# STUDIES IN EXPERIMENTAL SYPHILIS.

## VI. On Variations in the Response of Treated Rabbits to Reinoculation; and on Cryptogenetic Reinfection with Syphilis.*

By ALAN M. CHESNEY, M.D., and JAROLD E. KEMP, M.D.

*(From the Department of Medicine of the Johns Hopkins Medical School, Baltimore.)*

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The decision in any given case as to whether or not a syphilitic animal, treated and then reinoculated, has acquired a second attack of syphilis, will, of necessity, depend upon the criteria of a successful reinfection. In the older experimental work attention was directed mainly, if not entirely, to the occurrence of a local lesion (chancre) at the site of reinoculation. If no such lesion developed in the reinoculated animal it was assumed that a second infection had not been established. It is at once apparent that this assumption is justified only if syphilitic infection is always accompanied by the occurrence of a primary lesion at the portal of entry. That syphilis may occur in human beings without the development of a chancre is well known (1). The same has been shown to hold true for monkeys (2) and for rabbits (3–5). In view of these now well established facts it is clear that, in determining in the reinoculated animal whether or not a second infection has been established, one must take into consideration the possibility that second infections may occur without the appearance of any local lesion at the portal of entry. Such second infections might be characterised by dissemination of the treponemata throughout the body and their lodgement in distant organs, with or without the production of lesions at the site of such lodgement. Neisser (2) admitted the possibility of such a result from reinoculation in his discussion of superinfection,

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and Brown and Pearce (6) called attention to this point several years ago. As they very aptly expressed it, one must be able "to see beyond the reaction at the site of reinoculation." More recently the same question has been raised by other workers in this field (7–9).

During the past few years in the course of a series of experiments, some of which have been reported in other communications, we have had occasion to carry out a number of reinoculations in treated syphilitic rabbits. Keeping in mind the principle elaborated above, we directed our attention, in judging of the results of reinoculation, not alone to (*a*) the occurrence of a lesion at the site of reinoculation, but also to the possibility of (*b*) the establishment of infection without the production of a local lesion, to (*c*) the occurrence of dissemination of the virus under these conditions, and to (*d*) the production of a positive Wassermann reaction. The purpose of this communication is to bring together the results of reinoculation of treated rabbits studied from these points of view. The evidence afforded by this study indicates that it is possible successfully to reinoculate a treated rabbit without the production of any clinically detectible lesion at the site of reinoculation; furthermore, that the time at which treatment is begun and the method of reinoculation are of importance in bringing about this type of response to a second infection.

In this communication we shall deal only with the results in treated rabbits, reinoculated with homologous strains of *Treponema pallidum*. It is obvious that in untreated rabbits reinoculated with the same strains it would be a most difficult feat to demonstrate a successful reinoculation if such occurred without a lesion being produced at the second portal of entry.

In the first part of our report we shall discuss the results obtained when both first and subsequent inoculations were intratesticular, and in the second part we shall deal with the results obtained when the first inoculation was intratesticular and the second was made by inoculating a granulating wound produced on the back of the animal. The Nichols strain was used in all the experiments.

## *I. All Inoculations Intratesticular.*

### *Experimental.*

*First Inoculation.*—In each animal only one testis was inoculated with virus emulsion.

*Treatment.*—Arsphenamine was administered intravenously at weekly intervals for a total of six doses. The individual dose was 10 mg. per kilo in each instance. Treatment was begun at varying intervals after inoculation, ranging from 41 to 291 days. Lymph node transfer was performed in all but one of the animals *after* treatment but *before* reinoculation.

*Reinoculation.*—Reinoculations were in some instances performed only once and in others twice, and always in the testis opposite to that originally inoculated. They were carried out 53 to 69 days after treatment was concluded. Lesions developing at the site of reinoculation were, of course, examined for the presence of treponemata. If no lesion developed the testis was excised, an emulsion of the organ was made with normal saline solution and examined for treponemata by the dark-field method. In four instances where this emulsion was negative it was inoculated intratesticularly into normal rabbits. In every instance the reinoculated testis was studied by one or both of these methods.

