EXPERIMENTAL SYPHILIS IN THE RABBIT.

I. Primary Infection in the Testicle.

By WADE H. BROWN, M.D., and LOUISE PEARCE, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

Plates 45 to 52.

(Received for publication, January 29, 1920.)

The successful transmission of the virus of human syphilis to rabbits in 1906 (1) gave promise of an unusual opportunity for the investigation of problems relating to syphilitic infection by the use of experimental means, and during the years following, a great deal of time was devoted to the study of the animal infection with a view to adapting it to this purpose. Numerous methods of inoculation were devised and perfected and the resulting infections were studied in great detail, but the hope of obtaining an experimental infection by local inoculation which was closely analogous to the human disease was not fully realized. Isolated instances of generalized infection with the occurrence of lesions of various types have been reported from time to time, and while some investigators have obtained such evidence of generalization in as many as 50 per cent of the infected animals (2), these occurrences have been comparatively rare in the experience of most observers.

It should be remembered, however, that much of the work which has been reported was based largely upon the study of animals inoculated with virus recently isolated from human sources or with virus which had been carried in rabbits for only a few years, and that while most investigators are of the opinion that the virulence of *Treponema pallidum* for rabbits may be increased by passage or by adaptation, it is still uncertain how long this increase in virulence can be kept up or to what extent it may be carried.

The infection in the rabbit, as in man, is one which runs a prolonged course and is very variable in character, so that it would

necessarily require years of observation and comparison of infections in large series of animals to reach any definite conclusion upon these points. Thus far, it is doubtful whether any one has been in a position to carry out such experiments upon a scale which would enable him to say what form of disease might ultimately be established in the experimental animal or how closely this disease might be made to resemble the human infection. From the standpoint of the use of the experimental infection as a means of investigating problems of human syphilis, it is obvious, however, that the animal infection should be brought to a stage of development as near that of the human infection as possible. From our own investigations it appears quite likely that this condition is much more nearly attainable than results thus far reported might indicate.

Among the problems of human syphilis which have been attacked through the use of the experimental infection in the rabbit, that of therapy stands out as of foremost importance, and our interest in experimental syphilis grew out of the use of the rabbit infection as a means of studying the therapeutic action of drugs. While at no time have we been able to undertake the study of the experimental disease as an independent problem, we have, nevertheless, been forced to carry out a great deal of collateral investigation as the need for information of a given character arose. In addition, we have had the opportunity of studying a large series of animals infected with two strains of *Treponema pallidum* both of which have been carried in rabbits for a number of years.

What we have to report at present, therefore, is not the result of a series of experiments designed expressly for the solution of particular problems in experimental syphilis, but the results of a series of observations supplemented by experiments intended to give information of a particular character.

The chief needs for therapeutic work upon experimental syphilis are reliable means of propagating the infection and an accurate knowledge of the disease as it exists in the experimental animal. It is the purpose of these papers, therefore, to condense the results of 4 years observation into a series of brief reports dealing with clinical aspects of experimental syphilis in the rabbit with especial reference to the phases of the subject which are of importance in carrying out

therapeutic experiments. Detailed discussion of individual papers will be deferred until the series has been completed, when the various aspects of the subject will be considered together. Later, we hope to be able to take up other phases of experimental syphilis which cannot be included in the present group of papers.

It will be found that our description of the experimental infection in the rabbit differs materially from others in the literature, but this is not to be taken as evidence of conflicting observations. We have dealt with older strains of *Treponema pallidum* than have commonly been used, and different principles have been employed in the handling of these organisms, so that it is not unlikely that the infection produced by us has been of a different order. Whether or not one uses such organisms to begin with, it is well to have in mind the state of virulence to which an organism may be developed and the character of the infection which may be produced by these organisms.

Primary Infections in the Rabbit.

Three general forms of local inoculation have been successfully employed in the rabbit. These include inoculation into the anterior chamber of the eye, inoculation into the testicle, and inoculation of skin surfaces—usually the scrotum. Of these, the first is at present chiefly of historical and scientific interest. Testicular inoculation was introduced by Parodi (3) in 1907 and soon became the most widely used form of inoculation. It is generally recognized that testicular infections are especially well adapted to the maintenance of pure strains of the organisms, but they are on the whole of less value as objects of clinical study, since a large part of the process takes place in parts hidden from direct observation, and the infection is of a less stable character than that produced by skin inoculations. There are, however, certain features of the syphilitic reaction which are brought out to greatest advantage by this form of infection, so that the consideration of the experimental disease may logically begin at this point.

EXPERIMENTAL.

Organisms Used.—Two strains of *Treponema pallidum* were used in the work to be reported. One was obtained from Dr. Hans Zinsser and Dr. J. G. Hopkins and was isolated by them from a mucous patch on November 17, 1913, being Strain A of their series (4). The other organism was the Nichols nervous strain V for which we are indebted to Colonel M. A. Reasoner, Medical Corps, U. S. Army (2).

The first of these organisms has been carried in rabbits in our laboratory for 4 years and had been isolated 2 years before it came to us. The Nichols strain was isolated from the spinal fluid of a case of neurorecidive in June, 1912, and has, therefore, been carried in rabbits for more than 7 years.

Animals Used.—During the course of the work, many types of rabbits were used. There were many animals whose markings indicated either a pure stock or a strong admixture of such breeds as the Belgian hare, the Flemish giant, the Dutch belt, the Himalayan, the silver-gray, the Polish, the New Zealand red, the Angora, the English, and perhaps others. The majority of the animals, however, were of the common varieties of albino, gray, brown, brindle, black, blue or maltese, and animals of mixed or broken coloring. We have used animals of all ages and degrees of testicular development, from the young rabbit of 3 or 4 months in which the testicles were still but slightly developed to the very old rabbit with redundant scrotum and atrophic testicles.

From records showing the character of each rabbit used, its weight, testicular development, character of the scrotum, etc., it was found that in as far as the testicular infection alone was concerned, almost any type of animal with well developed testicles would give good results, but that if other features of the infection were to be considered also, the best results were obtained from the use of the small or medium sized albino and common gray or brown rabbits. As far as possible, animals were selected in which the testicles were well developed, with a preference for young rather than old animals.

Technique of Inoculations.—Testicular inoculations were carried out by the use of a virus emulsion prepared from an infected testicle or skin nodule. The animal which served as a source for the virus was anesthetized and the testicle or nodule excised with aseptic precautions. This material, or a portion of it, was then placed in a sterile mortar and finely minced with scissors, after which enough sterile salt solution (0.85 per cent) was added to moisten the entire mass. The material was then rubbed to a thick paste, more salt solution being added and thoroughly mixed with the contents of the mortar; the amount of salt solution to be added was determined by the dilution of the spirochetes in the emulsion as indicated by dark-field examination. The emulsion ordinarily used contained not more than two to three spirochetes to a microscopic field nor fewer than a single organism to two to three fields. Such an emulsion was usually obtained with from five to ten volumes of salt solution to one of tissue. The only object in attempting to control the strength of the emulsion was to insure

the presence of a sufficient number of organisms to produce a prompt reaction and to maintain some degree of uniformity in the dose of virus used in successive transfers.

The fluid emulsion was aspirated into a small glass syringe fitted with a 22 gauge needle $\frac{5}{8}$ inch in length. The scrotum of the animal to be inoculated was sponged with 50 per cent alcohol, more for cleanliness than for antiseptic purposes, and 0.2 to 0.5 cc. of the emulsion was injected into the center of each testicle. The exact dose used was determined by both the strength of the emulsion and the size of the testicle to be inoculated. The precaution was taken to see that the needles used were sharp and smooth to avoid laceration and that the testicle was not unduly distended by the fluid injected.

This technique is one intended for intensive inoculations and never failed to produce a take. From time to time the procedure described was modified in several respects for different purposes, but we found it very simple of execution and entirely satisfactory for routine inoculations.

Material Studied.—From November, 1915, to September, 1919, 83 transfers of the two strains of *Treponema pallidum* were made by testicular inoculation. All the inoculated animals became infected and developed characteristic testicular lesions. Some were used for subinoculations and others for therapeutic experiments, but the course of the local infection was followed in a large number of animals from the time of inoculation to the spontaneous disappearance of the local lesions, and some animals were kept under constant observation for a much longer period of time.

Local Reaction to the Infection.

The infections produced in the testicles of rabbits inoculated with *Treponema pallidum* differed in many important respects. There were differences in the time and mode of onset and in the character and progress of the reaction as well as in the nature of the lesions developed, but on the whole the course of these infections tended to conform to a given plan and the variations were largely those of detail. The characteristic feature of the reaction observed in this series of animals was a tendency to the occurrence of a succession of changes separable into periods or phases of progression and quiescence or regression which gave to the local reaction a cyclic or relapsing course not unlike that which characterizes the infections produced by the blood spirochetes. This type of reaction occurred not only in the testicles but was the fundamental pattern of every phase of the syphilitic reaction in the rabbit, and forms, therefore, a logical basis for a consideration of syphilitic infections.

