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## Production of Acute Pulmonary Edema by Ammonium Salts.*

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During the investigation of another problem,[1] in which guinea pigs were administered ammonium chloride by gavage, small overdoses of this chemical were found to result in pulmonary edema after a short interval.

In guinea pigs so treated, acute pulmonary edema appeared to be a consistent cause of death.[2,3] It was therefore considered worthwhile to experiment further with the thought of utilizing ammonium salts as a tool for a subsequent investigation of the pathogenesis of acute pulmonary edema.

*Methods.* Guinea pigs and white rats of both sexes were principally used. Six cats and 5 rabbits also were studied. Early in these studies, complete autopsies were done but when it became apparent that changes were limited to the chest, autopsy studies were restricted to the thoracic viscera.

Microscopic studies were carried out on all lungs in which the pathological diagnosis was in doubt, and also in a series of animals in which it was desired to study the histological changes of the grossly abnormal lungs. The lungs were fixed in one of 3 ways: by vascular

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† Presented to the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

1 Windle, W. F., Koenig, H., and Jensen, A. V., *Arch. Neurol. and Psychiat.*, 1946, **56**, 428.

2 Koenig, H., and Koenig, R., *Fed. Proc.*, 1948, **7**, 67.

3 Koenig, H., and Koenig, R., *Anat. Rec.*, 1948, **100**, 52.

perfusion[4] of 10% formalin in isotonic saline solution with 2.4% gum acacia added; by intratracheal injection of 10% formalin in isotonic saline solution; or by ligating and excising a pulmonary lobe, immersing it in 10% formalin in isotonic saline, and agitating it constantly on a shaking device for 24-48 hours. Immersion fixation of an entire lobe gave the best results for the demonstration of edema exudate. All lung sections were stained with hematoxylin and eosin.

A 6% solution of ammonium chloride in water (weight in volume) was used at all times with the exception of the animals administered other salts of ammonium to determine the specificity of the cation with regard to the lung changes. Control animals were treated like the experimental animals except for the administration of ammonium chloride. These animals were also anesthetized with pentobarbital ("Veterinary Nembutal") just before removal of the lungs. Further details of procedure will be given in the appropriate sections to follow.

*Results. A. Dosage and routes of administration. Guinea pig.* Most of our experience has been with this animal. We have seen typical pulmonary alterations with ammonium chloride given by the gavage, subcutaneous, intramuscular, intraperitoneal and intravenous routes of administration. The dose of ammonium chloride depended upon its mode of introduction. The slower the rate of absorption, the larger the dose had to be. Thus, a dose which would produce acute pulmonary edema by gavage would, when injected into the peritoneal cavity, usually kill the animal before sufficient time had elapsed for the evolution of these changes. Ammonium chloride could be successfully injected into the vein only by slow drip. Another factor of importance was the weight of the animal; the usual direct relationship between body weight and dosage appeared to apply.

The gavage and intraperitoneal routes were utilized almost exclusively because the results were more dependable. By stomach tube, the effective dose was 0.09 to 0.12 g/100 g of body weight; intraperitoneally, it was 0.05 to 0.07 g/100 g of body weight. Thirty of the 40 experimental guinea pigs succumbed to acute pulmonary edema. Wherever lung changes failed to appear, it could be attributed to one of two causes. If the amount of ammonium chloride was too great, or if it was too rapidly absorbed, death resulted from cardiac failure, or more frequently from respiratory failure, before the appearance of lung changes. On the other hand, if the dose was inadequate, or if absorption appeared retarded, the lung changes were not seen and other manifestations of toxicity were often completely absent. Our experience suggested that a definite concentration of the ammonium ion has to be attained and maintained in the blood for a period of at least 10 or 15 minutes for the development of pulmonary edema.

*Rat.* Ammonium chloride solution was administered to this animal by stomach tube and by intraperitoneal injection. The latter route proved more convenient and reliable. The effective dose for the intraperitoneal route was 0.04 g/100 g (0.66 cc of a 6% solution). Administration resulted in acute pulmonary edema in all of 10 rats.