*Dissemination of Virus.*—The possibility of occurrence of dissemination of the virus was ascertained by clinical observation and by transfer of a popliteal lymph node to the testes of normal rabbits. This was carried out from 90 to 101 days after reinoculation and it was not omitted in any animal reported upon in this paper. The period of observation following reinoculation was at least 90 days and in many instances longer. As judged by the behavior of the controls this period affords ample opportunity for the development of syphilitic lesions at the site of reinoculation.

*Wassermann Reaction.*—The Wassermann reaction was performed on the blood serum of many of the rabbits at frequent intervals before and after reinoculation, according to the technique outlined in the preceding paper of this series. The reaction was negative in every instance prior to reinoculation.

### *Results.*

We are able to report upon a total of twenty-three treated syphilitic rabbits reinoculated and studied as described above.

If it be assumed that reinfection may take place without the production of a local lesion at the site of reinoculation, but may be manifested by local infection without clinical phenomena, or by dissemination of the virus, then it is apparent that there are six theoretically possible responses to reinoculation, ranging from the production of a second infection which is entirely similar to the first,

to no infection at all. These six theoretical possibilities may be stated as follows:

*Response A.*—The production of a local lesion at the site of reinoculation accompanied by dissemination of the virus, in other words, a reaction which is identical with that of normal rabbits to syphilitic infection.

*Response B.*—The production of a local lesion at the site of reinoculation without evidence of dissemination of the virus.

*Response C.*—The persistence of virus at the site of reinoculation and dissemination of the virus but without the development of any local lesion.

*Response D.*—Dissemination of the virus without the production of a local lesion and without the persistence of virus at the site of reinoculation.

*Response E.*—Persistence of the virus at the site of reinoculation without dissemination and without the development of a local lesion.

*Response F.*—Absence of a local lesion and absence of virus at the reinoculation site together with no dissemination of the virus; in other words, a negative result.

It is, of course, questionable how far one may go in distinguishing Response D from Response C, since it is always conceivable that the virus may have been present at the site of reinoculation but have been overlooked due to insufficient examination. Negative results are notoriously misleading, but the theoretical possibilities exist, and it is of interest to see how closely the observed facts fit them, when reasonably diligent attempts are made to find the organisms at the site of reinoculation.

Of the six possible responses to reinoculation, examples of five were encountered in one or more rabbits in the group of twenty-three reported upon. The distribution of these rabbits among the five responses, or categories, as we shall call them, is shown in Table I, together with the duration of the infection as well as the duration of the initial orchitis at the time treatment was begun.

Consideration of Table I shows that in six of the entire series of twenty-three animals a characteristic local lesion developed at the site of reinoculation, followed by evidence of dissemination of the virus (Category A). The duration of the primary orchitis and the

TABLE I.

*Incidence of Various Types of Response to Reinoculation.*

| Category | Lesion at site of reinoculation | Virus at reinoculation site without lesion | Dissemination of virus (node transfer) | Rabbit No. | Duration of orchitis at time of treatment | Duration of infection at time of treatment | Wassermann reaction after reinoculation |
|---|---|---|---|---|---|---|---|
| | | | | | *days* | *days* | |
| A | + | 0 | + | 1 | 17 | 49 | Not done |
| | | | | 2 | 22 | 48 | " " |
| | | | | 3 | 16 | 48 | " " |
| | | | | 4 | 15 | 48 | " " |
| | | | | 5 | 16 | 41 | ++++ |
| | | | | 6 | 26 | 41 | ++++ |
| | | | | Total 6 | Average 18.7 | Average 45.8 | |
| B | + | 0 | 0 | None | – | – | |
| C | 0 | + | + | 7 | 23 | 41 | ++++ |
| | | | | 8 | 32 | 68 | ++++ |
| | | | | Total 2 | Average 27.5 | Average 54.5 | |
| D | 0 | 0 | + | 9 | 16 | 41 | ++ |
| | | | | 10 | 16 | 61 | ++ |
| | | | | 11 | 26 | 41 | ++++ |
| | | | | 12 | 26 | 41 | ++++ |
| | | | | 13 | 19 | 41 | ++++ |
| | | | | 14 | 38 | 68 | ++++ |
| | | | | Total 6 | Average 23.5 | Average 48.8 | |
| E | 0 | + | 0 | 15 | 167 | 193 | 0 |
| F | 0 | 0 | 0 | 16 | 179 | 209 | Not done |
| | | | | 17 | 196 | 209 | " " |
| | | | | 18 | 272 | 291 | " " |
| | | | | 19 | 27 | 56 | 0 |
| | | | | 20 | 182 | 193 | 0 |
| | | | | 21 | 174 | 193 | 0 |
| | | | | 22 | 174 | 193 | 0 |
| | | | | 23 | 171 | 193 | 0 |
| | | | | Total 8 | Average 172 | Average 192 | |

