SUPERINFECTION IN EXPERIMENTAL SYPHILIS FOLLOWING THE ADMINISTRATION OF SUBCURATIVE DOSES OF ARSPHENAMINE OR NEOARSPHENAMINE.*

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PLATES 73 AND 74.

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It is generally held that a person infected with the virus of syphilis becomes practically immune to a second infection—that, with the development of the initial lesion, a condition becomes established which makes it difficult or impossible to superimpose a second infection upon the one already present and that this refractory state is maintained as long as an infection exists. As far as they have been tested, human and animal experiences are in essential agreement upon these points.

In the absence of any evidence to the contrary, it has been assumed that the principles contained in this conception of syphilitic immunity apply to treated as well as to untreated cases of infection and many syphilographers have regarded the appearance of fresh lesions of the chancre type, under circumstances which would indicate a new infection, as the most conclusive evidence of the cure of the previous infection. There are some, however, who have questioned the validity of so called reinfections, and others who have objected to their interpretation as evidence of cure. Granting that the class of cases referred to represents instances of true, second infection, an alternative interpretation of such occurrences has recently been presented by Jacobi (1) in which it is suggested that the reaction to a second inoculation may be viewed more as an expression of an existing state of immunity than as evidence of the presence or absence of infection.

* The results of the experiments reported in this paper were demonstrated at the All-America Conference on Venereal Diseases, Washington, D. C., December 6 to 11, 1920.

The situation which exists with reference to the immunity of infected individuals, the possibilities of superinfection or reinfection, and hence the interpretation to be placed upon the appearance of manifestations of disease which give every evidence of being due to a new infection are both complicated and obscure. Until the introduction of modern methods of spirocheticidal therapy, instances of so called reinfection were comparatively rare, and interest in the subject was largely a theoretical one. Within the past few years, however, the literature has contained numerous reports of reinfection following treatment with arsphenamine or neoarsphenamine, and the interpretation to be placed upon these occurrences has become a matter of immediate practical importance. Where the evidence of a new infection seemed sufficient, the general tendency has been to accept such infections as proof of a cure, and this would appear to be the logical interpretation unless it can be shown that under circumstances such as have existed in these cases, superinfection becomes possible. An element of uncertainty is introduced by the treatment employed, since practically nothing is known concerning the influence of such drugs as arsphenamine and neoarsphenamine upon syphilitic immunity.

The point at issue, therefore, is not so much a question of the immunity conferred by syphilitic infection as it is the effect which given therapeutic agents may have upon the resistance of infected individuals, whether native or acquired, and upon any spirochetes which may survive their action. Presented in this form, the problem of reinfection may be approached experimentally as one of the influence of drug action upon immunity and infection, and experiments have been carried out in animals for the purpose of determining the effect of subcurative doses of arsphenamine and of neoarsphenamine upon the resistance of infected animals to reinoculation. These experiments were divided into two groups according to the stage or progress of the infection, and the work to be reported in this paper deals with the results of treatment and reinoculation of animals with early but marked primary lesions.

EXPERIMENTAL.

The experiments to be reported consisted of the infection and treatment of two sets of rabbits—one with arsphenamine and the other with neoarsphenamine—after which they were reinoculated for the

purpose of determining their susceptibility to a new infection as indicated by the production of lesions at the site of inoculation. The results of the experiments were controlled in four ways, (1) as to the effects of the treatment employed, (2) as to the immunity developed by the infection, (3) as to the relative susceptibility of normal animals as indicated by their reaction to the virus used in the reinoculation experiments, and (4) by the use of a virus of essentially the same virulence as that causing the existing infection.

All animals were kept under observation for a minimum of 8 weeks after treatment while the therapeutic controls were held for 4 months as a means of affording a more accurate estimation of the status of the infection existing after treatment.

