University of Nebraska Medical Center
BREAKTHROUGHS FOR LIFE

University of Nebraska Medical Center
DigitalCommons@UNMC

MD Theses

Special Collections

1957

Coxsackie virus

Robert Louis Stiehl
University of Nebraska Medical Center

This manuscript is historical in nature and may not reflect current medical research and practice. Search PubMed for current research.

Follow this and additional works at: https://digitalcommons.unmc.edu/mdtheses

Recommended Citation

Stiehl, Robert Louis, "Coxsackie virus" (1957). *MD Theses*. 2274.
https://digitalcommons.unmc.edu/mdtheses/2274

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact digitalcommons@unmc.edu.

COXSACKIE VIRUS

Robert Louis Stiehl

Submitted in Partial Fulfillment for the Degree of

Doctor of Medicine

College of Medicine, University of Nebraska

March 16, 1957

Omaha, Nebraska

TABLE OF CONTENTS

I. HISTORY

The discovery of Coxsackie viruses or "C viruses" was made by Dalldorf and Sickles (1) in 1947 when they isolated Coxsackie virus from suckling mice which were innoculated with stool extracts from two boys who were clinically diagnosed as paralytic poliomyelitis. Polio-myelitis virus was also isolated from the stools of these two boys. The filterable agent (Coxsackie virus) differed from Poliomyelitis virus in that it induced fatal disease with paralysis and destructive lesions of striated muscle in unweaned mice but not in adult mice, adult hamsters, or Rhesus monkeys. Dalldorf (1) proposed the term Cox-sackie virus for this filtrable agent that was isolated, because the two boys from whom the virus was first iso-lated lived in Coxsackie, New York.

In 1949 Dalldorf et al. (2,3) proposed the nomenclature for Coxsackie viruses. The classification proposed in 1949 is based on groups designated by capital letters and types identified by arabic figures. At the present this classification is widely used and was endorsed by an Ad Hoc panel, under the chairman of the Virus Subcommittee of the International Committee on Bacteriological Nomenclature, meeting during the session of the International Congress for Microbiology in Rome in 1953.

The interesting history of the clinical syndromes, and the history of the causative group of Coxsackie viruses as etiological agents will be discussed elsewhere in the paper.

II. CHARACTERS OF THE VIRUS

A. Physical Properties

In 1950 Robinson (4) reported that suspensions of certain strains of Coxsackie viruses are inactivated in thirty minutes between 53° C and 55° C. He also reported that Coxsackie viruses withstand pH 2.3-9.4 for one day and pH 4.0-8.0 for seven days. Melnick et al. (5) reported in 1950 that the activity of Coxsackie virus is not lost when specimens of infected tissue are stored in 50% glycerol or horse serum at room temperature for as long as 70 days and in a refrigerator for over a year. In 1952 Sulkin (6) demonstrated the isolation of C viruses from stools of patients that had been stored for at least 6 years in a dry ice cabinet. In 1951 Melnick et al. (7) found the particle size of four antigenically different C viruses to be 15-23 millimicrons by ultrafiltration and 24-32 millimicrons by sedimentation in the ultracentrifuge. They also noted that the Lansing strain of poliomyelitis virus was of the same magnitude as the Coxsackie virus. In 1950 Himmelweit et al. (8) found the particle size of

two serological unrelated strains of C virus to be 10-15 millimicrons by methods of filtration through Gradocal membranes. Quigley (9) in 1949 working with Dalldorf Type I C virus showed that the virus passed through an 18 millimicron membrane, indicating that the virus size would be 10 millimicrons or less. In 1952 Melnick and Curnen (10) stated that Warren and Breese in 1951 found Texas-1 type (Type A4) C virus to be 25-35 millimicrons in size using analytical ultracentrifuge and electron microscope methods. From this data it appears that C viruses are very small and range from 10-35 millimicrons in size. In 1950 Melnick (11) showed that C viruses passed through bacteria tight filters with no significant loss of titer and that these viruses were not inactivated in vitro or in vivo by penicillin, streptomycin, chloramphenicol, terramycin, or viscosin. His studies also revealed that the viruses were not inactivated by ethanol (70%), lysol (5%), roccal (1%), or ether, but were rapidly inactivated when treated with 0.1N HCL or 0.3% formaldehyde and that these viruses were precipitated by ½ saturation of ammonium sulfate. Melnick and Curnen (10) stated that

Kaplan and Melnick in 1952 reported that aqueous suspensions of certain strains of C viruses were inactivated between 50 and 55 C° when heated for 30 minutes and that temperatures 5-20 C° higher were required for inactivation

when the virus was suspended in milk, cream, or ice cream. Mc Kee et al. (12) reported in 1953 that nothing is known about the metabolism of this group of viruses.

B. Types

In 1955 Dalldorf (13) stated that thus far 24 C viruses have been discovered and that 19 types belong to Group A and 5 types belong to Group B. This differ-entiation into types was accomplished by neutralization and complement fixation methods through the work of Dalldorf, Melnick, Huebner, and their co-workers.

III. EPIDEMIOLOGY

A. Geographic Distribution

Since the discovery of C virus in 1947 by Dalldorf and Sickles (1), the C viruses appear to have a world-wide distribution and have been isolated wherever a valid attempt has been made.

In 1955 Pohjanpelto (14) compiled data on the geographic distribution of C viruses. In his compiled chart he revealed that C viruses had been isolated in Denmark, England, Finland, France, Germany, Hungary, Iceland, Italy, Netherlands, Sweden, Switzerland, Alaska, Brazil, Canada, United States, Australia, New Zealand, Japan, Egypt, Israel, and Transvaal.

From the numerous reports of isolation of C viruses in United States, it appears that these viruses

are wide spread within the United States.

B. Seasonal Incidence

The majority of C viruses appear to have been found in the summer and autumn. In 1955 Pohjanpelto (14) stated that only 16 of the 276 strains isolated all over the globe (6.) were found in the winter or spring, making a summer-autumn incidence 16 times higher than the winter-spring incidence. He feels that this ratio is probably incorrect, since presumably the majority of the samples investigated were collected in the summer and autumn. The reason that these specimens were collected in the summer and autumn was that many investigators were trying to find out if the C viruses played any role in patients with poliomyelitis, since C viruses are frequently found in patients who have poliomyelitis. In 1951 Cole et al. (15) reported that the seasonal occurence of Group A C viruses was in the late summer months and the early fall months. In 1950 and 1951 Huebner et al. (16,17) collected fecal specimens at different times from different inhabitants and noted that the isolation of C viruses was made almost exclusively in July, August, and September. Melnick and Curnen (10) stated that Melnick, Coffey, and Schoof collected specimens of sewage each month from different areas in the United States, and their studies indicated that C viruses were encountered more frequently and in a

greater quantity in the summer and fall. Melnick et al. (18) stated in 1954 that the C virus infections belong to the summer and fall diseases and that they do not occur evenly throughout the year.

The investigators cited in this section are all in agreement that C viruses are found most frequently in the summer and autumn (fall).

C. Age, Race, Sex, and Familial Incidence

In 1951 Cole et al. (15) reported that out of over 1000 samples studied in the United States, C viruses were found in 10% in persons under 10 years of age and in only 4% of those over 10 years of age. Dalldorf (19) in 1950 reported that in 433 cases that C viruses were found in 39.3% in children under 5 years, 35.7% in children from 5 years through 9 years, 21.4% from the ages 10 through 19, and in 3.6% of persons over 19 years of age. Pohjanpelto (14) in 1955 reported that C viruses for the most part have been isolated from children and that only 69 (27%) of the 285 virus strains isolated in different parts of the world were found in persons over 10 years of age.

It is evident from this survey of literature that C viruses are more frequently isolated from children 10 years old or younger.

Dalldorf and Gifford (20) in 1951 stated that

the disease (C virus infection) was twice as common in males as in females. Cole et al. (15) in 1951 noted no significant difference in sex incidence of diseases caused by C viruses. Thinnes (21) in 1954 stated that from the present data there does not seem to be any sex or race difference in people affected with C viruses.

In 1951 Cole et al. (15) reported on the multiple cases of Group A C viruses found within families. Numerous other investigators have reported on familial infections with C viruses, thus confirming that C virus infections are disseminated in family groups.

