CHEMOTHERAPY OF TRYPANOSOME AND SPIROCHETE INFECTIONS.

BIOLOGICAL SERIES. III.

THE THERAPEUTIC ACTION OF N-PHENYLGLYCINEAMIDE-*p*-ARSONIC ACID IN EXPERIMENTAL TRYPANOSOMIASIS OF RABBITS.

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PLATES 17 TO 37.

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Experimental trypanosomiasis of rabbits, as has been pointed out, presents many points of similarity with naturally acquired trypanosomiasis of human beings and animals, and since the treatment offers an opportunity of obtaining fundamental information which may be of practical importance in the treatment of trypanosomal disease as it exists in nature, we have endeavored to emphasize such fundamental points in the description of the therapeutic results with the amide of *N*-phenylglycine-*p*-arsonic acid reported in this paper.

EXPERIMENTAL.

Condition of Experiments.—The majority of therapeutic experiments were carried out with strains of *Tr. brucei* and *Tr. gambiense* and a smaller number of experiments with *Tr. equiperdum*, *Tr. equinum*, and *Tr. evansi*. The infection produced by all five strains was uniformly fatal in untreated rabbits. The infecting virus in all experiments was, with one exception, obtained from stock guinea pigs in which the trypanosome strains were continuously carried, thus conforming to the principle of the influence of the animal species upon the type of infection which has been discussed in a previous paper.[1] In addition,

[1] Pearce, L., and Brown, W. H., *J. Exp. Med.*, 1918, xxviii, 109.

in order to secure regularity of incubation as well as uniformity of character and termination of the infection in as far as this was possible, blood was taken from stock guinea pigs which showed a well developed infection and the inoculating doses of the blood suspension were given intravenously. With *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi*, 1 cc. per kilo of body weight of a + suspension was inoculated, while with *Tr. gambiense* and *Tr. equinum*, 1 cc. per kilo of a ++ to +++ suspension was used. Under these conditions of experimental procedure, rabbits infected with *Tr. brucei* showed initial signs of the disease in from 5 to 7 days and the untreated animals survived from 1 to 3 months, although the majority of controls died within the first 5 weeks, while the first signs of the infection with *Tr. gambiense* were noted 7 to 15 days after inoculation and untreated animals survived on an average of 8 weeks. The clinical course of the infection produced in rabbits by our strains of *Tr. equinum*, *Tr. evansi*, and *Tr. equiperdum* is, on the whole, comparable with that caused by *Tr. brucei*.

Rabbits thus infected were treated with the sodium salt of the amide of *N*-phenylglycine-*p*-arsonic acid at a time when the infection was well established, usually about 10 to 15 days after inoculation with *Tr. brucei* and at a somewhat longer period of time with the other strains of trypanosomes, when well marked clinical signs of the disease together with loss of appetite, weakness, and a concomitant loss of weight or even emaciation were present. In a certain number of cases, particularly in the treatment of relapses, rabbits showing a more advanced or even a chronic stage of trypanosomiasis were used.

With regard to the matter of dosage, it is important to recognize that since individual rabbits vary considerably in their reaction to trypanosomal infections as shown by the character and degree of the clinical signs, we have endeavored to utilize this factor by placing some of the animals most affected at the crucial dose levels, that is, within the curative range. The dose of the drug for each animal was calculated in grams per kilo of body weight, weighed on a balance, dissolved in sterile distilled water, and injected by a syringe into the marginal ear vein except in the few instances in which the drug was administered intramuscularly, subcutaneously, or *per os* through a stomach tube. The concentration of the drug solutions varied from 5 to 50 per cent, although in the majority of experiments a 5 per cent solution was used.

The immediate and ultimate therapeutic effect of the drug in the treatment of experimental trypanosomiasis in rabbits was closely observed throughout a period of at least 3 months and in many instances for a longer period of time, although the majority of relapses after non-curative doses of the amide of *N*-phenylglycine-*p*-arsonic acid occurred in our experiments within 2 to 5 weeks after treatment.

Determination of a Single Dose Curative Range.

Our initial experiments with the amide of *N*-phenylglycine-*p*-arsonic acid were largely concerned with the determination of the range of therapeutic action and a study of the effect of the drug within this range rather than the determination of a single curative dose or an attempt to ascertain the ideal method of treating and of curing experimental trypanosomiasis. Although the treatment of trypanosomiasis in this animal species resolves itself for the most part into the treatment of individuals, we have grouped together in Table I the results of a number of experiments which have certain features in common. In these experiments, thirty-four rabbits infected with *Tr. brucei* were treated 8 to 15 days after inoculation, at a time when all showed well marked clinical signs and symptoms of the disease. Some of the control animals died from 20 to 36 days after treatment, while others which showed extremely advanced signs at the end of 42 days were used for therapeutic purposes. The single doses of the drug employed in the treatment of these rabbits, as is shown in the table, ranged from 0.75 to 0.1 gm. per kilo of body weight and were administered intravenously in a 5 per cent solution.

There were no evidences of drug intoxication after treatment with single doses of this range and the clinical signs of trypanosomiasis were quickly influenced. Usually the acute manifestations of the infection, such as edema, capillary dilatation and congestion, and swelling of the base of the ears, eyelids, prepuce, and testicles, largely subsided within 24 to 48 hours, while the reduction of the more indurated and advanced lesions required from 2 to 4 days. The immediate clinical results of the treatment are illustrated in Figs. 1 to 7. Apart from the outward signs of the disease, the animal's general condition improved markedly following the injection of the drug as shown by

a rapid return of appetite and an increase in weight, so that within a few days emaciation had given place to a more or less well nourished condition, while in the succeeding weeks, a normal increase in weight occurred.

The final results in this series are given in Table I. There were no relapses among the twelve rabbits treated with doses of both 0.35 and 0.25 gm. per kilo of body weight, while with the intermediate dose of

TABLE I.

Results Obtained in the Treatment of Rabbits Infected with Tr. brucei with Single Doses of N-Phenylglycineamide-p-Arsonic Acid, Administered Intravenously.

Dose per kilo.	No. of rabbits.	No. of intercurrent deaths.	No. of relapses.	No. of cures.	Percentage of cures.
gm.					
0.75	1	0	0	1	100
0.4	2	0	0	2	100
0.35	6	2*	0	6	100
0.3	7	0	1	6	86
0.25	6	3†	0	6	100
0.2	10	1‡	3	7	70
0.1	2	0	2	0	0
Total.....	34				

* One was killed 71 days after treatment; extensive ringworm. One died 73 days after treatment; middle ear abscess.

† One died the day before the series was discarded; hemorrhagic septicemia. One died 71 days after treatment; acute pneumonia. One was killed 71 days after treatment; extensive ringworm.

‡ Killed 77 days after treatment; bacterial infection.

0.3 gm. per kilo, there was one relapse out of seven animals, or 86 per cent of cures. Furthermore, with a lower dose of 0.2 gm. seven out of ten rabbits were cured (70 per cent), and finally, the two rabbits treated with the very low dose of 0.1 gm. relapsed, although the marked therapeutic activity of the drug at this level is shown by the fact that with rabbits so treated, regression and healing of the lesions took place very rapidly and the relapses did not occur until 11 and 21 days after treatment. There were six incidental deaths among the thirty-four rabbits included in this table, but since they occurred over 10 weeks after treatment and were in no way associated with evidences

of trypanosomiasis, we have not excluded them from the number of cured animals.

The outstanding feature of the treatment of an acute, progressive, and well established *Tr. brucei* infection in rabbits with single doses of the amide of *N*-phenylglycine-*p*-arsonic acid as shown in Table I is a well marked sharply limited curative range of therapeutic action with doses varying from 0.2 to 0.35 gm. per kilo, and that within these limits, the percentage of cures is, on the average, high. Thus, there were twenty-nine rabbits treated with doses of 0.2, 0.25, 0.3, and 0.35 gm. per kilo of body weight and of these four relapsed and twenty-five, or 86.2 per cent, were cured. In addition, both upper and lower limits of action of the curative range are sharply marked—with doses of 0.2 gm., there were 70 per cent of cures and with the lower dose of 0.1 gm., there were no cures although this amount of the drug possessed a marked therapeutic effect, while, on the other hand, doses of 0.35 gm. or higher were uniformly curative. If we consider that the minimum lethal dose of the drug for rabbits is from 0.75 to 0.9 gm. per kilo of body weight when given intravenously and that the curative range of single doses is from 0.25 to 0.35 gm., we have a curative ratio of from one-third to one-half as expressed in fractions of the minimum lethal dose.

Much the same range of curative action is operative in rabbit trypanosomiasis produced by *Tr. gambiense* as is shown in Table II. In this series, eighteen rabbits were treated intravenously with doses of the amide of *N*-phenylglycine-*p*-arsonic acid of 0.35 to 0.15 gm. per kilo of body weight. There was a very noticeable regression of the signs of the infection on the 1st and 2nd days after treatment, and no detectable signs remaining on the 5th day, as may be seen in Figs. 8 and 9. Two out of the three rabbits treated with 0.15 gm. per kilo relapsed 20 and 33 days after treatment, and there was one relapse at 0.25 gm. 26 days after treatment, but the eleven rabbits treated with the other three doses of 0.2, 0.3, and 0.35 gm. per kilo were cured. Thus the curative range of the compound in *Tr. gambiense* infections of rabbits may be considered to be from 0.2 to 0.3 gm. per kilo when administered as single doses intravenously—or from one-fourth to one-third of the minimal lethal dose.

Our experience with the effect of this compound upon rabbit trypanosomiasis produced by other species of trypanosomes is limited

so that we are unable to draw final conclusions as to the ultimate curative range of the drug in these particular infections. At the time the experiments were done, the infection produced in these animals by our strain of *Tr. equinum* was unusually severe as was also the case in certain mouse experiments, and in addition, the upper range of the doses used was apparently too low. However, single doses as small as 0.2 gm. per kilo of body weight caused a complete disappearance of the acute signs of the disease in 3 days, although relapses eventually occurred with the doses 0.15, 0.2, 0.25, and 0.3 gm. per kilo. It was obvious that larger doses should have been used or a different system of treatment followed to effect a permanent cure in an infection as

TABLE II.

Results Obtained in the Treatment of Rabbits Infected with Tr. gambiense with Single Doses of N-Phenylglycineamide-p-Arsonic Acid, Administered Intravenously.

Dose per kilo.	No. of rabbits.	No. of intercurrent deaths.	No. of relapses.	No. of cures.	Percentage of cures.
gm.					
0.35	3	0	0	3	100
0.3	4	0	0	4	100
0.25	3	1*	1	1	50
0.2	5	1†	0	4	100
0.15	3	2‡	2‡	1	33⅓
Total.....	18				

* Died 6 days after treatment; bacterial infection.

† Died 20 days after treatment; gastroenteritis.

‡ Relapse plus bacterial infection 20 and 33 days after treatment.

severe as was the case in this particular experiment, since the control rabbit died the day after the animals in the series were treated and relapses occurred with such promptness and severity.

Two rabbits heavily infected with *Tr. equiperdum* which were treated with single doses of 0.5 and 0.3 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo of body weight, 36 days after inoculation, at a time when the disease was advanced, showed a prompt recovery from the clinical signs as is illustrated by Figs. 12 and 13. Both rabbits subsequently relapsed on the 47th and 57th days after treatment but were successfully retreated with repeated doses of the drug with no ultimate recurrence of the infection.

The therapeutic effect of the compound upon *Tr. evansi* in rabbits was studied in but one animal. The infection was well established on the day of treatment, 36 days after inoculation, as is shown in Fig. 14, when 0.3 gm. per kilo was administered intravenously. There was very little change in the clinical signs during the first 48 hours but on the following days, the improvement was marked (Figs. 15 and 16). Thick crusts about the nose and upper lips had shelled off in 10 days time and by the end of the 3rd week, the skin in these areas was healthy in appearance and the hair had begun to return (Fig. 17). There was no relapse and the rabbit was in excellent condition when it was discarded 113 days after treatment.

Summing up the results of the treatment of trypanosomiasis with single doses of the drug, we may say that with infections of average severity and duration produced by *Tr. brucei* and *Tr. gambiense*, the drug exercises a prompt and lasting therapeutic action in doses of from 0.2 to 0.35 gm. per kilo of body weight, and, furthermore, a marked therapeutic effect of some duration in doses of 0.1 and 0.15 gm. per kilo of body weight. Although the experimental evidence with regard to *Tr. evansi* infection in rabbits is insufficient for final conclusions, it is probable that the same range of curative action would apply to such rabbit infections as are produced by the particular strain of surra with which we have worked. On the other hand, single doses of the compound which fall within this curative range failed to cure *Tr. equinum* and *Tr. equiperdum* infections in rabbits. However, it should be pointed out that in both these experiments, especially with *Tr. equinum*, the disease was extremely marked and more advanced at the time of treatment than was the case with the other three infections, and it is possible that with more comparable experimental conditions, a certain proportion of cures could be obtained with single doses falling within the curative range of 0.2 to 0.35 gm. per kilo of body weight.

