THE PREVENTION OF DEATH FROM PHOSGENE POISONING IN THE FROG BY THE ADMINISTRATION OF SODIUM CHLORIDE

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PHOSGENE is an extremely poisonous gas. Inhalation of sufficient phosgene by man and other mammals produces an acute pulmonary œdema which is generally fatal in its fulminating form. The acute pulmonary œdema is of the irritative type and due to increased permeability of the pulmonary capillaries. In man and in other animals depending upon the lungs for oxygenation, increased permeability of the pulmonary capillaries leads to accumulation of fluid in the alveolar spaces and a mechanical blockage of oxygenation which of itself may be lethal if the respiratory surface of the alveoli not blocked mechanically by fluid becomes insufficient for oxygenation of the body. The importance of this mechanical blockage of respiratory surfaces as a cause of death in phosgene poisoning is emphasised in the experiments to be described below, which demonstrate that phosgene death can be readily prevented when there is no mechanical blockage to exchange of respiratory gases.

The experiments were performed upon phosgenised frogs. When frogs are maintained in water at a temperature below 15° C., the exchange of respiratory gases occurs entirely through the skin, the relatively primitive amphibian lung being inactive. This has been demonstrated by Dolk and Postma,[1] Adolph,[2] and Boyd and Mack.[3] In preliminary experiments, it was found that frogs kept at low temperatures could be killed by exposure to a sufficient concentration of phosgene. Assuming by analogy with the action of phosgene in mammals that the respiratory skin surface was the site of lethal action of phosgene in these frogs, it was obvious that death could not have been due to mechanical blockage of the respiratory surface by œdema fluid. The skin of the frog serves as a medium of exchange for many substances required in body economy. In addition to being a respiratory surface, large volumes of water may pass through the skin, as shown by Boyd, Clark and Smith.[4] These considerations prompted investigation of the possibility that exposure to phosgene had increased the permeability of the frog skin to some substance necessary to body economy and that loss of sufficient of this substance had been the cause of death. Further experimentation confirmed this hypothesis, and demonstrated that chloride was such a substance lost through the skin in phosgene poisoning of frogs and that administration of sodium chloride prevented death from phosgene in these animals.

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Exposure to Phosgene

The leopard frog, *Rana pipiens*, obtained from a dealer in the Province of Quebec, was used in these studies. The frogs were first acclimatised to low temperature by placing them in an aquarium containing 4 cm. of distilled water at 4° C. in a refrigerator room and leaving them thus for at least 12 hours. Under these conditions, the frogs gradually took up water to a maximum of 10 to 20 per cent. of body weight, reaching a plateau absorption by the end of 12 hours, a fact previously demonstrated by Boyd and Mack[3] and confirmed in the investigation herein described. Further studies revealed that the rate of loss of body chloride through the skin under these conditions required also a period of 12 hours for acclimatisation to a temperature of 4° C.

In preliminary experiments, it was noted that large frogs could survive exposure to a concentration of phosgene which was lethal to small frogs. Because of the relatively larger skin surface compared to body weight of the small frogs, this reflected the probable correctness of the original premise that the site of lethal action of phosgene in frogs at this low temperature was the skin. In all subsequent experiments, the frogs used in each comparison of the effect of phosgene were selected so as to be of approximately the same average body weight per group. The method used was to take frogs out of the aquarium in the refrigerator room at 4° C., dry their skin and weigh them to the nearest 0·1 g. on a trip scale balance. Each frog was then placed in an individual beaker with its body weight marked on the beaker. After a sufficient number of animals had been weighed, the beakers were aligned in order of increasing body weight of the frogs. Alternate beakers and their frogs were then assigned to control and treated groups. Each group therefore was composed of frogs of about equal average body weight and of the usual frequency distribution of body weight for this species.

Exposure to phosgene was accomplished by placing each frog in a battery jar of 3-l. capacity covered with a tightly fitting metal cap containing a small hole over which was pasted a piece of paper. The frogs thus assembled were removed to a thoroughly ventilated gassing room and for each 1 g. of frog, 0·30 ml. of phosgene, drawn at room temperature from a tank, was injected with the aid of an hypodermic syringe into the battery jar through the paper-covered opening in the cap. The frogs in the battery jars were then returned to the refrigerator room and phosgene allowed to act for a total period of 30 minutes. The actual initial concentration of phosgene in the battery jars averaged 1 in 500, the concentration varying with the weight of the frog. Under these conditions, 50 to 100 per cent. of frogs died as a result of exposure to this concentration of phosgene. With the same procedure but using a smaller battery jar, concentrations of phosgene less than 1 in 500 produced a comparable mortality amongst the frogs. These variations in lethal concentration appeared to be associated with variations in the rate of dispersal in the air of the battery jar of the injected phosgene and with the rate of hydrolysis of phosgene which was found to be rapid.

