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Association of Group B Coxsackieviruses with Cases of Pericarditis, Myocarditis, or Pleurodynia by Demonstration of Immunoglobulin M Antibody

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Tests for immunoglobulin M (IgM) antibody to group B coxsackieviruses were performed on sera from 259 patients with a clinical diagnosis of pericarditis, myocarditis, or pleurodynia on whom there were no definitive serological or virus isolation findings to establish a viral etiology, and on 259 "control" patients with clinical diagnoses of viral or mycoplasmal pneumonia or pneumonitis. IgM antibodies to coxsackievirus types B1, B3, B4, B5, and B6 were detected by a micro-immunodiffusion technique, and antibodies to virus type B2 were detected by reduction of neutralizing antibodies with ethanethiol. Of the patients with pericarditis, myocarditis, or pleurodynia, 27% (70) had IgM antibody to group B coxsackieviruses, as compared with 8% in the control group. On retrospective review of the clinical diagnosis, some of the patients in the control group with IgM antibody were found to have had additional clinical findings which could be attributed to a coxsackievirus infection. Coxsackievirus IgM antibody was demonstrable in 30% of 113 patients in the study group for whom virus isolation had been attempted with negative results. The presence of coxsackievirus IgM is discussed in relation to the time of serum collection, age of the patients, and month of onset of illness.

The ability of group B coxsackieviruses to produce pericarditis or myocarditis in man, particularly in infants, is well recognized, (1, 3, 8, 9, 17, 18, 28). However, a clear-cut laboratory diagnosis of group B coxsackievirus infection, based upon virus isolation or demonstration of a significant rise in antibody titer during the course of illness, can be established only infrequently in sporadic cases of pericarditis or myocarditis (2, 10, 11, 27). In cases of pleurodynia, recovery of coxsackievirus or significant rises in antibody titer, or both, are more frequently demonstrable, but there is still a fairly high proportion of sporadic cases in which there are no positive laboratory findings. For example, in 1970, the period covered by this report, specimens were submitted to this laboratory on more than 250 patients with a clinical diagnosis of pericarditis, myocarditis, or pleurodynia; a group B coxsackievirus was isolated from two patients with chest pain compatible with a clinical diagnosis of pleurodynia, and one patient with a clinical diagnosis of pericarditis showed fourfold or greater rises in neutralizing antibody titer to three types of group B coxsackievirus.

It has been speculated that pericarditis, myocarditis, or pleurodynia may occur relatively late in the course of coxsackievirus infections, at a time when antibody levels are already elevated and virus is no longer being shed or is combined with antibody.

Previous studies from this laboratory have shown that immunoglobulin (Ig) M antibody to group B coxsackievirus types 1, 3, 4, 5, and 6 and group A, type 9 can be demonstrated by immunodiffusion tests (24-26). IgM antibody reacts type-specifically with the intact virion of coxsackieviruses and forms an immunoprecipitate distinct from that produced by IgG antibody and empty capsids. It has also been shown that the initial antibody response to group B coxsackievirus infections consists of the production of IgM antibody, which is gradually replaced in 4 to 6 weeks by IgG (26). The possibility was considered that detection of IgM

antibody might be used to demonstrate current or recent group B coxsackievirus infections in patients with a clinical diagnosis of pericarditis, myocarditis, or pleurodynia who had no other laboratory findings to implicate coxsackieviruses or other viruses as the etiological agent. This report describes the occurrence of IgM antibodies to group B coxsackieviruses in sera submitted to this laboratory from patients with a clinical diagnosis of pericarditis, myocarditis, or pleurodynia, and in a control group of patients with clinical diagnoses of viral or mycoplasmal pneumonia.