In addition to the above experiments, a series of twelve rabbits with a very severe *Tr. brucei* infection of 23 days duration was treated intravenously with large single doses in order to ascertain the reaction of extremely ill animals to the drug as compared with that of normal animals. The dose of 0.9 gm. per kilo of body weight caused toxic symptoms in the three rabbits so treated, with death on the 2nd and

3rd days, while a similar result followed the dose of 0.75 gm. in two out of three rabbits; with 0.6 gm., only one rabbit of the three became toxic and eventually recovered, and the dose of 0.5 gm. caused no toxic disturbances whatever. From the above results, it is evident that the tolerance of very ill rabbits suffering from a severe acute trypanosomiasis infection compares very favorably with that of normal animals to single large doses of *N*-phenylglycineamide-*p*-arsonic acid administered intravenously.

Treatment of Initial Infections with Repeated Doses.

In many respects, the treatment of rabbit trypanosomiasis with repeated small doses of the amide of *N*-phenylglycine-*p*-arsonic acid more nearly meets conditions as they are found in nature, and in addition the control and ultimate cure of severe and long standing infections which might not yield to a single dose treatment may often be successfully accomplished by this system of therapeutic procedure. This is due in part to the fact that with the debilitated and weak condition of the animal host, it may not be wise to give a single dose of sufficient size and probably in part also to the chronic and indurated nature of the lesions present, the penetration of which may be difficult and may require considerable time. By observing the action of single small doses of the drug, we form an idea of their effect and duration. Obviously, the repetition of a dose should occur before the effect of the previous dose has ceased, and we consider the proper spacing of doses as important a factor in this connection as the number and size of the doses. Finally, all these factors must be largely determined by the physical state of the diseased animal as well as by the age, severity, and type of the infection.

We have treated a number of rabbits infected with *Tr. brucei* with three doses of the compound ranging from 0.2 to 0.05 gm. per kilo repeated at various intervals of time, of which the following are typical examples. Two rabbits infected with *Tr. brucei* were treated 11 days after inoculation, with three doses of 0.2 gm. per kilo repeated at 2 day intervals. The disease was well established with marked typical clinical signs at the time of treatment. Both animals recovered promptly with no signs of drug intoxication and no return of the

infection. Two other rabbits in the same series treated with three doses of 0.15 gm. at 2 day intervals likewise showed a prompt and complete disappearance of the clinical signs of the infection but one animal eventually relapsed 30 days later. Another animal which was given three doses of 0.1 gm. at 2 day intervals remained free from any signs of the infection for 186 days at which time it was discarded as cured. The last rabbit in this series which was given three doses of 0.05 gm. relapsed 13 days after treatment had been begun.

A modification of the spacing of repeated small doses was followed in the case of another animal which was treated 14 days after inoculation with *Tr. brucei*; the initial dose was 0.1 gm. per kilo. 8 days after treatment, the return of clinical signs of trypanosomiasis was observed about the head and nose, and the dose of 0.1 gm. per kilo was repeated 2 days later. The clinical signs cleared up promptly within 4 days and the animal remained in good condition without relapse during the period of observation following treatment.

In the experiments just cited, the infections were of an acute, rapidly progressive type. The effect of treatment with repeated doses of this arsenical upon a slowly developing severe infection of long standing is illustrated in Figs. 18 to 23. In this instance, the rabbit was inoculated with *Tr. brucei* in the usual manner but the infection was relatively slow in developing and treatment was not begun until 48 days after inoculation, when three doses of 0.2 gm. were given intravenously on succeeding days. At this time, the animal was extremely weak and emaciated, the ears, eyelids, face, nose, and external genitalia were swollen and indurated, and in addition, there were a mucopurulent exudate and scab formation about the eyes and nose (Fig. 18). An immediate improvement in the clinical manifestations of the disease was seen after treatment, although there was a loss of weight for the first 5 days, but by the 7th day this had largely been regained. Within the week following treatment, the scabs about the ears and around the eyes and end of the nose and lips had scaled off almost entirely, leaving a fairly healthy looking skin, the indurated swellings about the head and genitalia had greatly subsided, and the general condition of the rabbit had improved remarkably as is shown in Figs. 19 and 20. By the end of 3 weeks, the change in the appearance of the rabbit as contrasted with its condition on the day of treatment was

quite striking (Fig. 22), and in 6 weeks, a small bald patch at the base of the left ear was the only residual sign of a previous lesion (Fig. 23). The recovery from a severe and long standing infection under the system of three small doses of 0.2 gm. per kilo repeated at daily intervals was complete and lasting, for there was no subsequent relapse during the period of observation of 195 days.

The treatment of initial infection with *Tr. brucei* in rabbits, therefore, may be successfully accomplished by a system of repeated small doses provided that the repetitions occur well within the time of duration of action of the previous dose. The value of this system of therapy is especially marked in instances of greatly weakened animals or in those in which chronic indurated lesions are pronounced.

Treatment of Relapses with Single Doses.

The successful treatment of trypanosomiasis relapses in rabbits, many of which were intentionally produced as test objects, is based largely upon the severity and duration of the relapse and the general physical condition of the rabbit. While the factor of fastness of trypanosomes due to previous treatment unquestionably plays a part in the blood infections of mice and rats and must be considered to a certain extent in the tissue infection of rabbits, it is our opinion that the severity and duration of the relapse as manifested by the signs and symptoms of the actual infection, together with the general physical state of the rabbit host, play a more important rôle. It is obvious that the treatment of a relapse is almost wholly the treatment of individuals, and hence no rules of procedure can be laid down; but because of the inherent condition imposed by the infection, we believe that in most cases relapses can best be handled by a system of repeated doses, the size and time of repetition of which are necessarily determined by the extent, severity, and age of the disease together with the physical condition of the animal host. However, if a single large dose is used, it must of necessity cause no serious degree of intoxication or pathological injury, and yet at the same time, it must be of sufficient strength to penetrate diseased tissues of more or less induration and in like manner to act as a trypanocidal agent for a sufficient length of time. A number of relapses in rabbits have

been treated with single doses of this arsenical, of which the following will serve as examples.

The appearance of a *Tr. brucei* relapse of a week's duration at the time of retreatment is shown in Fig. 24. This rabbit was originally treated intravenously with three doses of 0.05 gm. per kilo of the drug which had served to clear up all outward manifestations of the disease, but 13 days later, definite signs of a relapse were noted. The infection was allowed to progress for a week at which time the clinical signs had rapidly increased both in degree and extent. The general physical condition of the rabbit was good. The entire left side of the face was greatly swollen and the left eye completely closed; the upper eyelid was very puffy, and protruding and escaping from between the margins of both lids, which were reddened with considerable loss of hair, was a slightly purulent exudate. The right upper eyelid, right cheek, and upper lip were also slightly swollen. Both ears, especially the left, which drooped markedly, were swollen, inflamed, and edematous and the right testicle was somewhat indurated. The dose selected for retreatment in this instance was 0.5 gm. per kilo of the amide of *N*-phenylglycine-*p*-arsonic acid given intravenously; it was followed by no toxic symptoms whatever. There was a most marked reduction of the clinical signs on the 2nd day; in a week's time the appearance of the rabbit was quite normal as is shown in Fig. 25, and this continued with no return of the infection during the period of observation of 166 days.

Another nagana rabbit which had been treated intravenously with the single dose of 0.29 gm. of the drug per kilo of body weight relapsed 26 days after treatment and the signs of the disease increased extremely rapidly for the succeeding 8 days. A single dose of 0.4 gm. per kilo of the drug was given intravenously. This was followed by a marked immediate improvement in the clinical signs of the infection and in 10 days the animal had regained its original weight and its appearance was entirely normal. There was no further relapse during the 149 day period of observation.

In addition to the treatment of rabbits infected with *Tr. brucei* which had relapsed after the initial treatment with the amide of *N*-phenylglycine-*p*-arsonic acid, a number of animals were treated that had relapsed from initial treatment with single doses of arsphenamine

and neoarsphenamine (German salvarsan and neosalvarsan), ranging from 0.005 to 0.02 gm. per kilo of body weight. For purposes of comparison, the original series which included these rabbits was conducted under the same conditions as were the parallel experiments in which the amide of *N*-phenylglycine-*p*-arsonic acid was used, and the infection was of the same acute nature with marked clinical signs at the time of treatment. The animals which relapsed after the treatment with arsphenamine and neoarsphenamine were held until the infection had progressed to an extreme degree with conspicuous clinical signs and a generally poor physical state of the animals; they were then treated with single doses of *N*-phenylglycineamide-*p*-arsonic acid. Five animals were given 0.5 and eight were given 0.4 gm. per kilo of body weight and all promptly recovered from the signs of the infection, with no evidences of drug intoxication and with a very noticeable and rapid improvement in their physical condition. Three rabbits in this group were killed 34, 47, and 56 days after treatment because of extensive ringworm or middle ear disease, but at autopsy no evidence of trypanosomiasis was found and none of the remaining ten rabbits suffered a relapse from the retreatment with *N*-phenylglycineamide-*p*-arsonic acid.

From the experimental evidence, therefore, it is seen that rabbits infected with our strain of *Tr. brucei* which have relapsed with severe clinical signs after insufficient treatment with the amide of *N*-phenylglycine-*p*-arsonic acid, arsphenamine, and neoarsphenamine may be permanently cured with single doses of 0.4 and 0.5 gm. per kilo of body weight of the amide of *N*-phenylglycine-*p*-arsonic acid.

Treatment of Relapses with Repeated Doses.

In the treatment of relapses with repeated doses, the chief factors of importance to be considered are first, the severity and type of the infection and the general physical condition of the rabbit. If the relapse is long standing with chronic indurated lesions, a single dose of the drug may not penetrate the lesions sufficiently or operate over the necessary length of time required for resolution of such lesions. Moreover, as is frequently the case with this type of chronic relapse, the physical state of the rabbit may be very poor and a single large dose

of the drug might under these conditions prove somewhat dangerous. Hence, from a consideration of these factors, it would seem that a system of small repeated doses, properly spaced, is the most rational system of treating chronic trypanosomal relapses of rabbits. The size of the initial dose or doses should be large enough to exert some influence upon the infection and its lesions, and subsequent doses should be given before the effect of the previous dose has completely worn off. Consequently a knowledge of the general effect and duration of action of various sized doses of this arsenical, as well as accurate and frequent clinical observations of the rabbits under treatment, is essential to a satisfactory outcome with this system of therapy. In this connection, it is important to bear in mind that rabbits as well as other animal species exhibit a high degree of tolerance for the drug as has been shown elsewhere,[2] and this attribute which exists in rabbits infected with trypanosomiasis as well as in non-infected animals may be used to great advantage in the treatment of trypanosomiasis, both of the initial infections and of the relapses. Our experience in handling relapses with repeated doses of this drug has been extremely varied because we have purposely endeavored to ascertain what might be accomplished with various sized doses given at various intervals of time under the many different conditions imposed by the infection rather than an attempt to effect a cure in every case. We have selected certain typical protocols which will illustrate some of the essential points in question.

Fig. 26 shows the appearance of an 8 day relapse in a rabbit which had been treated 34 days before with 0.02 gm. of arsphenamine (German salvarsan) per kilo of body weight. The signs of the relapse increased rapidly and at the time of retreatment with the amide of *N*-phenylglycine-*p*-arsonic acid, the rabbit was not in a particularly good general condition. The face was markedly swollen and indurated, both eyes were closed, the base of both ears was involved in the inflammatory process, both testicles were swollen and indurated, and the prepuce was extremely swollen and congested. Three doses of 0.15 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid were given intravenously on successive days. There were no evidences of drug intoxication whatever, the general condition improved immediately, and

[2] Brown, W. H., and Pearce, L., *J. Exp. Med.*, 1919, xxx, 417.

the clearing up of the clinical signs progressed rapidly as is seen in Fig. 27, which shows the appearance 6 days after treatment had been begun. At this time there remained only a slight swelling and induration of the face and a slight degree of resistance of normal size testicles. These residual signs had completely disappeared on the following day. Unfortunately no opinion of the ultimate therapeutic success of the treatment was possible in this particular case as the animal succumbed to a bacterial infection 24 days after treatment.

Fig. 28 illustrates the appearance of a relapse of 15 days duration in a rabbit that had been treated 49 days previously with 0.02 gm. of neoarsphenamine (German neosalvarsan) per kilo of body weight. There was a very marked swelling of the face and the base of the ears, particularly of the left, an indurated swelling of the eyelids with crust formation, a purulent exudate, loss of hair and complete closure of both eyes, a mucoserous nasal discharge, and a moderate involvement of the genitalia. In addition, the animal was very thin and weak. A dose of 0.5 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo was given intravenously and was followed by a marked general improvement and gradual disappearance of the signs of the infection with an accompanying gain in weight, although the rabbit still remained weak and middle ear disease developed. 2 weeks after the first dose of the drug had been given, a second dose of 0.5 gm. per kilo was given intravenously with no evidences of drug intoxication but with a decided improvement in the animal's general condition, although the local condition in the right middle ear continued to progress, causing a marked twisting of the head to the right. In the course of recovery from the trypanosomal infection, a large portion of diseased skin over the bridge of the nose and about the eyes gradually desquamated, leaving a healthy looking but bare surface denuded of hair. The growth of new hair in these areas was quite remarkable as is shown in Fig. 29. There was no later evidence of trypanosomiasis observed in this rabbit either clinically or at autopsy, and despite the middle ear abscess which was eventually the cause of death 59 days after the treatment of the relapse, the general physical condition remained excellent.

