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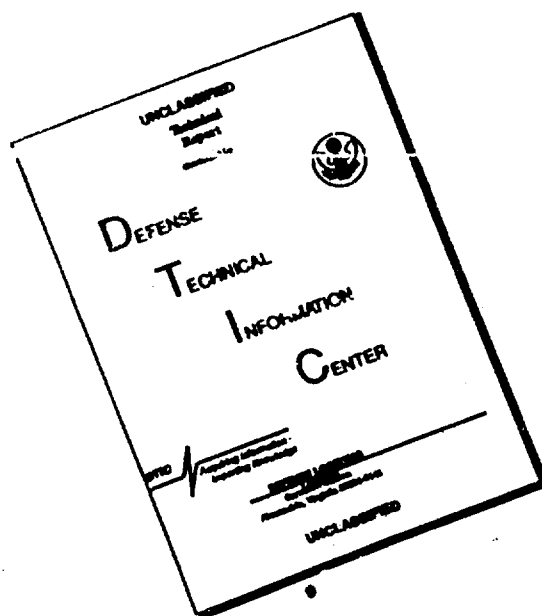
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Experimental Material on the Etiology of the Autumnal Form of Encephalitis, Report I,
by A. A. Shubladze

Among the diverse forms of encephalitis encountered on the territory of our country in the past few years there is observed a new infection, characterized by severe affections of the central nervous system and resembling, clinically, summer (Japanese) encephalitis. These infections differ from the tick spring encephalitis, studied in 1937 by Zilber, Ievkovich, Shubladze, Chumakov and Solovev, first of all by their seasonality. They began in late August and ended in October. This autumnal seasonality most distinctly characterized the epidemiology of the infection.

This report describes the results of studies conducted by myself in the fall of 1938 in an expedition organized by the N. K. Zdrav USSR for the study of this infection.

Taking into consideration the results of tests on the tick spring form of encephalitis, signifying that this might be one of the forms of infection of the central nervous system, we used the same methods used so effectively in the study of the tick spring encephalitis.

The basic experiments on the isolation of the virus were conducted on white mice. Material used for the infection of the animals was; brain of humans, having died from the infection, blood of patients, spinal fluid and urine. The brain of a corpse was utilized no later than 24 hours after death and, as is a rule, in those cases where death occurred in the first 5-6 days of illness.

Pieces from various regions of the brain (cortex, medulla oblongata,

and pons) were extracted in aseptic conditions, triturated in a beater with a Ringer solution into a 10% emulsion and after the emulsion set for 15 minutes, it was injected intracerebrally into 3-5 mice in quantities of 0.03 cm³.

Before our arrival, the brains of 16 people, who had died from this infection, were studied by V. D. Neustroev. In his tests, upon the injection of an emulsion of brain, from a human having died from this infection, into the cerebral of mice, no symptoms of infection were disclosed. These mice were transferred to me, and during successive passages of the brain, they were infected with a typical experimental encephalitis. In 2 cases the material of the corpse (particles of the brain) taken by V. D. Neustroev, was stored for 5-10 days in 50% glycerine on ice. Upon injection it caused an infection in mice with characteristic symptoms of affection of the central nervous system.

Besides this, I studied the brain material of 13 other corpses. Two of them with the help of A. A. Smorodintsev.

Thus the brains of 31 corpses were studied. In 29 cases a strain of virus was isolated, pathogenic for white mice, working well in passages (some strains have been used on more than 10 passages to this time) and easily separated from the brain of humans, dying on or before the 6th day of illness. In two cases, where the brain was obtained on the 11th and 14th days of illness, it was not possible to detect the virus, but there also was no confirmation of an encephalitis diagnosis upon autopsy.

The initial injection of the brain of dead patients into mice caused infection only in 4 cases, however, the first passage of the brain of these clinically uninfected mice, in an overwhelming majority of the cases, caused

a typical infection. The brain being studied did not give any bacterial growth on artificial feeding mediums (sugar bouillon and sugar agar).

The next series of tests were devoted to the analysis of the blood of patients for the presence of encephalitis virus in it.

In the first 5-6 days of illness, blood was taken from the elbow vein, defibrinated in a glass jar with "beads" and injected into 3-5 mice in a dose of 0.03 cm³. If the initial injection of the blood of the patients did not cause an infection of the mice, than 2 more passages were conducted.

