CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

Biological Series. IV.

The Action of N-Phenylglycineamide-*p*-Arsonic Acid upon Spirochete Infections.

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

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

Plates 38 to 44.

(Received for publication, June 18, 1919.)

A second group of conditions upon which the action of *N*-phenylglycineamide-*p*-arsonic acid has been studied is the infections produced in laboratory animals by spirochetes of the *recurrens* group and by *Treponema pallidum*. Early in the course of these studies, it was found that the action of the drug upon these organisms was not so pronounced as in the case of the trypanosomes. Accordingly, the experiments which were carried out were designed more for the purpose of studying the range and character of the action than as attempts to cure the infections.

Blood Spirochetes.

The action of A 63 upon the spirochetes of relapsing fever was studied in both rats and mice infected with *Sp. obermeieri* and with *Sp. novyi*. The animals for these experiments were inoculated intraperitoneally with blood suspensions of the organisms given in such doses as would produce an infection of the desired character—usually an infection which would show a few spirochetes in the peripheral blood within 24 hours after inoculation. The animals were then treated by intraperitoneal injection of single doses of the drug and the results followed from day to day by examination of tail blood under dark-field illumination. Finally, at the end of 60 days, surviving

animals were reinoculated as a means of determining the presence or absence of immunity such as usually develops in the course of untreated infections which terminate in spontaneous recovery, or in treated animals which have not been cured, and is absent in those which have been cured.

The results obtained from the treatment of rats infected with either of the spirochetes mentioned were almost entirely negative. The blood of these animals could not be cleared of spirochetes except by the use of doses of the drug which were nearly always fatal (1.5 gm. per kilo), while smaller doses exercised very little influence upon the course of the infection.

The action of A 63 appeared to better advantage in mice, however, due perhaps to the greater tolerance of these animals for the drug. With an average infection, the dose required to clear the blood of spirochetes temporarily was 1.5 gm. per kilo, or approximately the same as that found to be necessary in the case of rats, but no lasting effect was produced until the dose of the drug used reached upwards of 2 gm. per kilo of mouse, or the largest dose which could be used with safety.

The general character of the therapeutic effects produced may be seen by reference to Table I which shows the results obtained from the treatment of one series of mice with doses of A 63 ranging from 1.5 to 2.25 gm. per kilo. The results of blood examinations are given for the 1st week only with the final result obtained from reinoculation at the end of 60 days.

The infection in these mice might seem to have been a rather severe one since all four controls died within 8 days but the results obtained with the treated mice were of an average character. It will be seen from an examination of this table that the course of the infection was modified in all cases. Usually the progress of the infection was checked rather abruptly and the peripheral blood freed of spirochetes within 24 hours, or by crisis as it were. In a few instances (Mice 6 and 12), this effect was accomplished more slowly; the progress of the infection was first arrested and the infection then terminated by lysis instead of by crisis. In still other animals, the effect of the drug was manifested only by a reduction in the severity of the infection as indicated by the numbers of organisms in the peripheral blood. These effects were not always lasting but were followed in some instances by one or more slight relapses.

When the surviving mice were reinoculated at the end of 60 days, an interesting condition was found in that some mice known to have shown a recurrence of spirochetes in the peripheral blood were as susceptible to reinoculation as those in which no relapse had been observed. While the untreated controls in this experiment were all

TABLE I.

Results Obtained from the Treatment of a 24 Hour Infection of Sp. obermeieri in Mice.

Dose per kilo.	No. of mouse.	Results of blood examinations on days following inoculation and treatment.								Blood on days after reinoculation on 60th day.	
		1	2	3	4	5	7	8	60	2	3
gm.											
2.25	1	+	−	−	−	−	−	−	−	+	++
	2	+	−	−	−	−	−	++	−	+	++
	3	+	−	−	−	−	−	−	D.		
	4	+	−	−	−	−	+	−	"		
	5	+	−	−	−	−	D.				
2.0	6	+	+	+	−	−	−	−	−	−	++
	7	+	−	−	−	−	−	−	−	−	++
	8	+	−	−	−	−	−	−	−	−	++
	9	+	−	−	−	−	−	−	−	−	+
	10	+	−	−	−	−	+	+	−	−	++
1.75	11	+	−	−	−	−	D.				
	12	+	+	+	−	−	+	−	D.		
	13	+	−	−	−	−	++	−	"		
	14	+	−	+	++	−	−	−	"		
	15	+	−	−	−	−	−	−	"		
1.5	16	+	−	−	−	−	++	−	−	−	−
	17	+	+	++	−	−	−	−	−	−	−
	18	+	−	++	−	−	++	++	−	−	−
Controls.	19	+	+++	++++	++++	−	D.				
	20	+	+++	++++	++++	−	"				
	21	+	+++	++++	++++	−	−	D.			
	22	+	+++	++++	++++	−	−	"			

In the tables − indicates no spirochetes found, + few spirochetes present, ++ spirochetes fairly numerous, +++ spirochetes numerous, ++++ spirochetes present in large numbers, D. dead.

dead, the negative results obtained with the mice given only 1.5 gm. of the drug per kilo served as checks against the positive results obtained with the other animals.

Relapse and spontaneous recovery without immunity are almost a paradox, but there are a number of suggestions which occur to one as to how or why such anomalous conditions might arise. Whatever the explanation offered, however, it is obvious that the drug has had its effect even in these relapses and that any immunity developed was either so slight or of such short duration that these mice reacted to reinoculation after 2 months, just as if they had been promptly and completely freed of the spirochetes by the administration of the drug.

In estimating the curative effects of A 63, therefore, one can speak with assurance only of the results of reinoculation. On this basis, it was found that mice infected with *Sp. obermeieri* could be cured with doses of A 63 ranging from 1.5 gm. per kilo of mouse upwards, but with no dose of the drug were we able to obtain cures in more than 75 per cent of the animals treated.

Treponema pallidum.