MATERIALS AND METHODS

Sera examined. Tests were performed on sera routinely submitted to this laboratory for diagnosis of viral infection. Paired or single sera were tested from 259 patients with clinical diagnoses of pericarditis (148 cases), myocarditis (92 cases) or pleurodynia (19 cases) and on 259 patients with a clinical diagnosis of viral or mycoplasmal pneumonia. The sera were selected on the basis of a computer printout which listed all patients with these diagnoses (based on clinical findings given on the laboratory request form) and with an onset of illness during 1970. The study group included all myocarditis, pericarditis, or pleurodynia patients from whom sera were available in 1970 and the control group was selected to include an equal number of paired and single serum specimens from pneumonia patients during the same period, matching by sex. In each group 121 patients had paired serum specimens and 138 had only a single specimen. There were 145 males and 114 females in each group. Equal numbers of specimens from the study group and the control group were tested for IgM antibody in each run, and the individuals performing the tests were unaware of the group to which the patient belonged.

Immunodiffusion tests. Antigens for group B coxsackievirus types 1, 3, 4, 5, and 6 were prepared from infected HeLa cell culture fluids concentrated 300-fold by high-speed centrifugation. Satisfactory antigens for demonstration of IgM antibody required starting material with an infectivity titer of at least $10^{6.5}$ mean tissue culture dose per ml. Tests were conducted by a micro-method previously described (24). The gel consisted of 0.8% agarose in 0.01 M tris(hydroxymethyl)aminomethane buffer, 0.1 M NaCl, 0.001 M ethylenediaminetetraacetic acid and 0.05% sodium azide; it had a pH of 7.6. Sera were examined undiluted and unheated for the presence of precipitating antibodies.

Demonstration of IgM antibody to coxsackievirus type B2. Because it has not been possible to differentiate between IgM and IgG antibodies to coxsackievirus type B2 in immunodiffusion reactions (24, 25), treatment of sera with the sulfhydryl reducing agent ethanethiol (15) was used to demonstrate IgM antibodies to this virus type. A 1:4 dilution of test serum was mixed with an equal volume of 0.06 M ethanethiol, incubated at room temperature for 3 h and then heated at 56 C for 30 min to inactivate the serum and vaporize the sulfhydryl reagent. The treated serum was tested in a micro-metabolic-inhibition system (21) in parallel with untreated serum (inactivated at 56 C for 30 min), and a fourfold or greater reduction in neutralizing antibody titer of the ethanethiol-treated portion was considered to suggest the presence of IgM antibody. Reproducibility of a significant titer decrease in another run was regarded as a valid demonstration of IgM antibody to coxsackievirus type B2.

Virus isolation attempts. Attempts to isolate coxsackieviruses from clinical specimens were made by inoculation of test material into primary or secondary rhesus monkey kidney cell cultures and human fetal diploid cell cultures by procedures described elsewhere (21).

RESULTS

Occurrence of IgM antibody to group B coxsackieviruses. Of the patients in the pericarditis, myocarditis, and pleurodynia group, 27% had IgM antibody to group B coxsackieviruses as compared with 8% in the control group (Table 1). There was little difference between males and females in the percentage showing IgM antibody in either group. Table 2 shows the occurrence of IgM antibody by clinical syndrome. A higher proportion of patients in the pleurodynia group had IgM antibody than in the pericarditis or myocarditis groups, but there was a total of only 19 pleurodynia patients.

After testing was completed, it was found that when specimen submission slips on the control patients with coxsackievirus IgM antibody were reexamined in detail, six of the patients in the pneumonia group had clinical evidence of cardiac or central nervous system involvement suggestive of a coxsackievirus infection. The additional findings in these patients included clinical signs of heart failure, pericarditis, and encephalitis.

Type specificity of group B coxsackievirus IgM antibody. In 1970 there were no recognized outbreaks of group B coxsackievirus infections in California, and virus isolations were made only from sporadic cases, usually with clinical signs of aseptic meningitis, scattered throughout the state. The virus types most frequently recovered were B1, B2, and B4. The type specificity of the IgM antibody to group B coxsackieviruses demonstrated in the study and control groups is shown in Table 3. As previously noted (25, 26), some individuals were found to have IgM antibody to more than one group B coxsackievirus type. In both groups the greatest number of patients showed IgM antibody for coxsackievirus type B4, followed by types B1 and B2.

