in amounts equal to the optimum amount of the untreated material.

Further study of the numerous factors to be considered in the use of iodinated trypsin *in vivo* is in progress.

Summary. The coagulation of recalcified plasma, *in vitro,* is hastened considerably by trypsin which has been iodinated sufficiently to greatly decrease its hypotensive effect.

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Depth of Penetration of Nebulized Substances in the Respiratory Tree.*

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(Introduced by E. Ogden.)

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To effect a local concentration of penicillin in the pulmonary system, some physicians have been administering nebulized penicillin in the inspired air.

Administering penicillin mist by the usual method of inserting a spray into the oral pharynx uses two routes of administration simultaneously. The mist deposited on the pharyngeal mucosa is absorbed, diluted by the blood stream and distributed throughout the body; also some of the mist may follow the column of inspired air down the respiratory passages to effect a local surface action. Intramuscular injection of penicillin produces higher blood concentrations than is possible by mucosal absorption of the relatively small dosage used in nebulizers. Thus, the only advantage of nebulization is a possible local action on the surfaces of the respiratory passages.

The purpose of this study was to determine the magnitude of concentration and the depth of penetration down the respiratory passages, of a nebulized substance administered with inspired air.

Liquids, such as Lipiodol, pass rapidly to the terminal bronchioles and alveoli. It is known from unpublished war research that aerosol clouds in which the mass median diameter is carefully controlled will penetrate in small concentrations to the alveoli. Dusts, such as silica, over long periods of exposure may penetrate deeply. This study is not concerned with such phenomena, but only with conditions similar to those of aerosol therapeusis, *i.e.*, mixture, with commercial nebulizers, of mist with inspired air in the oral pharynx, and durations of exposure of 15-60 minutes of eupneic breathing.

Nebulized particles in inspired air tend to be deposited against the walls of the respiratory system by settlement due to gravity and by changes in the direction of air flow. The particles tend to be retained in the system by impingement on moist and adhesive surfaces. The deposition due to changes in direction of air flow increases with the velocity of air flow and turbulence, whereas settlement, due to gravity, is only effective where the velocity is low.

Hatch[1] states that maximum air velocities occur in the nasal passages. The mean velocity of the air column in the bronchioles is reduced to 1/1000, and at the entrance of the alveoli is further reduced to 1/100,000 of nasal velocity. Thus the greatest deposition of nebulized particles should be expected in the upper respiratory tree. The air velocity

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[1] Hatch, T. F., Behavior of Microscopic Particles in the Air and in the Respiratory System, p. 102, in *Aerobiology*, Moulton, F. R., editor, publication of the A.A.A.S., No. 17, Washington, D.C., 1942.

reaches zero throughout the system for a portion of each respiratory cycle during which period settlement by gravity is possible. However, it is generally accepted in standard texts[2] that respiratory movements do little other than ventilate the respiratory passages down to the finer subdivisions of the bronchioles (Henderson *et al.*[3]). A gas finds its way to the depth of the alveoli only by molecular diffusion. Since nebulized substances are much larger than molecules of a gas, the laws of diffusion do not apply. Nebulized particles, therefore, are transported only when there is an active movement of a column of air, at which time the forces of inertia and impingement causing deposition of such particles are greatest.

An additional factor preventing penetration and retention is the return of some of the particles in the inspired air to the outside atmosphere with expiration. Brown[4] has shown that retention of dust particles is inversely proportional to the respiratory rate for rates below 20 per minute, thus, the slower the respiration the greater the retention. He also concluded that the percentage retention was not affected by volume per respiration, vital capacity or relative humidity of inspired air.

It is often stated that a 5 micron particle will float airborne to terminal air passages. This statement is based on the fact that the terminal bronchioles are 300-400 microns in diameter, and thus it is assumed that a 5 micron particle should have no difficulty in making the passage. This assumption is open to criticism.

Van Wijk and Patterson[5] studied the proportion of particles of different sizes removed from dust-laden air by breathing. They found that 25% of 0.2 micron particles are removed, 80% of 2 micron particles are removed, and 95% of 5 micron particles are removed. They further state that the larger particles are deposited in the larger air passages, thus, the smaller the particle, the less the retention. Bryson and co-workers[6] made photomicrographs of penicillin aerosol reporting the average particle diameter to be 0.54 microns with a range of 0.24 to 1.18 microns.

