The full significance of these studies will not be realized until investigations of this nature are extended to other respiratory virus diseases. By examining viruses of varying epidemic potential and comparing such factors as infectious dose, clinical illness, virus-shedding patterns, airborne survival, etc. on a quantitative basis, a better knowledge of the underlying mechanisms of airborne transmission of virus will be gained. This information will be helpful in approaches to environmental control of respiratory disease.

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LITERATURE CITED


Discussion

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Gerone and his associates have presented observations on the production of small-particle virus aerosols with a Collison atomizer in a modified Henderson apparatus, information on the production of viral aerosols by persons infected with coxsackievirus A-21, and data acquired by large-volume air sampling in the environs of infected volunteers. Portions of those studies have been published in greater detail elsewhere (1, 2).

From these observations, certain inferences are made as to the significance of small particles in the transmission of naturally occurring disease due to coxsackievirus A-21, as to the suitability of
the exposure method for the inoculation of volunteers, and as to the usefulness of the large-volume air sampler in demonstrating virus in the environment of infected persons.

The purposes of large-volume air sampling may be twofold. First, one might theoretically detect airborne agents in one-thousandth the concentration detectable by conventional samplers. Second, the larger volume sampled might give one more confidence in an estimate of concentration than that derived by the smaller sample size conventionally obtained. The large-volume sampler has two inherent limitations. First, because of problems of evaporation of collecting fluid, only relatively short periods of sampling are possible. Second, no estimate is possible of particle size distribution of the aerosol sampled. The first problem might be solved by the introduction of sterile distilled water at a rate equal to the loss by evaporation, thus maintaining the integrity of the composition of the collecting fluid. The second, that of particle sizing, appears to be insoluble with the equipment.

The results presented indicate that the device has not attained its theoretical capability to meaningfully quantitate airborne virus. A 100-fold variation between estimates of the concentration of a given aerosol and a 20-fold average variation over the concentrations sampled reduce the device in its present state to a qualitative sampler whose negative results would be suspect.

The presented results of comparison of the large-volume sampler with the Porton all-glass impinger raise more questions than they answer. Greater than 10-fold differences in virus recovery versus tracer recovery are indicated for both samplers, under conditions where tracer recovery was remarkably consistent. These are not compatible with the stated accuracy and reproducibility of the virus assay procedure (standard deviation, 0.25 log\(_{10}\) TCID\(_{50}\) per ml).

The studies on s neezes and coughs establish two main points. First, the particle size distributions and particle volume distributions are markedly unlike that of the artificially generated aerosol used to infect volunteers. Second, no correlation can be made between titer of oral secretions and the amount of virus in a sneeze or cough.

The suitability of any method for the inoculation of volunteers by inhalation can be defined in terms of predictability of the dose to be administered and the site of deposition desired for the purpose of the experiment being conducted.

Predictability of the dose administered to man is influenced by stability of the agent in the spray suspension and in the airborne particulates generated, the uniformity of the aerosol generated, both qualitatively and quantitatively, and by the rate, manner, and volume of breathing of the test subject. It is also obviously dependent upon the accuracy of the assay procedures employed.

The lengthy training required to accustom volunteers to the highly stylized breathing cycle of nasal inhalation and oral exhalation required for mask exposures has long been recognized. Without this, marked variation in respiratory rates and tidal volumes will materially affect sites of deposition of airborne particles, yet not be reflected in the presented dose.

