SECTION ON MICROBIOLOGY

DECEMBER 21, 1949

I. EXECUTIVE SESSION

Reading of the Minutes

II. PAPERS OF THE EVENING

INFECTIONS WITH COXSACKIE VIRUSES

a. The Coxsackie viruses
Gilbert Dalldorf
State of New York
Department of Health, Albany

b. Clinical and epidemiological features of human disease associated with viruses pathogenic for infant mice (Coxsackie group)
Edward C. Curnen (by invitation)
Yale University School of Medicine

c. Studies on viruses pathogenic for infant mice (Coxsackie group); properties, distribution in nature, and immunological aspects
Joseph L. Melnick (by invitation)
Yale University School of Medicine

FRANK L. HORSFALL, JR.
Chairman
The Rockefeller Institute for Medical Research

HARRY MOST
Secretary
New York University, College of Medicine

*The Coxsackie Viruses**

GILBERT DALLDORF

It has now been repeatedly confirmed that some patients having the combination of symptoms we associate with poliomyelitis, harbor in their feces viruses that are pathogenic for suckling mice.[1-4] In a number of patients the appearance of neutralizing antibodies and the disappearance of fecal virus have been shown to coincide with recovery.[1,2] There is some evidence that these viruses do not occur in healthy individuals.[2] Laboratory infections have been described and similar viruses have been tentatively associated with illnesses other than poliomyelitis.[2] The viruses are presumably widely disseminated and appear to be rather common incitants of infection of man. I have set myself the tasks of defining and classifying these agents as completely as present knowledge permits and then of describing the circumstances under which we have found them.

Twenty-eight strains isolated in our laboratories have been studied experimentally to some degree; all have been compared histologically and a number serologically. Something is known of their physical properties.

The characteristics originally noted, the susceptibility of suckling mice and the muscle lesions, are common to all of our strains. Unweaned mice are still required for their isolation and propagation and, while it has been possible to produce focal lesions in the

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muscles of mice weighing as much as 10 to 12 grams, we have failed to propagate the virus in series in such animals or to adapt it to older mice by alternate passage through suckling and weaned mice.[5]

It is noteworthy that the resistance of mature mice is not due to the peripheral barriers that interfere with certain other virus infections,[6] for older mice are equally resistant to intracerebral inoculation. Indeed in testing for the presence of virus we routinely inoculate one group of mice intracerebrally and another intraperitoneally. At times the peripheral route is more effective than the central one.

Immature mice are known to offer somewhat similar practical advantages in the study of yellow fever[7] and St. Louis encephalitis[8] but have been little used in the isolation of viruses. We cannot foretell, therefore, whether others will be found to share this property of the Coxsackie viruses or how conclusive a test of their identity the age factor may be.

We knew from the first that the muscle lesions are not specific for we had long seen them in MM virus infection in hamsters and Doctor Pappenheimer showed me such lesions in mice inoculated with Theiler's virus. Similar lesions occur in the muscles in experimental scurvy and in vitamin E deficiency. Nevertheless, the morbid anatomy of Coxsackie virus infection is very helpful not only in identification but also in classification. It readily permits us to separate our strains into two groups that we refer to as A and B. A includes 20 of our strains and B, 8. The distinction may be helpful in reconciling divergent observations.

Differences between the groups are evident in the infected animals. Group-A mice become weak and then helplessly paralyzed. Their extremities are leaden or immobile and death soon follows. Those that do survive for a few days have whitish streaks in their muscles that may be easily seen once the animal is skinned. The Group-B mice on the other hand may be either spastic and tremulous or paralyzed. They usuallly survive longer and, when they are dissected, their brains are found to be softened and their fat pads conspicuously pale.

Histologic examination of Group-A mice shows only a generalized destruction of striated muscles. These are uniformly involved. The Group-B mice have similar lesions but they are focal and less extensive. In addition, Group-B mice have a severe encephalopathy. This involves the cerebral hemispheres and consists first of patchy dissolution of the neurones, followed by softening of the matrix, and ending in cystic degeneration. Smaller lesions occur along the neuraxis but usually end in the upper levels of the spinal cord. We have never found destruction of the anterior horns of the spinal cord and only infrequently have the olfactory lobes been involved although these are very prominent in the mouse.

