THE SPREAD OF TULAREMIA THROUGH WATER, AS A NEW FACTOR IN ITS EPIDEMIOLOGY

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Rodents are the chief sources of infection of tularemia and the reservoir of its virus in the external world. The rôle of the water-rat, which, in the U. S. S. R., is the principal source of tularemic infection for man is well known. In the United States the same part is played by the wild rabbit. In the U. S. S. R. the rôle of the hare, rabbit, and mouse,—in Norway, that of the wild rat—has been proved. Other rodents may serve as reservoirs of virus, for instance: *Apidemus silvaticus, Ondatra zibethica, Didelphis virginiana, Marmota flaviventer, Marmota bobac, Microtus ilaeus, gerbitus tamaricinus, Citellus beecheyi* Rich., *Citellus pygmeus* Pall, *Microtus californicus-aestuarinus*, etc.

Animals that do not belong to the rodent-class, such as opposums, foxes, and lemmings, have also been recorded as sources of infection.

The modes by which tularemia spreads from these sources of infection are also being carefully investigated. Contact with water-rats while hunting or flaying them has been well studied in the U. S. S. R. (Zarhy, Hatanever, Golov, Volfertz, Suvorov, Voronkova, Gauzner, Belitzer, Kniazevsky, Berdnikov, Tiflov and others). In the United States contact with wild rabbits during skinning and especially during the preparation of their carcasses is the cause of infection in 61 per cent of cases (Francis). The spread of tularemia through contact with the domestic rabbit was proved for the first time in the U. S. S. R. by Miller in collaboration with Kreutzer, Kvashnina and Grzebina. Its spread through contact with mice has also been proved by Miller con-

jointly with Volfertz, Korbon, Jampoladova and Grzebina. In this case the chief epidemiological factor is the soiling of food stuff by the excretions of the mice. The spread of tularemic infection through contact with hares, or rather with their carcasses and skins when preparing the killed hares and when making tinned food was also established first in the U. S. S. R. by Berezin and Nesgovoroff.

A number of infection-carriers or vectors have also been studied, such as mites, flies, horse-flies, fleas and water-rat lice.

Quite recently, Prof. Miller has pointed out another possible mode of spread of tularemia. In his paper on tularemia, read at the All-Russian Conference of Microbiologists and Epidemiologists, which took place in Leningrad on December 7 to 11, 1934,[1] as well as in his monograph "Tularemia," published in collaboration with Prof. Stradomsky, Prof. Miller points to water as a new possible mode of spread of tularemia. Thus, for instance, he says in his monograph "Tularemia": "Beside food stuff, water can also serve as a source of infection of tularemia. The investigations, which Dr. Somoff has carried out in my laboratory have established this fact on the ground of the study of one outbreak (the culture has been isolated from water) and of experimental data." We have no other references to this mode of spread of tularemia.

Having studied in 1935 one outbreak of tularemia we were able to prove by exhaustive evidence this new mode of spread of the disease in question. An epidemic due exclusively to water, broke out on meadows amongst people who were occupied in haymowing. In a few days, cases of disease became numerous and in a week's time nearly all the workers had been taken ill, with the exception of one group of mowers, who worked on a plot near by.

CLINICAL PICTURE

Forty-three patients only could be carefully examined and observed for some length of time. Some of the other patients had not been hospitalized at all, others had been discharged from the hospitals before our coming.

[1] This paper has been published in the Sovetskaya Vratschebnaya Gazeta, 1935, N. 3, under the title "Tularemia and new data in its epidemiology."

Disease appeared two to eight days after the people came to the meadow-lands.

All the patients noted the sudden onset of the disease with shivering on which followed a sensation of fever, headache—mostly in the frontal part and pains all over the body—pains in the back, the neck and the extremities being particularly noted. Often there was nausea, sometimes vomiting and pain in the substernal region. In some cases on the second or third day of illness there was a nasal hemorrhage. On the first day of sickness, and more often on the third to the fifth day (in some cases on the tenth day) most of the patients complained of painful swallowing. At about the same time the lymph glands behind the angle of the underjaw began to enlarge, as well as the cervical glands along the musculus sterno-cleido-mastoideus (oftener on one side than on both) and the submaxillary glands. Some patients did not show any phenomena related to the pharynx and glands and their main symptoms were fever and general malaise. In some cases inflammation of one eye was from the first a prominent feature.

