CHEMO-THERAPEUTIC TRYPANOSOME STUDIES WITH SPECIAL REFERENCE TO THE IMMUNITY FOLLOWING CURE.

By

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INTRODUCTION.

Immunity Following Treatment.—In 1904, Ehrlich and Shiga discovered an interesting method for producing immunity. On curing, by one or more injections of trypanred, mice infected with the parasites of caderas, they found that these could no longer be acutely infected if reinoculated with the same trypanosomes. As a result of the cure an immunity had developed. No signs of immunity, however, were observed among the untreated mice. There was never a spontaneous cure or even a chronic course of the disease.

Protection not Due to the Dye.—That the resistance to infection manifested by the treated mice was not due to unexcreted medication, Ehrlich and Shiga proved by treating normal mice and subsequently inoculating them with trypanosomes. They found that injections of virus made as early as one and two days after treatment could infect. In these cases, however, the incubation period might last eighteen days or longer. After the second day the protection due to the dye diminished rapidly, and in one of their

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Many of the results here given in detail were briefly reported on May 26, 1909, under the title "Immunity to Various Species of Trypanosomes Induced in Mice by the Cure of Experimental Infections," Proc. of the Soc. for Exper. Biol. and Med., 1909, vi, 118.

tables it is seen that mice inoculated on the fourth, fifth, or sixth day after treatment became infected as quickly as their normal controls, and died either at the same time or only one or two days later than these animals.

The Immunity Inefficient.—The immunity which followed cure was not, however, efficient. Even when mice were reinoculated as early as one to seven days after the curative treatment, none of them survived for any length of time. Two died negative for trypanosomes at dates too early to exclude the possibility of relapses, and, after incubation periods of twelve to fifty-three days, all of the others became infected and died.

The Immunity Temporary.—Their experiments also indicated that the immunity was of short duration. The sooner the tests were made after the curative treatment, the longer, as a rule, was the incubation period. Twenty days after the treatment the delay in infection was slight, and by the thirtieth day it was scarcely noticeable.

Loss of Immunity to Explain Relapses.—As the immunity was temporary, Ehrlich and Shiga used this fact to explain the relapses which occasionally followed the use of trypanred. Twenty, thirty, and, in one case, sixty-three days after curative treatment, the parasites reappeared. In these cases the organisms usually increased rapidly in number and soon brought about the death of the infected animal. If the reappearance of the trypanosomes coincided with the disappearance of the immunity, the duration of the latter varied from less than twenty to more than sixty days.

Duration of Effectual Immunity.—To Franke it seemed not improbable that an immunity sufficient to prevent a relapse might be present long after the animals had become susceptible to infection when inoculated with fully virulent trypanosomes. In order to determine the duration of the “effectual” immunity, Franke inoculated a series of mice with caderas, treated them, and, at varying intervals after treatment, repeatedly reinoculated them with the same parasites until infection was manifest. When thus tested, the duration of the immunity was found to be quite constant. It lasted only eighteen to twenty days.

Attempts to Prolong the Immunity.—Ehrlich and Shiga attempted to prolong the immunity to caderas by repeatedly infecting and curing mice in the course of a number of weeks. Their efforts in this direction, were, however, not very successful. In table X of their experiments, the longest interval between the disappearance of the trypanosomes and their reappearance is twenty-four days.

Immunity to Nagana.—Subsequently, Ehrlich studied the immunity which followed the cure of nagana, the virulence of which had been greatly increased by passage for years through mice. He found that the duration of immunity to this infection could sink to ten days.

Immunity Factors.—According to Ehrlich, the duration of the immunity depends upon two factors: (1) the height of the immunity reached, and (2) the virulence of the trypanosomes employed to overcome the immunity. The higher the virulence of the organisms, the shorter the immunity; and the lower the virulence, the longer the animals resist infection.

The Immunity Specific.—After confirming the discovery of Ehrlich and Shiga that an immunity follows the cure of caderas, Halberstaedter found that this immunity is specific. A mouse cured of caderas lost none of its susceptibility to dourine, and on being cured of dourine, it could be infected with nagana as readily as a normal animal.

Reaction Delicate.—Although recognizing that the reaction is specific in the sense that animals cured of one species acquired an immunity to that but none to other species, Ehrlich was not of the opinion that this reaction would suffice to show that given strains of trypanosomes belonged to different species. For this purpose it was apparently too delicate. After having rendered strains of trypanosomes of common origin resistant to various medicaments—atoxyl, fuchsín, trypanblue—Ehrlich found that the reaction enabled him to distinguish these strains. But in these


cases, also, the immunity was inefficient. Every test infected. Nevertheless, the immunity phase was always most striking for the strain with which the previous inoculation had been made.

The preceding summarizes the main points learned about the therapeutic immunity reaction before March, 1907, when, at the suggestion of Dr. Felix Mesnil, the problem of determining whether the reaction might serve to differentiate species was begun in his laboratory at the Pasteur Institute, Paris. After August of the same year, this and other problems were carried on independently at the Rockefeller Institute. As the work has covered a period of nearly three years, it seems desirable to indicate the condition of the problem when the present study was begun, reserving for another part of this paper all mention of the newer contributions.

**The Reaction Little Studied.**—From the history already given, it will be seen that before March, 1907, the reaction had been but little studied and that satisfactory answers to the following questions had not been made. Is the reaction a general one? Is it always elicited following cure? Can its strength be so increased that it completely prevents infection? Is an immunity acquired more easily against the less virulent than against the more virulent trypanosomes? May the reaction be of service in detecting contaminations of virus? Will it show the close relationship of surra of India and surra of Mauritius? Does the immunity to one species ever protect against other species? Does the strength of the immunity to a given species differ with the medicament employed to effect the cure? How early following cure does the immunity appear? Can the influence due to the immunity be distinguished from that of the medicament in the days immediately following treatment? Is the immunity influenced in any way by injections of virus that fail to infect? Can the immunity be prolonged? And, finally, is the immunity following cure by means of medicaments distinct from that which develops in certain resistant animals?

The preceding and other questions concerning the therapeutic immunity reaction have interested me greatly and all have been touched upon in my experiments. Before giving the results of these, however, it is necessary to say something about the virus, the
medicaments, the technique employed, and the outlines adopted for recording the results.

Virus.—In the following experiments were employed the trypanosomes of surra of India (T. evansi), surra of Mauritius, surra of Nhatrang, caderas (T. equinum), dourine (T. equiperdum), and nagana (T. brucei). The parasites of these infections had never been in contact with medicaments of any sort and will be referred to as normal trypanosomes to distinguish them from three nagana strains of common origin which had been rendered resistant to treatment. One of the latter was resistant to paraffuchsin, another to toluidin blue, and a third to both atoxyl and acetyl-atoxyl (arsacetine). The normal trypanosomes I owe to the kindness of Dr. Felix Mesnil, while for the resistant strains I am indebted to Dr. Paul Ehrlich. In preserving the virus for immunity tests, mice were employed almost exclusively.

Pathogenicity.—All of the above mentioned trypanosomes were exceedingly pathogenic. When one quarter of a cubic centimeter of a suspension containing five or more parasites per field was introduced intraperitoneally into mice, with very few exceptions these animals were visibly infected within twenty-four hours and died between the third and the fifth day after inoculation. When, however, the parasites were injected subcutaneously, infection was slower in becoming manifest, unless a relatively larger number of trypanosomes was introduced. Nevertheless, the ultimate result was the same in all cases. During the time the virus was preserved in mice, a recovery in an untreated animal was never seen.

The Medicaments.—The therapeutic agents employed were amidonaphtol disulphonic acid 1.8.3.6 plus dichlorbenzidine (symbol CL), acetyl-atoxyl (arsacetin, symbol AA), trypanred (Trypanrot, symbol TR), arsenophenylglycin (symbol APG), and various combinations of acetyl-atoxyl and dichlorbenzidine. For the dichlorbenzidine employed in these experiments I am indebted to Drs. Mesnil and Nicolle and to the Farbenfabriken, formerly F. Bayer and Co., Elberfeld, for the trypanred, to Dr. Simon

'The dichlorbenzidine from Elberfeld possessed trypanocidal properties comparable to that obtained from Paris, but was less easily soluble and the solution was blue, whereas the Paris dichlorbenzidine was a bluish purple. As the work
Flexner; and for the acetyl-atoxyl and arsenophenylglycin, to Dr. Paul Ehrlich.

Dosage.—In all instances the medicaments were weighed out and so diluted with distilled water (this was the only solvent employed) that in one cubic centimeter was contained the calculated dose for a mouse weighing twenty grams. Mice weighing more or less than twenty grams received correspondingly larger or smaller injections. The dose for a mouse weighing eighteen grams was .9 of a cubic centimeter, that for one weighing twenty-four grams was 1.2 cubic centimeter, etc. The strength of the solutions employed are indicated in the outlines which accompany the descriptions of the experiments.

In all instances the medicaments were injected under the skin of the back. To prevent loss by leakage, following each treatment a small spring hemostat was applied for fifteen to twenty seconds to a fold of skin at the site of inoculation.

Sterilization.—Before the aqueous solutions of the medicaments were introduced, they were placed in sterile test tubes and the latter were immersed in boiling water for two to five minutes. In the case of arsenophenylglycin the technique was slightly modified. The distilled water necessary for the dilution was sterilized, and while it was still almost boiling hot the arsenophenylglycin was dropped into it. The water was stirred, cooled rapidly to about 37° C., and injected at once.

The rapidity with which the trypanosomes disappeared after treatment varied with the medicament and with the strength in which this was employed. It took place in twenty-four to forty-eight hours after .5 per cent. trypanred and .2 per cent. arsenophenylglycin; in a little more than twenty-four hours in the majority of cases after 1 per cent. dichlorbenzidine (not infrequently in less than twenty-four hours, never after forty-eight); and in less than twenty-four hours after 2.5 per cent. to 4 per cent. acetyl-atoxyl, .5 per cent. to .6 per cent. arsenophenylglycin, and mixtures 1, 3, and 4. For the composition of the mixtures see the explanation of the outlines.

had been begun with the Parisian dichlorbenzidine, and as enough of this was at my disposal for the experiments, the medicament from Elberfeld was used but little.
Relapses were rarely seen after treatment with the above medicaments and then only after injections of dichlorbenzidine. The favorable results are attributed to the great efficiency of the arsenic preparations and the mixtures, to the fact that the dyes were employed by themselves only against the trypanosomes known to be susceptible to them (i.e., dichlorbenzidine in the treatment of the surras, and trypanred in the treatment of caderas), and in part to the prevention of leakage after treatment.

**Criterion of Cure.**—Mice which remained negative for fifty consecutive days following a curative treatment with one of the above medicaments are regarded as cured. Nevertheless, to be perfectly sure, the blood examinations were continued long after the fiftieth day, and the majority of the animals were kept under observation for six months. While keeping the mice for one hundred and eighty days may be useful in determining the toxic effects of the medicaments employed, this is not necessary to make certain of the cure. Relapses usually take place in the first three weeks, and in my experiments the longest observed interval between the disappearance of the parasites and their reappearance has been thirty days.

The records in some instances are incomplete, for on leaving the Pasteur Institute in August, 1907, observation of a number of animals had to be discontinued. Concerning their subsequent history I can make no statement, but in my outlines and descriptions the number of days these animals were followed is indicated.

**The Outlines.**—The influences capable of affecting the immunity following cure are so numerous that an adequate analysis of the results necessitated the finding of some form by which these factors could be briefly yet clearly expressed. After many trials, outlines were devised which proved so helpful that it seemed desirable to employ them in reporting my results. It was found, however, that a number of changes had to be made in order to adapt them for printing. In making these alterations, Dr. W. H. Manwaring rendered invaluable assistance.

**Explanation of Outlines.**—The outlines are read from left to right, each line representing a different mouse, no other animal being employed in the immunity tests. In order to avoid the printing of common fractions, these, in each instance, have been con-
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Verted into the nearest decimal, for example, \( \frac{1}{2} = 0.5 \), \( \frac{1}{3} = 0.3 \), \( \frac{1}{4} = 0.3 \), \( \frac{1}{6} = 0.2 \), \( \frac{1}{7} = 0.1 \), etc. The abbreviations are as follows:

**VIRUS.**

**NORMAL.**
- \( \text{CD} \) = caderas.
- \( \text{DN} \) = dourine.
- \( \text{NG} \) = nagana.
- \( \text{SI} \) = surra of India.
- \( \text{SM} \) = surra of Mauritius.
- \( \text{SNH} \) = surra of Nhatrang.

**RESISTANT.**
- \( \text{NG[A]} \) = nagana resistant to atoxyl and acetyl-atoxyl (arsacetine).
- \( \text{NG[P]} \) = nagana resistant to parafuchsin.
- \( \text{NG[T]} \) = nagana resistant to toluidin blue.

**MIXED.**
- \( \text{SI+CD} \) = surra of India mixed with caderas.
- \( \text{SI+SM} \) = surra of India mixed with surra of Mauritius.

**SEPARATED.**
- \( \text{CD[I]} = \text{CD} \) separated by the immunity reaction from SI.
- \( \text{SI[C]} = \text{SI} \) separated by the immunity reaction from CD.
- \( \text{SI[M]} = \text{SI} \) separated by the immunity reaction from SM.
- \( \text{SM[I]} = \text{SM} \) separated by the immunity reaction from SI.

**MEDICAMENTS.**

**ARSENIC PREPARATIONS.**
- \( \text{AA} \) = acetyl-atoxyl, or arsacetine.
- \( \text{APO} \) = arsenophenylglycin.

**DYES.**
- \( \text{CL} \) = dichlorbenzidine plus amidonaphtol disulphonic acid 1.8.3.6.
- \( \text{TR} \) = trypanred.

**MIXTURE OF MEDICAMENTS.**
- \( \text{Mx}1 \) = equal volumes of \( \text{AA} \) 2 per cent. and \( \text{CL} \) 1 per cent.
- \( \text{Mx}3 \) = equal volumes of \( \text{AA} \) 2 per cent. and \( \text{TR} \) 0.5 per cent.
- \( \text{Mx}4 \) = three volumes of \( \text{AA} \) 2 per cent. and one volume of \( \text{CL} \) 1 per cent.
Each of the above abbreviations stands for an injection of the corresponding virus or medicament. The day after the first injection of virus or medicament is regarded as the first day of the experiment.

**SIGNS.**

L (large or small) = living. The number of days lived are indicated by figures to the left of the L. "L shows that the mouse was still alive on the 64th day.