After exposure to phosgene for the 30-minute interval, the frogs were removed from the battery jar and placed in 400-ml. beakers containing 100 ml. of distilled water or of the solution to be studied. The movements of the animals were extremely sluggish at this low temperature of 4° C. so that it was seldom necessary to place a restraining cover over the beakers. Death due to phosgene occurred invariably within a period of 100 hours after exposure to the gas, so that this interval was selected for observation of the lethal effects of this gas.

In a typical experiment, of 34 frogs treated and phosgenised as described above, 60 per cent. died within 48 hours of gassing with no further delayed deaths on observation for 1 week. At or near death, there was a generalised congestion of the white skin over the belly and anterior and internal surfaces of the limbs, an appearance similar to that seen in the disease of frogs known as "red leg" in this locality. There was no change in body weight until death, after which there occurred an increase in body weight due to uptake of water. The latter was not characteristic of phosgene poisoning since a similar post-mortem absorption of water may be demonstrated in frogs left in water after death from other causes. Congestion of the respiratory skin surface would appear to be the characteristic lesion of phosgene poisoning in frogs, corresponding to congestion and œdema of the lungs in phosgenised mammals and man. There is another possible, though probably unlikely, explanation of the changes observed, namely that phosgene lowered the resistance of the frogs to red leg which is endemic amongst frogs in this locality and which can produce death rapidly. As a working hypothesis, it was assumed that phosgene had produced congestion and increased permeability of the frog skin and further investigation was directed toward ascertaining the nature of the assumed increased permeability and finding a way to combat it.

Baths of Ringer Solution, Isotonic Sucrose Solution and Frog Saline Solution

If phosgene had increased the permeability of the frog skin, it seemed reasonable that phosgenised frogs might live longer or even survive if placed in a bath the composition of which was more like that of frog extracellular fluid than was distilled water. Frog Ringer solution was selected for initial trial as a substitute for distilled water in the beaker baths. 2 weight-matched groups of 15 frogs each were both exposed to phosgene as described above. One group was then placed in a bath of distilled water and the second in a bath of frog Ringer Solution, each frog being in 100 ml. of the bath fluid at 4° C. in a 400-ml. beaker. The mortality rates of these two groups were: distilled water, 53 per cent.; frog Ringer solution, 0. In this experiment, the bath of frog Ringer solution gave complete protection against the lethal action of phosgene and suggested that death from phosgene had been due to loss of physiological salts through the skin.

Before investigating this concept in further detail, it was judged desirable to eliminate the possibility that the phosgenised frogs had survived in frog

Ringer solution because they were in a bath which was osmotically isotonic. Hence an experiment was performed upon 2 groups of 10 frogs each, both exposed to phosgene, then one group placed in baths of isotonic sucrose solution and the other in baths of distilled water. The mortality rates were:—distilled water, 80 per cent.; isotonic sucrose solution, 80 per cent. The frogs placed in baths of isotonic sucrose solution actually lived 24 to 48 hours longer than did the frogs placed in baths of distilled water. Isotonicity alone in the bath delayed but did not prevent death from phosgene which re-focussed attention upon physiological salts in the water bath.

Of the physiological salts contained in frog Ringer solution, it seemed likely that the most important in preventing death from phosgene would be sodium chloride. Hence a third experiment upon baths was performed using 3 groups of 10 frogs each, exposed to phosgene as described and then distributed as follows: frogs of group I were placed in beakers containing 100 ml. of distilled water in the refrigerator room; frogs of group II were similarly placed in 100 ml. of frog Ringer solution; frogs of group III were placed in 100 ml. of frog saline solution (0·75 per cent. of sodium chloride in distilled water) in a like manner. The mortality rates amongst these three groups were:—distilled water, 100 per cent.; frog Ringer solution, 0; 0·75 per cent. sodium chloride, 0. It is obvious that the significant factor in preventing death of frogs from exposure to phosgene was the presence of sodium chloride in the water bath.