Data have been presented showing a not unreasonable relationship between the concentration of coxsackievirus A-21 in spray fluids and in the aerosol generated in the device employed. The inconstancy of the relationship, as demonstrated by these data, deserves consideration. As examples, aerosol concentrations of approximately 10 TCID\(_{50}\) per liter were obtained with spray suspensions with concentrations ranging from 6.9 \(\times\) 10\(^8\) to 8.2 \(\times\) 10\(^8\) TCID\(_{50}\) per liter, and aerosols containing approximately 1,000 TCID\(_{50}\) per liter were obtained from suspensions containing 8.6, 9.5, and 10.5 \(\times\) 10\(^8\) TCID\(_{50}\) per liter. Conversely, from a single spray fluid concentration were generated aerosols containing less than 100 and over 1,000 TCID\(_{50}\) per liter. Thus, although the relationship, or so-called “spray factor” may be useful in generalized predictions, it does not have the constancy and precision required for individual dose determination. Reliance must still be placed upon after-the-fact estimation of doses presented by assessment of samples collected over the same periods as the volunteer exposures, from samplers located in immediate proximity to the exposure port of the aerosol-generating device.

Even were the problems of dose predictability resolved, suitability of an exposure method still remains dependent upon the purpose of the experiment. If the objective of a study is to determine whether or not man may be infected by an airborne agent in an essentially small-particle aerosol, the method employed by Gerone and his associates is quite appropriate. Similarly, the capacity of a virus to initiate disease in the lungs and tracheobronchial tree is susceptible to examination by this method, the artificially induced pneumonia and tracheobronchitis with strain 49882 HEK affirmatively answers such a question. Elucidation of the mechanisms of naturally acquired coxsackievirus A-21 infection and of the significance of particles of various sizes in natural transmission of disease is an altogether different matter.

Naturally occurring coxsackievirus A-21 illness
is an upper respiratory disease. Experimentally produced upper respiratory illness with this virus has been achieved by nasopharyngeal inoculation (3), by deposition of virus on selected sites in the upper respiratory tract, and by inhalation of large particle aerosols (4), which are primarily deposited in the upper respiratory tract.

In a series of experiments, Buckland and his associates circumvented the problem of precise location of deposition of airborne particulates by direct application of coxsackievirus A-21 to specific locations in the upper respiratory tract. Their findings showed the nasal mucosa to be exquisitely susceptible to infection, whereas the oropharynx and nasopharynx were refractory to doses several orders of magnitude greater. In subsequent studies, volunteers were infected with doses comparable to those directly instilled when presented in relatively large airborne particles, virtually all of which might be expected to be deposited on the nasal mucosa. These authors concluded that only particles retained in the upper respiratory tract are of significance in transmission of naturally occurring disease.

In attributing production of upper respiratory disease to the small particles generated with the Collison atomizer, Gerone and his associates have not rigorously excluded the contribution of that portion of the particles larger than 2 μ, which might be expected to be retained in the upper respiratory tract. From analysis of the particle size spectrum of the aerosol, approximately one-fifteenth the dose presented might be so retained (5). This may well be a significant quantity of virus, of itself capable of initiating infection.

Further experimentation, either by use of aerosols whose upper respiratory retention is negligible, or by bypassing the upper respiratory tract via an artificial airway, are needed if this matter is to be definitively resolved.

Most disappointing to this reviewer is the lack of information presented upon the airborne stability of coxsackievirus A-21 under varying conditions of relative humidity and temperature. The observations of Buckland and his associates indicate a biological decay rate of 50% per min for virus in small particles and roughly 25% per min in the larger particles, if decay is linear. Such values are compatible with droplet infection. Far greater airborne stability is required for significant airborne transmission under ordinary conditions. Valuable information could be obtained by sequential examination of static aerosols with slit samplers or impingers.

In summary, the authors have described an aerosol used to induce infection in man. This discussant believes that further, more critical examination is required to definitively establish the significance of deep respiratory deposition of small particles in production of upper respiratory disease, and hence the appropriateness of the model for the study of naturally acquired infection. It is hoped that further studies will clarify this. Similarly, improvements in high-volume sampling, combined with knowledge of airborne stability of this virus, will permit more critical evaluation of the role of airborne dissemination in coxsackievirus A-21 upper respiratory disease.

Literature Cited


Author’s Comments on the Discussion

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In Col. Gochenour’s discussion of our paper, several points were made with which we are in complete agreement. Other issues were raised, however, regarding which we would like to clarify the position or the conclusions that have been reached.