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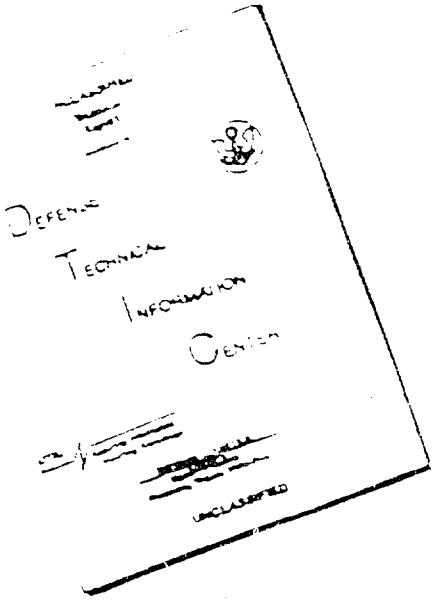

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PRECIPITATING ANTIGEN IN THE BLOOD OF MICE INFECTED WITH ARBOVIRUSES

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The possibility of using the reaction of the diffused precipitation in agar gel for the detection of virus (antigen) in the blood in experimental arbovirus infections was studied. In the result of the research of 12 arboviruses of group A, B and others, positive results were obtained with Semliki, Piksuna, Venezuelan Equine encephalomyelitis and Uukuniemi viruses.

The reaction of diffused precipitation in agar (RDP) is gradually gaining recognition in research with arboviruses. At first RDP was used for the study of antigen structure of arboviruses. As the methods were worked out for obtaining the precipitated antigen from the brain of infected mice or wool cultures, reports appeared concerning the application of RDP for the diagnosis of tick encephalitis [4, 5] and the detection of antibodies in horses to the Dengue virus, the equine encephalitis of Herreya, Kun'in [9] and Western Nile [6] and for the research of serums of domestic animals in centers of Japanese encephalitis [1].

All this research was based on the detection of precipitating antibodies in blood. Proceeding from the fact that the arbovirus infections, both clinically expressed and symptomless, are accompanied by viral families which do not often yield to an indicator of viral propogation in the brain, we faced the question of the possibility of detecting virus in the blood using RDP. In the case of a positive solution of the problem, the opportunity of speedy diagnosis of arboviral infections in the initial stages of illness is opened. There has been no work of a similar nature in available literature. Research has been carried out with 12 arboviruses of various antigen groups.

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MATERIALS AND METHODS

Seven viruses of antigen group A were utilized: Sindbis EgAr-339, Semliki, Piksuna BEAr 35 645, Mukambo BEAn 8, Chikungun'ya Ross, Venezuelan and Western American equine encephalitis (VEE and WAEE); 4 viruses of group B: Japanese encephalitis (JE) P-I, Western Nile (WN), Povassai and San Lui; viruses of Tribeh and Uukuniemi S-23. The viruses were injected into 2-3 day old suckling mice.

Ascites of immune mice containing antibodies to each of the viruses used in the work were the source of the antibodies as well as immune ascites to the viruses of group A and B. In separate experiments control serum immune to virus VEE and the serum of guinea pigs immune to Uukuniemi virus were employed. The mice were immunized with a suspension of the brain of suckling mice infected with the ad'yuvant of Freiida. The ascite was induced intra-abdominally by the introduction of cages of sarcoma 180/TG [2]. Rabbits and guinea pigs as well were immunized with the brain antigens of the corresponding viruses. The presence of antibodies was established according to RTGA and, in the case of Tribech and Uukuniemi, according to PSK.

The native serum of the blood of infected mice taken at the height of illness without any special treatment was used as the antigen for RDP. A titre of virus in the serum of blood was defined as titration in the culture of wool or in the mice with an infection in the brain. The culture of wool of chicken fibroblasts was utilized for viruses VEE, Sindbis and Tribech and 2-4 day old mice for viruses Mukambo, Piksuna, Chikungun'ya, Uukuniemi, Japanese encephalitis, San-Lui, Western Nile, Il'eus and WAEE. The titre of virus was exhibited in $lgLD_{50}/0.02$ ml.