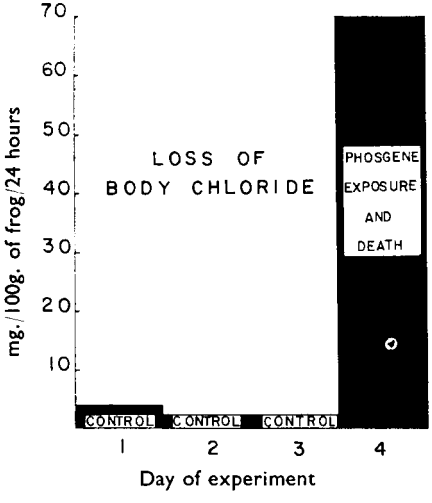

FIG. 1. The effect of exposure to a lethal dose of phosgene upon the rate of elimination of chloride through the skin of frogs at 4° C.

LOSS OF CHLORIDE BY PHOSGENISED FROGS

Since the presence of sodium chloride in the water bath prevented death amongst frogs previously exposed to phosgene, it seemed reasonable to suppose that death in a bath of distilled water had been due to loss of body chloride into the bath water. To investigate this hypothesis, 7 frogs were acclimatised to water at 4° C., and then placed in separate 400-ml. beakers each containing 100 ml. of distilled water also at 4° C. Every 24 hours for 3 successive days the frogs were each removed to another beaker containing 100 ml. of fresh distilled water and the accumulation of chloride during the previous 24 hours determined by measuring the chloride content of the distilled water from which the frog had been removed, by the method of Van Slyke.[5] As indicated by the data charted

in Figure 1, loss of chloride by the frogs into the distilled water of the bath reached a plateau level on the second and third day. At the end of 72 hours, therefore, the frogs were exposed to phosgene as described above. All of these frogs died toward the end of a 24-hour period after exposure to phosgene and when each animal died or was near death it was removed from the water bath, the chloride content of which was then determined. The output of chloride was expressed as mg. of chloride per 100 g. of frog per 24 hours, these values were averaged for the 7 frogs in each of the three days before and the day after exposure to phosgene and these means have been charted graphically in Figure 1.

By reference to this figure, it may be seen that the output of chloride attained a constant value of 2·2 mg./100 g. of frog/24 hours over the 48 hour period just before exposure to phosgene. During the 24 hour period after exposure to phosgene, there was a 32-fold increase in the output of chloride to a mean of 70 mg./100 g. of frog/24 hours. To eliminate the possibility that this tremendous increase in the output of chloride was a post-mortem change which occurred rapidly after death, similar chloride estimations were made upon 3 frogs before and after death other than from phosgene and there was no post-mortem increased output of chloride during the 24-hour period immediately after death. Therefore the conclusion seems justified that poisoning by phosgene at 4° C. produces a marked loss of body chloride in frogs assembled in distilled water.

The site of this augmented loss of body chloride would seem to be indicated as the skin, and the cause, an increased permeability of the skin produced by phosgene, a situation analogous to the increased permeability of the pulmonary alveoli and capillaries produced by phosgene in mammals and man. This postulated hypothesis is supported by some preliminary investigations made upon 5 frogs assembled in distilled water as were the frogs described above but with, in addition, a cannula and collecting tube ligated into the cloaca for the separate collection of cloacal contents. In these frogs, the major output of chloride was found to be in the distilled water of the bath and not in the cloacal contents.

Parenteral Injection of Sodium Chloride Solution

Finally, it was possible to demonstrate that the parenteral administration of a solution of sodium chloride to frogs assembled in distilled water after exposure to lethal concentrations of phosgene would effectively prevent death of the animals. For this experiment, 10 frogs acclimatised to water at 4° C. were exposed to phosgene and then placed in 400-ml. beakers containing 100 ml. of distilled water. Every other frog was given an injection of 0·5 ml. of 30 per cent. solution of sodium chloride in distilled water into the dorsal lymph sac just after gassing. None of the frogs which received this injection died as a result of the exposure to phosgene although each one became swollen, suggesting that the dose of sodium chloride probably could have been reduced. Of the remaining animals not protected by injection of the solution of sodium chloride, 80 per cent. died.

