THE COXSACKIE VIRUSES

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Coxsackie is a village on the banks of the Hudson River in upper New York State. In the course of investigating an outbreak of poliomyelitis there, Dalldorf and Sickles (1948) isolated a hitherto unrecognized filterable agent from the faeces of two children with lower limb paralysis. Neutralizing antibodies for this new virus appeared in the blood of both patients during convalescence. Attempts to isolate the poliomyelitis virus in rhesus monkeys were unsuccessful, but paralysis was induced in newborn suckling mice, although not in mice over twelve days old. This discovery heralded the advent of a new family of Coxsackie viruses.

Reports soon followed of the isolation of the Coxsackie virus in other epidemics of paralytic poliomyelitis (Dalldorf *et al.*, 1949), in non-paralytic poliomyelitis, or aseptic meningitis (Curnen *et al.*, 1949; Melnick *et al.*, 1949); pyrexia of unknown origin (Webb and Wolfe, 1950); summer 'grippe' (Sabin and Steigman, 1949; Melnick *et al.*, 1950), the Guillain-Barré-Landry syndrome (Armstrong *et al.*, 1950), influenza-like illnesses (Kilbourne, 1950), pleurodynia or Bornholm disease, and herpangina. The ubiquitous nature of this virus led to a spurious association between it and a variety of diseases. More recently a cautious attitude has been adopted in relating the Coxsackie family of viruses to human disease, especially non-paralytic poliomyelitis and aseptic meningitis. Isolation of the poliomyelitis virus is a technically difficult procedure, whereas the Coxsackie virus is comparatively easy to isolate. These technical points should be borne in mind in assessing their aetiological significance. Dalldorf has stated that we are in the anomalous position of having found the cause of the disease, but not the disease (Huebner *et al.*, 1950). Much work has still to be done before it is possible to assess the role, if any, of the Coxsackie viruses in relation to the poliomyelitis virus and non-paralytic poliomyelitis.

There are two clinical conditions in which all available evidence suggests that the Coxsackie virus is responsible for the disease, namely, epidemic pleurodynia (Bornholm disease) and herpangina.

Epidemic Pleurodynia

This condition was observed in 1930 by Sylvest as an epidemic of myositis occurring amongst the inhabitants of fishing villages on the island of Bornholm off the coast of Denmark. It has undoubtedly occurred in earlier times, and the original description is attributed to Finsen, who observed it in Iceland in 1856, and called it pleurodynia. It has been recognized in both epidemic and sporadic forms as an acute febrile, self-limiting disease, characterized by lower intercostal muscle pain or diaphragmatic pleurisy, and occasionally accompanied by pleural friction. Its importance from a clinical point of view lies mainly in its differential diagnosis from tuberculous pleurisy, pneumonia, renal and biliary colic, appendicitis, peritonitis, pulmonary infarct and coronary thrombosis.

Curnen, Shaw and Melnick (1949) drew attention to a patient with epidemic pleurodynia, in whom the Coxsackie virus was isolated and neutralizing antibodies to this virus were found in the serum. They also mentioned three laboratory workers who were affected by pleurodynia whilst studying the Coxsackie virus. Findlay and Howard (1950) strengthened the association between the Coxsackie virus and Bornholm disease still further by reporting the occurrence of laboratory infections in workers studying the virus, and also of an interesting human transmission experiment. A volunteer was inoculated intranasally with a suspension of the Coxsackie virus, which was known to be active in suckling mice. No symptoms occurred until 46 hours later, when sudden pain was felt below the right scapula. It radiated intercostally, and was aggravated by deep breathing and laughing. There was a transient pyrexia of 99.2° F., and the pain subsided after 48 hours. Following this attack, complement-fixing antibodies for a Coxsackie virus antigen were detectable in the serum although none had been present before the transmission experiment. Findlay and Howard also reported the isolation of the Coxsackie virus from the nasal washings, stools and blood of a patient with Bornholm disease.

In 1947, 114 cases of epidemic pleurodynia were observed at the Boston City Hospital (Finn *et al.*, 1949). Although no aetiological agent was

demonstrated at the time, fortunately some of the throat washings and sera were stored, and subsequently the Cocksackie virus was isolated from four specimens of these washings, and specific neutralizing antibodies against two strains of viruses were demonstrated (Weller *et al.*, 1950). It seems probable that this Cocksackie virus was aetiologically responsible for this Boston epidemic.

Strong evidence indicating that the Cocksackie virus is aetiologically responsible for Bornholm disease and that the patient is not a mere intestinal carrier of this virus is afforded by some interesting experiments undertaken by workers at the Institut Pasteur (Lepine, Desse and Sautter, 1952). They were able to isolate the Cocksackie virus from muscle-biopsies of two patients suffering from Bornholm disease. Histologically, the muscle-biopsy sections showed the hyaline degeneration and mononuclear cell infiltration, just as it is seen in the experimental infection in suckling mice.

Case Report.—The following case illustrates the association of Bornholm disease and the Cocksackie virus, and also underlines its differential diagnosis from an acute abdominal catastrophe.