D. Source, Host Range, and Mode of Infection

The first isolation of C viruses was made from the stools of two boys clinically suffering with paralytic poliomyelitis by Dalldorf and Sickles (1) in 1947. Curnen (22) in 1950 recovered C viruses from feces and pharyngeal swabbings from patients, and in one case C virus was demonstrated in both the throat and feces for more than two weeks. In 1951 Huebner et al. (16) isolated Group A C viruses from throat washings, rectal swabs, and in the stools of patients with Herpangina. In 1951 Cole et al. (15) were able to isolate Group A C viruses from the stools of one patient for 76 days after the onset of illness; however, they stated that the virus usually cannot be recovered from the stools for more than one month

after the first symptoms of the disease are observed. According to Carpenter and Boak (23), Rhodes and Van Rooyen (24), and Lazarus et al. (25), the best source of isolation of C viruses is from feces, as the viruses appear for only a few days after the onset of illness in pharyngeal washings or swabbing. In 1952 Melnick and Curnen (10) stated that Melnick, Coffey, and Schoof isolated C viruses from sewage and flies collected throughout the year. In 1950 Howitt (26,27) isolated C viruses from spinal fluid, blood, urine, and from CNS lesions from humans at post-mortem. This observation was doubted for some time but now has been shown to be true by other investigators. In 1952 Lepine et al. (28) reported isolation of C viruses from stools and muscle tissue obtained from muscle biopsies from patients with Bornholm's disease. Creveld et al. (29) in 1956 reported on culture and isolation of C viruses from the heart of infants obtained at post-mortem who died of interstitual myocarditis.

Dalldorf et al. (1,2) in 1949 were the first to show at that time that the host range was man, and suckling mice and hamsters. In 1950 Melnick and Ledinko (5) reported that following oral administration of C viruses an infectious state could be established in Cynomalgus monkeys. The animals developed a febrile response with no evidence of muscle or CNS lesions and became pharyngeal

and intestinal carriers. Sulkin (6) in 1952 reported that newborn guinea pigs were refractory to C virus infections. In 1952 Melnick and Penner (30) reported that C viruses can be detected for 12 days in the excreta of flies fed the viruses experimentally. Fischer and Syverton (31) in 1951 stated that the American cockroach will excrete C viruses for as long as 15 days following a single meal containing C viruses. Tobin (32) in 1953 stated that Lepine, Chaumont, and Blusson in 1952 revealed that young hamsters and merions were susceptible to C viruses. The adaptation of C viruses to tissue culture cells will be discussed later when the laboratory diagnosis is considered (33).

In 1951 Cole et al. (15) reported on the familial incidence of Herpangina and suggested that the mode of spread was from person to person. Huebner et al. (34,35) in 1952 noted the presence of C viruses in the majority of people who were close contacts, thus suggesting also that these viruses are spread from one another by contact.

Tobin (32) in 1953 stated that Findlay in 1952 suggested that droplet infection was the normal method of spread from case to case. Tobin (32) believed that it was more probable that these viruses were spread in the same manner as other enteric agents ie/ by infected fomites, food, and water; therefore, their incidence should be

reduced by high standards of personal and communal hygiene. Melnick et al. (18) in 1954 stated that it is not evident whether or not, the presence of C viruses in sewage and flies is a direct or even an indirect link in the chain which leads these viruses from one infected person to another. They also stated that even though the viruses have been found in flies, there is no evidence that multiplication of the viruses occurs within them. The same holds true for the isolation of C viruses from the American cockroach by Fischer and Syverton (31). Thinnes (21) stated in 1954 that spread seems to be by direct contact with all susceptibles becoming infect-ed ,but only 30-50% exhibit clinical symptoms. Carpenter (23) stated in 1952 that close contact is conducive to transmission of infection by C viruses and that infection may result from contact with persons who have asymptomatic cases, with persons in whom the disease is clinically apparent, or from carriers. Dalldorf (3) stated in 1955 that since proof is still lacking that current sanitary methods regularly destroy C viruses, it offers a challenge to sanitarians to help combat the spread of C viruses. He believes that C viruses are transmitted rather directly , and personal hygiene seems most important.

The exact spread of C viruses is still not known in man.

IV. PATHOLOGY

A. Experimental Animals

Gifford and Dalldorf (36) reported in 1951 the division of C viruses into two groups (Group A and Group B) by different histopathological features of the disease produced in suckling mice by the two groups of viruses which had no antigenic relationship to one another. A detailed description of their interesting discovery and findings will now be presented.

In their experiments 255 mice, not older than 10 days, and 2 suckling hamsters were used.

1. Findings with Group A Coxsackie viruses

"It was noted that the experimental animals infected with Group A C viruses produced flaccid paralysis of one or more limbs and generally died within a day or two following the first evidence of weakness. No signs of encephalitis were noted."

The only gross lesions that they observed in the infected animals with Group A C viruses were opaque whitish streaks in the skeletal muscles of the severely paralyzed ones. On the microscopic examination they noted extensive hyaline degeneration and active repair in most of the striated muscles. They have never observed these changes in normal mice of the same age. The degeneration started with the loss of finer details in the striated muscle fibers.

The process soon underwent hyaline degeneration, fragmentation and clumping. Soon after this process, active repair began which was manifested by the appearance of young mesenchymal elements that surrounded the muscle fiber fragments. They noted that repair was extreme in degree and that it might be due to the immaturity of the animals. Most of the young mesenchymal cells were myoblasts. They noted that the route of innoculation of the animals did not determine the morphologic response.

2. Findings with Group B Coxsackie viruses

Gifford and Dalldorf (36) noted that the experimental animals infected with Group B C viruses often showed generalized spasms as well as weakness and paralysis. They also noted that frequently the tail became tremulous and rigid, and dyspnea and cyanosis were often observed. In contrast to the experimental animals infected with Group A C viruses, it was noted that the animals infected with Group B C viruses may survive for as long as 10 days or may recover.

The gross findings that they noted in the animals infected with Group B C viruses were liquefaction of the cerebral hemispheres, and congestion or pallor of the fat pads between the scapulae. They have noted that no gross muscle lesions have ever been recognized with this group of C viruses.

On microscopic examination the skeletal muscles were noted to be involved in approximately two-thirds of the infected animals and that the lesions produced in the muscle differed from those produced by the Group A C viruses in being focal rather than generalized. The structural details of the lesions were indistinguishable from those produced by the Group A C viruses. They noted that the animals innoculated intraperitoneally tended to develop more severe muscle changes than those infected by the intracerebral route. The most conspicuous change noted by them in intracerebrally innoculated animals was in the cerebral hemispheres which was usually bilateral and symmetrical. The changes noted consisted of a patchy dissolution of the parenchymal cells which resembled early anemic necrosis. It was noted that death of neurons occured very quickly and was followed by widespread liquifaction and infiltration of a few PMN leukocytes. It was also noted in the animals that survived for a number of days that their cerebral hemispheres were transformed into cystic masses and that this process occured most commonly in the frontal lobes. The midbrain and thalmus were commonly involved, and the hippocampus was occasionally the site of the initial lesion. In all their infected animals except one, the olfactory bulbs were intact. They noted that the spinal cord (upper levels), pons, and medulla

might be affected and that outside the cerebral hemispheres the damage to the CNS was focal, and degeneration was limited to small areas or to particular nuclei. Occasional vascular lesions were seen by them in the CNS which consisted of dilatation accompanied by some thickening of the vascular endothelium. It was felt by them that this involvement was, if anything, more common and pronounced in the neuraxis. No other prominent features were noted in this respect. The most unique lesion noted by them was found in the fat pads and was most conspicuous in the large fat pad between the scapulae. It was also noted by them that usually other depots were simultaneously involved. They observed that the process commonly began at or near the periphery of the lobules and that about the necrotic zone was a fairly loose inflammatory reaction consisting of leukocytes and connective tissue elements with which were mixed regenerating fat cells. They noted that the general structure was somewhat like pancreatic fat necrosis in humans from which it differed in the incompleteness of its necrosis and in its inflammatory response. They found myocardial lesions in 3 of the 51 Group B infected animals in which the myocardial fibers appeared faded, and in the larger foci they appeared faintly hyaline in character. They also noted hydropic degeneration near the margin of muscle cells, and fragmentation and loss of muscle fibers were seen in relatively

large areas. They noted a few muscles in which acidophilic granules could be seen. They observed hepatitis of varying degree; no pancreatic disease was noted. In 1952 Kunz et al. (37) reported that pancreatitis developed in adult mice (mice over 3 weeks of age) that ate infected suckling mice with Group B C viruses. It was noted that the pancreatic acinar tissue became necrotic and was replaced by fat in the surviving animals and that pancreatitis does not occur during the suckling period in mice which is from the 4th. day of life to the 21st. day of life. Dalldorf (3) reported in 1955 that to date no virus particles have been identified in lesions produced by C viruses and that "Aumonier undertook to characterize the earliest changes and found that the sequence of events in striated muscle was loss of "Z" and "Z" lines, loss of "A" and "I" discs, swelling of the myofibrils, and, finally, complete loss of structure. He postulated that Group A virus may have a specific affinity for one or more of the constituents of the myofibrils."