Of a group of 22 examined patients (the first 3 were primarily examined by Neustroev with negative results), in 12 cases there was isolated a virus, analogical to that isolated from the brain of corpses. However, it is more difficult to isolate the virus from the blood of patients than from the brain of a corpse (only in 50% of the cases). But even this quantity of positive finds of virus in the blood of patients exceeds the positive finds during analogical studies of tick encephalitis (Zilber, Levkovich, Shubladze, Chumakov and Solovev). These observations testify of the massive accumulation of the virus and its great dissemination in the organism of the patient. The following tests on the spinal fluid and urine also inform of this.

The spinal fluid was obtained in four cases on the 3-6 days of infection. The fresh liquid was injected in quantities of 0.03 cm³ into mice intracerebrally. The mice, as in previous tests, were under observation for 8-9 days and, if they did not become infected, their brain was used on passages on fresh mice. In all 4 cases we obtain a virus - 2 on the first passages and 2 on secondary passages.

The urine was obtained from 13 patients on the 6-12 days of infection, in three cases it contained virus, because its injection, in doses of 0.03 cm³,

caused an infection in white mice upon the second passage and later brought about their death.

The ensuing Table summarizes the results of the study on the isolation of the agent from the organism of patients. (Table at end of translation).

Comparison of these results with those of tick encephalitis indicate that we were able to isolate the virus more often, even from the comparison of virus isolated from the blood, spinal fluid and urine of patients, and also the brain of those having died from the infection.

The isolated strains were studied for their pathogenicity to various animals.

The intracerebral injection of the virus to white mice, and causing an infection, indicates their high susceptibility to this virus. The introduction of 0.03 cm³ of a 10% emulsion of an incubated strain causes a typical infection in mice on the 4-5 day, with affection of the central nervous system. The virulence increases with passages, and the mice died upon the intracerebral introduction of a dilution of an incubated strain 1:1,000,000.

During intravenous injection of a 1% emulsion of an incubated strain in a dose of 0.15 cm³, infection occurred on the 4-5 day; an intraperitoneal injection of 0.25 cm of a 10% emulsion caused infection on the 7th day; an intranasal injection of this same emulsion in a 0.05 cm³ dose caused infection on the 5-6 day after injection; and a subcutaneous injection of 0.25 cm³ of the emulsion caused infection in the mice on the 9-10 day after injection.

The initial symptom observed in mice becoming infected was an increase in their sensitiveness. Upon irritation, even a light touch, the mice jumped,

and a secondary touch sometimes caused convulsions. Convulsions appeared even without touching them. In other cases there appeared paresis and paralysis of the rear extremities, prostration and death. All these appearances developed within hours. Thus, the chart of infection in mice during the form of encephalitis now being studied is very similar with experimental tick encephalitis.

Histological studies of the brain of white mice, having died from experimental encephalitis, according to the data of L. S. Leibin, indicate the presence of numerous inflammatory changes in the brain stem, such as the perivascular covering and a multitude of large gliotic knots, now and then interflowing.

The pathogenicity of the virus to guinea pigs was studied by the introduction of the aforementioned material from patients, in a majority of cases containing virus, which was confirmed by simultaneous injections into white mice, and also infection with an incubated virus strain. The material was inoculated intraperitoneally and intracerebrally into the pigs. In the first case 5 cm³ was injected and in the second - 0.2 cm³. Six of the pigs received blood of the patients in the belly. Six in the brain, 6 more in the brain with an emulsion of the brain of a corpse, 10 in the belly with urine of patients, 4 pigs in the brain with an incubated virus of mice.

The guinea pigs did not react to the injections. Only in one case, during intracerebral injection of a 10% emulsion of the brain of a corpse, of two pigs infected in such a manner was there observed a rise in temperature on the 6th day after injection. The temperature held for 5 days. In one other case one guinea pig had a rise in temperature on the 7th day after

injection with an incubated strain intracerebrally (strain "K"). The curves set on Graphs 1 and 2 demonstrate this reaction in guinea pigs. So, of 32 pigs, only 2 reacted with a weak temperature rise, which testifies of the low susceptibility of guinea pigs to the virus of encephalitis obtained by us. (Graphs set at end of text).

Six rabbits received three injections, 5 days apart, totaling 5 cm³ of a 10% emulsion of an incubated virus intraperitoneally and one injection of 5 cm³ of a 1% emulsion intravenously. The rabbits remained healthy, and 2 weeks after the last injection, the serum of these rabbits was tested in regard to its neutralization of the isolated strain K. It contained specific neutralizing antibodies to this virus.