In addition, a unique lesion may sometimes be found in the fat pads. The pad between the scapulae is a common site. Microscopic examination shows that this process begins on the periphery of the lobules and is necrotizing in nature. The lesion is not so punctate as pancreatic fat necrosis nor are the changes in the fat as complete. These lesions are quickly recognized in preparations stained by Giemsa's method.

There are other differences. Group A includes a number of serologically distinct types while our preliminary tests suggest that the Group-B strains are related. Group-A strains are as a rule easily isolated and established in mice. The adaptation of Group-B strains is sometimes precarious and several have behaved erratically.

These are the salient features of the experimental disease in our experience. Before describing the viruses, it may be well to comment on the pathogenesis of the muscle lesion. We have from the first interpreted it as a form of degeneration resembling Zenker's degeneration rather than a kind of myositis. In paralyzed mice one finds hyalin fragments of the muscle fibers surrounded by a heavy cellular infiltration largely composed of young muscle cells. The lesion represents an extreme degree of regeneration. This is easily verified if one examines mice early and late. In the early stages, one may see that degeneration of the muscle fibers precedes the cellular infiltration and, in the late preparations, one can easily recognize the young cells for

what they really are, for by then they are well differentiated. This distinction may be of some value. For one thing we should perhaps look to the muscle cell to learn more about the virus and, since muscle cells are relatively well understood, this seems a hopeful prospect. Furthermore, if muscle lesions occur in man, they may not resemble the mouse lesion which probably owes much of its cellular character to the age and species of the animal rather than to the process itself.

The size of one of the Group-A viruses has been measured by means of Elford membranes.[9] Infectivity has regularly been found in the filtrates of membranes having an estimated pore size of 18 mμ, membranes that consistently retain MM virus. Elford's correction factor[10] would place the diameter between 6 and 9 mμ which is not signicantly different from observations made by others of poliomyelitis virus.

The electron microscope has failed to reveal any particulate material in infective preparations that cannot be found in suspensions of normal tissues. This has been true of suspensions of muscle as well as brain. The failure may be due to the technical methods used in purification but it may also be accounted for by the size or density and thickness of the virus. Hillier[11] reports the limiting resolution of the electron microscope to be 5 mμ for viruses on collodion membranes. Our experience with Coxsackie virus parallels that of Rhian, Lensen, and Williams[12] who, working with the Lansing strain, concluded that poliomyelitis virus has never been unequivocally identified on electron micrographs.

The centrifugation studies also suggest that the virus is very small. Speeds of 550 r.p.s. for 30 minutes in a 6-inch rotor fail to sediment all the virus. Similar studies of the B Group are now in progress.

Group-A strains are inactivated at 53° to 55°C. in 30 minutes. They are relatively little affected by pH, being much more stable than MM virus and resembling in this respect the Lansing strain of poliomyelitis.[13] Suspensions have remained infective after standing at room temperature for seven days at pH 4 to 8.5. Coxsackie virus is stable, in the form of mouse brain, for at least one year in 50 per cent glycerol and for many months at —70°C. The limits have not been established.

Our major interest is poliomyelitis. Coxsackie virus was originally isolated from two young boys with symptoms of poliomyelitis. Both became ill in August, 1947, during a mild outbreak of what was considered to be poliomyelitis. The older, a boy of nine years, complained of headache, nausea, and pain in his legs. He was febrile, his cerebrospinal fluid contained an abnormal number of leukocytes and in the days that followed his trunk and back muscles became weak. Paralysis persisted for more than seven months.

The other patient was three and one-half years old. His left thigh became weak two days after prodromal symptoms of sore throat and lethargy. Paralysis disappeared within eight months but, when last seen, he still had measurable atrophy of the left calf muscles.