Melnick and Curnen (10) stated in 1952 that the distribution of lesions in mice is influenced by many factors including the type and dose of virus, the age and strain of mice, the route of innoculation, and the fixation of tissue for histological examination. "These variables make the histopathologic classification of C virus

suggested by Dalldorf more difficult and less certain than one based exclusive-ly on immunologic criteria."

B. Human

The literature is scant on the distinctive lesions that may be produced in man, because no deaths have been reported due to C viruses, unless the deaths from myocarditis neonatorum in which Group B C viruses were isolated are considered. The-se pathological findings will be mentioned when clinical syndromes are considered. The same will be done with the gross findings of Herpangina.

Lepine et al. (28) reported in 1952 that similar lesions were seen in muscle biopsy material from 2 cases of Bornholm's disease that resembled those seen in suckling mice experimentally innoculated with Group B C viruses. Focal areas of mononuclear and PMN infiltration, and degeneration of muscle fibers were seen in the human muscle biopsies. Group B C virus was recovered from the stools and from the muscle biopsies of the patients by the innoculation of suckling mice.

V. PATHOGENESIS IN MAN

Rhodes and Van Rooyen (24) reported in 1953 that little is known about the pathogenesis of C viruses in man, and speculated that the viruses entered the body through the pharynx, intestine, or nose. They also stated

that it was not known where the virus proliferates nor which organs are eventually affected. They thought that it would seem likely that the virus invades and proliferates in viscera, muscles, and in some cases the meninges. It was also speculated by them that at some stage the virus is presumbably blood borne. Keller and Vivell (38) proposed in their report in 1951 that the C viruses enter chiefly through the G-I tract and that the viruses pass via the lymphatics to the blood stream. Then after a short stage of viraemia, the virus is localized in the muscles producing myositis. They believe that the further course depends on the neutratropism of the virus and that Coxsackie A strains are only feebly neurotropic and tend to progress no further.

VI. IMMUNITY IN MAN

C viruses are capable of producing disease in humans and of stimulating an immune response. This immune response in the human host was clearly demonstrated by Shaw et al. (39) who reported in 1950 the studies of 6 laboratory workers who became infected while investigating C viruses. A strain of C virus was isolated from each laboratory worker during the acute febrile illness. Sera collected from these workers prior to the illness contained no antibodies against the infecting type of C virus while all sera collected during convalescence possessed type-

specific antibodies. Kraft and Melnick (40) in 1952 reported that the C-F tests with the sera from these 6 workers showed not only the development of antibodies against the infecting strain, but also the development of antibodies against unrelated strains. They believed that this represented an anamnestic reaction. Beeman and Huebner (41) stated in 1952 that this rise in heterologous antibody may be due to a group antigen contained in all C viruses. One can readily see that C-F tests with human sera have a limited value in the diagnosis of C viruses, because of this rise in heterologous antibody as well as the homologous one. Tobin (32) reported in 1953 that Curnen stated in 1952 that C-F antibodies do not persist for the same length of time as neutralizing ones and often disappear or drop to a low level within 6 months. He also stated that in human infection with C virus a rise in neutralizing antibody can be demonstrated provided that the first serum sample is taken within the first few days of illness, and the second sample taken
10 days or more after the appearance of the illness.

Tobin (32) stated in 1953 that antibodies appear on the 5th. to the 9th. day and increase rapidly, in many cases to their maximum within a few days, and persist for at least a year with the group B C viruses and for longer periods with those of group A C viruses. Dalldorf (19)

stated in 1950 that adult serums customarily contain antibodies for many types of C viruses. Mc Kee et al. (12) stated in 1953 that the incidence of antibodies against multiple types of C viruses increase with age, and the incidence of antibodies against the virus is lower among children than among adults. Melnick (11) in 1950 reported that neutralizing antibodies appear at or soon after the onset of the disease and that C-F antibodies appear to be demonstrable only after neutralizing antibodies have made their appearance. Curnen and Melnick (42) stated in that there is no evidence to suggest that individuals immune to poliomyelitis are immunized against infections caused by the C viruses or vice versa. Melnick and Ledinko (43) reported in 1951 that natural passive immunity is conferred from mother to child by way of milk, as measured by neutralizing and C-F antibodies and that these antibodies disappear from the serum after a few months.

The exact duration of immunity produced in humans from C virus infections is still not known.

VII. CLINICAL FEATURES

The general clinical features of C virus infections will be discussed in this section. The specific clinical features of the syndromes caused by C viruses will be discussed under clinical syndromes. One must realize that in many cases there are combinations of

these features, and they are by all means not clear cut.

In 1953 Yeager (44) and Rhodes and Van Rooyen (24) reported that the general clinical characteristics of Coxsackie infection were as follows:

General malaise which was very common at the beginning of the disease process.

Muscular pain or myalgia was a common finding which at times may be accompanied by muscle spasm or tenderness. This muscle pain and tenderness may resemble that seen in poliomyelitis, but no true muscle shortening, as seen in poliomyelitis, has been reported with C virus infections.

Meningismus and aseptic meningitis are also fairly common findings in people infected with C viruses. These patients usually develop signs of meningeal irritation. A moderate pleocytosis may be seen in the CSF. The cells are predominately lymphocytes. The WBC in the CSF may reach 150-200/cm. but rarely higher. The total protein in the CSF may also be elevated but usually not over 60 mg.%. The total sugar in the CSF is usually normal.

Pyrexia is usually present and moderate in intensity, but infants may develop extreme pyrexia and have convulsions. The fever is usually of short duration.

Transitory muscle weakness or paralysis has been reported in some cases infected with C viruses. The paralysis or weakness is transitory. No investigator has ever

determined the mechanism for this weakness or paralysis.

Generalized flushing and injection of all mucous membranes of the mouth, eyes, and upper respiratory tract may occur.

The patient may complain of a painful sore throat, and ulceration of the mucous membranes, particularly of the tonsillar pillars and soft palate, may occur.

Gastro-intestinal crises may be seen with C virus infections in the form of cramping, diarrhea, and vomiting. It has been frequently seen in familial contacts of Coxsackie disease that these people suffer from a short term gastro-entero-colitis. They recover without mani-festing other symptoms of Coxsackie infection. Yeager (44) is inclined to believe that these G-I upsets are abortive forms of the disease and states that this impression is supported by the fact that C viruses are so readily re-covered from the stools of patients with Coxsackie disease.

Incubation period appears to range from 2-9 days with a mean between 3 and 5 days according to Curnen (22), Huebner et al. (16), and Findlay and Howard (45).

Recovery in most cases is complete but in the past several years, several investigators have reported deaths of infants with interstitual myocarditis caused by Group B C viruses. This will be discussed later in detail. In general the course is short, but some cases of encephalitis have prolonged courses as will be seen later.

Treatment is entirely symptomatic and at the present time no anti-serum or vaccine for active or passive immunity is available.

VIII. CLINICAL SYNDROMES

A. Those caused by Group A C viruses

1. Herpangina

In 1920 Zahorsky (46,47) described what he called Herpetic sore throat which in 1924 he called "Herpangina". His original description of Herpangina was so exacting that later investigators could add very little to his description; therefore, his work will be cited.

Zahorsky (46,47), Parrott et al. (48), and Huebner et al. (16) agree that the disease affects children between the ages 3-10 and that the incubation period ranges from 4-10 days. These investigators also noted that the disease was very common in families. Zahorsky (46) in 1920 noted that smears taken from the lesions in the mouth revealed the same bacteriological picture which is usually obtained from secretions of the mouth and throat. This has been confirmed by numerous other investigators.

Zahorsky's (46) description of Herpangina will now be presented.

"The disease begins suddenly with a high fever (102-105° F). A marked chill was not observed. In one

child a severe convulsion ushered in the general symptoms. Vomiting was frequently present, but only once did it continue for 2 days. The children complain of being tired, have headache, and backache. Severe prostration did not occur. Diarrhea was observed only a few times and was not severe".

