# THE PULMONARY RETENTION OF AEROSOLS: A QUANTITA-TIVE METHOD OF MEASUREMENT USING SODIUM PARA-AMINO HIPPURATE

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The increasing importance of aerosols in industry, in the treatment of pulmonary conditions and more recently in the diagnosis and experimental study of bronchial asthma has intensified the need for a method permitting measurement of the amount of aerosol retained in the human lung. This problem has been approached in various ways. Landahl and Hermann (1) measured the difference in concentration of aerosol in the inspired and expired air. They showed that the relative amount of aerosol removed is markedly influenced by the size and physical properties of the particles and also by the rate and depth of respiration. Using aerosols containing radioactive sodium, Talbot, Quimby, and Barach (2) estimated the quantity of aerosol retained by using a Geiger counter over the axilla and the foot. Wilson and LaMer (3) extended this method to include the use of aerosol droplets of uniform size. They determined the total amount of radioactive sodium retained by the subject by measuring the difference in the amount delivered to the subject and the amount in the expired air. The quantity absorbed was estimated from the concentration of radioactive sodium in the blood. The use of blood concentrations as an index of the amount absorbed from the lungs requires assumptions regarding the distribution of the substance in the body. By assuming that the concentration of radioactive sodium in the interstitial fluid was 25 per cent of that found in the blood 15 minutes after exposure, Wilson and LaMer estimated that between 31 and 63 per cent of an inhaled aerosol composed of particles 0.7 microns in diameter was retained in the alveoli. In these experiments, 71 to 80 per cent of the administered aerosol was removed by the subject, the difference presumably being trapped in the upper respiratory tract. More direct methods for measuring the amount of material retained in the lung have not been described.

In the experiments to be described, we determined the pulmonary retention of inhaled aerosols by measuring the difference between the amount in the inspired and expired air and also by measuring the amount absorbed. By using two methods simultaneously, it was hoped that possible errors in measurement would be more easily detected and the validity of both methods could be determined.

#### METHODS

In order to avoid the necessity of making assumptions as to distribution in the body, a substance was sought which after absorption is quantitatively excreted in the urine. Phenol red (PSP), which has been used (4), was discarded by us since urinary excretion is incomplete and variable because the dye is destroyed in the liver. Sodium para-amino hippurate (NaPAH)<sup>1</sup> seemed to be satisfactory; it is well tolerated, rapidly absorbed and quantitatively excreted in the urine.

The apparatus used is shown in Figure 1. Twenty per cent NaPAH was nebulized from a large capacity nebulizer of our own design (A) by oxygen flowing at an accurately measured rate of about 16 liters per minute. The nebulizer contained a large quantity of solution in order to minimize concentration by evaporation. The rate of flow was measured by means of a flowmeter and manometer (B) and regulated by adjusting the reducing valve on the oxygen tank. The nebulum then passed over concentrated sulfuric acid in order to decrease the size of the aerosol droplets. Drying served to make the aerosol more stable without decreasing the amount of NaPAH in suspension, and removed about 95 per cent of the water from the solution aerosolized. Fourfold drying was effected by the oxygen alone and the concentrated sulfuric acid decreased the residual water fivefold. Rough tests indicated that the use of sulfuric acid reduced the loss of aerosol in the bottles and tubing. From the drying bottles, about two-thirds of the aerosol (about 10 liters per minute) passed to the patient via a flutter value (C), and the remainder (about 6 liters per minute) was sampled (see below). An oil-filled

<sup>1</sup>Kindly supplied by Dr. William P. Boger of Sharp and Dohme, Inc.



APPARATUS FOR QUANTITATIVE ADMINISTRATION OF AEROSOL



spirometer compensated for the intermittent demand of the subject's respiration. A three-way value (D) situated between the oxygen flowmeter and nebulizer permitted the sudden diversion of oxygen from the nebulizer when the amount of aerosol produced had exceeded that removed by the subject and the sampler (E). The sampler (E) consisted of two filter-paper thimbles in series. Aerosol was drawn through the sampler by a suction pump (F) at a rate (about 6 liters per minute) sufficient to ensure collection of a representative sample. The rate of flow was measured by a flowmeter and inclined manometer (G) and was kept constant by adjusting the opening of a bleeder (H) situated between the flowmeter and pump. By slowly closing the bleeder (H) during operation of the sampler, a constant rate of flow was maintained through the sampler (E), even though the filters became somewhat plugged as aerosol was deposited.

The expired aerosol passed through a second flutter value (J) to an impinger (K). A rubber bag (L) pre-

vented loss of aerosol during the period when the peak rate of expiration exceeded the flow through the impinger. A bleeder (M) enabled the impinger to operate continuously at full flow without drawing aerosol from the apparatus during inspiration. With the aerosol used, the impinger operated at about 99 per cent efficiency when the rate of flow was at least 25 liters per minute. A flowmeter (N) in the suction line indicated whether this flow was maintained.