D(large or small) = dead. The day of death is indicated by the figures to the left of the D. Where only one test was made, the course of infection dating from this test is shown to the right of the D. In 'D 8 ', the 8 shows that the mouse died on the eighth day, and the 1–5, that the animal became infected on the first day after the immunity test and died on the fifth day after this test.

o = animal microscopically negative (at least twenty fields examined). A few negative examinations are omitted. The day or days upon which examinations were made is indicated directly above the result of these. The same result on intervening days is shown by a dash connecting two numbers. 1-35 indicates that the mouse was microscopically negative from the first to the thirty-fifth day.

+= animal microscopically infected. The number of parasites present is not indicated except when the sign appears immediately to the left of the treatment, e. g., 'CL, or to the right of the virus, e. g., SM++'.

In these two positions, + = less than five trypanosomes per field; ++ = five to twenty trypanosomes per field; +++ = more than twenty trypanosomes per field. Thus, '+CL' shows that the mouse had 5 to 20 trypanosomes per field when it was treated on the third day with CL, and the single plus in SM++, indicates that the parasites injected were less than 5 per field. On the other hand, '+' the mouse is shown to have been infected from the fourth to the seventh day, but the number of parasites is not indicated.
— no examination or no control, according to position. Occurring in the course of infection, it means no examination; but appearing to the right below of the virus or treatment, it means no control.

Examples:
- = no examination on the 6th day.
Cl. = no control on the treatment with Cl.
SM = no control on the injection of SM.

DETAILS ASSOCIATED WITH THE VIRUS.

To the left above, the day of injection.
To the left below, the result of blood examination on same day.
Example: SI = this mouse was inoculated with SI on the 11th day, on which, previous to the inoculation, the microscopical examination had been negative.

To the right above, three points in the following order:
(1) the quantity of virus introduced (.1 = one tenth c.c., .2 = two tenths c.c., etc.);
(2) the mode of inoculation (s = subcutaneous, i = intraperitoneal);
(3) the number of parasites per field in fresh specimens about one red blood corpuscle in thickness (Zeiss, lens "D," ocular No. 4).

Examples: SI = .1 c.c. injected subcutaneously, the suspension containing 2 surra of India trypanosomes per field.
SI = .3 c.c. injected intraperitoneally, the parasites being 15 per field.

To the right below, the course of the virus control (or controls). Three points are indicated:
(1) The day infection was manifest, shown by initial number.
(2) The nature of the course, — = regular, and *= irregular (i.e., the mouse subsequently becoming spontaneously negative for one or more days).
(3) The day control died, shown by final number.

Examples:
SI = the control on the virus was positive on the first day, pursued a regular course, and died on the third day.
the first control was positive on the fifth day, had a regular course, and died on the 20th day; the second control was also positive on the fifth day, but pursued an irregular course, and did not die until the 217th day.

DETAILS ASSOCIATED WITH THE TREATMENT.

To the left above, the day on which it was injected.
To the left below, the blood examination on same day.

Examples: 

- :AA: = on the first day, the trypanosomes being less than 5 per field, the mouse was treated with acetyl-atoxyl.
- :CL: = on the fourth day, the trypanosomes being more than 20 per field, the animal was treated with CL.

To the right above, the strength of the solution employed (.5 per cent. = five tenths per cent.).

To the right below, the control on the treatment.

Examples:

- :AA: = two per cent. AA was injected on the first day and 1 per cent. CL on the 6th day; the control on this double treatment remained negative and was alive on the 180th day.
- :CL: = the control on an injection of 1 per cent. CL remained negative until its death on the 7th day.
- :CL: = the first control was negative to the 44th day and died on the 48th, no examination being made after the 44th day; the second control relapsed (+) on the 19th and died on the 21st day.

INTERVAL BETWEEN TREATMENT AND TEST.

This is so important that it is indicated by a large figure placed between the symbol for the treatment and that for the injection of the virus. The figure shows the number of days separating the two injections. For an example see the large 3 in the first outline given on page 14.

Outlines Illustrated.—The explanation of a few outlines will probably render the reading of the others quite easy. As examples, two prophylactic and three immunity experiments have been chosen. The explanation of each will be given immediately after its outline.
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In the above prophylactic test, the mouse was given an injection of 1 per cent. \( \text{CL} \) and, after an interval of three days, was inoculated with surra of India, receiving .3 c.c. intraperitoneally, the parasites being fifteen per field (.3 i 15). The virus control was positive on the first and dead on the third day (1-3). In the experimental animal following this inoculation, trypanosomes were found on the fourth, fifth, sixth, and seventh days \( \cdot \cdot \cdot \), the animal dying on the eighth day, \( \text{D} \). In order to facilitate comparison with the virus control, the course of infection in the experimental animal, calculated from the injection of the virus, is shown to the right of the \( \text{D} \). In this position 1-5 indicates that the experimental mouse was positive on the first and dead on the fifth day after the test.

In this prophylactic experiment an injection of acetyl-atoxyl 3 per cent. was followed five days later by an inoculation of 1 per cent. \( \text{CL} \). Three days after the last treatment the animal was tested with surra of Mauritius, receiving .3 c.c. intraperitoneally, the parasites being thirty per field. On the following day (the ninth), trypanosomes were detected in its blood and were seen also on the tenth and eleventh days, but from the twelfth to the eighteenth, the mouse was microscopically negative, and was found dead on the nineteenth day. Calculated from the injection of the virus, this animal was positive on the first and dead on the eleventh day (1 * II). The star between the I and the II to the right of the \( \text{D} \) shows that the course of infection was irregular. The control on the virus was positive on the first, and dead on the fourth day (1-4).

In the initial inoculation with surra of India the mouse received .1 c.c. subcutaneously, the parasites being eleven per field. On the third day, the trypanosomes being fairly numerous (i.e., five to twenty per field), this animal was treated with 1 per cent. \( \text{CL} \). It became negative and fifteen days later (18th day) the mouse was tested for its immunity by receiving subcutaneously .3 c.c. of a suspension containing four parasites per field (.3 s 4). From the 20th to the 35th day the blood remained negative and the animal died on the 62d day, having survived the virus injection by 44 days. There were three controls, two on the virus and one on the treatment. The virus controls were positive on the second day, but the first died on the fourth, the second on the fifth day. The treatment control became negative, remained so until the 44th day, and died on the 48th. No examination was made after the 44th day.

In this instance a mouse infected with surra of India was treated on the first day with \( \text{CL} \) and 16 days later was tested for immunity. As it resisted infection, a second test was made on the 28th. This also failed and the mouse was alive.
on the 160th day. All of the inoculations were controlled. On the initial injection of SI there were two controls. One was positive on the first and dead on the fourth day; the other was infected on the second and dead on the fifth day. The control on the treatment became negative and remained so until its death on the 52d day.

In the final example, we note that the mouse received a rich inoculation of caderas, and 24 hours later, the trypanosomes being few, it was treated with trypanred .5 per cent. Four days later it was first tested for immunity. As no infection took place, the virus was reinjected on the 8th, 11th, 14th, 17th, 20th, 23d, 29th, 32d, 46th, 49th, and 52d days. Following the last inoculation the mouse became infected and died on the 56th day. This animal was examined daily, but was continuously negative from the disappearance of the parasites following treatment to the 53d day. Many of these negative examinations were omitted from the outline in order to save space.

**SURRA OF INDIA.**

**PROPHYLACTIC EXPERIMENTS.**

All of the tests for immunity, subsequently to be described, were made in animals that had been injected with one or more medicaments. If the influence due to the latter is to be excluded, it is necessary to know, (1) in what way unexcreted medicament can affect subsequent inoculations of virus, and (2) how long this influence may be manifested. In order to secure this information, prophylactic experiments were carried out with various forms of treatment and with different species of trypanosomes. The results are given in full, for in them is to be found the means of differentiating protection due to immunity from that due to treatment alone.

*Dichlorbenzidine.*—In the prophylactic experiments with Cl and surra of India, infection was prevented, delayed, rendered irregular, or prolonged.

*The efficiency of Cl in the prophylactic and curative treatment of mice infected with surra of India was first pointed out by F. Mesnil, and M. Nicolle in an article entitled, "Traitements des trypanosomiases par les couleurs de benzidine." Seconde partie.—Etude expérimentale," *Ann. de l’Inst. Pasteur*, 1906, xx, 513.*
**Infection Prevented.**—Infection was completely prevented only when the inoculations of virus were made close to the treatment, that is, on or before the third day. It was observed five times (mice 1, 2, 3, 5, and 6). On the other hand, after the third day every injection of virus infected and killed. While the interval between treatment and test is undeniably very important, it is not the only factor in determining whether or not infection will take place. The number of parasites introduced and the method of inoculating these must also be considered. We observe that with one exception all of the injections that failed were subcutaneous, and that in the single intraperitoneal inoculation (mouse 5) the trypanosomes introduced were few. On the other hand, when the parasites were numerous and were injected intraperitoneally, infection took place even on the first day after treatment with Cl (see mouse 4).
Infection Delayed.—Delayed infection was the least characteristic sign of unexcreted CL. It was observed but twice. This influence, unlike the preceding, was not seen in the tests made very close to treatment, for three inoculations before the fourth day (mice 4, 7, and 8) infected within twenty-four hours. The injections which infected after their controls, were given on the fourth and fifth days (see mice 9 and 15). We note that in these two cases the trypanosomes introduced were comparatively few, and that infection was not greatly delayed. It took place one and two days respectively after the controls. It seems quite possible that the delay in these two cases was not entirely due to unexcreted medicament for in two other mice (16 and 17) infection took place one day sooner in the experimental animals than in their controls.

Irregular Infections.—In four instances after infection had become manifest the parasites disappeared again (mice 4, 7, 10, and 11). These irregular courses were observed only in animals inoculated comparatively close to the treatment, i.e., from the first to the fourth day after the CL. Three of the mice (Nos. 7, 10, and 11) relapsed, and the remaining one (No. 4) died early. The number of days that each remained infected before becoming negative, varied with the interval that separated the tests from the treatment. The mouse (No. 4) inoculated one day after treatment was positive for one day only, another (No. 7) tested three days after treatment was positive for five days, and in the tests made four days after the CL, one (No. 10) required five, the other (No. 11), seven days to become negative. The disappearance of the parasites in these animals is probably to be attributed to the formation of immune bodies.

Prolonged Infection.—Of the eighteen mice which became positive, thirteen survived their controls. Prolonged infection is, therefore, by far the most frequent manifestation of unexcreted medicament. In some cases the experimental animals outlived their controls, by one or two days only; in other instances the death of the former did not take place until six to thirteen days after the latter. As might be expected, the longer courses were usually seen in the mice inoculated comparatively early after treat-
ment, i. e., from the first to the fifth day (see mice 4, 7, 9, 10, 11, 12, and 13). Nevertheless, infection was prolonged following tests made as late as the seventh day after treatment: In fact, the influence of the medicament was apparently stronger on this than on the preceding day, for in the four tests on the sixth day, all of the animals died as quickly as their controls, while in the two inoculated on the seventh, death was delayed one to two days.

**Trypanred.**—When mice treated with trypanred were inoculated with *Surra* of India three, four, and five days later, every test infected and in each instance the parasites appeared within twenty-four hours after the inoculation of the virus.

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It is interesting that here also there was an apparent fluctuation course of infection was prolonged four days in the mouse tested three days after the injection of trypanred, and one day in the animal inoculated on the fifth day after treatment. On the other hand, the mouse injected on the fourth day died as quickly as its control.

**Acetyl-Atoxyl.**—In the prophylactic experiments with acetyl-atoxyl and *Surra* of India, the tests were made two to seven days after treatment. That some of the inoculations were close enough to the medicament to be influenced by it, is shown by the fact that two injections on the third day (mice 2 and 3) were followed in the strength of the prophylactic action of the medicament. The by irregular courses. It is important to note, however, that in no case was the incubation period of the infected animals prolonged.

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<td>1</td>
<td>1</td>
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<td>7</td>
<td>6</td>
<td>1</td>
<td>1</td>
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<tr>
<td>8</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>10</td>
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</table>
One mouse, tested on the sixth day (No. 7), failed to become infected. The explanation of this is rendered all the more difficult by the fact that infection took place within twenty-four hours and terminated promptly in other mice tested six days after receiving a double injection of acetyl-atoxyl (see the following experiment).

**Acetyl-Atoxyl, Double Injection.**—It had been thought that a double injection of the acetyl-atoxyl might influence the tests more profoundly than a single one. There is nothing, however, in the following experiment to support this view.

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<tbody>
<tr>
<td>1.</td>
<td>AA²⁻²</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>AA²⁻²</td>
<td>5</td>
</tr>
<tr>
<td>3.</td>
<td>AA²⁻²</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>AA²⁻²</td>
<td>6</td>
</tr>
<tr>
<td>5.</td>
<td>AA²⁻²</td>
<td>7</td>
</tr>
</tbody>
</table>

All of the mice were infected on the day after inoculation, and not one survived the fifth day.

**Arsenophenylglycin.**—In the prophylactic experiments with arsenophenylglycin, we observe that the inoculations before the fourth day, either failed to infect (3 cases, mice 4, 5, and 6), or were delayed (one case, mouse 1). After the third day, however, **every test infected within twenty-four hours.**

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<tbody>
<tr>
<td>1.</td>
<td>APG⁻²⁻²</td>
<td>3</td>
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<td>2.</td>
<td>APG⁻²⁻²</td>
<td>5</td>
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<tr>
<td>3.</td>
<td>APG⁻²⁻²</td>
<td>7</td>
</tr>
<tr>
<td>4.</td>
<td>APG⁻⁶⁻²</td>
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</tr>
<tr>
<td>5.</td>
<td>APG⁻⁶⁻²</td>
<td>3</td>
</tr>
<tr>
<td>6.</td>
<td>APG⁻⁶⁻²</td>
<td>3</td>
</tr>
<tr>
<td>7.</td>
<td>APG⁻⁶⁻²</td>
<td>4</td>
</tr>
<tr>
<td>8.</td>
<td>APG⁻⁶⁻²</td>
<td>5</td>
</tr>
<tr>
<td>9.</td>
<td>APG⁻⁶⁻²</td>
<td>6</td>
</tr>
</tbody>
</table>

**Mixture I.**—In the mice tested with surra of India four to seven days after a single injection of Mixture I (equal volumes of acetyl-atoxyl 2 per cent. and Cl 1 per cent.), the course of infection was scarcely influenced. In one case infection was somewhat prolonged (mouse 2), but **in no instance was it delayed.**
Chemo-Therapeutic Trypanosome Studies.

