lipids were analyzed in patients with hereditary spherocytosis, pernicious anemia, sprue, intermediate thalassemia, sickle cell anemia, and polycythemia vera using chromatography on silicic acid columns. The total lipid phosphorus concentration was increased in the patients with intermediate thalassemia and sickle cell anemia and may have been slightly decreased in the patients with pernicious anemia and sprue.

No consistent difference from normal in distribution of the individual phospholipid fractions was found. Variations in the absolute concentrations of the individual phospholipid fractions reflected differences in the total red blood cell lipid phosphorus concentrations.

ADDENDUM. The red blood cells of a 56-year-old lady with paroxysmal nocturnal hemoglobinuria were analyzed and found to contain 4.14 mmoles/l lipid phosphorus with a phospholipid distribution as follows: "cephalin"—44.4%; lecithin—34.1%; sphingomyelin—20.0%; and lysolecithin 1.5%.

1. Erickson, B. N., Williams, H. H., Bernstein, S. S., Avrin, I., Jones, R. L., Macy, I. G., *J. Biol. Chem.*, 1938, v122, 515.

2. Williams, H. H., Erickson, B. N., Bernstein, S., Hummel, F. C., Macy, I. G., *ibid.*, 1937, v118, 599.

3. Williams, H. H., Erickson, B. N., Bernstein, S. S., Macy, I. G., PROC. SOC. EXP. BIOL. AND MED., 1940, v45, 151.

4. Allison, A. C., Kates, M., James, A. T., *Brit. Med. J.*, 1960, 1766.

5. Kates, M., Allison, A. C., James, A. T., *Biochim. Biophys. Acta*, 1961, v48, 571.

6. Phillips, G. B., Roome, N. S., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 489.

7. Phillips, G. B., *Biochim. Biophys. Acta*, 1958, v29, 594.

8. Rhodes, D. N., Lea, C. H., *Biochem. J.*, 1957, v65, 526

9. Reed, C. F., Swisher, S. N., Marinetti, G. V., Eden, E. J., *J. Lab. Clin. Med.*, 1960, v56, 281.

10. Pauling, L., Itano, H. A., Singer, S. J., Wells, I. C., *Science*, 1949, v110, 543.

11. Marinetti, G. V., Erbland, J., Kochen, J., *Fed. Proc.*, 1957, v16, 837.

12. Farquhar, J. W., Oette, K., *ibid.*, 1961, v20, 279.

13. Winsor, T., Burch, G. E., *J. Am. Med. Assn.*, 1945, v129, 739.

14. Prankerd, T. A. J., *J. Physiol.*, 1958, v143, 325.

Received October 31, 1961. P.S.E.B.M., 1962, v109.

Complement-fixing Antibody Responses to ECHO Virus Types 12 and 19 of Patients with Enterovirus Infections.* (27204)

NATHALIE J. SCHMIDT, JUANITA DENNIS AND EDWIN H. LENNETTE
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

Instances of heterotypic complement-fixing (CF) antibody responses in individuals with various enterovirus infections have been cited by several investigators(1-6), some of whom utilized heat-inactivated antigens(1, 2,5), while others employed uninactivated antigens(3,4,6). In our recent study(7) of the antibody responses of individuals with ECHO virus infections, it was noted that a high proportion of the patients studied developed heterotypic CF antibodies to ECHO virus types 12 and 19 (uninactivated antigens); heterotypic rises in CF antibody to the 6 other ECHO virus types used as antigens were infrequently seen. An extension of that work to determine the frequency with which CF antibody to ECHO virus types 12 and/or 19 appears in individuals with enterovirus infections caused by a variety of immunotypes is reported here.

Materials and methods. Human sera examined. Sera examined for presence of CF antibodies to ECHO virus types 12 and 19 were from patients with enterovirus infections confirmed by isolation of the etiologic agent and, in most instances, by demonstration of a diagnostically significant increase

* This work was supported by a grant from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S., Dept. of H.E.W.

in neutralizing antibody (or CF antibody in the case of poliovirus infections) to the infecting virus type. All sera were inactivated at 60°C for 30 min. prior to examination for the presence of CF antibodies and at 56°C for 30 min. prior to examination in neutralization tests.