We utilized these serums further in tests of differentiation of the isolated strain from tick and Japanese encephalitis.

Several tests on the filtration of the isolated strains through the Zeitts filter and Berkefield candle disclosed the filterability of the virus: the obtained filtrate caused a typical encephalitis in mice upon intracerebral and intranasal tests.

Thus, of three animals tested, only the mice proved to be highly susceptible to the virus, the susceptibility of the rabbits and guinea pigs was low.

To establish the serological characteristics of the isolated virus in relation to virus of encephalitis, it was necessary to set tests of its neutralization by specific serums.

Three strains of virus were taken for these tests; a strain of Japanese encephalitis, a strain of tick (spring) encephalitis "C" and the isolated

strain K. All these strains were studied in neutralization with specific serums: (a) a mixture of serums of two convalescents, (b) rabbit immune serum, obtained during immunization with respective strains, and (c) normal serum of humans (control).

The tests were set according to a generally accepted form; the centrifuged emulsion of the brain of encephalitis mice in various dilutions was mixed with equal volumes of undiluted serum. The mixture remained in a thermostat at 37° for 2 hours, then it was injected intracerebrally, in doses of 0.03 cm^3 , into 3 fresh white mice. The judging of the neutralizing properties of the serums was according to the infectability of the mice after injection of the mixture. The virus was used in 7 various dilutions in the tests. Every dilution in the mixture with the serums held three mice.

Graph 3 (at end of text) shows the results of the tests of neutralization with Japanese tick (spring) viruses, and the virus obtained by us.

As Graph 3 shows, the virus of Japanese encephalitis is neutralized by rabbit serum, homologically specific in regard to Japanese strains, in a dilution of 1:25,000. Serum of patients, infected with the virus now being studied by us, also neutralizes this virus well. Mice injected with a mixture of serums of convalescents and virus of Japanese encephalitis in the dilution of 1:5,000, did not become ill, while a mixture with normal serums of the virus caused infection in one of three mice, even in a dilution of 1:3,125,000. Strain K, isolated by us, is similar analogically to Japanese encephalitis virus. Rabbit immune serum and serum of convalescents neutralizes it in dilutions of 1:25,000, while normal serums leave the virus active even

in dilutions of 1:3,125,000.

Tests of neutralization with strains of tick encephalitis gave completely different results. If the rabbit serum, specific to this virus, neutralized the tick virus strain C in the dilution 1:25,000, than the serum of convalescents, of the virus being studied, did not protect the mice from the virus, even during dilution at 1:3,125,000, just as normal serum did not.

These tests establish the close relation of the virus of Japanese encephalitis and the virus isolated by us and the clear difference of the latter from the virus of tick encephalitis.

On Graph 4 are set the tests of neutralization, made with 9 serums of convalescents of the infection being studied, and 11 serums of convalescents of tick (spring) encephalitis. The virus in these tests was taken in two dilutions - 1:2,500 and 1:12,500.

As the chart shows, the isolated virus is neutralized by almost all the serums of convalescents, taken in the test, during dilution of the virus 1:12,500 and in several cases in the dilution 1:2,500. It is neutralized also with serums of convalescents of tick encephalitis. However, the virus of tick (spring) encephalitis, neutralizing well with serums of tick encephalitis convalescents, is not neutralized by serums of convalescents of the infection being studied; of 9 cases, only one case showed neutralization in a dilution of the virus 1:2,500. Analogical studies were set by us with ten more serums. They gave basically the same results.

These data once more indicate the difference of the virus isolated by us and the virus of tick encephalitis. Serums specific to tick encephalitis, neutralize tick virus and our strain, but serums specific to our strain,

neutralize only those strains and almost completely leave the virus of tick encephalitis active.

Thus, the relation of the strain isolated by us to tick virus is fully in conformity with the relations established by us between the virus of Japanese and tick encephalitis.

Simple tests of cross neutralization indicated that serums obtained by immunization with virus of Japanese encephalitis, fully neutralize our strains, and on the reverse, serums obtained by our strains fully neutralize virus of Japanese encephalitis.

Thus, the isolated virus proved serologically identical with the virus of Japanese encephalitis.

