decreased variation there was a ten-fold increase in 
$^{14}$CO$_2$ produced from glucose-1-$^1$C (resting = 6.4 ± 
1.1; phagocytizing = 23.6 ± 1.7 nmol $^{14}$CO$_2$ produced 
from glucose-1-$^1$C/10$^8$AM/H) by both resting and 
phagocytizing cells.

The importance of this work centers around four 
considerations. The above-mentioned alterations in the 
assay of Stubbs and Karnovsky may lead to the elucidation 
of principles not possible with less efficient and less 
accurate methods. A second consideration is the maintain-
nance of resting CPM sufficiently above background 
levels in order to reflect actual phagocytizing/resting 
ratios. This may not be possible with less efficient assays. 
A third consideration is that the improved assay method 
facilitates calculation of stoichiometric relationships such 
as quantitative oxygen uptake. Finally, increased effi-
ciency and accuracy should make possible comparison of 
data produced by different investigators.

CONCLUSION

The observed variability in our earlier work was at 
least partially due to problems inherent in the assay 
system. There are numerous variables in this assay for 
monitoring cellular activation and HMPS activity, some 
of which can be controlled by available techniques. The 
ten-fold increase in efficiency of measuring $^{14}$CO$_2$ pro-
duction from glucose due to improved methods can aid 
in the study of cellular physiology.

ACKNOWLEDGMENT: Our thanks to Mary Horton for 
typing the manuscript and to Jane Drake and Chris Mason, 
our diligent technicians.

REFERENCES

1 Horton FO, Meyers FJ, McCallum RE, et al: Glucose 
metabolism in resting and phagocytizing human alveolar 

2 Stubbs M, Kuhner AV, Glass EA, et al: Metabolic and 
Med 137:537-542, 1973

3 Holmes B, Page AR, and Good RA: Studies of the metaboli-
c activity of leukocytes from patients with a genetic 
abnormality of phagocytic function. J Clin Invest 46:1428-
1432, 1967

4 Hoidal JR, Fox RB, Takiff HE, et al: Alveolar macro-
phages (AM) from young, asymptomatic cigarette smokers 
(CS) and nonsmokers (NS) use equal amounts of oxygen 
(O$_2$) and glucose (1-$^1$C), but smoker AM make more 
superoxide anion (O$_2^-$). Am Rev Respir Dis 119:(part 2) 
222, 1979

5 Hoidal JR, Beall GD, Rasp Jr FL, et al: Comparison of the 
metabolism of alveolar macrophages from humans, rats, 
and rabbits: Response to heat-killed bacteria or phorbol 

Physical Properties, Hygroscopicity 
and Estimated Pulmonary Retention 
of Various Therapeutic Aerosols*

F. Charles Hiller, M.D.; F.C.C.P.;** 
Malay K. Mazumder, Ph.D.;† Gary M. Smith;‡ and 
Roger C. Bone, M.D., F.C.C.P.§

Therapeutic aerosols are widely used in the manage-
ment of patients with obstructive lung disease. Their 
efficacy is determined by their quantity and site of 
deposition in the respiratory tract which is determined 
primarily by airway patency, inhalation technique, and 
by physical properties of the particles. Particle size, the 
most important property affecting deposition, is best 
expressed as aerodynamic diameter (Da) which accom-
modates all factors such as size, shape, density, and 
surface characteristics that affect the behavior of 
suspended particles. Da is defined as the diameter of a 
unit density spherical particle having the same terminal 
settling velocity as the particle in question. Aerodynamic 
size has been difficult to measure, especially for unstable 
particles containing volatile components such as water. 
Most previously used sizing devices required particle 
deposition, a process which provided only a coarse esti-
mate of size distribution and could not account for 
growth or decay of unstable particles during or after the 
deposition process. Growth of therapeutic aerosols by 
water condensation has been predicted, but no informa-
tion is available quantitating this growth.¹²

The purposes of this work were: (1) to measure the 
aerodynamic size distribution of several commonly used 
therapeutic aerosols, and (2) to determine the growth of 
particles at high humidity, similar to that found in the 
respiratory tract. For these studies we used the single 
particle aerodynamic relaxation time (SPART) analyzer 
unique in its ability to rapidly measure Da of single 
particles in real time over the so-called "respirable size 
range" (0.1-10.0 μm).³⁴ For two of the aerosols studied, 
aerodynamic size distributions were used to estimate 
the quantity of active ingredient which would deposit in 
the lower respiratory tract.