*Cat.* The cat proved to be more resistant to the pulmonary edema produced by ammonium chloride than the guinea pig or the rat. Vomiting and salivation were a common occurrence with toxic doses of ammonium chloride given intraperitoneally or by gavage, but care was taken to prevent aspiration. In later experiments, the trachea was cannulated following local infiltration with 1% procaine in order to circumvent these difficulties. Of the 6 cats used, pulmonary edema occurred in 3 animals. The animals in the latter group weighed 1950-2750 g each. Two were given 35 cc of a 6% solution of ammonium chloride intraperitoneally. A third was given 69 cc of a 6% solution of ammonium chloride intraperitoneally in 4 divided doses over a period of 2 hours.

*Rabbit.* In none of 5 animals did acute pulmonary edema occur after ammonium chloride was given intraperitoneally or by gavage in doses great enough to cause toxic symptoms culminating in death.

[4] Koenig, H., Groat, R. A., and Windle, W. F., *Stain Technology*, 1945, **20**, 13.

*B. Specificity of the ammonium ion.* In order to determine the specificity of the ammonium moiety of ammonium chloride in this phenomenon, 6 guinea pigs, each weighing about 350 g, were used. One was given 6 cc of 20% ammonium nitrate, a second was given 7 cc of 6.4% ammonium acetate, a third 7 cc of 10% ammonium bromide, a fourth 7 cc of 6% ammonium chloride, and a fifth 7 cc of 7.4% ammonium sulfate. The 4 latter solutions were roughly equivalent with respect to the concentration of ammonium. A sixth animal was given 7 cc of water to serve as a control. Gavage administration was used in these experiments. The first 5 animals all succumbed to acute pulmonary edema. The lungs of the control were normal.

Guinea pigs given the standard dose of ammonium chloride by gavage showed a blood pH of 6.8 to 7.2, 15 to 30 minutes later. Two mechanisms are involved in the production of this acidosis, acid hydrolysis of ammonium salts, and the conversion of ammonia into urea. The first mechanism does not operate with an ammonium salt of a weak acid, such as ammonium acetate. However, guinea pigs and cats rendered even more severely acidotic by gavage administration of a sodium lactate buffer, or a dilute solution of hydrochloric acid, never showed pulmonary edema. It is evident, therefore, that the ammonium ion rather than the acidosis is the primary factor in the pathogenesis of this form of lung edema.

*C. Syndrome of ammonium intoxication.* In the guinea pig and rat, the characteristic sequence of events transpired in 15 to 60 minutes, taking less than 30 minutes in most of the animals. A considerable increase in rate and depth of respiration occurred several minutes after the administration of ammonium chloride. Weakness and difficulty in locomotion soon appeared; at about the same time, hyperexcitability for tactile, auditory and painful stimuli was noted. Shortly thereafter, muscle fasciculations appeared over most of the body and could be best seen in the tongue. These usually preceded the onset of one or more generalized tonic convulsions. Occasionally the convulsions appeared before the fasciculations. Coma quickly supervened, the corneal reflex was absent and no stimulus however great would bring about a response. The respirations were by this time greatly reduced in frequency and were deep and gasping in character. Stridor was frequently heard. A serosanguineous, or rarely a serous, fluid exuded from the nostrils or mouth before death. Occasionally, no fasciculations or convulsions occurred, or perhaps only a terminal convulsion; yet acute pulmonary edema was present at autopsy.

The above description held also for the cat, with several exceptions. The dose required was greater and the time interval before lung edema appeared was longer, varying from one to 3 hours. Sialorrhea usually occurred and vomiting was common. This animal appeared to be on the whole more resistant to ammonium pulmonary edema than were the rodents. Another notable difference was the character of the edema fluid issuing from the upper respiratory tract. In the cat, the fluid was never blood-tinged, whereas, in the rat and guinea pig, blood-tinged edema fluid was the rule, with very few exceptions.