Infection of the Animals Used.—The animals used were inoculated in both testicles with 0.2 cc. of an emulsion containing one to three spirochetes to the microscopic field (Nichols strain). The object in view was the production of an intense infection which would confer a high degree of protection against reinoculation within a short period of time. The incubation period of the testicular lesions averaged about 10 days and the lesions developed very rapidly; at the end of 18 days the testicles were markedly enlarged and indurated, and in some instances there was an edema of the scrotum, indicating that the lesions were approaching the height of their first cycle of development. The animals were then divided into three groups according to the degree of development of the testicular lesions: Five animals with the most advanced infections were placed in the group to be treated and reinoculated; five others with the least advanced lesions were set aside as infected controls to be reinoculated at the same time as the treated animals; while a third group, consisting of animals showing various degrees of testicular involvement, were treated as therapeutic controls for the reinoculated animals of the first group.

Treatment.—Treatment was carried out 18 days after inoculation by the intravenous administration of a single dose of arsphenamine or neoarsphenamine. The products used were of the original German manufacture, being from Lots A 25819 and A 25884 respectively. The arsphenamine was neutralized by the addition of the theoretical amount of N sodium hydroxide to form the disodium salt, and both substances were administered in a 0.2 per cent solution.

The drugs were employed in the equivalent amounts as stated by the manufacturer. The doses used were 6 mg. of arsphenamine per kilo of body weight and 9 mg. of neoarsphenamine. The selection of these doses was based upon known values of therapeutic action for arsphenamine rather than neoarsphenamine and represented an attempt to use a dose of this drug which in the average animal of the group would yield a therapeutic effect of a definite character; namely, a regression of lesions approximating complete resolution with freedom from recurrence for 4 to 6 weeks followed by clinical relapse within a period of not more than 2 to 3 months. The ability to gauge these effects correctly was considered to be one of the two most essential features of the experiments, the other being reinoculation.

Twelve rabbits were treated with each of the drugs in the manner described; five of them were subsequently reinoculated and the seven others held as therapeutic controls.

Reinoculation.—Reinoculation of treated and untreated animals was carried out on the 24th day of the infection (5 days after treatment). In order that there might be as little difference as possible in the virulence of the organisms used for reinoculation and those producing the original infection, the virus used was obtained from an animal of the same series as those to be reinoculated. This was, in a sense, a measure of control. Each animal received an intracutaneous injection of 0.2 cc. of an emulsion containing one to three spirochetes to the microscopic field at two widely separated points, the ventral surface of the sheath and the base of the right ear in the region of the posterior marginal vein (see illustrations). It will be noted that one of these points was in close proximity to the original lesions and the other as far removed as was practicable. This choice of sites for reinoculation was determined in part by the susceptibility of skin areas and in part was used as a means of checking the extension of the immunity in control animals.

Reinoculations were timed both with reference to the possible retention of drug in a biologically active state and with reference to the progress of drug effects, the intention being to reinoculate as early as possible so as to give the best opportunity for the development of lesions before recurrence of the original lesions took place.

Methods of Control.—The results of the experiments outlined were controlled in four ways:

1. Therapeutic Controls.—In order to avoid any confusion which might arise from an attempt to interpret therapeutic effects obtained in animals which had been reinoculated, seven rabbits from each of the treated groups were held under observation for the purpose of determining as nearly as possible the effect which had been produced upon the original infection by the treatment employed.

2. Infected Controls.—Five infected rabbits of the same series as those used for treatment were reinoculated in the manner described with the same material as that used for reinoculation of the treated animals and for the inoculation of the normal controls.

3. Normal Controls.—The relative susceptibility of normal animals to the virus used for reinoculation was controlled by the inoculation of three normal rabbits carried out in the same manner as that of treated animals. These will be referred to as normal controls.

4. Virus Control.—As a means of insuring equality in the virulence of the spirochetes originally introduced and those used for the second inoculation, the reinoculations were made with material taken from a testicle of an animal of the same series as those to be reinoculated.

Effects of Treatment.

A consideration of the results obtained from the experiments outlined above should logically begin with the effects of the treatment employed. Following administration of the drugs, the testicular lesions began to regress, resolution proceeding somewhat more rapidly in the animals treated with neoarsphenamine than in those with arsphenamine. In some animals, the lesions disappeared completely by the end of 7 to 14 days, at which time the effect of the drug upon existing lesions practically ceased.