The investigation of the first group of patients, some of which we were able to examine in the first days of the illness, showed the following clinical picture.

Nikita P., aged 25. Has been ill for four days.

The patient looks feverish. The temperature is 39°C. The face is hyperemic. The scleral vessels and the conjunctiva are injected. The tongue is moderately furred, moist. The appetite is reduced. Acute hyperemia of the front arches of the pharynx and the uvula. Enlargement and hyperemia of the right tonsil. Behind the angle of the underjaw on the right an enlarged lymph gland, 4 cm. of length, not adherent to the skin and the subcutaneous cellular tissue, painful at palpation. The liver protrudes from under the costal margin to a finger's breadth. The spleen begins from the eighth rib, its margin is palpable in the right-side position. Both organs are somewhat painful on palpation.

As has been stated above, we were able to see only a few patients of this group, most of the patients examined being either in the period when the illness was fully developed or in that of falling temperature. On the whole we have examined 35 patients at this stage.

The main morbid symptoms in this group of patients were the following: the temperature, which up to the seventh or tenth day of the illness ranged from 38.5° to 39.5°C., diminished after this by 1° or 1.5° and in the period from the eleventh to the twentieth day it was either normal or subfebrile in the evening. Most patients experienced severe malaise all through the illness. There was no delirium. The appetite, which had been low during the feverish period was rapidly restored after the fall of the temperature. The angina appeared, as has been stated above in some cases from the first day, more often from the third to the fifth day of illness. It was mostly of the catarrhal type and manifested itself in a hyperemia of the soft palate, the uvula and one or both tonsils.

The tonsils, especially the left one, were often enlarged and friable. Some of the patients had also follicular or lacunar angina, or an angina with small patches of fur of diphtheritic character. In three cases there was observed purulent amygdalitis of the left tonsil, with discharge into the pharynx and pus secretion. As was stated above, the lymph glands were involved in the process either behind the underjaw angle or along the musculus sterno-cleido-mastoideus and mostly on one side. If the glands along the musculus sterno-cleido-mastoideus were affected, as many as two or three glands were involved. The glands were from three to seven cm. in size. At the first examination they were never seen to adhere to the skin and the underlying tissues. In two cases we observed further a lesser mobility of the glands and the skin was then also involved in the process. Palpation of the gland began to show the presence of fluctuation and, on puncturing, a greenish pus was obtained.

Seven of the forty-three patients examined had fever and lymphadenitis in the aforementioned regions, but did not complain of pain in the pharynx—and angina was not observed either at their admittance into the hospital or later on. In five cases the chief symptom was the rise of temperature up to 38.5°C., which lasted from twelve to sixteen days. The fall of temperature was a slow lysis. As a rule there was an enlargement of the liver and the spleen. The general state of these patients was

worse than that of the patients of the two first groups. Some of them were delirious. Neither angina nor lymphadenitis were observed. One of the patients complained during the fever and for some time after the temperature had fallen of pains in the stomach region. Palpation of the stomach was painful. We could not note anything particular on palpation, although the patient remained under control for some time afterwards (Clinic of infectious diseases at the University at Tomsk).

In one case there was another prominent symptom beside fever, and that was an affection of the right eye. There was a sharp photophobia, epiphora and hyperemia of the scleral vessels and the conjunctiva. On the conjunctiva of the under lid there were several enlarged yellowish grains. These ocular phenomena disappeared in the course of the first seven days. Several days after the beginning of the ocular phenomena there appeared near the pinna an enlarged lymph gland which disappeared in the course of twenty days.

In another case, beside fever, angina and enlargement of the lymph glands behind the underjaw angle there was an affection of the left eye with the phenomena described above and also an affection of the gland near the left pinna.