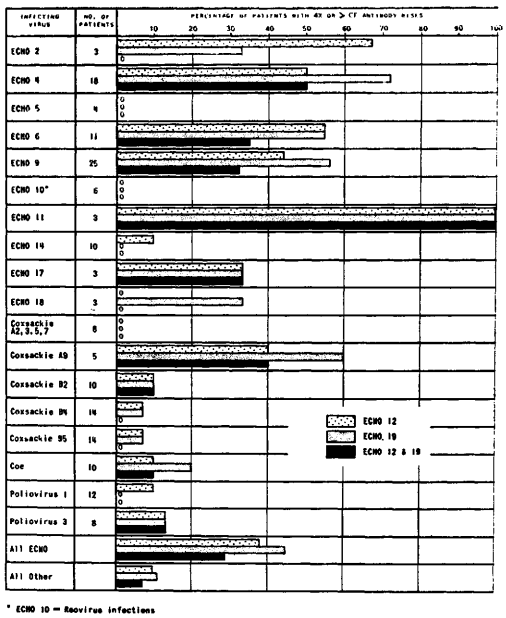
Virus isolation attempts. ECHO viruses, polioviruses, group B and type A9 Coxsackie viruses were isolated from stool suspensions in monkey kidney cell cultures(8), while isolations of Coe virus were made from throat washings inoculated into HeLa cell cultures (9). Group A Coxsackie viruses other than type 9 were recovered from stools inoculated into suckling mice less than one day of age (10).

Complement-fixing antigens. CF antigens for ECHO virus types 12 and 19 were produced in HeLa cell cultures by the method recently described(7). Poliovirus CF antigens were also prepared in HeLa cell cultures as described elsewhere(11). All CF antigens were employed without inactivation by heat or other methods. Two units of antigen, as determined by block titrations(11) were employed in all tests on human sera.

Complement fixation test procedure. The method routinely used in this laboratory for CF tests has been described(12). In instances in which the volume of serum available did not permit use of the standard volumes of reagents, one-half volumes were utilized.

Neutralizing antibody assays. Neutralization tests for antibodies to ECHO, Coe, and Coxsackie A9 viruses were conducted by a colorimetric procedure using HeLa cells(13), and neutralization tests for antibodies to the group B Coxsackie viruses were performed by a colorimetric method using the FL line of human amnion cells(14).

Results. The occurrence of 4-fold or greater increases in CF antibody level to ECHO virus types 12 and 19 in 167 patients with enterovirus infections is summarized in Table I and Fig. 1. Among the patients with ECHO virus infections, a high proportion of rises to ECHO types 12 and/or 19 was observed with sera from patients whose stools had yielded ECHO virus types 2, 4, 6, 9, 11, 17 and 18 (Table I). The small numbers of patients infected with ECHO types 2, 17 and 18 might lead to speculation as to the significance of the percentage of CF antibody rises in patients infected with these viral types. However, in the larger groups of patients infected with ECHO types 4, 6 and 9 there is obviously a high proportion of individuals showing 4-fold or greater rises in CF antibody to ECHO types 12 and/or 19. The antibody increases occurring in the ECHO 11 patients would appear significant, as all 3 patients showed rises to both viral types in the absence of corresponding neutralizing antibody, and the fact that 2 of the 3 patients were only one year of age would make it less likely that the CF rises represented anamnestic responses to viral types other than ECHO 11 with which the patients might have experienced previous infections.



* ECHO 10 = Reovirus infections

FIG. 1. Proportion of patients with enterovirus infections showing 4× or > rises in CF antibody to ECHO type 12 and 19 antigens.

It is of interest that none of the patients infected with ECHO type 5 or reoviruses showed rises in CF antibody to ECHO types 12 or 19, and only one of the 10 patients with ECHO 14 infections manifested a 4-fold rise to ECHO type 12.

None of the 8 patients infected with Coxsackie group A viral types 2, 3, 5 or 7 showed

TABLE I. Frequency of 4-Fold or Greater Rises in CF Antibody Titer to ECHO Virus Types 12 and 19 in Patients with Enterovirus Infections.