We have selected two examples of the treatment of nagana relapses of a more prolonged and chronic type which are generally considered to be much more difficult of permanent cure than the types previously

considered in this section. The first of these rabbits was originally treated intravenously with 0.2 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo of body weight which sufficed to clear up all signs of the infection. However, 27 days later, the diagnosis of a probable relapse was made because of signs about the eyelids, and although at the end of a week's time, the clinical signs of trypanosomiasis were outspoken, the relapse was allowed to progress and the rabbit was not treated until 22 days after the first signs had been noted. The appearance of the head of the rabbit on the day of retreatment is shown in Fig. 30. There was a marked edematous swelling of the face involving the cheeks and eyelids and the eyes were almost closed; both upper lips were extremely swollen and congested with some loss of hair and scab formation; the lower portions of the ears were considerably indurated and very hot to the touch and at the base of the right ear was a necrotic area the size of a silver half dollar. The prepuce was edematous, congested, and somewhat swollen and both testicles although not enlarged were indurated. The general physical condition of the rabbit was fairly good. Two doses of 0.4 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid given a week apart caused a marked improvement in the rabbit's condition as shown in Figs. 31 and 32, but 3 days after the second dose, there was a swelling of the left cheek and both left eyelids, with a slight mucopurulent discharge from the eye. The succeeding four doses (0.6, 0.5, 0.5, and 0.75 gm. per kilo) given over a period of 28 days were eventually effective in clearing up the clinical signs (Figs. 33 to 35), but a relapse ultimately occurred during the summer. Since the total amount of the drug administered was in our experience sufficiently large to cure a relapse of this duration and type, it would seem that the failure was due to the incorrect spacing of the doses which were too far apart. It is probable that if the first three doses had been given on successive or even on alternate days, a permanent cure would have been obtained.

As a contrast to the system of treatment of a relapse pursued in the preceding experiment which failed to effect a permanent cure, the following instance is given of the successful treatment of a chronic and very advanced relapse, a type of infection generally considered extremely difficult of cure. A rabbit infected with *Tr. brucei*, which had been treated in the spring, relapsed during the summer, and at the time of retreatment, about 3 months later, presented an extreme

picture of a long standing chronic relapse (Figs. 36 and 37). A large portion of the face including the area around both eyes and the bridge of the nose was practically devoid of hair. The skin had lost its elasticity, was quite firm, resistant, and indurated, and there were numerous small scabs over the surface, especially around the right eye. The base of the right ear was also swollen and indurated and there was a small raw thickened area on the right hind foot covered with reddish yellow scabs. The prepuce was markedly swollen and quite firm; both testicles although not enlarged were very firm and indurated. The rabbit was weak but in a fairly good state of nutrition. Treatment was begun with the injection of 0.75 gm. of the drug per kilo of body weight and repeated on the 4th and 6th days—a dose which we believed was unnecessarily large, yet the reaction and effect of which we wished to determine in this particular type of relapse.

The therapeutic effect of the drug upon the clinical lesions was most marked as is shown in Figs. 38 to 40. There was no loss of weight, the rabbit's general condition was very noticeably improved, and, moreover, there was no indication of any drug intoxication at any time. 1 week after treatment had been begun, most of the scabs about the base of the right ear, around both eyes, and over the face had scaled off, leaving a smooth, soft, elastic skin denuded of hair but otherwise quite healthy looking, and the involved area on the right hind foot had a similar appearance. The prepuce was practically normal and the testicles were rapidly becoming so.

The further changes in this rabbit during the succeeding 10 days were quite remarkable as are shown in Figs. 41 and 42. The eyelids, nose, face, and base of the right ear were no longer swollen or indurated and the rapid growth of hair in the bare areas was quite conspicuous. The affected area on the right hind foot was somewhat pinkish with a few yellowish pink scabs but with a beginning growth of fine hair. The external genitalia appeared normal, although on palpation the scrotum on both sides was still somewhat diffusely thickened and the testicles were atrophic. On the 11th and 14th days, two additional doses of 0.75 gm. per kilo of the drug were given intravenously for the purpose of determining any degree of acquired tolerance or any signs or symptoms of drug intoxication that these

additional large doses might cause, although from the point of view of the infection, we were confident that they were unnecessary. Apparently, however, they had no untoward effect whatever and the rabbit continued to improve very noticeably. By the end of the 3rd week, there were no residual lesions indicative of the trypanosomiasis infection except one or two small patchy areas below the inner canthus of each eye and just above the tip of the nose where the hair was thinner than elsewhere (Fig. 43), but in a short time, these areas had resumed a normal appearance. This particular rabbit has been held in the laboratory 3 years and there has been no return of the infection.

The treatment of trypanosomal relapses in rabbits with this compound depends largely upon two important factors, the general physical state of the rabbit and the extent and severity of the infection, and these conditions can in our opinion be most adequately handled by a system of repeated doses. Since the factor of tolerance for the amide of *N*-phenylglycine-*p*-arsonic acid is exhibited to a considerable degree in rabbits, the size of succeeding doses may be greatly increased if necessary and the number of dose repetitions may also be large. However, it is of essential importance that the succeeding doses should be repeated well before the effect of the previous dose has worn off.

Administration of the Drug by Routes Other than the Intravenous.

The great majority of experiments with the amide of *N*-phenylglycine-*p*-arsonic acid in the treatment of rabbit trypanosomiasis, including those described above, were carried out by the intravenous administration of the drug, since this route offers uniform and constant conditions from the point of view of actual administration as well as development of drug action. Under such conditions, a comparison of the effect of different sized doses and a final appraisal of the action of the drug from the point of view of a permanent cure may be more accurately made. However, since it may be desirable to employ routes of administration other than the intravenous, from the standpoint of actual usage and ease of manipulation, a few rabbits infected with *Tr. brucei*, of which the following are examples, were treated subcutaneously, intramuscularly, and *per os*, in order to ascertain the general effect and duration of certain selected doses upon initial infections and relapses.

Two rabbits with a 14 day nagana infection and showing well marked clinical signs of the disease were treated with 0.1 gm. of the drug per kilo of body weight subcutaneously. The acute inflammation and edema about the head and external genitalia were materially reduced and only slight signs remained 7 days after treatment, but on the 8th day, they became increased in the first rabbit. Consequently both animals were treated on the 8th and 10th days with 0.1 gm. per kilo, again subcutaneously. The clinical signs regressed completely in the first animal during the following week and were absent for 3 weeks, at which time they recurred with considerable severity; in the case of the second rabbit, in which the infection was still in abeyance at the time of the second and third doses, the clinical signs were absent for 6 days after the second treatment; they then recurred and steadily increased in severity and extent. Two other rabbits in this group were treated subcutaneously with a dose of 0.2 gm. of the drug per kilo of body weight which was not repeated. One rabbit was permanently cured and the other relapsed on the 36th day. A fifth animal with a long standing infection of 48 days duration showing marked chronic lesions about the head and external genitalia with considerable loss of weight was treated with 0.5 gm. of the amide of *N*-phenylglycine-*p*-arsonic acid per kilo subcutaneously. There were no signs of drug intoxication and no inflammation or induration about the site of injection; the regression of lesions proceeded somewhat more slowly than after intravenous administration of the drug, but at the end of 2 weeks they had subsided and the animal appeared normal except for a few bald patches about the head. No further signs of trypanosomiasis were observed for 41 days, at which time the outer margin of the right ear showed signs of early involvement and 2 days later a second dose of 0.25 gm. per kilo, one-half the original dose, was given subcutaneously for the purpose of ascertaining the effect upon the local lesion and upon the general course of the infection of a single dose much smaller than the original one. There was a very noticeable improvement of the ear lesion on the following day. A portion of the scab scaled off and the induration of the entire margin was distinctly decreased; on the 5th day after treatment, the ear appeared normal. There were no further indications of a relapse but unfortunately the rabbit developed an acute, extensive ringworm and

had to be killed 35 days after the second dose so that the question of a permanent cure is problematical. The rapid healing of the local ear lesion, however, and the fact that the relapse was treated soon after its appearance, although with a comparatively small dose, and that as long a time as 35 days elapsed without signs of a relapse either clinically or at autopsy are all factors indicative of a probable cure.

Seven rabbits were treated by the intramuscular route of administration. Two animals which were treated 14 days after inoculation with *Tr. brucei* received single doses of 0.2 gm. of the drug per kilo. The speed of action of the drug upon the well marked characteristic clinical lesions of the infection as seen in these two animals was apparently quite comparable with that observed after intravenous administration, and a permanent cure was effected in both rabbits. Two other rabbits of the same series were treated with an initial dose of 0.1 gm. per kilo which was repeated on the 8th and 10th days. One animal was cured but the other showed marked lesions of the disease 6 days after the last dose and was treated again with three doses of 0.1 gm. per kilo of body weight given intramuscularly on successive days with a marked, though temporary effect. In this instance, it is probable that the infection was originally not cleared by the first three doses and consequently either the second course of treatment was not given soon enough to effect a permanent cure or the amount of drug was not large enough. Another rabbit was treated 42 days after infection with *Tr. brucei* with three doses of 0.1 gm. per kilo of body weight intramuscularly on successive days. At the time of treatment, the lesions about the ears, eyes, face, nose, and lips were extremely marked and the animal itself was in only a fair physical condition. Regression and healing of the lesions about the head took place very satisfactorily and there were no signs of drug intoxication, but a subcutaneous abscess developed at the site of injection in the lumbar region and gradually increased in extent. The rabbit was killed 25 days after treatment. There were no clinical signs of a relapse at this time and the autopsy findings were negative. Finally, two rabbits with severe relapses after previous treatment—one with 0.015 gm. per kilo of neoarsphenamine given intravenously and the other with three doses of 0.1 gm. per kilo of the amide of *N*-phenylglycine-*p*-arsonic acid given subcutaneously—were treated intramuscularly with

larger doses of the latter drug. The first, which received 0.5 gm. per kilo of body weight, showed a rapid regression and healing of the lesions of the infection and was permanently cured; the other rabbit was given an initial dose of 0.3 gm. per kilo of body weight and 3 days later 0.5 gm. per kilo with no sign of drug intoxication and a very marked improvement in the signs of the infection. The ultimate result of the intramuscular administration of the drug cannot be determined in this case, for the succeeding treatment 11 days later was by mistake given intravenously.

Finally, an example of the therapeutic administration of the amide of *N*-phenylglycine-*p*-arsonic acid by mouth may be given for completeness, although the number of trypanosomiasis rabbits treated by this route is too small to admit of any conclusion regarding the effect and final result of various sized doses. A severe initial infection of 8 days duration with well marked clinical signs was treated with a single dose of 0.75 gm. per kilo of body weight given *per os* immediately after a small dose of sodium bicarbonate. There were no untoward effects following the administration of the drug; the clinical signs of the infection regressed and healed in the usual time and the rabbit was permanently cured.

While the number of rabbits treated with the amide of *N*-phenylglycine-*p*-arsonic acid administered by the subcutaneous, intramuscular, and *per os* routes is too few to admit of final conclusions as to their comparative value and usefulness, they demonstrate that even relatively small doses of the drug are highly active therapeutically when given intramuscularly and to a somewhat less extent subcutaneously, while in one instance a large dose given by mouth cured a severely infected animal.

SUMMARY.

In the treatment of experimental trypanosomiasis of rabbits with subsequent appraisal of the value of the therapeutic agent used, there are certain experimental factors including uniform infecting strains of trypanosomes and the observation of general procedures of method and time of inoculation conditioned by the infection itself which must be taken into account. The conspicuous and characteristic clinical

signs and symptoms seen in rabbit trypanosomiasis serve as criteria of the severity and duration of the disease, and it is obvious that the infection should be well established before treatment is instituted. For the same reason, before the question of a permanent cure can be established, treated rabbits should be kept under observation for a sufficient period of time, which with the species of organisms that we have used is at least 3 months.

The therapeutic results with the amide of *N*-phenylglycine-*p*-arsonic acid were obtained in rabbits which showed well marked clinical signs of a definitely established disease, and in many instances the infection was extremely advanced and of prolonged duration. The five species which we have employed, *Tr. brucei*, *Tr. gambiense*, *Tr. equinum*, *Tr. equiperdum*, and *Tr. evansi*, are uniformly fatal in rabbits. With the usual acute, actively progressing infection of from 1 to 2 weeks duration produced by our strain of *Tr. brucei*, the drug has a curative range of from 0.2 to 0.35 gm. per kilo of body weight, when administered intravenously in single doses, or from one-third to one-half the minimal lethal dose. Of the twenty-nine rabbits treated with doses falling within this range, twenty-five, or 86 per cent, were permanently cured and there were no relapses observed with doses above 0.3 gm. The infection produced by our strain of *Tr. gambiense* is controlled by a slightly lower dose, since there were no relapses with single doses of 0.3 gm. and a single dose of 0.15 gm. effected a cure in one of three rabbits so treated. The therapeutic experiments with *Tr. equinum*, *Tr. equiperdum*, and *Tr. evansi* are too few to admit of final conclusions, but apparently from the evidence at hand, much the same curative range is operative in *Tr. evansi* infections, while larger doses or a different system of treatment should have been employed in the treatment of rabbits infected with our strains of *Tr. equinum* and *Tr. equiperdum*.

In addition to the ultimate curative results obtained with single doses within the curative range, it is important to consider the marked therapeutic action with smaller single doses, as shown by the rapid regression and healing of the clinical lesions of the acute infections produced by all five species of trypanosomes together with a marked improvement in the general physical state of the animal. Moreover, large single doses, above those of the so called curative range, caused no disturbance of a toxic nature and were apparently well borne.