The action of drugs upon syphilitic infections has usually been studied by the use of some form of the infection which can be produced in rabbits by inoculation with *Treponema pallidum*, and these experimental infections have been used in various ways by different investigators. The lesions of the eye, testicle, and skin have all proved of some value but the scrotal chancre is generally regarded as the most serviceable lesion of the group. The effects produced by drugs upon these infections may be estimated conveniently in three ways: first, by the changes produced in the lesions themselves; second, by the effects upon the organisms present in the lesions; and finally, by the duration or permanence of any changes which may be induced.

In this way, the action of A 63 upon scrotal chancres was studied in a series of twenty-nine rabbits. The lesions were measured with calipers and in most instances were photographed before treatment and at various intervals thereafter in order to determine as accurately as possible the character, extent, and rate of the changes which took place. In like manner, the lesions were aspirated and the fluid was

examined by dark-field illumination before treatment and at intervals such as 1, 3, 5, 7 days, etc., after treatment as a means of following the effects upon the spirochetes. The treated animals were kept under constant observation for such periods of time as the purpose of the experiment demanded, which varied from 1 to 7 months with different animals. For the most part, treatment was carried out by the use of single doses of the drug given intravenously; one series of animals was treated by subcutaneous administration, a second by intramuscular, and a third by the use of repeated small doses of the drug given intravenously. Some study was also made of the action of the drug when given by mouth.

The nature of the results obtained from these experiments may be seen by reference to Table II which contains the abbreviated protocols of twelve rabbits treated by intravenous administration of single doses of A 63 ranging from 0.1 to 0.5 gm. per kilo of body weight.

In order to make these results more intelligible, certain details of the experiments must be supplied. Rabbit 1 of this series was inoculated November 20, 1916. The chancres developed rapidly and at the end of 50 days when treatment was carried out, they measured 2.4 by 2.4 by 1.5 cm. (8.64 cc.) and 2.6 by 2.3 by 1.6 cm. (9.568 cc.) on the right and left respectively. On the day following treatment, the chancres were somewhat edematous, and while a few non-motile spirochetes could still be found, they had disappeared entirely by the end of 48 hours and were never found again. The chancres regressed rapidly for 2 weeks after treatment, when the crusts were torn from the ulcers by the animal. The wounds became secondarily infected and healing was somewhat delayed. The animal was still negative when discarded 100 days after treatment.

The second rabbit of this group requires but little comment. It was an animal with moderately large chancres which reacted to treatment precisely as did Rabbit 1. This animal died from hemorrhagic septicemia 6 days after treatment.

The next two rabbits of the series present an important contrast in the effects produced by the drug. Rabbit 3 was inoculated November 20, 1916, and treated January 9, 1917. At the time of treatment the chancres measured 1.7 by 1.55 by 1.25 cm. (3.294 cc.) on the right and 2.1 by 1.8 by 1.5 cm. (5.67 cc.) on the left. They were markedly

TABLE II.

Results Obtained from the Treatment of Rabbits Infected with T. pallidum.

Dose per kilo.	No. of rabbit.	Testicle.	Chancres at time of treatment.		Results of treatment.						Length of observation.
			Volume.	No. of spirochetes.	Spirochetes.				Chancres.		
					24 hrs.	1 wk.	2 wks.	Recurrence.	Time of healing.	Recurrence.	
gm.			*cc.*						*wks.*		*days*
0.5	1	Right.	8.64	++	−	−	−	No.	5	No.	100
		Left.	9.568	++++	+	−	−				
	2	Right.	5.304	+++	−	D.					
		Left.	6.16	++	−						
0.4	3	Right.	3.294	+++	−	−	−	No.	3	No.	207
		Left.	5.67	++++	−	−	−				
	4	Right.	8.1	++++	−	−	−	Yes.	5	No.	147
		Left.	6.799	+++	+	−	−				
0.3	5	Right.	1.89	++++	−	−	−	No.	3	No.	207
		Left.	3.315	+++	−	−	−				
	6	Right.	3.956	+++	−	−	−	Yes.	3	No.	207
		Left.	2.925	++++	−	−	−				
0.2	7	Right.	1.3	+++	−	−	−	No.	3	No.	85
		Left.	3.22	++++	+	+	−				
	8	Right.	3.47	++++	+	+	−	Yes.	Not healed.	No.	85
		Left.	3.31	++++	+	−	−				
	9	Right.	4.817	++++	+	−	+++		D.		
		Left.	2.755	++++	+	++	+				
	10	Right.		++			+	Never clear.	3	On left.	D.
		Left.	3.27	+++	+++	+	++				
0.1	11	Right.	2.38	+++	+++	−	−	Yes.	2	Yes.	72
		Left.		No lesion.							
	12	Right.	1.586	+++	++	+	++	Never clear.	Not healed.	Yes.	
		Left.	1.42	+++	++		+				

indurated and growing very actively (Fig. 1). Spirochetes were numerous. On aspiration 24 hours later, there was a marked increase in the fluid content of the lesions and no spirochetes could be found nor were they observed at any subsequent examination. The lesions regressed rapidly and at the end of 3 weeks were completely healed with moderate residual thickening in the region of the scars on both sides (Figs. 1 to 4). This rabbit was kept under constant observation for 207 days during which time no lesions, local or general, were observed.

Rabbit 4 was treated 40 days after inoculation. On the day of treatment, the chancres measured 2.5 by 1.8 by 1.8 cm. (8.1 cc.) on the right and 2.1 by 1.85 by 1.75 cm. (6.799 cc.) on the left and were of essentially the same character as those of Rabbit 3 (Fig. 5). For the first 2 weeks, the effects of treatment appeared to be about the same as in Rabbit 3. Regression then proceeded more slowly and the lesions were not completely healed until the end of the 5th week (Figs. 5 to 8), and while there was considerable residual thickening about the scars, no spirochetes could be found. This diffuse thickening gradually diminished, but translucent, glistening, and slightly indurated patches developed at the site of the scars (Fig. 9), and on the 52nd day, numerous actively motile spirochetes were found in these patches. This condition persisted with but slight change for several weeks and then cleared up, as shown in Figs. 9 and 10. The point to be noted here is that although spirochetes were present in the scrotum of this animal at the site of the original lesions, the associated lesions were very slight and showed almost no tendency to growth.

