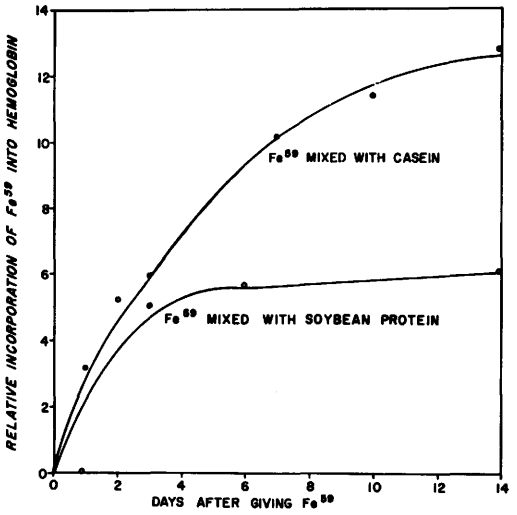

FIG. 3. Effect of isolated soybean protein on gastrointestinal absorption of iron. Incorporation of Fe^{59} into hemoglobin is expressed as cpm in 1 ml blood $\times 10^4$ divided by the cpm in the initial dose. During these studies hemoglobin decreased from 7.1 to 5.2 g per 100 ml. The study using soybean protein was done 36 days after the one using casein and appropriate corrections were made for residual radioactivity.

were microcytic and hypochromic, serum iron concentrations were low and serum iron binding capacities were high. Iron therapy, either parenteral or oral, produced a prompt remission of anemia. Since these diets contain as much iron as other diets that are adequate in this respect, gastrointestinal absorption of Fe^{59} was evaluated in the presence of soybean protein and compared to Fe^{59} absorption in the presence of casein. Soybean protein caused a significant reduction in gastrointestinal absorption of Fe^{59}.

We are indebted to Mr. Raymond A. Squires and the Little Rock Municipal Zoo for permitting us to study the Zoo monkeys and to Mr. Ralph S. Izard for technical assistance.

1. Sharpe, L. M., Peacock, W. C., Cooke, R., Harris, R. S., *J. Nutrition,* 1950, v41, 433.
2. Miller, C. D., Louis, L., *ibid.,* 1945, v30, 485.
3. Davis, P. N., Norris, L. C., Kratzer, F. H., *ibid.,* 1962, v77, 217.
4. Subrahmanyan, K., Sathe, V., De, S. S., *Ann. Biochem. and Exp. Med.,* 1950, v10, 13.
5. Dinning, J. S., Day, P. L., *J. Exp. Med.,* 1957, v105, 395.
6. Trinder, P., *J. Clin. Path.,* 1956, v9, 170.
7. Rath. C. E., Finch, C. A., *J. Clin. Invest.,* 1949, v28, 79.
8. Porter, F. S., Fitch, C. D., Dinning, J. S., *Blood,* 1962, v20, 471.
9. Morrison, A. B., Sarett, H. P., *J. Nutrition,* 1958, v65, 267.
10. O'Dell, B. L., Savage, J. E., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 304.
11. Hubbell, R. B., Mendel, L. B., Wakeman, A. J., *J. Nutrition,* 1937, v14, 273.

Received February 7, 1964. P.S.E.B.M., 1964, v116.

Destruction of Enteroviral Ribonucleic Acid Infectivity by Phosphodiesterase.* (29181)

H. ROUHANDEH AND G. R. DUBES (Introduced by H. A. Wenner)
Section of Virus Research, Department of Pediatrics, and Department of Microbiology, School of Medicine, University of Kansas, Kansas City, Kansas.

Colter *et al*(1) showed that poliovirus preparations after treatment with phenol contained ribonuclease-labile infective units. Infection of primary monkey kidney cultures with such units was found to be facilitated by inoculating calcium-depleted cultures with the infective ribonucleic acid (RNA) adsorbed to a facilitator (*e.g.,* talc)(2). The infection of such cultures with RNA from Coxsackie A23 (Vispo)(3), Coxsackie A7, A9, and B3, and enteric cytopathogenic human orphan (ECHO) 5, 7, 8, 11, 12, 19, and 26 (Rouhandeh, manuscript) viruses has since been found to be facilitated by this method, as also has the infection of mouse L-cells with encephalomyocarditis virus RNA (4).

