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THE PRODUCTION AND REMOVAL OF OEDEMA FLUID IN THE LUNG AFTER EXPOSURE TO CARBONYL CHLORIDE (PHOSGENE)

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Many investigations into the effects of exposure to irritant gases were made during the 1914–18 war (Dunn, 1918; *The Official History of the War*, 1923; Laquer & Magnus, 1921; The Medical Department of the United States Army in the World War, *Medical Aspects of Gas Warfare*, 1926; Underhill, 1920; Winternitz, 1920). Further experiments have been undertaken in an endeavour to clarify the phenomena involved in the production and removal of oedema fluid from the lungs of animals exposed to phosgene.

METHODS

Blood changes resulting from exposure to phosgene were investigated in rabbits, in dogs and in goats. All these animals were unanaesthetized. Blood samples were obtained from the ear veins of rabbits and from the external jugular veins of dogs and of goats. Oedema fluid was collected by inverting the animal immediately after death or when the animal was killed. The fluid was always clear and macroscopically free from blood contamination.

The plasma volume was estimated by the use of Evans Blue dye, the method followed being that described by Courtice (1943). Haemoglobin percentage was determined by the Haldane haemoglobinometer and plasma and oedema fluid proteins by micro-Kjeldahl digestion and Nesslerization (Wong, 1923). Albumin was similarly determined after precipitation of globulin with 22·5% sodium sulphate at 37° C. Non-protein nitrogen was estimated by precipitation of the proteins with trichloroacetic acid, micro-Kjeldahl digestion and Nesslerization.

The lymph flow from the lungs before and after exposure to phosgene was determined in dogs anaesthetized with 'Nembutal'. In the dog the lymph from the lungs and heart drains into the right lymph duct (Drinker, 1942; Warren & Drinker, 1942). To avoid opening the thoracic cavity and the use of artificial respiration, the right lymph duct was cannulated just above the pleura. As the normal composition of heart lymph and lung lymph is approximately the same, and as any increase in flow during pulmonary oedema probably comes from the lungs, but little error is introduced by collecting this mixed lymph and assuming it all to come from the lungs. Clotting of the lymph was prevented by introducing dry heparin into the cannula at intervals. The protein content was determined by the methods used for plasma proteins.

Blood samples were obtained from one femoral artery and the blood pressure was recorded from the other.

The animals were exposed to the phosgene in a static chamber, 10 cu.m. in volume. The concentration of phosgene was 440 mg./cu.m. and the time of exposure varied from 10 to 30 min. The

phosgene concentrations were determined by drawing a known volume of air from the chamber into a bubbler containing 15 c.c. of a solution of hexamine in sodium hydroxide. The resulting chloride was estimated by titration with mercuric nitrate solution. The over-all accuracy of the estimation was ±10%.

RESULTS

Blood changes

Rabbits. Twenty-four rabbits were exposed to phosgene in the following groups: four for 15 min., eight for 20 min., eight for 25 min. and four for 30 min. Increased time of exposure caused oedema of greater severity. The

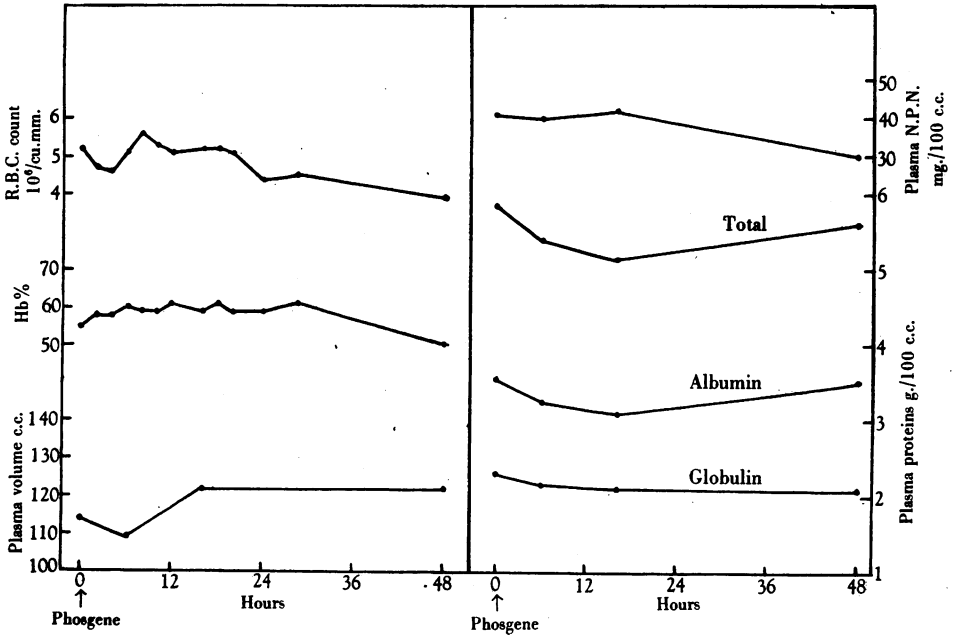

Fig. 1. The effects of exposure of rabbits to 440 mg. phosgene/cu.m. for 20 min. Average of three experiments.

mean results of experiments on three rabbits that survived out of a group of four, exposed for 20 min., are shown in Fig. 1. Although these rabbits survived, they all suffered from considerable respiratory distress. The changes in plasma volume were slight and there was but little haemoconcentration. In spite of such little change in the concentration of the blood, the plasma protein level fell and rose again when the animals recovered. Similar results were obtained in the group exposed for 15 min.