In a third case also, after having suffered from shivering, heat, nausea, and pain all over the body the patient had fever with inflammatory phenomena in the left eye, enlargement of the gland near the left pinna and small ulcers under the tongue. The patient noted these ulcers on the fourth day of illness. An examination made on the seventeenth day showed that under the tongue on each side of the frenum there was a small ulcer, 1 cm. long, with a clean bottom. On touching the bottom of the ulcers with a spatula a slight sensation of pain was experienced. Examination on the thirty-sixth day of illness showed that the ulcers had disappeared. This patient had also an enlarged gland (5 to 6 cm.) on the left side under the bottom of the buccal cavity which was painful on palpation. This enlargement was observed simultaneously with the appearance of the ulcers.

In the outbreak in question morbidity did not seem to bear any relation to sex or age. With a few exceptions nearly all those

were taken ill, who had in any way been exposed to the source of infection (which will be mentioned below). No death occurred, but after the fall of temperature all the patients noted a persistent weakness and at the time of the second examination many of them were still performing only easy jobs.

This clinical picture suggested tularemia with a portal of entrance through the ring of Waldeyer and the buccal and ocular mucosa and the observed cases could be classified as belonging to five different forms of the disease. The first group must be considered as belonging to the anginal form, which takes the aspect of the bubonic form; the second group belongs to the purely bubonic form, the third to the typhoid form; while two of the patients suffered from the glandular-ocular form and two other ones showed a combined anginal and glandular-ocular form.

LABORATORY TESTS PERFORMED IN ORDER TO CONFIRM THE CLINICAL DIAGNOSIS

In order to confirm the clinical diagnosis we carried out a number of laboratory tests.

During the first examination blood was taken for the agglutination-test with *Bacterium tularense* and for the infection of guinea pigs. The blood was taken from six patients on the thirteenth to the twenty-third day of illness. In the case of five patients the agglutination test made with the killed suspension of *B. tularense*[2] was positive in a dilution from 200 to 1000 (fifteenth to twenty-third day of illness). For one patient (thirteenth day of illness) the test was negative. Guinea pigs injected intraperitoneally with the blood of these patients did not fall ill. These results are not unexpected: infection of guinea pigs with the blood of patients gives positive results if it is performed in the beginning of the illness. As to the agglutinins, they appear in the second week of the illness and in a certain percentage of cases even later.

At the same time with these tests the leucocytes and also the

[2] The suspension was prepared at the Central Public Control Institute in the name of L. N. Tarassevitsch.

Shilling's formula were determined for three of the six patients. The following results were obtained:

1. Patient V. O. (a woman). Eighteenth day of illness. Anginal form. Total 8200 leucocytes: segmented leucocytes, 50 per cent; rod-shaped ones, 4 per cent; lymphocytes, 38 per cent; monocytes, 8 per cent; no eosinophils.

2. K. O. (a woman). Twenty-third day of disease. Anginal form. Total 6800 leucocytes. Of these: segmented leucocytes, 61 per cent; rod-shaped ones, 5 per cent; lymphocytes, 32 per cent; monocytes, 1 per cent; eosinophils 1 per cent.

3. K. E. (a woman). Eighteenth day of illness. Anginal form. Total 9800 leucocytes: segmented leucocytes, 50 per cent; rod-shaped ones, 7 per cent; lymphocytes, 33 per cent; monocytes, 9 per cent; eosinophils, 1 per cent.

In three cases with severe angina the slime and fur from the tonsils were examined bacteriologically. Fuchsin staining and Giemsa staining revealed a rich flora consisting of short bacilli accumulated in heaps, small cocci, diplococci and a considerable quantity of small coccobacilli.

After the second examination on the thirty-fifth to fortieth day of illness the agglutination test was made for 21 patients. In all these cases it was positive. The dilution was 1000 to 5000. At the second examination two of the patients showed fluctuation on palpation of the enlarged lymph glands. In both cases a puncture of the gland was performed, which yielded in each case 2 cc. of greenish pus. With this pus we infected guinea pigs subcutaneously and intraperitoneally and later obtained cultures of *B. tularense.*

The clinical diagnosis of tularemia was thus confirmed serologically and bacteriologically.