Of the twelve animals treated with neoarsphenamine, the testicular lesions were completely resolved in seven, four showed definite residual lesions in the form of diffuse thickenings or of circumscribed nodules, and in one the result was uncertain. In contrast to this, complete resolution occurred in only four of the animals treated with arsphenamine—a difference which is quite characteristic of the action of the two drugs in inducing resolution or healing of lesions in the experimental animal.

The first evidences of relapse among the therapeutic controls were noted between 14 and 17 days after treatment. Four of the seven animals treated with arsphenamine showed reinduration and gradual increase in the size of residual lesions or the development of new foci of infection by the end of the 3rd week, and the infection progressed at a normal rate. A fifth animal relapsed 45 days after treatment, while the condition in the two others was not clear. From the 3rd week onward, there were brief periods during which the testicles of these two animals appeared to enlarge somewhat, there were ill defined areas of thickening, and even a few tiny nodules in the testicles or tunics, all of which were suggestive of relapse, but none of these conditions developed into affections which exhibited the characteristic clinical appearance of syphilitic lesions. At the end of 3 months, test inoculations were made from popliteal lymph nodes of both animals with positive results, and one of them developed a testicular lesion shortly afterwards.

Clinical relapse among the animals treated with neoarsphenamine was more delayed. Three animals of the control group showed minor changes suggestive of relapse as early as 14 days after treatment, but outspoken lesions did not develop until towards the end of the 3rd month. Relapse occurred in the four others between the 24th and 39th days, but in two of these the growth of the lesions was again rather slow and irregular for from 4 to 6 weeks.

Infection was, therefore, shown to be present in all the therapeutic controls of both series. In other words, none of the animals were cured by the treatment received, and similar but less marked effects were produced by the treatment given the animals which were used for reinoculation.

Results from Reinoculation of Infected Controls.

The second point to be considered is the probable state of immunity which had developed in the treated and reinoculated animals. It was not possible to determine this with certainty, and in reality such a determination was not essential to the object of these experiments. However, as the best means of estimating the immunity in these rabbits and of controlling the results of reinoculation at the same time,

which was of more importance, animals with the most advanced infections were selected for treatment, while those showing the least progress of the infection were used as controls. In this way, the state of the infection existing at the time of reinoculation of the untreated animals was practically the same as that which had existed at the time of treatment of the other group.

The results from reinoculation of the infected controls may be given briefly. Within a few hours, all animals of the series showed a slight acute reaction at the site of inoculation which consisted first of an edema and then of a slight diffuse redness about the site of inoculation. This reaction subsided completely within 24 to 48 hours and in one animal was the only reaction observed. In the others, a diffuse or papular infiltration developed in the sheath and at the base of the ear. The lesions in the sheath reached their height in from 5 to 7 days and disappeared completely within 12 to 14 days. The papules measured from 3 to 6 mm. in cross-diameter; they were of a rose-pink color, firmly indurated, and of a slightly translucent appearance. The more diffuse lesions presented essentially the same characteristics. The ear lesions developed somewhat more slowly, and in three of the five animals were very slight, diffuse infiltrations lasting approximately 3 weeks. The majority of the lesions described were of the type of slight non-specific inflammatory processes, or they might be regarded as allergic reactions. It is possible that some of them were due to a slight but transient local infection, but no examination was made for spirochetes for fear that the trauma inflicted might induce regression.[1]

In two instances out of the ten inoculations (two injections in each animal), small, firmly indurated, and translucent papules developed at the base of the ear (Figs. 1 and 2). Clinically, the lesions presented the appearance of syphilitic granulomata. One of them disappeared within 3 weeks, and while the other never developed to more than 4 to 6 mm. in diameter, it persisted for 54 days.

These were the only instances in which reinoculation resulted in the production of skin lesions which gave evidence of being due to

[1] While trauma in some form appears to play a part in the distribution and even in the development of syphilitic lesions in the rabbit, scarification, cutting, or aspiration with a needle frequently causes them to regress.

infection, and they are reproduced in Figs. 1 and 2 for purposes of comparison with the lesions obtained by reinoculation of the treated animals. It is to be noted that both lesions occurred on the ear, while the foreskin was entirely negative, which is the reverse of the order of susceptibility of the two skin areas in normal rabbits.

Results from Reinoculation of Treated Animals and Normal Controls.