Incubation Period.

The time elapsing between inoculation and the development of testicular changes which could be recognized by external examination varied from 2 to 6 weeks, with occasional instances of longer or shorter periods of incubation. In fully 90 per cent of the animals, however, the incubation period fell within the narrower limits of from 3 to 4 weeks, and under properly chosen conditions of virus and rate of transfer, it was found that this time could always be brought within the 3 weeks period.

By dark-field examination of fluid obtained from the testicles, spirochetes were frequently demonstrated at a period well in advance of the appearance of clinical signs of infection. The same was found to be true of histological changes and not infrequently even gross alterations could be made out in the excised organ before any clinical sign of infection had become apparent. The time at which spirochetes and histological changes became demonstrable need not be reported in detail but was found to be within 7 to 10 days after inoculation.

Successive transfers did not necessarily shorten the incubation period to any appreciable extent. The dose of virus used, other things being equal, had a noticeable effect upon the speed of reaction, but this was not always the case. The vitality, or what may be termed the infectivity of the spirochetes, proved to be a factor of much greater importance than that of dosage. Thus, transfers made from actively developing lesions or from animal to animal as rapidly as the infection developed tended to produce or maintain a short incubation period, while inoculations made from old, inactive, or regressing lesions showed a relative prolongation of the incubation period irrespective of the dose of spirochetes used.

Mode of Onset.

The specific reaction in the testicle began in one of two ways, either as a small circumscribed focus of induration situated at the point of inoculation or as a diffuse swelling with increased tension of the entire testicle. When inoculations were made from old or inactive lesions or at relatively long intervals, the first form of reaction usually prevailed, but when transfers were made from one animal to

another in rapid succession, the reaction was more often of the second type. These differences in the character of the initial reaction were only temporary, as a rule, and tended towards a common type as the infection advanced.

Course of the Reaction.

Once the presence of a specific reaction became established, the progress of the infection was marked by certain changes such as enlargement and induration of the testicles which were common features of the reaction in all animals. The details of these changes necessarily varied in individual animals or even in the two testicles of the same animal, so that in general two extreme types of reaction with numerous modifications and variations became recognizable, depending upon the speed and sharpness with which successive changes occurred and the extent to which the several processes participated in the reaction.

In its highest form, the specific reaction in the testicle was characterized by a short incubation period, a diffuse onset and rapid development followed by a sharp crisis during which there was marked regression of the testicular lesions, a period of quiescence or inactivity, and one or more secondary cycles of active proliferation and quiescence or regression. Naturally, all these phases of the testicular reactions were rarely observed in their ideal form in one animal, but the several features of the reaction and some of its more common modifications may be illustrated by concrete examples.

Acute Exudative or Fulminating Reaction.—The first form of reaction to be cited is one which was characterized by an intense cycle of acute reaction terminating in crisis and followed by a slight secondary cycle of proliferative reaction. The case of orchitis shown in Figs. 1 to 6 furnishes an example of a reaction of this kind.

Fig. 1 shows the testicles at the time of inoculation. 15 days after inoculation, there was an increased tension and slight swelling of the testicles which progressed rapidly for about 6 days, the left testicle being more affected than the right (Figs. 1 to 3). At first, the scrotum was drawn tightly about the testicle, its vessels were slightly prominent, and there was a faint reddening of the skin surface (Fig. 2). On the 6th day, the reaction reached its height (Fig. 3), having

culminated in a diffuse congestion and marked edema of the scrotum most of which had developed within the last 24 hours.[1]

On the 7th day after the appearance of the specific reaction, there was a sharp change during which the congestion and edema of the scrotum subsided very rapidly and were followed somewhat more slowly by a decrease in the swelling of the testicle and a softening of the induration. Within 3 days, regression had reached the point indicated in Fig. 4. At the end of a week, the testicles had returned to approximately normal size and appeared as small, rather soft, atrophic masses with an area of thickening at the lower pole of the right testicle.

No further change was noted during the succeeding 10 days. Then on the 12th day, the mass at the lower end of the right testicle was found to be definitely enlarged and indurated. This marked the beginning of a second cycle of progressive reaction which at this time was limited to a circumscribed area in one testicle. 7 days later, the nodule in the right testicle had increased to nearly 1 cm. in diameter and a few small shotty nodules were palpable in the left testicle (Fig. 5).

The lesion on the right continued to develop for another week at which time it presented the appearance shown in Fig. 6. Meantime, the nodules on the left had disappeared completely and there was no further reaction in this testicle. During the next 2 weeks, the nodule on the right showed several short periods of quiescence and slight activity and then underwent complete resolution.

The difference in the intensity and extent of the reaction in the two testicles of this animal is of especial importance. It will be seen by comparing the photographs that the acute reaction in the left testicle was much more intense than that in the right, and that when the crisis came, regression in this testicle took place more rapidly and was more complete. In like manner, the secondary or proliferative reaction, while slight in both testicles, was decidedly less in the left than in the right. This relation between the intensity of the first cycle of reaction on the one hand, and the secondary reaction on the other was so constant as almost to establish a rule of inverse proportions between the first and second cycles of reaction in a given animal.

[1] At this stage of the infection, the scrotum and scrotal sac were filled with a gelatinous exudate or with a clear straw-colored or slightly blood-stained fluid which coagulated quickly on standing. The testicles also contained a fluid exudate of similar character which dripped freely when the organ was cut. Microscopic examination of these structures showed an inflammatory exudate composed chiefly of serum and fibrin with some polymorphonuclear leucocytes and red blood cells.

Chronic or Proliferative Reaction.—The second general type of reaction which occurred in the testicles of infected rabbits was one in which infiltration and acute exudative phenomena were subordinated to processes of proliferation. Reactions of this type showed, therefore, a more gradual onset and development of lesions with less marked cyclic alterations. In this group, the infection frequently began as a circumscribed focus of induration which gradually spread until the entire testicle became involved. Enlargement of the testicle took place more slowly as a rule than in the preceding group of cases and was associated with induration rather than increased tension of the testicle. The infection usually progressed steadily for upwards of 2 to 3 weeks by which time the testicle presented the appearance of a large, smooth or slightly nodular organ of extreme hardness. Congestion of the scrotum was comparatively slight, and edema, when present, was less marked than in the typically acute cases.

Instead of a pronounced crisis, progress of the reaction ceased more gradually and was followed by a period of inaction or of comparatively slow regression during which the testicles diminished in size and softened to a greater or less extent. If edema was present, it disappeared more rapidly, but at the end of the period of regression, the testicle still remained definitely enlarged and indurated. This residual induration was sometimes diffuse or uniform in distribution, while at others it was limited to certain areas or portions of the testicle.

Growth of the lesions was then resumed and continued with occasional remissions so that the progressive tendency of the reaction remained uppermost for a considerable period of time. The reaction in the testicles then subsided and the lesions resolved in much the same way as they had developed.

Two examples of this type of reaction are given in Figs. 7 to 9 and 10 to 12 which represent stages in the progress of the reaction in two animals of the same series. The incubation period in these two animals was 29 and 22 days respectively. In the first animal (Figs. 7 to 9), there were diffuse enlargement and induration which were first noted about 4 weeks after inoculation; the infection involved the scrotum as well as the testicles proper. The reaction reached its height in the left testicle 36 days after inoculation (Fig. 7) and in the right 39

days (Fig. 8). Both testicles then showed a slight regression (indicated in the photographs by a slight decrease in size) followed by renewed activity toward the end of the 7th week after inoculation (Fig. 9).

The reaction exhibited by the second animal differed somewhat from that of the first. There were again a diffuse enlargement and induration of both testicles, but, as indicated by a shorter incubation period, the reaction took place more rapidly, reaching its height during the middle of the 4th week after inoculation (Fig. 10). At the time the reaction was nearing its height in the first animal, a decided regression had already taken place in the second (Fig. 11). This regression was more marked than in the first case, and a well marked second cycle of reaction was not apparent until towards the end of the 7th week. The character of this reaction is indicated in Fig. 12 which shows the condition present 8 weeks after inoculation.

Variations in the Specific Reaction. Subacute Reactions.

The modifications and combinations of these two fundamental types of reaction were, as we have said, quite numerous, but a few specific examples will serve to indicate the character and direction of the more important variations.

