# THE COXSACKIE GROUP OF VIRUSES

## EPIDEMIOLOGICAL STUDIES

BY

*PIRKKO POHJANPELTO*

HELSINKI 1955

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ACADEMIC DISSERTATION

TO BE PRESENTED WITH THE ASSENT OF THE
MEDICAL FACULTY OF THE UNIVERSITY OF HELSINKI FOR PUBLIC
EXAMINATION IN AUDITORIUM XII ON MAY 17TH, 1955,
AT 12 O'CLOCK NOON

HELSINKI 1955

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# PREFACE

Docent N. Oker-Blom, M.D., Chief of the Bacteriological Laboratory of Helsinki started Coxsackie virus investigations in Finland in 1951. At his suggestion I began in autumn 1951 to study the epidemiology of the Coxsackie group of viruses. Docent Oker-Blom has supervised my work at its various stages and given me unstinted support and encouragement throughout the course of the work.

I have carried out my work in the Department of Virology, which during these early years of its activities has been connected to the Department of Serology and Bacteriology of the University of Helsinki. Professor K. O. Renkonen M.D., chief of the Department of Serology and Bacteriology has made my work possible by placing at my disposal test animals and technical assistance. It has been a pleasure to work in the stimulating scientific atmosphere created by him.

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The sera from healthy subjects I have collected in co-operation with Dr. S. Pere M.D. Docent E. Helske, M.D., Dr. J. Lehtinen,

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*Pirkko Pohjanpelto.*

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# INTRODUCTION

Coxsackie viruses were discovered by sheer coincidence in connection with a poliomyelitis investigation. In the autumn of 1947, Dalldorf and Sickles were experimenting with the cultivation of the poliomyelitis virus in new-born mice by injecting the faeces of poliomyelitis patients into them. Two out of ten faecal samples induced paralysis in the mice. It was soon discovered, however, that the cause of the paralysis was not the poliomyelitis but an entirely new virus. It was named Coxsackie after the small village in New York State where it was first isolated.

Ever since Dalldorf published his observations the Coxsackie virus has been the subject of intense investigation. It was early established that the question was not one of a single virus but of a whole group of viruses with a number of common characteristics but differing serologically. These viruses have been reported on in numerous papers from various parts of the world and evidently quite a common and wide-spread virus group is involved.

In the early stage of the investigation, Dalldorf stated »We are in the anomalous situation of having discovered the cause of the disease before discovering the disease». Subsequently Coxsackie viruses have been found in connection with several different diseases. The part they play in inducing diseases is, however, not quite clear, nor is it known whether the various types differ in this respect. As none of the usual run of test animals react to Coxsackie infection exactly like man, and since large-scale experiments on humans are out of the question the solution to these problems can only be sought through epidemiological investigations. The present investigation is an attempt to contribute towards the solution of the problem.

# SURVEY OF THE LITERATURE

## CHARACTERISTICS OF THE VIRUS

### DEFINITION OF COXSACKIE VIRUSES

The first Coxsackie virus isolated by Dalldorf induced paralysis in newborn mice and hamsters but had no effect on adult mice and hamsters. It produced no symptoms in the monkeys and guinea pigs also injected with it, nor was it possible to reproduce it in the developing chick embryo. The virus caused degeneration in the striated muscles of newborn mice (29, 30). The changes are not, however, peculiar to Coxsackie viruses; similar changes are produced in hamsters by the MM virus and in mice by the Theiler virus. They can also be seen in experimentally induced Vitamin C or E deficiency (24).

The distinguishing characteristic of the Coxsackie virus seemed to be its peculiar host range, and particularly the fact that the age of the animal was of decisive importance.

Later it was discovered that some Coxsackie types at least could live in hosts other than suckling mice and hamsters and that the effect of the different types on mice of varying ages differed to some extent. It was also found that the pathologic picture induced by the different types in suckling mice was somewhat different. But a characteristic common to all Coxsackie types is that *suckling mice are more susceptible to infection than the adult animals and that suckling mice, as symptoms of infection, show flaccid or spastic paralyses and degenerative muscular changes.*

### VIRUS TYPES

Sixteen serologically different Coxsackie types have been distinguished to date by neutralization and complement fixation

methods. Laboratories employ different names, which makes comparisons of the types difficult.

Dalldorf distinguished 14 types. He divided them into two main groups, A and B, according to the pathologic picture they produce in suckling mice. The viruses of Group A damage the muscular tissue only, while those of Group B induce changes in the muscles, the central nervous system, adipose tissue and organs of the abdominal cavity. The Group A viruses, total ten, are numbered from one to ten. The Group B viruses are four, numbered one to four (39). There seems to be every justification for this division into Groups A and B and the names are easy to remember. It is these names that will be used in the present paper.

