tional area being interspace, or to a large resistance in current flow through the cells. If the whole tissue were interspace, all the current would of necessity pass through the interspace and the expected specific resistance would be that of Tyrode's (44 ohm-cm(4)). However, the reported values for the interspaces of cardiac, skeletal, and smooth muscles, although variable, are not sufficiently different to account for the differences in the resistance changes (Table I). Therefore, the current flow paths through the cells of cardiac and smooth muscles appear to be of high resistance. This high resistance is more likely due to the high resistance of cell membranes than to high myoplasmic resistance.

Other studies predict that the current flow path through smooth muscle cells should involve a succession of high resistance cellular membranes(13). Our results lead to the conclusion that this may also be true of cardiac muscle. This is consistent with the observation that ventricular muscle specific resistance is larger than that of skeletal muscle. It would appear that cardiac muscle cells are separated from each other by high resistance cellular membranes and do not form a low resistance functional syncytium.

Summary. The D.C. specific resistances of dog and beef ventricular muscle strips were compared with those of cat intestine circular smooth muscle, which is a non-syncytial tissue, and of frog sartorius muscle where individual cells extend almost the full length of muscle. Resistances were measured in the direction of fiber orientation before and after the interspace ion concentration was reduced by soaking in isotonic 10% Tyrode's-sucrose solution. Cardiac and smooth muscle resistances were much more sensitive to interspace ion depletion than was skeletal muscle resistance. It is suggested that the current flow path through the cells of cardiac muscle is of high resistance due to large number of high resistance cellular membranes involved, as in smooth muscle. It was concluded that cardiac muscle cells are separated from each other by high resistance membranes and do not form a functional syncytium.

1. Fawcett, D. W., Selby, C. C., *J. Biophys. Biochem. Cytol.*, 1958, v4, 63.

2. Moore, D. H., Ruska, H., *ibid.*, 1957, v3, 261.

3. Sperelakis, N., Hoshiko, T., Keller, R. F., Jr., Berne, R. M., *Am. J. Physiol.*, in press.

4. Prosser, C. L., Sperelakis, N., *ibid.*, 1956, v187, 536.

5. Robertson, W. Van B., Peyser, P., *ibid.*, 1956, v184, 171.

6. Manery, J. F., Bale, W. F., *ibid.*, 1941, v132, 215.

7. Truex, R. C., Copenhaver, W. M., *Am. J. Anat.*, 1947, v80, 173.

8. Bozler, E., Lavine, D., *Am. J. Physiol.*, 1958, v195, 45.

9. Johnson, J. A., *ibid.*, 1955, v181, 263.

10. Levi, H., Ussing, H. H., *Acta physiol. scandinav.*, 1948, v16, 232.

11. Boyle, P. J., Conway, E. J., Kane, F., O'Reilly, H. L., *J. Physiol.*, 1941, v99, 401.

12. Johnson, J. A., *Am. J. Physiol.*, 1957, v191, 487.

13. Sperelakis, N., Prosser, C. L., *ibid.*, 1959, v196, 850.

Received May 26, 1959. P.S.E.B.M., 1959, v101.

Infectious Ribonucleic Acid Derived from Enteroviruses.* (25033)

KATHERINE SPRUNT, WINIFRED M. REDMAN AND HATTIE E. ALEXANDER
Babies Hospital (Presbyterian Hosp.) and Department of Pediatrics, Columbia University College of Physicians and Surgeons, N. Y.

In recent years the ribonucleic acid (RNA)-containing fraction of a number of viruses has been shown to be infectious. The evidence supports the premise that the active ingredient of this infectious portion is RNA; infectivity is inactivated by crystalline ribonuclease (RNAase) but not by gamma globulin prepared from specific antiserum or by desoxyribonuclease (DNAase)(1). In earlier ex-

* Aided by grant from National Fn.

periments *in vivo* technics in plants, animals and chick embryos were used to demonstrate infectivity of RNA preparations made from tissues infected *in vivo*. Recently, Alexander *et al.*(1), using the phenol extraction method of Gierer and Schramm(2), demonstrated that under certain conditions of molarity and pH infectious RNA derived from polioviruses regularly produced plaques in tissue culture, and that the infectious capacity of the RNA was, under these conditions, proportional to dilution. A simple quantitative tool was then available for the study of infectious RNA. Most of their experiments were carried out using highly purified or partially purified poliovirus preparations. The work to be reported here demonstrates that their methods are equally applicable to crude tissue culture-grown virus suspensions, and can be successfully used to prepare infectious RNA from other members of the enterovirus group. RNA derived from Coxsackie A7, B4 and B5, and Echo types 1 and 8 induced plaques in which the intact virus supplying the RNA could be demonstrated. Previous exposure of the RNA-containing fraction to crystalline RNAase destroyed its infectivity, whereas DNAase had no significant effect.