Coming to a still smaller dose of the drug, we again have two animals in which the effects produced were somewhat different. Rabbit 5 was treated 50 days after inoculation. The chancres at this time measured 1.4 by 1.35 by 1 cm. (1.89 cc.) and 1.7 by 1.5 by 1.3 cm. (3.315 cc.) on the right and left respectively. They were both actively growing and well indurated chancres; the one on the right showed a small depressed ulcer, while that on the left was not ulcerated. Spirochetes were numerous on both sides. The results of treatment were essentially the same in all respects as those with Rabbit 3. During 207 days observation, neither spirochetes nor lesions of any kind could be found.

Rabbit 6 was inoculated on November 24, 1916, and treated 46

days later. The chancres in this animal measured 2.15 by 1.6 by 1.15 cm. (3.956 cc.) and 1.95 by 1.5 by 1 cm. (2.925 cc.). Spirochetes disappeared and healing of the chancres took place in the usual manner. There was no sign of relapse for 7 weeks after treatment when several small nodules not more than 1 mm. in diameter appeared in the region of the scar and along the vessels in the dorsal fold of the scrotum on the left. Puncture of these nodules showed numerous actively motile spirochetes. Similar lesions developed later on the right. This animal was kept under close observation for 23 weeks after these lesions appeared, during which time the lesions showed very little change, until they finally regressed and disappeared.

In the next group of rabbits, there were four animals which gave a variety of results. Rabbit 7 responded to treatment by a gradual disappearance of the spirochetes and by rapid regression and healing of the lesions which was almost complete in 3 weeks (Figs. 11 to 14), and remained negative during a period of 85 days observation.

Rabbit 8 also showed a reduction in the spirochetes, and for a short time near the end of the 2nd week after treatment, no spirochetes could be found. In the meantime the chancres had decreased to less than half their original size, but at this point regression ceased. The chancres did not grow again, however, but after remaining stationary for a week or so, underwent spontaneous regression.

Rabbits 9 and 10 behaved still differently. At no time were the lesions of these animals free from spirochetes with the exception of one chancre of No. 9. Nevertheless, the chancres all regressed at a normal rate, and in No. 10, they were healed at the end of 3 weeks with slightly thickened, glistening patches at the site of the scars (Figs. 15 to 18). This rabbit lived only 36 days after treatment, but during this time, there was no renewal of the growth of the lesions, although numerous actively motile spirochetes were present all the while. The other rabbit, No. 9, died of hemorrhagic septicemia before the chancres had healed.

The final group of two rabbits gave results of much the same character as those of the preceding group. Rabbit 11 was inoculated November 1, 1916, but there was very little reaction for 2 months. The chancre on the right then developed rapidly and when treatment was carried out 97 days after inoculation (or about 40 days after the

beginning of the specific reaction) the main chancre mass measured 1.85 by 1.14 by 1.13 cm. (2.38 cc.). On the left, there was only a tiny nodule. Spirochetes disappeared from the chancre in 1 week after treatment and the lesion was healed with moderate residual thickening at the end of 2 weeks (Figs. 19 to 21). The animal was observed for 72 days during which time a small nodule developed in the deeper tissues of the scrotum beneath the scar. A similar nodule also appeared in the left scrotum but neither of these nodules grew and had almost disappeared at the time the animal was discarded.

Rabbit 12 was inoculated December 18, 1916, and treated 49 days later. The chancre on the right measured 1.48 by 1.24 by 0.87 cm. (1.586 cc.) and that on the left 1.41 by 1.2 by 0.84 cm. (1.42 cc.). They were both well indurated and actively growing chancres (Fig. 22). The effect of treatment was to reduce the spirochetes to a moderate extent and to cause considerable regression of the lesions lasting over a period of about 12 days (Figs. 23 and 24). Both chancres then began to increase in size (Fig. 25), in which respect the result differed from that usually obtained from the use of larger doses of the drug.

To the effects which have been described, one other type of reaction should be added; namely, that of the refractory animal or the refractory infection, which was encountered twice in our series of twenty-nine rabbits. The best example which we have of the infection which did not yield to treatment is that shown in Figs. 26 to 29. This animal was inoculated November 8, 1916. For 2 months, the growth of the chancres was rather slow and irregular, but after that they developed very rapidly and measured 1.82 by 1.57 by 1.3 cm. (3.715 cc.) and 1.75 by 1.7 by 1.35 cm. (4.016 cc.) 87 days after inoculation. Both chancres were markedly indurated and contained unusually large numbers of spirochetes. The rabbit was given three intravenous injections of A 63, 48 hours apart, each dose representing 0.1 gm. per kilo. The effect of this treatment was comparatively slight. The spirochetes were very little affected, but the chancres diminished in size for about 9 days with a maximum decrease of 0.4 cm. in all dimensions. They then began to increase in size and 3 weeks after treatment (Fig. 28) measured 1.67 by 1.93 by 0.9 cm. (2.901 cc.) and 1.5 by 1.37 by 0.9 cm. (1.85 cc.). The rabbit was then treated for the second time with a single dose of 0.3 gm. given intramuscularly

with an effect which was but slightly greater than that of the first treatment, the extent of which is shown in Fig. 29.

While the effects which have been described relate almost entirely to intravenous therapy, much the same results were obtained when the drug was given either subcutaneously or intramuscularly. The chief difference noted was that slightly larger doses of the drug were required by either of these routes to produce effects comparable with those obtained by intravenous administration. The drug was found to be active also when given by mouth, but this method of treatment was not used to a sufficient extent to warrant any statement as to its relative value.