duration of the infection at the time treatment was instituted averaged 18.7 and 45.8 days respectively. The Wassermann reaction of the two animals of this group in which it was performed became positive after reinoculation. None of these animals exhibited generalised lesions following reinoculation.

There were no instances in which a local lesion was produced at the site of reinoculation without dissemination of the virus (Category B). In our experience whenever a local lesion has been produced with the Nichols strain at the site of reinoculation, it is invariably accompanied by dissemination of treponemata and involvement of distant lymph nodes.

In two animals (Category C) no local lesion was produced but careful search revealed the presence of treponemata at the site of reinoculation and there was definite evidence of dissemination of the virus. The average duration of the primary orchitis and of the infection at the time treatment was instituted in these animals was 27.5 and 54.5 days respectively. Both animals developed positive blood Wassermann reactions after reinoculation.

In six animals (Category D) no local lesion developed following reinoculation nor was it possible by careful search to demonstrate the presence of treponemata *in situ*, nevertheless, there was evidence of dissemination of the virus in all of them and they all showed positive blood Wassermann reactions following reinoculation. The average duration of the primary orchitis and of the infection at the time treatment was begun was 23.5 and 48.8 days respectively.

We encountered one rabbit, No. 15 (Category E), in which no local lesion developed at the site of reinoculation and in which there was no evidence of dissemination of the virus as judged by lymph node transfer or the occurrence of generalised lesions. Careful search of the testis (left) in which the reinoculation was made showed no treponemata by dark-field examination, but the emulsion of testicular material was inoculated into two normal rabbits and both developed syphilis. It should be noted that this animal was originally inoculated in the right testis and a characteristic syphilitic inflammation of this organ developed, but up to the time treatment was instituted, namely, 193 days after the original inoculation and 167 days after the primary orchitis was manifest, no macroscopic lesions had appeared

in the opposite testis (left), which was the one reinoculated. Furthermore, this animal never developed a positive Wassermann reaction after reinoculation. The evidence is very suggestive that the virus recovered by animal inoculation from the reinoculation site was that introduced at the time of reinoculation. It would appear that the animal had been rendered highly, although not completely refractory to a second infection.

There were eight rabbits in which no local lesion developed at the site of reinoculation and in which the virus could not be demonstrated at that site (Category F). Furthermore, the virus could not be recovered from the lymph nodes of any of these animals following reinoculation and in all of them the Wassermann reaction remained persistently negative. Of the eight animals in this group, there were four in which the reinoculated testis was emulsified and, in addition to being studied by dark-field examination, was inoculated intratesticularly into each of two normal rabbits. In every instance this inoculation proved to be negative. In the remaining four animals the examination of the reinoculated testis was confined to dark-field examination of an emulsion of the organ. In this group, then, no evidence could be adduced to show that any of the animals had been successfully reinoculated. The average duration of the infection in this group at the time treatment was begun was 192 days. All but one of the animals falling in this group were treated late in the course of the disease. The single exception, No. 19, received its first treatment on the 56th day of the infection.