The experiments indicate that phosgene is not a deadly poison in the frog "breathing" through its skin at a temperature of 4° C. providing that loss of sodium chloride through increased permeability of the skin is prevented, either by parenteral administration of sodium chloride in solution or by allowing the animal to absorb sodium chloride from its water bath or preventing loss of sodium chloride into the water bath. The results suggest that the highly lethal property of phosgene when inhaled by man and mammals is due not to the increased permeability of the pulmonary alveoli and capillaries but to the accumulation of œdema fluid in alveolar spaces and a mechanical blockage of respiratory exchange.

Discussion

The information obtained in the experiments described above cannot be applied directly to the treatment of acute pulmonary œdema due to exposure to phosgene or other causes in man and mammals because of fundamental differences in the respiratory mechanism. They do suggest that phosgene of itself need not be particularly lethal if a means could be found of preventing the accumulation of œdema fluid in the pulmonary alveoli with its mechanical blockage of respiratory exchange. The results further suggest that if the œdema fluid were removed from the lungs, restoration of physiological substances so lost, particularly sodium chloride, might be indicated.

Acute pulmonary œdema is still one of the commonest causes of death due to respiratory failure. The condition can arise when one or more of the following three basic alterations in the physiology of the lungs appears in acute form:

(*a*) *Increased pulmonary intracapillary pressure*, such as occurs when the output of blood by the right ventricle is greater than that of the left ventricle, as was explained by Welch,[6] and as is seen in left ventricular decompensation from cardiac lesions, and bradycardia and reduced cardiac output from carotid sinus and aortic stretch hyperreflexia such as may occur in lesions of the brain and be relieved by sympathetic section[7] or sympatholytic drugs.[8] It may arise also from increased peripheral resistance such as occurs following intravenous injection of large doses of adrenaline hydrochloride, hypertensive vascular disease and intense neurogenic excitement which may be relieved by morphine and occasionally by suitable psychiatric therapy.[9] The pulmonary intracapillary hydrostatic pressure is normally low and of the order of 10 mm. of mercury,[10] which favours reabsorption of water from the alveoli by the opposing 25 mm. of mercury osmotic attraction of the plasma colloids, but if the latter figure falls below the former, a similar type of pulmonary œdema ensues.

(*b*) *Decreased intra-alveolar air pressure*, from the normal intra-pleural pressure of −2·5 to −6 mm. of mercury during quiet breathing, or from whatever pressure to which pulmonary adjustment has been made, may produce acute pulmonary œdema as in bronchial spasm and obstruction or sudden relief from obstruction.

(*c*) *Increased permeability of the pulmonary capillaries*, such as may

occur in inflammatory lesions of the lung, toxæmias, anoxæmia and inhalation of alveolar irritants such as phosgene. Obstruction of the right lymphatic duct is another possible cause, at least in theory.

Because of the decreased alveolar absorptive surface in acute pulmonary œdema, oxygen is indicated in treatment, without carbon dioxide and under a pressure of 6 cm. of water, which increases appreciably the volume of gas in the lungs,[11] perhaps with ethanol vapour as an anti-foaming agent.[12] The anxiety and fear of suffocation are allayed by morphine, thus lessening the demands for oxygenation. Further measures may be directed at specific contributing factors.

The basic abnormality in man and all animals dependent upon lungs for oxygenation would seem to be obstruction of the alveoli with œdema fluid. No rapid and completely effective means of removing this œdema fluid has been found so that the mortality rate from acute pulmonary œdema remains high. On the other hand, mechanical blocking of the breathing surface cannot occur in the phosgenised frog kept in water at low temperature and all that is therapeutically necessary is to restore salt lost through increased permeability of the skin. Phosgene poisoning, which is extremely lethal in mammals, can be readily cured in the frog.

Summary

1. Frogs maintained at a temperature of 4° C. and respiring entirely through the skin are killed when exposed to a sufficient dose of phosgene, due to congestion of the respiratory skin surface and a tremendously increased loss of chloride through the skin, but with no mechanical blockage of the respiratory surface by œdema fluid as in lung-breathing animals.

2. Under these conditions, death from phosgene is readily prevented by the parenteral administration of sodium chloride in solution or by maintaining the animals temporarily in a bath of sodium chloride solution.

3. The results emphasise that death from acute pulmonary œdema following exposure to phosgene in man and other lung-breathing animals is probably due entirely to mechanical blockage of the respiratory surfaces by œdema fluid.

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