Melnick and co-workers have distinguished 16 types, generally named after the place where they were isolated (17).

Huebner and co-workers have distinguished 7 types, also with special names (3).

Melnick and co-workers have compared the above types and found that the types isolated in different laboratories correspond to one another (17) (Table 1). The different types generally differ distinctly, i.e. each reacts to its own immune serum only. A slight

TABLE 1

THE CORRESPONDENCE OF COXSACKIE TYPES ACCORDING TO CONTRERAS AND BARNETT AND MELNICK (17)

| Dalldorf | Melnick | Huebner |
|---|---|---|
| A 1 | Easton —2 | Type 1 |
| A 2 | Type 2 (Fleetwood) | Type 2 |
| A 3 | Type 3 (Olson) | — |
| A 4 | Texas —1 | Type 4 |
| A 5 | Easton —14 | H 1 |
| A 6 | Israel —7 | H 4 |
| A 7 | Texas —15 | — |
| A 8 | Easton —10 | H 2 |
| A 9 | Boston | — |
| A 10 | Alaska —5 | H 3 |
| B 1 | Conn —5 | — |
| B 2 | Ohio —1 | — |
| B 3 | Nancy | — |
| B 4 | Texas —13 | — |
| — | Texas —12 | — |
| — | Texas —14 | — |

cross reaction has been observed between the following types: B 1—B 3, Texas 14—B 3, Texas 12—A 5, A 3—A 8. Heterologous immune serum, however, neutralizes only approx. 100 $ID_{50}$ viruses while homologous immune serum neutralizes approx. 10,000—100,000 $ID_{50}$ (4, 17).

In addition to the types mentioned, Coxsackie strains named independently and not compared with the types listed above have been isolated in several laboratories. It is possible, therefore, that there may be more than 16 Coxsackie types.

Nothing definite is known so far of how close the relationship is between the different Coxsackie types. Hence the properties established for one type cannot simply be given a general application covering the viruses of the Coxsackie group.

## PATHOLOGIC PICTURE

Only the pathologic features produced by Coxsackie viruses in suckling mice will be discussed as they alone have been studied in detail.

The pathologic features produced by all the virus types of *Group A* are highly similar. The muscle fibres lose their finer structure, hyaline degeneration, fragmentation and clumping occur. The lesions are confined to the striated muscles. The changes are seen over extensive areas and may appear in any striated muscle.

The viruses of *Group B* induce changes in the central nervous system, adipose tissue and some internal organs as well as in the muscles. The *muscular lesions* are similar in type to the Group A lesions but only occur sporadically in small foci. In the *brain* encephalomyelitis is observed. Clearly defined necrotic areas are seen both in the grey and white matter. They appear above all in the area of the brain stem, but often in the spinal cord too. Inflammatory changes around the necrotic areas are rare. The *adipose tissue* shows necroses which usually start from the periphery of the lobules and continue towards the centrum. Inflammatory signs are seen around the necrotic areas. These lesions are mostly found in the adipose tissue between the shoulder-blades. The *internal organs*, liver, pancreas, heart and kidneys are injured considerably less regularly than the above tissues. In this respect there may be differences between the types and it is possible that the strain of mice employed also plays a part (39, 41).

## PHYSICAL AND CHEMICAL PROPERTIES

Beautiful pictures of Coxsackie virus, types A 2, A 10 and B 3 have been made under the electron microscope (8, 9). These viruses are spherical particles which are notably uniform. The particles form clusters which are hexagonal or diamond arrays. Type A 10 formed pseudocrystals in 2—3 superimposed plates.

The Coxsackie viruses are among the very smallest of viruses. Types A 1, A 4, B 1 and B 2 have been studied by the ultrafiltration and sedimentation method. Their recorded size is 10—32 mm$\mu$ (42, 61, 77, 89). From electron microscope pictures the diameter of Types A 2, A 10 and B 3 have been given as 37 mm$\mu$ (8). The types do not seem to differ appreciably in size, though Type A 1 is believed to be smaller than the others (77).

The Coxsackie viruses are fairly resistant to heat and pH changes. Types A 1, A 2, A 3 and B 1 survive for 30 min. at +49°C (93), and a virus (type not mentioned) of Group A survived 7 days at room temperature and several months at +4°C (3). Types A 1, A 2, A 3 and B 1 keep for 24 hours at pH 2.3—pH 9.4, and for 7 days at pH 4.0—pH 8.0. Of these types, A 1 was more sensitive to acid and A 3 more sensitive to alkali than the others (30). No systematic investigation has been made into the effect of disinfectants on the various types but it seems that the Coxsackie viruses are insensitive to ether, lysol and ethyl alcohol but sensitive to 0.1 normal hydrochloric acid and formaldehyde (67, 100). Type B 2 differs from the others in being sensitive to ether.