MATERIAL AND METHODS

Trade names of the aerosols studied, listed in the order 
they appear in the tables are Bronkometer, Isuprel Mistome-
ter, Metaprel, Medihaler Iso, Vancoril, and Aarane. The 
active ingredient in the first five is aerosolized from a 
metered-dose device and for the last is aerosolized from a

*From the Pulmonary Division, University of Arkansas 
College of Medicine, and the Department of Electronics 
and Instrumentation, University of Arkansas Graduate 
Institute of Technology, Little Rock. 
Supported by NIH Grant HL 20024, and by a University of 
Arkansas College of Medicine Student Research Fel-
lowship.

**Assistant Professor of Medicine.
+Associate Professor of Electronics and Instrumentation. 
†Junior Medical Student.
‡Professor of Medicine and Chief, Pulmonary Division. 
Reprint requests: Dr. Hiller, University of Arkansas for Me-
dical Science, 4301 West Markham, Little Rock 72201

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Table 1—Physical Properties of Aerosols Measured at Low Humidity

<table>
<thead>
<tr>
<th>Aerosol</th>
<th>CMAD** μm ± SD</th>
<th>MMAD** μm ± SD</th>
<th>( \sigma ) †</th>
<th>Particles per dose ((\times 10^6)) ± SD</th>
<th>Aerodynamic mass/dose ((\mu g)) ± SD</th>
<th>n ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoetharine</td>
<td>0.74 ± 0.02</td>
<td>3.59 ± 0.73</td>
<td>2.0</td>
<td>123 ± 38</td>
<td>334 ± 164</td>
<td>10</td>
</tr>
<tr>
<td>Isoproterenol HCl</td>
<td>0.70 ± 0.03</td>
<td>3.21 ± 0.50</td>
<td>2.1</td>
<td>49 ± 2.5</td>
<td>89 ± 18</td>
<td>7</td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>0.68 ± 0.01</td>
<td>4.05 ± 0.32</td>
<td>2.0</td>
<td>288 ± 24</td>
<td>660 ± 54</td>
<td>7</td>
</tr>
<tr>
<td>Isoproterenol SO₄</td>
<td>0.70 ± 0.02</td>
<td>2.88 ± 0.24</td>
<td>2.0</td>
<td>140 ± 6</td>
<td>225 ± 23</td>
<td>7</td>
</tr>
<tr>
<td>Beclomethasone</td>
<td>0.62 ± 0.02</td>
<td>2.01 ± 0.51</td>
<td>2.1</td>
<td>41 ± 2</td>
<td>23.7 ± 6</td>
<td>8</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>1.41 ± 0.05</td>
<td>2.31 ± 0.07</td>
<td>1.7</td>
<td>383 ± 87</td>
<td>1230 ± 290</td>
<td>8</td>
</tr>
</tbody>
</table>

*Count median aerodynamic diameter—average of n studies
**Mass median aerodynamic diameter—average of n studies
†Geometric standard deviation—average of n studies
‡n = number of trials