* This research was supported by a grant from the National Foundation and from U. S. Public Health Service training grant to Dept. of Microbiology.

Holland *et al*(5), using a hypertonic solution of $MgSO_4$ for infecting HeLa cells with RNA, found that the infectivity of poliovirus 1 RNA was destroyed by snake venom phosphodiesterase (SVP). We have used the above-described facilitation system employing poorly soluble facilitators and primary monkey kidney tissue cultures for testing the effect of SVP on the infective RNA's from 3 other enteroviruses; for reference, poliovirus 1 RNA was also used.

Materials and methods. Preparation of cells. Primary rhesus monkey *(Macaca mulatta)* kidney cultures were grown under medium S, which is high-cystine altered Eagle's maintenance ($hcAE_m$) medium(6) containing 4% calf serum, in 60-mm Petri plates kept in a humidified incubator continuously flushed with 3% CO_2 and 97% air. Suitable sheets of cells developed in 4 to 6 days. Sheets to be inoculated with infective RNA were then depleted of calcium by washing 4 times, each time with 4 ml of PBS(7) without $CaCl_2$, with incubation at 37° for 30 minutes between the second and third washings; sheets to be inoculated with intact virus were washed twice, as above, but with PBS.

Preparation of inocula. The viral strains used were Brunhilde (poliovirus 1), Wallace (ECHO 7), Travis 2-85 (ECHO 12), and Bozek (Coxsackie A9). Stocks of these were prepared on primary rhesus kidney cultures under liquid medium $hcAE_m$. RNA preparations were obtained from these stocks using phenol; for Brunhilde an extractive phenol method(8) was used whereas for the other 3 viruses the monophasic method as used by Rouhandeh(4) was employed. The infectivity of each RNA preparation was ribonuclease-labile. Inocula of intact virus were obtained by appropriately diluting the virus into PBS. RNA inocula were prepared by diluting the RNA preparations into PBS without $CaCl_2$; RNA inocula also contained 0.5% talc as facilitator. RNA preparations were inoculated at concentrations ranging from 0.002 to 0.2.

Procedures for infection and plaque development. After receiving 0.3 ml inoculum, each cell sheet was kept at 37° for one hour and then was overlaid with 2-4 ml medium $hcAE_m$ containing 0.9% washed agar. The plates were then incubated in 3% CO_2 atmosphere (see above). Plaques were scored 3-5 days later after staining with neutral red.

Preparation of stock solution of SVP. A sample of SVP kindly supplied by Worthington Biochemical Corp., Freehold, N. J., was used. Enzyme was dissolved in sterile deionized water to a concentration of 1250 μg SVP per ml.

Preparation of 0.19 M *tris (hydroxymethyl) aminomethane (TRIS) pH 8.9 buffer.* This buffer was prepared by appropriately diluting 0.20 M TRIS with deionized water, using sufficient 1 N HCl to bring the pH to 8.9 ± 0.1.

Reaction of RNA preparations with SVP. Two volumes of deionized water, 21 volumes of the pH 8.9 TRIS buffer, 2 volumes of SVP solution containing from 1.25 to 1250 μg enzyme per ml, and 25 volumes of RNA preparation were mixed and kept at 25° for 5-60 minutes; water replaced SVP in the controls. After this incubation, samples from the reaction mixtures were diluted 2.5- to 250-fold into PBS without $CaCl_2$ containing the appropriate concentration of talc at 0°. Inoculations were made from these dilutions.

Titration of infective RNA. RNA titers, expressed in plaque-forming units (pfu) per ml, were calculated from the initial slopes of the curves showing arithmetic mean number of plaques per plate as a function of concentration of RNA preparation inoculated (titration curves). In the experiments for Fig. 1 the mean plaque count from plating a single concentration of an SVP-treated preparation was used for determining infective RNA concentration therein by interpolating on the titration curve obtained with the RNA preparation not treated with SVP in the same experiment.