TABLE 1. *Occurrence of IgM antibody to group B coxsackieviruses in patients with pericarditis, myocarditis, or pleurodynia and in patients in the control group*

Group	No. tested	IgM antibody to group B coxsackievirus	
		No.	%
Pericarditis, myocarditis, pleurodynia	259	70	27
Males	145	41	28
Females	114	29	25
Control[a]	259	21	8
Males	145	11	8
Females	114	10	9

[a] Patients with a clinical diagnosis of viral or mycoplasmal pneumonia.

TABLE 2. *Occurrence of IgM antibody to group B coxsackieviruses in various clinical syndromes*

Clinical diagnosis	No. of patients	IgM antibody to group B coxsackieviruses	
		No.	%
Study group			
Pericarditis	148	40	27
Myocarditis	92	23	25
Pleurodynia	19	7	37
Totals	259	70	27
Control group (pneumonia)	259	21	8

Relationship between time of specimen collection and presence of IgM antibody to group B coxsackieviruses in patients with pericarditis, myocarditis, or pleurodynia. Table 4 shows the occurrence of IgM antibody to group B coxsackieviruses by time interval after onset for patients in both the study and control groups. In neither group was IgM demonstrated in specimens collected later than 42 days after onset. Table 5 compares the number of patients in the study group with paired serum specimens and with single specimens who showed IgM antibody to group B coxsackieviruses, and it relates the presence of IgM antibody to the time of specimen collection. A larger proportion of patients with paired serum specimens showed IgM antibody than did those with a single specimen. Five of the patients showed IgM antibody only in the convalescent-phase serum, and these were all patients from whom the acute-phase serum was collected within the first 3 days after onset of illness. Seven of the patients had IgM antibody only in the acute-phase specimen, and there was wide variation in the collection times of the convalescent-phase sera from these patients in which IgM antibody was no longer demonstrable. None of the three convalescent-phase sera collected later than 42 days after onset had demonstrable IgM antibody, and all of the single serum specimens which contained IgM antibody were collected less than 36 days after onset of illness.

Virus isolation attempts on pericarditis, myocarditis, or pleurodynia patients. Efforts to recover a coxsackievirus from patients in the study group are summarized in Table 6. It is seen that virus isolation attempts were performed in 113 of the cases, none of which yielded a virus. Approximately equal numbers of patients without isolation attempts and with negative results had IgM antibody to group B coxsackieviruses. For the patients with negative results, most of the specimens tested were fecal specimens, and approximately one-half of the isolation attempts were made on specimens collected within the first week after onset of illness. Thus, negative virus isolation results were obtained despite the fact that appropriate specimens collected at suitable times were examined. This is in marked contrast to the experience in this laboratory with cases of aseptic meningitis due to coxsackieviruses in which the virus is readily recovered from stools and spinal fluid in a high proportion of cases. However, 30% of the pericarditis, myocarditis, or pleurodynia patients with negative isolation results possessed IgM antibody for group B coxsackieviruses.

Place of residence and months of onset for patients in study and control groups. Approximately 70% of the patients in both the study group and the control group were from eight counties in the San Francisco Bay area. The remainder of the patients in each group were distributed throughout 16 other counties in California. Of the patients who had IgM antibody to group B coxsackieviruses, 42 of 70 in the study group and 10 of 21 in the control group were from the San Francisco Bay area.