*Comment.*

The foregoing results indicate that when rabbits with syphilitic orchitis are treated with arsphenamine comparatively early in the course of the disease, that is to say before the 69th day, and are subsequently reinoculated intratesticularly in the opposite testis with the homologous strain of treponemata, the response to the second inoculation will vary within stated limits. Some animals may show a response similar to that of normal animals and are, therefore, to be regarded as non-immune. Other animals may show no local lesion at the reinoculation site but, nevertheless, will present evidence of having been successfully reinoculated as determined by the persistence

of the virus at the site of reinoculation and its dissemination to distant lymph nodes. Still others may show no virus at the reinoculation site but give evidence of its dissemination nevertheless. In general, it would appear that reinfection of treated syphilitic rabbits may take place without any local lesion, following intratesticular reinoculation, and that this type of response to a second inoculation is apt to occur when treatment is begun from 41 to 68 days after the first infection. It would appear to be almost constantly accompanied by the development of a positive Wassermann reaction in the blood. Animals responding in this manner to a second infection may conceivably be regarded as in part refractory. Occasionally, as exemplified by Rabbit 19, an animal may become apparently entirely refractory to a second infection early in the course of the infection and remain so even if treatment is begun before the 69th day of the infection.

The behavior of Rabbit 15 is of interest in that it indicates a high degree of resistance to a second infection, which, however, falls just short of being complete. This animal was treated late in the course of the disease (193 days) and when reinoculated gave no indication of dissemination of the virus, but the presence of the latter at the site of reinoculation could be demonstrated by animal inoculation. The animal, moreover, failed to exhibit a positive Wassermann reaction following reinoculation.

Where the treatment was postponed for 6 months or more there was, except in the one instance noted above, no evidence that a successful reinfection had taken place. This observation is in accord with those of Kolle (10) and Frei (11) and would indicate that the refractory state in such animals is probably complete.

The data presented in Table I would indicate that in general the type of response (A) to a second inoculation characterised by the development of the essential disease picture (local lesion and dissemination of the virus) is most apt to occur when the primary orchitis is interrupted relatively early (average 18.7 days) by treatment. If treatment is withheld until the orchitis is slightly farther advanced (average 24.5 days) the response tends to become that characterised by dissemination of the infection without the production of a local lesion (D and E). If treatment is withheld until a much later date

(6 months or more), when the animal has had opportunity to heal the initial lesion by virtue of its own defensive mechanism, no infection occurs following intratesticular inoculation, or at most a local infection without local lesion and without dissemination of the virus. These facts point to a gradually developing resistance, the first manifestation of which is the ability of the animal to suppress the local reaction.

It may be objected that the recovery of the virus from distant lymph nodes of treated reinoculated animals in which no local lesion is produced at the reinoculation site, is not conclusive evidence of the success of reinoculation, since it is conceivable that the virus recovered from such nodes may be that of the first infection. This objection may be met with the statement that this phase of the experiment was controlled in all but one of the animals by removal of a node *after* treatment but *before* reinoculation, and transfer of the latter to normal animals. All such transfers proved to be negative. Furthermore, study of other animals similarly treated but *not* reinoculated showed that treatment was uniformly effectual in rendering the nodes non-infectious. Even if nodes were removed from such rabbits as late as 201 days after the last dose of arsphenamine they always failed to convey the infection to normal animals. Similar results were obtained by Nichols and Walker (12) with even fewer doses of arsphenamine than we have used. In addition, the occurrence of a positive blood Wassermann reaction following reinoculation, when previously it had become negative under treatment, offers additional support for the view that such animals were in truth reinoculated successfully and that the virus obtained from the lymph nodes did not originate from the first reinfection but represented that introduced at the time of the second inoculation.

### *II. Reinoculations on Wounds.*

In a previous communication (13) we have called attention to the fact that a granulating wound (11 to 16 days old) on the back of a rabbit offers a particularly favorable site for the development of a chancre after inoculation with the Nichols strain of *T. pallidum*. When a testicular emulsion of the virus is allowed to drop on the exposed granulating surface of such a wound, infection readily takes

place and after the wound heals a characteristic chancre develops, which can even be made to conform to a predetermined pattern. It seemed of interest to determine how a rabbit, first infected by intratesticular inoculation and treated at a time when it could be presumed to be refractory to a second intratesticular inoculation, would react to a second infection in which the virus was deposited upon a granulating wound on the back, in the manner previously described.