1. Mxl 4 5
2. Mxl 5 6
3. Mxl 6 7
4. Mxl 7

Acetyl-Atoxyl Followed by Mixture 1.—After being treated first with acetyl-atoxyl, then with Mixture 1, the following two mice were tested with surra of India, three and five days respectively after the last treatment.

| 1 | AA₂₇₅ Mxl 3 5 | 2 | AA₂₇₅ Mxl 5 7 |

Both animals became infected, one (the first) only after a delay of five days, the other within twenty-four hours. As a delayed infection is characteristic of immunity, it is desirable that more than three days separate this particular form of treatment from the immunity tests, in order that the influence due to the treatment may be excluded. An interval of five days might be safe, for in the above experiment the animal thus tested became infected at once.

Mixture 1 followed by CL.—The prophylactic experiments with Mixture 1 followed by CL are of considerable interest for the reason that every test from the second to the eighth day showed the influence of the medicament.

| 1 | Mxl CL 9/16 2 5 | 2 | Mxl CL 9/16 3 6 |
| 3 | Mxl CL 1 4 7 |
| 4 | Mxl CL 1 5 8 |
| 5 | Mxl CL 4 9 |

In the mice inoculated two, three, and five days after the CL (Nos. 1, 2, and 4), irregular infections developed, and the first and the last of these animals recovered without further treatment. Although the virus inoculations were strongly influenced as late as the fifth day, we observe that the incubation period was in no case prolonged, for all of the mice were positive within twenty-four hours.

Mixture 1 Given Twice.—In all of the tests made three to six
days after the double treatment with Mixture I, the influence of the medicament may be seen.

1. Mx1 'Mx1 3 3°SI:1 2 9-30 3°D.' 4°°
2. Mx1 'Mx1 3 3°SI:1 2 6-8 9-11 12-26 3°D.' 4°°
3. Mx1 'Mx1 4 3°SI:1 2 + + + 3°D.' 4°°
4. Mx1 'Mx1 4 3°SI:1 2 1-6 8-9 9-11 3°D.' 4°°
5. Mx1 'Mx1 4 3°SI:1 2 8 9-12 3°D.' 4°°
6. Mx1 'Mx1 5 3°SI:1 15 1-6 9-10 3°D.' 4°°
7. Mx1 'Mx1 5 3°SI:1 15 8 9-11 3°D.' 4°°
8. Mx1 'Mx1 5 3°SI:1 15 9-30 3°D.' 4°°
9. Mx1 'Mx1 6 3°SI:1 15 9-11 3°D.' 4°°
10. Mx1 'Mx1 6 3°SI:1 15 10-11-16 3°D.' 4°°
11. Mx1 'Mx1 6 3°SI:1 15 10-11-16 3°D.' 4°°

In three cases (mice 6, 8, and 10) infection was regular but slightly prolonged, and in two (mice 1 and 9) it was entirely prevented. It is rather remarkable that one of the tests that failed (mouse 9), was made as late as the sixth day after the last treatment.

Although the influence of the medicament in this series was particularly strong and general, it affected the incubation period but little; for, of the animals tested subsequent to the third day, we note that only three (Nos. 3, 6, and 7) became positive after their controls, and in these cases the delay was only one day.

**HOMOLOGOUS IMMUNITY TESTS.**

**Strong Immunity after CL.**—When mice infected with surra of India were treated with a single injection of CL, they acquired an
immunity to the original infection. This is clearly shown in the preceding tests made five to seventeen days after the treatment.

In ten of the twelve mice, the immunity apparently prevented infection. Nevertheless, only four mice were followed long enough to exclude the possibility of a relapse (mice 1, 5, 7, and 8). Of the other six animals, three died early and three could not be kept under observation beyond the thirty-first or thirty-second day.

From mouse 3 we see that an immunity may also be acquired even when infection is apparently prevented. This animal received simultaneously an inoculation of virus and medicament and was never visibly infected. Nevertheless, it acquired a strong immunity, for an inoculation of surra of India on the sixth day after the CL failed to infect it.

How early the immunity to surra of India appeared in this series, or how long it lasted, can not be told with exactness. We note, however, that no test made before the tenth day infected and that one mouse inoculated as late as the fifteenth day after treatment remained negative.

Why did two of the animals become infected? In mouse 9 the immunity was probably overcome by the large number of parasites introduced, for it received the richest injection of trypanosomes in this series. In mouse 12, instead of one, there were apparently two factors: (1) a rich injection of parasites, and (2) a weakened immunity due to the long interval separating the treatment from the test.

The trypanosomes that appeared in mouse twelve on the twenty-ninth day showed not a trace of resistance to the medicament with which this animal had been treated, for on the thirtieth day, when the parasites were about forty per field, a single injection of CL caused the trypanosomes to disappear within twenty-four hours. The action of the medicament was, in fact, unusually rapid, for mice as richly infected as this often required a little more than twenty-four hours for the blood to become free of parasites.

It is important to observe that the two mice which became infected, did so only after a delay. In both, the incubation period was distinctly prolonged, mouse 9 becoming positive on the fifth day after inoculation (control on the first), and mouse 12 on the
The importance of the above observation will become evident if the incubation periods in the preceding and following tables are carefully studied, for we shall find that immunity nearly always delays infection, while, under similar conditions, unexcreted medicament does so only rarely.

That the immunity to surra of India develops very early after treatment with CL is shown by the following experiment.

<table>
<thead>
<tr>
<th>Test</th>
<th>CL Inoculum</th>
<th>Duration</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>4 days</td>
<td>Positive</td>
</tr>
<tr>
<td>2.</td>
<td>5</td>
<td>4 days</td>
<td>Positive</td>
</tr>
<tr>
<td>3.</td>
<td>5</td>
<td>4 days</td>
<td>Positive</td>
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<tr>
<td>4.</td>
<td>5</td>
<td>4 days</td>
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</tr>
<tr>
<td>5.</td>
<td>5</td>
<td>4 days</td>
<td>Positive</td>
</tr>
<tr>
<td>6.</td>
<td>5</td>
<td>4 days</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Of the tests made one, two, and four days after treatment (mice 1, 2, and 3), the first alone visibly infected. The failure of those made on the second and fourth days is attributed to the presence of immunity, for prophylactic experiments have shown that a rich intraperitoneal inoculation of surra of India may infect when given as early as twenty-four hours after CL. From the richness of the injections employed on the second and fourth days, it seems almost certain that the prevention of infection in these two mice was not due to unexcreted medicament, but to the development of immunity.

Furthermore, the early appearance of immunity is apparently shown by the mouse which became infected (No. 1), as well as by those that remained negative, for the first animal was positive for one day only, then recovered and was found to be immune when retested on the sixteenth day. It is very probable that in both the first and the second animals an immunity was present by the third day after treatment.

Following a single injection of CL, an immunity to surra of India was present in three mice tested as late as the twenty-eighth day (twenty-seven days after treatment). In the case of two (mice 4 and 5), the immunity was shown by the delay in infection. These animals became positive after incubation periods of six and seven days respectively, while the control was infected on the second day. In the sixth mouse the immunity was stronger, for
infection was prevented altogether and the animal was still alive on the 160th day.

Attention is called to the fact that these six mice were twice tested with surra of India. Only one of the first and two of the second series of inoculations infected. The resistance to infection in this last series was so strong that it seemed possible that the first inoculations which failed, had prolonged the immunity. That they really did so, is highly probable from experiments given elsewhere in this paper.

Immunity after Acetyl-Atoxyl.—An immunity to surra of India was also acquired when a mouse was cured of this infection by acetyl-atoxyl. In the animal thus treated, parasites were found only after an incubation period of six days, while the control was positive on the first day.

1. $\text{SI}^{2,18}$, $\text{AA}^{2,56}$, 6

Although an immunity followed the cure with acetyl-atoxyl, it was surprisingly weak when compared with that produced by treatment with CL.

Weak Immunity after Arsenophenylglycin.—The immunity which followed treatment with arsenophenylglycin (.6 per cent.) was also disappointing.

1. $\text{SI}^{3,14}$, $\text{APG}^{4,6}$, 6
2. $\text{SI}^{3,00}$, $\text{APG}^{4,6}$, 8
3. $\text{SI}^{3,00}$, $\text{APG}^{4,6}$, 12

In the tests made six, eight, and twelve days after treatment, the incubation period of the first animal was distinctly prolonged, and that of the second, probably. In the third mouse, however, infection took place as quickly as in the control. From this we see that in these few experiments the immunity to surra of India was of short duration as well as weak.

Acetyl-Atoxyl Followed by CL.—In mice infected with surra of India the immunity which developed after treatment with acetyl-atoxyl followed by CL, resembled that produced by a single cura-
tive injection of CL, but was stronger than that seen after a single treatment with either acetyl-a toxyl or arsenophenylglycin.

1. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 7 SI\textsuperscript{3} 1\textsuperscript{5} 12 - 8 H. 150 L.
2. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 7 SI\textsuperscript{3} 1\textsuperscript{5} 13 - 90 H. 150 L.
3. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 11 SI\textsuperscript{3} 1\textsuperscript{5} 1 - 90 H. 150 L.
4. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 15 SI\textsuperscript{3} 1\textsuperscript{5} 2 - 30 D. 30 - 10 D.

Three of the tests failed to infect, and a fourth, on the twentieth day (15 days after the CL), had an incubation period of five days (control 1).

**Immunity to Surra of India Prolonged.**—Tests which fail to infect, or which infect only temporarily, may prolong the immunity. Evidence for this is found in the following experiment and in a number of others to be described later.

1. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 4 SI\textsuperscript{3} 1\textsuperscript{5} 20 - 21 D.
2. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 4 SI\textsuperscript{3} 1\textsuperscript{5} 20 - 29 D.
3. SI\textsuperscript{3} 1\textsuperscript{5} + AA\textsuperscript{3} 3\textsuperscript{6} Cl\textsubscript{1} 1\textsuperscript{6} L. 4 SI\textsuperscript{3} 1\textsuperscript{5} 34 - 15 D.

Nearly all of the inoculations in these three animals were intra peritoneal and given at intervals of three days. Two of the mice (Nos. 1 and 2) were positive on the day after the second test, but recovered spontaneously and each subsequently received four injections of surra of India. One became infected on the twenty-seventh day; the other remained negative, but died on the twenty-eighth. In the third animal the immunity was stronger. In spite of the fact that it received eight inoculations of surra of India, it remained negative, and died on the thirty-third day, apparently of a staphylococcus infection.

**HETEROLOGOUS IMMUNITY TESTS.**

Caderas.—In the mice cured of surra of India by CL, all of the tests with caderas infected. Nevertheless, the quickness with which the parasites appeared in these animals seemed to depend largely upon the method of introducing the virus. The intraperitoneal injections infected within twenty-four hours, while the mice
receiving the parasites subcutaneously, did not become positive until four to five days after their controls (mice 4, 5, 6, and 7).

1. SI. 51  
2. SI. 52  
3. SI. 53  
4. SI. 54  
5. SI. 55  
6. SI. 56  
7. SI. 57

That a rich intraperitoneal injection of virus may infect without perceptible delay, even when given close to the introduction of the CL, is clearly shown by mouse 1. Twenty-four hours after treatment, this animal was inoculated with caderas, became infected on the following day and remained so until its death.

The mouse cured of surra of India by acetyl-atoxyl was as susceptible to infection with caderas as the normal animal which served as the control.

1. SI. 58  
2. SI. 59  
3. SI. 60

Following the intraperitoneal injection of the virus, both animals were positive on the first and dead on the fourth day after the test.

Mice immunized to surra of India by acetyl-atoxyl and CL offered no resistance to infection with caderas when tested four, five, and seven days after the last treatment, for all were positive on the day after inoculation.

1. SI. 61  
2. SI. 62  
3. SI. 63

The irregular course of infection in the first and second animals is probably attributable to the influence of unexcreted medicament. The reason for so thinking is explained in what follows.

Influence of CL Prolonged by Acetyl-Atroxyl.—Although acetyl-atoxyl is excreted rapidly, there is considerable evidence to show that it is capable of prolonging the influence of CL introduced five or more days afterwards. A similar effect may often be seen when a mixture containing acetyl-atoxyl is followed by the injec-
tion of a mixture containing CL. The unexcreted medicament usually acts, not by preventing or delaying infection, but by enabling the mice to become negative again after infection is manifest. Often the disappearance of the parasites is only temporary, but in some cases the recovery is complete. Attention is now called to the influence exerted by acetyl-atoxyl, in order that this point may be looked for in the subsequent experiments.

*Nagana.*—Apparently no immunity to nagana was acquired when mice were cured of surra of India by CL, for the experimental animals became infected about as quickly as their controls.

1. \( \text{SI}^{n-} \quad \text{CL}_{10^{6}} \quad 8 \quad \text{NG}^{-8} \quad 12-15 \quad \text{D}^{1-3} \)
2. \( \text{SI}^{3-1} \quad \text{CL}_{10^{4}} \quad 9 \quad \text{NG}^{-1} \quad 12-14 \quad \text{D}^{1-3} \)
3. \( \text{SI}^{3-1} \quad \text{CL}_{10^{4}} \quad 17 \quad \text{NG[A]}^{-3-0} \quad 19-22 \quad \text{D}^{1-3} \)

*Dourine.*—Mice cured of surra of India by arsenophenylglycin (.6 per cent.) were readily infected by dourine, when inoculated eight, twelve, and fifteen days after the treatment.

1. \( \text{SI}^{3-1} \quad \text{APG}_{10^{4}} \quad 8 \quad \text{DN}^{-13} \quad 10-20 \quad \text{D}^{1-3} \)
2. \( \text{SI}^{3-1} \quad \text{APG}_{10^{4}} \quad 12 \quad \text{DN}^{-10} \quad 16-10 \quad \text{D}^{1-3} \)
3. \( \text{SI}^{3-1} \quad \text{APG}_{10^{4}} \quad 15 \quad \text{DN}^{-11} \quad 17-20 \quad \text{D}^{1-3} \)

In the first and third animals, parasites were found on the day after the inoculation of the virus. In the second mouse, the appearance of infection was somewhat delayed (two days), but death occurred four days before that in the control.

*Surra of Nhatrang.*—In the single test with surra of Nhatrang, made eight days after treatment with CL, infection took place as quickly as in the control. We note, however, that the experimental animal subsequently pursued an irregular course and outlived its control by six days.