Materials and methods. Virus strains used were 1) a heat-resistant mutant picked from a type 1 poliovirus population (Mahoney strain); 2) Coxsackie B5 virus grown from spinal fluid of a newborn infant with meningoencephalitis and probable myocarditis; 3) Coxsackie B4 virus from stool of patient with aseptic meningitis; 4) Echo 1 and Echo 8 strains obtained through courtesy of Dr. Heinz Eichenwald, N. Y. Hospital; and 5) Coxsackie A7 virus obtained from Dr. Karl Habel, Nat. Inst. of Health. The polio and Coxsackie B strains were grown in HeLa cells and amnion cells in Scherer's maintenance solution with 10% horse serum (MS) and were tested on amnion cells in monolayers in 60 mm Petri dishes. Echo viruses and Coxsackie A7 virus were grown in HeLa cells and tested in HeLa cell monolayers in Petri dishes. *Cell lines*. HeLa cells used were the L5 line grown from a clone isolated by Leidy *et al.* and picked for sensitivity to polioviruses. Amnion cells were the Cold Spring Harbor passage line of amnion cells (Fernandes(3)). HeLa cells were grown in Puck's modification of a Weymouth solution(4) and amnion cells in Eagle's solution with double the usual concentrations of amino acids and vitamins. *Monolayers*. Monolayers were prepared in 60 mm Petri dishes by adding approximately one million cells/plate in 4 ml of Puck's solution. Plates were incubated in an atmosphere of 5% CO_2 for 3 days, washed 2 $\times$ with a Tris-buffered nutritive solution to remove antibody, and inoculated with 0.1 ml of virus. After a 30-minute adsorption period a 1% agar overlay was added and the plates incubated in CO_2 for 4 days. At this time a neutral red-containing agar overlay was added and plaques were counted 3 to 4 hours later. If the inoculum was whole virus both wash solutions and virus diluent consisted of modified Earle's solution with lactalbumin, yeast, glucose, and Tris buffer added at pH of 7.2. If the inoculum was RNA, the second wash solution consisted of 0.65 M NaCl and 0.03 M Tris solution buffered to pH 7.6, and RNA dilutions were made in 0.9 M KCl plus Tris solution buffered to pH 8.0. Otherwise, RNA and whole virus were similarly treated. *RNA preparation*. The virus for RNA preparation was grown in HeLa or amnion cells in MS and harvested when 3^+ to 4^+ cytopathogenic action became evident. Suspensions were routinely frozen before use. When thawed, they were centrifuged at 1,500 rpm for 10 minutes and the supernatant used as the whole virus from which the RNA was derived. The phenol extraction technic for removing protein from RNA, noted briefly below, is described in detail by Alexander *et al.*(1). The procedure was carried out with chilled materials and equipment through the ether washing stage. In a centrifuge tube 2.5 ml of distilled or washed Mallinckrodt reagent phenol in water-saturated solution was mixed 1:1 with virus preparation which consisted of 2 ml of virus suspension, 0.3 ml of 5 M NaCl and 0.2 ml of phosphate buffer at pH 7.3. Virus and phenol were shaken by hand 4 minutes and separated by quick centrifugation at 12,000 rpm. The aqueous phase was removed, mixed 1:1 with saturated solution of phenol, shaken for 2 minutes and centrifuged. The third ex-

TABLE I. Comparison of Titer of Intact Enteroviruses and Their Infectious "RNA."