Reinoculation of treated animals gave results which were strikingly different from those of the infected controls. In a word, all except two of them developed perfectly typical chancres, examples of which are given in Figs. 3 to 12; there was marked lymphadenitis such as is associated with primary lesions, and spirochetes were present in abundance. Within 7 days, every animal of the group showed a characteristic syphilitic reaction at the site of inoculation, either in the form of an elevated papule or of a flattened area of infiltration. Of the twenty points inoculated, nineteen were positive by the 7th day, and a lesion appeared at the one remaining focus on the 11th day after inoculation. The incubation periods of these lesions coincided with those of the normal controls.

The growth of both the sheath and ear lesions in four of the ten animals, including two treated with arsphenamine (Figs. 3 to 7) and two with neoarsphenamine (Figs. 8 to 12), was extremely rapid and practically uninterrupted until they reached the stages of development shown in Figs. 3 to 12, and some of them progressed beyond the points shown. On the other hand, none of the three normal controls developed lesions which were at all comparable to the sheath and ear lesions of these four treated animals.

In another animal (arsphenamine), the lesion on the sheath grew somewhat irregularly, but within 4 weeks formed a characteristic ulcerated chancre measuring 1 cm. in diameter. Growth then ceased for a short time, but the lesion was considerably larger and increasing actively when the animal was discarded. The ear lesion was of the nature of a papule surrounded by a zone of diffuse infiltration. It developed to approximately 8 mm. in diameter during the first 4 weeks but had practically disappeared before the animal was discarded.

The lesions of a sixth animal (neoarsphenamine) grew more rapidly than those of any other for about 3 weeks, but development ceased

at this point. On the sheath, there was a lenticular lesion measuring 5 to 7 mm. in thickness at its center and spreading diffusely over an area more than 1 cm. in diameter. A similar but less elevated lesion developed at the base of the ear, and while there was some exfoliation over the center of both lesions, neither of them underwent ulceration. They gradually subsided and had practically disappeared at the end of the 2 month period of observation. It is noteworthy that this animal developed a slight periostitis of the nasal bones 39 days after treatment and later a lesion of the cornea. There was also a marked popliteal lymphadenitis such as is commonly associated with focal infections in the drainage area.

In two other animals of the arsphenamine group, the reaction during the first few weeks after reinoculation was comparatively slight. During the 4th week, however, both the ear and sheath lesions of one animal began to increase rapidly and developed into characteristic chancre-like lesions of approximately 1 cm. in cross-diameter. The ear lesion of the other animal was first a papule, then a diffuse infiltration, but a typical chancre measuring 8 mm. in diameter developed on the sheath.

The lesions produced in the two remaining animals of the treated and reinoculated series were comparatively slight and consisted of small papules or diffuse infiltrations. They were more pronounced and more enduring than any lesion of the infected controls but less than those of the normal controls. The therapeutic response in one of these animals, both of which were treated with neoarsphenamine, was apparently less than that in any other animal of the series. The original lesions were never completely resolved, and a clinical relapse was recognized 17 days after treatment.

A feature of especial interest in these experiments was the fact that relapse of the original lesions occurred in eight of the ten reinoculated animals at about the time the superinfection lesions became well established. In most instances, the second chancres overgrew (inhibited) the recurrent lesions, but in other animals, both sets of lesions developed together.

An excellent illustration of this phenomenon of the double infection is furnished by Figs. 6 and 7. Inspection of Fig. 6 will show that there is a diffuse enlargement of the right testicle and two large nodules are well outlined at the positions indicated by the arrows. There is also a small patch of infiltration in

the left scrotum. The nodules in the testicle existed from the time of treatment, and although they were considerably softened and diminished in size, they became reindurated and began to enlarge by the end of the 2nd week. The development attained within 5 weeks (the time represented by this photograph) would of itself preclude the possibility of a metastatic origin. The further development of the two sets of lesions is shown in Fig. 7.

The results of the treatment and reinoculation experiments as a whole may be stated as follows: Of the five animals treated with arsphenamine, and then reinoculated, the original lesions were completely resolved in only one instance and relapse occurred within 33 days in four of the five animals, including the one animal whose lesions had been resolved. The other animal of the group showed no definite increase in the testicular lesions during the period of observation, and the lesions which resulted from reinoculation were less marked than those of the other animals. By reinoculation, characteristic chancres were produced in all the animals of the group. Three of the five animals developed well marked lesions at the base of the ear as well as on the sheath, while the ear lesions were comparatively slight in the two others.