RDP was drawn according to the method of Oukhterlon [10] using the modification of A. I. Gusev and V. S. Tsvetkov [3]. 10 ml of sulfur cleansed polysaccharide of 1% agar Difco in a borate buffer pH 9.0 were set on clean, skimmed glass plates with dimensions of 9 x 6 cm. After the agar has congealed on them holes were punched out with a press. The diameter of the center hole was 5 mm, the lateral holes 4 mm. The distance between the center and lateral holes was 10 mm. Along the perifery of the glass, holes were also punched out which were filled with a physiological solution so the agar would not get dry. After this, 0.04 ml of antigen were poured into the center holes, and 0.03 ml of immune liquid were poured into the lateral holes. The glass was maintained in a humid chamber at 37°. The reaction was observed after 12 hours.

RESULTS

All experiments were conducted on mice not more than 4-5 days old since it is known that viral families are the highest in animals of this age. Mice 4-5 days old were infected in the brain with a corresponding dose of 10^3-10^4 $LD_{50}/0.02$ ml. As soon as the first symptoms of the illness appeared, the animals were drained of their blood. The blood serum was cultivated in a

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normal manner. Part of the serum was separated for the titration of the virus. If the titration is not done immediately, the serum is kept at -70° until the time of the experiment. The antigen serum for the precipitation, which is usually conducted on the following day and repeated at a later date, is maintained at 4°. It was placed in the central hole and 4-6 radially arranged holes were filled with ascites immunized against the virus under test, viruses of the same group, polytypes of a given group and other groups were included as well for the control of the ascite toward the virus. During the staging of the experiment the possibility of interreactions between viruses closest to the antigen structure was taken into account. Influenced by this, in the experiments with the viruses of Mukambo, Piksuna and VEE, ascites to all these viruses were included. Such principles were observed in the experiments with the viruses of Sindbis and western equine encephalomylita and with the viruses of the Western Nile, Japan and San-Lui encephalitis. In the initially oriented experiments, the serum of the blood of suckling mice was investigated only in the reaction of precipitation without the titration of its infectiousness. In these experiments repeated positive results were obtained with viruses of Venesuelan equine encephalomylita, Piksuna, Semliki and Uukuniemi. In experiments with the other viruses taken up in the work, negative results were obtained.

Then research was carried out with the inclusion of one more test--the determination of the infected type of blood serum. The data of these experiments are given in the table. In all experiments with viruses of group A which gave positive results, the titre of virus was high. The threshold of the concentration of the infected virus is apparently between 7.75 and 8 $lgLD_{50}$. Positive results were not obtained during the investigation of group B viruses and the Tribech virus. The infected titres of these viruses are lower than 7.01 $lgLD_{50}$. The Uukuniemi viruses yielded a distinct precipitation with a virus titre higher than 6.5 $lgLD_{50}$. The serum-antigen of VEE, Piksuna and Semliki with immune ascites yielded a patch of the precipitation alone. This patch was rarely distinct and settled near the holes with the antigen (see figure a). Apparently, such an arrangement depends on a disparity of the concentrations of antigen and antibodies towards the last prevailing ingredient. The serum-antigen of Uukuniemi yielded 2 patches of precipitation with homologous ascites. The patches were formed near the hole with the antibodies. In the limited period of the experiments on the titration of the serum-antigen VEE and antibodies on a type of chess board, it was shown that, with the utilization of separate serums, the patch of precipitation is drawn to the side of the hole with the antibodies; however, the distance from the hole with the antigen still did not exceed one third. The titre of ascite VEE, utilized in the experiments, to RDP is equal to 1:32. The antigen reacted up to a cultivation of 1:4. Serum-antigen VEE reacted as well with the polytyped ascite of group A and with the Mukambo ascite. In the RTGA with the virus of VEE, these serums reacted correspondingly in titres of 1:2560 and 1:320. In the experiments carried out, the serum antigen VEE did not react with the Piksuna ascite, and, in its turn, the Piksuna antigen reacted only with a homologous ascite. The given models of ascites responded in such

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a manner in RTGA. The Semliki antigen reacted only with a homologous ascite and did not yield group reactions (see figure c). Very specifically, the Mukambo virus reacted with ascites of the same type.(see figure d). On the whole the reactions were registered very clearly; non-specific patches were not observed.

