THE MEDICAL AND PUBLIC HEALTH IMPORTANCE OF THE COXSACKIE VIRUSES

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The Coxsackie group of viruses derived their name from the Hudson River Town, Coxsackie, in New York State, where the first two members of this group were identified by Dalldorf and Sickles in 1947.[1] Both these viruses were isolated in suckling mice from the faeces of children acutely ill with paralytic poliomyelitis. The pathogenicity for suckling mice is one of the distinguishing features of this group of viruses, and their relative lack of pathogenicity for adult mice and other experimental animals accounts for their escape from recognition hitherto.

Subsequent studies have shown that there are 2 groups of Coxsackie virus, named groups A and B. Mice infected with group-A strains develop flaccid paralysis and on histological examination show only hyalin degeneration and active repair of the voluntary striated muscles. This diffuse muscle destruction and the absence of lesions elsewhere are characteristic of group-A strains. Mice infected with group-B strains develop weakness, tremors, spasms, and paralysis. The voluntary muscles show focal lesions. The pads of fat between the scapulae show whitish degeneration and on microscopic examination necrosis and an inflammatory reaction, followed later by calcium deposits in the necrotic areas. This lesion of the fat pad is unique and characteristic of Coxsackie-B virus infection. The central nervous system is often involved, showing a patchy dissolution of the parenchymal cells and associated with an inflammatory infiltration, particularly in the region of the blood vessels. The heart muscle may also show foci of necrosis and acute inflammation. Similar focal lesions may be found in the pancreas and liver. These histological pictures are of differential value in distinguishing Coxsackie group-A viruses from Coxsackie group-B viruses. Serological studies have revealed that there are at least 17 different immunological types of group-A virus and 5 of group-B virus.

When they were discovered and for some time afterwards it was not known what diseases the Coxsackie viruses caused. The cases from which the first two viruses were isolated were proved to be true cases of paralytic poliomyelitis infected also with poliovirus. As a result of more recent studies their pathogenicity has now been more clearly defined.[2] The group-A viruses have been incriminated as the cause of herpangina and, as the present studies show, are possibly related to a number of other illnesses. Coxsackie-B viruses have been incriminated as the cause of Bornholm disease and, as the present studies show, in parts of Southern Africa are important causes of aseptic meningoencephalitis and of myocarditis neonatorum. A study to assess the importance of these viruses in South Africa was begun about 5 years ago and this paper besides giving more general information based on the observations of others in other countries summarizes our findings in Southern Africa.

Incidence of Coxsackie Virus Infections

An epidemiological study of a group of Bantu (African) infants, living under slum conditions in an urban Native township, was undertaken to determine the incidence of these infections. This study has now extended over 4 years and the results will be described in detail in another paper. At present it is of interest to note that during this period most of the then known types of Coxsackie virus were isolated and identified. In addition several new types, confirmed as such by Dalldorf and Sickles, were brought to light. Unfortunately, it was not always possible to relate these infections to illness. However, other studies proceeding concurrently have defined the clinical conditions caused by these viruses. Both groups are of importance and will be considered separately.

COXSACKIE GROUP-A VIRUS INFECTIONS

Herpangina

Herpangina was first described in 1920 by Zahorsky.[3] More recently studies by Huebner[4] and others have shown that this condition is caused by Coxsackie group-A viruses. The patients are taken suddenly ill, often with high fever, at times accompanied by chilliness, fatigue,

nausea, anorexia, vomiting, and abdominal pain, and occasionally diarrhoea. They suffer from headache, pain in the neck, back and extremities, and muscle tenderness. They experience moderate pain on swallowing, but do not have respiratory symptoms. Papulovesicles, about 2-10 in number, are found in the posterior part of the mouth, varying in size from a grain of rice to a pea, often arranged in groups and surrounded by a marked reddened area. The lesions are confined to the soft palate, the uvula, and the anterior and posterior parts of the fauces, and they also occur in the posterior wall of the pharynx and the tonsils and are often associated with intense congestion of the fauces and tonsils. The vesicles often rupture to form superficial ulcers, covered with a thin greyish-white exudate. The illness is benign and the temperature returns to normal within 2-4 days after onset. The disease exhibits a seasonal peak during the summer and autumn months. It is common in South Africa, and Coxsackie-A virus has been isolated from cases in several outbreaks, thus confirming its aetiological role.