The first variation which may be mentioned is one concerning the acute type of reaction. The photographs reproduced in Figs. 13 to 15 illustrate a reaction which was characterized by an acute cycle of moderate extent associated with some diffuse induration in the testicle which persisted after the crisis. The reaction began in this animal as a circumscribed focus of induration in the posterior portion of the testicle, which was first detected 18 days after inoculation. Induration spread and the testicle enlarged rather rapidly, at the same time becoming diffusely indurated. The height of this change was reached 26 days after inoculation (Fig. 13). There was a moderate edema of the scrotum which lasted for a few days and then subsided, leaving the testicle still slightly enlarged as indicated in Fig. 14. There was some further regression of the lesions, and renewed activity did not set in until towards the end of the 7th week. This second cycle of reaction was both focal and diffuse in character and at the time the third photograph of the series was taken (Fig. 15) there were diffuse induration of both testicles and numerous nodules varying from a few mm. to approximately 0.5 cm. in diameter.

A second type of orchitis which was quite common in our series of animals is shown in Figs. 16 to 18. The onset of the infection in this instance was again of a circumscribed nodular character with an incubation period of 27 days. The testicles became diffusely indurated and showed a marked enlargement. This reaction took place somewhat more slowly than in the preceding case and did not

reach its full development until about 6 weeks after inoculation (Fig. 16). At the time the first photograph was taken (Fig. 16) the left testicle was retracted within the abdominal cavity and could not be brought through the inguinal canal, and there was very marked edema of both scrotal sacs which is best shown on the right. Edema subsided rather slowly, and at the same time the testicles diminished somewhat in size and became slightly softened. On the 49th day (Fig. 17) the scrotum showed a diffuse thickening with beginning induration over the ventral surface of both testicles; the testicles themselves were still considerably enlarged and markedly indurated. From this point onward, the reaction gradually shifted from the testicles proper to the scrotum with the production of chancre-like lesions, the beginning of which is indicated in Fig. 18.

The feature of especial interest in the reaction exhibited by this animal was the slowly progressive but pronounced character of the reaction, culminating in a diffuse swelling and edema of both the testicles and the scrotum and the subsequent transference of the center of reaction from the testicles to the scrotum—the latter condition being a frequent occurrence in testicular infections.

Another group of photographs illustrating an infection of somewhat the same character is reproduced in Figs. 19 to 22. This animal was an old albino with rather atrophic testicles and, as is usually the case with such animals, the reaction was slow to develop. Infection began as a circumscribed focus of induration which was recognized about 4 weeks after inoculation and gradually spread until the entire testicle was diffusely involved. There was a slow but steady increase in the size and induration of the testicles, extending over a period of about 2 weeks. The height of the reaction in the right testicle was reached 46 days after inoculation (Fig. 19) and a few days later in the left. There were moderate congestion and edema of the scrotum which lasted for several days. Crisis occurred, followed by regression, but at the end of this phase of the reaction, both testicles were still diffusely enlarged and indurated much as they appear in Fig. 20. Within 10 days after the crisis, there was renewed growth of lesions situated in the tail of the epididymis (Fig. 21). These lesions grew actively for another 2 weeks at which time they formed large indurated masses of irregular shape, involving the skin as well as the epididymis (Fig. 22). In the meantime, the reaction in the remaining portions of the testicle had completely subsided, leaving the testicles as small atrophic masses.

A fourth example of a more unusual reaction is given in Figs. 23 to 25. The earlier stages of the reaction in this animal were of an ordinary chronic proliferative type and had progressed to the point of the formation of a scrotal chancre on the left associated with induration of the testicle itself with marked enlargement and induration of the right testicle and slight extension to the scrotum at its lower pole, when, towards the end of the 7th week, there was an acute exudative reaction in the right testicle and scrotum, which reached its height on the 53rd day after inoculation. This reaction was followed by a typical crisis (Figs. 23 and 24), and both testicles then began to diminish in size and induration,

while the lesions of the scrotum continued to grow actively (Fig. 25). The feature of especial interest here was the occurrence of an acute exudative reaction late in the course of infection.

This small group of cases, including the more acute and chronic forms of reaction together with various modifications and combinations of these two fundamental types of processes, is typical of the reactions seen in practically all cases of outspoken testicular infection. Many of these modified reactions might be spoken of as subacute in the same sense in which the others are acute or chronic in character, since they combine to a greater or less degree the features of exudation and infiltration with those of proliferation of fixed tissue cells. It will be noted, however, that whatever the variation in the response of the individual animal, they all show an unmistakable tendency towards a reaction of a recurrent or a relapsing type.

It was found that these reactions could be influenced to a considerable extent in several ways. Thus, reactions of the more acute type were especially frequent when transfers were made from one animal to another in rapid succession or during the ascending phase of the acute reaction. In a small proportion of animals, the local infection terminated with a single cycle of reaction such as that described. As a rule, however, the acute cycle of reaction was followed by other changes leading to the formation of lesions differing in many ways from those originally produced. The occurrence of these secondary cycles of reaction depended to a considerable extent upon the character and extent of the first cycle. Thus animals in which there was an intense, acute reaction were, as a class, less apt to show secondary reactions of marked degree than those in which the process had been more gradual or less intense.

Reactions tending towards the chronic or proliferative type were quite common and occurred with especial frequency after inoculations made at long intervals or from chronic indurative lesions. They were frequently associated with the most persistent local infections and gave rise to some of the most conspicuous and destructive lesions of the testicles.

Late Developments of the Testicular Reaction and the Character of the Lesions Produced.

Most writers have treated the testicular infection of the rabbit from the standpoint of the lesions produced, but we prefer to regard these lesions more as manifestations of a reaction to infection and hence very little has been said concerning the lesions themselves. Following out their ideas of the importance of the lesion as an entity in itself, many investigators have attempted to differentiate between the various types of lesions produced, and several classifications have been proposed, based largely upon the location of the lesion and the character of the pathological process (5, 6). In our experience such differentiations would be very difficult to make except as applied to a particular lesion at a particular time or to what may be termed the residual lesions which ultimately come to be established.

Without entering into a detailed discussion of this phase of the subject, it may be said that while the infection in the animals studied by us began either as a circumscribed or as a diffuse process, neither of these conditions was permanent, and the same applied to the character of the pathological process. In practically all instances, the infection ultimately involved the entire organ with the production of lesions in the parenchyma, tunics, epididymis, and cord, and in many instances in the scrotum as well. This extension took place early, as a rule, and by the time the reaction had reached its height, there was what might be termed a panorchitis.

In many animals, this widespread involvement was of a perfectly uniform character as far as could be determined by the gross appearance of the organ or by palpation. In a second group of animals, there was a finely granular condition of the testicle, while in a third, distinct indurated nodules could be recognized, separated by tissue showing a lesser degree of involvement or an involvement of a different character. This differentiation into lesions of a particular type or lesions situated in certain localities came as a late development in the course of the infection and was, in our opinion, attributable to the character of the reaction against the local infection and the tendency on the part of the infection to extend in certain directions. During the later stages of the infection, therefore, one fre-

quently had to deal not with a widespread infection at the height of its activity but with an infection which was localized only in certain areas. The points of most frequent involvement were the globus minor and major, the tunics, the mediastinum testis, and the skin, or, in other words, the areas in which membranes or connective tissue in some form was most abundant.

These later developments and extensions of the testicular infections are of considerable interest both from the standpoint of the reaction to infection and the lesions which are produced. Several examples of this phase of the local reaction have already been given in the preceding illustrations, which may be supplemented by Figs. 26 to 37.

The first group of photographs in this series (Figs. 26 to 28) illustrates an infection which began as a diffuse indurative orchitis. At the period of infection represented in Fig. 26, 58 days after inoculation, a more or less diffuse extension of the infection to the scrotum of the right testicle had taken place, while on the left, the lesions in the testicle and scrotum were assuming a more nodular character, and these differences in the character of the changes in the two testicles persisted to the end. As the infection advanced, a large portion of the right testicle and scrotum underwent diffuse necrosis; on the left, the lesions began to be more circumscribed and two well defined scrotal lesions were formed (Fig. 27). Eventually, a large portion of both testicles underwent necrosis as indicated in Fig. 28, which shows the condition 80 days after inoculation. Even at this stage, however, the multinodular character of the lesions of the left testicle could still be made out, there being three fairly well defined centers of reaction.

In this animal, the feature of especial importance in the reaction was the tendency to widespread necrosis which eventually involved the entire skin surface and the testicle as well. This was not always the case, however, even when the reaction in the testicle was fully as marked as in this instance. In Fig. 29, a case of orchitis is illustrated in which the involvement of the testicle was also quite marked, but the necrosis and ulceration were confined to two more or less circumscribed areas producing effects more analogous to primary skin lesions. Lesions of this type were very common among our animals, and all gradations and transitions could be found between the lesion which was definitely a diffuse orchitis with necrosis and ulceration and lesions which were identical in character with primary skin lesions. The photographs reproduced in Figs. 30 to 32 are given to illustrate this point.

Fig. 30 shows a condition which is clearly an orchitis with skin involvement which has led to the formation of depressed ulcers with a definitely indurated collar such as is seen in primary skin lesions. In Fig. 31, taken only 6 days later, the picture has changed somewhat, and the whole mass in the left testicle

has filled out, while the right testicle now shows a single large ulcerated lesion identical with the large skin chancres which develop in the rabbit.