The experiments on virus VEE which were revealed in the reaction of the precipitation were repeated with another indicating system--the serum blood of immune rabbits and the serum blood of the reconvalescents (see fig. a, b, c). The results appeared analogous even though it is necessary to note that with the utilization of mice ascite the patches of precipitation appeared more quickly and were more distinct. Moreover, with the rabbit serum it was possible to observe the formation of additional patches of precipitation (see figure b) at the expense of the reactions between the types of antigen of mice and antibodies to mice brain as long as the rabbits were immunized with the infected brain of mice. This precipitation in our example, situated near the 5th hole, has the form of an arc as long as mice serum is in the center hole, mice ascite is in the 2nd hole and rabbit serum immunized against mice brain is in the 5th hole. The precipitated antigen in the serum blood is steady. As is shown in the experiments with virus VEE, this antigen is maintained at 4° for not less than 2 months.

DISCUSSION

The possibility of detecting a virus by the method of a reaction of the precipitation in the blood of experimentally infected animals depends, apparently, on three indications: the titre, the dimensions of the virus and the titre of the antibodies in the indicated system. The highest titre of virus (8 $lgLD_{50}$ and higher) was in the mice infected with virus VEE. In ascite, the titre of antibodies was also high: 2560 in RTGA. However, apparently the latter was not of decisive significance for the antigen was caught by the immune rabbit serum and by the serum of reconvalescents after a laboratory infection, and the titres of the antibodies in which RTGA is concerned were lower. Ascite cultivated 32 times also caught the antigen VEE. Not withstanding that the ascites to other viruses, in the example Mukambo, had such high titre of antibodies, the antigen of Mukambo was not caught since the titre of Mukambo virus in the blood was lower than 7.75 $lgLD_{50}$. At the same time, the ascite of Mukambo reacted with the representative of that sub-group of the antigen VEE, the titre of which was higher than 8 $lgLD_{50}$. On the whole a relation between RTGA and RDP was formed, but the specificity of the latter was significantly higher. In separate series of the serum,titre of antibodies to members of the antigen sub-group of RTGA varies significantly,but a cross-reaction is carried out almost always. In RDP, these cross-reactions appeared only with a high titre of antibodies to the heterologous virus. The results obtained by us show in principle the possibility of forming antigen in the serum of blood during virem in RDP. In this sense, the negative results with the series of viruses used are not final. It is highly probable that a high titre of

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antibodies can achieve positive results for the detection of viral families with an infectious titre less than 7.75 $lgLD_{50}$, which in the given research is the threshhold. Until now RGA has been well known from non-biological methods of the formation of arboviruses in the blood. To obtain hemagglutin antigen several arboviruses such as Semlike, Bun'yambera and viruses of group C use the serum of blood [10]. With this it is noted that antigen is obtained if the titre of virus in the blood is not less than $10^{-[illegible]}$ [6]. Srihongse [11] for quick identification of the virus of Venezuelan and Western American encephalomyelita with the isolation of that virus from nature or from people proposes to make a saccharose acetone antigen from the blood, but not from the brain of infected mice since the titre of it is high, but the frequency of formation is more regular than in the brain. However, the assumed method is hardly compatible with mass formation since it is necessary to subject rather labor-consuming treatments by acetone to the serum in order to obtain the hemagglutinin antigen. Besides the hemagglutinin obtained must be further identified in RTGA. The method of viral formation in RDP is very simple and available since neither the antigen nor the serum require treatment. Even if this method is not employed immediately for the formation of virus in material from infected people or invertebrates, then it can be used satisfactorily in the examination of blood from mice infected by this material. One further advantage of the method we propose is the prolonged maintainence of the antigen in the serum at 4°. This means that the examined material can be studied in RDP even when the infectious virus is not always distinguished by biological methods.