Acute Febrile Lymphadenitis

An outbreak of an illness clinically resembling glandular fever, affecting many of the residents of a school hostel in Middelburg, Transvaal, was studied in collaboration with Dr. I. M. Patz. This outbreak will be described in detail in a separate paper. At present it suffices to note that of 200 children from 5 to 13 years of age in this primary school hostel, 30 were affected in 3 distinct waves during the period from 8 August to 16 October 1953. These patients at the onset, which was sudden, complained of feeling feverish and of enlarged tender glands, sometimes associated with painful stiffness of the neck. The temperature ranged from 99°F to 103·4°F and in the majority of cases lasted from 24 to 96 hours, with a range from a few hours to 10 days. The majority of patients also complained of frontal headache. Only 20% complained of sore throat, particularly on swallowing. Blisters suggestive of herpangina were not seen and there was no membrane apparent. The fauces were slightly injected. Abdominal pain occurred in 2 cases and vomiting in 3. In 28 of these 30 cases lymphadenopathy was found, involving the cervical glands. In most cases more than one group of glands were involved, often successively. The glands were painful and tender. The axillary and inguinal glands were involved in only 2 of the cases. Enlargement of the spleen was detected in 3 of the patients. No rash was seen in these cases, but in some patients with a similar condition in Johannesburg at the same time a few roseolar spots, somewhat like the rose spots of typhoid fever, were seen on the abdomen. Relapses after an interval of 4 days and 6 weeks respectively occurred in 2 cases. The majority of patients made a good recovery. The Paul-Bunnell test gave negative results in 24 of the 25 children whose blood was taken for this test. The remaining case gave a titre of 1 : 160, suggestive of glandular fever, but unfortunately absorption tests were not done. However, it is clear from these findings that these cases in their serological tests did not conform with classical glandular fever. This opinion was supported by the results of the blood count made on 23 of these cases on the 3-4th day of illness and 1 week and 3-4 weeks later. The leucocyte count varied from 5,200 to 17,300 and the majority of cases (15 of 23) had initial counts of over 10,000 white cells per c.cm. with a neutrophilia. In none of the cases was the blood picture suggestive of glandular fever.

Coxsackie group-A virus was isolated from the stool of 5 of these patients and from the blood of one of them. These 6 viruses were typed and, somewhat unexpectedly, 3 were found to be Type-5 and 3 to be Type-6 Coxsackie-A viruses. The finding of the virus in the stool of a patient does not necessarily mean that it is responsible for the patient's illness. However, the suspicion that this condition was caused by Coxsackie-A virus is greatly strengthened by finding this virus in the blood of one of the patients.

Meningo-encephalitis

In a study of cases of meningo-encephalitis admitted to the Johannesburg Fever Hospital in the summer and autumn of 1953-54, it was noted that a Coxsackie group-A virus was isolated from the faeces of 5. It was pointed out then that this may have been a coincidental finding. However, from 3 of these 9 cases the same type of virus was also isolated from the cerebrospinal fluid. It therefore was clear that, in these 3 cases at least, the illness was caused by Coxsackie-A virus.

Since that time several similar cases have been investigated. These were admitted to hospital with a provisional diagnosis of poliomyelitis. Several of these patients were less than 2 years of age, but those old enough to complain said that their illness had a fairly sudden onset, with sore throat, slight nausea, and sometimes vomiting, muscle pain and headache. After they had been feverish for 2-3 days their headache became severe and painful stiffness of the neck and back developed. None developed paralysis.