1. \( \text{SI}^{3-1} \quad \text{CL}_{10^{6}} \quad 8 \quad \text{SNH}^{-13} \quad 12-19 \quad \text{D}^{1-3} \)

*Surra of Mauritius.*—Do mice acquire an immunity to surra of Mauritius when cured of surra of India? The answer to this question is of special interest, for it is highly probable that in 1901
surra of Mauritius was derived from surra of India. Furthermore, the historical evidence that these two sursas are of common origin has been supported by experiment. For example, Vallée and Panisset found that two Breton calves immunized to surra of Mauritius could not be infected with surra of India. "This experiment," they say, "establishes indisputably the identity of Indian and Mauritian surra." As both history and experiment seem to indicate that Indian and Mauritian surra have a common origin, it was of interest to determine whether the parasites of these two infections had become differentiated in the course of the five and one half years that had elapsed since the Isle of Mauritius became infected.

In order to determine this point, fifteen mice infected with surra of India were treated with CL and at intervals of six to seventeen days after the introduction of the medicament, each received an injection of surra of Mauritius. The result was quite surprising.

The experiments with surra of Mauritius are placed among the heterologous tests, not because surra of Mauritius is regarded as specifically distinct from surra of India, but for the sake of emphasizing the differences often manifested by these two strains. For an account of the infection of the Isle of Mauritius, see D. Nabarro, "Trypanosomes and Trypanosomiases," translated from the French of A. Laveran, and F. Mesnil, Chicago, 1907, p. 251.
for in some of the tests surra of Mauritius behaved as if it were specifically different from surra of India (mice 1 to 7), in others, as if it were related to this infection (mice 8 to 12), and in others still, as if it and the Indian disease were identical (mice 13, 14, and 15).

In the first seven mice, infection was regular, not a trace of resistance being seen. In fact, some of the animals seemed hypersensitive to infection, for in three (mice 1, 5, and 6), parasites appeared one to two days earlier than in their controls.

Immunity after Spontaneous Recovery.—In the following experiment a mouse immunized to surra of India became infected by a double injection of surra of Mauritius. It recovered and then possessed an immunity for both surra of India and surra of Mauritius, for when inoculated with a mixture of these parasites on the thirteenth day, no infection was manifest.

On reinoculating this animal with surra of Mauritius, on the sixteenth day of the experiment, infection took place only after an incubation period of six days.

SURRA OF MAURITIUS.

PROPHYLAXIS.

$C_L$.—The prophylactic experiments with $C_L$ and surra of Mauritius resemble those in which surra of India was similarly treated. In both, infection was prevented (mice 1 and 3), delayed (mice 2, 5, and 7), rendered irregular (mouse 4), and prolonged (mice 2, 4, 5, and 7).
We note, however, that the efficiency of the CL was apparently slightly less in the experiments with surra of Mauritius, for we see above that one of the animals inoculated simultaneously with surra of Mauritius and CL, and all that were tested after the second day, became infected and died.

As in the experiments with surra of India, a prolonged course of infection was the most frequent sign of unexcreted medicament, since, with one exception, all of the mice tested lived longer than their controls. On the other hand, we observe that in mice injected after the second day, the incubation period was lengthened only when the parasites were few and introduced subcutaneously. An intraperitoneal injection on the third, and a rich subcutaneous inoculation on the fifth day, infected within twenty-four hours. From this it would seem advisable, in immunity tests made shortly after treatment with CL, to avoid small subcutaneous injections of virus, in order that the distinction between protection due to medicament and that due to an inefficient immunity may be as sharp as possible.

**Acetyl-Atoxyl Followed by CL.**—Attention has already been directed to the fact that acetyl-atoxyl may prolong the influence of subsequently injected CL. This effect of acetyl-atoxyl is seen in the following experiment in which injections of surra of Mauritius were made three and four days respectively after the CL.

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<th>1 A</th>
<th>3</th>
<th>SM</th>
<th>D</th>
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<tbody>
<tr>
<td>A</td>
<td>4</td>
<td>SM</td>
<td>L</td>
</tr>
</tbody>
</table>

Both mice were infected within twenty-four hours; both, however, recovered without further treatment and the second animal lived long enough to exclude the possibility of a relapse. The influence of the medicament was shown, not by preventing or delaying infection, but by enabling the animals to overcome this after it had become manifest.

**HOMOLOGOUS IMMUNITY TESTS.**

**Unsatisfactory Immunity After CL.**—From the close relationship of surra of India and surra of Mauritius, one might expect that the immunity in mice following the cure of one of these infec-
tions would resemble closely that produced by the cure of the other, if the treatment and the method of testing were similar. That this expectation has not been realized will be evident if one contrasts the immunity in the experiments below with that which followed the cure of surra of India.

The immunity to surra of India was distinctly stronger than that to surra of Mauritius, for, of the fifteen mice infected with surra of India, treated with CL, and reinoculated with surra of India before the eighteenth day (see page 21, and Nos. 1 to 3, page 23), trypanosomes were subsequently found in only two; whereas, in the above experiments, nine out of seventeen became infected, and of those that remained negative, three (Nos. 2, 3, and 6) died too early for the result to be conclusive.

Compared with that of surra of India, the immunity to surra of Mauritius was also of shorter duration and less constantly present. In the experiments with surra of India, no test before the tenth day infected; while in certain animals in the table above, inoculations made as early as the fourth, seventh, and eighth days after treatment (mice 9, 10, 11, and 12) were followed by infection. In the tests with surra of India all of the animals inoculated were more
resistant than their controls. In those with surra of Mauritius, not only was no resistance seen in some of the animals, but in four, hypersensitiveness to infection was apparently present.

**Hypersensitiveness.**—Although hypersensitiveness was seen in only four of the above animals (mice 13, 15, 16, and 17), it deserves special mention. It was observed in mice tested eleven to seventeen days after treatment and was manifested, not so much by a shortened incubation period (although this was noted in two of the animals) as by a shortened course of infection. The latter was present in all four of the mice above referred to. These died one to seven days before their controls.

Attention is directed to the fact that the mice which were hypersensitive were not overpowered by large injections of virus. On the contrary, the number of parasites inoculated was small, varying from two per field to one in two hundred fields.

**Guinea Pig Passage.**—At the time the experiments above recorded were performed, it was not known that the passage of virus through guinea pigs might bring about changes detectable by the immunity reaction. Fortunately, however, virus that had been passed through guinea pigs was used but rarely. Nevertheless, to find the few cases in which the result might have been influenced by this factor, a careful search of the older records was made. This revealed that in four mice (Nos. 12, 13, 14, and 17) in the experiment given above, the result may have been determined by guinea pig passage. In these four instances, therefore, it is not surprising that infection took place, but no explanation is offered for the hypersensitiveness shown by mice 13 and 17.

**Acetyl-Atoxyl.**—One mouse, infected with surra of Mauritius, was treated with acetyl-atoxyl 4 per cent. and subsequently reinoculated with surra of Mauritius. The test showed that it had acquired immunity, for it remained negative for seven days, then died, apparently intoxicated.

1. **SM**
2. **Aa**
3. **SM**

**Prolonged Immunity.**—Can a prolonged immunity to surra of Mauritius be secured? In the attempt to find a method of treatment which would yield a strong and prolonged immunity, a series
of mice were infected with surra of Mauritius, treated in a variety of ways, and then repeatedly inoculated with surra of Mauritius.

While the number of the experiments is too small to be conclusive, some of the results are so suggestive and so much in accord with other observations in this paper that a brief analysis seems warranted.

Two mice treated with CL alone (Nos. 1 and 2) acquired an immunity which was efficient as long as they lived. Of these animals, the one that received two half doses (mouse 2) lived two days longer than the one that had a single full dose. Both animals, however, died early, apparently intoxicated.

The other mice were treated with acetyl-atoxyl, alone, repeated, or combined with CL. From what follows it will be seen that the immunity varied with the treatment and that increasing the effectiveness of the treatment from the curative point of view was usually not followed by an increase in the strength of the immunity. On the contrary, we observe that a single injection of acetyl-atoxyl (although curatively inferior) proved superior, from the point of view of immunity, to two injections of this medicament (mouse 3), to CL followed by two injections of acetyl-atoxyl (mouse 4), and to acetyl-atoxyl followed by a half dose of CL (mouse 5). A different result was, however, obtained when curative efficiency was secured in combinations which tend to be more slowly excreted. We note, for example, that the immunity following the single injection of acetyl-atoxyl (mouse 6) was inferior to that produced by treatment with acetyl-atoxyl followed by two half doses of CL (mouse 7), and was far weaker than that induced by an injection of acetyl-atoxyl followed by a full dose of CL.
Chemo-Therapeutic Trypanosome Studies.

This last form of treatment gave rise to the strongest and most prolonged immunity I have observed. As the course of the mouse (No. 895) receiving this treatment is of considerable interest, it will be given in some detail.

Prolonged Immunity to Surra of Mauritius.—Mouse 895 was inoculated with surra of Mauritius, treated with acetyl-atoxyl 3 per cent. on the first day and with CL 1 per cent. on the sixth. Between the tenth and the forty-sixth day this animal received eleven intraperitoneal injections of surra of Mauritius, but did not become infected. Not only did it remain negative microscopically, but several drops of its blood injected intraperitoneally into normal mice on the thirty-sixth and again on the forty-seventh day, failed to infect. That the parasites introduced into mouse 895 were fully virulent is beyond doubt, for every injection of the virus was controlled by an inoculation into a normal mouse. All of these became infected and died, only one living longer than five days.

The earlier injections of surra of Mauritius were borne with ease by mouse 895. Later, however, shortly after each inoculation it appeared sick. Following the tenth injection, the signs of distress were very marked and immediately after the eleventh, the animal appeared desperately ill. Fearing that it could not withstand another injection, the tests were stopped at this point.

From the alarming effects of the eleventh inoculation (forty-sixth day), mouse 895 recovered quickly and was kept under observation for more than eight months (265 days). During this time it was apparently in the best of health and all of the many blood examinations proved negative. After this long interval it seemed that every possibility of a relapse could be excluded.

As mouse 895 had been exceptionally resistant, it was desirable to test it again for its immunity. It received, therefore, on the 311th day its twelfth test (thirteenth inoculation) with surra of Mauritius. In spite of the fact that the injection of virus was small, contained few parasites, and was given subcutaneously, the mouse did not show a trace of immunity. It became infected on the 315th and died on the 319th day. Compared with its two controls, it was hypersensitive to infection.
The two controls became infected on the fifth day. One remained positive until its death on the twentieth; the other recovered and lived until the 217th day. The virus employed in this last test of mouse 895 was apparently attenuated by passage through guinea pigs, and has been described elsewhere.

A Possible Error.—The next few experiments show clearly that erroneous results may be obtained if, in the attempt to estimate the duration of immunity following cure, one repeatedly (and at short intervals) inoculates the experimental animal with the original virus, for an injection of virus which fails to infect may prolong the immunity.

Prolonging the Immunity.—In my experience the effect of the virus upon the immunity has been most evident when the first test was made within four to six days after treatment and when the others followed at intervals of about three days. If the first test is delayed, or if the interval between the subsequent tests is too great (and probably also if the number of parasites introduced is too large), the animal soon becomes infected. The following experiment illustrates this point.

1. SM:21, AA:21, CL:19, 14
2. SM:21, SM:21, AA:21, CL:19, 9
3. SM:21, AA:21, CL:19, 4
4. SM:21, AA:21, CL:19, 4
5. SM:21, AA:21, CL:19, 4
6. SM:21, AA:21, CL:19, 4

The mice inoculated nine and fourteen days after treatment (Nos. 1 and 2) became infected promptly. Even when only four days separated the treatment and the first test, infection took place at once after the second injection of virus, when an interval of five days was allowed to separate this from the first test (mice 3 and 4). On the other hand, where the interval between tests was three days (Nos. 5 and 6), one mouse received five, the other, six inoculations of virus before infection occurred.

The influence of frequent inoculations in prolonging the immunity may be seen also in the following experiment.

1. SM: 6
   SM: 1.D.
2. SM: 6
   SM: 1.D.
3. SM: 6
   SM: 1.D.

Beginning six days after the last treatment, the immunity tests were made at intervals of three days. As in the fifth and sixth animals in the previous experiment, infection was manifest on the twenty-fourth day in one case, and on the twenty-seventh in the other. The parallelism between the mice in this experiment and the last two in the preceding, is all the more striking because the treatment differed somewhat in these two experiments.

Although the treatment in the following three mice was not exactly alike, a comparison of the results obtained in tests at varying intervals after the last injection of medicament is of interest.

1. SM: 6
   SM: 1.D.
2. SM: 6
   SM: 1.D.
3. SM: 6
   SM: 1.D.

In the first mouse inoculated with surra of Mauritius six days after the Cl, infection took place at once. In the other two, the initial tests were made four days after treatment and here the resistance was greater. In one of these animals (mouse 2), seven days separated the first from the second test, while in the other the interval between inoculations was only three to four days. The former was positive two days after the second test, while in the latter the parasites were not seen until the third day after the eighth test. The prolonged immunity in this third mouse is attributed largely to the fact that the injections of virus were begun early and repeated at short intervals.

**HETEROLOGOUS IMMUNITY TESTS.**

**Nagana.**—A mouse cured of surra of Mauritius showed no immunity to nagana when tested seven days after the Cl, for both
the experimental animal and the control became infected on the same day.

1. SM\textsuperscript{1} \textsuperscript{17}: \textsuperscript{1}C\textsubscript{LO\textsubscript{16}}\textsubscript{60} 7 \textsuperscript{10}NG\textsuperscript{1} \textsuperscript{12} \textsuperscript{13-15} \textsuperscript{16}D.\textsuperscript{14}

Caderas.—Mice cured of surra of Mauritius by acetyl-atoxyl and CL apparently acquired no immunity to caderas, for when inoculated with this virus four and six days after treatment, they became infected at once.

2. SM\textsuperscript{1} \textsuperscript{18}: \textsuperscript{1}AA\textsuperscript{2} \textsuperscript{5}C\textsubscript{LO\textsubscript{16}}\textsubscript{bL} 6 \textsuperscript{8}CD\textsuperscript{11} \textsuperscript{9-10} \textsuperscript{11}D.\textsuperscript{1-3}

3. SM\textsuperscript{1} \textsuperscript{19}: \textsuperscript{1}AA\textsuperscript{3} \textsuperscript{5}C\textsubscript{LO\textsubscript{16}}\textsubscript{bL} 4 \textsuperscript{8}CD\textsuperscript{11} \textsuperscript{10-11} \textsuperscript{12}D.\textsuperscript{1-3}

Although there was apparently no initial immunity to caderas, one of the animals subsequently had an irregular course of infection. This in all probability is to be attributed to the medicaments employed and to the closeness of the test to the treatment (four days), for after remaining positive for two days the animal became negative and when next inoculated it was found to possess considerable immunity to caderas. It had an incubation period of eight days, while its control was positive on the first day.