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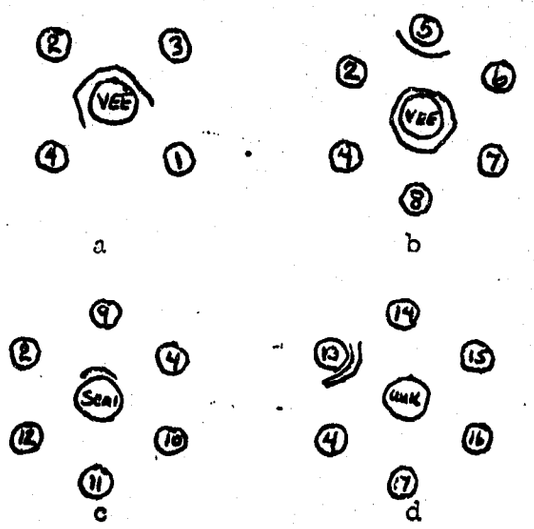
Appearance of antigen in the blood serum of infected mice

Virus	Virus titre in blood (in lg LD_{50}/0.02 ml)	Immune ascite from the indicated titre to the homologous virus in RTHA and RSK														
		Sindbis 1280	Semliki 2560	Piksuna 2560	VEE 1280	Mukambo 640	Chikungun'ya 320	WAEE 320	JE 320	Western Nile 320	San Lui 80	Il'eus 80	Tribech 40	Poly A	Poly B	Uukuniemi 80
Sindbis	7.5	N	N	N	N	.	N	N	.	.	.	.	.	N	N	.
Semliki	8.75	N	P	.	N	.	N	N	.	.	.	.	.	N	.	.
Piksuna	7.75	N	N	N	N	N	N	.	.	.	.	.	.	N	.	.
	8.00	N	N	P	N	N	N							N		
VEE	7.75	N	N	N	P	N	N							N	N	
	8.25	N	N	N	P	P	N	.	.	.	.	.	.	P	N	.
Mukambo	7.25	N	N	N	N	N	.	.	.	.	.	.	.	N	.	.
Chikungun'ya	5.00	N	N	N	N	.	N	N	.	.	.	.	.	N	.	.
WAEE	6.75	N	N	.	N	.	N	N	.	.	.	.	.	N	.	.
Japanese encephalitis	5.0	.	.	.	.	.	.	.	N	N	N	.	.	.	N	.
Western Nile	7.0	.	.	.	.	.	.	.	N	N	N	.	.	.	N	.
San-Lui	5.0	.	.	.	.	.	.	.	N	N	N	.	.	.	N	.
Tribech	4.0	N	N	.	N	.	.	.	.	.	.	.	N	N	N	.
Uukuniemi	6.5	.	.	.	.	.	.	.	N	.	N	.	.	.	N	P

Note: P-positive precipitation action; N-negative precipitation action; Period-no reaction.



Appearance of arboviral viruses in mice serum with the aid of immune-precipitation.

Antigens: a, b--VEE; c--Semliki virus; d--Uukuniemi virus.

1--ascite, immune to Piksuna virus; 2--to VEE virus; 3--to Mukambo; 4--to group A; 5--blood serum, immune to VEE virus; 6, 7, 8--serum of reconvalesents, which overcame VEE; 9--ascite, immune to Semliki virus; 10--to WAEE virus; 11--to Sindbis virus; 12--to Chikungun'ya virus; 13--to Uukuniemi virus; 14--guinea pig virus immune to Uukuniemi virus; 15--ascite, immune to Il'eus virus; 16--to San-Lui virus; 17 to group B viruses.