A system of repeated dose therapy may be employed with advantage in the treatment of both initial and relapsed infections in rabbits, especially in those instances in which there is induration or even necrosis of tissues with weakness and emaciation of the animal host. The factor of time of repetition or the spacing of doses is in our experience as important as that of size of the dose employed and depends upon the rate, degree, and duration of action of the particular dose of the drug in question. Since the amide of *N*-phenylglycine-*p*-arsonic acid apparently possesses the power of tissue penetration to a marked degree, it is desirable to give the second dose within a short time after the first in order that it may have a full opportunity for the immediate and complete development of its action. The repetition of small doses such as 0.15 gm. per kilo of body weight on successive or alternate days has given successful results as regards both the immediate regression and healing of lesions and ultimate permanent cures in severe, chronic infections. It is possible, however, to administer increasingly large doses, if this is necessary, since infected as well as normal rabbits exhibit a remarkable tolerance to repeated large doses of the drug. The therapeutic activity of small doses administered intramuscularly is quite comparable with that observed after similar doses given intravenously, as indicated by the rate of regression and healing of clinical lesions, while such effects proceed somewhat more slowly after subcutaneous injections. Permanent cures have been obtained in *Tr. brucei* infection with intramuscular and subcutaneous administration of single doses of from 0.2 to 0.5 gm. of the drug per kilo of body weight and in other instances with three repeated doses of 0.1 gm. per kilo given intramuscularly. One severely infected rabbit which received 0.75 gm. per kilo *per os* immediately following a small dose of sodium bicarbonate was also cured.

The therapeutic experiments here reported represent only a portion of those carried out with *N*-phenylglycineamide-*p*-arsonic acid and the scope of the present paper does not permit a detailed description of the many phases of the experiments or a full discussion of the various factors involved and the results obtained, all of which we hope to publish at some future time.

EXPLANATION OF PLATES.

The figures are reproductions of untouched photographs illustrating the effects produced upon experimental trypanosomiasis of rabbits by treatment with *N*-phenylglycineamide-*p*-arsonic acid. Objects are represented at about two-thirds their natural size, except where otherwise specified.

Figs. 1 to 17 illustrate the effect of single dose treatment upon acute and subacute initial infections.

PLATE 17.

FIG. 1. *Tr. brucei.* Acute infection of 15 days duration. Day of treatment with 0.35 gm. per kilo of body weight given intravenously. There is marked swelling of the face, eyelids, and base of both ears. General condition of animal good.

FIG. 2. 1 week after treatment. Appearance of rabbit normal. Permanently cured.

PLATE 18.

FIG. 3. *Tr. brucei.* Acute infection of 15 days duration. Objects represented at their natural size in Figs. 3 and 4. Day of treatment with 0.2 gm. per kilo of body weight given intravenously. The vulva and anus are markedly swollen and congested and are somewhat indurated. General condition of rabbit is good.

FIG. 4. 5 days after treatment. Rabbit permanently cured.

FIG. 5. *Tr. brucei.* Very severe, acute infection of 23 days duration. Day of treatment with 0.6 gm. per kilo of body weight given intravenously. The face, eyelids, and the lower portions of the ears are extremely swollen and indurated. The general physical condition of the rabbit is fairly good.

PLATE 19.

FIG. 6. 1 week after treatment. Slight residual fullness of the face. The hair over the nose and both upper lids is coming off. Animal's general condition is good.

FIG. 7. 29 days after treatment. Permanently cured.

PLATE 20.

FIG. 8. *Tr. gambiense.* Severe subacute infection of 36 days duration. Objects represented at their natural size in Figs. 8 to 11. Day of treatment with 0.2 gm. per kilo given intravenously. Both testicles are enlarged to about three times normal size and the scrotum is thickened, indurated, and cyanotic; on the right there are three large superficial ulcerations with scab formation. The testicles and cords are also enlarged and indurated. Rabbit's general condition is fairly good.

FIG. 9. 5 days after treatment. Scrotal scabs are separating.

FIG. 10. 19 days after treatment. There is a small residual adherent scab at the tip of the right scrotum. Testicle negative.

FIG. 11. 111 days after treatment—the day rabbit was discarded as permanently cured. External genitalia negative.

PLATE 21.

FIG. 12. *Tr. equiperdum.* Severe subacute infection of 36 days duration. Day of treatment with 0.3 gm. per kilo of body weight given intravenously. Rabbit is very thin and weak. There is marked swelling of the face with moderate involvement of the ears. Both eyes, especially the right, are partially closed and the upper eyelids are swollen and reddened.

PLATE 22.

FIG. 13. 5 days after treatment. There are no signs of trypanosomiasis and the animal's general condition is excellent. Ultimate recurrence.

PLATE 23.

FIG. 14. *Tr. evansi.* Severe subacute infection of 36 days duration. Day of treatment with 0.3 gm. per kilo of body weight given intravenously. Both ears are involved nearly to the tip, the left more than the right; the face is swollen with thickening of the tissues over the bridge of the nose and of the lips. All four eyelids are swollen and reddened; there is a mucopurulent discharge from both eyes, which are almost closed, and numerous yellowish scabs adherent to the lids. General physical condition of rabbit is good.

FIG. 15. 5 days after treatment. Very marked improvement. General condition good.

PLATE 24.

FIG. 16. 15 days after treatment. Scabs over end of nose and lips have desquamated. Beginning growth of hair.

FIG. 17. 19 days after treatment. The hair over the nose and lips is growing rapidly. Rabbit's condition is good with a considerable gain in weight. Permanently cured.

PLATE 25.

Figs. 18 to 23 illustrate the effect of repeated dose therapy upon a chronic initial infection.

FIG. 18. *Tr. brucei.* Extremely severe chronic infection of 48 days duration. Day of beginning treatment with three doses of 0.2 gm. per kilo of body weight repeated at 24 hour intervals and given intravenously. There is a marked and extensive indurated swelling of the tissues of the ears, face, eyelids, nose, and

lips with a purulent exudate from the eyes and nose. Rabbit is weak and emaciated.

FIG. 19. 6 days after treatment had been begun. Marked improvement in the local clinical signs of the infection and in the rabbit's general condition.

PLATE 26.

FIG. 20. 6 days after treatment had been begun.

FIG. 21. 13 days after treatment had been begun.

PLATE 27.

FIG. 22. 21 days after treatment had been begun. There is a very marked growth of hair in the bald areas of the face and ears.

FIG. 23. 40 days after treatment. Permanently cured.

PLATE 28.

Figs. 24 and 25 illustrate the effect of single dose treatment upon an acute relapsed infection.

FIG. 24. *Tr. brucei.* Acute relapse of 7 days duration. Day of treatment with 0.5 gm. of the drug per kilo of body weight given intravenously. The entire left side of the face including the eyelids and ears is swollen. Good general physical condition.

FIG. 25. 1 week after treatment. Rabbit appears entirely normal. Permanently cured.

PLATE 29.

Figs. 26 to 44 illustrate the effect of repeated dose treatment upon acute, subacute, and chronic relapsed infections.

FIG. 26. *Tr. brucei.* Severe acute relapse of 8 days duration. Day of beginning treatment consisting of three doses of 0.15 gm. per kilo of body weight repeated at 24 hour intervals and given intravenously. The face is markedly swollen and indurated and both eyes are closed. The base of both ears is similarly involved. General condition of the rabbit is fair.

FIG. 27. 6 days after treatment had been begun. Slight residual swelling over the bridge of the nose. General condition excellent. Probable permanent cure.

PLATE 30.

FIG. 28. *Tr. brucei.* Severe relapse of 15 days duration on the day of beginning treatment with 0.5 gm. per kilo of body weight given intravenously and repeated 14 days later. There is marked involvement of the face, ears, eyelids, nose, and lips and a mucoserous nasal discharge. Animal very thin and weak.

FIG. 29. 49 days after treatment. Appearance of rabbit normal except for the twisting of the head to the right. Probable permanent cure.

PLATE 31.

FIG. 30. *Tr. brucei.* Severe relapse of 22 days duration on the day of beginning treatment with 0.4 gm. per kilo of body weight given intravenously. The face, base of both ears, eyelids, nose, and upper lips are markedly swollen and edematous and there are thick scabs on the lips.

FIG. 31. 7 days after treatment had been begun. There is a marked improvement in the clinical signs.

PLATE 32.

FIG. 32. 9 days after treatment had been begun. Improvement continues with increase of weight.

FIG. 33. 15 days after treatment had been begun. Clinical signs of the infection have recurred.

PLATE 33.

FIG. 34. 21 days after treatment had been begun. Marked improvement with desquamation of the scabs and necrotic tissue in the involved area. General condition excellent.

FIG. 35. 28 days after treatment had been begun. Further improvement with rapid return to normal appearance. Ultimate relapse.

PLATE 34.

FIG. 36. *Tr. brucei.* Advanced chronic relapse of about 3 months duration on the day of beginning treatment with repeated doses of 0.75 gm. per kilo of body weight given intravenously. There is marked involvement of the face, eyelids, base of ears, and nose with loss of hair and scab formation. Rabbit is weak but not markedly emaciated.

FIG. 37. *Tr. brucei.* Day of treatment. Objects represented at their natural size. The prepuce is markedly swollen and indurated; the testicles and scrotum are very firm and indurated although not enlarged.

PLATE 35.

FIG. 38. 3 days later.

FIGS. 39 and 40. 1 week later. The appearance of the head and the external genitalia is normal except for the absence of hair and a few tiny scabs around the right eye. In Fig. 40 objects are represented at their natural size.

PLATE 36.

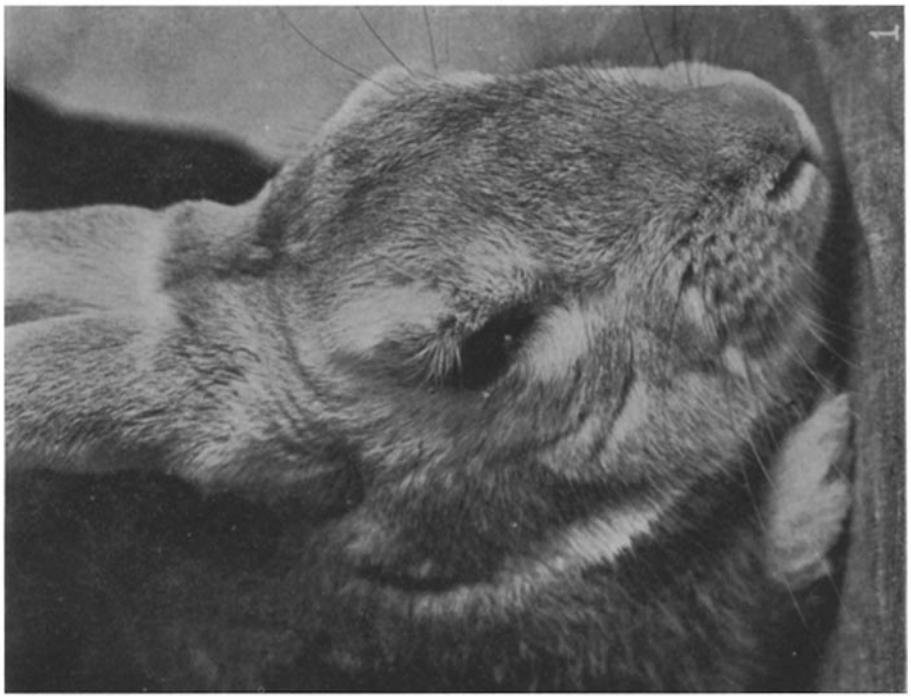
FIG. 41. 12 days later. There is a growth of fine hair on the bald patches about the head.

FIG. 42. 17 days later. Rapid growth of hair continues.

PLATE 37.

FIG. 43. 22 days later. Rabbit's appearance is entirely normal except for a tiny bald spot below the inner canthus of the right eye.

FIG. 44. 1 year later. Animal permanently cured.



(Pearce and Brown: Trypanosome and spirochete infections.)