The effects of toxic doses of ammonium would appear to be chiefly on the central nervous system, at least until pulmonary edema supervenes. They were manifested by muscle fasciculations, tonic convulsions and

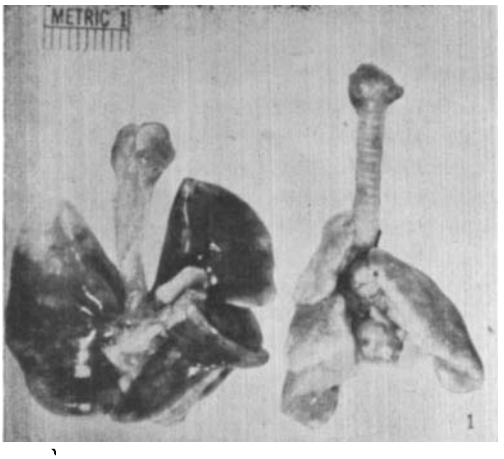


FIG. 1.

Photograph of gross specimens. On the left are the trachea and lungs from a rat that died 20 minutes after an intraperitoneal injection of ammonium chloride. The lungs are swollen and present pleural surfaces which are discolored by areas of hemorrhage and congestion. At the right are the trachea and lungs from a control rat.

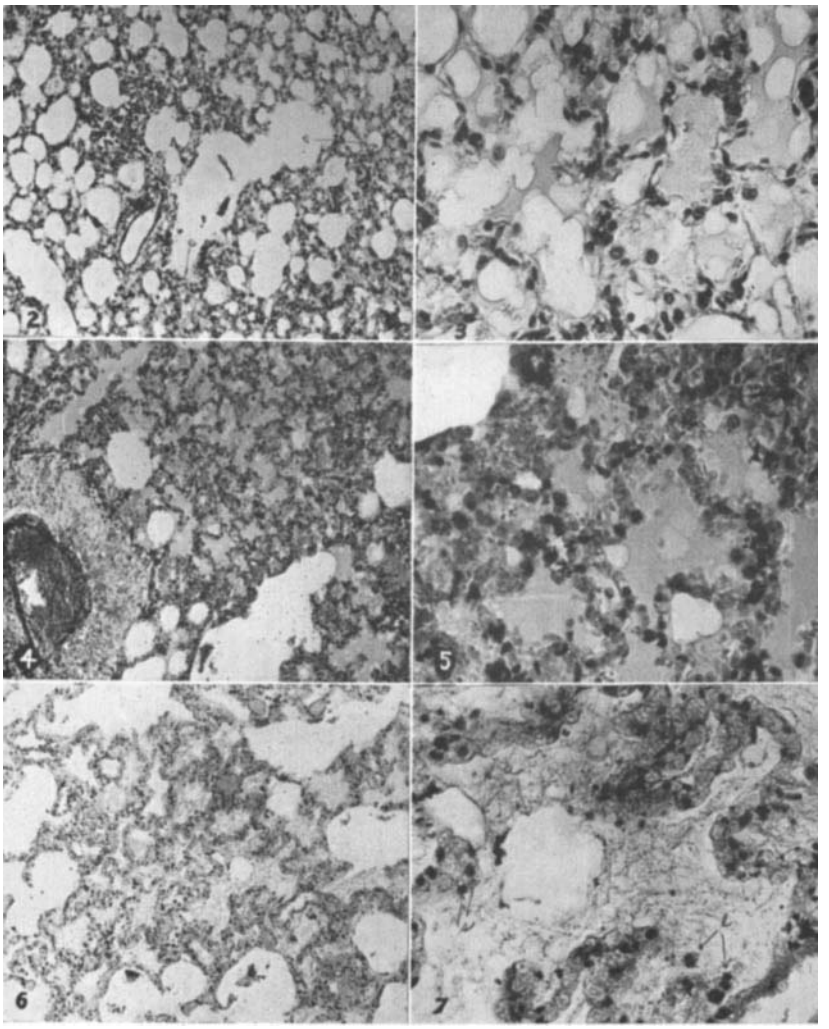


FIG. 2. Photomicrograph of a typical area from a section of lung from a guinea pig which succumbed to acute pulmonary edema 30 minutes after gavage administration of ammonium chloride. Many of the alveoli are filled with a homogeneous precipitate of edema fluid (e). × 64.