The next step in the series of transition is shown in Fig. 32. The infection in this animal began as a diffuse indurative orchitis which later became nodular with lesions such as those shown in Fig. 21, except that there was an active nodule situated in the tunic of the left testicle. The nodule in the epididymis of the right testicle extended to the skin, forming a chancre-like mass seen in Fig. 32. On the left, the nodule in the tunic was the one to develop most actively, forming a skin lesion still more like the ordinary chancre. These lesions healed in the course of about 4 weeks, leaving an active nodule in the epididymis of the left testicle (Figs. 33 and 34).

A final transition in the course of the testicular infection may be illustrated by Figs. 35 to 37. The infection in this animal began as a circumscribed focus of induration which quickly developed into a diffuse orchitis (Fig. 35). In time, the diffuse induration resolved, leaving small focal lesions in the tail of the epididymis of both testicles. Foci of infection then appeared in the skin and developed into the lesions shown in Fig. 36 (77 days after inoculation). Upon removal of the testicles and skin lesions, the testicles were found to be small atrophic masses entirely free from any gross evidences of active infection (Fig. 37). On the right, there was an indurated lesion in the epididymis which in part was continuous with the skin lesion; on the left, the two processes showed less connection and one of the two lesions in the scrotum was entirely distinct from the other lesions present (Figs. 36 and 37).

In this animal an infection which began as a circumscribed focus of induration first became diffuse, was then transformed into a nodular epididymitis, and the seat of active infection was finally transferred to the scrotum, leaving the body of the testicle free from active lesions.

Atypical and Low Grade Reactions.

Before leaving the subject of the local reaction in the testicle, mention should be made of another class of infections. There were, in our series, a few animals which showed some peculiarity in their response to infection, such as the development of slight or otherwise atypical lesions. These infections may be spoken of as atypical, on account of such peculiarities in the reaction, or as low grade reactions, in the sense that the reaction lagged or that the lesions which developed were of a minor character.

The common instances of this kind were slight reactions of short duration and slight but persistent reactions. Of the latter class, there were two main groups of infections, one in which a diffuse or

circumscribed focus of reaction developed very slowly and never resulted in more than a slight diffuse thickening in the testicle or a small nodular induration. The second group included instances in which a slight diffuse reaction subsided with the formation of residual nodules or in which an initial focus of infection was later supplemented by the appearance of secondary foci of a similar character. Minor lesions such as these not infrequently persisted for months without showing any especial sign of activity or growth.

Most of the infections of this class occurred during the earlier part of the work and have rarely been seen during the last 2 years. We are inclined to attribute occurrences of this kind to the circumstances under which the transfers were made.

From our present knowledge of conditions which influence the specific infection in the rabbit, we recognize this class of cases as attributable to certain definite causes, mainly to an attempt to inoculate with spirochetes at a time when their vitality, or infectivity, had been materially reduced by cyclic immunological reactions (page 492), but also to peculiarities in the response of individual animals to the specific infection.

Accessory Skin Lesions.

For the sake of completeness, mention may be made also of the occurrence of accessory lesions in the scrotum which develop along the path of the needle as a direct result of the process of inoculation (Figs. 2 and 4). At times, these lesions appeared before any reaction in the testicle had become recognizable. Without going into a discussion of this feature of testicular infections, which properly belongs in the section on scrotal chancres, it may be said that these foci of infection appeared in one of three forms, as small gelatinous swellings in the skin, as translucent pearly nodules in the superficial layers of the skin, or as opaque points or plaques in the depths of the scrotum, usually upon the outer surface of the tunica vaginalis. They rarely developed to lesions of any considerable extent. In some instances, however, they did develop into large lesions either as independent foci of reaction or as indistinguishable parts of the testicular reaction.

The Spirochete Reaction and the Spirochete Content of Lesions.

The above observations upon the course of the specific reaction in the testicle were supplemented by parallel dark-field examinations of fluid taken from the testicular lesions at different periods of the infection in order to determine what changes, if any, could be demonstrated among the infecting organisms while the changes in the testicles were in progress. From these examinations, it was found that as the infection progressed, the spirochetes exhibited changes analogous in character to those described in the testicles themselves. As we have already mentioned, spirochetes began to multiply and were present in considerable numbers before any gross manifestation of infection had become recognizable. This increase in spirochetes continued parallel with the development of the lesions, so that by the time the lesions had reached the height of their first cycle of development, actively motile spirochetes were present in large numbers.

At this point, the spirochetes suddenly began to lose their motility and to collect in tangled masses. In cases of acute orchitis, these changes coincided roughly with the development of edema of the scrotum and were followed promptly by regression of the testicular lesions.

Following the phenomenon of agglomeration, the spirochetes rapidly diminished in numbers, so that within a few days, organisms were difficult to find in fluid aspirated from the testicles, and those seen were either degenerated or showed but slight signs of motility; in many instances no organisms could be found.

After passing through a crisis such as this, actively motile spirochetes again appeared in the testicular fluid, and increased in numbers, presaging a renewed activity on the part of the lesions or at least a cessation of regression for the time being. These parallel changes continued throughout the existence of the local infection—the change in spirochetes usually occurring slightly in advance of the changes in the lesions.

As in the case of the lesions, cyclic changes were at times very sharp and easily recognizable, while at others they were less marked and might readily have escaped detection had it not been for the example of the sharper type of reaction.

During the latter part of the infection, the spirochetal content of lesions was found to be more difficult of estimation and somewhat uncertain. Where a single lesion was present, diffuse or circumscribed, the spirochetes in one area might diminish or disappear while they were present in considerable numbers or were actively increasing in other areas. These changes in the spirochetal content had their parallel, however, in the shifting centers of growth in the lesions themselves or, in the case of multiple lesions, in the resolution of one lesion while another was undergoing active development. By careful study of the lesions, it was found, however, that actively growing lesions or portions of lesions always contained actively motile spirochetes, and the same was true of many lesions which were merely quiescent but not regressing. The spirochetal content of lesions which were regressing was more variable; in many instances, spirochetes could not be detected by dark-field examination, while in others, they could still be demonstrated in fair numbers which diminished as the lesions resolved.

The changes affecting the spirochetes within the lesions will be recognized as entirely analogous to those which occur in blood stream infections with such organisms as *Spirochæta recurrentis*. In order to assure ourselves that the apparent immobilization, agglomeration, and degeneration described, entailed some actual alteration in the pathogenic properties of these organisms, tests were carried out by animal inoculation. For this purpose, inoculations were made with organisms taken as nearly as possible at the height of the first cycle in cases of intense, acute reaction, or more properly at the beginning of the crisis, and the tests were controlled by a parallel series of inoculations made with actively motile spirochetes taken from an early stage of testicular infection.

From these tests it was found that the infecting power of such organisms was markedly diminished. In one experiment, a series of animals inoculated with approximately ten times the dose of immobilized and agglomerated organisms that was used in the controls showed an incubation period of 6 weeks as contrasted with 3 weeks in the controls, while the lesions were slow to develop and were less pronounced than in the control animals. Similar observations as to the infectivity of the spirochetes at different periods of the infection have

been made many times and there is no doubt in our minds as to the significance of the cyclic reactions described.[2]

Duration of the Local Infection.

The duration of the local infection as determined by the presence of active lesions was as variable as the course of the infection itself, and no fixed limits can be given either for the several phases of the local reaction or for the infection as a whole. The period of active infection varied anywhere from 1 to more than 12 months. In some animals, the entire reaction was represented by one intense cycle of acute reaction which terminated within 4 to 6 weeks after inoculation; in others, the infection continued through successive cycles of reaction, but the period of active infection was rarely longer than 2 to 4 months. Inactive or latent lesions which showed occasional periods of slight activity frequently persisted much longer, and residual lesions in the epididymis, skin, and tunics not infrequently persisted for from 4 to 8 months. In general, the duration of the local infection was inversely proportional to the intensity of the local reaction.

CONCLUSIONS.

The conclusions which might be drawn from this series of observations are very numerous, but we shall refer briefly to only a few of the more important points.

From the standpoint of a pathological process, it is important to note that the local response is not altogether a granulomatous reaction. In fact, it appears that exudation and infiltration are the fundamental processes and that proliferation is a secondary phenomenon.

