RESPIRATORY TRACT FLUID AND INHALATION OF PHOSGENE

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Increase in the permeability of the respiratory membrane of rabbits after exposure to phosgene was followed by measuring the output and composition of respiratory tract fluid. During the latent period, levels of sodium and chloride in respiratory tract fluid were elevated but volume was normal. During the early symptomatic period, the levels of sodium and chloride were definitely elevated but volume was still normal. Just before death in the late symptomatic period, respiratory tract fluid poured out of the lungs at a rate which averaged 60 times the normal and its sodium, chloride, and lipid levels were identical to those of blood plasma. Rabbits in the last group had an intense pulmonary oedema, haemoconcentration, and little or no resistance to the lethal effect of urethane.

In a previous communication to this Journal, Boyd and Stewart[1] reported that death in frogs exposed to phosgene at 4° is due to increased permeability of the respiratory skin surface to sodium chloride. The objective of the work to be described in the present report was to determine when and to what extent inhalation of lethal doses of phosgene by rabbits increases the permeability of their respiratory membranes. This was done by measuring the composition of respiratory tract fluid and comparing this with the corresponding composition of blood plasma at intervals after exposure to phosgene. At the same intervals the degree of pulmonary oedema was estimated by histologic and chemical examination of the lungs.

Methods

The animals were healthy, adult, male rabbits. They were exposed to phosgene by the static method in a chamber of 400 l. capacity at an initial concentration of 0·27 mg./l. An exposure for 30 minutes was found in preliminary trials to kill 80 to 100 per cent of rabbits, anaesthetized as noted below. This dose is within the range of the median lethal dose of phosgene in unanaesthetized animals as reported by Spector[2].

The animals were anaesthetised to the upper level of Plane 1 of surgical anaesthesia with urethane given intraperitoneally, and arranged for the collection of respiratory tract fluid by techniques described by Boyd[3]. Thirty-one rabbits were so arranged immediately after gassing. A further 31 were gassed late in the afternoon and the survivors anaesthetised for collection of respiratory tract fluid 16 hours later. Eighteen rabbits served as controls given no phosgene, but otherwise treated as were the animals treated with phosgene.

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The output of respiratory tract fluid was measured at hourly intervals and expressed as ml./kg./24 hours. Samples collected at or near death were analysed for their content of sodium[4], chloride[5], and lipids[6]. Similar analyses were made upon samples of oxalated blood plasma collected at the same time. The haemoglobin content and per cent volume of erythrocytes (haematocrit) were determined upon these samples of oxalated blood since haemoconcentration has been reported in dogs[7–9], and guinea pigs[10] treated with phosgene. At autopsy were measured the histopathology, water, chloride, and iron[11] content of the trachea and of the hilus and periphery of the lungs, divided after the technique of Boyd and Johnson[12], and in part after the recommendations of Hemingway[13], and Poulsen[14]. Statistical analyses of data were made as described by Croxton[15] and Waugh[16].

RESULTS

Graphical arrangement of data upon the 62 rabbits indicated that values for most measurements fell into three frequency distributions.

TABLE I

THE HISTOPATHOLOGY OF THE LUNGS AND TRACHAE*

The results are expressed as mean ± standard deviation arbitrary (1+ to 4+) units

Site	Measurement	Group I	Group II	Group III
Alveoli	Oedema	0·9 ± 0·9 A	3·5 ± 0·9 A, B	4·0 ± 0·0 A, B, C
	Congestion	0·8 ± 0·9 A	1·0 ± 1·4 A	0·0 ± 0·0 B, C
	Emphysema	0·7 ± 0·7 A	1·4 ± 1·2 A, B	0·0 ± 0·0 B, C
	Haemorrhage	0·2 ± 0·5	1·0 ± 1·5 A, B	0·0 ± 0·0 C
	Contracted arteries	1·3 ± 1·3 A	0·3 ± 0·6 B	0·0 ± 0·0 B, C
Bronchioles ..	Oedema	0·3 ± 0·3 A	0·1 ± 0·3 B	0·0 ± 0·0 B
Trachea	Oedema	1·0 ± 1·3 A	0·1 ± 0·3 B	0·3 ± 0·5 B
	Congestion	0·7 ± 1·2 A	0·3 ± 0·5	0·3 ± 0·5

* A value of $P < 0.05$ by a t test that the mean of Groups I, II, or III equalled that of the controls is indicated by A, that the mean of Groups II or III equalled that of Group I by B, and that the mean of Group III equalled that of Group II by C.

