endothelial cell suspension is fully as active as brain extract or platelets in these two tests. It is thus rich in cephalin and much thrombin is formed in its presence.

It has been suggested that the sub-endothelial mast cells secrete a heparin-like substance which may play a part in keeping the blood liquid. All the live intact cells so far studied do not inactivate heparin[1] although intact endothelial cells have not been studied. The ability of the endothelial cell fragments to inactivate heparin was investigated as follows :

The prolongation of the clotting time caused by the addition of a given amount of heparin to a thrombin clotting system containing plasma as a source of co-factor is determined. If the addition of the cell suspension to the heparin results in a shorter thrombin clotting time it must be concluded that some of the heparin has been inactivated and is no longer 'available' to prolong the thrombin clotting time. Table 3 shows that much heparin was inactivated when the cell suspension was added. It is concluded that damaged endothelial cells can inactivate considerable quantities of heparin.

Table 3. HEPARIN INACTIVATION BY ENDOTHELIAL CELL FRAGMENTS

	Saline	Heparin 0·125 u/ml.	Heparin 0·25 u/ml.
Saline	22	51	60
Endothelial Cell Fragments	19	19	30

0·1 ml. of all reagents was used. All tubes contained platelet-poor plasma and saline or endothelial cell suspension. To these tubes was added either another volume of saline or heparin in the strength indicated. A strong thrombin solution was added and the clotting time recorded in seconds.

If endothelial cell damage or death occurs *in vivo* the two attributes of damaged endothelial cells reported above may play a vital part in thrombus formation ; these attributes are the weak brain-like activity including 'available' cephalin and the ability to inactivate heparin. Particularly may the brain-like activity be important since it initiates coagulation and is independent of any other trigger mechanism such as contact with glass.

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VIROLOGY

Infectious Ribonucleic Acid from Mouse Brains infected with Tick-borne Encephalitis Virus

INFECTIOUS ribonucleic acid has been prepared from several virus-infected tissues and from purified animal viruses[1–4] using the phenol extraction method of Gierer and Schramm[5]. Assuming that the tick-borne encephalitis virus contains a nucleic acid of ribose type, we have succeeded in preparing by the same method infectious ribonucleic acid from mouse brains infected with the *Hypr* strain of tick-borne virus isolated in Czechoslovakia. The infectivity of such preparations was about 10^3–10^5 times lower than that of virus. To be sure that the infectivity of ribonucleic acid was not caused by contamination with virus particles, the acid and virus were titrated after different treatment. The titrations were carried out in white mice by intracerebral injection of 0·03 ml. using serial ten-fold dilutions. Clarified 10 per cent (w/v) suspension of infected brains was designated as undiluted virus for convenience in comparing the infectivities of virus and ribonucleic acid. The results are shown in Table 1.

Table 1. INFECTIVITY OF TICK-BORNE ENCEPHALITIS VIRUS AND ITS RIBONUCLEIC ACID AFTER DIFFERENT TREATMENTS

Treatment	No. of exp.	LD_{50}/ml. Virus	LD_{50}/ml. RNA
None	I	$8·3 \times 10^8$	$1·3 \times 10^4$
	II	$1·3 \times 10^9$	$3·3 \times 10^3$
	III	$1·4 \times 10^8$	$6·6 \times 10^4$
Ribonuclease 45 µgm./ml. 37° C., 25 min.	I	$1·4 \times 10^5$	$< 2·1 \times 10^{4}$†
	II	$1·3 \times 10^8$	$< 2·1 \times 10^{4}$†
	III	$1·0 \times 10^9$	$< 2·1 \times 10^{4}$†
Heating 37° C., 25 min. (ribonuclease control)	I	—	$6·6 \times 10^3$
	II	—	$3·3 \times 10^3$
	III	—	$2·1 \times 10^4$
Neutralization with immune γ-globulin	I	$1·7 \times 10^4$	$2·1 \times 10^3$
	II	$1·0 \times 10^5$	$6·6 \times 10^2$
	III	$1·6 \times 10^5$	$1·4 \times 10^4$
Precipitation by ethanol	I	$< 1·0 \times 10^{8}$*	$3·3 \times 10^4$
	II	$< 1·0 \times 10^{8}$*	$5·6 \times 10^3$
	III	$1·6 \times 10$	$1·0 \times 10^4$
Heating 37° C., 4½ hr.	I	$3·3 \times 10^6$	$3·3 \times 10^3$
	II	$7·1 \times 10^7$	$<1·0 \times 10^{8}$*
	III	$1·0 \times 10^6$	$6·6 \times 10^2$

No infectivity could be demonstrated in 0·03* (0·015†) ml. of undiluted inoculum.
—, Not done.

The infectivity of ribonucleic acid was completely destroyed by the action of ribonuclease, while that of virus was reduced significantly only in experiment 1. Control experiments have shown that ribonucleic acid is destroyed really by the enzyme and not by heat. Neutralization by specific immune horse γ-globulin lowered the titre of virus 500–12,600 times, while that of ribonucleic acid only 5–6 times. The latter remained fully infectious after precipitation with two volumes of ethanol. The same treatment destroyed the infectivity of virus. Heat inactivation did not show marked difference between ribonucleic acid and virus.

Tick-borne encephalitis virus derived from the brains of mice which died following the intracerebral injection of ribonucleic acid was identified by neutralization tests with specific immune γ-globulin. In such brains typical lesions of viral encephalitis were observed by histological examination.

The ultra-violet absorption spectra of preparations of ribonucleic acid were typical for nucleic acid. The D_{258}/D_{280} ratios were 1·94–2·14 showing that preparations of ribonucleic acid contained only very small, is any, amount of protein impurities.

The results lead us to conclude that tick-borne encephalitis virus contains ribonucleic acid which if infectious when tested by intracerebral inoculation in mice.

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