Patient group	Infecting virus type	No. patients	No. patients with 4× or > rise to E12	E19	Both	% of patients with 4× or > rise to E12	E19	Both
ECHO virus infections	ECHO 2	3	2	1	0	(67)*	(33)	(0)
	" 4	18	9	13	9	50	72	50
	" 5	4	0	0	0	(0)	(0)	(0)
	" 6	11	6	6	4	55	55	35
	" 9	25	11	14	8	44	56	32
	" 10†	6	0	0	0	(0)	(0)	(0)
	" 11	3	3	3	3	(100)	(100)	(100)
	" 14	10	1	0	0	10	0	0
	" 17	3	1	1	1	(33)	(33)	(33)
	" 18	3	0	1	0	(0)	(33)	(0)
Sub-totals		86	33	39	25	38	45	29
Other enterovirus infections	Coxs. A2,3,5,7	8	0	0	0	(0)	(0)	(0)
	" A9	5	2	3	2	(40)	(60)	(40)
	" B2	10	1	1	1	10	10	10
	" B4	14	1	1	0	7	7	0
	" B5	14	1	1	0	7	7	0
	Coe	10	1	2	1	10	20	10
	Polio type 1	12	1	0	0	10	0	0
	" " 3	8	1	1	1	(13)	(13)	(13)
Sub-totals		81	8	9	5	10	11	6

* Percentages in parentheses based on less than 10 cases.
† ECHO 10 = Reovirus infections.

rises in CF antibody titer to the 2 ECHO antigens, but a relatively high proportion of the few Coxsackie A9 patients displayed 4-fold or greater rises to these antigens. However, as indicated below, the results of neutralization tests on the sera of these latter patients cast doubt upon the significance of the rises in antibody titer encountered in this group to the 2 ECHO virus antigens.

Of the patients infected with group B Coxsackie, Coe or polioviruses, 4-fold rises in CF antibody to the ECHO virus antigens were seen in no more than 2 patients in each group.

Previous investigations on heterotypic CF antibody responses in human enterovirus infections have not ruled out the possibility that the rises in CF antibody level encountered might have actually represented dual infections rather than heterotypic responses. Accordingly, sera from all individuals who showed 4-fold or greater rises in CF antibody to ECHO types 12 or 19 were examined for the presence of neutralizing antibodies to these 2 viral types. As a part of another investigation, the sera were also tested for the presence of neutralizing antibodies to ECHO 6 virus. Results of the neutralization tests are summarized in Table II. Few of the rises in CF antibody to ECHO types 12 and 19 were associated with rising, or even stationary, titers of neutralizing antibody to these viral types, and thus appear to represent true heterologous CF antibody responses.

On the other hand, neutralizing antibody assays indicated that the rise in CF antibody level to ECHO 12 and 19 viruses in the patients with Coxsackie A9 virus infection was not associated with this infection; one of the 3 patients showed a 4-fold increase in neutralizing antibody titer to ECHO 12, while another showed a 4-fold increase in neutralizing antibody to ECHO 6 which, on the basis of information obtained in this study, would appear to elicit heterotypic CF antibody responses to ECHO types 12 and 19. Thus, it would not appear that the Coxsackie A9 infections *per se* elicited the rises in CF antibody to types 12 and 19.

Other rises in CF antibody titer to ECHO types 12 and/or 19 which could be related to the presence of neutralizing antibody for one of these viral types were seen in one ECHO 2 patient, 2 ECHO 4 patients, 2 ECHO 6 patients (who showed a 4-fold rise

TABLE II. Neutralizing Antibody Responses of Patients Showing Four-fold or Greater Rises in CF Antibody Titer to ECHO Virus Types 12 and 19.

Infecting virus type	No. of patients tested	No. of patients with: ≥4× * CF rise to 12 only	Neut. antibody ≥4× 12	Neut. antibody ≥4× 19	Neut. antibody Stat.† 12	Neut. antibody Stat. 19	≥4× CF rise to 19 only	Neut. antibody ≥4× 12	Neut. antibody ≥4× 19	Neut. antibody Stat. 12	Neut. antibody Stat. 19	≥4× CF rise to both 12 & 19	Neut. antibody ≥4× 12	Neut. antibody ≥4× 19	Neut. antibody Stat. 12	Neut. antibody Stat. 19
ECHO 2	3	2	1			1	1									
" 4	18	0					4					9	1		1	
" 6	11	2					2	2				4				
" 9	25	3					6					8				
" 11	3											3				
" 14	10	1			1											
" 17	3											1				1
" 18	3						1									
Coxs. A9	5						1					2‡	1			
" B2	10											1				1
" B4	14	1					1									
" B5	14	1‡					1									
Coe	10						1					1				
Polio 1	12	1														
" 3	8											1				
Totals	59	11	1		1	1	18	2				30	2		1	2

* Four-fold or greater rise in antibody titer.

