the cerebral tissue outside the circulating blood, since it is well known that there is apt to be a spirochetemia in the early period of syphilitic infection in the rabbit.

EXPERIMENTAL.

Two groups of adult male rabbits, of various breeds, comprising in all ten animals, were used. All the animals were inoculated in the right testicle with a virulent strain of Treponema pallidum (Nichols strain), and all developed syphilitic orchitis in the usual manner. One group, of four animals, was untreated, the other group of six animals was treated with arsphenamine. The drug was given in doses of 10 mg. per kilo; each animal received six intravenous injections, one each at weekly intervals. Treatment was begun 181 to 194 days after inoculation, at a time when previous experience had shown that such animals would be refractory to a second inoculation. In all six of these animals the initial phenomena of syphilitic infection had by this time subsided and the animals were not showing any signs of activity of the disease. From 279 to 384 days after inoculation, the
animals in both treated and untreated groups were anesthetized with ether and
the heart's blood aspirated with a sterile syringe and inoculated at once, without
defibrinating and before clotting could take place, into the testes of two normal
rabbits. Sometimes one testis, sometimes two were inoculated, depending upon
the amount of blood aspirated, but never less than 1.0 cc. and frequently 1.5 cc.
was injected into a single testis. The internal organs and the popliteal lymph
nodes were then removed from the rabbit under aseptic precautions and por-
tions of the former were emulsified in physiological salt solution and inoculated
separately into one testis of each of two normal rabbits. The organs selected
for transfer to normal rabbits were as follows: heart, liver, brain, spleen and
bone marrow mixed, and the testicle which was originally inoculated and in which
syphilitic orchitis had developed and subsided spontaneously. The popliteal
nodes were emulsified in salt solution in toto and the entire amount of emulsion
was inoculated into the testes of two normal rabbits. Thus, including the heart's
blood, for each rabbit studied, seven pairs of normal rabbits were inoculated.
In the case of the treated animals, tissue transfers were carried out 49, 65, 70, 76,
76, and 168 days after the last dose of arsphenamine.

There was one animal in the treated group in which syphilitic keratitis developed
in one of the eyes both before and after treatment. At the time of organ transfer,
49 days after the last dose of arsphenamine, this keratitis was at its height.
Accordingly, when the animal was etherized for organ transfer the cornea was
excised, emulsified in physiological salt solution, and the emulsion was examined
with dark-field illumination and also inoculated into the testes of two normal
rabbits.

All the animals to which organs were transferred were kept under observation
for a period of 90 days before discarding them as negative. We regard this period
as ample for the particular strain of treponemata with which we are working,
as it is a highly virulent one and has never in our experience failed to produce
infection when inoculated intratesticularly in normal rabbits, the orchitis being
manifest well within 90 days.

The results of the experiment are shown in Table I.

Table I shows that in the case of the untreated rabbits virulent
treponemata were recovered from the lymph nodes of all four animals;
from the heart's blood once, from the liver three times, the mixed
spleen and bone marrow twice, the originally inoculated testicle three
times, but in no instance from the brain or heart muscle. The recovery
of the treponemata from the heart's blood of Rabbit 1, which was
untreated, does not, of course, permit of any deductions as to the
presence of the microorganisms in the tissues of that animal. The
fact that the heart's blood was infectious 297 days after inoculation
is of considerable interest from the standpoint of the possibility of
### TABLE I.
Result of Organ Transfer from Untreated and Treated Syphilitic Rabbits to Normal Rabbits.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Untreated group Rabbit No.</th>
<th>Treated group Rabbit No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Heart's blood.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(45)(52)</td>
<td></td>
</tr>
<tr>
<td>Heart muscle.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liver.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(55)(48)</td>
<td>(49)</td>
</tr>
<tr>
<td>Mixed spleen and bone marrow.</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(45)</td>
<td>(65)</td>
</tr>
<tr>
<td>Testicle.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Brain.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymph node.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cornea.</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>(47)(54)</td>
<td></td>
</tr>
</tbody>
</table>

The figures in parenthesis indicate the day of appearance of syphilitic orchitis in the inoculated rabbits. Results are not reported as positive unless treponemata could be demonstrated. Each + or - sign refers to a single rabbit.

* The animal died before 90th day after inoculation.

† Only one animal inoculated.
fresh blood stream invasions late in the course of syphilitic infection. Neisser has recorded a similar result in apes. He demonstrated the infectiousness of the blood of one of his animals 283 days after inoculation (4).