"On examining the children nothing is found except the characteristic appearance of the mouth and throat. On superficial inspection a tonsillitis will be diagnosticated, since the tonsil is always swollen, the pillars of the fauces congested, and a slight grayish exudate is often found protruding from the crypts of the tonsils. But on more careful inspection the characteristic vesicles or ulcers will be seen. They are not usually numerous: 2-6 is the rule. In one case I counted 10. They are generally situated on the soft palate, most frequently on the free-hanging margin between the tonsils and uvula, or on the anterior pillar of the fauces, but one or more vesicles or ulcers may be found on the posterior part of the buccal mucous membranes. I have several times found one or more vesicles or ulcers on the surface of the tonsils. Often one or more vesicles are located on the pharyngeal wall. These vesicles are the size of a pea, the center of which has an elevated grayish appearing blister. Most commonly the large blister bursts and

leaves a punched out ulcer surrounded by a gray ring of dead epithelial layer, and this is surrounded by a dark red areola. The surface of the ulcer often shows a thin grayish deposit. The fever subsides in 2-5 days and the ulcers in the mouth heal rapidly."

During the summers of 1946, 1947, and 1948 in Northern Louisiana, Cobb et al. (49) observed the epidemic occurence of an acute febrile illness in children which was characterized by high fever, headache, and brief duration. They termed this illness "Three Day Fever". They also noted vesicular studding over the soft palate. Cole et al. (15) reported in 1951 that they made the same clinical observations and stated that it was pathognomic of a well defined syndrome "Herpangina" described and named by Zahorsky in 1924.

It was not until 1951 that Huebner et al. (16) reported that Herpangina was caused by Group A C viruses. They carried out etiological investigations of Herpangina from 1949 thru 1951 and were able to show that Herpangina was definitely associated with the presence of one of 6 Group A C viruses in the feces or throat secretions, or both in 85 of the 99 cases of Herpangina that they studied.

In 1952 Beeman et al. (50) suggested that Herpangina was a fairly common disease, because of the relatively high number of human beings showing antibodies

against one of the Group A C viruses.

B. Those caused by Group B C viruses

Dalldorf (3) stated in 1955 that at the present time more attention is paid to the Group B than the Group A C viruses, because the Group B viruses are more important agents of disease in man and isolated more easily by tissue culture methods. Only 5 of the 19 Group A strains are cytopathogenic. He now believes that the Group B strains will intrude more into the study of poliomyelitis than the Group A strains.

1. Bornholm's Disease (Epidemic Pleurodynia)

In 1943 Howard et al. (51) reported that the first description of Bornholm's disease was made in 1872 by Daae in Norway who spoke of the disease as epidemic muscular rheumatism. They stated that Daae in 1872 wrote the following description of the disease which is still the best descripton.

"As a rule the patient has a stitch in one side of the chest, most often without any precursory ailment, but sometimes after an attack of chills; the stitch is often accompanied by pains in the back, shoulders, epigastrium and abdomen, and these pains are described sometimes as oppressive or sticking, sometimes as shooting or aching; less frequently these pains are felt also in the back, neck, legs, arms and even out in the fingers. There

is considerable difficulty in moving the affected parts, especially the chest; therefore, the respiration is laborious, sometimes to such an extent that the patient feels as if he were to be strangled. Usually the general condition is not greatly affected. There is as a rule some headache, anorexia and thirst; the bowels usually are sluggish. The tongue is generally coated. The pulse is normal or a little frequent. There is seldom any cough, so cough does not appear to go with this disease. Physical examination of the chest reveals no abnormality."

"There is a great difference in the severity of the attack in the various patients. Some have a fairly mild attack and have to rest only a very short time; in others the attack is so violent that one might expect them to die at any minute. In a few of the most severe cases the patients are thus confined to bed continuously for up to fourteen days. As a rule the patients have got up and walked about just as soon as they have been able to do it. A good many of them have had a relapse, some-times repeatedly. No case has terminated fatally. Many of the patients are exhausted after the disease, emaciated and feeble; sometimes they feel a stitch or stabbing pain now and then for several weeks after they have been able to begin to work".

Howard et al. (51) stated that Finsen in 1874

reported having observed epidemics of this nature in Iceland in 1856 and 1863 and was the first to speak of this disease as epidemic pleurodynia and that little was heard about this disease until 1930 when Sylvest reported on an epidemic in Bornholm. Since the disease was so common around the Bornholm Island, the disease has also been referred to as Bornholm's disease. Howard et al. (51) also stated that Dabney reported on an epidemic of epidem-ic pleurodynia in Virginia in 1888, and it was one of Dabney's patients who dubbed the disease "The Devil's Grip".

It was not until 1948 when Curnen (22) first showed that Group B C viruses were associated with epidem-ic pleurodynia. In 1949 Shaw et al. (39) observed epidem-ic pleurodynia in laboratory workers and showed a relation-ship of Group B C viruses. Weller et al. (52) in 1950 stated that they were able to isolate Group B C viruses in a high percentage of stool specimens collected during the 1947 outbreak of epidemic pleurodynia in Boston. Additional descriptions of laboratory infections with Group B C viruses, accompanied by symptoms resembling those seen in epidemic pleurodynia, have been recorded by Findlay and Howard (45) in England in 1950. Lazarus et al. (25) reported in 1952 on an outbreak of epidemic pleurodynia in 1950 in Washington State with demonstration

of the presence of Group B C viruses and evidence of a signific nt rise in antibody titer during convalescence. Lepine et al. (28) in 1952 reported on the isolation of Group B C virus from human muscle biopsies from two patients suffering with epidemic pleurodynia. They also stated that these biopsies showed similiar lesions as those seen in infected mice with Group B C viruses and that they yielded Group B C virus. Dalldorf (3) stated in 1955 that orchitis was a common complication with epidemic pleurodynia. Nichamin (53) reported in 1952 the difference of symptoms seen in children compared to those seen in adults with epi emic pleurodynia. He observed that children under 5 years of age seldom complain of headache but usually complain of abdominal pain and that the younger the child the more likely the pain would be referred to the periumbilical region. He also observed that epidemic pleurodynia in adults has a more abrupt onset than in children and that fever is not always present. He noted that rarely a child complained of lower thoracic or upper abdominal pain that are so characteristic of the disease in adults and that substernal "smothering" sensations are rarely described by children. His studies also reveale-d that only occasionally a child will complain of difficulty or pain in taking a deep breath and that nausea or vomiting have

not been prominent symptoms except in insta-nces in which persistent, intractable vomiting prevails.

2. Aseptic Meningitis

In 1948 Curnen et al. (54) described clinical features of aseptic meningitis or nonparalytic poliomyelitis in 10 patients from whom C virus was isolated or whose serum neutralized it. They reported that the onset may be gradual or sudden and that fever, nausea, abdominal pain, headache, and malaise are common early prodromal constitutional symptoms and that these symptoms usually preceded those suggestive of meningeal irritation. It was noted that the temperature range-d to a maximum of 40.3 °C with a mean of 39.4 °C and usually lasted 1-10 days with a mean of 5.6 days. They observed that the febrile course was occasionally diphasic.

On physical examination they noted that the patients did not appear particularly ill and revealed only a few objective indications of disease. Hyperemia of the pharynx was observed by them in some of the patients during the acute stage. They also noted positive Brudzinski or K rnig signs in most of the patients after the prodromal constitutional symptoms and that stiffness of the back or neck were usually evident which rarely persisted longer than the fever. They observed that signs of muscular weakness or spasm of the hamstring

muscles were equivocal or lacking and that examination of the reflexes revealed that they remained normal.

On examining the CSF they noted a pleocytosis of leukocytes which were mainly lymphocytes and that in many instances it did not exceed 100 cells/cm.. They noted that the protein content of the CSF was normal or only slig tly elevated and that the WBC count and differential were usually within normal limits.

In their studies they noted no complications and complete recovery.

Dalldorf (3) stated that Gsell was the first to report in 1949 an association of aseptic meningitis and epidemic pleurodynia and that the works of Johnson and Gard reported in 1954 le-ft little doubt that their observations of aseptic meningitis in Sweden were due to Group B (Type 3 C virus. Dalldorf (3) also stated that Johnson noted that pleurodynia was the common symptom of adult infection with Group B C virus and aseptic meningitis of children and that Gard postulated that the introduction of a Group B C virus into a population with previous experience might result in an epidemic of aseptic meningitis among children while in a nonimmune population adults would also be susceptible, and the epidemic would resemble pleurodynia.

Dalldorf (3) stated that Gard made the first

isolation of Group B C virus from the CSF in 1951 from patients with aseptic meningitis and that Gear isolated Group B C virus from the nervous tissue of fatal cases with aseptic meningitis. Since Hummeler et al. (55) in 1954, Mc Leod et al. (56) in 1956, Beale et al. (57) in 1956, and other investigators have isolated Group B C viruses from the CSF of patients with aseptic meningitis in which no other virus was isolated, thus adding to the evidence that Group B C viruses are definitely a cause of aseptic meningitis.