Lumbar puncture revealed a clear fluid, but on microscopical examination a pleocytosis with a variable proportion of polymorphonuclear cells and monocytes was noted. The routine tests for the presence of poliovirus in the faeces gave negative results. However, in 7 such cases Coxsackie group-A virus was isolated from the faeces in 3, from a throat swab in one, and from the cerebrospinal fluid in 3. These latter findings clearly indicate that the meningo-encephalitis of these patients was due to Coxsackie group-A virus.

This conclusion is supported by Johnsson and Lindahl[6] who, in a study of herpangina occurring in Stockholm, Sweden, noted that one of their cases presented signs of encephalitis and another the signs of aseptic meningitis. From both these cases Coxsackie group-A virus was isolated from the faeces. There thus is little doubt that Coxsackie group-A virus is a cause of meningo-encephalitis.

Association with Poliovirus in cases of Paralytic Poliomyelitis

It will be recalled that the first two Coxsackie viruses identified were isolated from cases of paralytic polio-

myelitis, from which poliovirus was also isolated. It is now clear that this association frequently occurs in cases of paralytic poliomyelitis. It has been suggested that it is purely coincidental, and it may well be. However, Coxsackie group-B viruses are rarely found in association with poliovirus.

In our studies carried out since 1953, of 80 cases from which Coxsackie group-A virus was isolated poliovirus was also isolated from 27; i.e., in 1/3rd of the cases found to be infected with Coxsackie virus there was also an infection with poliovirus. Conversely, from 25% of the cases proved to have poliomyelitis by the isolation of poliovirus, Coxsackie group-A virus was also isolated. There is thus no doubt that these two viruses frequently occur in association. Some patients with paralytic poliomyelitis have also been found to have lesions resembling herpangina in their throats, presumably due to the coincident Coxsackie-A virus infection. It seems possible that Coxsackie-A virus and poliovirus may have a synergic action in some cases of paralytic poliomyelitis. This possibility certainly merits further study to define the relationship between these two viruses in such cases.

Guillain-Barré Syndrome

Clinically this syndrome manifests itself as a symmetrical weakness or paralysis, often associated with paraesthesias. It is the condition most often confused with poliomyelitis. Pathologically it is characterized by changes in the nerve roots consisting of cellular infiltration and fragmentation of the myelin sheaths. The obstruction to the flow of cerebrospinal fluid at the nerve roots probably accounts for the increase in protein content without a corresponding increase in the cell count of the cerebrospinal fluid, the characteristic clinico-pathological finding. Many cases occur as post-infective complications of the acute specific fevers and within the last 2 years 2 cases following chicken-pox and one following Q fever have been seen in the Johannesburg Fever Hospital. Other cases appear to be related to an auto-allergic reaction to drugs, particularly the sulpha drugs. Some cases appear to be the result of infection. It is apparent then that the aetiology of the syndrome remains uncertain. It is therefore of interest to note that in a consecutive series of 7 cases studied in these laboratories Coxsackie group-A virus was isolated from the faeces of 4. It is possible therefore that in these cases there was an aetiological relationship. It is our belief that most cases of this syndrome are not related to Coxsackie-A virus infections and have some other basis. On the other hand some cases may result from infection of the cells of the supporting tissue with Coxsackie-A virus. Further study is necessary to define the significance of the finding of this virus in these cases.

Bell's Palsy

During the 3-year period of this study, Coxsackie group-A viruses were isolated from the faeces of 2 cases, and the throat swab of another case, of Bell's palsy. Again it is possible that the virus had a causal relationship to the patients' illness, but further study will be necessary to prove this.

Miscellaneous Conditions

Coxsackie group-A viruses were isolated from the faeces of a number of cases clinically diagnosed as myositis. One of these patients, a boy 15 years old, had a febrile illness lasting several weeks and was clinically diagnosed as a case of dermatomyositis. He was treated with cortisone for some weeks. It seemed possible that this treatment aggravated and prolonged his illness, but he eventually recovered completely.