Finally, one series of rabbits was treated by the use of repeated small doses of A 63 given intravenously, mainly for the purpose of getting an idea of the size of the dose and the interval between doses which would be required to maintain a continuous therapeutic effect. Without going into the details of these experiments, it may be said that the smallest dose which appeared to exercise a definite influence upon the infection was 0.1 gm. per kilo and that such doses could not be spaced more than 48 hours apart if the effect was to be made continuous. The experiments were not carried far enough to determine just how many such doses would be required to produce a given end-result.

From these experiments one may gain a fair impression of the effects produced by the amide of *N*-phenylglycine-*p*-arsonic acid upon experimental infections of *Treponema pallidum*. As seen by examination of fluid drawn from the lesions, the effect upon the spirochetes is to produce an impairment of motility or a complete loss of motion followed by degeneration and gradual disintegration so that the spirochetes eventually disappear from the lesions if the dose used is sufficiently large. One may be reasonably certain, therefore, that the drug possesses some measure of spirocheticidal action, but the dose required to produce such an effect as that described is comparatively large. At least 0.1 gm. per kilo is necessary to produce any appreciable effect upon the spirochetes; and the effect is usually not very pronounced until the dose used reaches upwards of 0.3 gm. per kilo, and even 0.4 gm. does not always insure a permanent disappearance of the infecting organisms.

The effect of the drug upon the lesions themselves is much more definite and apparently out of proportion to the effect produced upon the spirochetes. As we have seen, complete resolution and healing of scrotal chancres may be accomplished with doses as small as 0.1 gm. per kilo, and this is the usual result obtained with doses as large as 0.2 to 0.3 gm. whether spirochetes are destroyed or not. Further than this, the effects, as far as the lesions are concerned, appear to be more enduring. In our experience, lesions which have once healed, have shown little or no tendency to recur even though actively motile spirochetes were present. In a way, this peculiar type of action reminds one of the process of spontaneous control of *pallida* infections in rabbits; but it is not the same, and whatever the explanation of these effects may be, they are definitely referable to the action of the drug.

CONCLUSIONS.

To summarize the results obtained from these experiments, one may say that *N*-phenylglycineamide-*p*-arsonic acid is capable of exercising a very definite effect upon the course of infections produced by spirochetes of the *recurrens* group and by *Treponema pallidum*. It is more difficult to say, however, just how these effects should be interpreted. In the case of the blood spirochetes, the infection is ameliorated, and even though the spirochetes are not immediately destroyed, the infection is frequently brought to a termination which leaves the animal in a condition not unlike that produced by more powerful spirocheticidal agents. That is, the infecting organisms are either affected in such a way that they eventually die off or are destroyed by the host in such a way that no lasting immunity is developed in consequence of their destruction.

Apparently much the same type of reaction occurs in the treatment of rabbits infected with *Treponema pallidum*. It is possible that when very large doses of the drug are used, these organisms may be completely destroyed, but it is certain that in other cases, where complete healing of the lesions is accomplished as a result of treatment, the organisms are not destroyed. Moreover, it appears that such a result can be accomplished in the presence of numerous actively motile spirochetes, and once the effect of the drug has reached this

point, either the capacity of the spirochetes for stimulating reaction on the part of the tissues is lowered or else the reactivity of the tissues is reduced. At any rate, living spirochetes may remain in the tissues for considerable periods of time without giving rise to the usual tissue reaction which characterizes these infections.

With either group of organisms, therefore, *N*-phenylglycineamide-*p*-arsonic acid appears to act in a manner somewhat different from that of the usual spirocheticidal agents. While it does possess a considerable degree of spirocheticidal action, its chief effect is seen in the peculiar manner in which it modifies or controls the course of these infections.

Further than this, we shall not attempt to go at present. As set forth in the literature, the facts and principles upon which such experiments depend are so few as to offer almost no basis for an interpretation of these experiments. When we have had an opportunity of reporting our own experience in dealing with these infections, we may return to a consideration of the facts here reported.

EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs illustrating the effects produced upon scrotal chancres of rabbits by treatment with *N*-phenylglycineamide-*p*-arsonic acid. Objects are represented at their natural size.

PLATE 38.

FIGS. 1 to 4. Rabbit 3. Effect produced by 0.4 gm. per kilo of body weight.

FIG. 1. Chancres at time of treatment.

FIG. 2. 1 week after treatment.

FIG. 3. 2 weeks after treatment.

FIG. 4. 3 weeks after treatment. The lesions are healed with some residual thickening especially noticeable on the right, but no spirochetes could be found.

PLATE 39.

FIGS. 5 to 10. Rabbit 4. A peculiar therapeutic effect following the administration of a dose of 0.4 gm. per kilo.

FIG. 5. Chancres at the time of treatment.

FIG. 6. Chancres regressing rapidly.

FIG. 7. 4 weeks after treatment. Chancres unhealed but still regressing slowly.

FIG. 8. 5 weeks after treatment. Chancres are healed but the scars are thickened and translucent, especially that on the right.

FIG. 9. 9 weeks after treatment. The lesions are still healed but scars are now represented by translucent, highly refractile plaques which contain numerous actively motile spirochetes.

FIG. 10. 147 days after treatment. The foci of infection have cleared spontaneously and the scrotum is normal.

PLATE 40.

FIGS. 11 to 14. Rabbit 7. The effect of a single dose of 0.2 gm. per kilo.

FIG. 11. Chancres at the time of treatment. That on the right is an unulcerated globular mass.

FIG. 12. 1 week after treatment; very rapid regression of the lesions.

FIG. 13. 3 weeks after treatment. The chancre on the right has entirely disappeared; that on the left still shows a small thickened mass.

FIG. 14. 4 weeks after treatment. Resolution complete.

PLATE 41.

FIGS. 15 to 18. Rabbit 10. An instance of the healing of chancres with a single dose of 0.2 gm. per kilo while actively motile spirochetes were constantly present.

FIG. 15. Chancres at the time of treatment.