Table 2—Physical Properties of Aerosols Measured at High Humidity

<table>
<thead>
<tr>
<th>Aerosol</th>
<th>CMAD** μm ± SD</th>
<th>MMAD** μm ± SD</th>
<th>( \sigma ) †</th>
<th>Particles per dose ((\times 10^6)) ± SD</th>
<th>Aerodynamic mass/dose ((\mu g)) ± SD</th>
<th>n ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoetharine</td>
<td>0.93 ± 0.08</td>
<td>3.79 ± 0.63</td>
<td>1.9</td>
<td>447 ± 79†</td>
<td>1908 ± 566†</td>
<td>8</td>
</tr>
<tr>
<td>Isoproterenol HCl</td>
<td>0.92 ± 0.12‡</td>
<td>4.03 ± 0.38‡</td>
<td>1.9</td>
<td>202 ± 68‡</td>
<td>1016 ± 297‡</td>
<td>8</td>
</tr>
<tr>
<td>Metaproterenol</td>
<td>0.80 ± 0.04‡</td>
<td>5.22 ± 0.60‡</td>
<td>1.9</td>
<td>364 ± 52‡</td>
<td>2215 ± 839‡</td>
<td>7</td>
</tr>
<tr>
<td>Isoproterenol SO₄</td>
<td>0.71 ± 0.08</td>
<td>3.26 ± 0.43</td>
<td>2.2</td>
<td>209 ± 51‡</td>
<td>372 ± 91‡</td>
<td>9</td>
</tr>
<tr>
<td>Beclomethasone</td>
<td>0.63 ± 0.02</td>
<td>2.68 ± 0.51‡</td>
<td>2.2</td>
<td>78 ± 20‡</td>
<td>60 ± 20‡</td>
<td>8</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>1.52 ± 0.10‡</td>
<td>3.02 ± 0.36‡</td>
<td>1.9</td>
<td>152 ± 72‡</td>
<td>746 ± 240‡</td>
<td>8</td>
</tr>
</tbody>
</table>

*Count median aerodynamic diameter—average for n studies
**Mass median aerodynamic diameter—average for n studies
†Geometric standard deviation—average for n studies
‡n = number of studies
14*Probability that mean is different from that for aerosol studied under dry conditions is: 1) P ≤ .05; 2) P ≤ .01; 3) P ≤ .005; 4) P ≤ .001 (using two-tailed Student's t test for two means)
mass of active ingredient (Mp) in the respirable size range must be calculated from Ma. This can be done by subtracting from Ma the fraction estimated to be inactive ingredient and then converting Ma to Mp as follows: \( Mp = Ma/\rho \).

For cromolyn, the Ma of 1.23 mg shown in Table 1 is probably all active ingredient. The only other constituent of the capsule is lactose powder, the particles of which are much larger than the respirable size range and therefore not measured by the SPART analyzer. Since the density of cromolyn (at the humidity used) is 1.55 gm/ml, \( Ma = 0.99 \) mg. Of this, the computer-estimated deposition fraction for gas exchange regions is 34.2 percent and for conducting airways is 5.2 percent. The total deposition is 39.4 percent or 0.39 mg.

For beclomethasone a small portion of the Ma shown in Table 1 is oleic acid (a dispersing agent) and chlorofluorocarbon. These components probably do not contribute more than 10 percent to the total Ma (Dr. M. D. Yudis, Schering Corp., personal communication) but must be subtracted from 23.7 \( \mu \)g, leaving 21.3 \( \mu \)g of active ingredients. The density of beclomethasone is not known so conversion of Ma to Mp was accomplished using an assumed \( \rho \) of 1.17 gm/ml which is the density of other steroids. The calculated Mp for beclomethasone is 19.7 \( \mu \)g. For beclomethasone, deposition is estimated at 28.5 percent in gas exchange regions and 5.2 percent in conducting airways, or 6.7 \( \mu \)g total below the larynx.

**DISCUSSION**

These data indicate that the size distribution of all aerosols tested are, at low humidity, in a size range appropriate for pulmonary deposition. The increase in mass at high humidity for all metered-dose aerosols, and in CMAD and MMAD for cromolyn indicates that all aerosols tested grow when exposed to high humidity.