In the second place, it is quite clear that the reaction to infection in the testicle, and hence the course of the infection itself, are of a periodic or relapsing character, analogous in this respect to other spirochete infections, or for that matter present certain protozoan

[2] We have evidence sufficient to indicate that rabbits inoculated in the testicles or scrotum with *Treponema pallidum* always show a blood stream invasion, and that these organisms are subject to changes analogous to those which occur in the testicles. The details of this work will be reported later.

characteristics. To what extent this infection is influenced by a local reaction and to what extent by a systemic reaction are at present unknown, but as far as the local infection is concerned, it appears to be subject more to local than to general conditions. Further than this, it appears that the character of the reaction which takes place in a given case is itself significant. The relation between the reaction in the individual animal and the duration of the infection represents a constant. When the local reaction assumes the character of a chronic proliferative process, the life of the infection is prolonged and the extent of the reaction which takes place before the infection is brought under control is proportionately increased; when, however, the reaction assumes the form of an intensely acute reaction, the life of the local infection is promptly terminated. This relation between the local reaction and the duration of an active infection, together with the determination of the character of the experimental infection in the rabbit, are perhaps the two most important deductions to be drawn from this series of observations in that they touch every phase of the experimental infection.

SUMMARY.

A study was made of the infections produced in rabbits inoculated in the testicles with two strains of *Treponema pallidum* which had been carried in rabbits for several years. Infection resulted in all instances; the incubation period varied as a rule between 2 and 6 weeks and under properly chosen conditions could be reduced to approximately 3 weeks or less.

The resulting infection pursued a typically cyclic or relapsing course which affected both the spirochetes and the associated lesions in the testicle. The spirochetes in the local lesions exhibited periodic changes less marked and less regular but identical in character with the changes which occur in the blood in cases of relapsing fever. The lesions in the testicle also showed periods of active development and quiescence or regression which followed closely upon the changes exhibited by the spirochetes.

The specific reaction in the testicle showed considerable variation in the speed and sharpness with which successive phenomena occurred

as well as in the character and extent of the processes themselves. These reactions were of two fundamental types. In one group of animals, the reaction was characterized by an intense cycle of acute exudation and infiltration with a lesser degree of proliferation, followed by crisis and subsequent recurrence of secondary cycles of proliferative reaction of a minor degree. In the other group of animals, the reaction was more chronic in character and consisted largely of infiltration and proliferation. The progress of the reaction was more gradual, and sharp alterations in its course were absent. The infection progressed by a succession of stages with slight and irregular remissions.

In a third group of animals, the reaction was subacute, combining at the same time the processes of exudation, infiltration, and proliferation. The first cycle of reaction was fairly acute and terminated in a definite crisis with moderate regression which in turn was followed by recurrence and more or less pronounced secondary cycles of proliferation.

In all cases of outspoken infection, there was diffuse involvement of testicle, tunic, epididymis, and cord, but as the infection progressed, the lesions underwent many transformations, so that a variety of lesions was formed from processes which in the beginning were of a common type. Eventually, the reaction became more irregular and the infection became centered in one or more foci which were commonly situated in the epididymis, tunics, scrotum, or mediastinum testis. These centers served as residual foci of infection.

The duration of the testicular process was found to be very variable. In some animals, the entire reaction consisted of but a single sharp cycle, and the local infection was terminated by crisis within 4 to 6 weeks after inoculation. As a rule, the period of active infection was from 2 to 4 months, and quiescent or inactive lesions not infrequently lasted for from 4 to 6 months. In exceptional instances, local infection persisted for more than a year.

BIBLIOGRAPHY.

1. Bertarelli, E., *Centr. Bakteriol., Ite Abt., Orig.*, 1906, xli, 320; 1907, xliii, 238.
2. Nichols, H. J., *J. Exp. Med.*, 1914, xix, 362.
3. Parodi, U., *Centr. Bakteriol., Ite Abt., Orig.*, 1907, xliv, 428.
4. Zinsser, H., Hopkins, J. G., and McBurney, M., *J. Exp. Med.*, 1916, xxiii, 329.
5. Uhlenhuth, P., and Mulzer, P., *Arb. k. Gsndhtsamte.*, 1913, xliv, 307.
6. Reasoner, M. A., *J. Am. Med. Assn.*, 1916, lxvii, 1799.

EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs which represent the objects at their natural size. The statements of time refer in all instances to the time after inoculation, unless otherwise stated.

PLATE 45.

FIGS. 1 to 6. An acute diffuse reaction in the testicles and scrotum with crisis and regression followed by a slight secondary cycle of reaction and the formation of circumscribed nodular lesions.

FIG. 1. The normal testicles.

FIG. 2. 20 days. Well advanced, acute orchitis with beginning congestion and edema of the scrotum.

FIG. 3. 24 hours later. The height of the first cycle of reaction with intense congestion and edema most marked in the left testicle.

FIG. 4. 72 hours after the crisis. Note the greater regression of the left testicle.

FIG. 5. 48 days. The second cycle of reaction and the formation of nodular lesions in both testicles.

FIG. 6. 56 days. The lesion in the tail of the epididymis and scrotum of the right testicle is still increasing, while the lesions have disappeared from the left.

PLATE 46.

FIGS. 7 to 9. Chronic proliferative orchitis with scrotal involvement.

FIG. 7. 36 days. The height of the first cycle of reaction in the left testicle. Note the perfectly uniform enlargement of the testicle extending even to the cord. The scrotum of both testicles is involved, and on the right, there is an area of beginning necrosis.

FIG. 8. 39 days. The height of the first cycle of reaction in the right testicle; the crisis in the left has passed and the lesions are regressing.

FIG. 9. 51 days. The second cycle of reaction in progress in both testicles. Note the smooth, tense scrotum of the right testicle indicative of diffuse involvement, while in the left the lesions are now assuming a multinodular character.

FIGS. 10 to 12. Chronic proliferative orchitis in another animal of the same series as that in Figs. 7 to 9.

FIG. 10. 24 days. Well marked diffuse induration of both testicles approximately at the height of the first cycle of reaction.

FIG. 11. 36 days. Decided regression of the lesions of both testicles.

FIG. 12. 56 days. An early stage of the second cycle of reaction.

PLATE 47.

FIGS. 13 to 15. An acute diffuse reaction of moderate degree followed by crisis and a second cycle of chronic proliferation, diffuse and focal in character.

FIG. 13. 26 days. The height of the acute reaction with moderate edema of the scrotum. The left testicle is larger than the right.

FIG. 14. 28 days. Regression. The left testicle is still larger than the right due to a greater degree of proliferation in the initial reaction. Compare Figs. 13 and 14 with Figs. 3 and 4.

FIG. 15. 53 days. Moderate enlargement and induration of both testicles.

FIGS. 16 to 18. Subacute orchitis characterized by marked induration and edema of both testicles and scrotum and gradual shifting of the center of reaction from the testicles to the scrotum.

FIG. 16. 41 days. The left testicle is retracted within the abdominal cavity.

FIG. 17. 49 days. Some regression has taken place, but the scrotum is thickened and both testicles are still diffusely enlarged and indurated.

FIG. 18. 52 days. The lesions in the testicles are still slowly diminishing, but focal lesions are beginning to develop in the scrotum.

PLATE 48.

FIGS. 19 to 22. A subacute reaction with diffuse involvement of both testicles followed by a second cycle of reaction localized in the tail of the epididymis.

FIG. 19. 46 days. The height of the first cycle of reaction in the right testicle.

FIG. 20. 53 days. The extent of the regression following the crisis in the reaction is shown. Note that at the lower end of the right testicle, there is a nodule which has not regressed to the same extent as the rest of the testicle.

FIG. 21. 60 days. Continued regression of the testicular lesions with development of focal lesions in the epididymis.

FIG. 22. 76 days. Focal lesions of the epididymis and scrotum.

PLATE 49.

FIGS. 23 to 25. An acute exudative reaction occurring during a late stage of the local infection.

FIG. 23. 53 days. Chronic proliferative changes in both testicles with localized lesions of the scrotum with acute congestion and edema on the right.

FIG. 24. 2 days later. Regression on the right with slight swelling of the testicle and increasing growth of the scrotal lesion on the left.

FIG. 25. 61 days. Regression of the testicular lesions with simultaneous growth of the lesions in the scrotum of both testicles.

PLATE 50.

FIGS. 26 to 28. Chronic orchitis with extensive necrosis of testicles and scrotum. Right diffuse; left nodular.

FIG. 26. 58 days. Diffuse induration of the right testicle and scrotum with beginning skin necrosis and multinodular lesions of the left testicle.

FIG. 27. 73 days. Diffuse necrosis of the right testicle and scrotum; focal necrosis of the left.

FIG. 28. 80 days. Later stage of same lesions. Note lines of demarcation between the three masses on the left.

PLATE 51.

FIG. 29. Chronic diffuse orchitis with circumscribed lesions of the skin resulting from metastatic infection of the testicles.

FIG. 30. Chronic diffuse orchitis with multiple chancre-like lesions of the skin.

FIG. 31. Same testicles 6 days later showing transformation of the lesions. Note especially the single large chancre-like mass in the right testicle.

FIGS. 32 to 34. Late transformations in a case of testicular infection.

FIG. 32. Chancre-like extensions to the scrotum.