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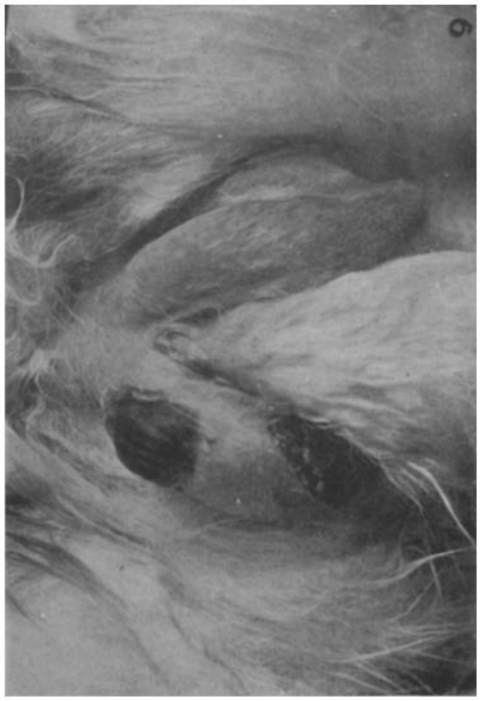

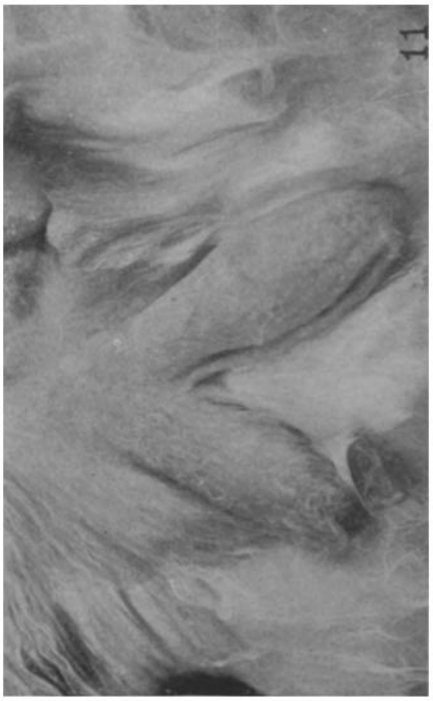

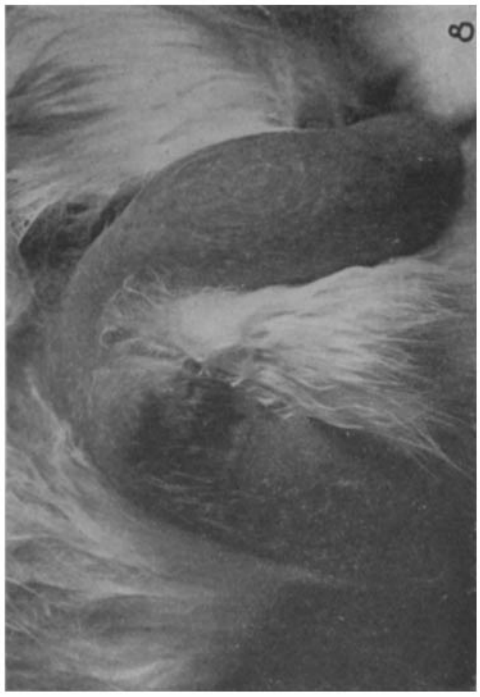

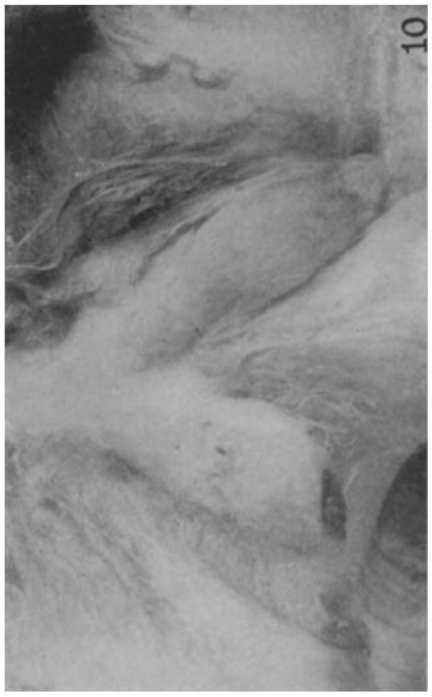

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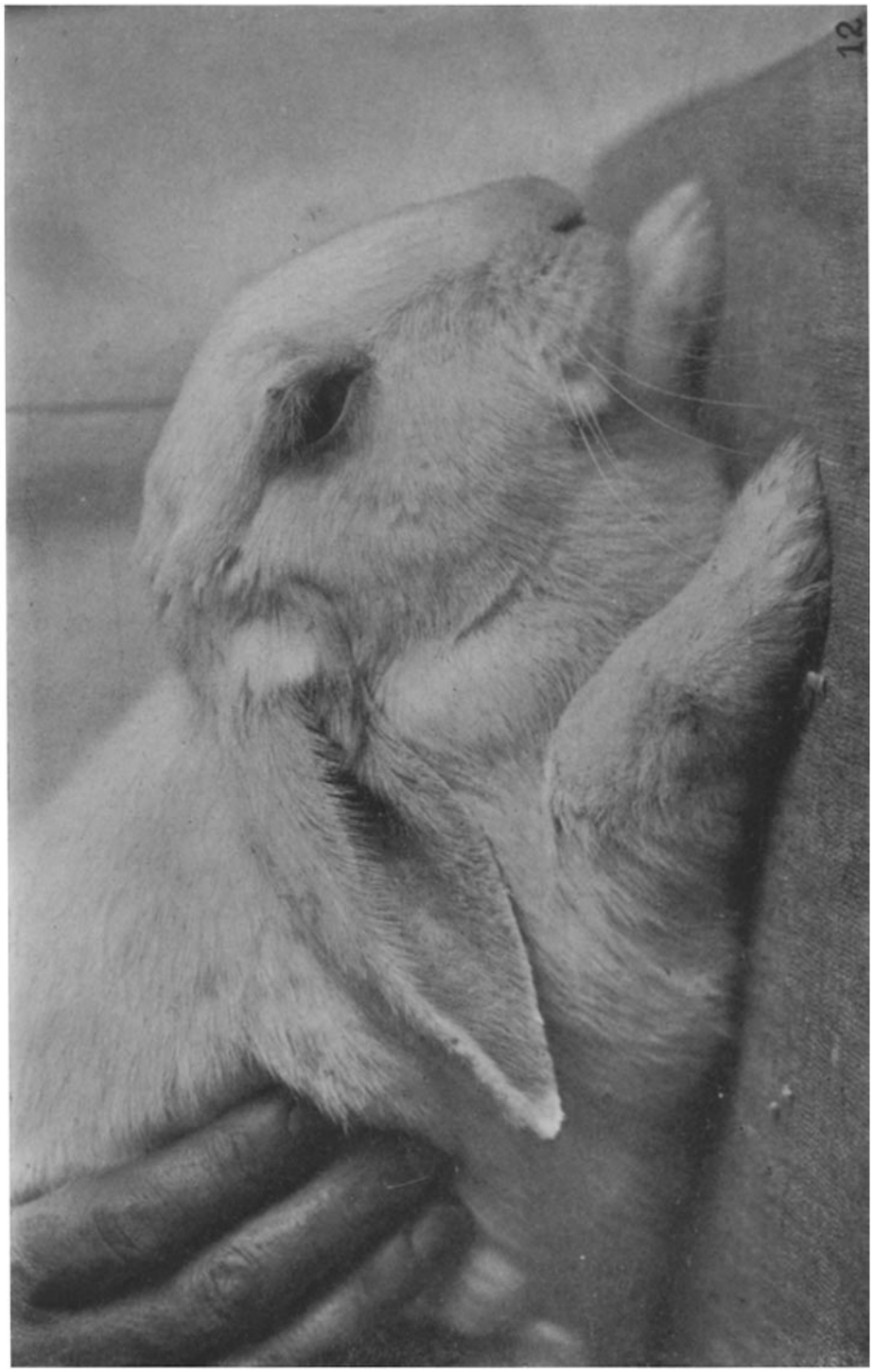

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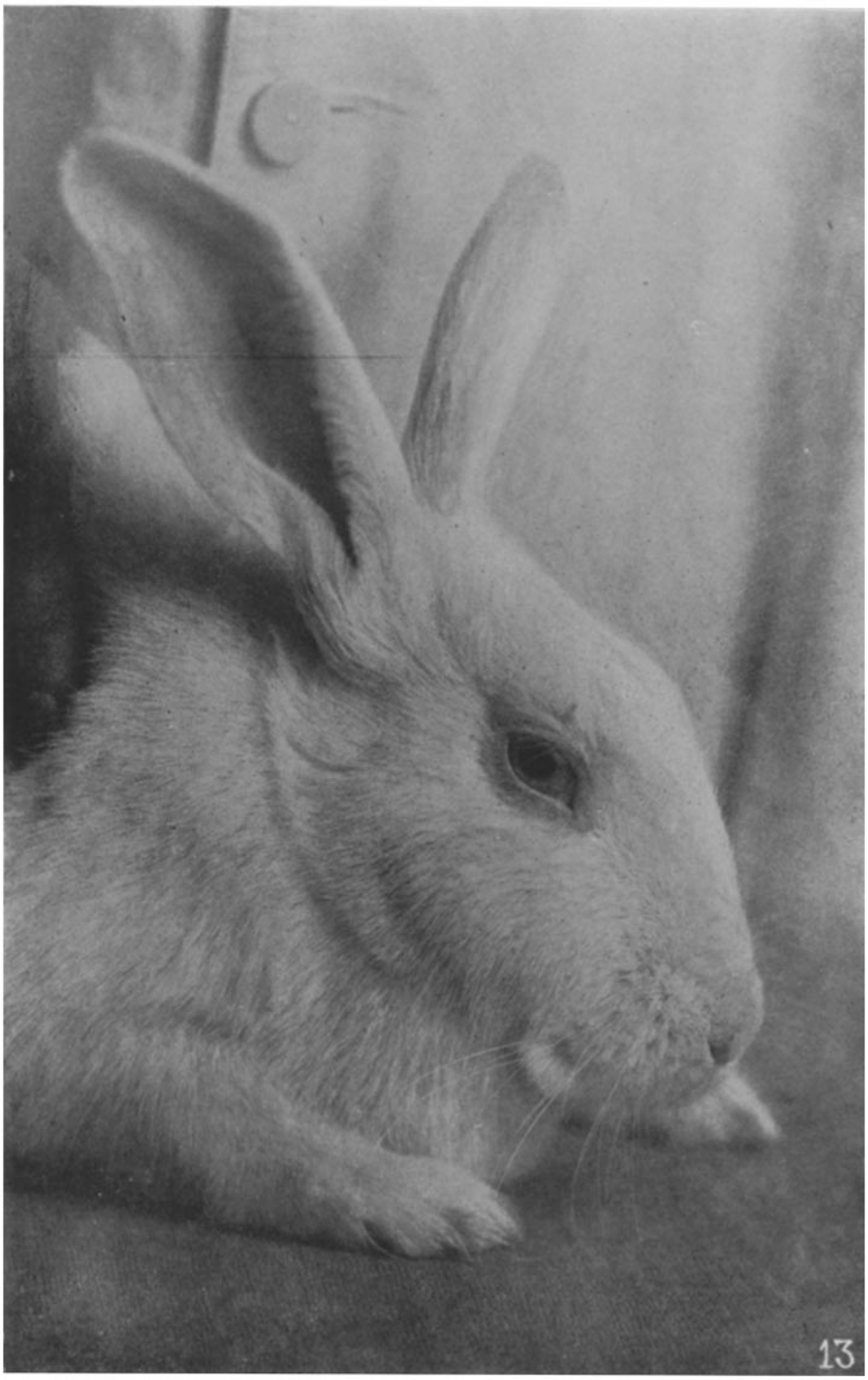

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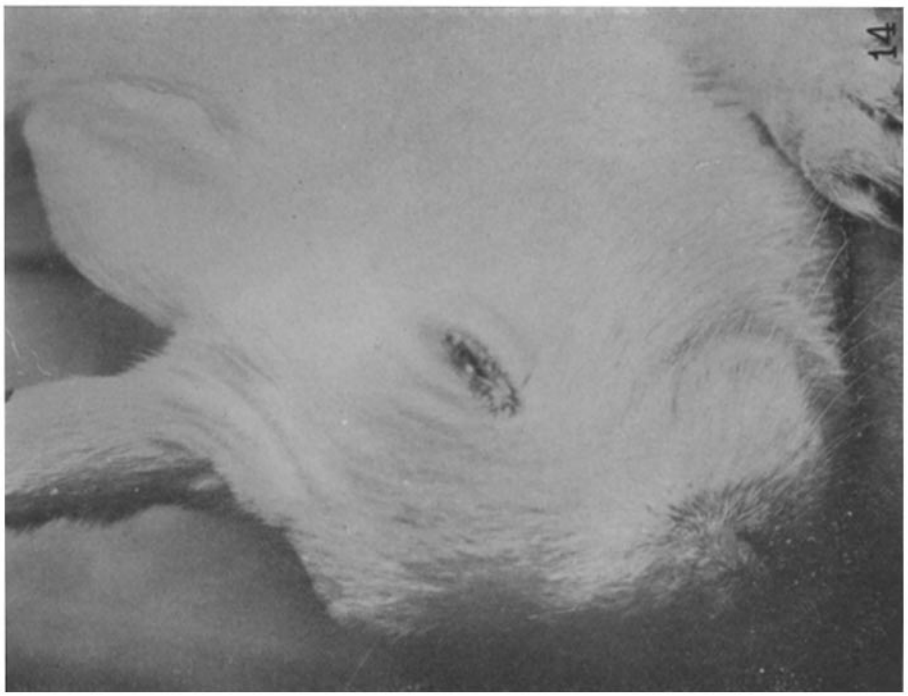