It is at once apparent that such an experiment might throw light upon the question of the extent to which the refractory state, which develops in syphilitic rabbits in the course of time, is a property of the animal body as a whole, that is to say, a general resistance, as against a local resistance only. There is in the literature some evidence to show that not all of the tissues of the rabbit share equally in the resistance toward a second infection which syphilitic rabbits unquestionably acquire.

Tomasczewski (14) found that if rabbits were inoculated in the cornea with syphilitic virus and a syphilitic keratitis developed, such rabbits, in most instances, could be successfully infected a second time by subscrotal inoculation as late as 128 days after the appearance of the keratitis. He also found that if the first infection were produced by subscrotal inoculation it was possible to produce a syphilitic keratitis in 76 per cent of his animals by inoculating the cornea from 39 to 105 days after the first inoculation. Apparently he reinoculated with an homologous strain of treponemata. Uhlenhuth and Mulzer (15) also succeeded, in three of four experiments, in obtaining second infections by intratesticular inoculation when the first infection was produced by corneal inoculation. In contradistinction to Tomasczewski, however, they were unable to obtain, in eleven of thirteen experiments, a second successful infection by the corneal route when the animals had been previously inoculated intratesticularly and a characteristic syphilitic orchitis had been produced. In two of their animals, however, they did succeed in producing a keratitis following testicular inoculation. The interval elapsing between first and second inoculations in their experiments ranged from 40 to 170 days. The two successful second infections were obtained when the second inoculation was carried out 40 and 61 days, respectively, after the first. These investigators were apparently using homologous strains for first and second inoculations.

Zinsser (16), largely on the basis of these experiments together with some of his own, has formulated the principle that the refractory state which develops in syphilitic rabbits is in reality a local phenomenon dependent upon previous infection at the site of reinoculation. Thus, his conception of syphilitic immunity is restricted even more than that of Kraus and Volk (17) who have suggested that it is a tissue immunity restricted to certain tissue groups and not shared to the same extent by all tissues.

## *Experimental.*

Sixteen animals were inoculated intratesticularly with the Nichols strain of *T. pallidum* and the infection was permitted to run its course for a period of more than 5 months. All the animals were then treated with arsphenamine intravenously in exactly the same manner as in the preceding experiment. Treatment was begun 159 to 201 days after inoculation, the average being 169 days.

TABLE II.

*Reinoculation of Treated Rabbits.*

| Rabbit No. | Wassermann reaction before reinoculation | Result of reinoculation | | | |
|---|---|---|---|---|---|
| | | Lesion at site of reinoculation | Generalised lesions | Wassermann reaction | Node transfer |
| 24 | Negative | None | None | Negative | Negative |
| 25 | " | " | " | " | Positive |
| 26 | " | " | " | " | Negative |
| 27 | " | " | " | " | Positive |
| 28 | " | " | " | " | Negative |
| 29 | " | " | " | " | " |
| 30 | " | " | " | Positive | " |
| 31 | " | Abscess | " | " | Positive |
| 32 | " | Chancre | " | " | " |
| 33 | " | None | " | Negative | Negative |
| 34 | " | Abscess | " | Positive | Positive |
| 35 | " | None | " | Negative | " |
| 36 | " | " | " | " | " |
| 37 | " | " | " | " | Negative |
| 38 | " | " | " | " | Positive |
| 39 | " | " | " | Positive | Negative |
| Virus controls | | | | | |
| 40 | " | Chancre | " | " | Not done |
| 41 | " | " | " | " | " " |
| 42 | " | " | " | " | Positive |
| 43 | " | " | " | " | Not done |
| 44 | " | " | " | " | Positive |