FIG. 33. Healing of the skin lesions with an active nodule persisting in the epididymis of the left testicle.

FIG. 34. 93 days later. Skin lesions practically healed, while the nodule in the epididymis is still active.

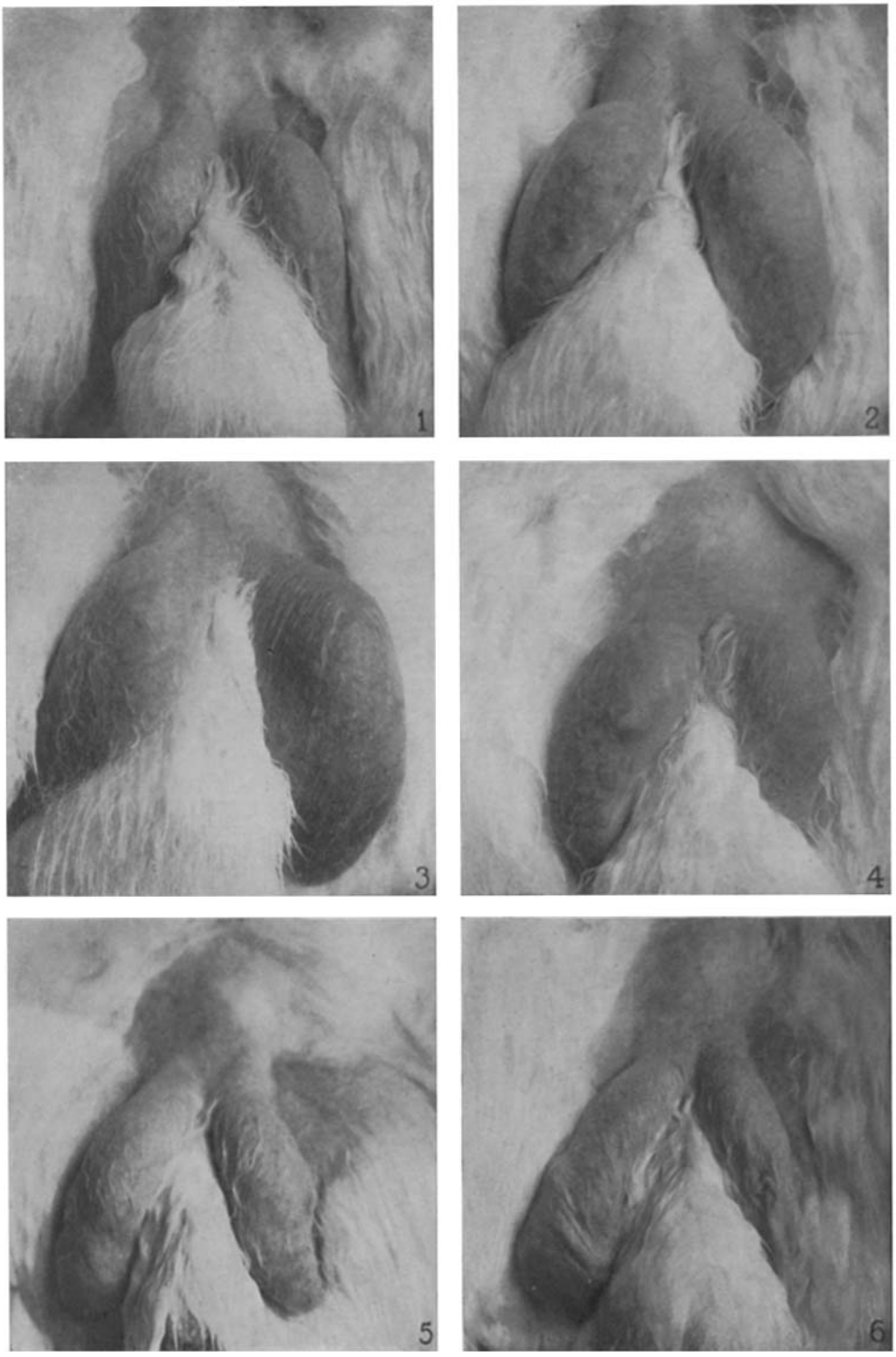
PLATE 52.

FIGS. 35 to 37. Diffuse orchitis with eventual transference of the local infection from the body of the testicles to the epididymis and scrotum.

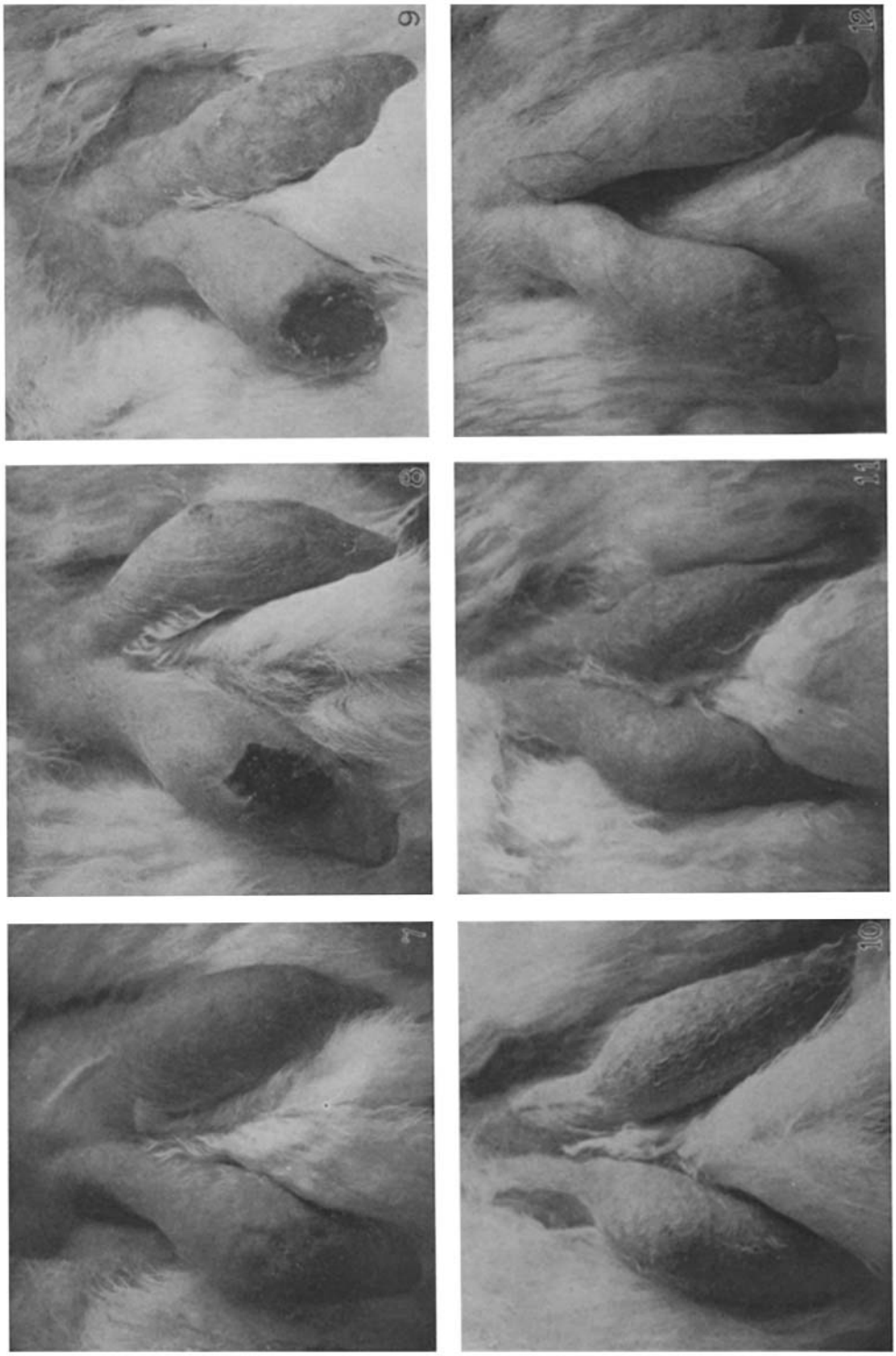
FIG. 35. Diffuse indurative orchitis of both testicles.

FIG. 36. The final lesions in the epididymis and scrotum.