Our second group of specimens was collected in Wilmington, Delaware, where an epidemic of 122 cases of poliomyelitis occurred during the summer of 1947. The epidemic was remarkable because of the extremely low fatality rate (1.6 per cent) and the failure to identify viruses pathogenic for monkeys in suitable specimens. In other respects, it seems to have been typical and 32 per cent of the patients were paralyzed. Four patients had bulbar symptoms.[14]

Dr. Robert Ward sent us three fecal specimens that he had tested in monkeys and found free of poliomyelitis virus. One of these, a concentrate of two pooled fecal suspensions, yielded in suckling mice a second serologic type of Group-A virus. We then secured from Miss Beatrice Howitt additional specimens including 14 acute-phase fecal specimens. Four of these yielded virus in mice. Three were serologically similar to the type recovered from the specimen submitted by Doctor Ward; the fourth was like the original strain. Fortunately, we were able to demonstrate that this patient had a serologic response to the virus isolated from his own feces.

Miss Howitt had tested 12 fecal specimens, including 9 of those given to us, and 2 naso-pharyngeal washings in rhesus mon-

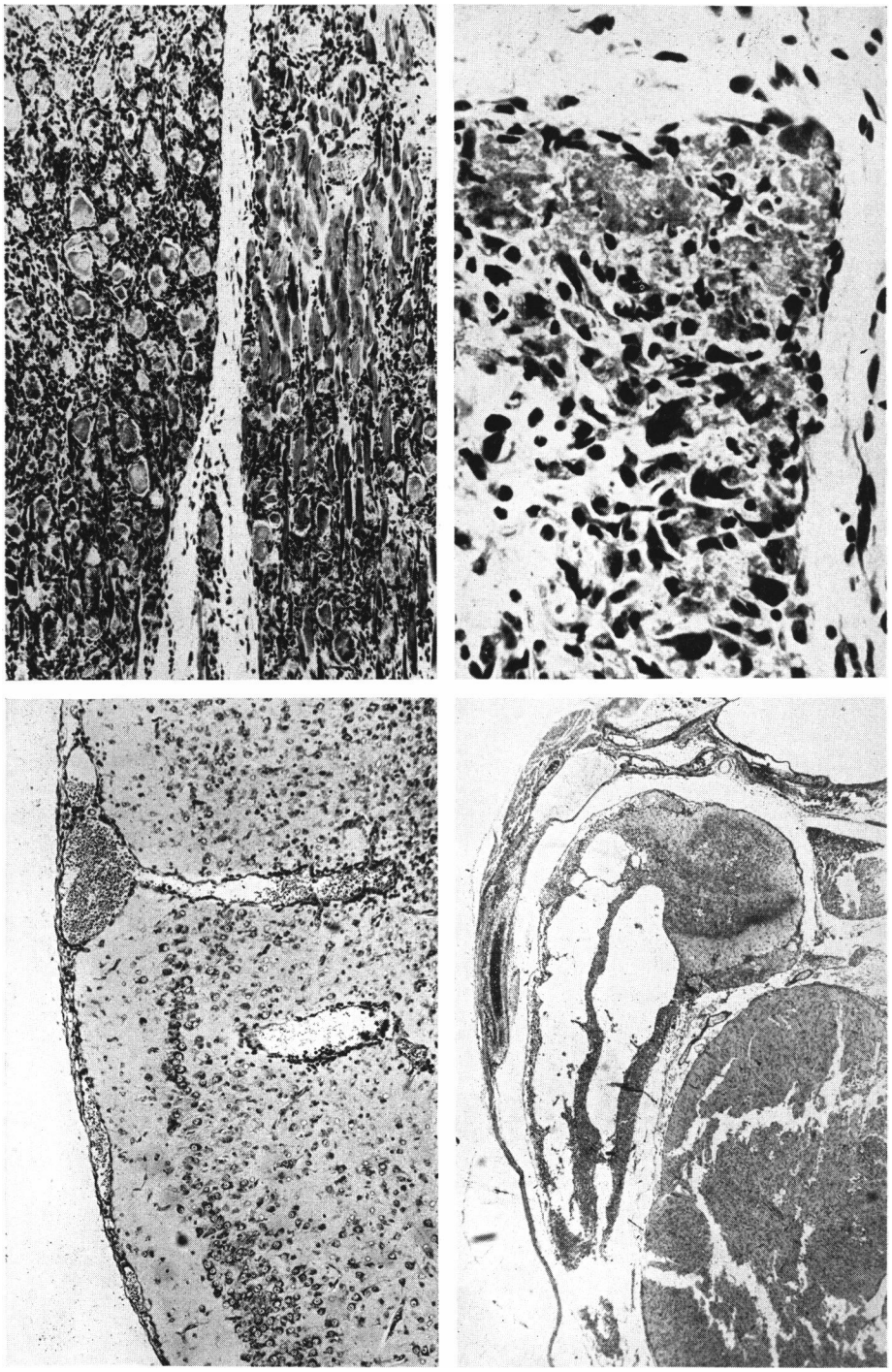

PLATE I—(*Legend on next page*)

keys with negative results. Thus, 16 specimens from the Wilmington epidemic suitably tested for poliomyelitis virus had been found to be noninfective for monkeys. One isolation of poliomyelitis virus had been made in Delaware during the summer of 1947 but that specimen consisted of a pool of three fecal suspensions, one from a patient in the suburbs of Wilmington, one from a child later recognized to have osteomyelitis rather than poliomyelitis, and the third from a patient from Milton, Delaware, 70 miles south of Wilmington. Poliomyellitis virus was again found in Milton during the following winter.

Three of the Wilmington patients from whom Coxsackie viruses were isolated were also listed as paralytic but the disability seems to have been slight. The two that were re-examined the following December had completely recovered.