	Intact virus diluted 1:1 with				RNA diluted 4:1 with				RNA dilution		
	BSS*	RNAase	NMS†	Specific antiserum	Dilution‡	RNAase	DNAase	NMS	10^{-1}	10^{-2}	10^{-3}
Type 1 polio (4×10^{-6})§	41‖	35	70	0	40¶	0	56	0		17	0
	44	27	60	0	72	0	92	0		11	0
	50	46	47	0	88	0	41	0		10	0
	57	30	60	0	94	0	131	0			
T =	1.2×10^8	9×10^7	1.5×10^8		7.4×10^3		8×10^3			1.3×10^4	
ECHO 1 ($1 \times 10^{-4.5}$)	14	28	25	0	28**	0	14	0	18	0	0
	23	32	21	0	30	0	59	0	18	4	0
	10	23	23	0	66	0	62	0		4	
	22	30	17	0	16	0	56	0			
T =	5×10^6	1.4×10^7	7×10^6		7×10^2		1×10^3		1.8×10^3	3×10^3	
ECHO 8 (2×10^{-4})	6	2	23	0	5**	0	17	0	2	0	0
	11	6	15	0	11	0	16	0	1	0	0
	2	6	15	0	9	0	13	0		0	0
	1	1	4	0	24	0	18	0			
T =	2.5×10^5				2.4×10^2		3.2×10^2		2×10^2		
Coxsackie A-7 (2×10^{-4})	16	11	14		39**	0	3+	0	5		
	10	14	18		16+	0	22	0	1		
	18	14	20		22+	0	6+	0			
		11	17		12+	0	6+	0			
T =	7×10^5	6×10^5	7×10^5		4.4×10^2		1.8×10^2		3×10^2		
Coxsackie B-4 (2×10^{-4})	9	6	10	0	17**	0	24	0	7	0	0
	15	15	9	0	18	0	13	0	12	0	0
	14	8	17	0	11	0	19	0		0	0
	15	9	11	0	1	0	12	0			
T =	6×10^5	5×10^5	6×10^5		2.4×10^2		2.8×10^2		1×10^3		
Coxsackie B-5 (4×10^{-6})	72	63	95	0	50+¶	0	126	0		12	8
	97	64	91	0	88	0	102	0		18	2
	47	63	94	0	88	0	129	0		26	0
	77	62	99	0	99+	0	117	0			
T =	1.8×10^8	1.6×10^8	2.4×10^8		9×10^3		1.2×10^4			1.9×10^4	3×10^4

* Modified balanced salt solution. See *Materials and methods.*
† Normal monkey serum.
‡ 0.9 M KCl + Tris solution, pH 8.
§ Titer of intact virus inoculum.
‖ No. of plaques on monolayers.
¶ RNA diluted 1:9 initially.
** " " 1:1 " .

traction duplicated the second. This final aqueous phase was washed by shaking 5 × with more than equal quantities of cold ether to remove residual phenol, the ether being decanted and discarded. Residual ether was removed by bubbling nitrogen through final solution for 5 minutes. This final solution was diluted promptly 1:1 or 1:9 in 0.9 M KCl plus Tris at pH 8.0, since infectious RNA seems to be stable in such solutions(1). *Normal serum* was undiluted normal monkey serum free of specific antibody to the virus being tested. Specific antisera were monkey serum for poliovirus and rabbit serum obtained from Microbiological Associates for the Coxsackie B viruses and Echo viruses. Coxsackie A7 antiserum was not available. *Enzymes.* Crystallized ribonuclease of bovine origin was obtained from Worthington Biochemical Corp. and made to contain a final test dilution of 2 μg/ml in 0.9 M KCl Tris solution for RNA testing, and Tris-buffered isotonic balanced salt solution for whole virus. This variable was introduced because RNA titers are greatly reduced in isotonic solution and whole virus titers are somewhat reduced in hypertonic solutions. Desoxyribonuclease was obtained from Worthington Biochemical Corp. and made to contain final test solution of 5 μg/ml in 0.9 M KCl Tris solution with 0.003 M $MgCl_2$ added. *Tests.* In each instance, original whole virus preparation was titered on same day in same lot of plates with the same serum and enzyme dilutions as the RNA preparation. The single exception is the variable in the RNAase dilutions which were made in hypertonic solution for RNA testing and in isotonic solutions for whole virus testing.

Results. RNA-containing fractions of 6 enteroviruses were prepared from crude tissue culture virus suspensions by the phenol extraction method of Gierer and Schramm. The intact virus was compared simultaneously with its free RNA for their respective titers of plaque-forming agents in human cell monolayers. The intact virus titer was compared after exposure to salt solution, RNAase, normal serum and type-specific antiserum before seeding on monolayers, and in the same experiment the plaque titer of its RNA preparation was measured in the same lot of monolayers in the presence of hypertonic salt solution, RNAase, DNAase, and normal serum.

The Table is designed to point up the differences between whole virus and its RNA, and to show some of the reasons why the plaque-forming agent in RNA preparations is considered to be infectious ribonucleic acid and not small quantities of residual whole virus. Intact virus infectivity is not significantly affected by RNAase and normal serum, but is totally inactivated at the dilutions used by specific antiserum. RNA, on the other hand, is totally inactivated by RNAase and normal serum and not affected by DNAase. The RNAase content of our specific antisera made them unsatisfactory for testing their neutralization of the plaque-forming agent in the RNA preparation. All serum tested in our laboratory contained at least some RNAase, and RNA is routinely inactivated by normal or immune serum. If gamma globulin is prepared from immune serum this RNAase-free antibody will inactivate whole virus and not RNA(1). Gamma globulin was not used here, however.