Summer Diarrhoea

Coxsackie group-A viruses have been isolated from a number of cases of summer diarrhoea in infants. Again it is possible that there is a causal relationship, but this too awaits proof.

It is apparent that where infections are as prevalent as the Coxsackie-A virus infections it is often difficult to define the significance of these findings. However, they are placed on record, and as further studies are undertaken it will be possible to define their significance more accurately.

COXSACKIE GROUP-B VIRUS INFECTIONS

Five serological types of Coxsackie group-B virus have been identified. Four of these 5 have been shown to occur in Southern Africa. It is of interest to note that so far Coxsackie group-B Type-1 virus, which appears to be prevalent in the United States, has not been isolated in Southern Africa.

Bornholm Disease

The Coxsackie group-B viruses have been incriminated as the cause of epidemic myalgia, Bamle disease or Bornholm disease. In Southern Africa Coxsackie group-B viruses have been isolated from outbreaks of Bornholm disease in Middelburg, Transvaal,[7] in Johannesburg, in Salisbury, and in Bulawayo. This clinical syndrome was first observed in 1856 in Iceland by Finsen, who called it pleurodynia. Since then epidemics have been recognized in all parts of the world. Many of them have been recorded in Scandinavia. In a classical monograph[8] on the subject Sylvest gave this condition the name Bornholm disease after the Danish island Bornholm in the Baltic. The same disease has been called by various other names, some based on the geographical site of its occurrence, such as Skien disease, Bamle disease, Drangedal disease, and others based on the most prominent clinical symptoms, such as epidemic diaphragmatic spasm, epidemic benign pleurisy, and devil's grip.

The disease occurs in epidemic form usually in the summer and autumn. The incidence of infection is usually greatest in children and young adults. The incubation period is 2-4 days. The onset is sudden, usually with pain in the region of the diaphragm, which may be very severe and associated with protective splinting of the lower chest and upper abdomen. It is aggravated by coughing, sneezing, laughing and even breathing. Pain and tenderness of the muscles of the trunk and limbs may also occur. The patient develops fever, a rapid pulse, generalized aches and frontal headache. The condition is characterized by fever, which shows a tendency to one or more relapses,

during which the patient complains typically of severe pain in the lower part of the thorax and epigastrium, but it may occur elsewhere too. In children abdominal pain, nausea and vomiting and occasionally diarrhoea are more frequently seen than the lower thoracic pain. In the relapses the original symptoms recur in most cases with equal severity. Abdominal pain may be so severe that an acute abdominal emergency is suspected, but as a rule marked rigidity of the muscles is not found. In patients with severe chest pain, pleuritic in character, pleural friction rubs may be heard.

Meningo-encephalitis

Bornholm disease may be complicated by the development of meningo-encephalitis. Studies recently carried out in the Laboratories of the Poliomyelitis Research Foundation have shown that Coxsackie group-B virus is the commonest cause of meningo-encephalitis in this region.[5] Similar cases occurring in Rhodesia have also been found to be associated with Coxsackie group-B virus infection.[9] Most of these cases were admitted to hospital with a provisional and quite justifiable diagnosis of non-paralytic poliomyelitis. Many of them gave no previous history suggestive of Bornholm disease, but in a number the signs and symptoms of this disease preceded the development of meningo-encephalitis. Most patients gave a history of sore throat, many had abdominal pain, and some had nausea and vomiting. These initial symptoms were often followed by a remission lasting 1-7 days and then the patient again developed fever associated with severe headache, stiff neck and back, and often anorexia, nausea, and vomiting. On admission to hospital the patients had fever and complained of severe headache, stiff neck and back, and pains in the limbs; occasionally they also complained of weakness affecting one or other limb. On examination the outstanding signs were stiff neck and back and slight enlargement of the lymph glands. Most had normal reflexes, but in a few the reflexes were diminished or were unequal on the two sides.

