Patients were excluded if they had: 1) a personal or family history of asthma; 2) a history of eczema or allergic rhinitis; 3) ≥ 30 percent reversibility in flow rates with bronchodilator inhalation when in a stable condition; 4) pneumonia on the admission chest roentgenogram. Treatment consisted of intravenous aminophylline, antibiotics, inhaled isoproterenol, chest physiotherapy, and either methylprednisolone 0.5 mg/kg or placebo q6h IV for 72 hours. Bedside spirometric tests, including forced vital capacity (FVC), and forced expiratory volume in 1 second (FEV1) were obtained pre- and postbronchodilator inhalation three times daily. Arterial blood gas determinations were obtained twice daily. The groups were similar by all clinical and laboratory parameters on admission. The changes observed in the FVC and ABGs were not significantly different in the two groups. The steroid-treated group had a greater improvement in both the pre- and post-bronchodilator FEV1 (Table 1, P < 0.001). Additionally, more steroid-treated patients had large improvements in the FEV1, as 12/15 patients (80 percent) whose FEV1 improved ≥ 40 percent from the admission value were in the steroid-treated group (P < 0.01). One steroid-treated patient died of cardiac arrest; one patient developed upper gastrointestinal bleeding in each group. In this population, steroid therapy results in a greater improvement in the FEV1.

Q: Can this effect be maintained in the chronic setting?
A. (Albert): The long term use of steroids was reviewed by Sahn (Chest) in 1978. There are no reliable data on this question.
Q. (Flenley): Why were these patients hospitalized?
A. (Albert): They were hospitalized for one of two reasons. Six from each group had Pco2 greater than 45 mm Hg with pH less than 7.35. The others had acute hypoxemia.

Variable Glucose Metabolism in
Resting and Phagocytizing Human
Alveolar Macrophages (HAM)
Measured by 14CO2 Produced From
Glucose-1-14C*

Frank O. Horton, III, M.D.; R. E. McCallum, Ph.D.; and
Robert M. Rogers, M.D., F.C.C.P.

Alveolar macrophages (AM) along with polymorphonuclear leukocytes (PMN) are thought to produce increasing quantities of elastase and collagenase with increasing levels of stimulation. Elastase has been used to produce emphysema in animals and is thought to be important in the production of COPD in humans. One of the characteristics of a stimulated macrophage is elevated hexose monophosphate shunt (HMP) activity measured by the production of 14CO2 from glucose-1-14C. The particulate load of smoking, along with increased numbers of AM, could lead to emphysema in susceptible individuals. This postulated activation response in AM of smokers may exhibit an accelerated or heightened response to stimuli such as infectious agents or other particulate matter, thereby giving rise to increased release of elastase. Alternatively, the AM could increase influx of PMNs and monocytes into the lungs of certain smokers via release of chemoattractive factors. Stimulation to AM, leading to increased production of chemoattractive factors for PMNs and monocytes, may be reflected by altered glucose metabolism. If one is to correctly discern nuances of glucose metabolism in AM from smokers, it is mandatory to have an assay which is efficient, sensitive, stable and mimics in vivo events.

We previously reported values for glucose metabolism in HAM from normal non-smoking adult males1 with a large coefficient of variation (Fig 1, resting = 0.44 ± 0.14; phagocytizing = 1.74 ± 0.41 nmol 14CO2 produced from glucose-1-14C/106 AM/H). The assay employed at that time was based on the method of Stubbs and Karnovsky,2 although Dulbecco’s phosphate buffered saline solution with glucose (DPBS) was substituted for Kreb’s ringer phosphate (KRP). The value for resting phagocytes reported by Stubbs and Karnovsky2 (0.77 nmol 14CO2 produced from glucose-1-14C/106 macrophage/H) was similar to that obtained in our original work.3 Variability in the glucose assay was not indicated by these investigators. Subsequently other researchers have reported varying ranges of glucose catabolic rates (Fig 1). Utilizing the method of Holmes et al.5 Hoidal et al.6 substituted Hank’s balanced salt solution (HBSS) for KRP. Two separate reports by this group yielded varying values for glucose catabolism in HAM. This inconsistency confirmed our earlier observation.