The animals inoculated with lymph node or testicular emulsions developed syphilitic orchitis earlier and with greater uniformity than those inoculated with heart's blood or internal organs. Inasmuch as we have shown (9) that the richer the inoculum the shorter is the incubation period, it may be suspected that the inocula composed of lymph node or testicular emulsion contained more virus than did the inocula composed of heart's blood, liver, or mixed spleen and bone marrow.

In the case of the six treated animals, tissue transfer to normal animals was negative in every instance except one; namely, in the case of Rabbit 10. This animal, as stated previously, had keratitis prior to treatment. Following treatment keratitis reappeared in the same eye. The involved cornea, excised at the time that the other organs were removed, was emulsified in physiological salt solution and the resultant emulsion examined for *Treponema pallidum*. Several typical examples were seen with the aid of dark-field illumination and the emulsion was then inoculated into the testes of two normal animals with a subsequent positive result in each. In spite of the fact that the animal was not cured, as evidenced by a relapsing keratitis following treatment and the recovery of the organisms from the cornea, the internal organs appeared to have been sterilized by the treatment.

**DISCUSSION.**

It is generally agreed that as syphilitic infection develops in man, apes, or rabbits, the host gradually loses his susceptibility to a superimposed infection until finally there comes a time when it is impossible to bring about a successful second infection. This refractory state persists for a considerable time after the early manifestations of the disease have subsided. At first this refractory state was thought, from observations upon man, to represent a true acquired immunity similar to that seen after such acute infections as typhoid fever, and it was believed to indicate complete subsidence of the
infection. Neisser, however, was able to show that in apes this refractory state was associated with the presence of the first infection in the host. The ape infected with syphilis is able to overcome the local lesions in the course of time but is not able in most instances to rid its body of the infection, although it is refractory to a second infection. In this respect the behavior of the rabbit is quite similar to that of the ape.

Treatment of syphilitic rabbits with arsphenamine apparently does not influence this refractory state, acquired as a result of the infection. Both Kolle and ourselves have shown that when rabbits are treated with arsphenamine at a time when they are known to be refractory to a second infection, they remain refractory for an appreciable time after treatment has ceased. Is the maintenance of this refractory state dependent upon the persistence of the first infection in spite of treatment or has the original infection been eliminated by the aid of drugs? If it has been eliminated then the acquired refractory state may be conceived of as not necessarily dependent upon the persistence of the first infection but may survive its abolition.

It seems to us that our experiments favor the latter view. Of six syphilitic rabbits treated late in the course of the infection, at a time when they would be uniformly refractory to a second infection, the popliteal lymph nodes, heart's blood, and selected internal organs were found to be incapable of transmitting syphilitic infection to normal rabbits. In one of these six animals, in which keratitis occurred before and after treatment, the involved cornea remained infected in spite of treatment, although the lymph nodes and internal organs appeared to have been rid of the infection. In four control untreated animals the infection persisted in the lymph nodes and in many instances in one or more internal organs. Thus, while all four of the untreated animals continued to harbor the infection, five out of six of the treated animals, or 83.3 per cent, were entirely rid of their infection as judged by tissue transfer. It would appear then, under the conditions of the experiment, that when arsphenamine is administered to syphilitic rabbits late in the course of the infection, the lymph nodes and internal organs may be sterilized in the majority of instances. Judged by such a criterion, biological cure of syphilis is possible in the rabbit, even if the treatment be postponed 6 months or more after inoculation.
It must be admitted that the possibility exists that there remain in other untested tissues of these treated animals foci of syphilitic infection which have not been reached by the arsphenamine. Again, it may be that in the tested organs of the treated animals the infection has been suppressed to the point where it is no longer capable of being transmitted to normal animals but has not been completely eradicated. Such might be the case if there were resistant forms in the life cycle of the parasite. In answer to the first of these objections, we can say that we have selected for study representative tissues, which in the light of previous experience of other workers and of our knowledge of the course of syphilitic infection in man, might reasonably be expected to harbor the infection. There are obvious limits to the tissue transfer method. It is manifestly impossible to emulsify an entire rabbit and inoculate it into normal rabbits, hence, it is extremely difficult, if not impossible, absolutely to exclude syphilitic infection in an animal. In answer to the second objection it may be said that it would be difficult to recognize the existence of an active syphilitic infection in a tissue that gave no macroscopic evidence of disease and yielded only negative results on inoculation. Not even the demonstration of spirochetes in such tissue by histological methods would guarantee the viability of those microorganisms. As yet no detailed histological studies of the organs of the animals in this experiment have been made. They were all free of gross evidence of syphilitic infection.