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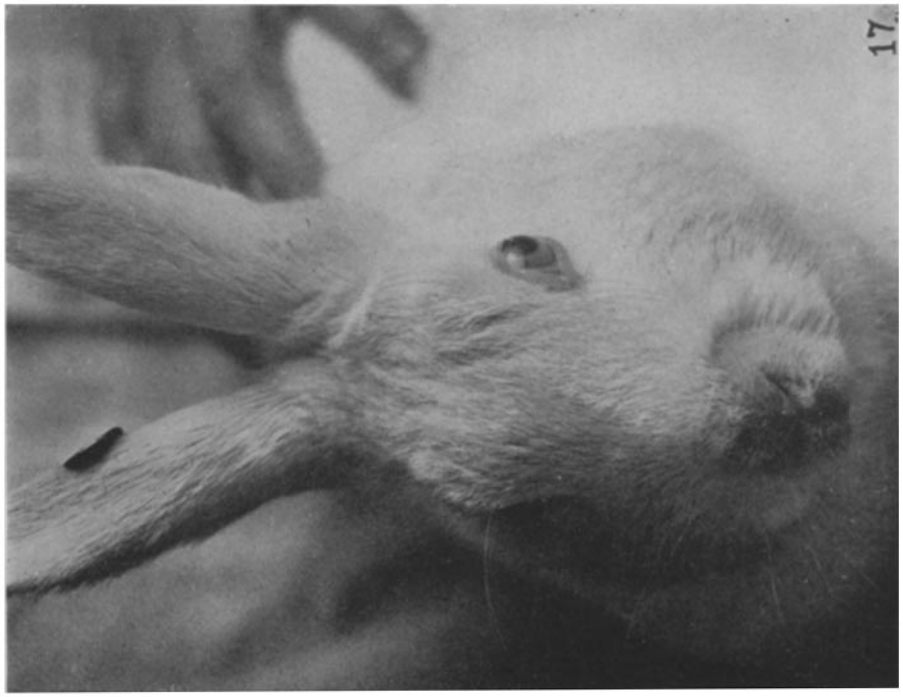

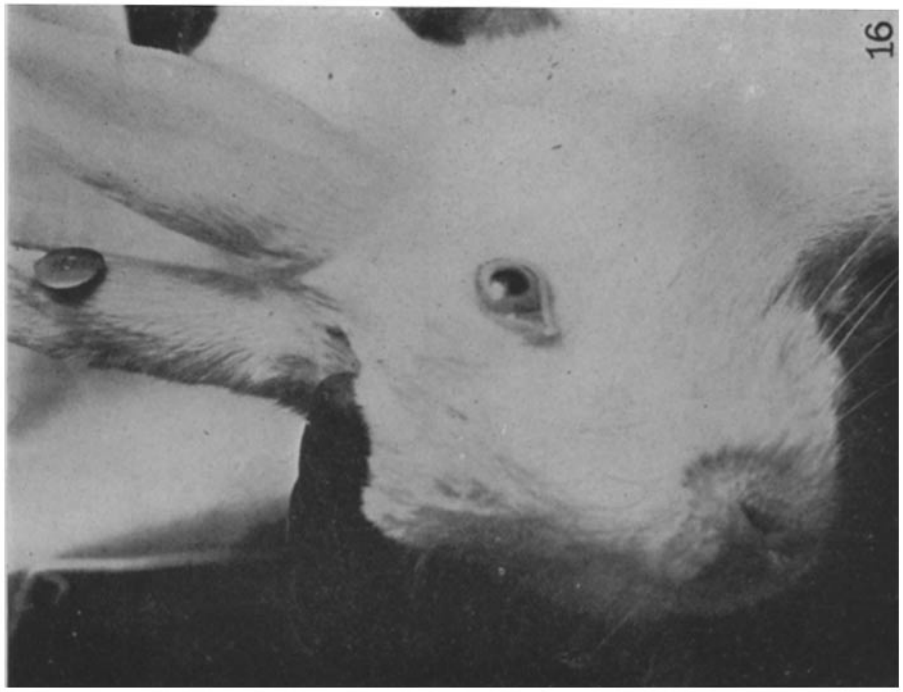

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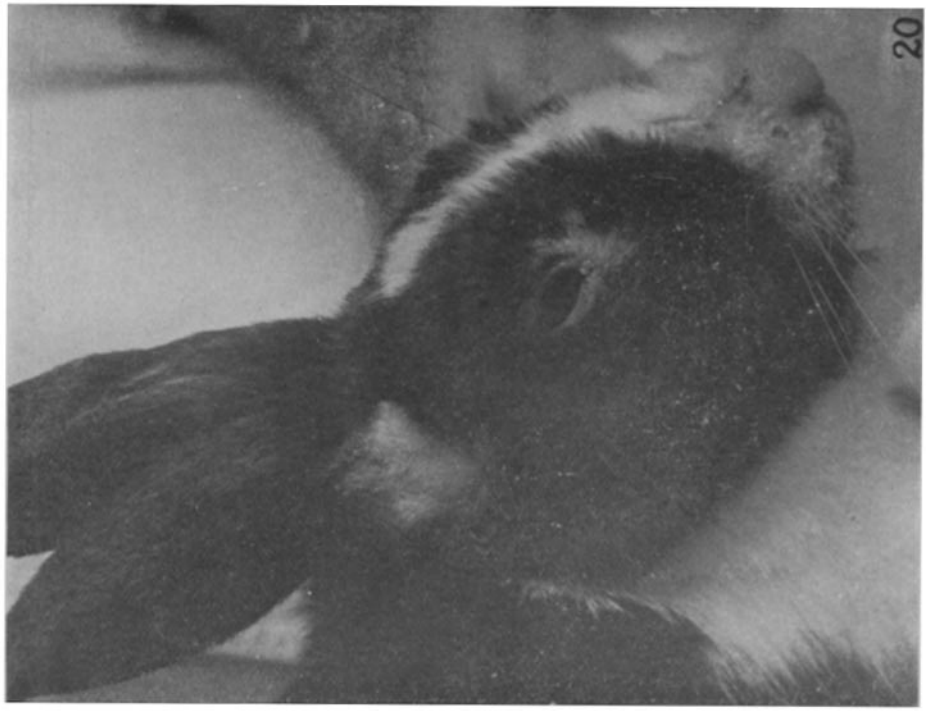

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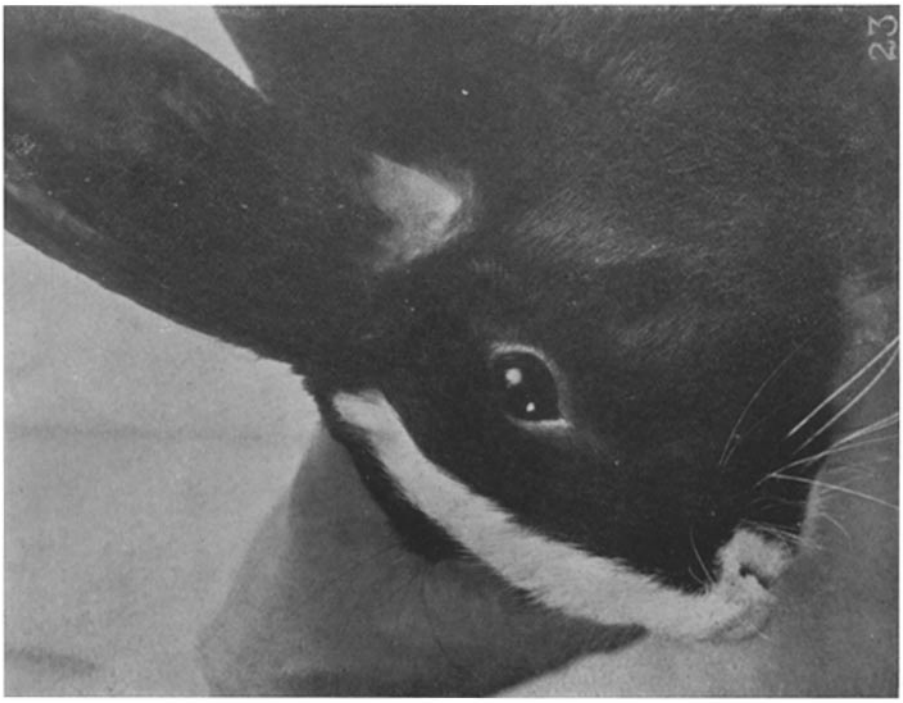