Mc Leod et al. (56) pointed out that aseptic meningitis may be caused by "Orphan" or Echo viruses and that in the individual case it was not possible to distinguish between aseptic meningitis caused by Coxsackie Group B, Poliomyelitis, or "Orphan" viruses.

Though no cases of death have been associated with aseptic meningitis due to Group B C viruses, and since they are widespread, one can readily see that these viruses offer a great diagnostic problem in patients with aseptic meningitis.

3. Encephalitis

In Australia in 1951 Stanley et al. (58) reported on an outbreak of Encephalitis due to Coxsackie Group B Type 3 virus. At first they believed that they had discovered a third group of C virus. The predominant

symptoms in this epidemic were fever, headache, stiff neck, muscle pains, sore throat, abdominal pain, lassitude, and muscle weakness. They noted that sequelae were common and consisted of severe headaches, photophobia, nystagmus, and dizziness. The majority of the patients were adults, and there was a prolonged period of hospitalization. It was noted that the signs of encephalitis were not great, and Dalldorf (3) believed that this epidemic resembled aseptic meningitis rather than epidemic pleurodynia.

4. Interstitual Myocarditis

Of much interest is the exact role of Group B C viruses in myocarditis of infants. The first observations we-re made by Javett et al. (59). They observed an outbreak of myocarditis in infants in Johannesburg late in 1952. Ten babies became ill while in, or soon after discharge from the maternity home in Johannesburg in which they were born. Six of these babies died after an acute fulminating illness lasting 3-8 days which ended in circul tory collapse. They noted that the general symptoms were those of a severe infection. That is, sudden onset, high temperature, cyanosis, dyspnea, tachycardia, profound toxemia, and a falling blood pressure. In some of the infants they noted that death supervened within a few hours of the apparent onset. They observed that this epidemic in the newborn babies usually took the

form of diarrhea and less commonly of pneumonia or viremia. The main clinical evidence of myocarditis noted by them was tachycardia above 200 beats/min., and an abnormal T-1 in the electrocardiogram. They heard no heart murmurs, and cardiac enlargement was not known until autopsy.

Post-mortem examination on three of the six babies that died showed that the outstanding lesion was a patchy myocarditis, and in one baby examined a focus of encephalitis was seen. Bacteriological examination revealed no cause for these findings.

The brain tissue of two of these infants yielded a Group B C virus as did the feces. This strain was identified as Type 3. They stated that Group B C virus has been isolated from the heart muscle of a newborn infant admitted to the Transvaal Memorial Hospital which had signs and symptoms of myocarditis that died soon after admission. Histological examination of the heart showed a myocarditis like that observed in their studies.

The myocarditis seen in these infants hearts are not unlike those seen in suckling mice infected with Group B C viruses studied by Gifford and Dalldorf (36) in 1951.

Creveld and De Jager (29) in 1955 observed 4 lethal cases of myocarditis in newborns in a 2 months

period of "summer grippe". Some of the mothers showed symptoms of influenza. In all 4 cases at post-mortem, they found an interstitual myocarditis. In some of the cases they found foci of inflammation in the liver and brain. In one case Group B-4 C virus was isolated from the brain and heart muscle. In all the other three cases a Group B-4 C virus was isolated from the heart muscle. Verlinde et al. (60) reported in 1955 that a fatal experimental infection resulted in a newborn cynomalgus monkey in which a Group B-4 C virus was isolated from the brain and heart. A local myositis was observed at the site of innoculation, and slight degeneration of the heart muscle was seen. Kibrick and Benirschke (61) reported in 1956, an intra-uterine infection with a Group B-3 C virus in an infant in whom acute myocarditis and meningoencephalitis developed. At autopsy a diffuse meningencephalitis and myocarditis were found. These changes were also seen in suckling mice innoculated by them with the virus. The virus was recovered from the thoracic spinal cord at the autopsy.

Kibrick and Benirschke (61) pointed out that it is now known that some of the cases of idiopathic myocarditis are caused by viruses. Of much interest in their case was the fact that C viruses can pass the placental barrier thus leading to intra-uterine infections

with C viruses. As they pointed out, this may help to explain some of the unexplained congenital abnormalities found in infants.

In all the investigators cases of interstitual myocarditis cited in this section, no other agent or agents were isolated that could explain these fatal cases. Prior to the identification of interstitual myocarditis, Coxsackie virus infection had never been proven to be an immediate cause of death.

IX. DIFFERENTIAL DIAGNOSIS

It will be the purpose of this section to acquaint oneself with some of the entities that may be confused with Coxsackie virus infections.

Aseptic menirgitis caused by Coxsackie viruses may be confused with aseptic meningitis caused by Arthropod-borne encephalitis, Infectious lymphocytosis, Infectious mononucleosis, Mumps, Lymphocytic choriomeningitis, Herpes simplex, Lymphogranuloma venereum, Leptospirosis, Nonparalytic poliomyelitis, and from the Echo viruses (56,62). The diagnosis in many cases may depend of appropriate serological tests and in some instances on the recovery and identification of the responsible agents. The laboratory diagnosis of the viruses that may cause aseptic meningitis is at times difficult and laborious. In some instances the patient

At times the abdominal pain has been so severe, that some physcians have operated on these people with pleurodynia for an acute abdomen.

In most cases the superficial character of the pain, the absence of deep abdominal pain or rectal tenderness, the relatively normal leukocyte count, and the absence of abnormal roentgenologic findings should aid in the diagnosis of infection by C virus which can be established eventually by recovery of the virus and demonstration of a type-specific antibody response against it in the patient's serum (10).

Herpangina at times may be confused with Herpes simplex. The two are usually easily identified by the typical lesions that they produce. In differentiation, the lesions of Herpes simplex are more commonly, but not always, located at mucocutaneous borders while those in Herpangina usually appear on the soft palate or buccal mucous membrane (10,46). If the differentiation is not possible by that means, they can be identified by isolation and serological methods.

From this discussion one can readily see that Coxsackie infections can be confused with many disease entities and at times, the diagnosis of the causative agent may be extremely difficult or even impossible.

X. LABORATORY DIAGNOSIS

For the laboratory diagnosis of Coxsackie viruses, a fecal specimen and paired blood specimens should be obtained. The first blood specimen should be obtained within 3-4 days from the onset of the acute stage, and the second specimen 7-10 days after the first specimen was obtained. The specimens should be frozen and packed in dry ice and then sent to the laboratory as early as possible. At the laboratory attempts will be made to: 1. Isloate the virus; 2. Identify the virus by sero-logical means; and 3. Demonstrate that the isolated virus is involved in the current infection by a concomitant rise in antibody titer from the acute to the convalescent phase of the disease.

Rather than report on the vast literature on the laboratory diagnosis of Coxsackie viruses, I thought that it would be more interesting to outline the laboratory methods used at the Reiharts laboratory at the University of Nebraska College of Medicine. The following description of the laboratory diagnosis of Coxsackie viruses was outlined to me by Mrs. O.F. Reihart (33).

5-10 grams of the frozen stool specimen are ground in a mortar with sterile alundum until a smooth emulsion is obtained. To this mixture four volumes of Hank's Balance Salt solution, containing a known amount of penicillin and gstreotomycin, are gradually added. These antibiotics are added to reduce b cterial contamination, as C viruses, and other viruses are resistent to them.

This mixture is then further diluted with equal volumes of a maintenance media. The 10cc. mixture is then placed in a plastic container in a high speed refrigerated centrifuge and spun for ½ hour at 19,000 R.P.M.. The supernatant media is then removed and put into tubes.

The next proceedure is to take 2 grown out monolayered tissue culture cell tubes, and to one tube is added 1 ml.of its specific maintenance media (designated tube number 1), and to the other tube is added 1½ ml. of its specific maintenance media (designated tube number 2). The different types of tissue culture cells used in the Reiharts laboratory are He La cells and trypsinized human and monkey kidney cells, human embryo cells, and human amnion cells of the placenta. They have also used trypsinized human skin and uterine cells.

To tube number 1 is added 0.1 ml. of the 10% supernatant media and to tube number 2 is added 0.5 ml. of the 10% supernatant media. These tubes are diluted in this way to try to dilute out toxic stools. A control tube is also set up which contains the same maintenance media and tissue culture cells of the same age and type. These tubes are then placed in an incubator and at the end of an hour tube no. 2 is removed and observed under the microscope for cytotoxic affect from the stools.