Since 1947 we have tested more than 400 specimens for the presence of Coxsackie virus. I cannot now report the results of that survey for some records are still incomplete and others should be verified but we have tabulated 245 cases in which the record indicates that paralysis was or was not present. Ninety-two or 37 per cent were classified as paralyzed. Of the 27 patients that have yielded virus and from whom we have information regarding the presence of paralysis, 10, or again 37 per cent, were classified as paralytic. Six of the 27 have or did have residual paralysis and several are still in orthopedic hospitals. The conclusion seems unavoidable that Coxsackie viruses may be recovered from patients with paralysis as well as from patients without paralysis. It does not prove that Coxsackie virus may *cause* paralysis in man. Most of our specimens have been collected at times when and places where poliomyelitis virus infection is presumably common. We have recently succeeded in causing paralysis in both young cynomolgous monkeys and suckling mice inoculated with the same fecal suspension. The lesions in the monkeys were confined to the central nervous system while those in the mice were limited to the muscles. Material from the monkeys failed to produce the signs of Coxsackie virus infection in suckling mice. These studies are still in progress.

Perhaps our experience during the past two years is an intimation of the relative importance of the two diseases. During this past summer when New York suffered its fourth largest epidemic of poliomyelitis, we isolated Coxsackie viruses only half as frequently as during 1948 when a fourth as many cases of poliomyelitis were reported.

In our experience, therefore, the two agents are intimately associated in the field and individual cases require very thorough study with both viruses in mind. One of our positive fecal specimens induced a sharp immune response but no paralysis in a young cynomolgous monkey that had received a single intracerebral injection of a fecal suspension. Thus, monkey testing of strains of Coxsackie virus should not be neglected.

I have tabulated the age distribution of the cases we have tested and those that

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PLATE I.

The lesions in mice infected with Coxsackie virus.

Upper left shows a typical, well-developed degeneration of striated muscles. The interstitial cells are largely young muscle cells. Hyalin degeneration of the fragmented muscle fibers is prominent.

The upper right photograph is of the margin of a fat pad and shows the kind of necrosis that is sometimes seen.

The lower left photograph is of an early lesion of the cerebrum following infection with a Group B strain. The parenchymal cells have disappeared from one segment of the cortex.