Table 7 shows the month of onset of illness for patients in the study group and the control group and the occurrence of IgM antibody by month of onset. The number of pericarditis, myocarditis, or pleurodynia patients was high in January, decreased during the spring and summer months, and increased in the autumn and early winter months. For the most part, the occurrence of IgM in the study group followed this same pattern. The demonstration of IgM antibody in the control group was also more

TABLE 3. *Type specificity of group B coxsackievirus IgM antibody detected in patients with pericarditis, myocarditis, or pleurodynia and in control group*

Group	No. positive/ no. tested	Virus type	No. of patients with coxsackievirus IgM antibody to				
			1 type only	2 types	3 types	4 types	5 types
Pericarditis, myocarditis, pleurodynia	70/259		56	11	1	1	1
		B1	8	7	1		1
		B2	6	5			
		B3	3		1	1	1
		B4	36	8		1	1
		B5	1	1	1	1	1
		B6	2	1		1	1
Control (pneumonia)	21/259		18	3			
		B1		3			
		B2	2				
		B3		1			
		B4	14	2			
		B5	1				
		B6	1				

TABLE 4. *Occurrence of IgM antibody to group B coxsackieviruses by time interval after onset in patients with pericarditis, myocarditis, or pleurodynia and in control group of patients with pneumonia*

Days after onset	Pericarditis, myocarditis, pleurodynia patients			Pneumonia patients		
	No. of sera tested	IgM +	IgM −	No. of sera tested	IgM +	IgM −
1-3	86	26	60	65	4	61
4-7	80	17	63	80	7	73
8-14	49	10	39	84	7	77
15-21	72	21	51	84	9	75
22-28	36	12	24	36	3	33
29-35	27	12	15	15	0	15
36-42	5	1	4	7	0	7
43-49	6	0	6	6	0	6
50>	19	0	19	3	0	3
Total sera	380	99	281	380	30	350
Total patients	259	70	189	259	21	238

TABLE 5. *Time of specimen collection for patients with pericarditis, myocarditis, or pleurodynia showing IgM antibody to group B coxsackieviruses*

Serum specimens examined	No. of patients	No. of patients positive	Days after onset							
			1-3	4-7	8-14	15-21	22-28	29-35	36-42	≥43
Paired	121	41 (34%)								
First serum										
IgM +			17	8	6	3	2			
IgM −			5							
Second serum										
IgM +					3	14	9	7	1	
IgM −						2	1		1	3
Single	138	29 (21%)								
IgM +			9	9	1	4	1	5		
Totals	259	70								
IgM +			26	17	10	21	12	12	1	0
IgM −			5	0	0	2	1	0	1	3

TABLE 6. *Results of virus isolation attempts on pericarditis, myocarditis, or pleurodynia patients*

Isolation results	All patients (259)	Patients with IgM antibody to group B coxsackieviruses (70)
Not done	146	36
Negative	113	34
Specimen tested		
Fecal	108	32
Pericardial fluid	4	2
Autopsy tissue	1	0
Days after onset specimen collected		
1-3	18	7
4-6	35	11
7-10	40	10
>10	20	6

frequent in cases occurring during the months of August to November. Over the past years the recovery of coxsackieviruses from clinical materials in this laboratory has also shown a seasonal pattern, with the greatest number of virus isolations being made in late summer and the autumn months.

Relationship between age and occurrence of IgM antibody to group B coxsackieviruses. Table 8 shows the ages of patients in the study and control groups and the occurrence of IgM coxsackievirus antibody in each age group. There was a greater number of control patients than study patients in children through 10 years of age, but only one of 30 control patients showed IgM antibody as compared with three of 12 in the study group. In the age group from 11 through 20 years there were roughly comparable numbers of patients in each group, and similar numbers showed IgM antibody to group B coxsackieviruses. Of particular interest is the greater number of pericarditis, myocarditis, or pleurodynia patients in the 21- through 60-year age groups showing IgM antibody to group B coxsackieviruses as compared with control patients in the same age groups.

DISCUSSION

The detection of IgM antibody to group B coxsackieviruses provided evidence of a current or recent infection in just over one-fourth (27%) of a group of 259 patients with clinical diagnoses of pericarditis, myocarditis, or pleurodynia for whom there were no other laboratory findings to confirm a viral infection. This would appear to offer a promising approach for further investigations on the possible role of coxsackieviruses in other clinical syndromes suspected to be of viral etiology.