The results after treatment with neoarsphenamine were not so uniform. The testicular lesions were quickly resolved in two of the five animals. The testicles of a third animal were left slightly enlarged and diffusely thickened, but no further change occurred, either in the way of regression or progression of the lesions during the period of observation, and while well marked lesions were produced by reinoculation on both the ear and the sheath, they were of shorter duration than those of the other animals. Clinical relapse occurred in all other animals of the group at from 14 to 24 days after treatment. Characteristic chancres were obtained from the second inoculation in three of the five animals on both the sheath and ear; the other two gave only diffuse or papular infiltrations.

The three normal controls developed characteristic chancres on the sheath which measured from 1 to 1.5 cm. in diameter. One of them developed a large nodule in the subcutaneous tissues at the base of the ear in addition to a diffuse infiltration of the skin and the usual lymphadenitis. In the two others, there were small papules surrounded by a zone of diffuse infiltration, but none of them developed chancre-like lesions in this location.

DISCUSSION.

In attempting to give an interpretation of the experiments reported, the first point to be considered is that while one group of animals with well developed primary lesions of the testicles proved to be extremely refractory to a second cutaneous inoculation with a virus of equivalent virulence, a second group of animals from the same series, after treatment with arsphenamine or neoarsphenamine, was highly susceptible and with two exceptions reacted to the second inoculation with the development of characteristic manifestations of a primary infection. In fact, if susceptibility may be gauged by the reaction at the site of inoculation, some of the treated animals (four out of ten) were even more susceptible to infection than the normal controls. Disregarding for the moment the therapeutic result in as far as the original infection is concerned, it is certain that this difference in the reaction of the treated and untreated animals can be attributed to no other cause than the treatment employed.

In the second place, it is practically certain that none of the animals was cured. In the majority of them, the original lesions were not completely resolved, but there still might be some doubt as to whether the relapses which occurred in the reinoculated animals were true relapses of the original infection or were lesions arising from the second inoculation. In several instances, the clinical history of the relapse appeared to be sufficient in itself to exclude the latter possibility, since within a time too short to permit of the development of metastatic lesions, reinduration and growth occurred in existing lesions. In order to remove any possible confusion which might arise from this source, however, fourteen therapeutic controls were set aside—infected and treated with the same material and in the same manner as the reinoculated animals—and none of these was cured.

The results of the experiments may, therefore, be reduced to a very simple statement; namely, that treatment of animals with marked primary lesions of the testicles altered their resistance to such an extent as to render them susceptible to a second cutaneous infection without having effected a cure of the original infection. It is clear, therefore, that under the circumstances existing in these experiments, not only is superinfection of rabbits possible but animals treated in the manner

described may be rendered even more subject to a new infection than a normal animal.

It should be emphasized that these findings do not conflict in any way with established facts of syphilitic immunity. The conflict, if there is one, is with the assumption that the same conditions obtain in treated as in untreated infections or that an immunity once established cannot be altered.

It has been clearly shown by the work of Finger and Landsteiner (2) and of others that immunity to a second infection is not always absolute—that by resorting to the use of large doses of virus, it is possible, even in advanced cases of syphilis, to produce superinfection, or lesions at the site of inoculation which tend to assume the form of those characteristic of the stage of infection during which reinoculalation is carried out. It is also well known that superinfection with the production of typical chancres is comparatively easy during the first incubation period, and multiple chancres and autoinoculation with chancre-like lesions are explainable upon this basis.

The facts enumerated have been found to apply to both human and animal infections. From the data available, the chief differences which appear to exist in untreated infections are that protection against reinfection develops much more quickly, is more marked, and more enduring in the rabbit than in man. These few facts furnish all the basis necessary for an understanding of the phenomena of a second infection following treatment which is not curative. It is only necessary to consider that treatment with such substances as arsphenamine and neoarsphenamine may cause an infection to revert to the condition which existed during the first incubation period when spirochetes were present but no immunity had developed; further, that the spirochetes which survive the action of the drug employed may be so attenuated or enfeebled for the time being as to be incapable of arousing an antagonistic reaction on the part of the host, thus favoring the propagation of more vigorous organisms introduced from without and the production of characteristic primary lesions at the site of the new infection.