FIG. 3. Photomicrograph, at higher magnification, of part of the area shown in Fig. 2 illustrating the homogeneous edema exudate which contains many air bubbles. Hemorrhage is not a prominent feature here, but occasional small groups of extravasated red blood cells are present in the alveoli. × 263.

FIG. 4. Photomicrograph illustrating a typical area of a section of lung from rat which died 25 minutes after an intraperitoneal injection of ammonium chloride. Most of the alveoli in this field are filled with a homogeneous eosinophilic exudate in which air bubbles and occasional blood cells are included. A branch of the pulmonary artery in the lower left hand field is surrounded by a wide "collar" of edema exudate containing many red blood cells. × 64.

FIG. 5. Photomicrograph at higher magnification of a portion of the field shown in Fig. 4. The edema exudate and the dilatation and intense congestion of the alveolar capillaries are prominent features. × 263.

FIG. 6. Photomicrograph of a section taken from the lung of a cat which died of acute pulmonary edema 2½ hours after the intraperitoneal injection of ammonium chloride. A fibrillar exudate fills many of the alveoli. A number of small veins are seen dilated and filled with blood cells. × 64.

FIG. 7. Photomicrograph at higher magnification of part of a single alveolus in the field shown in Fig. 6. Many fibrillar strands are in the edma exudate. A small bubble (a) of air still remains in the center of the alveolus, another smaller one is at the side. Occasional macrophages (septal cells(s)), and leucocytes (l) appear in what was formerly the alveolar space. Capillaries in the alveolar septa are turgid with blood cells. × 263.

dyspnea. The fasciculations suggested repetitive discharging of primary motor neurons of the brain stem and spinal cord. The tonic convulsions indicated a greatly heightened reflex excitability of the spinal cord. The dyspnea which occurred early had a two-fold origin. Acidosis was an important factor, which probably stimulated respiration reflexly through the chemoreceptors[5] and through an increase in $CO_2$ tension in the blood which resulted from a depression in pH. In addition, ammonium is a direct respiratory stimulant;[6] we noted dyspnea with ammonium acetate, a salt which produces no immediate depression of blood pH.

*D. Anatomical changes.* Gross pathology found at necropsy was observed only in the chest and upper respiratory tract. The nasal cavity, larynx, trachea and bronchi were filled with a frothy fluid, usually blood-tinged in the guinea pig and rat, but serous in the cat. These passageways were otherwise unobstructed. Pleural fluid was never seen. The lungs were increased in size (Fig. 1), collapse being prevented by the fluid in the lung parenchyma. The pleural surface of the lungs was always discolored; in some animals there was only a petechial distribution of deep red spots, in others a massive hemorrhagic involvement, but an appearance intermediate between these extremes was more common. The changes showed no predilection for lobes or parts of lobes. On section, the lung was deep red in many places and a frothy serosanguineous fluid could be readily expressed. Congestion of blood vessels and parenchyma was marked. The right atrium, right ventricle and the venae cavae entering the heart were usually moderately dilated, but the left ventricle and left atrium appeared normal.

Histological alterations were limited to the lung and the brain.‡ By far the most important change was the massive pulmonary edema with variable degrees of hemorrhage. The alveolar spaces and smaller air passages were filled with a precipitated pale eosinophilic material in which air bubbles and red blood cells were often included. This edema exudate was usually homogeneous in the guinea pig (Figs. 2 and 3) and rat (Figs. 4 and 5) when the lung was fixed by total immersion, but was distinctly fibrillar in the cat. Interstitial edema was not a prominent finding except in the rat where periarterial "collaring" or "cuffing" of edema exudate was always present. Hemorrhage into the alveoli and the interstitial tissues was always present, but varied greatly in extent and degree. Vascular congestion was intense and involved not only the larger vessels, but also the capillaries. In the cat (Fig. 6 and 7) it was not unusual to find alveolar capillaries that were seven or eight red blood cell diameters in width and filled with densely packed blood cells.