The volume of gas flowing through a flowmeter depends not only on the flowmeter reading but on the pressure of the gas delivered. This was checked in the case of the oxygen supplied to the nebulizer by means of a manometer (P), and in the case of the sampler by manometer (Q). The increase in the negative pressure at Q as the filters collected aerosol was shown to be insufficient to alter significantly the calibration of the sampler flowmeter (G) under the conditions of the experiments.

Experiment	Amount of PAH found in:				PAH*	PAH	PAH	Total recovery	
	Sampler (1)	Impinger (expired air) (2)	Valves and mouth washings (3)	Urine (4)	Breathed by subject (5)	by subject (5) $-(2) -(3)$ (6)	from lungs (4) ×1.05† (7)	(2) +(3) +(7) (8)	Per cent of quantity delivered (5) (9)
1 2 3 4 5	mg 63.0 114.2 124.0 78.4 75.0	mg 17.3 29.6 23.3 13.8 17.5	mg 18.3 23.9 29.3 28.2 30.4	mg 31.1 42.9 53.7 45.5 53.4	mg 72.1 109.5 117.1 91.1 114.6	mg 36.5 56.0 64.5 49.1 66.7	mg 32.6 45.0 56.4 47.8 56.1	mg 68.2 98.5 109.0 89.8 104.0	% 94.6 90.0 93.0 98.6 90.7

TABLE I

\* Actually the amount delivered to the inspiratory value. (5) = (1)  $\times \frac{\text{Volume Breathed}}{\text{Volume Sampled}}$ 

† Allowance made for loss during hydrolysis of acetylated PAH in urine. (See text for explanation.)

The duration of an experiment was measured with a stop watch, during which time the subject breathed aerosol and the sampler (E) and the impinger (K) operated continuously. The total time of aerosol production (which was not continuous) was timed separately. The rate of oxygen flow, sampler flow, and impinger flow were checked with the spirometer for each experiment. During an experiment, the nose was clamped and the subject kept his mouth closed around a rubber mouthpiece attached to the valve assembly (C and J). The subject did not swallow and allowed his saliva to drip into the valve assembly. The position of the spirometer was noted at the beginning and at the end of the exposure. After the experiment, the mouth was rinsed with water and the washings were combined with the valve washings. As will be shown, absorption from the mouth was negligible. Urine was collected over a period of 12 hours following each experiment.

The amount of PAH in the sampler, the valve as sembly and mouth washings, and the impinger was de termined by the method of Smith and colleagues (5 The amount of PAH in the subject's urine was deter mined by Newman's modification of this method (6) i order to include acetylated PAH. About half the PAH in the urine was acetylated, the fraction increasing wit the interval between absorption and excretion, and ha to be hydrolyzed before colorimetric determination. W found that hydrolysis entailed some loss of PAH, an therefore the total amount administered could not be recovered even with the utmost care. For example, it was found on two occasions that after the intravenous ir jection of 60 mg. of PAH, only about 95 per cent could be recovered in the urine. Therefore, under the cond tions of the inhalation experiments, the amount of PAI detectable in the urine must have been less than that al sorbed by at least 5 per cent, and for this reason w have added 5 per cent to the amount of PAH found the urine for purposes of computation.

## RESULTS

The results of five experiments are shown in Table I. The duration of exposure was 10 minutes in all but Experiment No. 4, where it was 9 minutes and 28 seconds. The flow through the nebulizer was 16.0 to 16.3 liters per minute and that through the sampler was 4.31 to 4.36 liters per minute. The volume delivered to the subject varied from 52.8 to 65.8 liters. The first four columns in the table show the amount of PAH collected in the sampler, impinger, valve assembly and mouth washings, and urine. The concentration and character of the aerosol produced varied from experiment to experiment because of unavoidable variations in the adjustment of the nebulizer. Sample calculations for Experiment No. 5 are shown below.<sup>2</sup> The amount of PAH delivered to the subject was determined by multiplying the amount sampled by the ratio of volume breathed to the volume sampled. Because of the position of the sampler in the circuit, this calculation actually gives the amount delivered to the inspiratory valve rather than that inhaled by the subject. We could not satisfactorily avoid this difficulty because the sampler had to be located upstream to the inspiratory valve in order to avoid sampling expired air. The PAH absorbed by the subject was computed by adding 5 per cent to that found in the urine, as explained above. The amount re-

