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THE TAXONOMIC POSITION OF COMMON COLD VIRUSES AND SOME OTHERS

During recent years workers in several countries have isolated a number of "new" viruses from upper respiratory infections. For some of these viruses claims have been made that they cause common colds. Before accepting such claims it is well to be sure of two things: i) that the viruses can be recovered with reasonable frequency from typical common colds in adults; and ii) that they will reproduce typical common colds in volunteers. The second criterion is more easily fulfilled. Workers at the Common Cold Unit at Salisbury have produced cold-like symptoms in volunteers with the following viruses propagated in tissue cultures or in fertile eggs: Influenza A; Parainfluenza 1 and Parainfluenza 3[1]; ECHO 11[2]; ECHO 20; ECHO 28 (JH or 2060)[3]; Coe virus[4]; Adenovirus[5].

With most of these viruses, the colds produced were not quite typical, particularly when attention was given to the whole group of inoculated persons and not only to individuals. For instance, the adenoviruses and parainfluenzas gave rise to numerous cases of fever; ECHO 11 and 20 caused gastro-intestinal as well as respiratory symptoms (usually sore throats). The Coe virus, however, produced typical colds; so too did Echo 28, though the earlier results were hard to interpret.

On the other hand, most of these viruses were not isolated from typical colds. The parainfluenzas in particular came mainly from minor respiratory infections in children, while the adenoviruses, Coe virus, and some others were most frequently from outbreaks in recruits. During fifteen years' work at Salisbury, none of these viruses has been recovered from nasal washings from typical naturally-occurring colds in adults, though such washings will reproduce colds in a high proportion of volunteers. Several strains of Echo 28 have recently been isolated, however, from washings from colds in children.

All things considered, the Salisbury workers did not feel that typical common cold viruses[6] were being dealt with. Then, in 1960, it was reported that some rather different viruses had been cultivated from colds

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in adults and that these had strong claims to be the agents which were being sought[7]. These newly discovered agents, for which the name Rhinovirus is proposed, are the subject of this paper.

PROPERTIES OF RHINOVIRUSES

(i) *Size.* Two strains have passed through gradocol membranes of average pore size 0.100μ, being retained by those of 0.69μ. This result might suggest a virus diameter of 64 mμ. However it is well known that much virus, if it is of low titre, may be retained by filters; since the virus titres in these instances were only $10^{1.5}$ and $10^{2.5}$, it is likely that the virus' true size is much less, very possibly in the range of most enteroviruses, about 25 to 30mμ.

(ii) The viruses tested have resisted treatment with 20% ether overnight and were inactivated when heated 30 minutes at 56°. They have been readily purified by blending with fluorocarbon (Arcton 23) and centrifuging.

(iii) The type of cytopathic effect in culture has been very similar to that produced by enteroviruses.

(iv) The rhinoviruses tested (three strains) have not produced symptoms on intranasal or intracerebral inoculation into suckling mice, nor has any other experimental animal proved to be susceptible to the agents.

In all these respects rhinoviruses resemble ECHO viruses and it could be argued that, when their properties have been worked out a little further, they should be allotted numbers in the enterovirus or ECHO virus series. There are, however, cogent reasons against adopting this course.

DIFFERENCES FROM ENTEROVIRUSES

The term enterovirus implies a habitat in the intestine and the "E" of ECHO virus has the same connotation. The rhinoviruses appear to multiply in the nose—hence the name; inoculation even on to the posterior pharyngeal wall is less successful than into the nose. They have not yet been isolated from faeces, though it will not be surprising if a little virus manages to survive passage through the gut now and again. Even so, the name enterovirus would seem to be wholly misleading. The rhinoviruses seem certain to form a family with numerous serological types, some already known, many other yet to be described. One can hardly imagine a course leading to more confusion than mixing them up in a numerical series with enteroviruses of entirely different habitat and pathogenicity. Fortunately, the rhinoviruses have characters other than habitat which permit their separation from ECHO or other enteroviruses. These are not fundamental, structural, stable characters such as serve to separate viruses into

their major divisions[8] but secondary characters of the kind useful in subdividing for convenience the major groups. These characters concern the conditions for growth in tissue culture and depend upon a necessity, at least on primary isolation, for (i) a lower temperature than is conventional, that is 33°C, rather than 36° or 37°; (ii) a rather acid pH in culture; and (iii) a high oxygen tension. The last is shown in part by the need to keep cultures rotating. All three conditions are fulfilled in the virus' natural habitat, the nose; and the rhinoviruses can be conceived of as enteroviruses which have become adapted to life in this environment.