*Discussion.* The lung changes produced by toxic doses of ammonium salts appear to have escaped notice. Underhill and Kapsinow[7] determined the minimum lethal dose for a series of ammonium salts in white rats, producing death within three hours. They concluded that the toxicity of these compounds, with a few exceptions, varied directly with the ammonium content of the compound. However, these investigators did not report the presence of pulmonary edema, nor did they state the cause of death in the animals studied. Chenoweth and his colleagues[8] studied the toxicity of ammonium gluconate

[5] Schmidt, C. F., Macleod's Physiology in Modern Medicine, Philip Bard, C. V. Mosby Co., St. Louis, 9th edition, 1941, 561, 567.

[6] Brassfield, C. R., Hansen, E. T., and Gesell, R., *Fed. Proc.*, 1946, **5**, 10.

‡ Pathological changes have been seen in the brain and are described elsewhere.[1]

[7] Underhill, F. P., and Kapsinow, R., *J. Biol. Chem.*, 1922, **54**, 451.

[8] Chenoweth, M. B., Civin, H., Salzman, C., Cohen, M., and Gold, H., *J. Lab. and Clin. Med.*, 1941, **26**, 1574.

which was given intravenously to cats, but they did not record the presence of pulmonary edema at autopsy.

It is in the clinical literature that one finds a hint of this toxic effect of ammonium salts. Fazekas[9] described 2 cases of suicide resulting from the ingestion of ammonium chloride. One woman died 10 hours afterward and autopsy revealed, among other findings, an advanced pulmonary edema. The brain changes which were described were characteristic of anoxia. The amount of ammonium chloride ingested was unknown.

Abnormal increases of ammonium in the blood are occasionally encountered in disease. McNeil and Levy[10] found that this occurred most often in conjunction with acute disease processes involving the liver. In view of the important role the liver normally plays in the transformation of ammonium to urea, this is not surprising. While there is apparently little danger of ammonium intoxication with the usual therapeutic doses of ammonium salts, the possibility exists that large doses, such as are occasionally given to cardiac patients to promote diuresis (up to 40 g a day)[11] in the presence of an hepatic insufficiency, may result in a blood concentration of ammonium great enough to cause toxic manifestations—even pulmonary edema. Such an occurrence would ordinarily be imputed to the cardiac disorder rather than the ammonium.

*Summary.* (1) Ammonium salts in toxic doses produced an acute pulmonary edema in the rat, guinea pig and cat. The cat was more refractory to this effect than the rat and guinea pig. The rabbit did not appear to be susceptible to ammonium lung edema. (2) While a number of routes of administration of ammonium chloride were found effective for the production of acute pulmonary edema, the gavage route (0.09-0.15 g per 100 g of body weight) was most dependable for the guinea pig, and the intraperitoneal route (0.04 g per 100 g of body weight) for the rat. (3) The ammonium ion was found to be the agent responsible for the lung edema. Many ammonium salts produced this effect. Acidosis was ruled out as the primary factor, because acidosis produced in other ways did not result in lung edema. (4) The syndrome of ammonium intoxication consisted chiefly of dyspnea, muscle fasciculations and convulsions, terminating in an early acute pulmonary edema. (5) Gross changes at autopsy were limited to the thoracic cavity. They consisted of pulmonary edema and congestion with variable degrees of hemorrhage and moderate dilatation of the right ventricle, right atrium, and entering venae cavae. The microscopic appearance of the lungs was one of edema, congestion and hemorrhage.

[9] v. Fazekas, G. I., *Deutsch. Ztschrft. f.d. ges. gericht. Med.*, 1934, **23**, 225.

[10] McNeil, H. L., and Levy, M. D., *J. Lab. and Clin. Med.*, 1917, **3**, 18.

[11] Keith, N., quoted by Sollmann, T., A Manual of Pharmacology, W. B. Saunders, Co., 7th edition, 1948, 776.