

INTRA-STOMACHAL AND INTRA-INTESTINAL
INOCULATIONS OF TRYPANOSOME VIRUS
WITH TESTS FOR IMMUNITY.*

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Introduction.—In unpublished experiments of Dr. Frederick G. Novy, relapsing fever spirochætæ were introduced directly into the stomachs of rats. As a result of these inoculations, either no infection at all, or only a slight infection was observable. When the rats that had received the virus intra-stomachally were subsequently injected with the same spirochætæ, it was found that all, even those that apparently had not been infected, had acquired a strong immunity.

As rats normally possess no immunity to these spirochætæ, but acquire it after recovering from infection, it was natural to inquire whether the immunity noted in the rats inoculated intra-stomachally, had not arisen as the result of an infection too mild to be detected by microscopical examination.

This question was rendered the more pertinent because rats show very decided differences in the way in which they react towards inoculations of the same spirochætæ. Some have very severe infections and die, while in others the course of the disease is quite mild. On account of the great differences observable in rats infected with spirochætæ, it seemed desirable to repeat the experiments with parasites which show less variation in their action.

For this purpose, pathogenic trypanosomes preserved exclusively in rats seemed to be admirably suited, for when certain species of these parasites infect rats at all, they invariably kill if the animals receive no curative treatment. At the suggestion of Dr. Novy,

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therefore, the work here reported was carried out in the Hygienic Laboratory of the University of Michigan, in the summer of 1908. To Dr. Novy my thanks are due, not only for the problem and virus, but for aid and suggestions during the course of the work.

INTRA-STOMACHAL INOCULATIONS.

Virus.—The trypanosomes employed in the following experiments were those of nagana (*T. brucei*), surra of India (*T. evansi*), dourine (*T. equiperdum*), and caderas (*T. equinum*).

Technique.—Rats alone were employed. In the animals bled for virus, the parasites were, as a rule, 100 or more per field (Zeiss, lens "D," ocular No. 4), in fresh specimens about one corpuscle thick. In securing this virus the rat selected was, under ether anesthesia, bled to death from the heart, the blood was defibrinated, cooled quickly to almost 0° C., and kept at this temperature until used. In most of the experiments the injections of blood were made in less than three hours after it was obtained, and usually within one and one half hours, never later than six hours. In all cases the trypanosomes were still actively motile when injected.

After shaving and disinfecting the skin of the abdomen, a laparotomy was performed under ether anesthesia and the stomach of the rat was drawn through the incision. An injection of one fourth to one half cubic centimeter of richly infected blood was then made directly through the thin cardiac part of the stomach wall. To prevent the escape of blood through the hole in the stomach and to disinfect the wound, a small pledget of cotton that had been thoroughly squeezed out in 2 per cent. lysol was placed on the stomach around the hypodermic needle before this was withdrawn. By raising the cotton from time to time, it could be determined when the puncture in the stomach remained clean. As a rule, the stomach was kept out of the abdominal cavity only a few minutes. When all hemorrhage had stopped, the organ was allowed to slip back into its place and the incisions in the peritoneum, muscles, and skin were sutured with silk.

The results of the intra-stomachal inoculations are given in table I.

TABLE I.

Virus.	Inoculated.	Not infected.	Per cent. not infected.
Surra (<i>T. evansi</i>)	5	2	40
Nagana (<i>T. brucei</i>)	16	7	44
Dourine (<i>T. equiperdum</i>)	13	6	46
Caderas (<i>T. equinum</i>)	7	4	57
	41	19	

Table I shows conclusively that trypanosomes may be inoculated directly through the stomach wall without infecting. Of forty-one rats inoculated in this way, nineteen, or nearly 50 per cent., failed to become infected.

The trypanosomes used in these experiments were fully virulent, as was shown by the course of the controls. Most of these controls received minimal inoculations of virus to imitate the infection that might be produced in the experimental animal if only a few trypanosomes succeeded in making their way through the puncture in the stomach into the peritoneum or blood vascular system. For example, one rat received intra-stomachally one half cubic centimeter of nagana blood so diluted that it contained ten trypanosomes per field. As controls on this animal, three other rats were inoculated intra-peritoneally, each with 1 cubic centimeter of diluted nagana blood. The dilutions were 1 to 100,000, 1 to 500,000 and 1 to 1,000,000. All three controls became infected and died, the rat receiving the dilution of 1 to 1,000,000 becoming infected after the others, but one day before the animal inoculated intra-stomachally.

Table I seems to show also that the result of the inoculations varied with the virus employed. Surra of India was apparently the most virulent, infecting 60 per cent. of the rats, and caderas the least virulent, infecting 43 per cent. Nagana and dourine occupied intermediate positions infecting 56 and 54 per cent. respectively.