The estimated depositions for beclomethasone and cromolyn are similar to the quantities shown or estimated to be deposited by previous investigators. *In vitro* studies of beclomethasone using simple airway models suggest that 15 percent is deposited, similar to the 13.7 percent estimated in this study. From drug excretion studies of cromolyn, approximately 5-10 percent of cromolyn is deposited in normal subjects. Another study of cromolyn in asthmatic patients estimated that 3.2 percent of the inhaled dose was deposited in the lung. Our estimation of 2 percent deposition for cromolyn is somewhat below that of other investigators. Our estimation for both aerosols is based on the dry aerosol without consideration of any increase in deposition which would occur as a result of particle growth, which we have shown to occur at high humidity.

The following discussion is a summary of our postulates for explaining our observations. Further data are needed to establish the validity of these postulates. The increase in particle number at high humidity for all metered-dose aerosols is probably due to growth of small particles, \( \rho \), those < 0.1 \( \mu \)m into the SPART analyzer sizing range. The increase in mass at high humidity for all metered-dose aerosols is, in some cases, out of proportion to the observed increase in MMAD. The increase may therefore be due to the addition of new particles to the measurable size range as well as growth of particles initially in the measurable size range.

For cromolyn, there are probably few particles outside the SPART analyzer sizing range at low humidity. The CMAD is well within the area of best resolution of the instrument and particle number falls at a size well above the lower resolution limits. The fall in particle number and mass at high humidity is probably due to growth of particles to sizes larger than can be resolved by the SPART analyzer without, as proposed for metered-dose aerosols, an accompanying shift of very small particles into the measurable range.

Deposition estimation by these methods approximates the results of other studies. Our estimation based on size at low humidity would be expected to be low. Our data show that growth at high humidity occurs for all aerosols tested and would increase deposition above that esti-
ACKNOWLEDGMENT: The authors thank J. D. Wilson for engineering assistance, R. G. Renninger for programming aid, L. A. Higgins for artwork, M. Smith for secretarial help, and Dr. M. K. Testerman for constructive criticism.

REFERENCES

5 Lippman M: Regional deposition of particles in the human respiratory tract. In Handbook of Physiology (Lee D, ed), Bethesda, American Physiological Society, 1977

Q. (Butler): How rapidly do these particles grow?
A. (Hiller): We don’t know, but our preliminary data show that growth probably occurs within milliseconds.

Long-Term Patterns of Obstructive Lung Disease in Cystic Fibrosis

R. Menendez, M.D.;** F. Mather, Ph.D.;
W. W. Waring, M.D., F.C.C.P.

Cystic fibrosis (CF), the most common chronic destructive pulmonary disease of childhood, produces significant airways obstruction in a majority of patients by late adolescence. Little information, however, is available about the patterns of deterioration in pulmonary function. Several investigators1, 2 found large differences in the pulmonary function among CF patients of the same age. One study, 3 a review of five to seven years of pulmonary function data, concluded that in spite of great variability, there was an exponential rate of decline. In addition, they noted that some patients had little change of function during the observation period.

To identify patterns that may account for some of this variability, we analyzed pulmonary function data on a group of CF patients followed for as long as 12 years.

MATERIAL AND METHODS

At the New Orleans CF Clinic, pulmonary function values have been obtained for each patient since 1965. Of 83 patients with at least three sets of values, 34 were excluded because they had less than four years of follow-up. The remaining 49 patients, 21 males and 28 females, constituted our study group. The majority of these patients born after 1960 were diagnosed before five years of age. Pulmonary function data available for analysis included peak expiratory flows, forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and the percentage of the ratio of FEV1 to FVC (%FEV1/FVC).

The patterns of obstructive airways disease were examined as independently as possible of lung growth, by use of the ratio of %FEV1/FVC as the index of obstructive disease. Although this ratio may underestimate the actual amount of obstructive disease, it should parallel the progression of obstruction in the lungs. It, however, would be insensitive to synchronous slowing of the FEV1 and FVC, as might occur with growth retardation or in purely restrictive disease. We plotted the %FEV1/FVC as a function of age for each patient. In normal subjects, this value varies little with age and, in general, remains above the 75 percent level throughout childhood and adolescence.

Figure 1. Graphic representation of the patterns of change with age of %FEV1/FVC in 49 CF patients. The number of patients in each group is shown in parentheses.