The lower right photograph is of a late cerebral lesion and shows cystic degeneration in a mouse that survived several days of illness.

yielded virus. While our total specimens were rather equally drawn from the four age groups, the infected individuals were predominately children. Seventy-five per cent were younger than ten years. Thus Coxsackie virus infection appears to be a disease of the very young and to differ in this respect from poliomyelitis as it now occurs in New York where roughly one-fifth of the cases are in adults.

TABLE I—CLASSIFICATION BY AGE OF INDIVIDUALS TESTED AND OF PROVEN CASES OF COXSACKIE VIRUS INFECTION

	Individuals tested		*Proven cases*	
Age	*No.*	*%*	*No.*	*%*
Yrs.				
Less than 5	114	26.3	11	39.3
5 to 9 inc.	118	27.2	10	35.7
10 to 19 inc.	112	25.9	6	21.4
Over 19	89	20.6	1	3.6
Total	433	100.0%	28	100.0%

A breakdown by sex shows that our samples were evenly drawn from males and females while among our positive cases males were twice as common as females. This was also true in Connecticut[2] and is one of the most constant epidemiologic features of poliomyelitis. It is therefore of no value in distinguishing between the two diseases.

Some of you may consider the testing of hundreds of specimens for the presence of virus a rather laborious undertaking. There is no satisfactory alternative. Serologic testing is complicated by the number of strains involved. In the case of type 2, in our experience the most common strain, there is a further difficulty in that all of our patients have had homologous neutralizing antibodies in their acute-phase sera. In the latter respect, type 2 infection is similar to poliomyelitis. We are indeed skeptical of the significance of antibodies to any of the Group-A strains since reacting sera are so common. Pooled adult sera effectively neutralize all of these strains.

We may conclude, I believe, that we have learned to identify a rather common infectious disease, a disease that may simulate poliomyelitis, that occurs in close association with poliomyelitis and that has some characteristics in common with poliomyelitis. We hope that it will somehow serve to explain poliomyelitis. For the present we should be satisfied to observe and describe and suspend judgment.

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*Human Disease Associated with the Coxsackie Viruses**

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INTRODUCTION

Within the past 2 years a group of viruses with unusual and distinctive properties has been encountered and identified with human disease. The first isolation was reported in 1948 by Dalldorf ond Sickles[1] who discovered the agent in feces obtained during the acute stage of illness from 2 boys with muscular paralysis. These patients resided in Coxsackie, New York and were observed during a small outbreak of poliomyelitis. Additional isolations were subsequently reported by Dalldorf and his associates[2,3] and by other investigators.[4,5]

These viruses are characterized by their capacity to cause paralysis and death and to induce "severe destructive lesions of the striated muscles, with or without encephalomalacia in immature mice and hamsters."[6] They appear to differ immunologically from poliomyelitis viruses and do not cause the distinctive signs or lesions of poliomyelitis when injected into monkeys.[2,4] Dalldorf has suggested that the term "Coxsackie virus" be used as a provisional designation for agents of this type.[6] For convenience, in reference, we have used an abbreviation of this term, "C virus."

It is the purpose of this paper to review some preliminary observations concerning the epidemiology and clinical features of human diseases associated with these agents.

RELATION OF COXSACKIE VIRUSES TO HUMAN DISEASE

Epidemiology of Cases in Southern New England: During the summers of 1947 and 1948 scattered outbreaks of acute febrile illnesses occurred in southern New England[7-9] as well as elsewhere.[10-12] Many of the cases which were observed resembled nonparalytic forms of poliomyelitis, and in certain instances, strains of poliomyelitis virus were recovered.[4,12,13] It seemed possible, however, that some of the illnesses might have been caused by a different agent.

In order to investigate this possibility a study was made of 157 patients with a diagnosis of poliomyelitis or aseptic meningitis, cause unknown, who were admitted during 1948 to hospitals in Connecticut and Rhode Island.[8] All of these patients had pleocytosis of the cerebrospinal fluid and none died. Forty-four (28 per cent) of the cases were classified as paralytic and 113 (72 per cent) as nonparalytic. The ratio of males to females was approximately 2 to 1 in the paralytic group and almost 3 to 1 in the

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