The trypanosomes most frequently used were those of nagana and dourine. Nagana was selected because of its extreme virulence, and dourine because it is generally thought to possess a characteristic not usually ascribed to nagana, *i. e.*, the ability to pass through unbroken mucous membranes. From the table, however, we see that dourine introduced into the stomach proved slightly less infectious than nagana.

Distended Stomachs.—One point of some practical importance should be mentioned, *i. e.*, that rats having stomachs greatly distended with food are unfavorable for the intra-stomachal inoculations. In every instance in which this organ was markedly distended, even though there was no obvious leakage from it, infection followed the inoculation. Even moderate distention was unfavorable, for only one rat with a stomach in this condition escaped infection.

In order to avoid distended stomachs, it was not sufficient merely to withhold food for a number of hours before operation, for rats often bury food under the saw-dust or shavings in their jars, and dig it up again when hungry. To prevent their doing this, it was necessary to place the experimental animals in jars in which it was known that there was no food, and to keep them there for ten to twenty-four hours before the laparotomy. By this procedure it was possible to obtain stomachs that were empty or nearly so.

Immunity.—It was now of interest to ascertain whether the animals that had escaped infection in table I, had acquired an immunity. To determine this point, all of the rats that had escaped infection were reinjected subcutaneously with small quantities of the virus with which they had been originally inoculated. The object of using small quantities of virus was to detect the presence of even a slight immunity. The trypanosomes injected varied from two per field to less than one in 1,000 fields, and the quantity of suspension inoculated was never more than one fourth of a cubic centimeter and usually only one twentieth of a cubic centimeter. In the beginning the immunity tests were made twenty-eight days after the intra-stomachal inoculations. Subsequently the interval between the original inoculation and the immunity test was shortened to twenty-four, then to twenty, sixteen, and finally to twelve days. The result was the same, however, in every instance. Every animal inoculated became infected and died. Not a trace of immunity was seen.

Hypersensitiveness.—Not only was no immunity observable in the rats that survived the intra-stomachal inoculations, but in the case of surra and nagana there seemed to be a slight hypersensitiveness to infection, for both of the surra rats and five of the seven

nagana rats died before their controls. On the average, the nagana rats died two days earlier than their controls.

INTRA-INTESTINAL INOCULATIONS.

Dourine.—Using technique similar to that employed in the intra-stomachal inoculations, one half cubic centimeter of dourine blood containing ten trypanosomes per field was introduced into the intestines of two rats, in each case within one centimeter of the stomach. Both rats subsequently became infected and died, one as quickly as its control, the other two days later.

Unfortunately, for lack of time, further experiments along this line could not be carried out. No statement, therefore, can be made as to whether animals inoculated intra-intestinally can, even in a small per cent. of the cases, survive this form of injection.

THE VIABILITY OF TRYPANOSOMES IN THE STOMACHS OF RATS.

Attempts to ascertain the influence of the stomach upon trypanosomes introduced into it were made in two ways. (1) The effect upon motility was studied by withdrawing samples of blood from time to time and examining these under the microscope. (2) The retention or loss of virulence was tested by inoculating into normal animals blood that had been withdrawn from the stomach.

Caderas.—With caderas no early tests were made, but two rats inoculated intra-stomachally, each with one half cubic centimeter of blood containing fifty trypanosomes per field, were examined after two and three hours respectively. In the stomachs of neither, however, could a trypanosome be found.

Dourine.—Two rats were inoculated with dourine. Into the stomach of the first rat was introduced one cubic centimeter of blood containing 100 trypanosomes per field. Blood removed from the stomach by needle puncture revealed actively motile parasites during the first forty minutes, but in this interval the number of motile organisms had decreased to one in two fields. By the fiftieth minute the parasites were sluggishly motile and only one was found in ten fields. After this no motile parasites could be detected although a few non-motile organisms could be seen. Eighty min-

utes after the inoculation of the blood, 0.1 cubic centimeter of the stomach contents of this animal was inoculated subcutaneously into another rat, but failed to infect it.

Into the stomach of the second rat, three fourths of a cubic centimeter of blood containing 200 dourine trypanosomes per field was injected. In this rat the parasites remained active for forty-three minutes, but had decreased in this time to five per field. Seven minutes later not a motile trypanosome could be found although the non-motile forms were fairly numerous. Seventy-eight minutes after the introduction of the parasites, 0.1 cubic centimeter of the stomach contents was inoculated subcutaneously into another rat but failed to infect it. From this we see that the virulence of the dourine parasites introduced into the stomach had apparently disappeared in less than eighty minutes.