Variable Immunity to Surra of India.—As we have already seen in mice immunized to surra of India and subsequently inoculated with surra of Mauritius, the close relationship of these infections may, or may not, be indicated. In some of the tests the parasites of surra of Mauritius behaved as if they were identical with those
of surra of India, and in others, as if they and the Indian trypanosomes were specifically distinct. The same remarkable differences are seen in the experiments in which mice infected with surra of Mauritius were treated with CL and subsequently tested with surra of India.

In the first five mice in the outline above, infection took place as quickly as in the controls; in the next five, it was markedly delayed; and in the last three, it was completely prevented.

Mice cured of surra of Mauritius by acetyl-atoxyl and CL were inoculated with surra of India four, six, and eleven days after the CL. In these tests the surra of India infected as quickly as if it were specifically distinct from surra of Mauritius.

The influence of unexcreted medicament upon infections following early tests for immunity has been frequently seen when the treatment has consisted of an injection of acetyl-atoxyl followed by CL, and is apparently plainly shown in the following experiment, although the possibility that the result was affected by the close relationship of surra of India and surra of Mauritius, must not be ignored.

That the influence of unexcreted medicament may make itself manifest nearly a week and a half after treatment is seemingly indicated by the first animal, for in this mouse the parasites, after being present for three days (fourteenth, fifteenth, and sixteenth) disappeared on the tenth day after the last injection of medicament and were not again found during the life of the animal.

The second mouse received a rich intraperitoneal injection of surra of India and became infected at once. But the parasites after being present for one day only disappeared, and the animal acquired a strong immunity to surra of India, for it was given six inoculations of this virus before becoming infected (thirty-second
day). The strength and duration of the immunity in this case is attributed to the joint influence of unexcreted medicament and frequent inoculations of virus at short intervals.

CADERAS.\textsuperscript{12}

PROPHYLAXIS.

In the following prophylactic experiment with caderas, the virus injections were begun early. We note, nevertheless, that in no case was the incubation period lengthened.

\begin{itemize}
  \item 1. Mx3 $\text{CL}^{1\text{st}}$ 2 $\text{CDI}^{3\text{rd}}$ 6-10 \text{L.}
  \item 2. Mx3 $\text{CL}^{1\text{st}}$ 3 $\text{CDI}^{3\text{rd}}$ 6-9 \text{D.}
  \item 3. Mx3 $\text{CL}^{1\text{st}}$ 4 $\text{CDI}^{3\text{rd}}$ 7-10 \text{D.}
  \item 4. Mx3 $\text{CL}^{1\text{st}}$ 5 $\text{CDI}^{3\text{rd}}$ 8-10 \text{D.}
  \item 5. Mx3 $\text{CL}^{1\text{st}}$ 8 $\text{CDI}^{3\text{rd}}$ 11-14 \text{D.}
\end{itemize}

Every test infected within twenty-four hours, but the first animal inoculated two days after treatment was positive for one day only. It then became negative and recovered completely. After the second day the influence of the medicament was slight.

HOMOLOGOUS IMMUNITY TESTS.

One of the few attempts to produce an efficient immunity to caderas was successful.

\begin{itemize}
  \item 1. $\text{CDI}^{3\text{rd}}$ $\text{TR}^{5\text{th}}$ $\text{A}_{\text{O}_{1}}^{5\text{th}}$ 5 $\text{CDI}^{3\text{rd}}$ 6-9 \text{L.}
\end{itemize}

This mouse was treated first with trypanred, then with acetyltoxyl, and was tested five days after the last treatment. It resisted infection and was living on the 180th day.

Prolonged Immunity.—As we have already seen, in securing prolonged immunity to surra of India and to surra of Mauritius,

\textsuperscript{12} Additional evidence of the delicacy of the immunity reaction has been given by the writer (\textit{Jour. Exper. Med.}, 1909, xi, 802), who has shown that two strains of caderas of common origin, preserved in duplicate in guinea pigs for one year can be differentiated. Similar observations were made with surra of India.

Under slightly different conditions, Schilling and Jaffé (see footnote on nagana, page 43) have obtained a result which is quite comparable.
three factors were apparently of importance. These were (1) a suitable form of treatment; (2) a short interval between the treatment and the first test; and (3) repeated tests at short intervals. In the following experiments with caderas these factors seem to be equally important (see mice 3, 4, and 5).

1. CDi:12 Mx3 C l:16 6 21.02 7 CDi:3 13-17 16-21 D.
2. CDi:12 Mx3 C l:16 6 21.02 5 CDi:4 13 36 15-17 D.
3. CDi:11 AA 2.5% C l:12 3 CDi:2 11-14 1 CDi:1 15 D.
4. CDi:11 AA 2.5% T RoiD 3 CDi:2 11 16 1 CDi:1 17 CDi:1 34 D.
5. CDi:11 TRoiD 4 CDi:12 16 1 CDi:1 16 1 CDi:1 11 CDi:11 1 CDi:1 3 D.

It is interesting and possibly significant that in this experiment, the simplest, and from the curative point of view probably the weakest form of treatment (see mouse 5), was followed by the most prolonged immunity. We note, for example, that mouse 4, treated with both acetyl-atoxyl and trypanred, became positive (thirtieth day) after the seventh inoculation of virus, while mouse 5, treated with a single injection of trypanred did not become infected until after the twelfth test (fifty-second day).

That the immunity to caderas was less prolonged in other mice cured of this infection by a single injection of trypanred and similarly tested is possibly due to the fact that in these cases (see page 41) comparatively rich injections of parasites were too early resorted to.

**HOMOLOGOUS AND HETEROLOGOUS TESTS.**

A most surprising result was obtained when mice infected with caderas were twice treated with Mixture I, reinforced with an inoculation of caderas, and tested six days after the last treatment by injections of caderas, surra of Mauritius, surra of India, nagana, and dourine. Although immunized to caderas, these animals be-

12 C. Schilling, in the *Arch. f. Schiff- u. Tropen-Hyg.*, 1909, xiii, 1, reports an immunity of only three days in mice cured of caderas by trypanred. He found, furthermore, that the immunity to this species was short (nine days), when the cure was effected by atoxyl, but that it could be prolonged by repeatedly infecting and curing the mice.
came infected more quickly with caderas than they did with surra of Mauritius or surra of India.

On comparing the experimental animals with their controls (all of which were positive on the first day), we see that the infection due to caderas (mouse 1) was delayed three days (i.e., with reference to its control), that due to surra of Mauritius (mouse 2), seven days, while the animal inoculated with surra of India (mouse 3) did not become infected at all and was living on the 180th day. On the other hand, the tests with nagana and dourine infected at once. The resistance of surra of India and surra of Mauritius in this experiment is attributed to their sensitiveness to the form of treatment employed.

The third animal in this series makes an interesting contrast with the last one. The treatment in these two cases was almost identical, but one was tested six days after treatment, the other eight. As we have just seen, the former failed to become infected while the parasites appeared in the latter as quickly as in the control.

Arserophenylglycin and Trypanred.—In the following experiment the immunizing power of arserophenylglycin is compared with that of trypanred.
We note that the main difference between these two forms of treatment was not apparent early, for, with two exceptions (Nos. 3 and 8), all of the animals became infected on the ninth day. Nevertheless, after infection was manifest the subsequent course in the two series differed. In the mice treated with arsenophenylglycin, little resistance to the further development of the parasites was seen. In these animals the trypanosomes increased steadily and killed the mice six to seven days after the second series of tests. On the other hand, in the mice treated with trypanred, a similar course was present in only two of the five animals, the other three showing a strong resistance to infection. Two that were infected (Nos. 6 and 7) became negative, and subsequent tests indicated that they had again become immune, while the third mouse (No. 8) was inoculated three times before parasites were found in its blood. It was positive from the twelfth to the seventeenth day, then became negative and remained so until the twenty-third day, when parasites reappeared, apparently as a result of the fourth inoculation on the twenty-first day.

Two points of considerable interest were observed when mice, cured of cadera in various ways, were inoculated with surra of India at intervals of three to five days after the last treatment.

We note, for example, that all of the animals manifested a distinct resistance to surra of India. This observation is apparently of importance, for it seems highly improbable that the delay in infection can be explained by the action of unexcreted medicament, for this almost never affects the incubation period if the trypanosomes are introduced as late as the third day after treatment, are given intraperitoneally, and the injections are rich enough to infect the controls within twenty-four hours. As the conditions for prompt infection were present, the delay seems significant; espe-
cially so in the first and second animals, for prophylactic experiments with trypanred have shown that surra of India infected within twenty-four hours, when inoculated into mice three, four, and five days after the medicament. In the first and second mice in the outline, the tests were made four and five days after treatment, but in neither was infection observed until the fourth day after the inoculation of the virus. It seems not impossible, therefore, that the cure of caderas gave rise to a weak immunity to surra of India.

We note, also, that in the fifth mouse above we have, in all probability, a striking example of the influence upon immunity of frequent injections of virus. In this animal the failure of the first test apparently gave rise to an immunity which the subsequent inoculations merely served to prolong, for this mouse received eight injections of virulent surra of India without becoming visibly infected.

NAGANA.14

PROPHYLAXIS.

Acetyl-Atroxyl.—Although acetyl-atoxyl is usually excreted rapidly, the prophylactic experiment with nagana which follows,

14 Since 1907, most of the contributions to our knowledge of the therapeu tic immunity reaction have come from the study of nagana. The following points should be mentioned:—

New evidence of the delicacy of the reaction has been given by Ehrlich and by Schilling and Jaffé. The former (Münchener med. Woch., 1909, lvi, 217) has shown that the immunity distinguishes strains of common origin if one of them is rendered resistant to serum, and the latter (Arch. f. Schiff's- u. Tropen-Hyg., 1909, xiii, 525) have pointed out that mice and rats which were immune to a nagana preserved exclusively in mice were not immune to another nagana of common origin that had been preserved for two years in guinea pigs.

In the immunity experiments of Schilling (Arch. f. Schiff's- u. Tropen-Hyg., 1909, xiii, 1) and in those of Schilling and Jaffé, arsenophenylglycin was employed to cure mice and rats. Following the curative treatment with this medicament, the results were very variable. In some instances scarcely a trace of immunity could be detected, and in others an immunity of inconstant duration was produced. At times it disappeared after the ninth day, but in other animals it lasted much longer and in one case a single injection of the medicament sufficed to prevent infection with the original virus for an interval of 140 days. In the case of rats Schilling was able to secure a certain amount of immunity by inoculating these animals simultaneously with the medicament and the trypanosomes. Schilling’s attempts to strengthen the immunity by frequently injecting the virus
shows that the medicament may exert a marked influence on tests made as late as the third day after treatment.

1. \( \text{AA}^{3^{\text{th}}} \) 1 \( \text{NG}^{3^{\text{rd}}} \) 2-97 \( \text{D}^{1^{\text{st}}} \) 0
2. \( \text{AA}^{3^{\text{th}}} \) 2 \( \text{NG}^{3^{\text{rd}}} \) 3-10 \( \text{D}^{1^{\text{st}}} \) 0
3. \( \text{AA}^{3^{\text{th}}} \) 3 \( \text{NG}^{3^{\text{rd}}} \) 4-7 \( \text{D}^{1^{\text{st}}} \) 0
4. \( \text{AA}^{3^{\text{th}}} \) 3 \( \text{NG}^{3^{\text{rd}}} \) + \( \text{D}^{1^{\text{st}}} \) 0
5. \( \text{AA}^{3^{\text{th}}} \) 3 \( \text{NG}^{3^{\text{rd}}} \) 4-7 \( \text{D}^{1^{\text{st}}} \) 0
6. \( \text{AA}^{3^{\text{th}}} \) 4 \( \text{NG}^{3^{\text{rd}}} \) + \( \text{D}^{1^{\text{st}}} \) 0
7. \( \text{AA}^{3^{\text{th}}} \) 5 \( \text{NG}^{3^{\text{rd}}} \) + \( \text{D}^{1^{\text{st}}} \) 0

In the mice inoculated one and two days after the acetyl-atoxyl (Nos. 1 and 2), the influence of the medicament was so strong that neither became infected, and in one of the three animals tested on the third day (mouse 3), the parasites were present for four days only, then disappeared again. While this mouse died too early to exclude the possibility of a relapse, it is interesting to note that several drops of blood drawn from it on the second day after the disappearance of the parasites (ninth day), failed to infect another mouse.

In this table, also, one is struck by the fact that, when tests infected at all, they usually did so at once. In only one instance (mouse 6) was the incubation period at all prolonged and in this case it was only one day longer than that of the control.

**HOMOLOGOUS AND HETEROLOGOUS IMMUNITY TESTS.**

In the following experiment mice cured of nagana by acetyl-atoxyl acquired a distinct but inefficient immunity to nagana (incu-
were, however, not successful. On introducing the parasites daily into animals that had been cured one or more times, he detected no perceptible increase in the immunity. For the details of Schilling’s numerous experiments, the two papers above cited should be consulted.

Browning (*Jour. Path. and Bact.*, 1908, xii, 166), working in Ehrlich’s laboratory, found that the number of parasites present when treatment was begun was of importance. If the mice were treated when the parasites were few (i. e., twenty-four hours after the inoculation of the virus) the immunity lasted not more than ten days. It was of longer duration, however, if the treatment was not instituted until the trypanosomes were numerous in the blood (i. e., forty-eight hours after the virus injection).
bation period prolonged five days), but none whatever to surra of India or to an atoxyl-resistant strain of nagana.

\[
\begin{align*}
1. & \text{NG}_2^{3 \times 10} \quad +^{2} \text{AA} \quad 4 \\
2. & \text{NG}_2^{3 \times 10} \quad +^{2} \text{AA} \quad 4 \\
3. & \text{NG}_2^{2 \times 10} \quad +^{2} \text{AgCI}_2 \quad 9
\end{align*}
\]

\[
\begin{align*}
& \text{Ng}^{2 \times 10} \quad +^{2} \text{A} \quad 4 \\
& \text{SI}^{2 \times 10} \quad +^{2} \text{A} \quad 4 \\
& \text{Ng}^{2 \times 10} \quad +^{2} \text{A} \quad 9 \\
& \text{Ng}^{2 \times 10} \quad +^{2} \text{A} \quad 9
\end{align*}
\]

Nagana Strains Differentiated.—That, in the above experiment, the two nagana strains (one normal, the other resistant to acetylatoxyl) were as sharply differentiated from each other as nagana was from surra of India, is not surprising in the light of the work of Ehrlich and Browning, who have shown that the immunity reaction readily distinguishes between strains of common origin, if one or both have been rendered resistant to treatment.