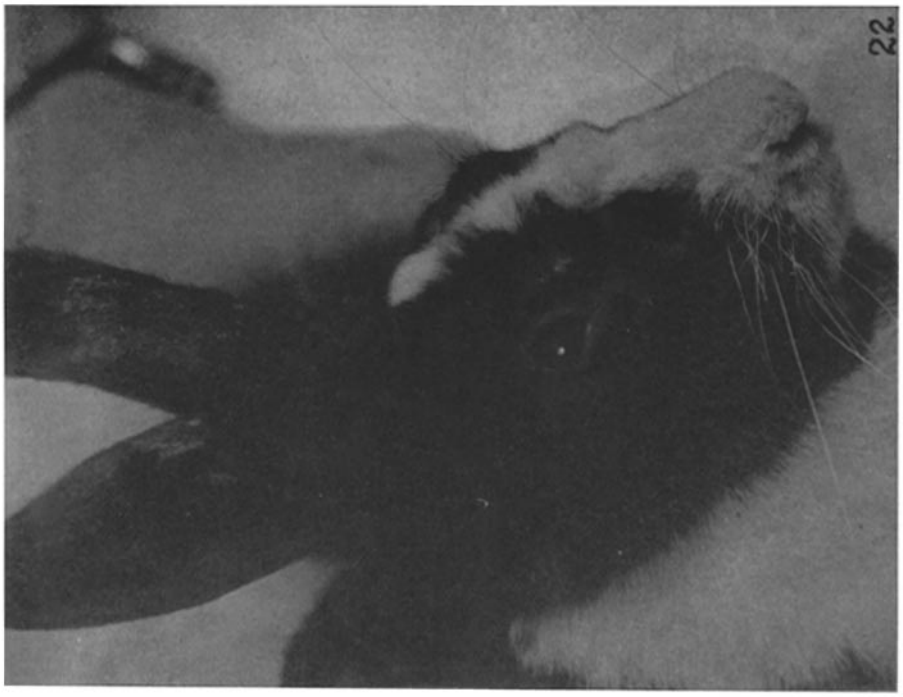

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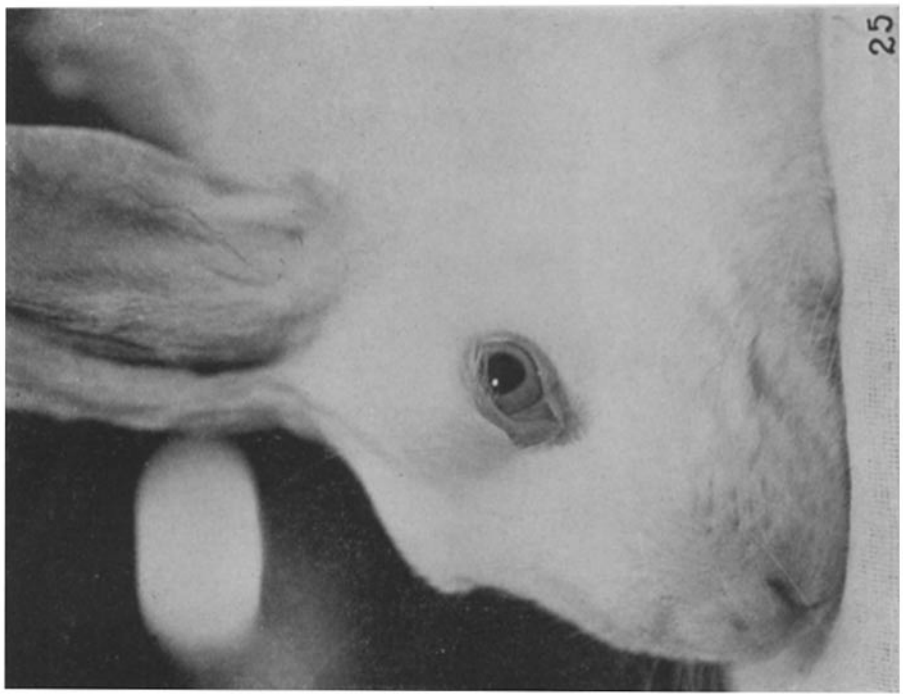
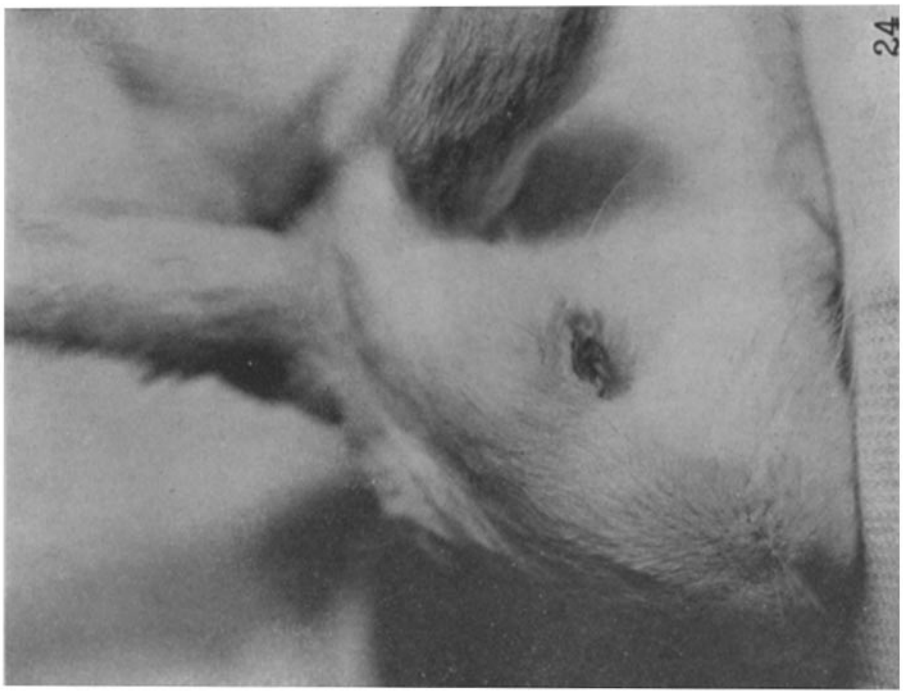
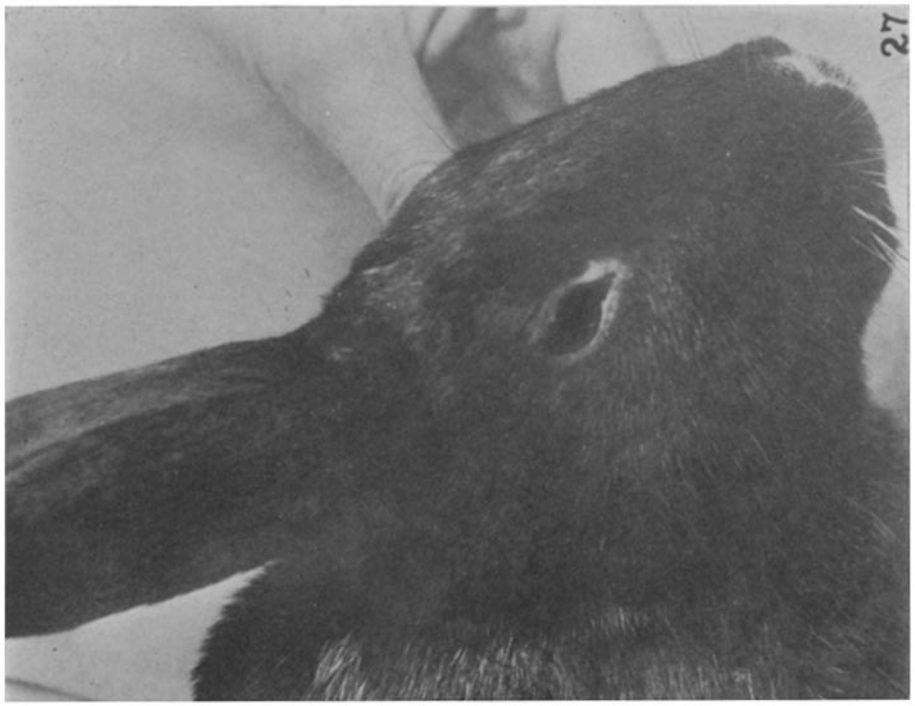
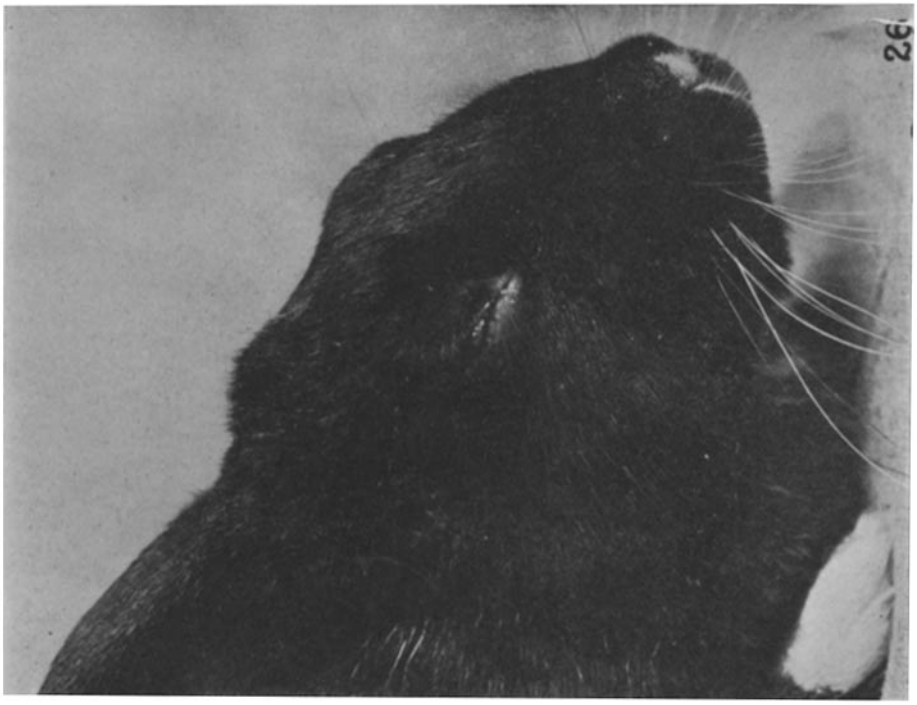




(Pearce and Brown: Trypanosome and spirochete infections.)





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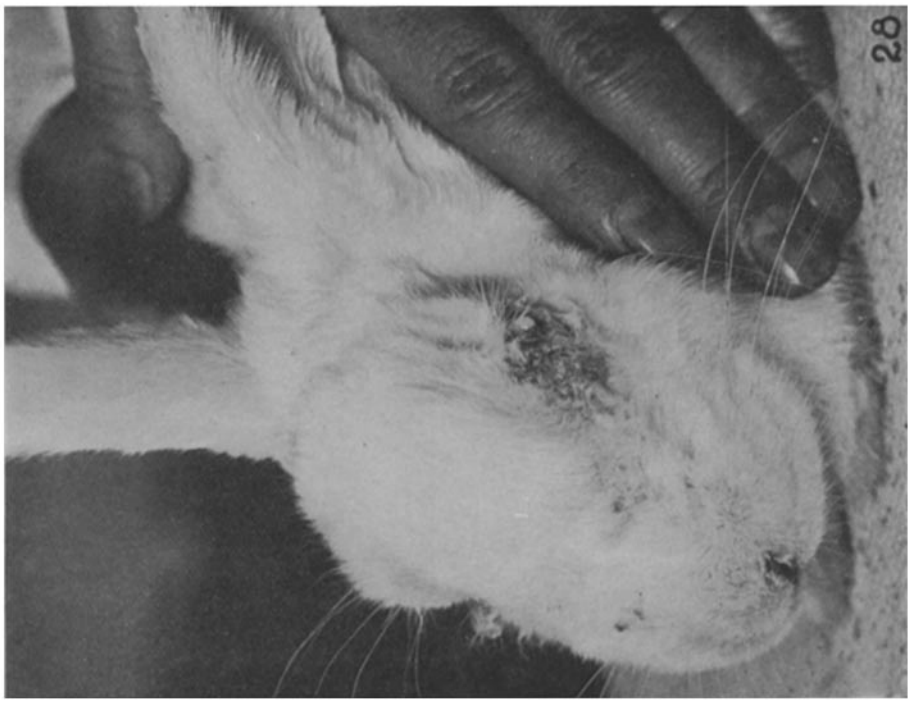

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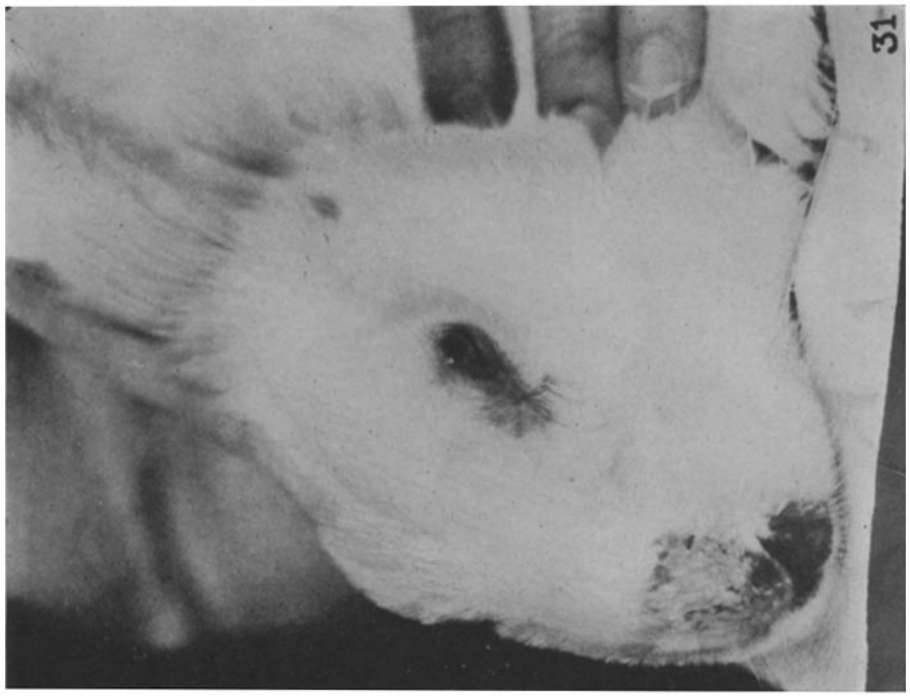

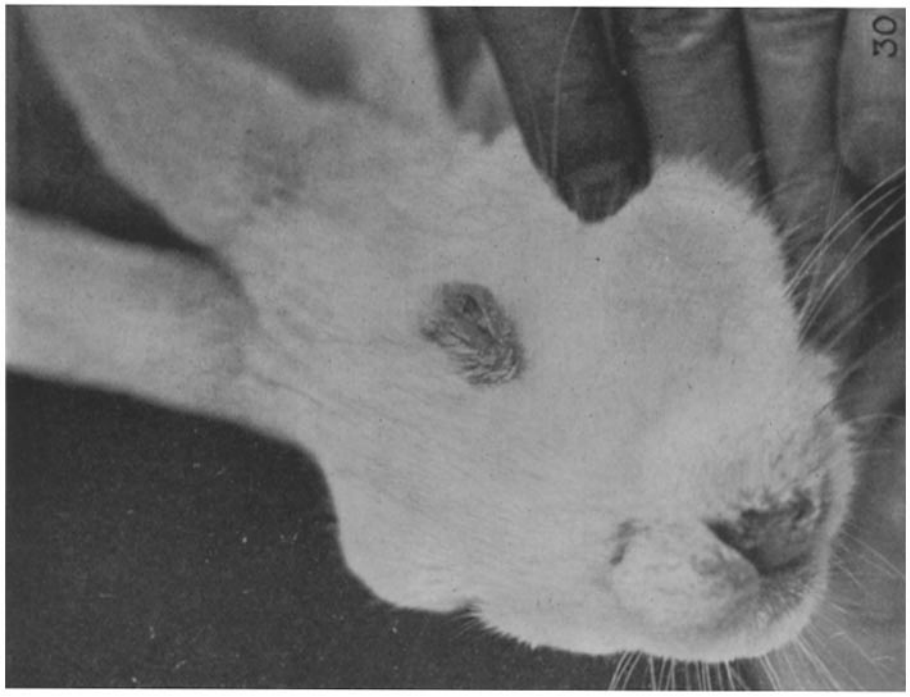

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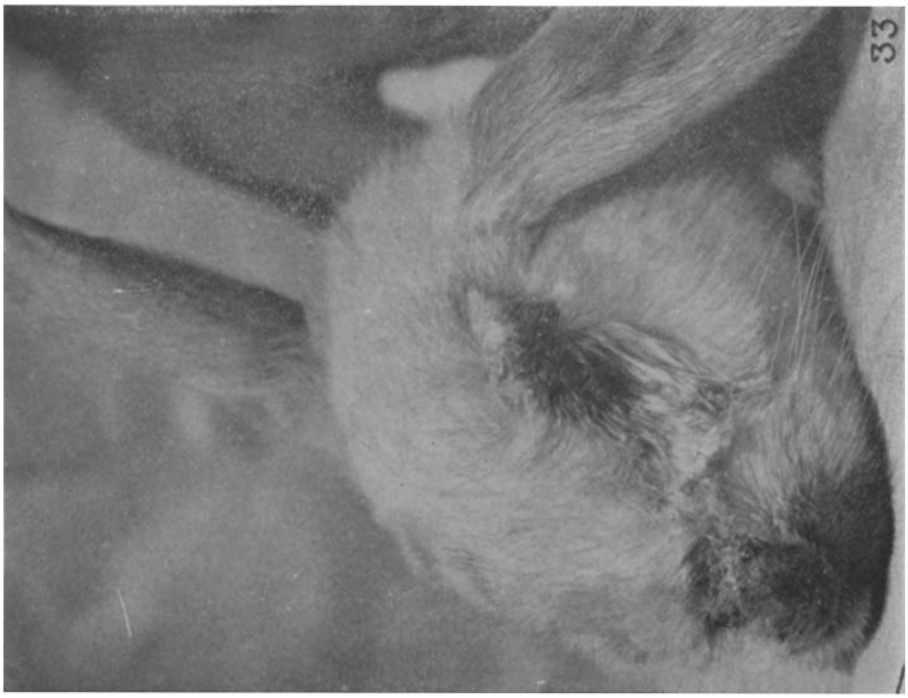