In every instance reported here, plaques produced by RNA contained infectious particles which were unaffected by normal serum but were neutralized by antiserum specific for the intact virus from which the RNA was prepared. No antiserum for Coxsackie A7 was available, but the virus grew in the presence of normal serum. In brief, the infectious RNA entered host cells and directed the cells to produce type-specific whole virus. This provides additional evidence that the nucleic acid fraction carries some, if not all, of the genetic potentialities of the whole virus.

The data presented show further that the plaque titer of RNA was usually between 3 and 4 logs lower than that of whole virus, a differential which is not yet understood. It is of interest, however, that the differential is consistent throughout the series.

A simple, and probably generally applicable tool is now available for studies of virus RNA. Infectious RNA can be prepared with ordinary laboratory equipment from small quantities of crude tissue culture virus in less than one hour. The only requirements are that

there be at least 10,000 infectious particles present in the original virus suspension and that the virus be an RNA-containing one.

Several attempts have been made to produce infectious nucleic acid by the same method from vaccinia, a presumed DNA-containing virus. So far all such attempts have been unsuccessful despite the fact that some were carried out in parallel with successful enterovirus experiments, using similar quantities of virus and the same materials throughout.

Summary. 1) RNA-containing fractions have been prepared from crude tissue culture virus suspensions of 6 varieties of enterovirus by the method of Gierer and Schramm. Each RNA preparation has produced plaques on human cell monolayers. The plaque-forming agent is completely inactivated by RNAase but not by DNAase. RNA is, therefore, an essential component for the infectivity of the preparation. 2) The plaques formed by RNA fraction contain intact virus from which RNA is prepared. The RNA directs the cell to replicate not only the specific RNA, but also a new highly specific protein needed for synthesis of intact original virus.

1. Alexander, H. E., Koch, G., Mountain, I. M., Van Damme, O., *J. Exp. Med.*, 1958, v108, 493.

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

3. Fernandes, M. V., *Texas Rep. Biol. and Med.*, 1958, v1b, 48.

4. Puck, T. T., Marcus, P. I., Cieciura, S. J., *J. Exp. Med.*, 1956, v103, 273.

Received June 30, 1959. P.S.E.B.M., 1959, v101.

Assay and Properties of Serum Inhibitor of C'1-Esterase* (25034)

LAWRENCE R. LEVY† AND IRWIN H. LEPOW‡
Institute of Pathology, Western Reserve University, Cleveland

Evidence has been presented that the first component of human and guinea pig complement (C'1) exists in serum as a proenzyme which may be activated by antigen-antibody complexes(1-5). Human C'1 was also activated by plasmin(1,6) and, in a partially purified state, by autocatalysis(6,7). Two activities were demonstrable for human "activated C'1" (C'1-esterase): hydrolysis of certain synthetic amino acid esters, of which N-acetyl-L-tyrosine ethyl ester (ALTEe) was most susceptible(6-8); and inactivation of the second and fourth components (C'2 and C'4) of human complement(3,7). Both activation of C'1 and enzymatic activity of C'1-esterase were inhibited by a property of fresh human serum which was non-dialyzable, heat-labile (56°-30 min.), and unrelated to any of the 4 recognized components of complement(6,8). However, the precise relationship of the serum inhibitor of activation and of esterolysis could not be defined in the absence of purification studies. The present investigation was undertaken to provide information for subsequent purification of the serum inhibitor of C'1-esterase. The assay and properties of this inhibitor in normal serum are described and a comparison is given of levels of inhibitor in various mammalian sera.

Materials and methods. C'1-esterase—Partially purified human C'1 was prepared by published procedures(7) and activated autocatalytically by adjustment of physico-chemical conditions (pH 7.4, ionic strength 0.15, 37°, 15 min)(6,7). Rabbit C'1 and C'1-esterase were prepared from normal rabbit serum by identical procedures. *ALTEe*—N-acetyl-L-tyrosine ethyl ester, synthesized in the Dept. of Chemistry of Western Reserve Univ., was used as a substrate for C'1-esterase. The ester was dissolved in 2-methoxyethanol (methyl cellosolve) to a stock concen-

* Supported by research grant, National Inst. of Allergy and Infect. Dis., U.S.P.H.S.

† Work carried out in partial fulfillment of requirements for M.D. degree, and performed in part during tenure of Lederle Medical Student Research Fellowship.

‡ Research Fellow, Cleveland Area Heart Soc.