We therefore posed the question, “Is the observed variability inherent to the assay or to differences between subjects tested?” The purpose of this investigation was to examine in detail the assay for measurement of 14CO2 produced from glucose-1-14C. By utilizing the modifications detailed below we were able to decrease the coefficient of variation and increase the efficiency approximately ten-fold.

Methods

We modified the original assay of Stubbs and Karnovsky2 in the following manner: 1) Gey’s balanced salt solution (GBSS) containing 0.5 percent bovine serum albumin (BSA) and 5.56 mM glucose in 2.5 ml was substituted for...
KRP; 2) 2 µCi of glucose-1-$^{14}$C was substituted for 0.5 µCi of glucose-1-$^{14}$C; 3) protocol (20 µl) saturating a filter paper strip was substituted for 0.2 ml of 20% KOH in a hanging center well; 4) 25 ml Erlenmeyer flasks were used rather than 10 ml flasks; 5) flasks contained 2 X 10$^6$ AM compared to 1 X 10$^7$; 6) zymosan granules exposed to UV light for 10 minutes, opsonized for one hour at 37°C in fresh serum and sonicated post-opsonization were used as phagocytic particles instead of polystyrene spherules; 7) Econofluor, containing 10% methanol was substituted for Buhler's solution. Addition of acid at the end of the one hour incubation period was found to be sufficient to stop the reaction and allow for maximum CO$_2$ release; 8) flasks were allowed to equilibrate at room temperature for one hour after addition of acid as opposed to 30 minutes.

**Cell Free Experiments**

Since this assay is dependent upon CO$_2$ release and trapping, NaH$_4$CO$_3$ was used in a cell-free system initially to study factors inherent in the assay components which might influence variability of CO$_2$ production. Additionally, an attempt was made to increase the efficiency and sensitivity of the CO$_2$-trapping system.

**RESULTS**

Figures 2 and 3 refer to CO$_2$ in CPM obtained in a system identical to the modified assay, except that NaH$_4$CO$_3$ was substituted for glucose-1-$^{14}$C and macrophages were absent. As can be seen in Figure 2, the flasks containing HBSS produced a fraction of the CPM produced by flasks containing modified GBSS. The GBSS used throughout this assay was modified by deleting NaHCO$_3$ and adjusting the pH with HCl or NaOH. The series of data points for HBSS did not seem to be influenced by Ca$^{++}$ + Mg$^{++}$. The data points for calcium and magnesium free (CMF)-HBSS were identical to HBSS and are not shown in Figure 2. As can also be seen in Figure 2, DPBS with Ca$^{++}$ + Mg$^{++}$ prevented complete uptake or release of CO$_2$ produced from NaH$_4$CO$_3$ following the addition of acid. When CMF-DPBS was used in this cell-free system, a value similar to GBSS and KRP was obtained.

**Trapping CO$_2$ by Different Scintillation Fluids**

The efficiency of trapping CO$_2$ by different scintillation solutions was examined and the results are shown in Figure 3. It is clear from the data shown in this figure that the total CPM diminished with time when Aquasol was used instead of Econofluor + 10% methanol. Employing Aquasol II rather than Econofluor + 10% methanol revealed a similar decrease but not as severe as that seen with Aquasol (not shown in Fig 1). Our earlier work$^1$ was performed using DPBS and Aquasol or Aquasol II as scintillation fluid.