It is noteworthy that, although group B coxsackieviruses are the principal agents suspected in virtually all of the cases of pericarditis or myocarditis on whom clinical specimens are submitted to our laboratory for virological studies, nearly three-fourths of the cases in the present study failed to show evidence of a current or recent coxsackievirus infection. It should be emphasized, however, that clinical diagnoses in this study were taken from laboratory request forms and accompanying correspondence and were not further substantiated.

IgM antibody for coxsackievirus types B1, B3, B4, B5, and B6 can be demonstrated relatively simply by micro-immunodiffusion techniques which lend themselves to large-scale studies. However, antigens used for detection of IgM antibody must be prepared from infected tissue culture fluids with high infectivity titers, since it is the intact virion with which the IgM antibody reacts (22, 26). Demonstration of IgM antibody for coxsackievirus type B2 is more complex, since it is based upon dissociating the IgM antibody with a sulfhydryl reducing agent and then assaying for neutralizing antibodies. However, the use of a micro-colorimetric neutralization test (21) greatly facilitates this procedure.

The fact that IgM antibody to group B coxsackieviruses was found in a relatively high proportion of patients from whom it was impossible to recover virus would be in keeping with the view that pericarditis and myocarditis are likely to be late events in the course of coxsackievirus infections, at a time when virus excretion has ceased or virus is masked by antibody. The possibility also exists that coxsackievirus infections complicated by pericarditis, myocarditis, or pleurodynia may result in a prolonged production of IgM antibody. Prolonged rubella IgM antibody production has been noted in rubella infections complicated by thrombocytopenic purpura or carpal-tunnel compression (7), and prolonged persistence of IgM antibody to measles virus has been found in some patients with subacute sclerosing panencephalitis (5) and multiple sclerosis (14).

Several other investigations have suggested the association of group B coxsackieviruses with a high proportion of sporadic pericarditis or myocarditis cases (2, 11, 20, 27), but in each of these studies there were very few cases in which virus was isolated or significant increases in antibody titer could be demonstrated. Instead, the possible association of coxsackieviruses was based, in most cases, upon the presence of

TABLE 7. *Month of onset of illness for patients with pericarditis, myocarditis, or pleurodynia and of control group*

Month of onset (1970)	Patient group			
	Pericarditis, myocarditis, pleurodynia		Pneumonia, pneumonitis, mycoplasma	
	All patients	Patients with IgM antibody to group B coxsackieviruses	All patients	Patients with IgM antibody to group B coxsackieviruses
January	38	11	17	1
February	14	3	14	0
March	16	0	10	0
April	12	4	8	1
May	13	1	5	2
June	13	4	6	0
July	17	6	26	1
August	13	8	32	4
September	30	10	40	2
October	34	8	34	7
November	33	10	37	3
December	26	5	30	0
Totals	259	70	259	21

TABLE 8. *Age of pericarditis, myocarditis, or pleurodynia patients and of control group*

Age range (years)	Patient group			
	Pericarditis, myocarditis, pleurodynia		Pneumonia, pneumonitis, myocoplasma	
	All patients	Patients with IgM antibody to group B coxsackieviruses	All patients	Patients with IgM antibody to group B coxsackieviruses
<1	6	0	9	0
1-10	12	3	34	1
11-20	23	10	25	9
21-30	59	15	45	5
31-40	52	24	47	1
41-50	39	6	37	1
51-60	45	7	36	1
61-70	16	4	18	3
>70	7	1	8	0
Totals	259	70	259	21

moderate to high levels of neutralizing antibody to a coxsackievirus type or upon the demonstration of a fourfold or greater decline in antibody titer over a period of time following illness, both of which are of speculative value for establishing a temporal relationship between infection and illness. Moderate to high levels of neutralizing antibody to one or more group B coxsackievirus types from past, frequently subclinical, infections are often encountered in the normal population and in individuals with other illnesses unrelated to a coxsackievirus infection (13, 19). Further, the decay of neutralizing antibodies to enteroviruses occurs at such variable rates (4, 12, 23) that the demonstration of a declining titer may be coincidental, the antibody having been elicited by a long-past infection not associated with the patient's present illness. Thus the diagnostic significance of stationary or declining levels of neutralizing antibody to coxsackieviruses in patients with cardiac disease is highly equivocal, especially in the absence of comparative data on antibody levels in a control group.