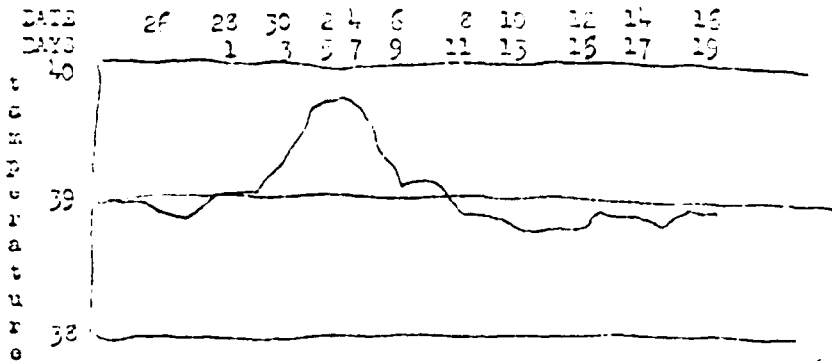
Further studies should show whether these viruses are identical in other relations, in particular, in relation to pathogenicity for monkeys and sheep and in relation to immunogenic properties. But data on hand at this time lead us to believe that the isolated virus is the virus of Japanese encephalitis or very near to it.

It was known that mosquitoes are the vectors of Japanese encephalitis, so we set tests to determine the presence of virus in mosquitoes, gathered in the areas of infection. These tests were done with mosquitoes obtained by me from Prof. P. G. Sergiev and K. P. Chagin. The tests gave positive results; 4 strains of virus were isolated, identical with that described above. These studies will be published later.

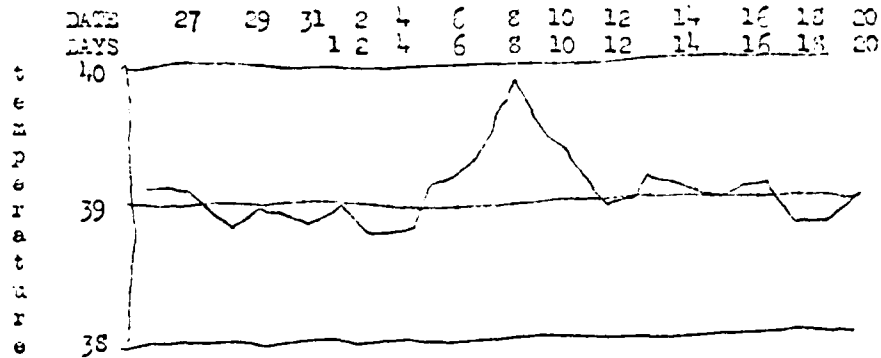
NOTE: Graph 4 explanation; tests of cross neutralization of viruses of autumnal and spring encephalitis with conformable serums. Figures mean: 1. dilution 1:2,500, 2. dilution 1:12,500. White squares - healthy mice, X squares - infected mice.

TABLE for text-- Experimental material on the etiology of the unusual form of enceph.

MATERIAL FOR INFECTION OF MICE	DAYS FROM BEGINNING OF ILLNESS	NUMBER OF CASES	NUMBER OF STRAINS ISOLATED BY			GRAND TOTAL NUMBER OF STRAINS
			INITIAL INFECTION	FIRST PASSAGE	SECOND PASSAGE	
BRAIN	3, 4, 5, 6,	31	4	21	4	29
BLOOD	3, 4, 5, 6,	22	0	8	4	12
SPIRAL FLUID	3, 4, 5, 6,	4	0	2	2	4
URINE	4, 5, 7, 9, 11, 12	13	0	0	3	3



Graph 1. Guinea pig No 29, 28 Sep 38, 5 cm³ of a 10% emulsion of the brain of a corpse injected into belly



Graph 2. Guinea pig No 30, 1 Nov 38, intracerebral injection of 0.2 cm³ of 10% emulsion of incubated strain 'X'.

Graph 3. Tests of neutralization of viruses with serums. Rabbit serums, of rabbits immunized to homologous strains. The figures define the dilution of the strain: I--1:1000; II--1:5000; III--1:25000; 4--1:125 000; 5--1:625 000; 6-- 1:3 125 000; 7--1:15 625 000. I, II, III, strain of Japanese encephalitis; IV, V, VI-- strain of autumnal encephalitis; VII, VIII, IX--spring encephalitis; Clear squares-- healthy mice; Marked squares-- ailing mice.