EPIDEMIOLOGY OF THE OUTBREAK

The meadows on which the epidemic broke out are situated between the villages of M. and N. The country is varied in topography. There are four eminences, divided by valleys, through

which flow the river S. and three brooks, which have no particular names. One group of mowers used the water of the river S. for drinking and in this group there did not occur a single case of illness; the other mowers, amongst whom the illness rate attained 100 per cent used the water of one of the brooks, which at the time in question was running freely.

An epidemiological survey of the area was carried out after we had clinically examined the patients and diagnosed the disease. This examination was accompanied by a close questioning of the patients as well as of the managers of the hay-mowing organizations. As this questioning showed, the patients as well as the managers had observed, that all the workers, who had drunk unboiled water from the brook, were taken ill, whereas in the group of workers who used water from the river S., no cases of illness occurred. The managers of one of the mowing organizations took this fact into account and a second brigade of workers, which came to replace the group that had been taken ill, received directions to take water out of the river S. (although this was at some kilometers from their mowing place) and to boil the water before drinking. This second group finished mowing and returned to their collective farm, without having had a single case of illness, whereas in another group where these measures were not strictly followed, some of the new-coming workers were taken ill in their turn.

Questioning of the patients showed, that near the brook out of which they took their drinking-water, they had seen, as they said, some "black rats" (water rats) and had even killed one of them, but all the people who had been in the fields declared these to be but isolated examples. Beside the rats a few field mice had also been observed.

Proceeding to survey in detail the locality of the outbreak we analyzed the clinical picture of the illness as well as the data obtained from questioning and came to the definite conclusion that it was through water that the infection had been spread. The fact that such a great number of people had fallen ill in a short period of time and the presence of a definite portal of entry (tonsils, conjunctiva and buccal mucosa) from the first excluded sev-

eral of the usual vehicles of infection. There was no industry and moreover the portal of entry excludes this mode of spread. The possibility of the infection being disseminated by biting insects (such as gnats, horse-flies, flies, etc.) is also excluded by the determinate portal of entry. Spread through food we also from the first considered as impossible and the subsequent experimental and bacteriological tests have fully proved the correctness of this view. And even if we did not have at our disposal such proofs as those we have gathered, this mode of spread requires the presence of a great number of rodents, under conditions which enable them to pollute food stuffs with their fecal masses.[3] The fields in question did not contain any rodents in great quantities, this fact having been subsequently verified by our survey. Moreover, rodents would have resorted to the camp of the brigade of workers, in which there was no case of illness as well as the others and cases of illness should have occurred in this group as well.

Consequently there remains but one mode of spreading the infection, the evidence for which stands out; this is spread through water.

In surveying the meadows we paid the greatest attention to the unknown brook N., which formed the source of this infection.

This brook springs from the upper marshy part of the valley and flows into the river. The place where it empties into the river is covered with thick vegetation and is hardly to be noticed from the opposite side of the river. All along the brook, which measures about 2.5 km., its banks are thickly grown with birches, aspens, bird-cherry trees, bushy willows and some needle-trees and currant bushes. In some places there are footpaths in the thick grass on which the workers used to go and fetch water. The brook is only 5 to 10 cm. deep and its bottom is covered with crushed stone and pebbles. At the places at which water had been drawn, there are artificial holes, up to 0.5 m. deep. The water in the brook is good flowing water, at places one hears its murmur. Neither fish nor other cold-blooded animals could be observed. Nor could we notice any rats or other animals, though

[3] Prof. Miller points to house-mice, but in this case even field-mice could scarcely have played that part.

in several places we saw otter-holes with fresh tracks leading to them.

LABORATORY TESTS OF WATER

In order to test the etiological rôle of water in spreading the infection, we injected samples of this water into guinea pigs on the thirty-first day after the beginning of the epidemic. We took some water for bacteriological and chemical analysis and for the purpose of testing it as to the presence of *B. tularense.* The temperature of the water measured in the brook on a cloudy day was 15°C. The water for bacteriological and chemical analysis was taken at the place where the drinking water had been drawn.

