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INHIBITORY EFFECT OF HEPARIN ON AFRICAN HORSE SICKNESS VIRUS

by

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In a report Nahmias & Kibrick (1) have demonstrated that heparin from various animal sources have an inhibitory effect in Herpes Simplex virus. This finding was confirmed by Vaheri & Cantell (2). Identical observations were recorded by Agol & Chumakova (3) in case of d-variant of Poliovirus or by Takemoto & Liebhaber (4) for minute-plaque of encephalomyocarditis virus. This report is concerned with a similar finding with African Horse Sickness virus (A H S V). The virus used in these experiments were neurotropic strains V R Y (type 4) and S 2 (type 9) adapted to hamster kidney cells (H K C) or to monkey kidney cells line (M S) as we have described in previous works (5, 6). The stock virus comprised of total harvest of viruses cultivated in M S cells, lyophilized and kept in -40° C

M S cell monolayers in tube were infected with 10 to 100.000 TCID/50 of virus. After 45 minutes, maintenance medium containing 2,5% inactivated calf serum and various amounts of heparin was added. All tubes were duplicated. For titration of viruses a set of tubes without heparin were included in each experiment. The cultures were kept at 36°C. Heparin without preservative in lyophilized form was kindly supplied by Choay Laboratory (Paris). A daily observation of cultures revealed after 5 days the absence of C P E in tubes containing 100 gamma g/ml or more of Heprin. The cytopathic effect in tubes containing 10 to 100 gamma g/ml of Heparin was present but in a lesser extend comparing with controls.

The results of one experiment are reflected in table I

Table I - Titration of A H S V in M S cells in the presence of Heparin

Heparin	T C I D 50/ml	Inhibition
gamma g/ml	log.	log.
5000	0	6.0
1000	0	6.0
100	2	4.0
10	5	1.0
1	5.5	0.5
0.1	6.0	0
None	6.0	0

It is interesting to note that when using Heparin at the level of 1000 to 5000 gamma g/ml, regardless of the size of inoculum, whitin the limited used, C P E and infectivity were absents. The infectivity was assayed in M S cells. The cultures were quickly frozen, thawed and centrifuged 5 minutes at 2,000 r.p.m. The clear supernate was diluted and tested for infectivity. There was a decrease in infectivity when 10-100 gamma g/ml of Heparin was added and no changes in infectivity were recorded by using 1 gamma g/ml or less of Heparin. The toxicity of Heparin for M S cells was slight. In the presence of 5000 gamma g/ml of Heparin these cells showed an slight change but the lower doses had no ill-effect on M S cells.

In another experiments it was found that 100 gamma g/ml of Heparin has no effect on the intracellular multiplication and plaque formation of A H S V.

The activity of Heparin on A H S V in-Vivo was also studied. It was ascertained that the product at the level usually used as anticoagulant had no inhibitary effect on virus when heparinized blood was injected intracerebrally to mice. Wether the inhibitory effect of Heparin on A H S V is due to its anticoagulant activity or to its prevention of the early interaction between virus and cells, the phenomenon may be of practical importance. As a matter of fact on several occasions we were unable to isolate virus in T C from the heparinized blood of horses died of horsesickness.

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