## HOST RANGE

Coxsackie viruses have been found in man, but they apparently also occur in some animals as well. O'Connor and Morris isolated a Coxsackie virus of Type A 4 from the blood of a wild rabbit (16). Melnick and co-workers were able to recover Coxsackie viruses from flies. No evidence was obtained, however, of Coxsackie viruses propagating in flies (68, 76). The examination of a large number of wild individuals of different animal species revealed the presence of antibodies neutralizing A 1 and/or A 7 and/or A 3 and/or A 4 in several rabbits, one marmot and one fox (81). In addition, A4 neutralizing antibodies have been established in four monkeys imported direct from Africa. In spite of the antibodies, however,

these monkeys became virus carriers after they had been fed with virus of Type A 4 (70).

In the laboratory it has been possible to propagate Coxsackie viruses in several experimental animals and some strains also in the developing chick embryo and tissue cultures. In most cases, however, the Coxsackie virus has been apathogenic or only slightly pathogenic.

Fatal infection is induced by Coxsackie viruses in suckling mice and suckling hamsters. Similarly two Coxsackie strains isolated in France (type not determined) were found highly pathogenic to suckling gerbils, an animal of the rodent class (62).

*In adult mice* the Coxsackie viruses usually induce no disease. An exception is Type B 1. It may damage the pancreas, liver and adipose tissue of adult mice but has not been found to produce muscular changes (44, 60, 85). The sensitivity of mice to this virus shows a wave-like fluctuation. It is greatest at birth, decreasing during the first week of life. After that it increases again to a second peak at the age of 3—4 weeks, to decrease gradually afterwards (44). However, the pathogenicity of the different B 1 strains for adult mice seems to vary. An interesting observation has been made: a virus strain transferred in suckling mice from pancreas to pancreas induced lesions in the pancreas of adult mice too, whereas another strain transferred in suckling mice from brain to brain had no such effect (28). Cortisone treatment has been found to increase the sensitivity of mice to Coxsackie. For instance, young mice weighing 7 g were found to grow more sensitive to viruses A 2, A 3, A 4 and B 2 after cortisone administration. Only types B 1 and B 3, however, were fatal to 15 g adult mice (55).

Coxsackie virus injected into *monkeys* or *suckling rats* does not produce disease in the animals but makes them virus carriers. Monkeys show a rise in antibodies after infection, and small areas of the muscles of suckling rats reveal changes similar in nature to those of suckling mice (6, 70).

The first Coxsackie strain isolated by Dalldorf did not grow in the *developing chick embryo* (30). But types A 2, A 4, A 8 and A 10 of the viruses discovered later have been made to propagate in the chick embryo (9, 40, 49, 94). Yet differences on this point may appear even between virus strains identical in type. Usually, Coxsackie infection does not kill the chick embryo.

It has been possible to cultivate several Coxsackie strains in human and animal *tissues in vitro*. The De Mole strain of Group B was grown in human embryonal brain and intestinal tissue and mature kidney, but not in embryonal cutaneous and muscular tissue. Type A 4 grew in embryonal brain tissue only (111). Type B 1, but not A 1, could be made to grow in the muscular tissue and adipose tissue of newborn mouse (99).

### SEROLOGIC REACTIONS

Coxsackie antibodies can be shown both by the neutralization and by the complement fixation method (56, 73).

In the *neutralization test* the experimental animals employed are suckling mice. The neutralizing antibodies seem to be specific in mice and monkeys but not quite specific in men (4, 73). The application of the neutralization test is restricted by the heavy consumption of mice.

In the *complement fixation test* a virus suspension purified with acetone-ether (12), protamine (18), bentonite (82) or by freezing and melting (18) is employed as the antigen. Complement fixing antibodies are specific in mice (19), but Coxsackie-infected monkeys show a rise in both homologous and heterologous Coxsackie antibodies (58). The same phenomenon is found with man (4, 57). The advantage of the complement fixation test is that a large number of sera can be examined in a short time and considerably fewer mice are needed.

*Hemagglutination.* — The effect of types A 1 and A 2 on human 0 cells and on the blood corpuscles of sheep, chicken and guinea pig at different temperatures has been tested (13). The effect of the C 2 strain, isolated in England, on human 0 cells has also been studied (32). All the hemagglutination tests gave a negative result.

## EPIDEMIOLOGY

### VIRUS SOURCES

Coxsackie viruses have been found above all in man but they have also been isolated from sewage and flies (14, 68).

The richest human source of virus is the *faeces*. Viruses are