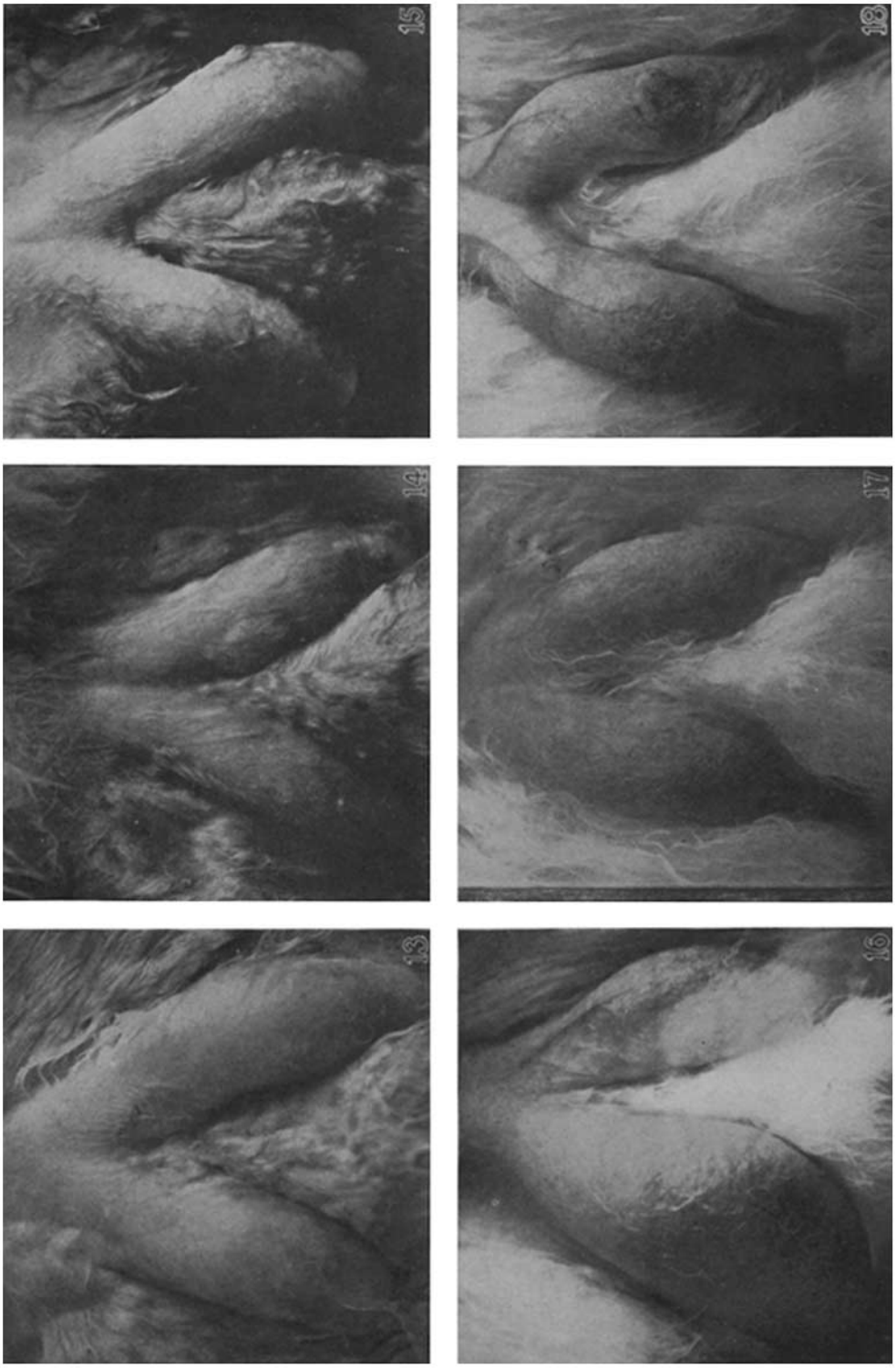
FIG. 37. The same lesions excised and sectioned. Both testicles are atrophic; on the right, the nodule in the tail of the epididymis fits into a hollow beneath the ulcer in the skin, but the two lesions are not entirely fused with each other; on the left, there is only a small nodule in the epididymis which is fairly distinct from the two lesions in the scrotum.



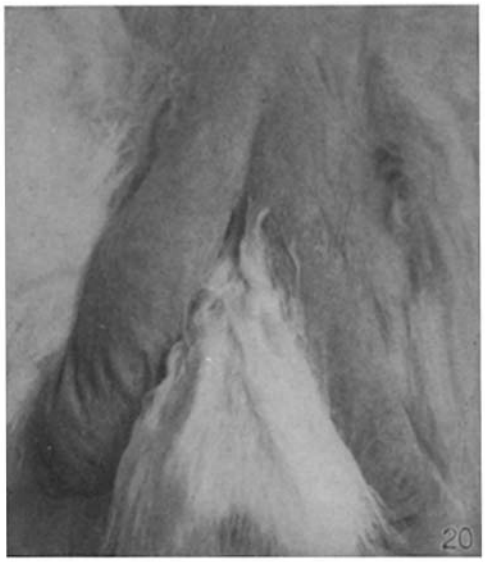
(Brown and Pearce: Experimental syphilis in the rabbit. I.)

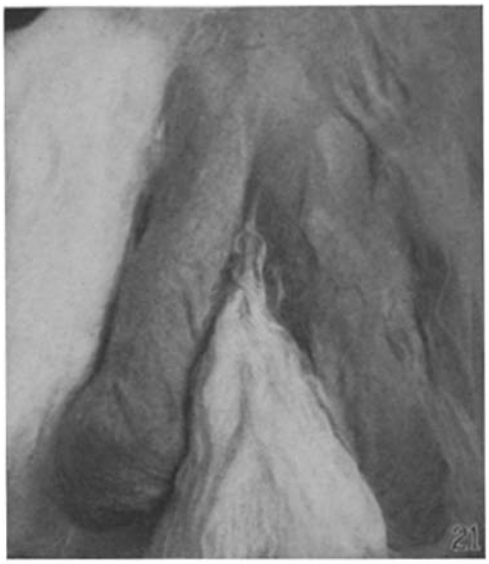


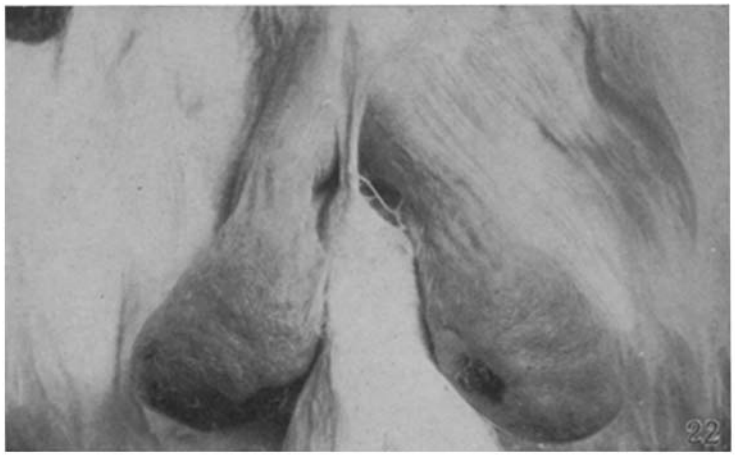
(Brown and Pearce: Experimental syphilis in the rabbit. I.)



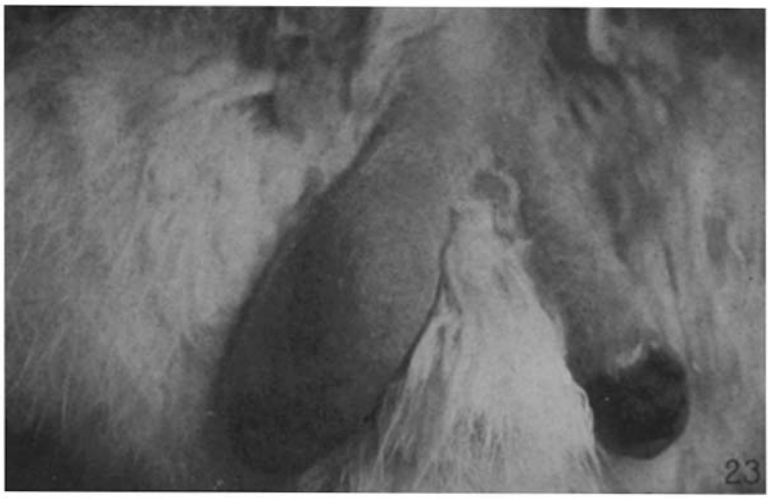
(Brown and Pearce: Experimental syphilis in the rabbit. I.)