It would appear from these purely theoretical considerations that large concentrations of aerosol mists will not penetrate deeply into the respiratory tree because

1. The forces of inertia and impingement causing deposition are greatest in the larger branches;
2. The moving air column does not penetrate to the alveoli;
3. The mist will not diffuse as will a gas;
4. There is a return to the outside of some of the mist; and
5. The larger particles which are most likely to be retained are most likely to be deposited in the larger bronchioles.

The literature reveals only one experimental study which separates clearly the factor of pharyngeal absorption from actual penetration of mist. Krueger and co-workers[7] confined an adult monkey in an atmosphere of dilute India ink for 60 minutes. On gross examination, following autopsy, carbon was found in large amounts in the nasal passages, larynx and stomach, in smaller amounts in the trachea and bronchi. Microscopic sections showed that some inspired material had penetrated into the alveolar spaces. The following experiments were undertaken to investigate the apparent lack of agreement between the theoretical considerations and the experimental result.

Methods. The depth of penetration and magnitude of concentration of various nebulized substances in the respiratory tree was determined experimentally in 26 animals (2

2 a. Bachmann, G., and Bliss, A. R., Jr., *Essentials of Physiology and Pharmacodynamics,* Blakiston Co., Philadelphia, 1940; b. Best, C. H., and Taylor, B. T., *The Physiological Basis of Medical Practice*, 3rd ed., p. 512, Williams and Wilkins, Baltimore, 1939.

3 Henderson, Y., Chillingworth, F. P., and Whitney, J. L., *Am. J. Physiol.*, 1915, **38**, 1.

4 Brown, C. E., *J. Indust. Hyg.*, 1931, **13**, 285; *ibid.*, 1931, **13**, 293.

5 Van Wijk, A. M., *J. Indust. Hyg.*, 1940, **22**, 31.

6 Bryson, V., Sansome, E., and Laskin, S., *Science*, 1944, **100**, 33.

7 Personnel of Naval Laboratory Research Unit No. 1, Command of Krueger, A. P., and Lyons, W. R., *Am. J. Med. Sci.*, 1944, **207**, 40.

TABLE I.

1	2	3	4	5	6
Angle of bend in glass tube	No bend	one 30° angle	one 90° angle	one 120° angle	two 20° angles
% of mist removed from air column	defined as 0%	30-35%	75-80%	90-95%	50%

mice, 11 rats, 2 rabbits and 11 dogs). A commercial nebulizer ("glaseptic"—Parke-Davis & Co.) connected to a half-liter pressure bottle was used to obtain a fine mist of diluted India ink (1:1 or 1:5 aqueous dilution), gentian violet (1%-5% aqueous solution) or ferric ammonium sulfate (1%-5% aqueous solution). It was found that a hand bulb gave a more sustained mist with smaller droplets than did oxygen at 8 to 10 liters per minute; so the former was used exclusively after the first 4 animals studied.

The size of the nebulized droplets sprayed from the pressure bottle was directly observed by comparison with red cell smears to include particles as small as 0.5 microns with 80% being 1 to 3 microns in diameter.

The animals were lightly anesthetized with pentobarbital sodium (Nembutal). The mists were introduced to the respiratory tree by a glass tube placed in the oral-pharynx, or in some animals by a tracheal cannula placed in the same axis as the trachea. The rate and depth of respirations were noted. The animals were exposed to the mists for periods of from 15 to 60 minutes. They were immediately sacrificed and gross and microscopic observations were made.

Preliminary experiments on the action of suspensions passing through a system of branching glass tubes demonstrated the removal of particulate matter from the air column at the points where the direction of the air column changed. The amount of nebulized suspension of dilute India ink passing through a straight tube was accepted as a measure of 0% removal (Table I). As the angle of bend in the tube increased, and as the number of bends in the tube increased, there was an increase in the removal of particulate matter from the air column. The carbon was deposited largely on the walls opposing flow at the bends in the tubing.

Results. 1. The first group of one rat and 2 mice was not anesthetized nor was an oral cannula used. The animals were placed in an atmosphere of a mist of 50% India ink for 60 minutes. Carbon particles were found in the nasopharynx and stomach of each animal. No carbon was seen grossly or microscopically in the larynx, trachea, bronchi, bronchioles, or alveoli.

2. Because the nasal passages appeared to be removing most of carbon from the inspired air in the first group, a second group of 7 rats and 2 rabbits, lightly anesthetized, was exposed to India ink, gentian violet or ferric ammonium sulfate solutions for periods of from 15 to 30 minutes. The mist was introduced to the inspired air by an oral cannula. The nose was occluded and the tongue pulled forward to insure mouth breathing.