† Stationary titer of antibody.

‡ One patient displayed a 4-fold increase in neutralizing antibody titer to ECHO 6 virus.

in CF antibody to ECHO 19 virus, but a 4-fold rise in neutralizing antibody to ECHO 12) and the only ECHO 14 and Coxsackie B2 patients who showed rises in CF antibody titer to the 2 ECHO antigens. The Coxsackie B5 patient who showed a rise in CF antibody to ECHO 12 virus showed a 4-fold increase in neutralizing antibody titer to ECHO 6 virus, and the rise in CF antibody may well have represented a heterotypic antibody response to ECHO 6 rather than Coxsackie B5 virus.

Discussion. When it was noted that an appreciable proportion of patients infected with ECHO viruses was giving heterotypic responses in CF antibody to ECHO 12 and 19 viruses, the identity of the CF antigens with respect to immunotype was checked by examining them in monolayer neutralization tests; each was completely neutralized by its homotypic immune serum, thus ruling out the possibility that contamination of the antigens with other viral types could account for their heterotypic reactivity.

The only good evidence of possible immunologic relationships between other enteroviruses and ECHO types 12 and 19 was seen with sera from patients infected with certain ECHO virus types. The relatively high proportion of rises to ECHO types 12 and 19 in the Coxsackie A9 patients would seem to be more apparent than real; results of neutralization tests on the sera of these few patients illustrate the fallacy of assuming the existence of immunologic relationships between viral types based upon examination of too few patients and failing to substantiate the results of CF tests with neutralization test data. Although a few of the patients with Coxsackie, Coe and poliovirus infections showed rises in CF antibody to ECHO 12 and 19 viruses, the proportions in each group were too small to permit valid conclusions concerning any immunologic relationship between these viral types. The single patient with a B2 infection who showed a rise in CF antibody to the 2 ECHO types was found to possess neutralizing antibodies for ECHO 19 virus, and the patient with a B5 infection who showed a rise in CF antibody level to ECHO 12 virus was found to have a 4-fold neutralizing antibody titer rise to ECHO 6 virus. It is conceivable that the few other rises in CF antibody to ECHO 12 and/or 19 viruses in patients with Coxsackie, Coe or poliovirus infections might have been heterologous responses to ECHO virus types ostensibly related to types 12 and 19, *e.g.*, types 4, 6, 9, 11, or to others for which a relationship has not yet been indicated.

In recapitulation, therefore, while evidence is accumulating that the antigenic moieties of different enterovirus types responsible for formation of CF antibodies in man may be immunologically related, testing generally has not been extensive enough to provide a clear definition of the antigenic relationships which exist. However, it would appear on the basis of examining sera from larger numbers of patients that the heterotypic rises in CF antibody to ECHO virus types 12 and 19 in patients infected with ECHO virus types 4, 6, 9 and possibly 11, may be evidence of an antigenic relationship between these viral types and ECHO types 12 and 19.

Summary. Sera from 167 patients with various enterovirus infections were tested against uninactivated CF antigens to ECHO virus types 12 and 19. Four-fold or greater rises in CF antibody titer, unassociated with the presence of neutralizing antibody to these 2 ECHO virus types, were seen in an appreciable proportion of patients infected with ECHO 4, 6, 9 and 11 viruses, possibly indicating antigenic relationships of the viral moieties responsible for production of CF antibodies in man. No evidence was seen of a relationship between ECHO types 12 and 19 and ECHO 5, ECHO 10 (reoviruses) or ECHO 14, nor was there any good evidence of a relationship between these 2 ECHO virus types and the Coxsackie group A, Coxsackie group B or poliovirus types studied.

The excellent technical assistance of Miss Inta Kumins is gratefully acknowledged.

1. von Zeipel, G., Svedmyr, A., *Arch. Virusforsch.*, 1957, v7, 355.