In the present study on IgM antibody to coxsackieviruses, the spectrum of patients available for study did not permit an exact matching with respect to age and dates of onset in the study and control groups; however, the two groups were not greatly disparate and the likelihood of finding coxsackievirus IgM antibody would appear to have been weighted in favor of the control group, since it contained a greater number of patients in childhood age

groups in which enterovirus infections most frequently occur and a greater number of patients with onsets in months in which coxsackievirus infections are most prevalent. However, there were over three times as many patients in the study group showing coxsackievirus IgM antibody as were seen in the control group.

The finding of IgM antibody to group B coxsackieviruses in 21 patients (8%) in the control group may in part represent a background level of unrelated recent coxsackievirus infections occurring in patients with respiratory disease, but in six of these patients (included in the controls on the basis of a clinical diagnosis of pneumonia) additional clinical findings of cardiac or central nervous system signs might be considered equally suggestive of a coxsackievirus infection. Although a control group of pneumonia patients was the best available to us and was used on the basis that coxsackieviruses have not been frequently implicated as etiological agents of lower respiratory tract disease, such an association may occasionally occur (6, 16). Also, overlapping symptoms such as cough and chest pain may occur in pleurodynia or pneumonia, and in the absence of more precise clinical records and X-ray findings allowance should be made in our findings for possible inaccuracies in the clinical diagnosis.