Particles were seen grossly to be present in rather large quantities in the oral pharynx and larynx of all animals. Below this point the nebulized substances were noted in small quantities, mostly at the point of branching of the bronchi. Microscopic sections indicated no evidence of foreign material in the alveoli. Only one cell out of every one or two thousand lining the bronchi showed any evidence of foreign material.

3. Because of the negative results in rats and rabbits, a third group of 4 dogs was exposed to India ink suspensions (1:5 aqueous dilution) and ferric ammonium sulfate (1% aqueous solutions) with exposures of from 30 to 60 minutes. The results were essentially the same as with the second group of animals except that in two animals there were minute particles in the larger bronchioles which may have been either artifact or inspired material.

4. In order to by-pass the screening of the inspired air by the pharynx and larynx, the mist was directly introduced through a tracheal cannula into a rat and 2 dogs. This fourth group of animals showed tracheobronchial distribution similar to the other groups, but

larger quantities of carbon and iron were deposited. No particulate matter was demonstrated in the smaller bronchioles or alveoli.

5. Because a satisfactory microscopic picture—for a comparison control—of the deposition of carbon, dye or iron in the alveoli had not yet been produced, an attempt was made to force particulate matter into the alveoli. The lungs and trachea were removed in one block from 2 dogs and 2 rats and were artificially inflated by the nebulizer. A free flow of mist was insured by cutting the tip from one of the lower lobes. The lungs were inflated and deflated beyond physiological limits for a period of 15 minutes. Microscopic sections indicated only a minute amount of foreign material in the bronchi. None of the alveoli was seen to contain foreign material. Even those alveoli adjacent to the free flow of mist at the cut tip were free of particulate matter.

6. The breathing of one dog was stimulated by an intermittent mixture of CO_2 with the inspired air. Dilute India ink mist was introduced into the oral pharynx for a period of 30 minutes. The gross and microscopic distribution was essentially the same as in dogs during the eupneic breathing. No carbon was distributed in the smaller bronchioles or alveoli.

7. Abramson[8] has suggested that 25% glycerol be added to the carbon suspension to lower vapor pressure and thus make a more stable mist. Two dogs were exposed to a glycerol mist containing carbon for 30-minute periods. There were tracheobronchial depositions of carbon in larger quantities than when aqueous suspensions were used. In a dependent lobe of one dog there was a sharply localized area 2 cm in diameter in which carbon was deposited in the alveoli. However, in this dog there was an excess of free flowing fluid in the oral pharynx and the dog coughed and "gurgled" several times during the procedure. It is believed that this localized alveolar deposition was due to aspiration and flow rather than to inspiration of nebulized mist.

Discussion. A careful search of the literature fails to bring to light any actual experimental confirmation of Krueger's experiment[7] in which the factor of pharyngeal absorption is clearly separated from the factor of inspiratory penetration.

Using nebulized sulfonamide solutions, Mutch[9] found that only 7 to 14 percent of the drug leaving the nebulizer reached the blood stream. Later, working with nebulized penicillin, Mutch and Rowell[10] found that only 25% of the mist was absorbed into the blood stream. Neither of these studies determined what proportion of the blood concentration was due to pharyngeal absorption. We have been able to demonstrate only a minimal penetration to the larger bronchi, and a questionable penetration of nebulized substance into the deeper respiratory passages.

Although penicillin aerosol may be adequate for effecting some degree of pharyngeal absorption, it does not yield adequate surface action on the respiratory passages. It is the impression of the authors that the concentration of the substance on the surfaces of the trachea, bronchi and bronchioles would not be sufficient for therapeutic effectiveness.

Summary. 1. An attempt has been made to determine the depth of penetration, and the amount of deposition in the respiratory system of nebulized dilute India ink, aqueous gentian violet solution and aqueous ferric ammonium sulfate solution in 2 mice, 11 rats, 2 rabbits and 11 dogs.

2. The substances were found to be deposited in decreasing amounts in the oropharynx, larynx, bifurcation of the trachea, the bronchi and bronchioles of the animals. The amounts of deposited substances in the bronchi and bronchioles were so small as to make microscopic detection difficult. In no case was any nebulized substance found to have reached the alveoli.

3. There is no evidence to support the theory that pulmonary lesions can be treated by topical application with commercial nebulizers.

8 Abramson, H. A., *Ann. Allergy*, 1946, **4**, 440.

9 Mutch, N., *Lancet*, 1944, **2**, 775.

10 Mutch, N., and Rowell, R. E., *Lancet*, 1945, **1**, 650.