## <sup>2</sup> Calculations for Experiment No. 5

s-	Observations	
e-	Time of breathing and sampling	10.0 min.
).	Time of aerosol production (oxygen flow)	6.83 min.
r-	Rate of aerosol production (oxygen flow)	16.0 L/min.
in	Rate of sampling	4.31 L/min.
H	Correction for position of spirometer at	
h	end of test	0.5 L
d	NaPAH in	
Te	Sampler	75.0 mg.
d	Impinger	17.5 mg.
	Valves and mouth washings	30.4 mg.
	Urine	53.4 mg.
15 1-	Calculations	
ld	Volume of aerosol produced =	
i-	$6.83 \text{ min.} \times 16 \text{ L/min.} =$	109.3 L
н	Volume sampled =	
b-	$10 \text{ min.} \times 4.31 \text{ L/min.} =$	43.1 L
/e	Volume of aerosol breathed $=$	
in	109.3 - 43.1 - 0.5 =	65.7 L
	NaPAH delivered to subject =	
	NaPAH sampled $\times \frac{\text{volume breathed}}{\text{volume sampled}} =$	
n	75.0 mg. $\times \frac{65.7 \text{ L}}{43.1 \text{ L}} =$	114.6 mg.
1-	NaDAH metained has autient	
9	delivered amount evolved (implement)	
ıe	- amount in values and mouth wash	)
d	inge =	-
rs	114.6 - 17.5 - 30.4 =	66.7 mg.
)-	NaPAH absorbed from lungs $=$ amount in	ı
ır	urine + estimated loss by hydrolysis =	
Н	$53.4 \text{ mg.} \times 1.05 =$	56.1 mg.
v	Total NaPAH recovered = amount in ar	
·	pired air + amount in valves and mouth	-
1- 1	washings + amount absorbed =	•
a	17.5  mg. + 30.4  mg. + 56.1  mg. =	= 104.0 mg.
1-	Dem east of any stitus deliver of the st	
	rer cent of quantity delivered that is re-	-

covered =  $104.0/114.0 \times 100 =$ 

90.7%

covered is the sum of that absorbed and that found in the valves and the impinger. Although each of these quantities varied markedly from experiment to experiment, the totals agreed fairly well with the amounts delivered and recovery was almost complete.

In these experiments an average of 47 per cent (41.1 to 52.5 per cent) of the quantity delivered to the valve system was absorbed by the subject. If we assume that about half of the material found in the valves was removed by the inspiratory valve, as indicated by two tests in which each valve was washed separately, 55 per cent of the amount reaching the subject's mouth was absorbed, and approximately 40 per cent failed to be absorbed and was collected in the expired air, in the saliva, and mouth washings. At the end of Experiment No. 2, the PAH in the saliva and mouth was measured separately from that in the valve assembly. The amount found was 6 mg., which would suggest that in experiments of this kind, an appreciable quantity of aerosol is trapped in the mouth. As shown below, this is not absorbed.

### DISCUSSION

The results indicate that all but a small portion of the aerosol delivered to the subject can be accounted for in the valves, the expired air, and by that absorbed as indicated by the amount in the Since the average of the sum of these urine. amounts (the amount recovered) equals 93.4 per cent of the amount delivered with a standard deviation of 1.7 per cent, there would seem to be a significant fraction which is unaccounted for. Nevertheless, the agreement in per cent recovery is surprising in view of the necessity of making two crucial measurements of air flow and several determinations of PAH in carrying out each experiment. Because of the possibility that some errors may cancel out, the real error may have been larger than one would be led to expect from the results. The amount of PAH actually found in the urine, uncorrected for loss during hydrolysis, was only about 15 per cent less than the difference between the amount delivered and that found on the valves and impinger. The amount actually retained must have been at least as great as the amount in the urine and not more than that which disappeared from the apparatus and is thus fairly well delimited for the conditions of the experiment.

With this method of measuring pulmonary retention of aerosols, absorption from the mouth would seem to be an obvious source of error. In order to determine if this occurred, an experiment was performed in which 90 mg. of PAH were applied to the mucosa with a swab. The subject then swirled his saliva about for 10 minutes, gargled intermittently, but did not swallow. The mouth was then rinsed thoroughly with gargling and the collected washings were found to contain 86 mg. or 96.0% of that placed in the mouth. In the succeeding 10 hours, less than 1 mg. was excreted in the urine. These results show that absorption from the mouth is negligible even with a much larger amount of PAH in contact with the buccal mucous membrane than the subjects were exposed to during the inhalation experiments. It would seem reasonable to conclude that absorption takes place below the pharynx, whether chiefly from the trachea and larger bronchi or from the lung parenchyma cannot be stated on the basis of these studies.

#### SUMMARY AND CONCLUSIONS

A method for the quantitative measurement of the pulmonary retention of aerosols is described using sodium para-amino hippurate. The use of this substance permits determination of the amount of aerosol retained by: 1) the difference between the amount inspired and that expired and trapped in the mouth; and 2) the amount which is absorbed and appears in the urine. The first requires a cumbersome apparatus; the second, collection of urine over a period of six to 12 hours. In either case, the method could readily be modified for study of the pulmonary retention of other substances given as aerosols by incorporating sodium para-amino hippurate in the solution to be nebulized.

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