If the cell layer shows no cytotoxic affects, the media is removed and 1 ml. of the fresh specific maintenance is then added, and the tube is then placed back into the incubator. If tube no. 2 initially shows cytotoxic affects, the tube is placed back in the incubator and allowed to stand overnight. If the cytotoxic affect from the stool is too great, all the culture cells may be destroyed. If all the tissue culture cells are not destroyed in tube no. 2, 1 ml. of the media in this tube is passed to a fresh culture tube containing trypsinized human kidney cell. The tube is then incubated for forty five minutes and then again observed for cytotoxic affects. If no cytotoxic affects are seen, the tube is returned to the incubator; however, if cytotoxic affects are seen, the media is again changed and then the tube is placed in the incubator and observed in less than 45 minutes for cytotoxic affects. This proceedure may go on many times until the cytotoxic affects of the stools is diluted out. When tube no. 2 has severe cytotoxic changes then the media on tube no. 1 must be changed, so the virus is not lost. One can see that from these cytotoxic affects from toxic stools that at times the media must be frequently changed, and in some instances transfers must be made to fresh tissue culture tubes. If this happens one can see how laborious the isolation of these viruses becomes.

In some instances the cytotoxic affect may have been so severe in both tubes that the tissue culture cells were destroyed. When this happens the whole proceedure must be repeated from the point where the supernatant fluid was added to the tissue culture cells plus their specific maintenance media. If no cytotoxic changes are observed or after they are diluted out, the maintenance media is changed on the tubes at least every five days or sooner if necessary.

It is unusual to have the virus show up in less than 48 hours, and at times the virus may not show up until 10-14 days after incubation. After the cytotoxic affects have been ruled out, and the tissue culture cells show rounding up, pyknotic changes or fall off, a small amount of the media from tubes no. 1 and 2 is removed and passed to fresh tissue culture tubes. This is done in hope-s of speeding up the reaction of the virus. Whe-n ½ of the tissue culture cells have fallen off the side of tube no. 2, a neutralization test is set up. The media from tube no. 2 is serially diluted in accordance with the degree of reaction seen in the culture cells. To these sets of serial diluted tubes and control tubes are added 1/1500cc. of the known antipoliomyelitis serum which is diluted 1:20. Type I is added to one set and control and the same is done with the other two types and

a mixture containing all 3 types. The tubes are then allowed to stand at room temperature for one hour. Then 0.2 cc. of the above serial diluted tubes with their antipoliomyelitis serum are added to tissue culture cell tubes containing 0.8 cc. of its specific maintenance media and placed in the incubator. The reaction then shows up in 2-4 days. If the virus was not a poliomyelitis virus, then a cytopathogenic reaction will be seen in all the sets of the tubes. Now ½ of a litter of suckling mice are innoculated intracerebrally with 0.02 cc. of the media from the tissue culture tubes showing a cytopathogenic reaction. The other ½ of the lit-ter is innoculated subcutaneously with 0.05 cc. of the media in the intrascapular fat pad. If C viruses are present, then in 5-7 days the suckling mice develop tremors of the head and/or tail, ataxia, weakness or paralysis, or spasticity. They also are usually dead in 2 weeks after the innoculation. Whether the mice are positive or negative, a neutralization test is set up gainst the Coxsackie antisera that is available. At the present time these are Types B-1, B-2, B-4, B-5, and A-9.

If no obvious growth was noted in the initial tissue culture cell tubes, then four passes to fresh tissue culture tubes are tried. All negative stools are repeated at least 3 times to help rule out false negatives.

Stools that were negative on He La cells are then repeated on monkey kidney cells and if negative there, they are repeated on human kidney cells.

If acute and convalescent serum from the patient have been sent to the laboratory, then neutralization and C-F tests are set up in an attempt to demonstrate a specific rise in antibodies in the patient's acute and convalescent serum from the causative virus.

Dalldorf (13) pointed out that Coxsackie virus infection is unique in that it is most easily diagnosed by isolation of the virus and that difficulty in isolation occurs if both a Group A and B virus are present. If this situation does occur then the Group A virus must be neutralized with type-specific antiserum to afford the Group B virus an opportunity to develop, to be recognized, and isolated.

In all, one can readily ascertain how laborious and at times how difficult or impossible the isolation of the virus may become .

XI. MUTUALITY AND COMPARATIVE STUDIES OF COXSACKIE AND POLIOMYELITIS VIRUSES

It has been known, since the discovery of C viruses in 1947 (1) that many of the late summer enteric infections are mixed and that Coxsackie and Poliomyelitis viruses may occur simultaneously in the same patient. Numerous investigators have since tried to prove whether

or not these two viruses influence one another. It is now generally accepted that paralytic poliomyelitis is caused by poliomyelitis viruses, but this has not been proven so with non-paralytic or abortive poliomyelitis. Numerous investigators have reported cases clinically diagnosed as non-paralytic poliomyelitis where only a Coxsackie virus was isolated.

Howitt and Nichols (63) were the first to report on research studies for mutual effects of the 2 viruses. Their studies revealed that Cynomolgus monkeys injected by various routes with Group A C viruses were not immune to a subsequent intracerebral innoculation with Brunhilde or Lansing poliomyelitis viruses. Melnick (11) was unable to show interference in mice, monkeys, or chimpanzees when mixtures of poliomyelitis and Group B C viruses were titrated simultaneously in them. Dalldorf (64) reported that when mice (5-8 days of age) were innoculated intraperitoneally or subcutaneously with Group B C viruses and then innoculated intracerebrally 4-10 days later with the Lansing strain of poliomyelitis virus, that 45% of the mice survived and only 1/5 of them manifested paralysis in contrast to a paralytic attack rate of 97% among the controls. In similiar experiments in mice 9-13 days of age, which are less responsive to C virus infections, none of the animals survived and 60%

became paralyzed. Sulkin et al. (65) and Stanley (66) also demonstrated int-erference of poliomyelitis virus with Group B C viruses of the same nature as that seen in Dalldorf's (64) experiments. From these experiments it is now generally agreed that the Group A C viruses have little interference or an opposite affect on poliomyelitis viruses in experimental animals and that interference with poliomyelitis viruses does occur if the animals are inf cted several days prior to the innoculation of poliomyelitis virus and if Group B C viruses are used. Dalldorf (3) stated that Le Bouvier has shown interference of Group B C infections on the course of poliomyelitis in tissue cultures. This interference was not noted with Group A C viruses. This information along with the information from experimental animals has led many investigators to wonder if this interference occurs in patients infected with the Group B C viruses and poliomyelitis viruses, since Pohjanpelto (14) and Dalldorf (3) have pointed out that the types of C viruses found in connection with poliomyelitis have for the most part belonged to Group A. Also because it is a rare event to isolate Group B C viruses and poliomyelitis viruses from a single stool specimen of a patient suffering from poliomyelitis. It has also been observed that when Group B Coxsackie infections have been prevalent, poliomyelitis

has occured infrequent or not al all (3,58). Beale et al. (57) reported that they have never isolate Group B C viruses from cases of paralytic poliomyelitis. In a survey of 484 fecal specimens collected from 282 cases of paralytic poliomyelitis and 202 cases of non-paralytic poliomyelitis, it was noted that 54 Group A strains were isolated from the paralytic cases while the non-paralytic cases yielded 22 Group A and 29 Group B strains (3).

This infrequent finding of Group B C viruses along with poliomyelitis viruses may be due to the relative sensitivity of different technics or may be masked by the possibility of interferences in the laboratory tests (3).

In closing this portion of this section, I thought that it would be appropriate to quote Dalldorf's views on this subject.

"Attention should be directed to the simultaneous occurrence of Group B Coxsackie and poliomyelitis viruses in carriers and patients for an interference, if it occurs in man, might be most apparent in the enteric phase of the disease where it could lead to a depression of infectivity and carrier rates. This would fit our observation that years of high Bornholm and low poliomyelitis incidence have frequently been followed by years of epidemic poliomyelitis which might be expected if subclinical infection and immunization had been suppressed by the Group B

infections. With the newer technics it may prove possible to investigate more thoroughly whatever relationships exist among the enteric viruses".

Both Coxsackie and poliomyelitis induce infections in man along with specific antibody responses. They occur most frequently in the late summer and in the fall. Both viruses may occur in the same patient, and in the human host the viruses may cause apparent or inapparent infection. These viruses may also be carried for some length of time in the human host. They are both resistant to antibiotics and many germicidal agents. Both viruses may be recovered from flies and sewage. These are just a few of the many simularities that the 2 viruses have and a detailed chart has been compiled from the works of Dalldorf (13) and Melnick (67).

See Chart 1 for these details.