So far, rhinoviruses have been cultivated only in primate epithelial cells. Most of them, the H strains, have grown as yet only in human embryonic kidney; others, M strains, also in monkey kidney and in several continuous cell lines of human origin. The M strains so far isolated fall into 2 serological types. It is uncertain how many types of H strains there will be, as it is difficult to make antisera against them; there are at least four, probably many more.

POSITION OF ECHO 28 VIRUS

Among strains recently isolated in Britain, there have been a few which behave like the M strains of rhinovirus but clearly fall, by serological tests, with ECHO 28. They differed from the other M strains in growing readily from the beginning at 36°C. Quite obviously, the ECHO 28 viruses form a link between the rhino-and ECHO viruses. There has been only one recorded isolation of ECHO 28 from faeces. The strains differ from the rhinoviruses in having less exacting temperature requirements. On the other hand they do resemble them in preferring a slightly acid medium for growth, and rolling of culture tubes. Further, washings containing these viruses have produced more typical colds than have other ECHO viruses such as 20. If the separation of rhinoviruses from ECHO viruses comes to be accepted, it might be more logical to class the JH-2060 viruses as aberrant rhinoviruses than as aberrant ECHO viruses. The point is not, however, important when we come to consider the taxonomy of enteroviruses as a whole.

CLASSIFICATION OF ENTEROVIRUSES

Only four years have passed since the general recognition that Poliomyelitis, Coxsackie, and ECHO viruses form a single family. The discussions at the Fourth International Poliomyelitis Congress held in Geneva in July 1957 made this grouping seem likely. In the summary at the end of that Congress, I said "We are beginning to see, as a whole, a family of

viruses which is characterized by a habitat in the intestinal tract of mammals, very small size, spherical shape, resistance to ether and perhaps a definite range of stability to varying pH's."[9]

In 1957, the American ECHO virus Committee changed its name to Enterovirus Committee, recognising that it had to deal with one virus family[10]. Since about that time, it has been growing harder to draw the line

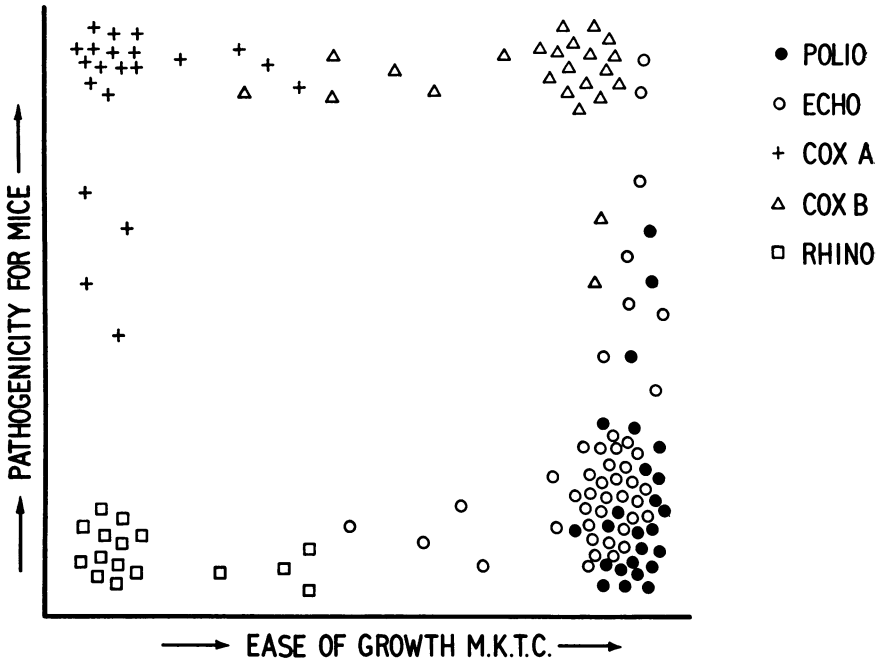

FIG. 1. The viro-astronomical approach to classification: grouping of certain *"Nanivirus"* constellations based on their pathogenicity for suckling mice and ease of growth in monkey kidney tissue cultures.

between Coxsackie and ECHO viruses. The main distinction lies in pathogenicity or otherwise for suckling mice; yet there are representatives of single serotypes, some of which will kill baby mice and others will not; or again some will do this when freshly isolated, others after varying numbers of mouse-passages, others not at all. The distinction is so illogical that many would like to abandon the attempt at separation and to call them indiscriminately "enteroviruses". It may become equally hard to distinguish between polioviruses and other enteroviruses, a distinction based chiefly on pathogenicity for the central nervous system of primates. This, too, is illogical, for attenuated poliovirus strains no longer possess this distinguishing

character. And there is a virus which Russian workers[11] have called poliovirus Type IV but which is recognised elsewhere as Coxsackie A type 7.