Results. Effect of SVP on infectivity of 4 enteroviral RNA's and on their corresponding intact viruses. In Table I are shown data from 5 experiments indicating that for each of the 4 enteroviruses tested the infectivity of the RNA but not that of the intact virus was destroyed by SVP.

Effect of different concentrations of SVP

TABLE I. Effect of SVP on Infectivity of Enteroviral RNA's.

Virus	Viral material treated*	Exp	Plaques/plate† (mean ± standard error) After control incubation in buffer	After incubation with SVP
Poliovirus type 1	Intact	a	64 ± 3	72 ± 6
	RNA	b	57 ± 4	0‡
		c	21 ± 2	0
Coxsackie type A9	Intact	a	27 ± 3	37 ± 2
	RNA	c	18 ± 3	0
		d	22 ± 2	0
ECHO type 7	Intact	a	45 ± 6	40 ± 4
	RNA	d	27 ± 2	0
		e	37 ± 3	0
ECHO type 12	Intact	a	51 ± 8	39 ± 0.9
	RNA	c	8 ± 1	0
		d	31 ± 4	0

* Intact virus stocks were plated at concentrations calculated from prior titrations to give 35 plaques per plate; RNA preparations were inoculated at concentration 0.05.

† Viral materials were incubated with SVP at 50 μg/ml at 25° for 30 min.; for the control incubation, enzyme was replaced by water.

‡ For groups with mean of zero, number of plates/group ranged from 5 to 8.

on ECHO 7 RNA infectivity. Various concentrations of SVP were tested to determine the relationship between concentration of SVP and the fraction of infective ECHO 7 RNA surviving the incubation with SVP. These fractions were determined as described in "materials and methods"; the results are given in Fig. 1A. At 2 μg SVP per ml 90% of the infective RNA was inactivated in 0.5 hour at 25°.

Rate of inactivation of ECHO 7 RNA by SVP. ECHO 7 RNA was reacted with 2 μg SVP per ml. Samples were withdrawn at various times after starting the reaction, and infective RNA titers of these samples were then determined (see *Materials and methods*). The results (Fig. 1B) indicate that the inactivation occurs without a lag and that rate of inactivation decreases with time.

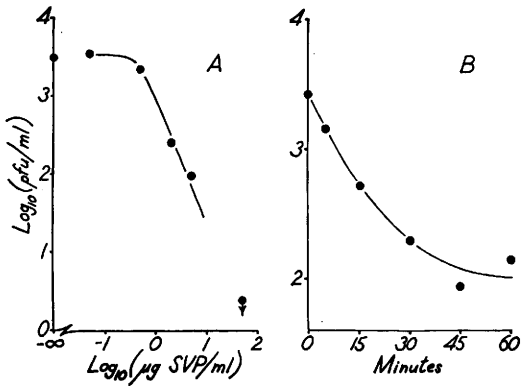

FIG. 1. A. Inactivation of ECHO 7 RNA by various concentrations of SVP. Incubation was at 25° for 30 min. B. Rate of inactivation of ECHO 7 RNA by SVP. Incubation was with SVP at 2 μg per ml at 25°.

Discussion. That the inactivation of ECHO 7 RNA by SVP proceeds without a lag suggests that only one critical internucleotide bond need be broken by SVP to remove the infectivity of the ECHO 7 RNA strand. However, the number of such SVP-hydrolyzable critical bonds per ECHO 7 RNA strand is not known. Nor do we know whether the critical internucleotide bonds broken by the SVP are terminal, nonterminal, or both. Holland *et al*(5) found that poliovirus 1 RNA also was inactivated by SVP without a lag.

Recently Singer and Fraenkel-Conrat(9) have set up conditions under which the action of SVP on tobacco mosaic virus (TMV) RNA, which is unphosphorylated at both ends, is predominantly exonucleolytic. With this procedure these workers have initiated the sequential analysis of the TMV RNA. Such an analysis may prove to be similarly useful for the enteroviral RNA's.