The following data were obtained from analysis:

The water was colorless and inodorous, with a slight flaky brown-red sediment. The reaction was alkaline. Oxidization—12 mgm. for 1 liter. Traces of ammonia and nitrous acid. Nitric acid, sulphuric acid, Cl and Fe are not found. General hardness, 14.5; removable, 14.0; constant hardness, 0.5. Dense sediment, 20.0 mgm. The bacteriological test showed the presence of a considerable quantity of microörganisms (up to 2000 colonies in 1 cc.) and many anaerobes. No intestinal bacilli were found in 10 cc.

Both the chemical and the bacteriological analyses suggested that the water was somewhat polluted.

In order to reveal the presence of *B. tularense* we immediately injected samples of the water into guinea pigs which we had brought with us. The water was injected subcutaneously into five guinea pigs. Each of the pigs received 10 cc. of water. On the next day all the guinea pigs showed infiltration at the site of injection. On our return to the Institute we noted that the guinea pigs had become noticeably thinner (they had not been weighed before being injected as our work was carried out under field conditions). On the ninth to the twelfth day all the guinea pigs died. Three or four days before death all the guinea pigs showed the presence of a number of enlarged lymph glands in the hypodermic tissue of the abdominal wall, in the form of "beads"

running from the site of injection to one or both groins. Autopsy revealed the presence of pathologico-anatomical changes in the organs of the guinea pigs as are characteristic for tularemic infection.

As an example of this we are giving here the shortened autopsy record of one of the guinea pigs.

Guinea pig 3. (Autopsy performed by the veterinarian consultant of the Institute, docent Urborsky.)

Water was injected on August 20. Death on August 29. Autopsy on August 29. The hair is easily pulled out. The hypodermic tissue is feebly pronounced. The visible mucosae are pale, dry, somewhat cyanotic. The lymph glands of the thoracic region are enlarged (one above the ensisternum on the left, the other beneath it on the right). Both glands are as large as a middle-sized pea. On palpation they are thick, on section they show a yellowish color and stratified consistency. The glands are but slightly adherent to the underlying hypodermic tissue. In the region of the white abdominal line the lymph glands, seven in number, are lentil-sized and are disposed in the form of a string of beads. Groin glands—one on each side, the right one as large as a middle-sized bean, the left one lentil-sized, brown-red on section. In the peritoneal cavity there is some bloody liquid. The mesenteric glands are slightly enlarged, juicy, of a dark brown color. The spleen is 4 by 2 by 0.4 cm., of brown-red color. The capsule is strained. Under it there are multiple, scattered, small, limited whitish-yellow foci, of the size of a poppy-seed or larger. On the surface of the porta of spleen there are similar foci. The consistency is thick and brittle. The surface of the section shows the same foci, of a grayish-yellow color. The liver is considerably enlarged, brown-red. The lobulation is levelled. The right lobule has one large hempseed-sized focus and a multitude of small grayish-yellow foci, as large as a poppy-seed or smaller. The left and middle lobules show the same changes. On section there are to be seen disseminated foci of the same color. The left renal capsule is somewhat enlarged, the left one normal in size. The limit between the cortical layer and the medullar one is pronounced. On being sectioned these layers show three yellow white foci of a thick, crumby consistency as large as a millet-seed. The bladder mucosa shows injected vessels and hemorrhagic dots. The findings in the other organs being normal, we do not mention them here.

These extracts from the autopsy record show a pathological-anatomical picture characteristic for tularemic infection as regards the lymphatic system, the spleen, the liver and the renal capsules. I. V. Toropzev, assistant in pathological anatomy in the Medical Institute at Tomsk, made histo-pathological preparations from all these organs as well as from the heart and the kidneys. (We take this opportunity of thanking him.)

The microscopy of the preparations showed the following data:

Lymph gland. Focal infiltration, developing in some places into suppurative liquefaction, with a pronounced reaction of the reticular cells and of the cells of the endothelium. The inflammatory process is not only localized in the parenchyma of the nodule, but goes over to the nodular capsule. Consequently, there is an acute periadenitis.