CHART 1 (13,67)
(COMPARATIVE STUDIES)

	Polio. Virus	C Virus
Virus Properties		
No. of Known Immurological Types	3	2h
Resistant To:		
Ether	Yes	Yes
Penicillin	Yes	Yes
Streptomycin	Yes	Yes
Chloromycetin	Yes	Yes
Phenol and pH changes	Yes	Yes
Heat Inactivation (Aqueous Medium for 30 Minutes)	50-55 °C	60 °C
Survival at Room Temp. and Frozen State for Days	Yes	Yes
Sedimentation Constant (Svedberg Units, S_{20})	120	120
Filtration Diameter	8-17mu	15-23mu
Experimental Disease		
House:		
Susceptibility:		
Virus Titer in Newborn	Y-SK, $10^{-1.5}$	10^{-5}-10^{-8}
Virus Titer in Adults	$10^{-3.6}$	0-10^{-3}
Virus Distribution	CNS	Muscle, CNS, Feces, Blood Etc.
Chief Lesion	Myelitis	Myositis
Monkey: Cynomolgus	Myelitis with Paralysis	Fever, Pharyngeal, and Intest. Carriers
Antibodies after Feeding Virus:	Yes	Yes
Chimpanzee after Feeding Virus:		
Apparent Disease	No (Occ. Yes)	No
Viremia	?	Yes
Pharyngeal Carrier	?	Yes
Intestinal Carrier	Yes	Yes

CONTINUENCE OF CHART 1

Experimental Disease	Polio. Virus	C Virus
Early antibody Rise	Yes	Yes
Homotypic Immunity	Yes	Yes
Heterotypic Susceptibility	Yes	Yes
Chick Embryo	No	No
Tissue Culture (Human, Monkey, He La Cells, and Mouse Embryo)	Yes	Yes
Interference with Polio. Virus		?
Interference with C Virus	No	
Natural Disease in Man		
Incidence	Common	Common
Virus in Throat and Stools	Yes	Yes
Persists Longer in Stools than Throat	Yes	Yes
Neutralizing Antibodies Present (Early-At Onset of Disease)	Yes	Yes
C-F Antibodies	Yes	Yes
Clinical Features:		
Fatalities	Yes	Yes
Paralysis	Yes	?
Pleocytosis in CSF	Yes	Yes
Stiff Neck and Back	Yes	Yes
Chest and Abdominal pain	No	Yes
Epidemiological Factors		
Family Outbreaks	Yes	Yes
Late Summer and Fall Disease	Yes	Yes
Virus Found in Sewage and Flies	Yes	Yes
More Common in Children	Yes	Yes
Antibodies in Normal Gamma Globulin and in Normal Sera	Yes	Yes

XII. SUMMARY

Coxsackie viruses are world wide in distribution and are a common cause of disease in man. Infections with C viruses occur chiefly in children although infections may occur in other age groups. The C viruses are small in size and extremely resistant to antibiotics, pH changes, and most germicidals. Infection with C viruses occurs chiefly in the late summer and fall. In these respects C viruses are like poliomyelitis viruses. It is postulated that C viruses are spread like other enteric viruses, and familial infections are common with this virus. Active and passive immunity occur with these viruses, but the duration of the immunity is not known. Coxsackie viruses are unique in that they produce lesions, paralysis, and death in suckling mice and that they are identified most easily by isolation. The exact pathogenesis in man is unknown.

Coxsackie viruses are capable of causing Herpangina, Bornholm's disease, Aseptic Meningitis, Encephalitis, and Interstitual Myocarditis in humans. Until the last few years, no deaths due to C viruses were reported. It is now known that fatal Interstitual Myocarditis may occur in infants infected with Group B C viruses. Since most infections with C viruses are self-limited and complete recovery occurs, their importance is not so much the disease itself, but the diagnostic problem that they present.

It has been postulated that C viruses may produce interference with poliomyelitis viruses, since it is a rare occasion when Group B C viruses and poliomyelitis viruses can be isolated from the same stool of a patient suffering from poliomyelitis. It has also been observed that epidemics of poliomyelitis are infrequent or absent when epidemics of Group B infections are present.

With the modern day use of antibiotics more and more cases of unexplained infections are arising. Probably a good share of these unexplained infections are caused by viruses. At present we have many antibacterial drugs but no satisfactory antiviral drugs. It is my belief that the newer tissue culture methods will reveal many unknown aspects of C viruses as well as other viruses, thus opening up a new field of medicine that will help explain many diseases that a-t the present are unexplained.

XIII. BIBLIOGRAPHY

1. Dalldorf, G., and Sickles, G.M., An unidentified filterable agent isolated from the feces of children with paralysis, Science, 108:61-62, (July 16) 1948.

2. Dalldorf, G., Sickles, G.M., Plager, H., and Gifford, R., A virus recovered from the feces of "Poliomyelitis" patients pathogenic for suckling mice, J. Exp. Med., 89:567-582, 1949.

3. Dalldorf, G., The Coxsackie viruses, Ann. Review of Microb., 9:277-292, 1955.

4. Robinson, L.K., Effect of heat and of pH on strains of Coxsackie virus, Proc. Soc. Exper. Biol. and Med., 75:580-582, (Nov.) 1950.

5. Melnick, J.L., and Ledinko, N., Infection of cynomalogus monkeys with the Ohio type Coxsackie virus (C virus), J. Immunol., 64:101-110, (Febr.) 1950.

6. Sulkin, E.S.,The Coxsackie viruses; Properti s, Immunologic l As cts and Role in Human Disease, So. M. J., 45:516-521, (June) 1952.

7. Melnick, J.L.,Rhian, M., Warren, J., and Breese, S.S., Jr., The size of Coxsackie viruses and Lansing poliomyelitis determined by sedimentation and ultrafiltration, J. Immunol., 67/2:151-162, 1951.

8. Himmelweit, F., Findlay, G.M., and Howard, E.M., The size of Coxsackie viruses as estimated by filtration through Gradocal membranes, Brit. J. Exper. Path., 31:809-812, (Dec.) 1950.

9. Quigley, J.J., Ultrafiltration and ultracentrifugation studies of Coxsackie viruses, Proc. Soc. Exp. Biol. and Med., 72:434-435, 1949.

10. Melnick, J.L., and Curnen, E.C., Viral and Rickettsial Infections of Man, Philadelphia, J.B. Lippincott Company, 1952. 2nd. Ed., Chapter 14.

11. Melnick, J.L., Studies of Coxsackie viruses; Properties, Immunological Aspects and Distribution in Nature, Bull. N.Y. Acad. Med., 26:342-356, 1950.

12. Mc Kee, A.P., Layton, J.M., Jeter, W.S., and Porter, J. R., The Coxsackie group of viruses, J. Iowa State M. Soc., 43:143-146, (Apr.) 1953.

13. Dalldorf, G., Introduction to Virology, Springfield, Charles C. Thomas, 1955. Chapter VI.

14. Pohjanpelto, P., The Coxsackie group of viruses; Epidemilolgical studies, Ann. Med. Exper. Et. Biol. Fenniae, 33:1-75, 1955.

15. Cole, R.M., Bell, J.A., Beeman, E.A., and Huebner, R.J., Studies of Coxsackie viruses; Observation on epidemiological aspects of Group A viruses, Am. J. Pub. Health, 41:1342-1358, (Nov.) 1951.

16. Huebner, R.J., Cole, R.M., Beeman, E.A., Bell, J.A., and Peers, J.H., Herpangina; Etiological studies of a specific infectious disease, J.A.M.A., 142.2: 628-634, 1951.

17. Huebner, R.J., Armstrong, C., Beeman, E.A., and Cole, R.M., Studies of Coxsackie viruses; Prliminary report on occurence of Coxsackie virus in a Southern Maryland Community, J.A.M.A., 144:609-613, 1950.

18. Melnick, J.L., Emmons, J., and Coffey, J.H., Seasonal distribution of Coxsackie viruses in Urban Sewage and Flies, Am. J. Hyg., 59/2:164-184, (Mar.) 1954.

19. Dalldorf, G., The Coxsackie viruses, Bull. N.Y. Acad. Med., 26:329-335, 1950.

20. Dalldorf, G., and Gifford, R., Clinical and epidemiologic observations of Coxsackie virus infection, New. Eng. J. Med., 244:868-873, (June 7) 1951.

21. Thinnes, J.L., Jr., The Coxsackie viruses in clinical practice, Ohio M. J., 50:39-42, (Jan.) 1954.

22. Curnen, E.C., Human disease associated with Coxsackie viruses, Bull. N.Y. Acad. Med., 26:335-342, 1950.

23. Carpenter, C.M., and Boak, R.A., Coxsackie viruses, Calif. Med., 77/2:127-130, (Aug.) 1952.

24. Rhodes. A.J., and Van Rooyen, C.E., Textbook of Virology, Baltimore, Williams and Wilkins Company, 1953. 2nd. Ed., Chapter 38.