2. Hammon, W. McD., Yohn, D. S., Ludwig, E. H., Pavia, R. A., Sather, G. E., *J.A.M.A.*, 1958, v167, 727.

3. Johnsson, T., Lycke, E., Wictorin B., Jönsson, B., *Arch. Virusforsch.*, 1958, v8, 285.

4. Johnsson, T., Böttiger, M., Löfdahl, A., *ibid.*, 1958, v8, 306.

5. Neva, F. A., Malone, M. F., *Jour. Immunol.*, 1959, v83, 645.

6. Halonen, P., Rosen, L., Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 236.

7. Schmidt, N. J., Dennis, J., Hagens, S. J., Lennette, E. H., *Am. J. Hyg.*, in press.

8. Schmidt, N. J., Lennette, E. H., *ibid.*, 1957, v66, 119.

9. Lennette, E. H., Fox, V. L., Schmidt, N. J., Culver, J. O., *ibid.*, 1958, v68, 272.

10. Lennette, E. H., Wiener, A., Hoshiwara, I., Woodie, J., Magoffin, R. L., *J. Lab. and Clin. Med.*, 1961, v58, 634.

11. Schmidt, N. J., Lennette, E. H., *Am. J. Hyg.*, 1957, v66, 1.

12. ———, *J. Exp. Med.*, 1955, v102, 133.

13. Schmidt, N. J., Shinomoto, Tak T., Dennis, J., Hagens, S. J., Fox, V. L., Lennette, E. H., *J. Lab. and Clin. Med.*, in press.

14. Schmidt, N. J., Midulla, M., Lennette, E. H., *J. Immunol.*, 1958, v80, 454.

Received November 1, 1961. P.S.E.B.M., 1962, v109.

Correlation of Mechanical Properties of Infant Lungs with Surface Activity of Extracts.* (27205)

PETER GRUENWALD,† RUDOLPH P. JOHNSON, ROBERT F. HUSTEAD‡ AND JOHN A. CLEMENTS§ (Introduced by J. H. Wills)

Department of Pathology and Division of Anesthesiology, Johns Hopkins Hospital, Baltimore, and Clinical Investigation Branch, Clinical Research Division, Directorate of Medical Research, Army Chemical Center, Md.

Much so-called elasticity of lungs is due to forces acting at the surfaces of small terminal air spaces, and is modified by presence at the gas-liquid interface of a film of surface-active material. Such material extracted from normal lungs has the property of changing surface tension to a great extent when surface area is changed(1). This property has been assumed to be related to the hysteresis characteristically present in pressure-volume curves of lungs when inflated with air (but absent when filled with saline) and to the stability of aeration of the lungs in expiration. The material presumably responsible for altering surface tension has been called "anti-atelectasis factor"(1).

In lungs obtained from newborn infants (both stillborn and those that lived a short period), there are high incidences of poor stability of aeration when artificially expanded and of decreased surface-activity in extracts examined with a surface balance of variable area. Similar information about a smaller volume of more diverse material was reported recently(2).

Numerical indices have been calculated for lung stability (L) from the deflation part of the pressure-volume (P-V) curve and for extract activity (*S*)¶ from the compression part of the tension-area curve. The lung stability index is:

$$L = \frac{V_5 - V_0}{V_M - V_0} + \tfrac{1}{2}\frac{V_{10} - V_0}{V_M - V_0}.$$

V_M denotes maximum lung volume, V_5 and V_{10} the volumes at 5 and 10 cm H_2O and V_0 the volume of trapped gas at 0 cm H_2O. L varies between 0 and 1.5. Specimens tending to collapse on return to low pressure have low values of L. The extract activity index is defined as change of surface tension divided by average surface tension(2):

$$S = 2\ (\gamma_{max} - \gamma_{min}) \ /\ (\gamma_{max} + \gamma_{min})$$

γ_{max} and γ_{min} denote maximal and minimal surface tensions of the extract. *S* lies between

* This investigation was supported in part by research grants from United Cerebral Palsy Research and Educational Foundation and Nat. Inst. of Neurol. Dis. and Blindness, P.H.S.

† Present address: Dept. of Pathology, Sinai Hospital, Baltimore, Md.

‡ Present address: Dept. of Anesthesiology, Univ. of Kansas Medical Center, Kansas City, Kan.

§ Present address: Cardiovascular Research Institute, University of California Medical Center, San Francisco.

¶ *S*=S with diacritical mark in Fig. 1 and 2.