These were the postulates which formed the basis for the experiments reported, and the results would appear to justify the conclusion that, in as far as early infections of the rabbit are concerned,

treatment with arsphenamine or neoarsphenamine may alter the immunological status of the animal to such an extent as to favor the propagation of a second infection without having accomplished a cure of the first. In advanced infections, conditions are more complicated, and it is more difficult to obtain chancres from second inoculations, but experiments now in progress indicate that even here superinfection is still possible of attainment.

Apart from any bearing which these experiments may have upon problems of syphilitic immunity, they serve to emphasize the necessity for a careful consideration of the influence which any therapeutic agent or any system of therapy may exert upon the mechanism of animal resistance as well as the spirocheticidal action of the agents employed.

CONCLUSIONS.

From the facts presented, it may be concluded that the existence of an infection with *Spirochæta pallida* does not constitute a bar in itself to the introduction and propagation of a second infection in the same animal; that, just as there is a period following a first inoculation during which a second infection may be implanted with the production of characteristic primary lesions, conditions may again arise in animals which have once become refractory to a second inoculation, that will favor the introduction of a new infection with the formation of lesions presenting the characteristics of an original or first infection.

Experimentally, such a state may be induced in rabbits with early but well marked primary lesions of the testicles by treatment with either arsphenamine or neoarsphenamine, hence, treated but uncured animals may be rendered as susceptible to a second cutaneous inoculation as a normal animal, and the manifestations of disease resulting from the second infection may be indistinguishable from those of a first infection.

SUMMARY.

Experiments were carried out on rabbits for the purpose of determining the effects of subcurative doses of arsphenamine and of neoarsphenamine upon the resistance of infected animals to reinoculation with *Treponema pallidum* and hence the possibilities of the occurrence of a second infection in treated but uncured cases of infection.

All the animals used were inoculated with the same virus, and the experimental tests were carried out when the first cycle of testicular reaction was nearing its height. The animals with the most marked testicular lesions were used for the basic experiment of treatment and reinoculation. The results of this experiment were controlled from four different standpoints: (1) the effect of the treatment employed upon the existing infection; (2) the immunity present at the time of treatment; (3) the virulence of the organisms used for reinoculation as compared with those causing the existing infection; (4) the comparative susceptibility of normal animals to the virus used for reinoculation.

The results obtained showed (1) that the treatment employed was insufficient to cure any of the therapeutic controls; (2) that the infected controls were highly refractory to a second inoculation; (3) that the treated animals were highly susceptible to a second inoculation and although not cured of their original infection, reacted to the second inoculation with the formation of lesions indistinguishable from those of a first infection; (4) that in certain instances the treatment given had rendered infected animals more susceptible to infection than the normal controls.

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EXPLANATION OF PLATES.

The illustrations are reproductions of photographs which represent the objects at their natural size.

PLATE 73.

FIGS. 1 and 2. Reinoculation lesions of control animals.

FIG. 1. A lenticular papule at the base of the ear 15 days after reinoculation. The lesion did not progress beyond the point shown.

FIG. 2. 30 days after reinoculation. There is an indurated papule in the same location. These were the most marked lesions produced by reinoculation of the infected controls.

FIGS. 3 to 7. Reinoculation lesions following treatment with arsphenamine.

FIGS. 3 and 4. Characteristic chancres on the ear and sheath of a treated animal as they appeared 42 and 37 days after reinoculation.

FIGS. 5 and 6. Ear and sheath lesions of an animal with clinical relapse of the original lesions—37 days after reinoculation. The right testicle (Fig. 6) is diffusely enlarged and indurated and contains two well defined nodules; the left testicle is still atrophic but there is a small area of infiltration in the scrotum.