25. Lazarus, A.S., Johnson, E.A., and Galbraith, J.E., An outbreak of Epidemic Pleurodynia with special reference to the laboratory diagnosis of Coxsackie virus infections, Am. J. Pub. Health, 42:20-26, 1952.

26. Howitt, B.F., Isolation and diffenentiation of the Coxsackie group of Viruses, Fed. Proc., 9:574-580, (Sept.) 1950.

27. Howitt, B.F., Recovery of the Coxsackie group of viruses from human sources, Proc. Soc. Exper. Biol. and Med., 73:443-448, (Mar.) 1950.

28. Lepine, P., Desse G., and Sautter, V., Histological examination and isolation of Coxsackie virus from muscle biopsy material of cases of Epidemic Myalgia (Bornholm's Disease), Bull. Acad. Nat. Med. (Paris), 136/5-6:66-69, 1952.

29. Creveld, Van S., and Jager, H. De., Myocarditis in Newborns, caused by Coxsackie virus, Ann. Paedi., 187/½:100-112, (Juli/Aug.) 1956.

30. Melnick, J.L., and Penner, L.R., The survival of Poliomyelitis and Coxsackie viruses following their ingestion by flies, J. Exper. Med., 96:255-271, 1952.

31. Fischer, R.G., and Syverton, J.T., Cockroaches as an experimental vector of Coxsackie virus, Am. J. Trop. Med., 31:238-242, (Mar.) 1951.

32. Tobin, J.OH., Coxsackie viruses, Brit. M. Bull., 9/3:201-205, 1953.

33. Reihart, O.F. (Mrs.), Personal communication to the author.

34. Huebner, R.J., B eeman, E.A., Cole, R.M., B-eigelman, P.M., and Bell, J.A., The importance of Coxsackie viruses in human disease, particularly Herpangina and Epidemic Pleurodynia, N. Eng. J. M., 247/7:249-256, (Aug. 14) 1952.

35. Huebner, R.J., Beeman, E.A., Cole, R.M., Beigelman, P.M., and Bell, J.A., Continuence of the above subject, N. Eng. J. M., 247/8:285-289, (Aug. 21) 1952.

36. Gifford, R., and Dalldorf, G., The morbid anatomy of experimental Coxsackie virus infection, Am. J. of Path., 27/6:1047-1055, (Nov.-Dec.) 1951.

37. Kunz, L.J., Richardson, S., and Pappenheimer, A.M., Pancreatic disease in mothers of suckling mice infected with Conn.-5 type of Coxsackie virus, Proc. Soc. Exper. Biol. and Med., 79:488-491, (Mar.) 1951.

38. Keller, W., and Vivell, O., Occurence of infection due to Coxsackie viruses in Germany, Klin. Wschr., 29/43-44:741.-745, 1951.

39. Shaw, E.W., Melnick, J.L., and Curnen, E.C., Infection of laboratory workers with Coxsackie viruses, Ann. Int. Med., 33:32-40, (July) 1950.

40. Kraft, L.M., and Melnick, J.L., Complement fixation tests with homologous and heterologous types of Coxsackie virus in man, J. Immunol., 68:297-310, 1952.

41. Beeman, E.A., and Huebner, R.J., Evaluation of serological methods for demonstrating antibody responses to Group A Coxsackie (Herpangina) viruses, J. Immunol., 68:663-673, 1952.

42. Curnen, E.C., and Melnick, J.L., Poliomyelitis and Coxsackie viruses in Paralytic Polio., Ped., 8:237-248, 1951.

43. Melnick, J.L., and Ledinko, M., Social serology; Antibody levels in a normal young population during an epidemic of Poliomyelitis, Am. J. Hyg., 54:354-382, 1951.

44. Yeager, W., Clinical Coxsackie disease, Miss. Doctor, 31/5:147-148, (Oct.) 1953.

45. Findlay, G.M., and Howard, E.M., Coxsackie viruses and Bornholm disease, Brit. Med. J., 1:1233-1236, (May 27) 1950.

46. Zahorsky, J., Herpetic sore throat, So. M. J., 13:871-873, 1920.

47. Zahorsky, J., Herpangina (a specific disease), Arch. Pediat., 41:181-184, 1924.

48. Parrott, R. ., Ross, S., Burke, F.G., and Rice, E.C., Herpangina; Clinical studies of a specific infectious disease, New Eng. J. Med., 245:275-280, 1951.

49. Webb, C.H., Wolfe, S.G., and Howitt, B.F., "Three day fever"; An acute febrile disease of childhood (further observation), Am. J. Dis. Child., 80:245-253, 1950.

50. Beeman, E.A., Cole, R.M., and Huebner, R.J., Studies in Humans of neutralizing antibodies against Group A Coxsackie (Herpangina) viruses; Prevalence of antibodies in a community in 1949 and 1950, Am. J. Hyg., 56:215-231, 1952.

51. Howard, T., Neymuller, C.A., Edson, J., Ittner, E., Watson, J., and Cassidy, M.L., Epidemic Pleurodynia in Brooklyn in the summer of 1942, J.A.M.A., 121/12:925-929, (Mar. 20) 1943.

52. Weller, T.H., Enders, J.F., Buckingham, M., and Finn, J.J., Jr., The etiology of Epidemic Pleurodynia; A study of two viruses isolated from a typical outbreak, J. Immunol., 65:337-346, 1950.

53. Nichamin, S.J., Pleurodynia in Children, J.A.M.A., 148.2:1002-1005, (Mar.) 1952.

54. Curnen, E.C., Shaw, E.W., and Melnick, J.L., Disease resembling Nonparalytic Poliomyelitis associated with a virus pathogenic for infant mice, J.A.M.A., 141:894-902, (Nov. 26) 1949.

55. Hummeler, K., Kirk, D., and Ostapiak, M., Aseptic Meningitis caused b-y Coxsackie virus from isolation of virus from cerebral spinal fluid, J.A.M.A., 156.1:676-680, (Oct. 16) 1954.

56. Mc Leod, D.L., Beale, A.J., Mc Naughton, G.A., and Rhodes, A.J., Clinical features of Aseptic Meningitis caused by Coxsackie-B virus, Lancet, Lond., 271 (6945):701-703, (Oct. 6) 1956.

57. Beale, A.J., Duncan, D., Stackiw, W., Dempster, G., and Rhodes, A.J., Further observations on the laboratory diagnosis of Aseptic Meningitis caused by Group B Coxsackie virus, Canad. J. Pub. Hlth., 47:179-187, (May) 1956.

58. St-anley, N.F., Dorman, D.C., and Ponsford, J., A hitherto undescribed Group of Coxsackie Viruses associated with an outbreak of Encephalitis, Austral. J. Exptl. Biol., 31:31-40, 1953.

59. Javett, S.N., Heymann, S., Mundel, B., Pepler, W.J., Lurie, H.I., Gear, J., Measroch, V., and Kirsch, Z., 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., 48.1:1-23, (Jan.) 1956.

60. Verlinde, J.D., Van Tongeren, H.A.E., and Kret, A., Myocarditis in newborns due to Group B Coxsackie virus; Virus studies, Ann. Paediat., 187:113-118, 1956.

61. Kibrick, S., and Benirschke, K., Acute Aseptic Myocarditis and Meningoencephalitis in the newborn child infected with Coxsackie virus Group B, Type 3, N. Eng. J. Med., 255/19:883-889, (Nov. 8) 1956.

62. Huebner, R.J., Cecil and Loeb Textbook of Medicine, Philadelphia, W.B. Saunders Company, 1955. 5th. Ed., p. 69.

63. Howitt, B.F., and Nichols, V.J., Innoculation of Cynomolgus monkeys with Coxsackie virus alone, combined, or with Poliomyelitis virus, J. Immunol., 68:599-608, 1952.

64. Dalldorf, G., The sparing affect of C virus infections on experimental poliomyelitis, J. Exp. Med., 94/1:65-71, 1951.

65. Sulkin, S.E., Wallis, C., and Murphy, T.P., Jr., Mixed infections with Coxsackie and Lansing Poliomyelitis viruses in mice, Proc. Soc. Exptl. Biol. Med., 84:184-189, 1953.

66. Stanley, N.F., Attempts to demonstrate interference between Coxsackie and Poliomyelitis viruses in mice and monkeys, Proc. Soc. Exptl. Biol. Med., 81:430-433, 1952.

67. Melnick, J.I., The Poliomyelitis, Encephlomyocarditis, and Coxsackie group of viruses, Bact. Rev., 14:233-244, (Sept.) 1950.