Spleen. Acute inflammatory hyperplasia. The organ is affected by a focal leucocyte infiltration, which in some places develops into abscesses.

Liver. Focal necroses with a pronounced inflammatory reaction on the periphery of the foci. Suppurative infiltration round the central veins developing here and into abscesses.

Renal capsules. Phenomena of fatty degeneration in the cells of the organ.

Kidneys. Congested plethora of the glomerular capillaries and degenerative changes in the epithelium of the tubules accompanied by phenomena of karyolosis.

Heart. Degenerative changes of albuminous character in the parenchyma.

From the liver, the spleen and the lymph glands of guinea pigs, which had died after having been injected with 10 cc. of water, we prepared emulsions and with these emulsions six guinea pigs were injected subcutaneously and intraperitoneally. The guinea pigs which had received the emulsion intraperitoneally, died on the third day, those which had been infected subcutaneously, showed a gradual loss of weight and died on the fifth day.

The necropsy of the pigs which had died from intraperitoneal infection revealed in all cases an enlargement of the groin glands and of the mesenteric glands; the changes in spleen and liver

(enlargement of the organs and presence of foci) were, quantitatively speaking, much less pronounced than in the case of pigs infected subcutaneously. Phenomena of peritonitis, perihepatitis and perisplenitis were to be observed in all the cases (autopsy records are not quoted in order not to lengthen this paper). The necropsy of the pigs infected subcutaneously with emulsions from the organs shows changes in the lymphatic system, the spleen and the liver which are as pronounced as in the case of the guinea pigs which had died from an injection of water (see autopsy record quoted above). In the case of the emulsion infection, however, considerably greater changes were to be observed in the mesenteric glands.

From the liver, the spleen and the lymph glands of the guinea pigs which had died from water injection and from these same organs of guinea pigs, which had died of an emulsion injection of organs (liver, spleen and lymph glands) taken from pigs infected with water injection, sowings were made on the medium of McCoy and that of Chapin. Smears were prepared and stained after Giemsa. The microscopy of the smears showed an enormous quantity of small bacilli, mostly in the form of coccobacilli. At places the microörganisms form a thick layer. For the most part they are free, but some are included in the leucocyte protoplasma.

On the media of McCoy and Chapin there could be observed, after an interval of forty-eight to seventy-two hours (rarely after twenty-four hours) a slight hardly noticeable growth of non-confluent colonies. After prolonged gram-staining, the culture showed gram-negative coccobacilli and short bacilli.

In order to test the specificity of the isolated cultures they were injected into guinea pigs subcutaneously and intraperitoneally at the rate of 0.2 cc. of culture from a test-tube. All the guinea pigs showed phenomena of diffuse peritonitis and died twenty-four to forty-eight hours after the intraperitoneal infection. Some of them showed such changes in the glands and the liver as are characteristic for the tularemic microörganism. The following short autopsy findings may illustrate this fact.

The guinea pig 11 was injected on August 31 intraperitoneally

with 0.2 cc. of the emulsion of the pure culture 3. Died on September 2.

On the abdomen, to the right of the white line the lymph gland is pea-sized. To the left there are two lentil-sized glands. Enlargement of the inguinal and the mesenteric glands. Perihepatitis, perisplenitis (the surface of the liver and the spleen covered with fibrin clots and grayish white films, which are easily taken off). The liver is enlarged. On its left lobe there are two small, white-yellow foci. On the middle-lobe there are several small foci. The spleen is enlarged but without any noticeable changes.

The guinea pigs infected subcutaneously with pure culture all died on the fifth day after emaciation and characteristic changes in the lymph glands, the liver and the spleen. This may be illustrated by extracts from one of the autopsy records.

The guinea pig 15 received on August 31 0.2 cc. of pure culture emulsion subcutaneously. Died on September 5. It had grown very thin. Weight before infection, 500 grams; on the day of the death, 370 grams.