FIG. 16. 1 week after treatment; marked regression of the lesions.

FIG. 17. 2 weeks after treatment.

FIG. 18. 3 weeks after treatment. The lesions have practically disappeared, but actively motile spirochetes are fairly numerous in the scars of both sides. There was a slight recurrent patch in the left scrotum.

PLATE 42.

FIGS. 19 to 21. Rabbit 11. An effect which may be accomplished by a dose of 0.1 gm. in lesions which are easily influenced.

FIG. 19. Chancre at the time of treatment.

FIG. 20. 1 week after treatment.

FIG. 21. 2 weeks after treatment.

PLATE 43.

FIGS. 22 to 25. Rabbit 12. The extent of the effect usually produced by a dose of 0.1 gm. per kilo.

FIG. 22. Chancres at the time of treatment.

FIG. 23. 8 days after treatment.

FIG. 24. 2 weeks after treatment. The chancre on the left is healed, but there is a considerable mass of unresolved tissue in the scrotum; that on the right is increasing.

FIG. 25. 3 weeks later. Both chancres growing actively.

PLATE 44.

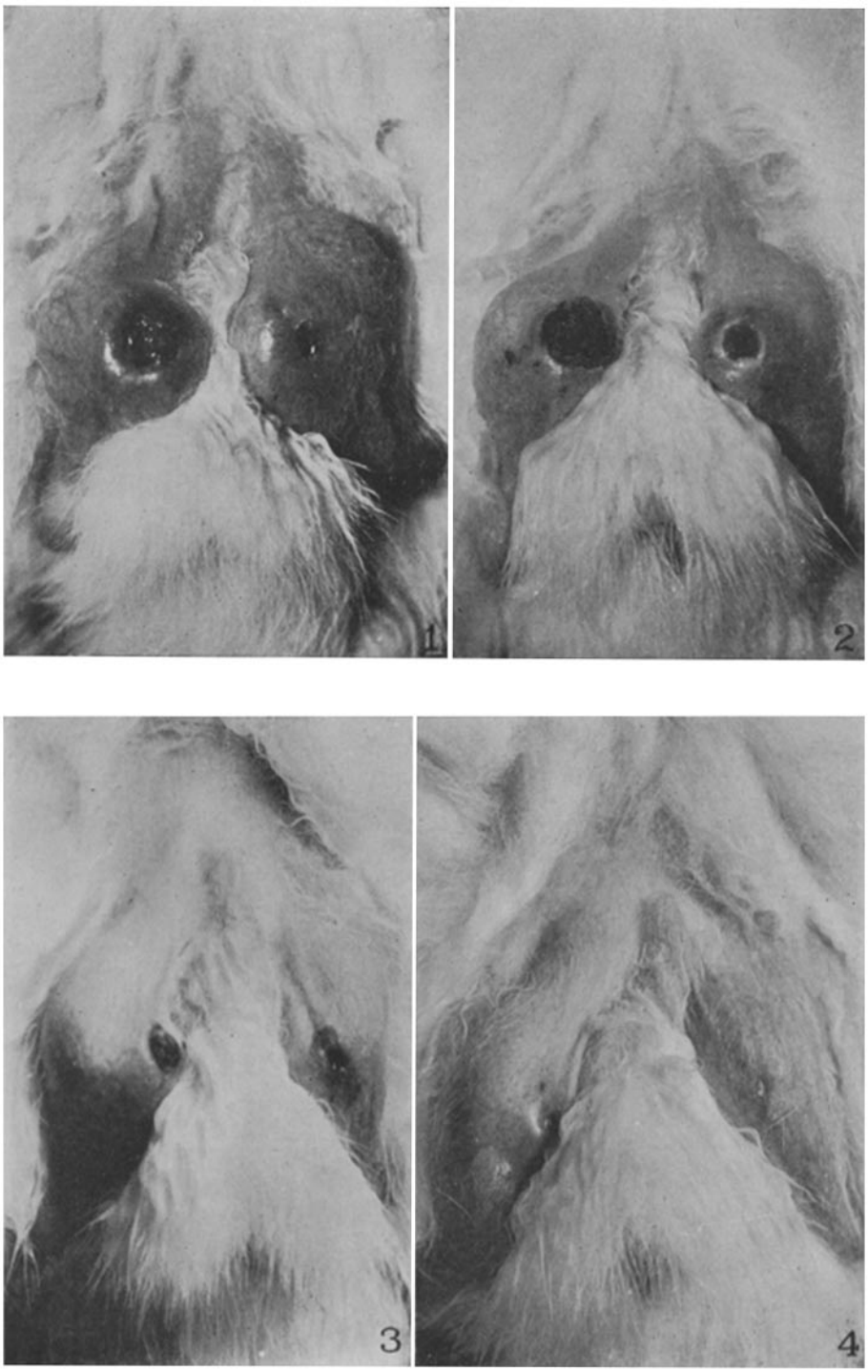
FIGS. 26 to 29. Chancres which proved refractory to treatment with this drug.

FIG. 26. Chancres at the time of first treatment which consisted of three doses of 0.1 gm. per kilo given intravenously at intervals of 48 hours.