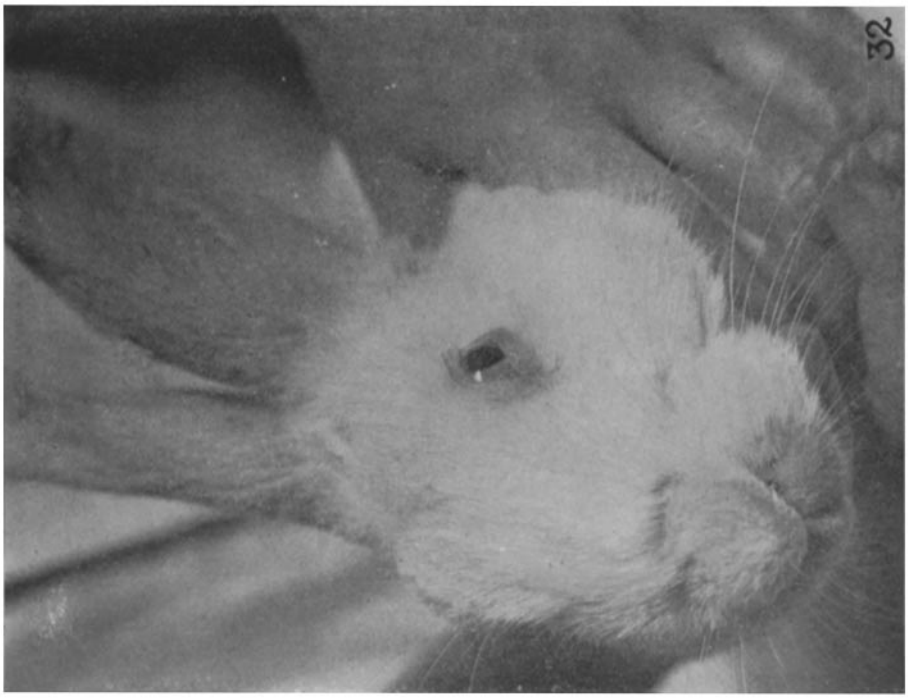

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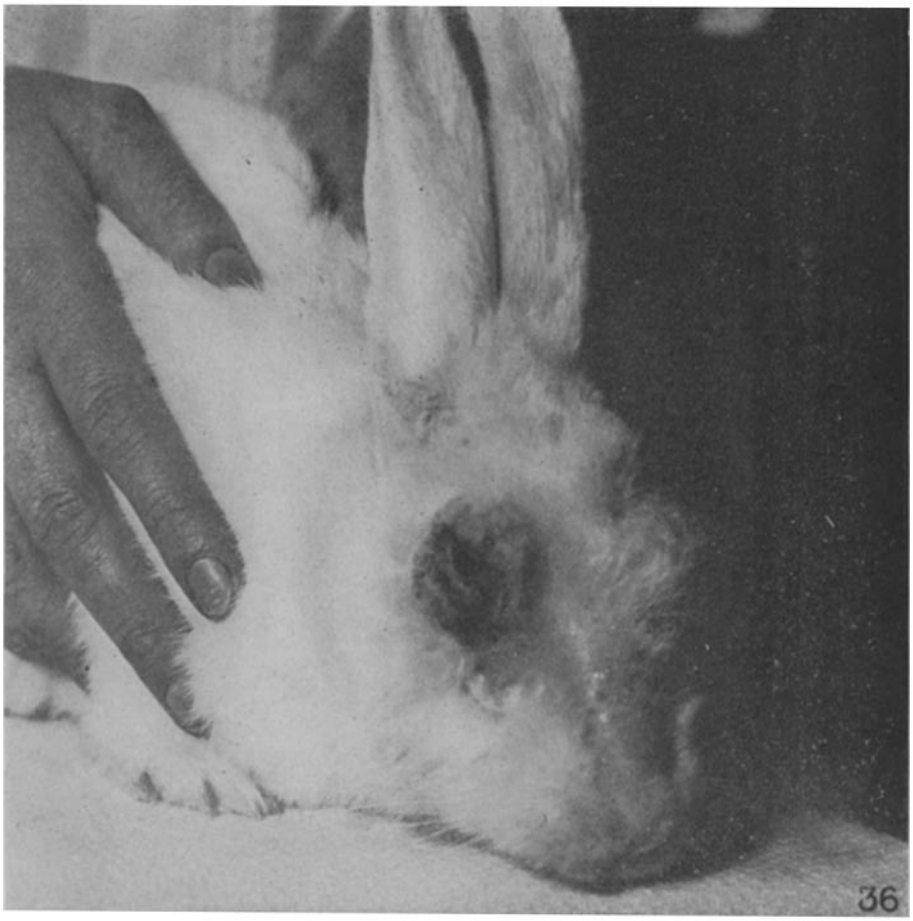

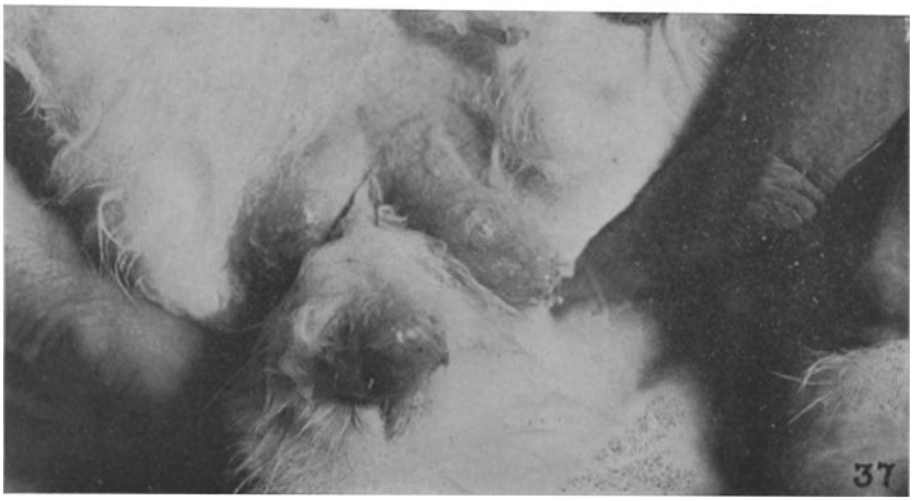

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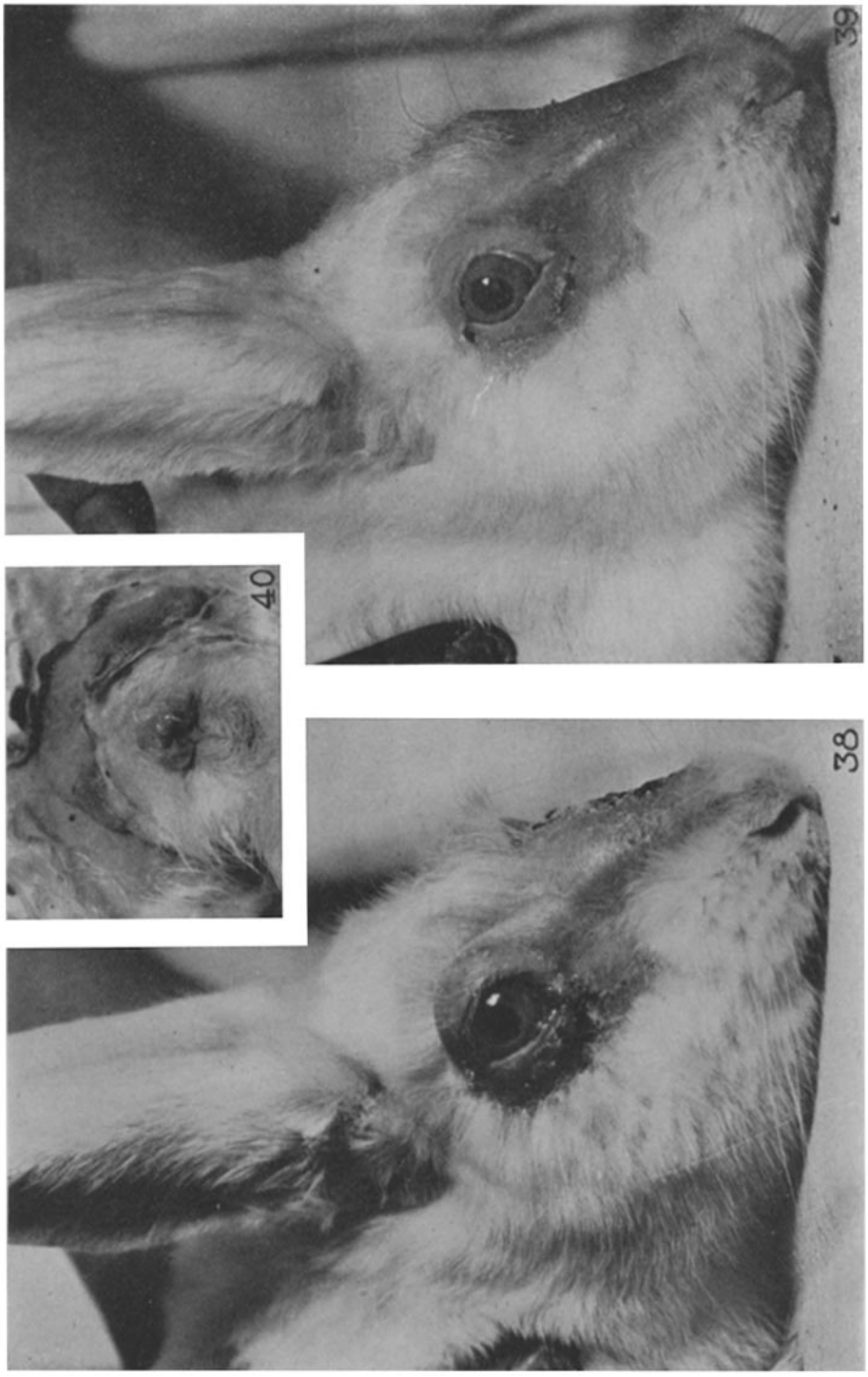

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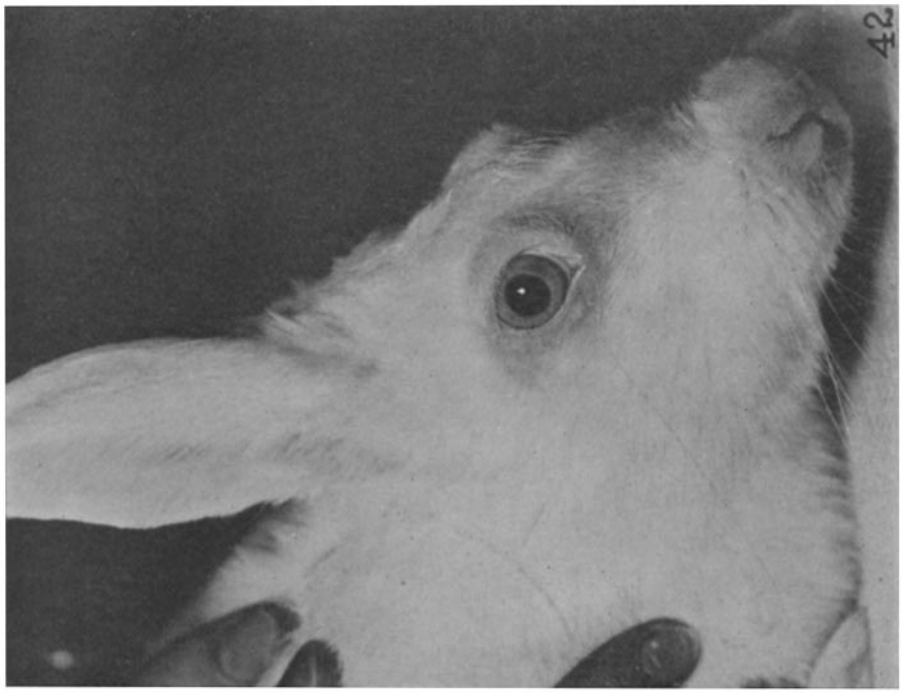

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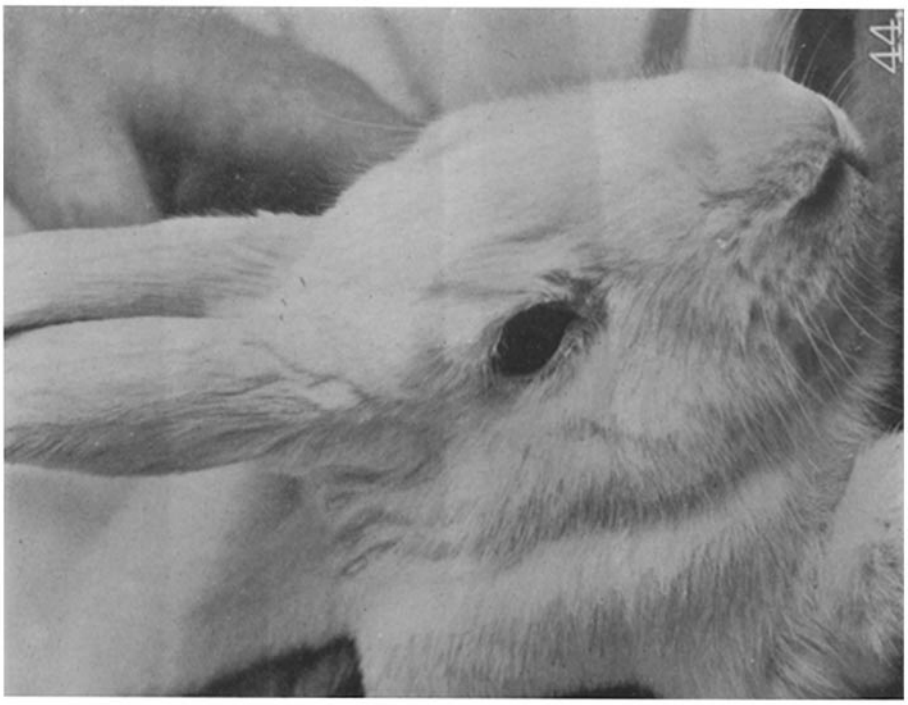

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