Efficient Immunity.—That an efficient immunity to nagana may be produced is shown in the following experiment (mouse 1).

\[
\begin{align*}
1. & \text{Ng}^{2 \times 10} \quad +^{2} \text{Mx1} \quad 6 \\
2. & \text{Ng}^{2 \times 10} \quad +^{2} \text{Mx1} \quad 6 \\
3. & \text{Ng}^{2 \times 10} \quad +^{2} \text{Mx1} \quad 6 \\
4. & \text{Ng}^{2 \times 10} \quad +^{2} \text{Mx1} \quad 6 \\
5. & \text{Ng}^{2 \times 10} \quad +^{2} \text{Mx1} \quad 6
\end{align*}
\]

One also sees that there was apparently no immunity for any of the other species, for the inoculations with surra of India, caderas, dourine, and surra of Mauritius infected within twenty-four hours.

Exceptional Course.—The course of infection in mouse 2 inoculated with surra of India was exceptional. After remaining positive for five days this animal recovered completely and was living on the 180th day. The recovery is attributed, at least in large part, to the sensitiveness of surra of India to the treatment employed. This sensitiveness of surra of India to unexcreted medicament has been noted both in prophylactic and immunity experiments in which mice have received the double treatment with Mixture 1. In both (see mouse 9, page 21, and mouse 3, page 41), injections of surra of India made six days after the last treatment failed altogether to infect.

\[\text{Browning, C. H., Chemo-Therapy in Trypanosome Infections: an Experimental Study, Jour. Path. and Bact., 1908, xii, 166.}\]
RESISTANT NAGANA STRAINS.

In the experiment below two resistant nagana strains of common origin were distinguished from each other and from the following normal strains: nagana, caderas, dourine, surra of India, and surra of Mauritius.

<table>
<thead>
<tr>
<th>No.</th>
<th>Strain Description</th>
<th>Immunity Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>2.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>3.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>4.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>5.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>6.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>7.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>8.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>9.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>10.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>11.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
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<tr>
<td>12.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
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<tr>
<td>13.</td>
<td>Ng[P]</td>
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<td>14.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
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<td>15.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
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<tr>
<td>16.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>17.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
<tr>
<td>18.</td>
<td>Ng[P]</td>
<td>Na[T]</td>
</tr>
</tbody>
</table>

The mice cured of Ng[P] (nagana rendered resistant to paraphenylenediamine, Nos. 1 to 9) acquired a distinct but slight resistance to the same strain (Nos. 1 and 3), but none to the other trypanosomes. In the mice cured of Ng[T] (nagana resistant to toluidin blue, Nos. 10 to 18), the immunity to the same strain was comparatively strong, that to Ng[P] was weak, while there was none whatever for the other strains.

Attention is directed to two interesting points in the above experiment: (1) that an efficient immunity was secured even in the case of one of the resistant strains (mouse 12); and (2) that in the mice immunized to Ng[T] the close relationship of this to Ng[P] was apparently indicated by the presence of an immunity to both.16

16 In Ehrlich's experiments no such relationship has been shown.
DOURINE.

PROPHYLAXIS.

Arsenophenylglycin.—In the prophylactic experiments with dourine and arsenophenylglycin, two strengths of the latter were employed. In the tests with these, the strongest effect of the medicament was seen on or before the third day.

With the .2 per cent. solution (mice 1 to 3), a markedly prolonged course of infection was observed only in the mouse tested on the third day (No. 1). With the .6 per cent. solution (mice 4 to 8), an inoculation on the second day failed and another on the third was followed by an irregular course of infection.

Attention is again directed to the fact that the incubation period was but little affected by the medicament in any of these experiments. Of the mice which became infected, all but one (No. 2) were positive as quickly as their controls, and in the exceptional case, infection was observed only one day later than in the control.

IMMUNITY EXPERIMENTS.

Homologous Tests.—While against surra of India, surra of Mauritius, caderas, nagana, and a toluidin blue resistant strain of nagana, an efficient immunity has been secured with comparative ease, all attempts thus far have failed to produce an immunity of equal strength against dourine. In the trials with dourine, twelve mice were employed. In spite of the fact that these were treated in a variety of ways, were tested early (most of them between the fifth and the eighth, not one later than the eleventh day
after treatment), and received only moderately rich injections of trypanosomes, all of the animals became infected and died.

1. DN31.1 1APG6% 8 DN31.2 10-16 D1
2. DN31.1 1APG6% 6 DN31.1 8-12 13-16 D6-10
3. DN31.1 2Mx1 8 DN31.5 12-14 14-18 D3-5
4. DN31.1 2Mx1 5 DN31.1 10-15 16 D2-5
5. DN31.5 2Mx1 2Mx1 11 DN31.1 17-19 19-23 D3-5
6. DN31.5 2Mx1 2Mx1 8 DN31.1 14-15 16-20 D3-5
7. DN31.5 2Mx1 2Mx1 5 DN31.1 11-13 14 D4-5
8. DN31.1 2AA6% 2Mx40 ISO L 7 DN31.1 12-14 15-21 D11
9. DN31.1 2AA6% 2Mx40 ISO L DN31.5 7 DN31.1 12-16 17-20 D6-10
10. DN31.5 2Mx1 2Mx1 7 DN31.1 14-17 18-21 D5-9
11. DN31.1 2Mx1 2Mx1 ISO L DN31.1 5 DN31.1 9-12 13 14-21 D4-14
12. DN31.5 2Mx1 2Mx1 5 DN31.1 11-19 21-27 D11-18

The immunity to dourine was of short duration as well as weak, for one mouse tested on the eleventh day after a double treatment with Mixture 1 (mouse 5), and another, eight days after arsene-phenylglycin (mouse 1), became infected as quickly as their controls. That the test in these cases were made after the immunity had disappeared is suggested by the fact that, following the same forms of treatment, a weak immunity was detected when the tests were made earlier. We note, for example, that in the seventh and second animals, the incubation periods exceeded those of their controls by three and five days respectively.

Reinforcing Inoculations.—In the dourine experiments, the reinforcing inoculations (mice 9 to 12) seem to have prolonged the immunity. For on comparing the course of infection in mice which in all other respects were similarly treated and tested, it is seen that those which received an inoculation of the original virus immediately after the last treatment, remained negative longer than those in which this was not given. For example, mouse 9 (immunity reinforced) had an incubation period of six days, mouse 8, one, of four. In the case of the twelfth and seventh animals, the difference was still more striking. The former (immunity reinforced) had an incubation period of eleven days, the latter one of four.
HOMOLOGOUS AND HETEROLOGOUS TESTS COMPARED.

The exceptional weakness of the immunity to dourine is also well seen in the following outlines where the result of the tests with this virus are placed by the side of those with surra of India, surra of Mauritius, caderas, and nagana.

1. DN\textsuperscript{3,5} Mx1 8 DN\textsuperscript{3,5} 15-16 + D\textsuperscript{1-3} 8
2. DN\textsuperscript{3,5} Mx1 8 SI\textsuperscript{2-4} 13-15 + D\textsuperscript{2-4} 8
3. DN\textsuperscript{3,5} Mx1 Mx1 8 DN\textsuperscript{17} 14-15 + D\textsuperscript{3-0} 8
4. DN\textsuperscript{3,5} Mx1 Mx1 8 SI\textsuperscript{1-3} 14 15-17 + D\textsuperscript{2-3} 8
5. DN\textsuperscript{11} AA\textsuperscript{2-7} Mx4 180L 7 DN\textsuperscript{210} 12-14 + D\textsuperscript{2-11} 7
6. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L 7 SI\textsuperscript{1-7} 12-14 + D\textsuperscript{2-12} 7
7. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L 7 CD\textsuperscript{1,3} 12-14 + D\textsuperscript{2-7} 7
8. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L 7 SM\textsuperscript{1,2} 12-16 + D\textsuperscript{2-9} 7
9. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L 7 NG\textsuperscript{1,1} 12-12 + D\textsuperscript{2-7} 7
10. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L DN\textsuperscript{1,5} 7 DN\textsuperscript{11} 12-16 17-17 + D\textsuperscript{2-10} 7
11. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L DN\textsuperscript{1,5} 7 SI\textsuperscript{1,7} 12 17-17 + D\textsuperscript{2-9} 7
12. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L DN\textsuperscript{1,5} 7 CD\textsuperscript{1,2} 12-14 15-17 + D\textsuperscript{2-7} 7
13. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L DN\textsuperscript{1,5} 7 SM\textsuperscript{1,2} 12-15 + D\textsuperscript{2-5} 7
14. DN\textsuperscript{21} AA\textsuperscript{2-7} Mx4 180L DN\textsuperscript{1,5} 7 NG\textsuperscript{1,1} 12-13 + D\textsuperscript{2-3} 7
15. DN\textsuperscript{11} Mx1 Mx1 180L DN\textsuperscript{1,1} 5 DN\textsuperscript{11} 9-11 13 14-21 + D\textsuperscript{1-11} 5
16. DN\textsuperscript{11} Mx1 Mx1 180L DN\textsuperscript{1,1} 5 SI\textsuperscript{1,2} 9-61 + 180 L
17. DN\textsuperscript{11} Mx1 Mx1 180L DN\textsuperscript{1,1} 5 SM\textsuperscript{1,24} 9-16 17-21 + D\textsuperscript{2-16} 5
18. DN\textsuperscript{11} Mx1 Mx1 180L DN\textsuperscript{1,1} 5 CD\textsuperscript{1,2} 9-11 + 17 D\textsuperscript{2-3}
19. DN\textsuperscript{11} Mx1 Mx1 180L DN\textsuperscript{1,1} 5 NG\textsuperscript{1,10} 9-10 + D\textsuperscript{1-3}

We observe that mice cured of dourine at times offered greater resistance to infection with other species than to dourine itself. While infection with dourine (mice 1, 3, 5, 10, and 11) was in no case delayed beyond the sixth day, a mouse injected with surra of Mauritius (mouse 17) did not become positive until the ninth day after the test, and two inoculated with surra of India (Nos. 6 and 16) failed altogether to become infected.

The mice immunized to dourine showed also a certain resistance to infection with caderas (Nos. 7 and 12), for two of the three inoculations with this species infected only on the fourth day (controls positive on the first).

Dourine Differentiated.—In spite of the fact that in the tests made before the ninth day after treatment (see above), a more or
less marked resistance to caderas, surra of Mauritius, and surra of India was manifested by mice immunized to dourine, the latter species was in one respect clearly differentiated from all of the others, for in every mouse inoculated with dourine, infection was delayed one or more days. On the other hand, trypanosomes appeared within twenty-four hours in at least one of the tests with each of the other species, and in the three with nagana (mice 9, 14, and 19), not a trace of resistance was detected.

DOUBLE IMMUNITY.

When mice infected with both surra of India and surra of Mauritius were cured, they acquired a double immunity.

1. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 5 10$SI^{-21}^{+}$ 
2. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 5 10$SIM^{-34}^{+}$ 
3. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 10 10$SI^{-14}$ 
4. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 10 10$SM^{-31}^{+}$ 
5. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 19 10$SI+SM^{-21}^{+}$ 
6. $SI+SM^{-21}^{+}$ $AA^{25%}$ $Cl^{15%}$ 5 10$CD^{-3}^{10}$

This is shown by the fact that no infection took place when the animals were tested with surra of India (mice 1 and 3), with SI[M] (mouse 2), with surra of Mauritius (mouse 4), or with a mixture of surra of India and surra of Mauritius (mouse 5). There was, however, little or no immunity to caderas (mouse 6), for this animal was positive twenty-four hours after its control.

ON THE SEPARATION OF ORGANISMS MIXED IN VITRO.¹⁷

Trypanosomes mixed in vitro can apparently be separated by means of the immunity reaction. In order to do this, the mixture of parasites is inoculated into mice immunized to one of the strains. Under these conditions, infection takes place with the trypanosomes against which no immunity was produced.

In the following experiments the attempt was made to separate

¹⁷ The experiments here reported should not be confused with those of Browning in which strains of trypanosomes rendered resistant to various medicaments were separated after being mixed in vitro. Browning (Brit. Med. Jour., 1907, ii, 1405) effected the separation, not by immunity, but by treating a double infection in mice with a medicament against which the trypanosomes of one of the infections were resistant.
(1) strains that are considered to be specifically distinct, i.e., surra of India and caderas, and (2) those that are regarded as having had a common origin, i.e., surra of India and surra of Mauritius.

**TRYPANOSOMES SPECIFICALLY DISTINCT.**

*SI[C], or Surra of India Separated from Caderas.*—In order to separate surra of India from caderas, the mixture of parasites was inoculated into mice immunized to caderas. Both animals became infected, presumably with surra of India. In subsequent tests the parasites derived from these animals will be referred to as SI[C].

1. $\text{CD}_{1-5} + \text{TR}_{2-7}$
2. $\text{CD}_{1-5} + \text{TR}_{2-7}$
   
Some Protection Against Surra of India.—In the above experiments we apparently have additional evidence that mice cured of caderas acquire a certain resistance to surra of India, for we note that in both tests infection was retarded, and that one of the mice (No. 1) recovered completely after being infected as late as the tenth and eleventh days after treatment. The recovery in this instance is all the more remarkable because it was in an animal that had received only one injection of trypanred, and in prophylactic experiments, this medicament has failed to protect, even when surra of India was inoculated as early as the third, fourth, and fifth days after treatment.

*CD[I], or Caderas Separated from Surra of India.*—In separating caderas from surra of India, the mixture of trypanosomes was inoculated into mice immunized to surra of India.

1. $\text{SI}_{1-10} + \text{AA}_{2-5}$
2. $\text{SI}_{1-10} + \text{CL}_{1-5}$
3. $\text{SI}_{1-15} + \text{AA}_{2-5}$
4. $\text{SI}_{1-15} + \text{CL}_{1-5}$

All of the inoculations were intraperitoneal and infected within twenty-four hours. The parasites from these mice will be referred to as CD[I].
Purity of $\text{SI}[C]$.—In the single experiment to test the purity of $\text{SI}[C]$, mice were immunized to this virus and then inoculated with surra of India, caderas, and other strains.

1. $\text{SI}[C]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{SI}[C]$ $9-14$ $9-12$ $10D$
2. $\text{SI}[C]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{SI}[C]$ $9-14$ $9-12$ $10D$
3. $\text{SI}[C]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{SI}[C]$ $9-14$ $9-12$ $10D$
4. $\text{SI}[C]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{SI}[C]$ $9-14$ $9-12$ $10D$
5. $\text{SI}[C]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{SI}[C]$ $9-14$ $9-12$ $10D$

We note that infection with surra of India was completely prevented (mouse 1), that it was delayed in the case of surra of Mauritius (mouse 2), but that in the tests with caderas, dourine, and nagana it took place at once (mice 3, 4, and 5). From this it would seem that surra of India had been separated in purity from caderas.