At the site of injection there is a considerable infiltration, 5 by 4 cm. large. On both sides of the abdominal line, the lymph glands are enlarged; they are bean-sized on the right side and pea-sized on the left. The liver is enlarged and has on its left lobe a large compact focus, which fills nearly one third of the lobe. Section shows a grayish-yellow color. When scraped the surface of the cut crumbs easily. On the right and the middle lobes of the liver there are eight foci, ranging in dimensions from the size of a millet seed to that of a lentil, of the same color and consistence as the foci on the left lobe. The spleen is 4 by 2 by 0.4 cm. large. Its borders are rounded, the capsule is strained. The surface of the organ is covered with a multitude of small foci, as large as poppy seeds. Owing to the great quantity of these foci, the spleen has a grayish-white color. A section of the spleen shows that its pulp is also penetrated by these foci.

These records show, that intraperitoneal injection leads to death from peritonitis. The subcutaneous injection of the culture gives a characteristic pathological-anatomical picture.

From the organs of the guinea pigs which had died from infection with pure culture we obtained *B. tularense.*

All the isolated cultures (19 strains)—those obtained from guinea pigs which had died from the injection of water, as well as those cultivated from the organs of guinea pigs which had died after being injected with emulsion of the first pigs, were agglutinated with five sera of patients from one of the collective farms. All the cultures showed a pronounced agglutination with all the sera in dilutions from 1500 to 2000.

All these data definitely prove the etiology of the isolated cultures, and their relation to the described outbreak.

TESTING OF PATHOLOGICAL MATERIAL FOR THE PRESENCE OF B. TULARENSE

Glandular punctures were made on two patients (patient O. on the thirty-eighth day of illness and patient T. on the thirty-sixth day); and with the pus of each of the patients we infected two guinea pigs. Out of the four infected guinea pigs two (one of each pair) died; one on the eighth day, the other on the thirteenth. The necropsy revealed characteristic pathological-anatomical changes and from the liver of both guinea pigs we obtained cultures of *B. tularense.* Both the patients in question were placed in the Clinic for infectious diseases of the Medical Institute at Tomsk (Director prof. G. F. Wogralik) and on the fifty-second and fifty-fourth day of illness another glandular puncture was performed. Pus was obtained, in one case (patient O.) 4 cc., considerably mixed with blood, in the other (patient T.) 17.5 cc. The pus of the latter patient was thick and had a greenish color. The pus of patient O. was injected in equal parts into two guinea pigs and another pair of pigs was infected with the pus of patient T. at the rate of 5 cc. each. The infection was performed subcutaneously and intraperitoneally. One of the pigs infected with the pus of patient O. died on the tenth day, and one of those infected with the pus of patient T. died on the twelfth day. The necropsy revealed pathological-anatomical changes characteristic for infection with *B. tularense.*

These data show that in suppurated but not discharging

lymphatic glands *B. tularense* can maintain its vitality for a long period.

The study of the outbreak does not end here, as some links are still lacking in the epidemiological evidence. At present investigation is being carried on, in order to discover the source of infection of the described outbreak. A number of important data are already available, but the studies being not complete, we shall not dwell upon this question for the present.

Our observations lead us to the following conclusions:

1. In the outbreak in question it was the tonsils, the mouth, the mucosa and the conjunctiva that formed the portal of entrance of infection.

2. In this outbreak the anginal form was predominating and, the typhoid form came next while a number of patients showed the glandular-ocular form of illness.

3. We did not observe any correlation between morbidity and sex or age.

4. A new mode of spread of tularemia was proved experimentally and bacteriologically, i.e., its spread by water.

5. The quantity of microörganisms in the infected water courses may sometimes be so considerable, that their isolation does not meet with great difficulties.

6. In our experiments 100 per cent of the guinea pigs infected with the water died and their organs showed characteristic pathological-anatomical changes. From the organs of all these guinea pigs by water we obtained cultures of *B. tularense.*

7. The cultures that were isolated from water did not differ in regard to virulence from those that were isolated from the suppurated lymph glands of the patients. When these cultures were tested on guinea pigs, death followed in a 100 per cent of cases and the organs showed changes characteristic of tularemic infection.