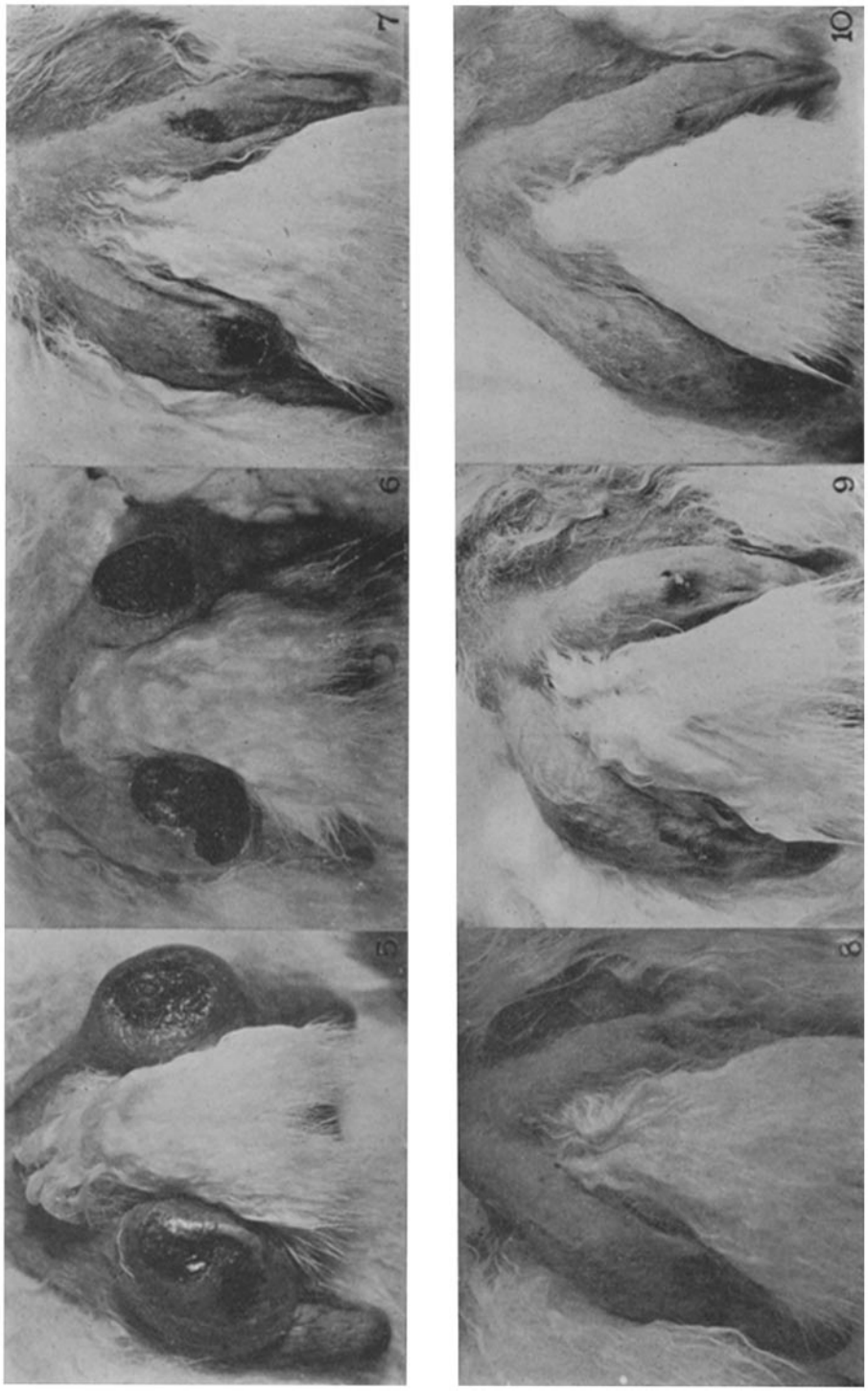
FIG. 27. 1 week after treatment had been commenced; practically the full extent of the reduction of the lesions is shown.

FIG. 28. 2 weeks later; the growth of the chancres in the interim is shown. Animal retreated with 0.3 gm. per kilo given intramuscularly.

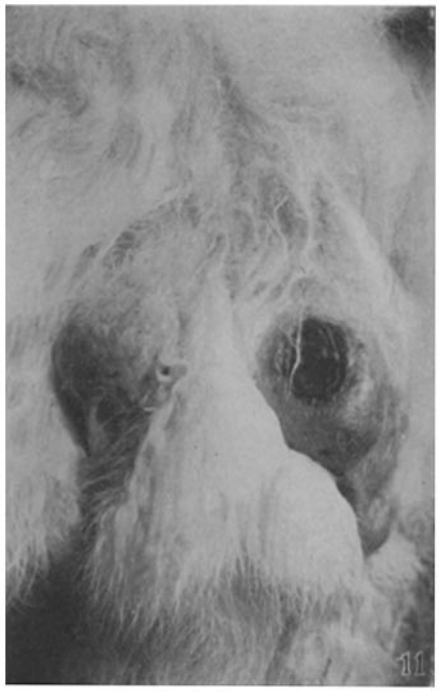
FIG. 29. 1 week later; a decided effect is shown, but renewed activity set in almost immediately.

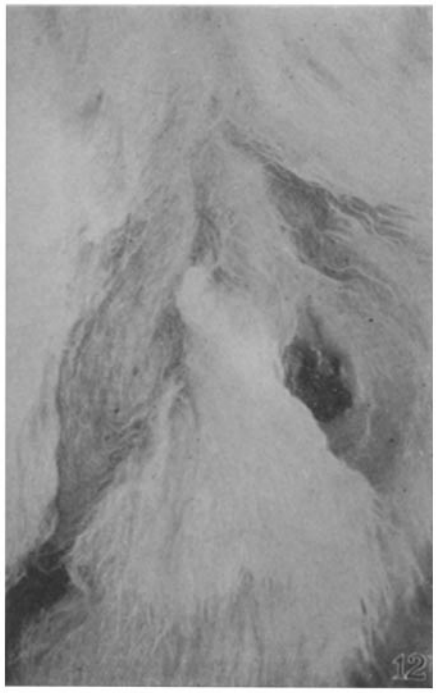


(Brown and Pearce: Trypanosome and spirochete infections.)

