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TRANSLATION NO. 87

DATE: Sept 1968

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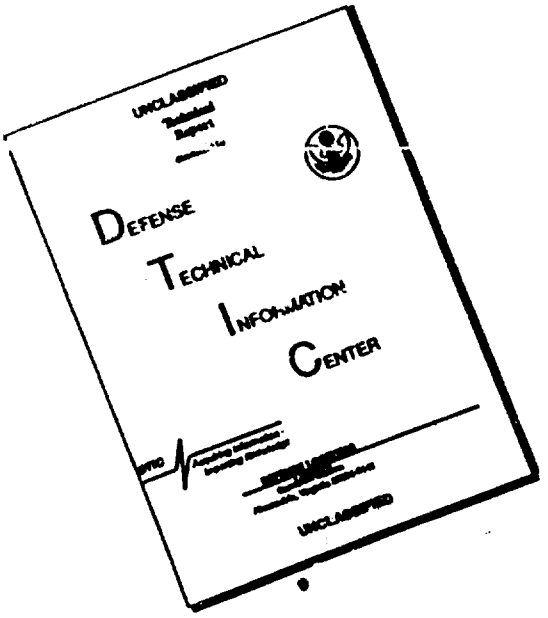

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Bulletin of Experimental Biology and Medicine
No. 6, 1956; Pages 48-51

Cultivation of Neurotropic Viruses in the Ascites-carcinoma of Mice.

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Tests on the cultivation of virus in the tumor of mice have been conducted by us since 1949. In the first series the grippe virus was studied (strain PR8), it went through 38 passages in a carcinoma. During this we were not able to obtain a variant of an analogical strain or a strain with a lowered degree of infectiousness for susceptable animals; in reverse, during the later passages through the tumor, the pathogenic properties of the strain were notably increased. Cultivation of neurotropic viruses (tick and Japanese encephalitis, poliomyelitis) in ascites-carcinoma has been conducted by us since 1950.

Ascites-carcinoma as a medium for the growing of viruses 'in vivo' creates optimal conditions for the multiplication of these intracellular parasites.

Literary data refer to the cultivation of viruses in the solid tumors of animals, therefore, our studies on the fluid tumors, in the first stages, had a searching characteristic.

In the process of the experimental work we were convinced that the ascites-carcinoma, as a medium for the growing of viruses, has a number of advantages over the solid tumors, and also over brain tissue of susceptable animals and chicken embryo.

The basic experiments were with viruses of tick and Japanese encephalitis. For successive incubations in the ascites-carcinoma we, in all cases, used a transparent fluid fraction derived from cellular

elements, it was always verified in biological assays for content of agents suitable for passage.

As a rule, besides the qualitive biological sampling of the materials of each passage, we systematically, after 5-6 passages, determined the titer of virus; this reached 10-7 to 10-8 for tick and Japanese encephalitis after 48 hours. With the virus of poliomyelitis only qualitive biological assays were made; done by infecting cotton rats and white mice with the passage material. The virus of poliomyelitis was maintained in a liquid fraction until the 15th passage through the tumor, after a determined incubation period it caused a characteristic infection in susceptable animals. The studies on poliomyelitis were conducted by us in cooperation with E. S. Sarmanov.

The virus of tick and Japanese encephalitis, as well as the strain of poliomyelitis virus by us, multiplies violently in the liquid tumor; their basic neurotropic properties do not weaken during this.

In the process of a lengthy cultivation of strains of tick and Japanese encephalitis in such tumors, we as yet have not been able to obtain a variant with a lowered pathogenicity for susceptable animals. But, we constantly observed some increase of the pathogenicity of the strains in a process of lengthy cultivation in tumors. This, evidently, is because the multiplication of the virus in the tumorous substrata of susceptable animals does not become isolated from the general physio-biological processes which take place in the organism of the host.

A close interaction exists between the organism and the tumor, this is the most advantageous medium for the virus. Therefore, it is quite possible, as some authors claim, to obtain only pathogenic strains

if they are cultivated in the tumor of an organism which is susceptable to the virus being cultivated.

Several series of tests were run for the determination of the dynamics of accumulation of the virus in the tumor, in dependence on the concentration and time of multiplication of the agent.

Table 1 contains data which **domfirm the intensive accumulation** of the virus in the fluid fraction. After only 48 hours, the concentration of virus becomes very high, while during introduction of an **analogical** dose into the brain of an animal the virus in this period of time is almost undetectable, even with the application of so called 'blind passages'.