The resistance manifested in the test with surra of Mauritius is probably to be explained by the close relationship of surra of Mauritius and surra of India.

Purity of $\text{CD}[I]$.—In testing the purity of $\text{CD}[I]$, only one experiment was carried out. In this the mice were immunized to $\text{CD}[I]$ and then inoculated with caderas, surra of India, surra of Mauritius, dourine, and nagana.

1. $\text{CD}[I]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{CD}[I]$ $6$ $10D$
2. $\text{CD}[I]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{CD}[I]$ $6$ $10D$
3. $\text{CD}[I]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{CD}[I]$ $6$ $10D$
4. $\text{CD}[I]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{CD}[I]$ $6$ $10D$
5. $\text{CD}[I]$: $\text{Mx1}$ $\text{Mx1}1000L$ $\text{CD}[I]$ $6$ $10D$

From the above one might think that the separation of caderas from surra of India had not been effected in purity and that we are here dealing not with caderas, but with a mixture of caderas and surra of India, for we note that the tests with surra of India failed as completely as those with caderas. Nevertheless, for the following two reasons it seems quite probable that this view is not correct:

18 As in well-stained blood specimens the trypanosomes of caderas are distinguishable from those of surra of India. It had been planned in this experiment to determine the purity of the separation by means of the microscope. Due to an oversight, however, this test was omitted.
1. In the conditions of the experiment in which the separation was attempted, there is nothing to explain a failure. We note that the tests were made early, that the injections were intraperitoneal, and that the number of trypanosomes introduced was great enough to infect within twenty-four hours but not large enough to give any ground for suspecting that the immunity had been overpowered. From this we see that all of the conditions for a successful separation were present.

2. It is unnecessary to assume a failure in order to explain the result, for, as we have already seen, to the form of treatment here employed, surra is unusually sensitive, and in one instance in the prophylactic experiments, an inoculation of this virus on the sixth day after treatment, failed to infect (mouse 9, page 21). If the second mouse in the above experiment were equally resistant, the result would be fully explained without assuming that the separation had failed. Moreover, we apparently have evidence that in this experiment the influence of unexcreted medicament was quite strong, for although the mouse tested with surra of Mauritius had no initial resistance (infection within twenty-four hours), the parasites were present in it for one day only, then disappeared and the animal recovered completely. The quickness with which this mouse became negative seems to indicate that the influence of the medicament was very strong.

TRYPANOSOMES OF COMMON ORIGIN.

While the separation of surra of India from surra of Mauritius seems to be attended with no great difficulty, the tests of the purity of the separated strains are most uncertain, for even when known to be pure the parasites of these two infections react towards each other in an inconstant way. As a consequence, even if the trypanosomes are separated successfully, we must expect that the tests will at times indicate the contrary. The experiments with the two surras follow.

SI[M], or Surra of India Separated from Surra of Mauritius.—To effect the separation, the mixture was inoculated into mice immunized to surra of Mauritius.
Every injection infected. In subsequent tests the parasites derived from these mice will be referred to as SI[M].

Attention is called to the fact that in three of the mice the virus was introduced intraperitoneally (Nos. 1, 4, and 5) and that here infection took place at once. On the other hand, when the virus was introduced subcutaneously (Nos. 2 and 3) infection was delayed. As delayed infections may be easily confused with relapses, it seems advisable to make the immunity tests intraperitoneally.

*SM[I], or Surra of Mauritius Separated from Surra of India.*—In attempting to separate surra of Mauritius from surra of India, the mixture was inoculated into mice immunized to surra of India.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parasite 1</th>
<th>Parasite 2</th>
<th>Parasite 3</th>
<th>Parasite 4</th>
<th>Parasite 5</th>
<th>Parasite 6</th>
<th>Parasite 7</th>
<th>Parasite 8</th>
<th>Parasite 9</th>
<th>Parasite 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. SM²⁺⁺</td>
<td>5Cl⁻⁻</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>14</td>
<td>16</td>
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<td>22</td>
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<tr>
<td>2. SM²⁺⁺</td>
<td>5Cl⁻⁻</td>
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<tr>
<td>3. SM²⁺⁺</td>
<td>5Cl⁻⁻</td>
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<td>14</td>
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</tr>
<tr>
<td>4. SM²⁺⁺</td>
<td>5Cl⁻⁻</td>
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</tr>
<tr>
<td>5. SM²⁺⁺</td>
<td>5Cl⁻⁻</td>
<td>6</td>
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</tr>
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</table>

As in the preceding experiments, every inoculation infected. The trypanosomes appearing in these mice will be referred to as SM[I].

*Purity of SI[M].*—The result of the experiments with SI[M] follow.

(I) Mice immunized to surra of India are immune to SI[M].

1. SI⁻⁻⁻ | AA⁺⁺⁺ | 5Cl⁻⁻ | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 |
2. SI⁻⁻⁻ | AA⁺⁺⁺ | 5Cl⁻⁻ | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 |

For two other experiments in which mice immunized to surra of India resisted infection with SI[M], see homologous immunity tests with surra of India, first and third animals (page 23).
An inoculation on the eleventh day (mouse 1) failed, and another on the sixteenth (mouse 2) infected only after an incubation period of eight days (control positive on the first day). As the starred virus differs in no important respect from SI[M], the results of tests with these two strains are at times given together, nevertheless these strains are always distinguished.

(2) Mice cured of surra of Mauritius possessed little or no immunity to SI[M].

All of the tests infected, and in four of the five animals, parasites appeared within twenty-four hours. In the mouse in which infection was delayed, the trypanosomes were found on the day after they were seen in its control.

(3) Mice immunized to SI[M] acquired a perfect immunity to surra of India (Nos. 1 and 5), reacted in a variable way toward surra of Mauritius (Nos. 3 and 6), and became infected within twenty-four hours when tested with SM[I]. cadenas, dourine, and nagana.

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20 This virus resembles SI[M] in that it was obtained by infecting with surra of India a mouse immunized to surra of Mauritius. It differs from SI[M] in that the immune mouse was inoculated, not with a mixture of surra of India and surra of Mauritius, but with surra of India alone.
With surra of Mauritius two inoculations (Nos. 3 and 6) were made. Following one of these (No. 3) infection was only slightly delayed, the parasites appearing forty-eight hours after the first test. After being positive for one day, however, this mouse recovered completely. In the second animal injected with surra of Mauritius (No. 6), the appearance of the parasites was greatly delayed, the incubation period being eleven days (control one day). With SM[I] only one test (mouse 4) was made. In this, infection took place at once, but subsequently the animal became negative and was not killed by the parasites until the seventeenth day after inoculation. In view of what has already been said about the relationship of surra of India and surra of Mauritius, the resistance shown by the third, fourth, and sixth mice is not surprising and can not be taken to prove that the separation of surra of India from surra of Mauritius was not effected in purity.

Purity of SM[I].—The experiments with SM[I] resulted as follows:

(1) Mice immunized to surra of Mauritius were immune to SM[I].

- **SM[I] 3110** + **CL 75% 010L** 12 **SM[I] 3110** + **D. 010**
- **SM[I] 3111** + **AA 5% 0CL 100L** 11 **SM[I] 3110** + **L.**
- **SM[I] 3111** + **AA 5% 0CL 100L** 15 **SM[I] 3110** + **D. 016**

Of the three animals inoculated, only one became infected and this (No. 3) was tested on the fifteenth day. Even then it had a strong immunity, for it did not become positive until the twelfth day after inoculation (control infected on the first day).

(2) Mice immunized to surra of India were not immune to SM[I].

- **SI 3110** + **CL 010** 4 **SI 3111** + **D. 119**
- **SI 3113** + **CL 010** 5 **SI 3110** + **D. 117**
- **SI 3113** + **CL 010** 6 **SI 3112** + **D. 117**
- **SI 3110** + **CL 100L** 10 **SI 3110** + **D. 117**
- **SI 3110** + **CL 100L** 10 **SI 3110** + **D. 117**

Every one of the five animals became infected and in only two (Nos. 4 and 5) was the appearance of the parasites delayed. In these two cases, the trypanosomes introduced were very few, not
one being seen in one hundred fields of the injected suspension (controls positive on the second day).

(3) In the prophylactic experiment with SM[I], this virus behaved more like surra of Mauritius than surra of India.

One mouse (the first) resisted infection and the other had a relapse on the twentieth day. In similar tests with surra of India both animals remained negative; and in those with surra of Mauritius one remained negative, the other relapsed, just as in the experiment above.

In the other tests, SM[I] acted anomalously, but scarcely more so than pure surra of Mauritius had done. In some, it behaved like surra of India (see first and second experiments below), in others like neither surra of India nor surra of Mauritius. The experiments which resulted anomalously follow.

1. Mice immunized to surra of India were immune to SM[I].

2. Mice immunized to SM[I] acquired an immunity to surra of India (No. 4) but none to surra of Mauritius (No. 1) or to caderas (Nos. 2 and 3).

3. A mouse immunized to both surra of India and surra of Mauritius offered little or no resistance to infection with SM[I].

In this test infection was manifest on the day after the parasites were found in the control. We note, however, that the mouse sub-
sequently became negative and did not die until the twentieth day after it was injected.

4. Mice cured of SM[I] acquired no immunity to surra of India, to surra of Mauritius, or to any other of the normal strains with which I was supplied.

While it seems probable that the SM[I] employed in the last experiment had been preserved exclusively in mice, it is impossible, due to an omission in the records, to exclude the possibility that several months previously it had passed through a guinea pig.

Exceptional Cases.—In the study of immunity following cure, exceptional results were occasionally encountered. Where infection and death were expected, in some animals infection followed by a spontaneous recovery was observed; in others, no infection whatever was seen. The outlines of the exceptional cases of this kind have been collected into a single table in order that their significance may be more readily studied.
In this table we note, (1) that surra of India and surra of Mauritius immunized against each other (mice 1 and 2, 5 and 6); (2) that mice cured of caderas, CD[I], and dourine (Nos. 8, 9, 11, and 12) failed to become infected when tested with surra of India five and seven days after treatment; and (3) that infection, followed by spontaneous recovery, took place in the third, fourth, seventh, tenth, and thirteenth animals.

From these observations two points are indicated, the first being both more obvious and certain than the second:—

(1) That trypanosomes probably identical in origin, but preserved apart for years, may, under certain circumstances, immunize perfectly against each other. The failure to infect in these cases is all the more remarkable for the reason that in other tests under conditions which outwardly seemed the same, mice immunized to one of these strains have offered no resistance to infection with the other.

(2) That certain forms of treatment may exert a profound influence upon trypanosomes introduced five or more days after the last injection of medicament. In nearly every instance, the treatment which has been followed by this prolonged influence, has been a double one, consisting of an inoculation of acetyl-atoxyl (or a mixture containing this, namely, Mixture 1) followed by an injection of CL (or a mixture containing this, namely, Mixture 1 or Mixture 4). From what has been learned in other experiments of the action of this double treatment, and from its frequence in the above table (it was employed eight times), it seems not improbable that the results in mice 3, 4, 8, 9, 10, 11, 12, and 13 may be explained, in whole or in part, by the prolonged action of the medicament.

The reasons for not attributing the above results to the presence of a non-specific immunity, are as follows: (1) No undoubted case of a strong non-specific immunity has yet been reported (surra of India and surra of Mauritius being regarded as specifically identical). (2) The assumption of such an immunity would not explain all of the cases. We note, for example, that four of the mice became infected (Nos. 3, 4, 10, and 13). If a strong, non-specific immunity had been present, infection
would have been delayed. In three of the mice, however, infection took place at once (Nos. 3, 10, and 13), and in the fourth animal, the delay was short, the parasites being found forty-eight hours after the first test (mouse 4).

The recovery of mouse 7 is particularly difficult to explain, for trypanred is excreted rapidly, usually exerting little influence on inoculations made three or more days after treatment. Perhaps in this animal the result was due to the combined action of a little unexcreted medicament and a weak non-specific immunity.

Puzzling Results.—As we have seen in the last table, following forms of treatment which exert a strong and prolonged influence, infection has occasionally been prevented or rendered irregular. In a few instances the same forms of treatment have been followed by delayed infection. Delayed and prevented infections due to unexcreted medicament are very confusing, for in animals properly tested they are usually seen only in the presence of immunity. It follows, therefore, that when these particular forms of treatment are employed, the influence due to immunity and that due to unexcreted medicament can at times be distinguished merely by the fact that the former is regarded as specific, the latter as non-specific.

Subinoculated Mice.—In attempting to differentiate these two influences, twenty-four hours after the immunity tests were made, blood was drawn from each of fifteen mice which had failed to become visibly infected, and was injected into the same number of normal animals. In the table on page 61 the result of each of these subinoculations is given just below the animal from which the blood was obtained, the connection between the two being indicated by an arrow.

In the experimental animals it seems probable that immunity prevented infection in tests with surra of India (No. 1), caderas (No. 6), and nagana (No. 9), and delayed it following injections of caderas (No. 3) and dourine (Nos. 10 and 14); and that unexcreted medicament prevented infection with surra of India (Nos. 5, 8, and 15) and delayed it after injections of surra of Mauritius (Nos. 2, 4, and 12), caderas (Nos. 11 and 13), and nagana (No. 7). The result seems, therefore, to have been determined by immunity in six cases, and by unexcreted medicament in nine.
Differentiation not Successful.—Although, as we have just seen, the results in the experimental animals were apparently determined in some cases by immunity and in others by unexcreted medicament, we find nothing in the subinoculated mice to distinguish the influence of the former from that of the latter. To the rule that the subinoculated mice always behaved like the animals from which they were inoculated, there were but two exceptions (Nos. 14 and 15).
It is evident, therefore, that subinoculation did not differentiate protection due to immunity from that due to medicament.

Although failing to differentiate influences which are at present regarded as distinct, the subinoculated mice bring out several points of interest. We note, for example, that the similarity of the protection due to immunity and to medicament is emphasized. With but two exceptions, if either was strong enough to prevent infection, infection in the subinoculated animals was also prevented, and if either sufficed merely to delay infection, a delayed infection was found in the subinoculated mice. We observe, also, that when strong enough to prevent infection, the two influences acted with considerable rapidity. This is shown by the fact that of the six experimental animals in which no infection was observed (Nos. 1, 6, 9, 5, 8, and 15) the blood of only one (the last) proved infectious when introduced into other mice twenty-four hours after inoculation.