The blood count varied considerably. It was of value in diagnosis in that the very high white-cell counts with a very high neutrophilia, such as occur in the case of bacterial meningitis, were not seen. The examination of the cerebrospinal fluid was of crucial value. Most cases had less than 100 cells. High counts of over 500 cells per c.mm. were not found. In the first lumbar puncture neutrophil leucocytes and lymphocytes occurred in about equal proportions. In the second lumbar puncture done about 2 weeks after the first it was still abnormal in over 60% of the cases, but now there were more lymphocytes than leucocytes. In the diagnosis of this form of meningo-encephalitis various other forms, including bacterial meningitis, fungal meningitis, protozoal infection such as toxoplasmosis and malaria, have to be considered and excluded.

Several other viruses known to cause meningo-encephalitis have also to be considered in the differential diagnosis. Meningo-encephalitis due to the mumps virus may occur either before or after the typical parotitis or orchitis, and many cases are seen without either parotitis or orchitis. The nervous forms of glandular fever often resemble meningo-encephalitis due to Coxsackie group-B virus. The diagnosis can only be established with certainty by the isolation of the virus. In most cases it is isolated from specimens of faeces. In a study carried out on the cases admitted to the Johannesburg Fever Hospital, Coxsackie group-B virus was isolated from the faeces of 20 cases, and from 9 of these 20 the same type of virus was also isolated from the cerebrospinal fluid. There is thus no doubt about the aetiological relationship of these viruses to these cases of meningo-encephalitis. Indeed, Coxsackie group-B virus emerged as the most frequently identified cause of meningo-encephalitis in this region.

Myocarditis Neonatorum

In October 1952 an outbreak of an acute fulminating illness occurred in a maternity home in Johannesburg.[10] Of 10 babies affected 6 died after an acute febrile illness ending in circulatory collapse. Post-mortems carried out on 3 of them revealed that the cause of death was acute heart-failure resulting from a focal but extensive myocarditis. In one case a focus of inflammation in the brain and of one suprarenal gland was found, suggesting that the myocarditis was a part of a generalized infection.

Coxsackie group-B virus was isolated from the faeces of 2 of the 4 patients who recovered. Baby mice inoculated with a suspension prepared from the brain and the heart of 2 of the fatal cases showed lesions of the fat pad and brain similar to those caused by Coxsackie group-B virus.

A similar outbreak affecting 3 babies soon after birth in a maternity home in Umtali, Southern Rhodesia, occurred in the autumn of 1954.[11] One of these babies died on the 12th day after birth. The other two recovered after being ill for about 1 week. Microscopic examination of the organs in the fatal case showed congestion but no other pathological change in the brain. The heart, which macroscopically had not appeared grossly abnormal, on microscopic examination revealed scattered foci of inflammation in the substance of the muscle. The muscle showed degeneration and fragmentation associated with a surrounding inflammatory-cell infiltrate mostly of mononuclear cells, histiocytes and lymphocytes, but also including polymorphonuclear leucocytes. The suprarenal showed marked congestion of the medulla, in which a few foci of inflammatory cells were also detected. Viruses resistant to the action of ether and pathogenic to baby mice were isolated from the faeces and caecal contents of the baby who died and from the faeces of one of the babies who recovered. These viruses were successively established on passage in baby mice. Microscopic examination of their tissues revealed an acute necrosis and inflammation of the fat pad. In a proportion of these mice, lesions of the brain and focal necrosis and inflammation of the heart muscle and voluntary muscles were also detected. These lesions resemble those produced by Coxsackie group-B virus. Cross-immunity tests carried out with sera prepared against Dalldorf's

classical strains revealed that this virus was a Coxsackie group-B Type-4 virus. More recently, from the heart of a baby who was admitted to the Transvaal Memorial Hospital for Children 9 days after birth, and who died shortly after admission, a Coxsackie group-B virus has been isolated. This virus has also been typed and found to be a group-B Type-2 virus. The isolation and identification of the virus from the heart of this case provides the final proof that this condition of myocarditis neonatorum is caused by Coxsackie group-B virus. Unlike the other manifestations of Coxsackie group-B infection, this condition is serious and a large proportion of the babies affected may die.