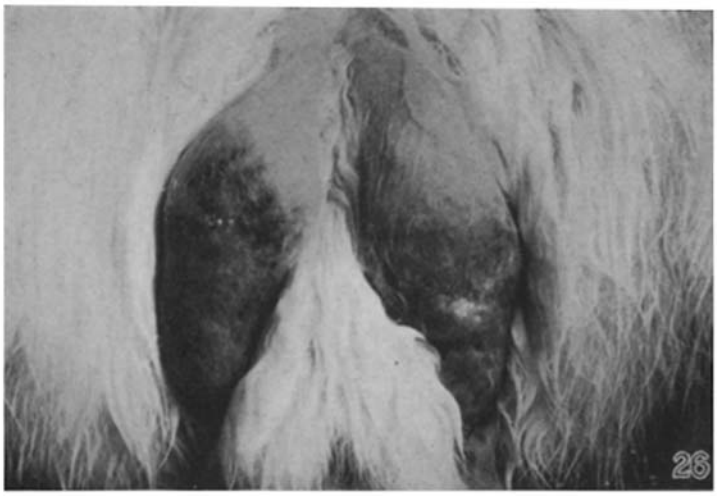


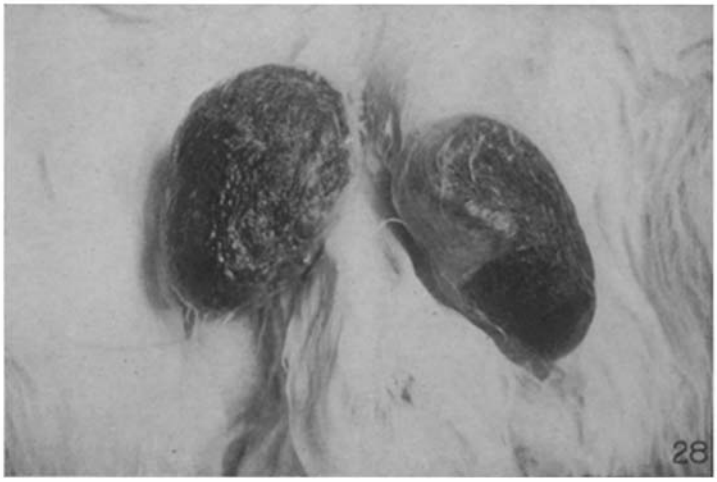


(Brown and Pearce: Experimental syphilis in the rabbit. I.)

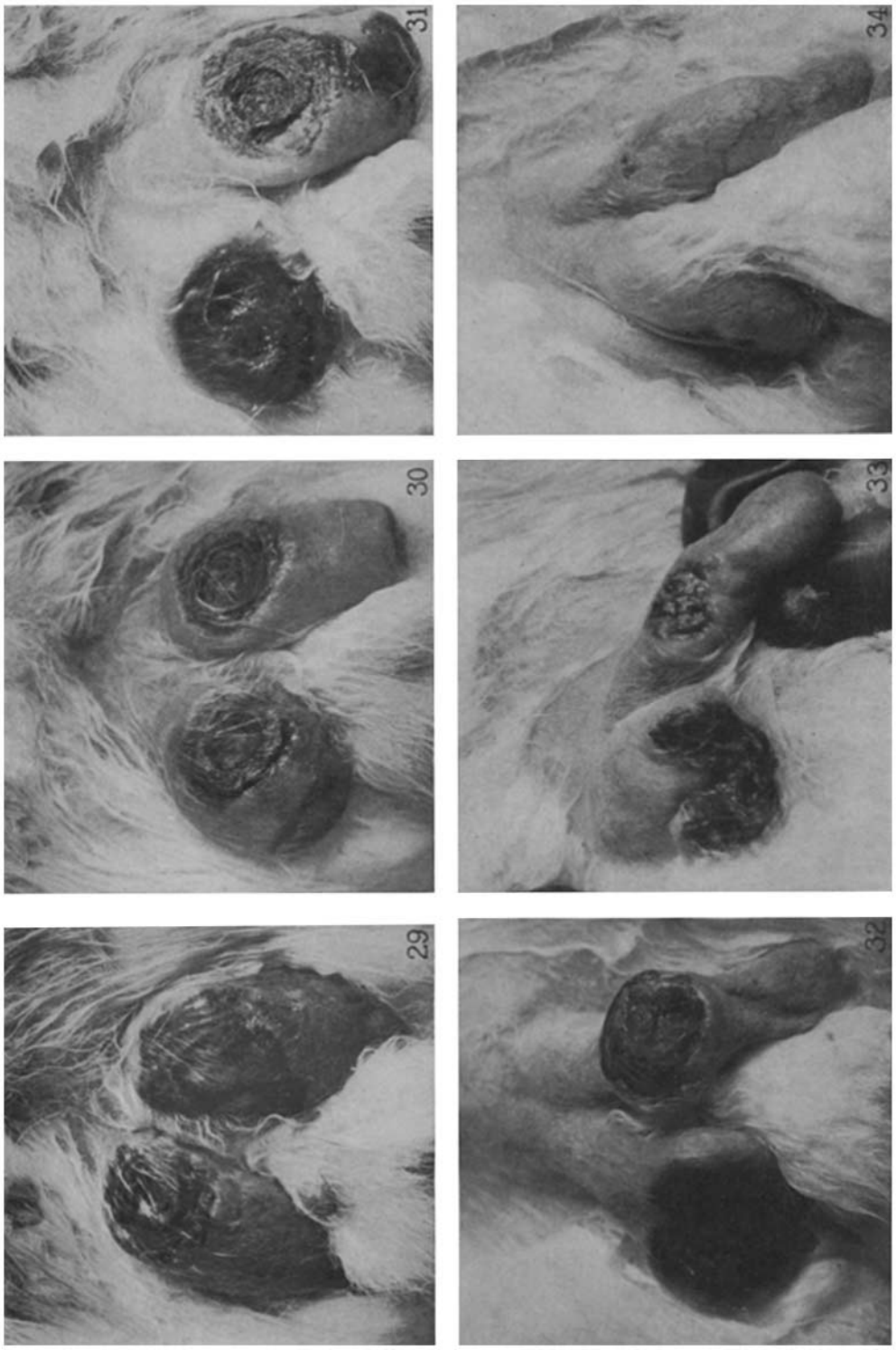


(Brown and Pearce: Experimental syphilis in the rabbit. I.)

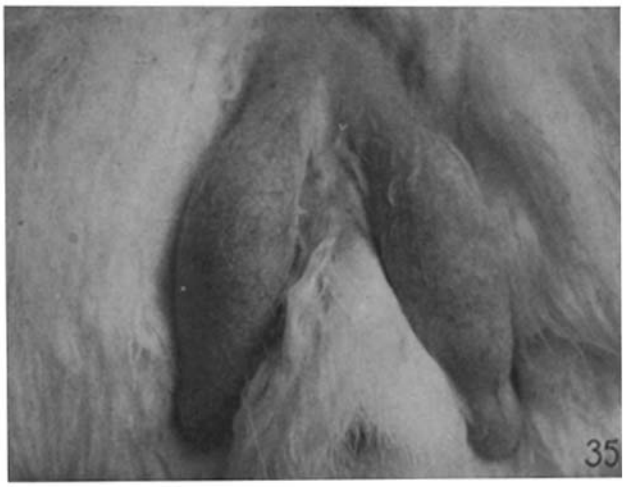


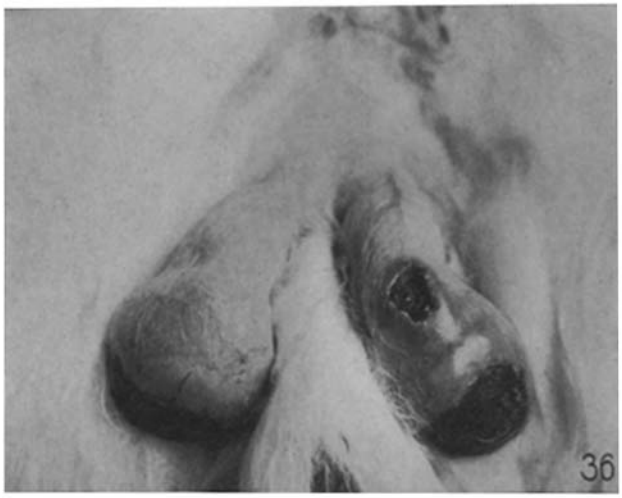


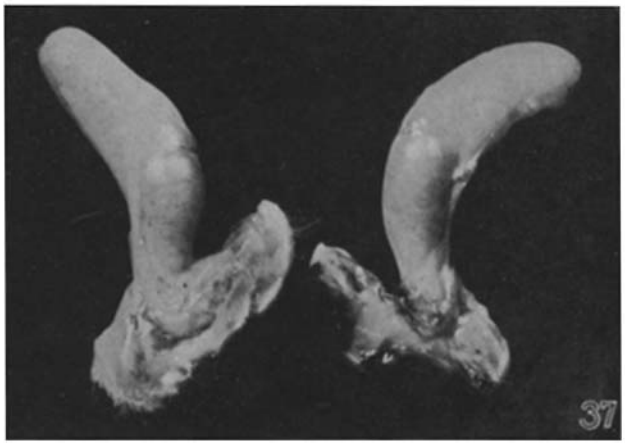
(Brown and Pearce: Experimental syphilis in the rabbit. I.)



(Brown and Pearce: Experimental syphilis in the rabbit. I.)







(Brown and Pearce: Experimental syphilis in the rabbit. I.)