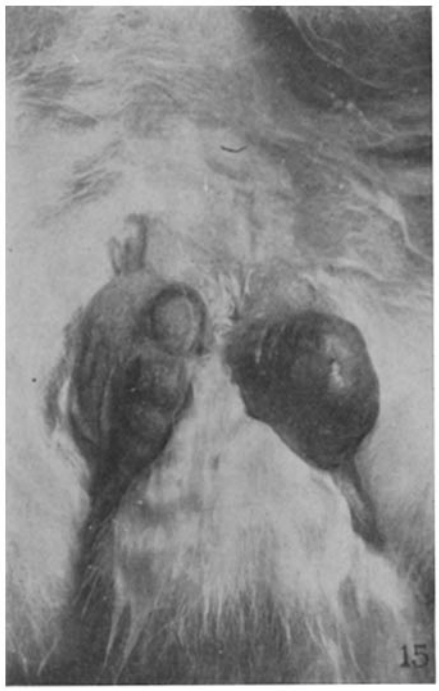


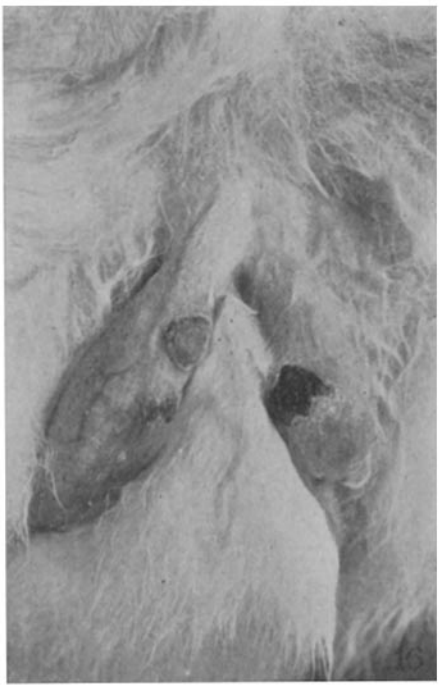
(Brown and Pearce: Trypanosome and spirochete infections.)

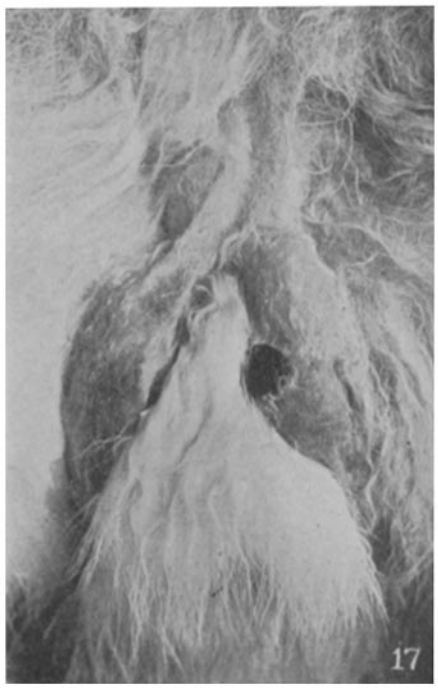


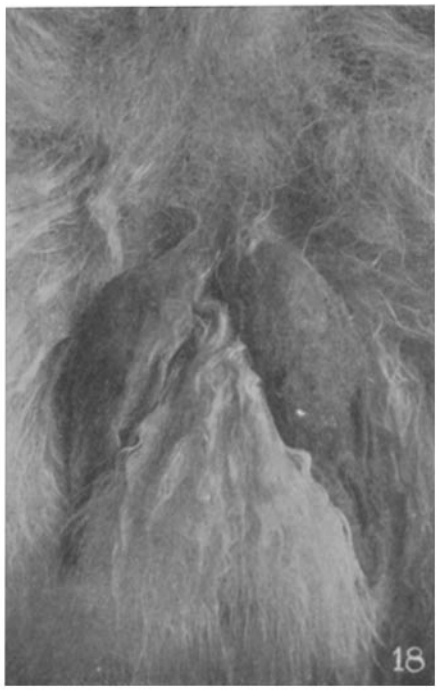


(Brown and Pearce: Trypanosome and spirochete infections.)