As a last point, attention is directed to the fact that in the case of the two mice which pursued courses different from those of the animals inoculated from them, species were differentiated by the subinoculated mice but not by the experimental animals (Nos. 14 and 15); for we see that in testing these, both of which were immunized to dourine, the inoculation with dourine infected, while the one with surra of India failed to infect. On the other hand, the mouse subinoculated after the dourine test remained negative, while the one inoculated after the injection of surra of India became infected. In this instance, blood removed by subinoculation from the further influence of both immune bodies and unexcreted medicament distinguished species, while the animals from which it was derived failed to do so.

SUPPLEMENTARY SERUM EXPERIMENTS.21

Soon after my work at the Pasteur Institute began, it was observed that mice immunized to surra of India possessed little or no immunity to surra of Mauritius. This seemed so surprising that,

21 The history of the experiments with immune sera has been so fully given by Mesnil and Brimont that it may be omitted here. The reader is referred to their article "Sur les propriétés protectrices du sérum des animaux trypanosomiés; races résistantes à ces sérum," Ann. de l'Inst. Pasteur, 1909, xxiii, 129.
at the suggestion of Dr. Felix Mesnil, the following serum experiments were carried out as controls on the purity of the surra of India and surra of Mauritius employed in the mouse experiments.

In these tests the serum of three goats was employed. For this serum and for the histories of the animals that furnished it, I am indebted to Dr. Mesnil. Goat 1 was immunized to both surra of India and surra of Mauritius, Goat 2 to surra of Mauritius alone, and Goat 3 was normal. The histories of the first and second animals follow.

Goat 1 was inoculated with surra of India on July 13, 1906. It became infected, recovered, and was then found to be immune, when on March 8, 1907, it was reinjected with the same virus. Nevertheless, this animal contracted a light infection when inoculated with surra of Mauritius on May 3 and 7, 1907. In the following experiments the serum derived from this animal is marked (I + M).

Goat 2 was inoculated with the trypanosomes of surra of Mauritius on May 7, 1907. Although it was microscopically negative from the tenth to the twentieth of this month, the animal was infected, for injections of its blood on May 18 killed two mice. Other inoculations into mice were made on June 18 and July 4, but these failed. Nevertheless, the goat still harbored parasites at the time its serum was employed in the following experiments, for twenty-five c.c. of its blood proved infectious when injected into a dog on August 24, 1907. On October 10, and again on November 15, of the same year, the blood of Goat 2 was inoculated into another dog, but failed to infect it. In the outlines which follow, the serum of this animal is marked (M).

Technique.—In the following experiments, serum, in varying quantities (usually one fourth, one half and three fourths c.c.), was placed in sterile test glasses and to each glass was added a given quantity (usually one tenth to one fourth c.c.) of a suspension of trypanosomes in citrated physiological salt solution. The serum and suspension were thoroughly mixed, and the mixtures, after standing at room temperature for two to four minutes, were injected subcutaneously into mice. As controls on these experiments, similar quantities of virus were measured into empty sterile test glasses, and after corresponding intervals were injected into normal mice. The details are recorded in the following outlines.

Serum Outlines.—The outlines of the serum experiments resemble closely those with which we have already become familiar. There are, however, some new factors. For instance, in these we find indicated the goat from which the blood was drawn, the date of the bleeding, and the quantity and age (in days) of the serum employed. The quantity of the virus suspension added to the serum and the number of trypanosomes in the former before mixing is also shown.

The explanation of a single outline will make the others clear.

The history of this goat is given by Mesnil and Brimont, who describe it as “chevre surra,” Ann. de l’Inst. Pasteur, 1909, xxiii, 135. The above account is less complete.
Chemo-Therapeutic Trypanosome Studies.

1. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SM}\textsuperscript{54\textdegree} \textsuperscript{-7} \text{ C} 3-11 60 L.

The above means that the serum employed came from the goat immunized to both surra of India and surra of Mauritius. Of this serum .75 c.c. (one day old) was mixed with .25 c.c. of a suspension containing three parasites in ten fields. The mixture was then injected subcutaneously into a mouse, and a similar quantity of virus (without serum) was introduced into another animal. The former remained negative from the third to the thirty-first day and was still living on the sixtieth day, while the latter became positive on the third day and was dead on the seventh.

When the serum from a single bleeding was used on different days, it was kept in the ice chest in the intervals between tests.

\textit{Goat 1.}—The experiments with the serum of Goat 1 follow.

\textbf{GOAT 1}

\textbf{BLEDT JUNE 11 1907}

1. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SM}\textsuperscript{23\textdegree} \textsuperscript{-3} \text{ C} 3-11 60 L.
2. \textbf{Serum\([I+M]\)} \textsuperscript{55\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SM}\textsuperscript{34\textdegree} \textsuperscript{-7} \text{ C} 3-11 60 L.
3. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SM}\textsuperscript{34\textdegree} \textsuperscript{-7} \text{ C} 3-11 60 D.
4. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.
5. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.
6. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.
7. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.
8. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.
9. \textbf{Serum\([I+M]\)} \textsuperscript{75\textdegree} \textsuperscript{C} \text{ DAY} + \textbf{SI}\textsuperscript{2 x 005} \textsuperscript{-9} \text{ C} 3-11 60 L.

From the above it is evident that the serum of this goat exerted a strong preventive action against the two strains of surra of India and surra of Mauritius employed in my experiments. Of the nine mice tested, only one (No. 9) became infected and this animal received the minimum quantity of serum and a comparatively large number of trypanosomes. Even in this case, however, there was some protection, for the incubation period was seven days, that of its control two days.

\textit{Goat 2.}—Goat 2 was twice bled. As the results of the two bleedings are very much alike, they will be considered together, the experimental evidence for both being given on page 65.

The tests with the serum of this goat seem to indicate that no contamination of virus had taken place in the strains used in my experiments, and show clearly the very close relationship between
Goat 2

Bled June 22, 1907


Bled July 4, 1907

11. Serum [M] 25 cc + Ng 1-17 = 1-17

Surra of Mauritius and surra of India, for of the six tests with surra of India and the eight with surra of Mauritius, not one infected.28 On the other hand, under similar conditions the maximum quantity of serum (three fourths cubic centimeter) failed to prevent infection when mixed with the parasites of caderas or nagana. We note, however, that in the case of caderas there was a distinct delay in infection.

In view of the fact that the serum of the goat that had recovered from surra of Mauritius alone, protected perfectly against both surra of India and surra of Mauritius, it is interesting to recall that the first goat, after becoming immune to surra of India was susceptible to infection with surra of Mauritius.

Goat 3.—The following experiment with the serum of Goat 3 serves as a control on those with the serum from Goats 1 and 2.

28 A result exactly similar to this has been reported by Mesnil and Brimont, who characterize it as “Proof new and superfluous of the identity of these two viruses,” Ann. de l’Inst. Pasteur, 1909, xxiii, 139.
and shows that the protective properties of the first and second sera are not manifested by normal serum, even when the latter is employed in quantities of .75 to 1.75 cubic centimeters.

GOAT 3
BLED JULY 4 1907

1. SERUM (NORMAL) + NG 1-2 'D.'
2. SERUM (NORMAL) + SM 3-6 'D.'
3. SERUM (NORMAL) + SI 7-13 'D.'
4. SERUM (NORMAL) + SI 9-6 'D.'

When mixed with the parasites of nagana, surra of Mauritius, and surra of India, normal goat serum not only did not prevent infection, but in three of the four tests the appearance of the parasites was not at all delayed, and in the fourth, although the incubation period was distinctly prolonged, death occurred earlier than in the control.

Goat and Mouse Immune Bodies Contrasted.—Trypanosome virus derived from guinea pigs was employed in testing the following mice: 1 to 6, Goat 1; 1 to 3, Goat 2 (bled June 22); and 1 to 5, Goat 2 (bled July 4). As we have just seen, in the goat experiments this virus was indistinguishable from the original strains with which the goats had been inoculated. We know, however, that the passage of virus through guinea pigs renders it serum resistant, and that a serum resistant strain is readily distinguished from the original, if mice cured of one of these strains are inoculated, some with one strain and some with the other. Since the tests made in mice show a degree of specificity not seen in goats that recover slowly without receiving treatment, it seems that this may be explained by assuming that the goat serum contains antibodies which are directed against a large number of serum resistant strains, while in the mouse, the antibodies formed are directed solely against the organisms of the original infection.

Report Incomplete.—It is clearly recognized that the present report is incomplete. This is due to the fact that in the course of the study several unavoidable but prolonged interruptions have occurred. As a consequence it has been impossible to test adequately some of the points here suggested. Nevertheless, in order
that those interested may have access to the work, it has seemed desirable to publish the results thus far obtained. A summary follows.

**GENERAL SUMMARY.**

When mice infected with various species of trypanosomes are given curative doses of a number of different medicaments, an immunity to the species cured is usually demonstrable in the tests made several days later. While the interaction of trypanosomes and some form of treatment seems essential for the production of this immunity, it is not necessary for the animals to be visibly infected.

In tests made three or more days after the last treatment, the protection due to immunity is usually distinguished from that due to unexcreted medicament by the fact that, when the parasites are numerous and are introduced intraperitoneally, infection is delayed or prevented by immunity, but takes place at once, as a rule, in the presence of unexcreted medicament.

The immunity is specific in the sense that mice immunized to one species, show, as a rule, no resistance to infection with other species. The differentiating power of the reaction is, however, so delicate that in a number of instances (compare surra of India, surra of Mauritius, and the resistant strains) it has easily distinguished strains of the same species.

In a few instances, however, it was difficult to decide whether a given result was due to an unusual influence of unexcreted medicament or to a non-specific immunity. Examples of a possible non-specific immunity were observed in mice immunized to surra of India and tested with caderas and vice versa, and also in mice immunized to dourine and tested with caderas.

The production of immunity in mice following the cure of experimental trypanosome infections seems to be a general phenomenon, for it has been possible to demonstrate its presence against every strain thus far tested.

The immunity develops early, being detected at times between the second and third day after treatment.

The immunity following cure is temporary. In most instances it seemed strongest four to six days after treatment.
however, it disappeared completely in eight to eleven days, and in animals tested but once it was unusual to find much resistance twenty days after treatment.

Some of the attempts to prolong the immunity were encouraging, the factors of most importance being apparently the following: (1) a virus against which immunity is readily obtained, (2) a suitable form of treatment, (3) a short interval between the treatment and the first test, (4) frequent tests at short intervals, and (5) the employment of only moderately rich injections of trypanosomes in the earlier tests.

In my experiments a strong immunity has been obtained with greater ease against the more virulent than against the less virulent trypanosomes. The infection against which it has been easiest to secure immunity was surra of India. With the less virulent dourine, on the other hand, the results have been much less satisfactory. Against the latter infection, twelve attempts to produce an efficient immunity have thus far failed.

The strongest immunity was usually obtained by employing one of the dyes, either alone or in combination with acetyl-atoxyl.

While acetyl-atoxyl is usually rapidly excreted, it is evident that an injection of this medicament has, in a number of instances, prolonged the excretion of Cl (dichlorbenzidine plus amidonaphtoldisulphonic acid 1.8.3.6.) given four or more days afterward.

Surra of India was particularly sensitive to Cl., employed alone or in combination with acetyl-atoxyl. By one of these combinations, the virus was so readily influenced that infection was at times completely prevented in tests made six days after the last treatment.

In treating mice with Cl a strong immunity was at times obtained following the injection of small quantities of the medicament. In one instance, half the usual curative dose gave rise to a strong immunity against surra of India.

Additional evidence that the action of Cl is indirect seems to have been furnished by the fact that rich intraperitoneal injections of surra of India and caderas were capable of infecting mice when introduced as early as twenty-four hours after the medicament.

Increasing the efficiency of the treatment was not usually attended by an increase in the strength of the immunity produced,
unless curative efficiency was secured in combinations known to be excreted slowly.

The results of the immunity tests differed with the strength of the immunity. If this was very strong, no infection whatever took place. If it was weaker, infection was delayed, and if it was very weak, infection was merely slightly prolonged.

In some instances infection followed by spontaneous recovery was observed. These cases were seen, when, due to the presence of unexcreted medicament, there was opportunity for the development of immunity after the test had been made.

In prophylactic and immunity experiments where infection followed by spontaneous recovery took place, the number of days intervening between the appearance of the parasites and their disappearance, usually varied directly with the number of days separating the treatment and the test. From this it seems that the stronger the influence of the unexcreted medicament, the more quickly the parasites are banished from the blood.

The strength of the immunity was often sufficient to prevent completely infection in the tests made comparatively soon after treatment. Such an immunity was secured in mice cured of surra of India, surra of Mauritius, caderas, nagana, and a toluidin blue resistant nagana strain.

At times, varying degrees of hypersensitiveness to infection were noted. It was seen many months after treatment in a mouse that for forty days had proved unusually resistant to infection with surra of Mauritius. It was also observed comparatively early after treatment in the case of four other mice cured of surra of Mauritius and subsequently tested with the same virus.

It is of interest to note that in three of these five instances of hypersensitiveness, the surra of Mauritius that infected had been passed through one or more guinea pigs.

When a double infection was treated, a double immunity was secured.

By means of the immunity reaction it was apparently possible in a number of instances to separate in purity organisms that had been mixed in vitro.

If subsequent investigation shows that strains of trypanosomes
preserved exclusively in mice undergo no alteration detectable by
the immunity reaction, it seems not improbable that this reaction
may be resorted to in order to detect contaminations of virus.
The experiments with goat serum gave no indication that con-
tamination had taken place in the surra of Mauritius and surra of
India with which I was working. When employed in the proper
quantity, the serum of the goat immunized to both surra of India
and surra of Mauritius protected perfectly against the strains of
similar name employed in my mouse experiments. Furthermore,
the serum of the goat immunized to surra of Mauritius alone
afforded absolute protection against both surra of India and surra
of Mauritius, delayed infection with caderas, and exerted no influ­
ence against nagana. The protection against surra of India and
surra of Mauritius exerted by serum from goats 1 and 2 was
perfect even when these strains had been passed in the meantime
through guinea pigs. Finally, normal serum in maximum quantity
failed to prevent infection with nagana, surra of India, or surra of
Mauritius.

In conclusion, I wish to express my thanks and appreciation to
all who have assisted by supplying me with virus, medicaments, or
suggestions. I am under special obligation to Dr. Felix Mesnil, Dr.
Paul Ehrlich, Dr. Simon Flexner, and Dr. W. H. Manwaring.