Nagana.—In the first fifty minutes that followed the intra-stomachal injection of three fourths of a cubic centimeter of nagana blood containing 100 trypanosomes per field, the number of motile parasites had decreased to two per field. After this no motile organisms were seen although a few non-motile trypanosomes were present as late as 105 minutes after the injection of the blood. Some of these were inoculated subcutaneously into a rat but failed to infect it.

Surra.—The parasites of surra were apparently more resistant to the injurious influences of the stomach than were the trypanosomes of the other species. From the stomach of a rat that had received three fourths of a cubic centimeter of blood containing 100 surra trypanosomes per field, samples of blood were withdrawn 5, 15, 30, 50, and 120 minutes after their introduction. In all of these, actively motile trypanosomes were found, although the number of the parasites had decreased from twenty per field at the first withdrawal to one in three fields at the last. From this it is seen that surra trypanosomes may remain actively motile for at least two hours after being placed in the stomach of a rat.

To test the virulence of the trypanosomes, 0.05 cubic centimeter of each of the five samples just described was introduced subcutaneously into rats and in each instance the animal became infected and died. This indicates that surra of India may remain infec-

tious for at least two hours after being introduced into the stomach of a rat.

THE VIABILITY OF TRYPANOSOMES IN THE INTESTINES OF RATS.

Dourine.—One cubic centimeter of blood containing 200 trypanosomes of dourine per field was introduced into the intestine of a rat. Forty-two minutes later blood withdrawn by needle puncture from the intestine contained but one active trypanosome in eight fields, and seventy-five minutes after the introduction of the blood only one actively motile parasite could be found in fifty fields. The animal was now killed by ether, and the intestine was removed and cut into sections one and a half inches long. On examining the section that had been nearest the stomach, two very active parasites were found in searching many fields. The bloody contents of the second section were then taken up in a little salt solution and 0.1 cubic centimeter was inoculated subcutaneously into a rat, but failed to infect it, although a microscopical examination of the fluid in the syringe showed one actively motile parasite in twenty fields. From the intestine of this rat most of the trypanosomes disappeared very rapidly, and the virulence of those that remained was apparently lost in less than an hour and a quarter after their introduction.

Nagana.—One half cubic centimeter of blood containing 100 trypanosomes per field was introduced into the duodenum of a rat, and again the disappearance of the parasites took place with great rapidity. After forty minutes, only two very active flagellates could be found in 100 fields, and after sixty minutes, no motile form of any sort could be discovered. The intestine was then removed and cut into sections an inch and a half long. On slitting open and examining the various sections in order, beginning at the stomach, a few motile trypanosomes were found in the first and third. The other sections were negative.

Surra.—Into the duodenum of a rat, four fifths of a cubic centimeter of blood containing 100 surra trypanosomes per field was injected. On examining the intestine of this animal, seventy-five and ninety minutes later, neither motile nor non-motile parasites

could be discovered. One hundred and fifteen minutes after the injection into the duodenum, it was noted that some blood had made its way into the stomach, so this organ was opened and its contents examined. Rather to my surprise, a number of sluggishly motile trypanosomes were found in the mixture of blood and stomach contents. Here again was shown the exceptional resistance of surra of India to the harmful influence of the stomach.

As far as the writer is aware, experiments exactly like those here described have not been reported, although the feeding experiments of others, *e. g.*, Kanthack, Durham, and Blandford,¹ agree, as far as they go, with the results here recorded, in that they show (1) that trypanosome virus may often be introduced into the alimentary tract without infecting, and (2) that the animals that escape infection do not acquire immunity.

SUMMARY.

In nearly 50 per cent. of the cases, virulent trypanosome virus inoculated directly through the stomach wall of rats failed to infect these animals.

The percentage of infections after inoculation of this kind seemed to vary with the virus. Surra of India proved to be the most virulent, and caderas the least. Nagana and dourine occupied intermediate positions.

The rats that escaped infection showed no trace of immunity when tested with small quantities of the same virus between the twelfth and the twenty-eighth days after the intra-stomachal inoculations.

The majority of the rats tested for immunity with surra of India and nagana seemed to have acquired, instead of an immunity, a certain *hypersensitiveness* to infection. Five of the seven nagana rats died before their controls.

The results of testing the vitality of the trypanosomes of caderas, dourine, and nagana introduced directly into the stomachs and intestines of living rats seem to show that both motility and virulence are usually lost in less than two hours. Surra of India proved

¹ Kanthack, Durham, and Blandford, On Nagana, or Tsetse Fly Disease, *Proc. Roy. Soc.*, 1898-99, lxiv, 100.

exceptionally resistant to the harmful influences of the stomach, for in one case, surra trypanosomes introduced into the *intestines* were found to be sluggishly motile in the *stomach* one hour and fifty-five minutes later, and in another instance, surra parasites injected into the stomach were found, when removed from that organ two hours later, to be actively motile and infectious.