Summary. Infectivity of RNA from ECHO

7 and 12 and Coxsackie A9 viruses was destroyed by snake venom phosphodiesterase (SVP) whereas the infectivity of the corresponding intact viruses was unaffected by this enzyme. At 25° using ECHO 7 RNA and 2 μg SVP per ml, rate of inactivation was constant through approximately 20 minutes, then progressively decreased.

1. Colter, J. S., Bird, H. H., Moyer, A. W., Brown, R. A., *Virology,* 1957, v4, 522.

2. Dubes, G. R., Klingler, E. A., Jr., *Science,* 1961, v133, 99.

3. Faas, F. H., Rouhandeh, H., Lamb, R. D., Kelly, D. G., Chapin, M., Lucas, T. A., Dubes, G. R., *Genetics,* 1963, v48, 889.

4. Rouhandeh, H., *Biochem. Biophys. Res. Comm.,* 1963, v12, 329.

5. Holland, J. J., McLaren, L. C., Hoyer, B. H., Syverton, J. T., *J. Exp. Med.,* 1960, v112, 841.

6. Klingler, E., Jr., Chapin, M., Dubes, G. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 829.

7. Dulbecco, R., Vogt, M., *J. Exp. Med.,* 1954, v99, 167.

8. Gierer, A., Schramm, G., *Z. Naturforsch.,* 1956, v11b, 138.

9. Singer, B., Fraenkel-Conrat, H., *Biochim. Biophys. Acta,* 1963, v72, 534.

Received February 10, 1964. P.S.E.B.M., 1964, v116.

Effects of Norepinephrine on Myocardial K, Na, Cl and H_2O in Hemorrhagic Shock.* (29182)

VINCENT V. GLAVIANO AND BERNELL COLEMAN†

Department of Physiology, Stritch School of Medicine, Loyola University, Chicago, Illinois

Clinical investigators do not generally agree on the efficacy of exogenous norepinephrine (NE) for treatment of hemorrhagic shock. In addition to not extending the survival period, administration of this sympathomimetic amine has caused hepatic, renal and cardiac damage(1,2). Treatment of shock with NE has also failed to correct the inability of the heart to utilize pyruvate or prevent the decline in myocardial efficiency(3). On the other hand, maintenance of cardiac output and an increase in survival time have been reported for NE treated animals in shock(4, 5). These beneficial effects have been attributed to the stimulating action of this catecholamine on the myocardium(6).

Dogs in irreversible hemorrhagic shock were recently reported to undergo increases in myocardial interstitial K sufficient to cause significant lowering of the resting membrane potential(7). Since NE has been observed to increase the intracellular/extracellular ratio of K in normal cardiac muscle(8,9), myocardial levels of K, Na, Cl and H_2O were compared in normal dogs with dogs in hemorrhagic shock, treated and not treated with exogenous l-NE.

Methods. Thirty mongrel dogs anesthetized with 35 mg/kg of sodium pentobarbital were divided equally into 3 groups. One group served as controls, a second group was subjected to hemorrhagic shock and a third group was treated with l-norepinephrine after inducing shock. The trachea was cannulated and the left common carotid artery was connected to a mercury manometer for monitoring mean arterial pressure. The left femoral artery and vein were cannulated for bleeding and reinfusion of blood respectively. Shock was induced by bleeding the animal (50 ml/min) until mean pressure stabilized at 40 mm Hg. Following the maintenance of this level of pressure for 4 hours, the total bled volume was reinfused. Prior to inducing shock, a control arterial blood sample was taken for analysis of plasma K, Na, Cl and H_2O. Coagulation of blood was prevented by initial administration of 5 mg/kg of hep-

* These studies were aided by contract between Office of Naval Research, Dept. of the Navy and Loyola Univ. Stritch School of Medicine.

† Predoctoral Research Fellow, Nat. Inst. of Health; present address: St. Louis Univ. School of Med., St. Louis, Mo.