The first frequency distribution was composed of 31 animals anaesthetised immediately after treatment with phosgene and was termed Group I. The second distribution was termed Group II; it contained 19 animals anaesthetised 16 hours after being treated with phosgene and in which the volume output of respiratory tract fluid was at no time greater than that of the controls or the rabbits in Group I. In the third group, Group III, there were 12 rabbits, anaesthetised 16 hours after phosgene treatment and in which there was a marked increase in the volume output of respiratory tract fluid just before death.

The rabbits of Group I survived a mean (± standard deviation) of 3·3 ± 1·9 hours from the time they were anaesthetized and arranged for collection of respiratory tract fluid. The corresponding survival time in Group II was 3·1 ± 2·9 hours. In Group III, the survival time was significantly less, averaging 0·7 ± 0·4 hours. The control animals were killed for measurements at the end of the day, some 6 to 8 hours after they were anaesthetised. Most normal rabbits live for 36 to 48 hours under the degree of urethane anaesthesia and surgical manipulation involved in collecting respiratory tract fluid.

A summary of histopathologic observations is presented in Table I. The measurements were made semi-quantitative by assigning to each an arbitrary value of 0 or 1+ to 4+, depending upon the visual estimate of each measurement. Values for the control rabbits were zero and have not been listed in Table I.

The immediate response to phosgene during the first 6 or 7 hours after exposure consisted of a mild oedema of the trachea, bronchioles, and alveolar tissues, associated in the latter, with some congestion, emphysema and contraction of arteries. At 16 to 20 hours in the animals of Group II, the pulmonary oedema had markedly increased, there was more emphysema and some haemorrhage, the pulmonary arteries were less contracted and there was less oedema in the bronchioles and trachea. In the animals of Group III with a premortal gush of respiratory tract fluid, pulmonary oedema was maximal, the arteries were not contracted, and there was no congestion, emphysema, or haemorrhage.

TABLE II

MEASUREMENTS UPON RESPIRATORY TRACT FLUID*

The results are expressed as mean ± standard deviation

Measurement	Units	Control rabbits	Rabbits treated with phosgene		
			Group I	Group II	Group III
Volume output	ml./kg./24 hr.	2·3 ± 1·7	3·0 ± 1·5	2·2 ± 2·2	136 ± 166 A, B, C
Sodium	mg./100 ml.	28·2 ± 24·1	46·3 ± 44·1	121 ± 115 A, B	329 ± 41 A, B, C
Chloride	mg./100 ml.	37·0 ± 30·1	—	206 ± 123 A	400 ± 69 A, C
Total lipid	mg./100 ml.	65·2 ± 24·1	—	—	345 ± 176 A
Neutral fat	mg./100 ml.	18·6 ± 25·3	—	—	68 ± 50 A
Total fatty acids	mg./100 ml.	42·3 ± 17·7	—	—	202 ± 70 A
Total cholesterol	mg./100 ml.	13·7 ± 22·6	—	—	87 ± 78 A
Ester cholesterol	mg./100 ml.	8·6 ± 14·1	—	—	45 ± 57
Free cholesterol	mg./100 ml.	5·1 ± 4·3	—	—	42 ± 28 A
Phospholipid	mg./100 ml.	28·3 ± 16·2	—	—	158 ± 111 A

* A value of $P < 0{\cdot}05$ by a t test that the mean of Groups I, II, or III equalled that of the controls is indicated by A, that the mean of Groups II or III equalled that of Group I by B, and that the mean of Group III equalled that of Group II by C.

Measurements upon respiratory tract fluid have been collected in Table II. The volume output was at no time significantly different from control values except in Group III where the mean output was increased sixtyfold. The concentration of sodium was above the normal range in a few of the animals of Group I, was significantly increased in the animals of Group II, and was further elevated in all animals of Group III. Concentrations of chloride were above normal in Group II and further increased in Group III.

These results suggested that permeability of the pulmonary capillaries and alveolar endothelium to the constituents of blood plasma was partially increased at some 6 hours after exposure to phosgene and considerably increased at 16 to 20 hours after exposure. In some 40 per cent of the latter animals (Group III), permeability to blood plasma apparently became complete, resulting in an outpouring of blood plasma into respiratory tract fluid.