If it be concluded, from the results of tissue transfer as carried out in this experiment, that treatment of rabbits with arsphenamine late in the course of the infection brings about a biological cure in most instances, then it must be concluded that failure to reinoculate a treated syphilitic rabbit cannot be interpreted as evidence of the persistence of the first infection. In other words, the reinoculation test in such an animal could not be utilized as a criterion of cure, but rather as an index of resistance only. Furthermore, as our experiments perhaps indicate, such a resistance may, in the case of the rabbit, persist after the elimination of the first infection. Whether such a state of resistance to second infection in the absence of the first infection is to be regarded as true immunity or not, will depend entirely upon one's definition of that term. It matters little whether
one speaks of such a state as one of immunity or refractoriness. What is important is to realize that it is a property which the animal body acquires as a result of syphilitic infection, and failure to acquire it can be governed by proper selection of the time at which arsphenamine is given. It is the expression of a biological reaction between host and parasite and if that reaction is interfered with before there has been time for its consummation, the refractory state does not appear. Indeed, there is evidence to show that the animal may be rendered less resistant (10). On the other hand, if the animal is allowed to react against the invading organism without interference in the shape of therapeutic agents, so that the primary orchitis runs its course and the pathological process subsides by virtue of the inherent defensive mechanism of the host, then this biological reaction is completed and the refractory state is established. Treatment at this point will not alter it.

Whether or not the establishment of the refractory state results in eradication of the infection is another matter, likewise to what extent the persistence of the infection is necessary for the indefinite maintenance of the refractory state is still perhaps debatable. The point which we wish to emphasize is, that, in our opinion, it is the opportunity which is afforded the host of reacting against the syphilitic infection without extraneous aids which determines his acquisition of the refractory state and his behavior toward a second infection, rather than the persistence of the infection.

The experiments recorded in this and the previous papers in the series offer evidence which strongly suggests that the refractory state is capable of surviving the abolition of the infection. If this is true, the reinoculation test must be regarded as no valid criterion of cure, and, accordingly, the prospect of curing syphilis in the rabbit becomes more hopeful than the deductions drawn from recent experimental work based upon the success or failure of reinoculation, would indicate.

If the reinoculation test cannot be accepted as a criterion of cure, the question may be raised, to what extent can the sterility of lymph nodes following treatment be regarded as an index of cure? We have too few experiments to permit of generalizations on this point. In the untreated animals the popliteal lymph nodes were infectious in every instance in which the internal organs were infectious. On
the other hand, these nodes were infectious in one instance when the above mentioned tissues were non-infectious. Among the treated animals, however, there was one in which keratitis recurred after treatment, and in this animal the lymph nodes were non-infectious, while the involved cornea was proved to be infectious. It would appear from this observation that sterility of lymph nodes does not in every instance indicate that the animal is free of syphilitic infection, particularly if there has been a preexisting keratitis. Lymph node sterility is, therefore, no absolute indication of cure of syphilis and perhaps the same may be said of the internal organs, but it is probably the best single index that we have. We incline to the view that study of the lymph nodes or internal organs of treated animals is essential in the ultimate evaluation of any chemotherapeutic agent in experimental syphilis, but continued observation of the treated animals to detect recurrent syphilitic lesions is fully as important and cannot be neglected. Mere observation of the behavior of the initial lesion as well as the disappearance of the treponemata from it is obviously insufficient for the purpose.

**SUMMARY.**

Simultaneous transfers to the testes of normal rabbits of circulating blood, heart muscle, liver, brain, spleen and bone marrow (mixed), inoculated testicle, and popliteal lymph nodes from a series of untreated syphilitic rabbits, demonstrated the persistence of the original infection uniformly in the lymph nodes and less regularly in the liver, mixed spleen and bone marrow, and testis originally inoculated. In one instance the circulating blood was found to be infectious. Transfer of similar tissues from syphilitic rabbits treated with arsphenamine late in the course of the disease failed to disclose syphilitic infection of any of these tissues. In one animal, in which keratitis developed both before and after treatment, the blood, internal organs, and lymph nodes were found to be non-infectious in spite of the fact that the cornea was shown to be the site of a syphilitic inflammation. Transfer of lymph nodes or internal organs of treated syphilitic rabbits is probably the best method of evaluating an antisyphilitic agent, but it must be supplemented by careful observation of treated animals over an appreciable interval of time following treatment.
The results of this study support the idea that failure to reinoculate a treated syphilitic animal does not necessarily mean the existence of the first infection but might be interpreted as indicating the presence of an acquired resistance which persists in rabbits in which no trace of the first infection can be demonstrated.

BIBLIOGRAPHY.