89 days after the last dose of arsphenamine was administered the animals were reinoculated on the back. This was accomplished by first excising an elliptical area of skin, under ether anesthesia, exposing the subcutaneous tissue. The wounds were not dressed in any way and 14 days after operation, when granulation tissue was well established, the crusts were removed with as little trauma as possible and 2 drops of a testicular emulsion, rich in actively motile treponemata, were allowed to drop on the exposed granulating surface. The same

strain (Nichols) of treponemata was used for reinoculation. The virulence of the inoculum was controlled by inoculating five normal rabbits with wounds of the same age and prepared in the same way. Reinoculation was performed 248 to 290 days after the first inoculation. Wassermann reactions were performed at frequent intervals both before and after reinoculation. In addition a single popliteal node was removed from each test animal and from two of the virus controls 60 to 63 days after reinoculation; it was emulsified in normal saline solution and the entire emulsion inoculated into the testes of each of two normal rabbits. The test animals were observed for a period of 195 days following reinoculation before the experiment was terminated.

*Results.*

The results are shown in Table II.

In all of the animals the wounds healed at about the rate expected in uninoculated rabbits. In two of the test animals abscesses developed and these were incised and drained. No treponemata could be demonstrated in the exudate from these infected areas. In one animal (No. 32) a characteristic chancre developed after an incubation period of about 25 days. Treponemata could be demonstrated with ease in the serum obtained from this lesion. It is of interest that this chancre began to regress spontaneously 47 days after its appearance, long before there was any sign of spontaneous regression in the lesions which developed in the controls. There were never any signs of generalised syphilis in this rabbit but the Wassermann reaction became positive after reinoculation and the lymph node transfer was also positive. In no other rabbit did a syphilitic lesion appear at the site of reinoculation. It is true that several animals exhibited transient induration of the scar at about the time of complete healing, but serum from these areas did not show treponemata by dark-field examination and no lesions developed subsequently. In five rabbits, including the one in which the chancre developed, the Wassermann reaction became positive. Lymph node transfer was positive in three of these five animals. Altogether, in eight of the sixteen test animals lymph node transfer was positive and in only one of these did a syphilitic lesion develop at the site of inoculation. In none of the sixteen test animals did any generalised syphilitic lesions make their appearance.

In the five control animals a typical chancre appeared in each

instance. These went through the ordinary course of development such as we have described previously, and finally healed spontaneously. In all five the Wassermann reaction became positive and lymph node transfer in the two instances in which it was done was positive. No generalised lesions were noted in the controls during the period of observation.

*Comment.*

It is clear that in but one of these sixteen treated and reinoculated rabbits did there develop a characteristic chancre following reinoculation upon a granulating wound. The remaining fifteen failed to show anything at the site of reinoculation which might be considered as syphilitic, and yet, in seven of these fifteen, lymph node transfer was positive, indicating that the animals were syphilitic at the time transfer was performed. For reasons outlined above we are inclined to the view that the virus obtained from these lymph nodes was that introduced at the time of reinoculation, and we interpret the experiment as indicating that at least half of the test animals had been successfully reinfected by inoculating a granulating wound on the back although in only one did a syphilitic lesion develop at the portal of entry.

The high incidence of successful reinoculations obtained in late treated syphilitic rabbits when the virus was deposited upon a granulating wound on the back (50 per cent), as contrasted with the comparatively low incidence of successful reinoculations obtained when the virus was injected intratesticularly, calls for explanation. One possibility that has to be taken into consideration involves the contention of Neisser that acquired immunity to syphilis is dependent upon the persistence of active foci of syphilitic infection somewhere in the body of the host, and furthermore that the response of a treated animal to reinoculation can be taken as an index of cure. In previous publications we have discussed this theory and need not therefore consider its validity at this point. Assuming it to be true, the results outlined in the second experiment are susceptible of but one interpretation, namely, that although treatment was begun late in the disease half of the animals were cured while half were not. On the same basis it would have to be concluded that the same mode of

treatment carried out in another group of syphilitic rabbits at a comparable period in the course of the disease (Category F, Table I) had failed to bring about cure in any, since they were all refractory to a second inoculation. This explanation does not seem logical and has the disadvantage in that it gives no clue as to why identical treatment should fail completely in one experiment and be 50 per cent effective in another, other things being equal. Nevertheless, if it is correct, then the experiment necessarily indicates that treatment late in the course of the infection in rabbits will apparently cure at least half of the animals.