A 42-year-old widow was admitted to the Middlesex Hospital under the care of Dr. G. E. Beaumont. Twenty-four hours before admission she was walking along the promenade in Brighton when she was suddenly seized with severe pain over the right lower ribs. It was intensified by deep breathing and it radiated to the right side of the abdomen. It passed off slowly in the course of the next half hour. Four hours later it gradually returned and was now present in the right loin and right iliac fossa. The severity of the pain prevented her from turning over in bed and she spent a sleepless night in considerable pain. On the following morning she felt sick and feverish. The pain was temporarily relieved by 100 mg. of pethidine administered by her doctor, but this relief lasted only 3 hours. When it returned, she likened the pain to a 'band' across the abdomen, aggravated by movement and deep breathing.

On examination, she was lying very still in bed, afraid to move because of the pain. Her temperature was 99.4, pulse 80, and respiration 20. There was marked tenderness and rigidity over the whole abdomen, with gross rebound tenderness. However, if the hand were allowed to rest on the abdomen, in the course of 1 minute it would sink gradually into a softer abdomen. Bowel sounds were faintly audible, and there was no tenderness on rectal examination. Examination of the chest revealed that the breath sounds were slightly weaker at the right base posteriorly. A well-marked feature was that the

pain was severely aggravated by sniffing. A chest X-ray revealed normal lung fields, but the right diaphragm was high and could well have been 'in spasm.' Unfortunately her clinical condition precluded screening at the time.

It was suggested that she might have a peritonitis due to a perforated peptic ulcer or ruptured appendix, or possibly an acute pancreatitis. Against these diagnoses were a normal pulse and blood pressure; rectal and vaginal examination caused no tenderness; bowel sounds were audible, a white cell count was 10,600 c.mm. with a normal differential count, and the erythrocyte sedimentation rate was 11 mm./hour (Westergren). During the first 24 hours in hospital her condition remained unchanged. On the second hospital day the pain was easier and had disappeared by the end of the third day. The most likely diagnosis seemed to be a severe Bornholm disease. The onset with sharp pleural pain, aggravated especially by sniffing, the mild pyrexia of 99.4°, the high right diaphragm, and finally the benign subsequent course were in favour of this diagnosis.

Nasopharyngeal washings and faeces were obtained for virus isolation, and acute-phase and convalescent-phase sera were examined for virus-neutralizing antibodies. From both nasopharyngeal washings and faeces a Cocksackie virus of the B type was isolated. The acute phase serum revealed antibodies to a titre of 1 in 10, and the convalescent-phase serum contained neutralizing antibodies to a titre of 1 in 500. This was a significant rise in neutralizing antibodies.

This case illustrated a more than usually severe clinical picture of Bornholm disease, from it a Cocksackie virus was isolated and the blood showed a significant rise in homologous neutralizing antibodies.

Davies and Warin (1951) reported an epidemic of Bornholm disease in Oxford. Patients presented with thoracic or abdominal pain, or meningitic symptoms. The diagnosis should also be borne in mind if appendicitis seems to strike a family simultaneously.

Significant rises in antibody titre to the Cocksackie virus have been found in patients who have been diagnosed clinically as coronary thrombosis, but in which the electrocardiogram was normal (Findlay, 1951).

Herpangina

Huebner and his colleagues (1951) made aetiological studies on six children suffering from an acute pharyngitis, in which small punched-out ulcers with greyish bases and a surrounding red areola were seen on the anterior faucial pillars. These children also had a fever of short duration, headache and abdominal pain; several had ulcers

on the hard and soft palate, and one of them on the tongue.

Bacteriological studies were negative, but the Cocksackie virus was isolated from throat washings and faecal suspensions quite easily. Neutralizing antibodies against the particular strain isolated were found in a significant rising titre in convalescent-phase sera.

Epidemiological data on a further 31 scattered cases revealed that it occurred in the summer; was probably spread by direct person to person contact in childhood; and that the incubation period was about 3-5 days.

This condition has been variously termed vesicular pharyngitis (Levine *et al.*, 1939) and aphthous pharyngitis (Breese, 1941), and is characterized by the appearance of small yellowish-white vesicles surrounded by a red areolâ in the throat. Virus studies were undertaken in two of Levine's cases and in one of Breese's series, but were unsuccessful. The Cocksackie virus was unknown at this time, and efforts were directed towards isolation of the herpes simplex virus. This had been shown (Dodd *et al.*, 1938) to be the causative agent in herpetic or aphthous stomatitis.

Since the herpes simplex virus can cause vesicular stomatitis, it seems unfortunate that the term 'herpangina' is perpetuated in association with the Cocksackie virus. Further studies must be made to determine the relative importance of the herpes simplex and Cocksackie viruses in the aetiology of this condition.

The Experimental Disease

Infant mice are susceptible to the virus when inoculated intracerebrally, intraperitoneally, subcutaneously or orally. Newly-born mice or those up to 4 days old are most susceptible. After an incubation period of 2-10 days, signs of muscular weakness and paralysis of the limbs appear in these suckling mice, and death ensues usually in 24 hours.