To investigate the suggestion that respiratory tract fluid collected from the animals of Group III was practically pure blood plasma, samples

were measured for their lipid content. The lipid composition of blood plasma is characteristic and different from that of any other tissue or fluid of the body. Sufficient respiratory tract fluid for lipid analysis could be collected from the controls and from the animals of Group III but not from the animals of Groups I and II. As shown by data summarised in Tables II and III lipid levels in respiratory tract fluid of rabbits in Group III were some fivefold the levels of the controls and identical to lipid levels in blood plasma of these animals.

Measurements upon blood have been summarised in Table III. The haematocrit and chloride levels were augmented over control values in Group II. The haematocrit and levels of chloride and all lipids except neutral fat were above control levels in Group III, some values being significantly higher than in Groups I and II.

TABLE III

MEASUREMENTS UPON BLOOD PLASMA*

The results are expressed as mean ± standard deviation units per 100 ml. of blood plasma

Measurement	Units	Control rabbits	Rabbits treated with phosgene		
			Group I	Group II	Group III
Sodium	mg.	261 ± 20	260 ± 13	264 ± 17	269 ± 16
Chloride	mg.	327 ± 13	331 ± 32	341 ± 25 A	362 ± 26 A, B, C
Total lipid ..	mg.	297 ± 80	312 ± 121	333 ± 139	356 ± 111
Neutral fat ..	mg.	118 ± 58	126 ± 87	140 ± 103	86 ± 85
Total fatty acids	mg.	201 ± 74	205 ± 97	221 ± 116	205 ± 75
Total cholesterol	mg.	58 ± 17	62 ± 25	67 ± 22	104 ± 51 A, B, C
Ester cholesterol	mg.	26 ± 13	31 ± 19	31 ± 16	54 ± 30 A, B, C
Free cholesterol	mg.	32 ± 97	31 ± 14	36 ± 13	50 ± 27 A, B
Phospholipid ..	mg.	96 ± 12	101 ± 36	101 ± 43	129 ± 49 A
Haemoglobin ..	g.†	11·4 ± 1·6	10·8 ± 0·7	12·4 ± 2·3 B	12·6 ± 2·1 B
Haematocrit ..	ml.†	35·0 ± 4·2	33·8 ± 7·0	41·3 ± 10·1 A, B	39·6 ± 6·2 A, B

* A value of $P < 0{\cdot}05$ by a t test that the mean of Groups I, II, or III equalled that of the controls is indicated by A, that the mean of Groups II or III equalled that of Group I by B, and the mean of Group III equalled that of Group II by C.

† Measured as units per 100 ml. of whole blood.

The water, chloride, and iron content of the lung periphery, hilus, and trachea were measured at autopsy to obtain a further estimate of the degree of oedema and congestion. The results are summarised in Table IV. Water and chloride levels in the periphery were elevated above control values in Groups I and II and increased further in Group III. The results agree with the histological data on alveolar oedema (Table I) but suggest that there was a greater diffusion of water and salt into the lung parenchyma and stroma at 6 to 7 hours than appeared as oedema in microscopic examination.

Data in Table IV indicate that the blood vessels of alveolar tissues contained more blood at 6 to 7 hours than controls, which corresponds with the congestion noted in Table I. The amount of blood (iron) was significantly lower in Group II than in Group I and this did not correspond with the microscopic interpretations noted in Table I. The findings of less iron in the periphery of rabbits in Group III than in all other groups corresponds to the absence of congestion noted microscopically (Table I).

The water content of the hilus was increased in the rabbits subjected to phosgene, but not to the same extent as in the periphery except in

Group II. This suggests that oedema of the bronchi occurred in Group II but not in Group I and III. Shifts in the iron content of the hilus were similar to those of the periphery, indicating that there was probably no significant change in the iron content of the bronchi.

The water content of the trachea was lower in the rabbits of Group III than of Group I which agreed with the histological findings. The iron content of the trachea was increased above the controls in Group I which also agreed with the microscopic findings.