A second and perhaps more plausible explanation which is suggested is that the resistant state which develops in syphilitic rabbits as a result of their infection is not absolute but is capable of being broken down in part by resort to a method of inoculation which appears to be particularly favorable to the inciting agent. It is possible that this breaking down of the animal's resistance is accomplished through failure of the granulation tissue of these healing wounds to share in the resistant state to the extent as does the testis for example, whether or not the latter organ has previously been the seat of a syphilitic inflammatory process. Nevertheless it would seem that the newly formed granulation tissue does share to some extent in the immune process since half of the reinoculated animals were, so far as we could judge, not reinfected, and since in only one of the remaining half, which apparently were successfully reinfected, did a characteristic chancre develop in the wound.

It is entirely possible that the tissues in these granulating wounds in reality did possess a high degree of resistance to a second infection but that the trauma incidental to removal of the crust of the wound preparatory to inoculation resulted in the opening up of lymph channels and thus facilitated migration of the treponemata and invasion of the blood stream before any local defensive process could effect a complete destruction of all the treponemata. If this is the correct explanation of the apparently successful reinfection of half of the animals, it would speak against a humoral immune mechanism in syphilitic rabbits, at least of great magnitude.

It is necessary to consider also the possibility that there is no real resistance to reinfection in these granulating wounds in late treated

syphilitic rabbits, that is to say no active local defensive mechanism. It may be that all that is being observed is an instance of acquired inability of tissues to react in the customary manner to syphilitic virus, that is, an indifference or state of anergy in the sense that Neisser used the term. If such is the correct view then we must consider the possibility of a humoral factor in acquired resistance to syphilis since at least half of the test animals were apparently refractory to a second infection. Regardless of whether one considers this inability to produce a characteristic chancre by inoculating granulating wounds of late treated syphilitic rabbits as evidence of tissue indifference or anergy, or as evidence of the existence of a local active defensive mechanism more or less complete, it must be admitted that the acquired property of the granulation tissue not to react is not dependent upon a preexisting local syphilitic infection. By no stretch of the imagination can one conceive of such granulation tissue having been previously infected with syphilis since the wounds were made after the animals had been thoroughly treated. The more or less refractory state of the granulating wound in the immune animal may be dependent upon either a humoral defensive mechanism, or a mechanism limited to cells, or a combination of both factors. Whichever factor is operative, the experiment shows clearly that the resistant state which develops in rabbits during the course of syphilitic infection is in part at least conferred upon newly formed granulation tissue (with all that that term implies) and suggests that the immune mechanism, if cellular, is capable of being inherited by, or imparted to, newly formed cells. The experiment suggests also that a method is at hand for successfully reinfecting syphilitic rabbits which from past experience one would be justified in assuming to be refractory to an intratesticular inoculation.

Whatever is the correct explanation of the facts that have been observed, it seems clear that in considering the results of reinoculation of treated syphilitic rabbits the experiments cited above would indicate that it is essential to bear in mind that the time at which treatment is begun and the mode of reinoculation are important factors in determining the character of the response to a second infection. Furthermore the evidence is strong that under the conditions of the experiments a second syphilitic infection with the homologous strain

of treponemata may be produced in a large proportion of treated rabbits without the development of any syphilitic lesion at the site of reinoculation.