Based on the pathological findings of the experimental disease, Dalldorf (1950) placed the many different strains of the Cocksackie family into Groups A and B. Group A infection causes extensive myositis with hyaline degeneration of the muscle fibres, oedema, and diffuse interstitial mononuclear cell infiltration. Group B infection, in addition, causes severe encephalopathy with patchy neuronal degeneration, ending in cystic degeneration, and also causes a peculiar necrosis of the interscapular fat pads. A typical Group B virus was isolated from the case described above, and this group is the one commonly associated with epidemic pleurodynia.

Host Range

Apart from man and infant mice, the Cocksackie viruses have been successfully cultivated in suckling hamsters, and in tissue culture medium consisting of minced embryonic mouse tissue (Slater and Syverton, 1950). It has been propagated with extreme difficulty in the chick embryo, but needed alternating mouse-egg passages (Huebner *et al.*, 1950). *Cynomolgus* monkeys and chimpanzees, when fed with Cocksackie viruses, develop mild febrile illnesses, followed by a carrier state with neutralizing antibodies in the serum (Melnick and Ledinko, 1950). This subclinical infection with an early carrier state may well represent the usual way in which man responds to Cocksackie infection.

When cockroaches are fed a single meal containing the Cocksackie group of viruses, they continue to excrete virus daily for up to 15 days (Fischer and Syverton, 1951). The excreted virus was able to paralyse and kill infant mice, showing that its pathogenic properties were unaltered. Flies have been incriminated as vectors of the disease, but it seems possible that cockroaches may also be able to maintain and disseminate these viruses freely.

Diagnosis

The clinical picture afforded by Bornholm disease when it occurs in its epidemic form is sufficiently striking to enable a confident diagnosis to be made. This disease should also be borne in mind in the differential diagnosis of sudden severe pain in the side, radiating to the abdomen, simulating perforation of a peptic ulcer or appendix, biliary and renal colic, and coronary thrombosis. However, the diagnosis of Cocksackie virus infection cannot be made clinically with certainty when patients present with aseptic meningitis or vesicular pharyngitis. The laboratory tests to confirm the diagnosis are (1) isolation of the virus from throat washings, blood, spinal fluid, or faeces, (2) serological evidence of an immunological response either (a) by specific neutralizing antibodies to the virus isolated, or (b) by significant rises in titre of the complement-fixation test.

(1) *Isolation of the virus.* Routine laboratory diagnosis by isolation of the virus is not a practical procedure because of the complexity of the procedure, the time involved, and the numbers of infant mice needed. However, isolation may be undertaken to establish identity of the virus in any given epidemic.

(2) (a) *Neutralization test.* The demonstration of specific homologous antibodies, which neutralize the disease in test mice, is a reliable index of infection with a particular strain. In the case presented above, the acute phase specimen of serum pro-

tected mice to a titre of 1 in 10, whereas the convalescent phase serum protected mice to a titre of 1 in 500. The virus was a typical B virus, and cross-immunized with Connecticut 5 serum.

(2) (b) *Complement fixation test.* This is the most convenient method, at present, for confirming the clinical diagnosis of Coxsackie virus infection, because it can be undertaken conveniently in a hospital laboratory without recourse to animal work if antigen is already provided. Five ml. clotted blood should be provided as soon as the disease is suspected, and again after an interval of about 10 days. A significant rise in titre, at least four-fold in extent, between acute-phase and convalescent-phase sera should be the minimal criteria of an immunological response on the part of the patient.

Kraft and Melnick (1952) have published recently a warning against misinterpreting the results of the complement-fixation test. They observed that apart from a rise in the homologous antibodies, there was frequently a significant rise in titre of heterologous antibodies, although only a single strain of the Coxsackie virus had been isolated. This could possibly be explained on the basis of an anamnestic reaction. It might, therefore, be expected that other infections could provoke this non-specific antibody response, leading to erroneous serological conclusions. No such non-specific response is observed when the neutralization test is performed and it gives a reliable index of homologous antibodies only.

Treatment

The Coxsackie viruses are resistant to penicillin, streptomycin, aureomycin, terramycin and chloramphenicol. This lack of response to antibiotics is a feature in common with most other viruses. The newer antibiotics are only effective against the large viruses of the lymphogranuloma-psittacosis group and the unknown agent of 'primary atypical pneumonia', whereas the Coxsackie viruses are probably of small size. In our present state of imperfect knowledge of the mode of action of antibiotics on viruses, the size of the virus is a rough index of its response to antibiotics.

Treatment is, therefore, symptomatic, for the pain of Bornholm disease. Fortunately it is self-limiting and lasts only 40-70 hours.

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CORRIGENDUM

"Textbook of Surgical Treatment," Edited by C. F. W. Illingworth.

The review of this book in November mentioned that "Professor Dick has recruited, etc." This should have been "Professor Illingworth, etc." We apologise.