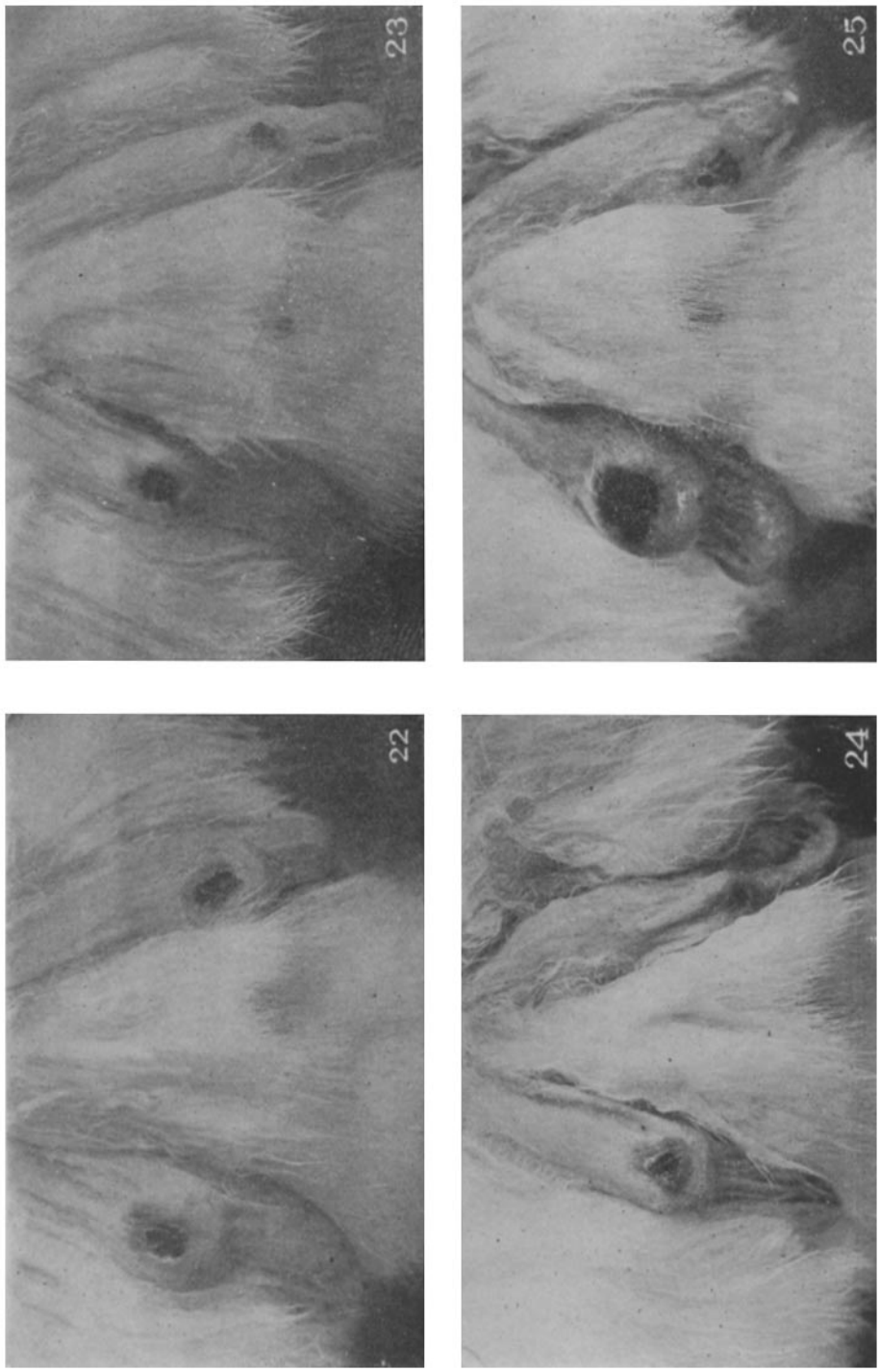




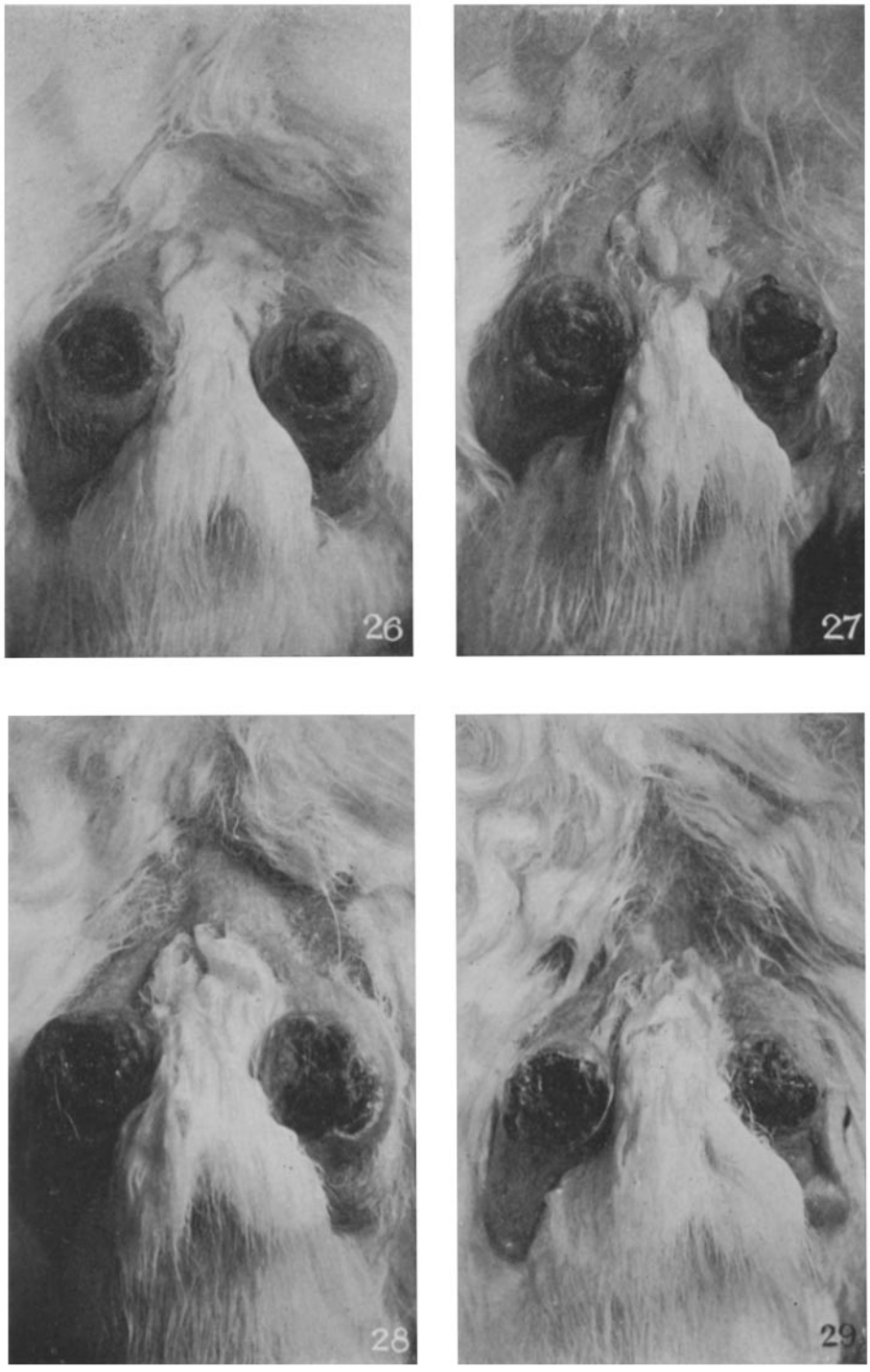


(Brown and Pearce: Trypanosome and spirochete infections.)

(Brown and Pearce: Trypanosome and spirochete infections.)



(Brown and Pearce: Trypanosome and spirochete infections.)



(Brown and Pearce: Trypanosome and spirochete infections.)