**Viability of Cells**

The ability of the suspension medium to maintain
viability of AM over a period of time comparable to the assay period is necessary if one is to compare results from separate experiments. We determined cell viability by Trypan blue dye exclusion immediately prior to incubation, whereas, at the end of the assay period viability was determined with acridine orange dye. We found it necessary to add protein to the incubation medium for maintenance of viability. Bovine serum albumin (0.5% Cohn fraction V) was selected since it is well defined and relatively pure in comparison to fetal calf serum. The use of GBSS + 0.5% BSA resulted in greater than 95 percent viability over a two hour period of incubation in comparison to approximately 75 percent viability obtained with DPBS and glucose alone.

Phagocytic Particle

In our original work we used Listeria monocytogenes, but switched to zymosan granules because Listeria metabolized appreciable amounts of glucose-1-14C when greater than 10^7 bacteria were in the flask without macrophages. Although not considered in our earlier work, a uniform ratio of phagocytotic particle to phagocyte (75:1) was maintained. This was accomplished by employing optical density measurements and sonication to disperse clumps of particles after opsonization.

Pipetting

Finally, mechanical pipettors and Lang-Levy constriction pipettes were employed for accurate addition of constituents to each flask to decrease inter-flask variations. We found these techniques superior to manual pipetting and injection by tuberculin syringes.

Coefficient of Variation and Efficiency

As can be seen in Figure 1, employing the modifications described above resulted in a decrease in the coefficient of variation compared with our earlier work. The values displayed represent five experiments with rat alveolar macrophages in suspension. Each experiment employed cells pooled from three rats. A major benefit of the modified assay was the ten-fold increase in efficiency readily apparent compared with the ordinate scales of the other graphs in Figure 1. The increased values obtained were a result of increased efficiency since the ratio of phagocytizing to resting levels was unchanged when compared with our earlier work. Phagocytizing activity was 416 percent and 389 percent of resting activity, respectively.

Discussion

There are several possible mechanisms by which the above noted increase in efficiency and decrease in variability were effected. Although many of the modifications of the assay were based on empirical findings it is possible that CO_2 upon release from NaHCO_3 in HBSS competes with 14CO_2 produced from glucose-1-14C for attachment to the Protosol-saturated filter paper strips. Secondly, the calcium and magnesium contained in DPBS may form complexes with CO_2 and prevent attachment to the Protosol-saturated filter paper strips.

We feel several factors resulted in the marked increase in efficiency and decrease in variability: 1) an efficient and stable scintillation fluid, such as Econofluor + 10 percent methanol; 2) a high molecular weight amine base such as Protosol for trapping 14CO_2; 3) an incubation medium (GBSS + 0.5 percent BSA) which maintained viability but did not appear to interfere with the assay; 4) a reproducible technique for addition of constituents to each flask, along with rapidity in closing the flask and initiation of incubation; and 5) addition of acid at the end of the incubation period to stop the reaction and release bound 14CO_2.

Use of these techniques resulted in a marked decrease in variability (resting, CV = 0.4; phagocytizing, CV = 0.2) in our recent experiments in comparison with our previous work (resting, CV = 1.1; phagocytizing, CV = 0.8). The marked variability seen in our HAM experiments was also observed in earlier unpublished experiments with rat alveolar macrophages and was apparently not due to variation between subjects. In addition to
decreased variation there was a ten-fold increase in
$^{14}$CO$_2$ produced from glucose-$^{14}$C (resting = 6.4 ±
1.1; phagocytizing = 23.6 ± 1.7 nmol $^{14}$CO$_2$ produced
from glucose-$^{14}$C/10$^3$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 mainte-
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$_2$
production from glucose due to improved methods can aid
in the study of cellular physiology.

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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
diameter 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.

The purposes of this work were: (1) to measure the
eaerodynamic 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).4 For two of the aerosols stud-
ied, 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 Brometer, Isuprel Mistome-
ter, Metaprel, Medihaler Ilo, Vancoril, and Aarane. The
active ingredient in the first five is aerosolized from a me-
tered-dose vial 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.
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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 Med-
ical Science, 4301 West Markham, Little Rock 72201

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