LABORATORY DIAGNOSIS

Both Coxsackie group-A and group-B viruses are found in the throat and the faeces of cases of the above-described conditions. In many they also occur in the blood stream but, as in most virus diseases, their occurrence there is of short duration. In some cases they occur in the cerebrospinal fluid. They may be detected in the faeces for several weeks.

For the laboratory investigation of these infections and for laboratory confirmation of a clinical diagnosis, the following specimens should be sent to the virus laboratory:

1. *Acute phase*
   (i) Blood with no preservative or anticoagulant
   (ii) Faeces
   (iii) Throat swab
   (iv) CSF (if meningo-encephalitis is suspected and a lumbar puncture is done)
2. *Convalescent phase*
   (i) Blood specimen for antibody titration in comparison with the acute-phase blood

In the laboratory, after appropriate preparation and purification, samples of the acute-phase specimens are inoculated into litters of 1-day-old baby mice and into tissue-culture tubes of human or monkey cells. The mice are observed for 2 weeks for signs of illness, especially for tremors, weakness and paralysis. If any develop the mice are killed with ether. Portions of their organs are fixed in Bouin's solution prior to the preparation of histological sections. Half the brain and the carcase, after the viscera have been removed, are kept and prepared for passage. It is often necessary to passage a suspected isolate 2-3 times before the characteristic clinical and pathological picture is found.

The identification of a virus as belonging to the Coxsackie group depends first on finding the characteristic histological lesions in baby mice. It will be recalled that with Coxsackie group-A viruses this is a diffuse acute myositis characterized by hyalin eosinophil degeneration of the voluntary muscle fibres, with which is associated an acute inflammatory and repair reaction. With Coxsackie-B viruses, only a focal myositis is found and it is not always apparent. The characteristic finding is an acute necrosis of the interscapular fat pad, associated with an acute inflammatory infiltration.

The final identification and typing of the virus is achieved in a serum-neutralization test in which the virus is tested against specific antisera of each of the known types of the corresponding group of Coxsackie virus.

CONCLUSION AND SUMMARY

From this survey it is apparent that the Coxsackie viruses are important pathogens of Man. They are very prevalent, particularly the group-A viruses and give rise to specific illnesses as well as to some conditions which may simulate other infections.

Coxsackie group-A viruses are the cause of herpangina and are one of the causes of meningo-encephalitis, and of a pyrexial illness of relatively short duration. Evidence has also been obtained suggesting that they may cause an illness clinically resembling glandular fever, but not associated with the characteristic blood picture of that condition and giving a negative result in the Paul-Bunnell test.

Some cases of the Guillain-Barré syndrome have been found to be associated with Coxsackie group-A virus infection. The significance of this association has not been determined, but it is believed that most cases of this syndrome have some other basis.

The relationship of Coxsackie group-A infections to summer diarrhoea and enteritis in infants has also still to be assessed, although these viruses have frequently been isolated from patients with these conditions.

Coxsackie group-A virus is found so often in association with poliovirus in cases of paralytic poliomyelitis that it is suspected that there may be a synergic action of these two viruses in such cases.

Coxsackie group-B viruses cause Bornholm disease and in Southern Africa are the commonest identified cause of meningo-encephalitis. They have also been incriminated in the present series of studies as the cause of outbreaks of myocarditis neonatorum, a serious condition which may end fatally.

An accurate specific diagnosis of these diseases is important from the medical and more particularly from the public-health point of view. This can now be achieved with a considerable degree of certainty in a properly equipped virus-laboratory.

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