Consequently, by using the method of passing material, which contains a minimum dose of an agent, through a **tumor**, it is possible to obtain in comparatively short periods a tumorous substrata already enriched with virus; this insures a larger percentage of detection of the agent in a smaller group of test animals.

Using the results of the biological tests, in which the quick enrichment of the fluid fraction by **the virus** was established, as a basis, we tried to utilize this as an antigen in the complement fixing reaction in order to hasten the diagnosis and preparation of the inactivated antigens for a retrospective serological analysis of serums. In order to do this, the liquid fraction obtained from each animal (infected with virus and **non**-infected), freed from cellular elements, was studied separately.

The individual fluctuations of the titers were quite insignificant, and only in concentrated antigens (1:5) is a non-specific content of hemolysis sometimes noted. During dilution of 1:10, as a rule, a clear specific reaction is revealed.

Mixtures, from 5-10 mice, of virus-containing and control antigens were tested by analogical means. Such mixtures are fully valuable as antigens for the complement fixing reaction.

The next phase of our work was the preparation of the inactivated fluid and dried antigens for diagnosis of tick and Japanese encephalitis so as to insure an expansive utilization and free transportation of them in and to all labs. Such antigens are already being prepared by us and have undergone experimental verification with animal serums.

In Table 3 are the results of the complement fixing reaction with inactivated antigens. As a rule we obtained very clear specific reactions; the dried antigens according to concentration did not differ from the corresponding fluid. It is true that until now we still do not have any standard antigens (due to several defects in inactivation and of technology of drying), but we can say with full confidence that the dried antigens in a 1:10 dilution are fully valuable diagnostic antigens. It is very important that 1 ml of them, according to concentration, is respective to a volume of 10 ml of antigens prepared from a 10% brain suspension of an animal. During utiliztion in the complement fixing feaction, 1 ml of the dried antigen is diluted in 10 ml of a physiological solution.

Antigens prepared this way are not complicated and quite economical; 6-10 ml of concentrated antigen can be obtained from one mouse with a tumor, this corresponds to 60-100 ml of antigen which is valid for the complement fixing reaction. In this work we do not report on a detailed method of preparing the inactivated antigens; this is a separate phase of our work which will be reported later.

Table 1. Results of titration of the liquid fraction of a tumor and brain of a mouse 48 hours after infection of them with various doses of tick encephalitis virus.

Dilution of material	Dose introduced 10-2 Brain	10-2 Fluid fraction	10-4 Brain	10-4 Fluid fraction	10-7 Brain	10-7 Fluid fraction	10-9 Fluid fract.	10-10 Fluid fraction
10-1	2/3	3/3	0/3	3/3	0/3	3/3	3/3	3/3
10-2	0/3	3/3	0/3	3/3	0/3	3/3	2/3	2/3
10-3	0/3	3/3	0/3	3/3	0/3	2/3	1/3	0/3
10-4	0/3	3/3	0/3	2/3	-	1/3	0/3	0/3
10-5	0/3	2/3	0/3	3/3	-	1/3	0/3	-
10-6	0/3	3/3	0/3	2/3	-	0/3	0/3	-

Mice becoming ill/Mice taken in test.

Table 2. Titers of virus of Japanese encephalitis during initial infection of mice with tumor. Material analysed 72 hours after infection.

Material for Infection...	Mode of infection and dose: Tumor-0.25 ml 10-3	Subcut-0.25 ml 10-3	Brain-0.03 ml 10-3
Fluid fraction	10-6	10-5	10-4
Brain tissue	0	0	10-2

Table 3. Complement fixing reactions with inactivated fluid and dry diagnostical antigens of tick encephalitis

Source of serums and in dilutions of 1:12	Doses of complement	Antigens: Virus containing: Fluid 1:10	Fluid 1:20	Fluid 1:40	Dried 1:10	Dried 1:20	Dried 1:40	Fluid 1:10	Fluid 1:20	Fluid 1:40	Dried 1:10	Dried 1:20	Dried 1:40	Control: Fluid 1:10	Fluid 1:20	Fluid 1:40	Dried 1:10	Dried 1:20	Dried 1:40	Control tis serums
Immune guinea pigs	1.5	p4	p4	p4	p4	p4	p2	p4	p4	p2	p4	p4	p1	-	-	-	-	-	-	-
	2	p4	p3	p1	p4	p3	-	p4	p2	p1	p4	p3	p1	-	-	-	-	-	-	-
Control guinea pigs	1.5	All the remaining chart had -(minus) marks which meant negative results...																		
	2																			
Contral of antigen	1.5																			
	2.																			

Explanation; p4 means ++++ p3- +++ p2- ++ p1- + p1- ± ; degree of content of hemolysis