There is an alternative to abandoning the well-known groupings into poliovirus, Coxsackie A, Coxsackie B, and so on. I call this the viro-astronomical approach. Astronomers perceiving a sky dotted higgledy-piggledy with stars did not abandon the effort to classify them but grouped them, quite illogically, into constellations, groupings which are still useful after hundreds of years. Similiarly, enteroviruses can be grouped into constellations by considering together any two properties, say the ability to grow in monkey kidney tissue culture and pathogenicity for suckling mice. If this is done as shown in figure 1, we see in the right hand lower part of the chart a cluster of viruses readily cultivated in monkey kidney, but of low pathogenicity for baby mice; these are the polio-and ECHO viruses. At the upper right are the Coxsackie B viruses, most of which can be cultivated in monkey kidney and which are also pathogenic for mice; at the upper left are Coxsackie A viruses, growing well in mouse brains, but poorly in monkey kidney cultures. Finally the rhinoviruses (lower left) do well in neither respect. The figure shows, however, that the differences are not absolute: some polioviruses will infect mice; some Coxsackie A viruses will grow in monkey kidney cultures, as will some rhinoviruses. A similar chart could employ any other two useful characters; for instance use of monkey pathogenicity would serve to separate polioviruses and ECHO viruses. However, it is not likely that use of more than very few characters would be necessary to separate all the important groups. We may conclude that it is probably fruitless to try to put every "new" enterovirus definitely into one group or another. Nevertheless the names currently used to denote clusters or constellations of viruses still have a significance and a worthy place in virology. After all we find the terms hillock, hill, and mountain very useful, even though we cannot sharply distinguish between them.

THE NANIVIRUSES

It is worth looking a little more widely than just at the enteroviruses affecting man. These seem to be members of a large natural family of viruses, all small, ether-resistant viruses which so far as is known contain RNA and have a similar structure. Besides the viruses already discussed there are those causing Teschen disease of pigs, encephalomyelitis of mice, epidemic tremor of chickens, and the very many viruses isolated from the intestinal tracts of monkeys, pigs, cattle, cats, and birds. Those unassociated with disease have been named according to the host species ECMO (enteric cytopathic monkey orphan), ECSO (in swine) ECBO (in bovines) etc.

on the analogy of ECHO. Such names can have but ephemeral value for there are but 26 letters in the alphabet and many thousands of known vertebrates. There is also the virus of foot-and-mouth disease, which, incidentally, in its pathogenic effects on mice, closely resembles Coxsackie virus[12]. Finally there are the encephalomyocarditis (EMC) viruses differing from the others in their pathogenicity for a wide range of hosts.

The characteristic feature of all these agents is their small size; hence the suggested name *"Nanivirus"* from the Latin *nanus* = a dwarf[8]. If one looks at these as a whole, it becomes much more reasonable to place within the large family a section—rhinoviruses—having the characters described earlier. It will then appear that it does not matter greatly if they grade into the ECHO viruses, for these in turn grade not only into Coxsackies but also into ECMO, ECSO, etc; ECSO into Teschen virus and so on.

With an agglomeration of viruses such as this, the taxonomist who would designate and name species is confronted with an almost impossible task. The criteria he has for subdividing his major groups are largely characters, such as specificity for particular hosts or culture-systems, which are rather readily manipulated in the laboratory. In the writer's view the right course to pursue at present regarding nomenclature is to give names to only a few major, fundamentally-different, groups of viruses. These could be validated by the international nomenclature committee. Names for lesser groupings, such as rhinovirus and enterovirus, will prove useful for some time but should not be officially blessed for the present.

There is a practical aspect of all this. With all the many new viruses that are being discovered, there is bound to be confusion if workers in different countries give different names to the same virus. The World Health Organization has accordingly begun to designate International Reference Laboratories in hope of keeping these matters under control. A beginning has been made with an Enterovirus Reference Laboratory at Baylor University in Texas and a Respiratory Virus Reference Laboratory at Salisbury in England. If enteroviruses are claimed to cause respiratory infections, there could arise difficulty in determining the spheres of influence of different laboratories within the virus field. Fortunately it is more likely that there will be fruitful co-operation in this matter between workers in many lands. Such co-operation will be helped if the viruses concerned can be viewed in some sort of taxonomic proportion.

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