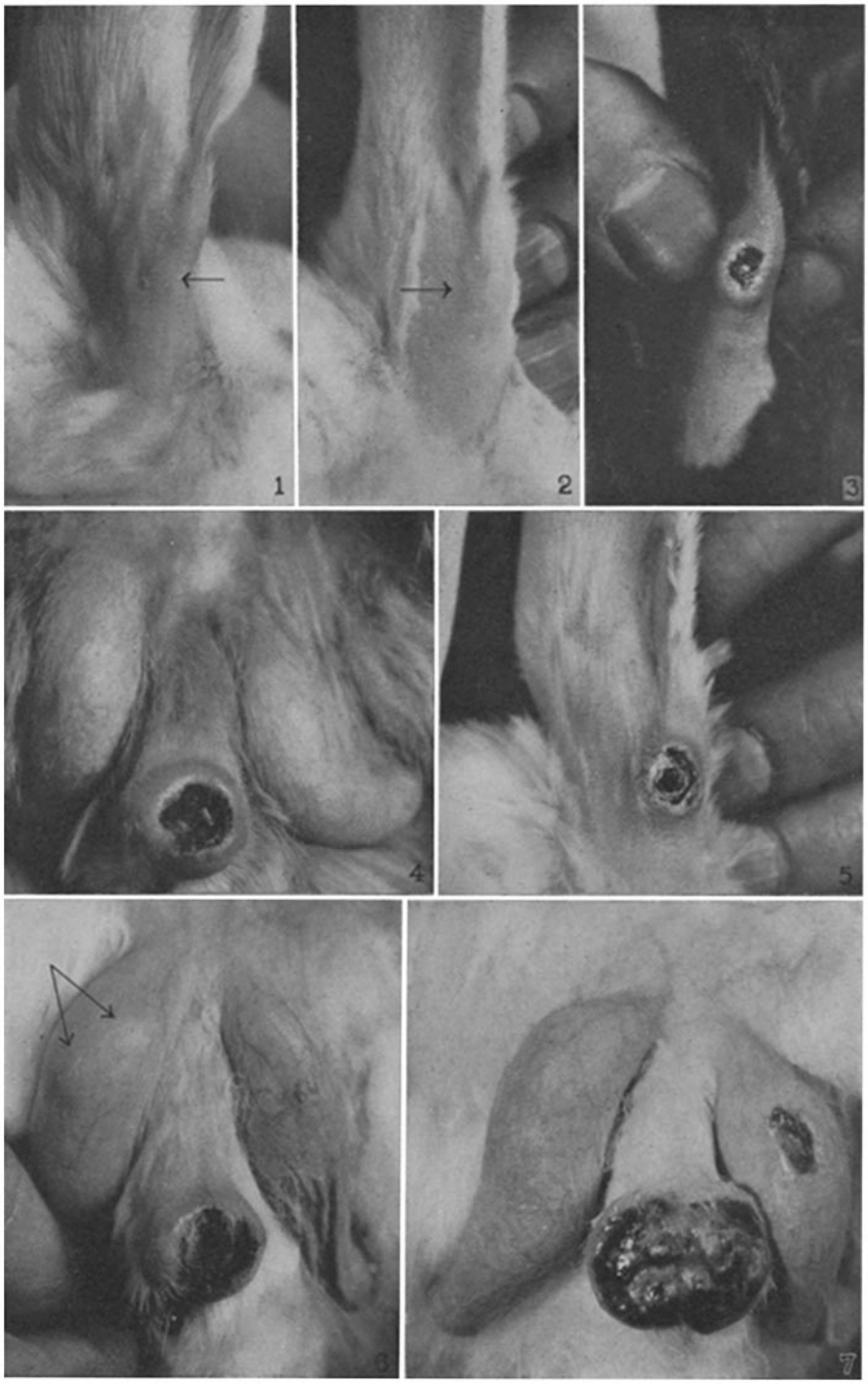
FIG. 7. The genital lesions of the same animal 12 days later. Note the marked increase of all lesions and the extensive necrosis of the lesion on the sheath.

PLATE 74.