The results of the experiment recorded in Table II, when contrasted with those outlined in Table I, seem to throw additional light upon the question of the advisability of using the reinoculation method as a criterion of the cure of syphilis. Our previous experiments, in conformity with those of Kolle and of Frei, have shown that when rabbits are infected by the intratesticular route and are treated with arsphenamine late in the course of the infection (181 to 291 days) they are almost uniformly (over 90 per cent) refractory to a second infection introduced in the testis. According to the reinoculation test these animals were not cured. On the other hand, in a group of sixteen animals originally inoculated in the testis and treated 159 to 201 days after infection with the same drug and the same dosage, successful reinoculations (with the same criteria) were apparently accomplished in eight, or half of the animals, when the second inoculation was made upon a granulating wound on the back of the animal. Using the reinoculation test as a criterion of cure one would have to assume that half of these animals had been cured and the other half had not. If comparable groups of rabbits treated in exactly the same manner can give such different results when reinoculated in different ways (90 per cent or more against 50 per cent), how is it possible in such experiments to draw any deductions, from the result of the reinoculation, as to the persistence or absence of the first infection in the body of the host, that is to say, cure or failure to cure? Who is to say which method of reinoculation is the one by which to judge? One can scarcely escape the conclusion that, in view of the variable results given by different methods of reinoculation, such a procedure cannot be accepted as a valid method for determining the possibility of cure of syphilitic infection.

It is perhaps not amiss to point out that the experiments outlined above may have clinical significance. If it is true that under certain conditions a treated syphilitic rabbit can be successfully reinfected without the occurrence of a local lesion at the site of reinoculation, but, nevertheless, with the development of a positive Wassermann reaction in the blood and with dissemination of the treponemata,

then one may well raise the question whether such a phenomenon ever occurs in human beings. Every syphilologist is familiar with the patient who comes under treatment relatively early, who is systematically treated, whose Wassermann reaction becomes negative, and who subsequently, months or years after the treatment has ceased, suddenly shows a completely positive Wassermann reaction in the blood without any signs of syphilis anywhere. Such cases have in the past been regarded usually as instances of a serological relapse, and, by inference, of a failure to cure. In the light of our experiments it may well be that such cases in some instances represent examples of a second infection without signs of syphilis, rather than instances of Wassermann reaction recurrence, and that this conceivable modification of the usual response to syphilitic infection, which they exhibit, is the result of the influence of the first infection. It is universally admitted that second attacks of syphilis with all the characteristic phenomena of a first infection do occur, although rarely. It is not such a far cry to imagine that second infections may take place in treated individuals, with no more evidence of their existence than the development of a positive Wassermann reaction. The importance of deciding whether such cases are really instances of a new infection, rather than examples of a relapse of the old, must be manifest so long as reinfection in humans is regarded as adequate evidence of cure. The clinical differentiation of a case of Wassermann reaction relapse from that of a reinfection without manifest signs but attended by the development of a positive Wassermann reaction, assuming that it occurs in man, would probably be an almost insurmountable task.

## SUMMARY.

Syphilitic rabbits inoculated intratesticularly and treated with arsphenamine before the 69th day of the disease, when reinoculated with the same strain of treponemata and in a manner identical with that of the first inoculation, are capable of responding to the infection in at least five different ways. In addition to exhibiting a local lesion at the site of reinoculation, accompanied by dissemination of the virus, they may show no local lesion at all but present evidence of dissemination of the virus together with the development of a positive Wassermann reaction. In some instances the virus may

be recovered from the reinoculation site although no local lesion is produced there and no dissemination of the virus can be shown to take place. An occasional animal treated before the 69th day of the disease remains completely refractory to a second infection.

When treatment is postponed to 6 months or more after the original inoculation, reinfection carried out by intratesticular injection is almost always impossible and such animals appear to be entirely refractory. However, if rabbits treated late are reinoculated with the homologous strain by depositing the virus upon a granulating wound on the back, successful reinfections can be accomplished in at least 50 per cent of the test animals. The resistance which develops in rabbits in the course of syphilitic infection is, then, not absolute but relative.

It is pointed out that these results cast discredit upon the validity of the reinoculation method as a test of cure in syphilitic infection. It is also suggested upon the basis of these experiments, that the subsequent occurrence of a positive Wassermann reaction in patients with early syphilis in whom the Wassermann reaction has become negative under treatment may not always represent a relapse in the the disease but possibly in some instances a new infection without clinical signs.

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