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LITERATURE CITED

1. Artenstein, M. S., F. C. Cadigan, and E. L. Buescher. 1965. Clinical and epidemiological features of Coxsackie group B virus infections. Ann. Intern. Med. **63**:597-603.
2. Bell, E. J., and N. R. Grist. 1970. Further studies of enterovirus infections in cardiac disease and pleurodynia. Scand. J. Infect. Dis. **2**:1-6.
3. Berkovich, S., R. Rodrigues-Torres, and J.-S. Lin. 1968. Virologic studies in children with acute myocarditis. Amer. J. Dis. Child. **115**:207-212.
4. Bussell, R. H., D. T. Karzon, A. L. Barron, and F. T. Hall. 1962. Hemagglutination-inhibiting, complement-fixing and neutralizing antibody responses in ECHO 6 infection, including studies on heterotypic responses. J. Immunol. **88**:47-54.
5. Connolly, J. H., M. Haire, and D. S. M. Hadden. 1971. Measles immunoglobulins in subacute sclerosing panencephalitis. Brit. Med. J. **1**:23-25.
6. Eckert, H. L., B. Portnoy, M. A. Salvatore, and R. Ressler, 1967. Group B Coxsackie virus infection in infants with acute lower respiratory disease. Pediatrics **39**:526-531.
7. Haire, M., and D. S. M. Hadden. 1970. Immunoglobulin responses in rubella and its complications. Brit. Med. J. **3**:130-132.
8. Helin, M., J. Savola, and K. Lapinleimu. 1968. Cardiac manifestations during a Coxsackie B5 epidemic. Brit. Med. J. **3**:97-99.
9. Javett, S. N., S. Heymann, B. Mundel, W. J. Pepler, H. I. Lurie, J. Gear, V. Measroch, and Z. Kirsch. 1956. Myocarditis in the newborn infant. A study of an outbreak associated with Coxsackie group B virus infection in a maternity home in Johannesburg. J. Pediat. **48**:1-22.
10. Johnson, R. T., B. Portnoy, N. G. Rogers, and E. L. Buescher. 1961. Acute benign pericarditis. Virologic study of 34 patients. Arch. Intern. Med. **108**:823-832.
11. Koontz, C. H., and C. G. Ray. 1971. The role of Coxsackie group B virus infections in sporadic myopericarditis. Amer. Heart J. **82**:750-758.
12. Lennette, E. H., and N. J. Schmidt. 1957. Studies on the development and persistence of complement-fixing and neutralizing antibodies in human poliomyelitis. Amer. J. Hyg. **65**:210-232.
13. Lennette, E. H., T. T. Shinomoto, N. J. Schmidt, and R. L. Magoffin. 1961. Observations on the neutralizing antibody response to group B Coxsackie viruses in patients with central nervous system disease. J. Immunol. **86**:257-266.
14. Millar, J. H. D., K. B. Fraser, M. Haire, J. H. Connolly, P. V. Shirodaria, and D. S. M. Hadden. 1971. Immunoglobulin M specific for measles and mumps in multiple sclerosis. Brit. Med. J. **2**:378-380.
15. Murray, E. S., J. M. O'Connor, and J. A. Gaon. 1965. Differentiation of 19S and 7S complement fixing antibodies in primary *versus* recrudescent typhus by either ethanethiol or heat. Proc. Soc. Exp. Biol. Med. **119**:291-297.
16. Niitu, Y. 1965. Coxsackie B1 virus associated with atypical pneumonia found in a mass chest X-ray survey among school children in Sendai in 1960. Sci. Rep. Res. Inst. Tohoku Univ. Ser. C Med. **12**:75-85.
17. Null, F. C., and C. H. Castle. 1959. Adult pericarditis and myocarditis due to Coxsackievirus group B, type 5. N. Engl. J. Med. **261**:937-942.
18. Plager, H., R. Beebe, and J. K. Miller. 1962. Coxsackie B-5 pericarditis in pregnancy. Arch. Int. Med. **110**:735-738.
19. Ray, C. G., G. W. Sciple, P. Holden, and T. D. Y. Chin. 1967. Acute febrile CNS illness in an endemic area of Texas. Pub. Health Rep. **82**:785-793.
20. Sainani, G. S., E. Krompotic, and S. J. Slodki. 1968. Adult heart disease due to the Coxsackie virus B infection. Medicine **47**:133-147.
21. Schmidt, N. J. 1969. Tissue culture technics for diagnostic virology, p. 79-178. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections, 4th ed. American Public Health Assoc., Inc., New York.
22. Schmidt, N. J., J. Dennis, L. H. Frommhagen, and E. H. Lennette. 1963. Serologic reactivity of certain antigens obtained by fractionation of Coxsackie viruses in cesium chloride density gradients. J. Immunol. **90**:654-662.
23. Schmidt, N. J., J. Dennis, E. H. Lennette, H. H. Ho, and T. T. Shinomoto. 1965. Antibody responses of rhesus (Macaca mulatta) monkeys experimentally infected with coxsackieviruses of group B and group A, type 9. I. Antibody responses within the coxsackievirus group. J. Immunol. **95**:54-69.
24. Schmidt, N. J., and E. H. Lennette. 1962. Gel double diffusion studies with group B and group A, type 9 Coxsackie viruses. I. The technique and reactions obtained with hyperimmune animal sera and human sera. J. Immunol. **89**:85-95.
25. Schmidt, N. J., and E. H. Lennette. 1962. Gel double diffusion studies with group B and group A, type 9

Coxsackieviruses. II. Serologic diagnosis of Coxsackie virus infections by the gel double diffusion technique. J. Immunol. **89**:96-105.
26. Schmidt, N. J., E. H. Lennette, and J. Dennis. 1968. Characterization of antibodies produced in natural and experimental coxsackievirus infections. J. Immunol. **100**:99-106.
27. Smith, W. G. 1970. Coxsackie B myopericarditis in adults. Amer. Heart J. **80**:34-46.
28. Walker, S, J., G. A. McNaughton, and D. M. McLean. 1959. Coxsackie B5 virus infections in children: Toronto, 1958. Can. J. Pub. Health **50**:461-467.