TABLE IV

MEASUREMENTS UPON THE LUNGS AND TRACHEA*

The results are expressed as mean ± standard deviation

Measurement	Units	Control rabbits	Rabbits treated with phosgene		
			Group I	Group II	Group III
Periphery, water	g./100 g. wet wt.	78·8 ± 2·4	82·4 ± 2·8 A	81·4 ± 2·1 A	84·6 ± 1·4 A, B, C
Periphery, chloride	mg./g. dry wt.	8·7 ± 0·9	13·7 ± 2·8 A	13·4 ± 3·0 A	16·6 ± 4·6 A, B, C
Periphery, iron	µg./g. dry wt.	420 ± 61	502 ± 180 A	391 ± 98 B	280 ± 86 A, B, C
Hilus, water	g./100 g. wet wt.	78·6 ± 1·9	79·8 ± 3·5	80·7 ± 3·1 A	82·0 ± 3·5 A
Hilus, iron	µg./g. dry wt.	404 ± 69	495 ± 186 A	352 ± 65 A, B	330 ± 82 A, B
Trachea, water	g./100 g. wet wt.	69·6 ± 5·9	71·4 ± 4·7	70·0 ± 7·5	67·6 ± 3·9 B
Trachea, iron	µg./g. dry wt.	191 ± 77	401 ± 308 A	187 ± 84 B	151 ± 56 B

* A value of $P < 0.05$ by a t test that the mean of Groups I, II, or III equalled that of the controls is indicated by A, that the mean of Group II or III equalled that of Group I by B, and that the mean of Group III equalled that of Group II by C.

DISCUSSION

The clinical course of phosgene poisoning is characterised first by a latent period of several hours following exposure. The rabbits of Group I were examined during this interval. Much pathological change had occurred during the latent period. Inhalation of the gas produced a mild irritation of the trachea which was temporary and not seen the next day. Irritation of the alveolar sacs was evidenced by mild oedema, congestion, and, probably compensatory, emphysema. Contraction of the pulmonary arteries was pronounced in many rabbits. Pulmonary vasoconstriction may have been due to increased susceptibility of the pulmonary arteries to, or to increased output of, noradrenaline which can also produce pulmonary congestion[17]. The condition made the animals susceptible to the lethal effect of urethane anesthesia.

The symptomatic phase of phosgene poisoning follows the latent period. Rabbits examined in this stage could be divided into two groups, one with advanced pulmonary oedema and a normal volume output of respiratory tract fluid (termed Group II), and a second with extreme pulmonary oedema and a tremendous premortal gush of respiratory tract fluid (termed Group III). The symptomatic phase is divided clinically into an early stage, a blue stage, and a grey stage[18]. The rabbits of Group II were in what corresponded approximately to the

early stage or first part of the blue stage in human poisoning. The animals of Group III were approximately in the late blue stage or grey stage.

The rabbits of Group II had the same resistance to urethane anaesthesia as the animals of Group I. Microscopically pulmonary oedema had progressed markedly but the water and chloride content of the lungs were not greater than in Group I. Respiratory tract fluid was normal in volume but contained markedly increased levels of sodium and chloride. There was more compensatory alveolar emphysema. The arteries were appreciably less contracted and there was a significant amount of pulmonary haemorrhage. The bronchioles and trachea had returned approximately to normal in microscopic appearance and water and iron content. The haematocrit and blood haemoglobin were appreciably elevated. In this group, therefore, pulmonary oedema had progressed considerably, but compensatory mechanisms made the rabbits no less resistant to urethane anaesthesia than the rabbits of Group I.

Resistance to urethane anaesthesia was significantly less in the rabbits of Group III. Respiratory tract fluid poured out of the lungs at a volume which averaged 60 times the normal rate. Respiratory tract fluid appeared to have become defibrinated plasma since its concentrations of sodium, chloride, and lipids were the same as those of blood plasma. Histologically there was a 4+ pulmonary oedema in all of these rabbits with no congestion, no emphysema, no haemorrhage, and no contracted arteries. The water and chloride levels of the lungs were greater than in the rabbits of Group II. The iron content of the lungs was even lower than in the controls, indicating that there was less blood than normal in the lungs. Levels of chloride and most lipids of blood plasma were elevated above normal and above the levels in Groups I and II. In this terminal group, therefore, the pulmonary capillaries had lost their semipermeability, blood plasma appeared in respiratory tract fluid, the pulmonary stroma was filled with oedema fluid, the blood supply of the lungs was reduced, haemoconcentration occurred, and resistance to urethane anaesthesia was reduced almost to zero.

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