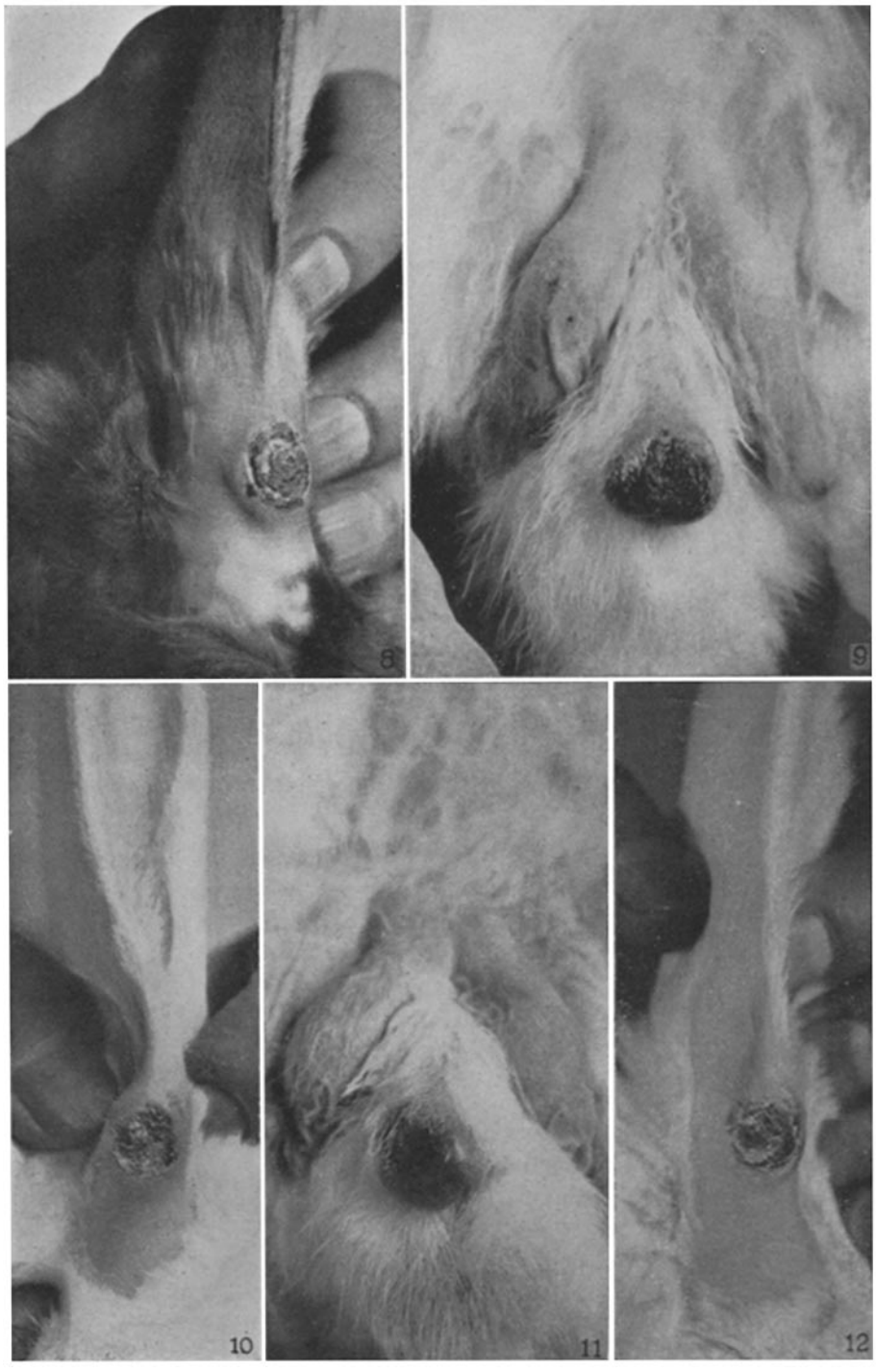
FIGS. 8 to 12. Reinoculation lesions following treatment with neoarsphenamine.

FIGS. 8 and 9. These figures are from the same animal and represent conditions as they existed 42 days after reinoculation. The two lesions are about equally developed and show marked necrosis.

FIGS. 10 to 12. From a second animal. Figs. 10 and 11 are 42 days, and Fig. 12, 49 days after reinoculation.



(Brown and Pearce: Superinfection in experimental syphilis.)



(Brown and Pearce: Superinfection in experimental syphilis.)