With a longer exposure, 30 min., rabbits usually died quickly with massive pulmonary oedema, and yet showed little or no concentration of the blood. In Table 1 are given the haemoglobin percentages, red cell counts and the lung/heart weight ratio in a typical case. The mean results of twelve experiments in

which the animals were exposed for 25 or 30 min. show that the haemoglobin percentage was 66 before exposure and 67 just before death, while the lung/heart weight ratio was 7·6. Death occurred in seven animals from 4 to 7 hr. after exposure and five were killed 6½ hr. after exposure. Assuming a normal lung/heart weight ratio of 1·8, the average excess fluid in the lungs was 31 c.c. In four of these animals the average plasma volume was 116 c.c. before exposure and 117 c.c. just before death.

TABLE 1. Effects of exposure of rabbits to phosgene, 440 mg./cu.m. for 30 min.

Time	Hb%	R.B.C.	Lungs/heart
Before phosgene	82	5·74	—
After phosgene: 1 hr.	83	5·89	—
2 hr.	79	5·83	—
3 hr.	75	5·78	—
4 hr.	78	5·88	—
5 hr.	80	5·95	—
5¼ hr.	Died	—	6·0

It thus appears from these experiments that, in the rabbit, oedema develops rapidly, and the loss of fluid from the blood stream into the lungs is equally rapidly compensated by a withdrawal of tissue fluid into the circulation. As a result of this compensation, the plasma-protein concentration falls considerably. An examination of the proteins in the oedema fluid reveals that plasma protein passes out into the lungs. Table 2 gives the average changes produced in ten rabbits. It is evident that the oedema fluid contains a high protein concentration, lower than the initial plasma-protein level but higher than the final level.

TABLE 2. The proteins, g./100 c.c., of the plasma and of the oedema fluid in the lungs of rabbits about 6 hr. after exposure to phosgene and just before death. Means and S.E. of means of ten experiments

	Before exposure	After exposure
Plasma proteins	5·57±0·12	4·37±0·18
Plasma albumin	3·22±0·04	2·63±0·09
Plasma globulin	2·35±0·12	1·74±0·13
Oedema fluid proteins	—	4·79±0·24
Oedema fluid albumin	—	3·71±0·26
Oedema fluid globulin	—	1·08±0·18

The loss of plasma proteins from the blood stream is approximately balanced by the gain of protein in the oedema fluid. In a group of ten rabbits in which the plasma volume and plasma-protein concentration were measured, the average fall in total circulating plasma protein was 1·3 g. The average volume of oedema fluid at death about 6 hr. after exposure was 32 c.c. containing 1·5 g. protein. As the time during which these changes took place was short, the amount of protein replacement or regeneration was probably not very great. These results, therefore, suggest that plasma protein is lost from the circulation only into the lungs.

If rabbits were kept on a drier diet than normal for a few days before exposure to phosgene, the blood usually concentrated much more as the pulmonary oedema developed. This is shown in Table 3. It seems, therefore, that the rabbit normally has a readily available store of fluid with which to replace the lost plasma, and, if this store is reduced, haemoconcentration occurs as pulmonary oedema develops.

There is also some evidence that haemoconcentration after exposure to phosgene is a protecting mechanism and reduces the risk of death. Ten normal rabbits and ten rabbits that had been on a drier diet than normal for 3 days were exposed together to the same concentration of phosgene for the same time. Eight of the control rabbits died and only three of those that had been on the drier diet.

TABLE 3. Effect of previous water restriction on haemoconcentration in rabbits after exposure to phosgene

	Haemoglobin %			
	Rabbit 573	Rabbit 574	Rabbit 575	Rabbit 576
Start of water restriction				
22. iii. 41	53	60	58	52
24. iii. 41	72	78	73	68
25. iii. 41	68	80	75	68
26. iii. 41	72	86	88	70
27. iii. 41	70	83	88	70
28. iii. 41	74	90	88	68
Exposed to phosgene				
2 hr. after	63	88	86	64
4 hr. after	80	88	82	58
6 hr. after	76	80	96	80
24 hr. after	85	110	106	100
30 hr. after	82	110	108	93

Dogs. The behaviour of dogs after exposure to phosgene is in some respects different from that of rabbits. The mean results of experiments on nine dogs that survived out of a series of twelve are shown in Fig. 2. The plasma volume has fallen considerably 12 hr. after exposure, and is at about the same level 24 hr. after exposure. It then gradually rises to normal.

Associated with this fall in plasma volume is a rise in haemoglobin percentage and in red cell count. The onset of haemoconcentration and of symptoms is relatively slow. Three hours after exposure the blood is slightly more dilute, but 6 hr. after exposure haemoconcentration begins and rapidly develops. Up to 6 hr. there are no obvious respiratory symptoms, but at 12 hr. the animal shows signs of respiratory distress and cyanosis. These signs continue until about 36 hr. after exposure. During this time, from 12 to 36 hr. after exposure, the blood is very concentrated, the gums and tongue are cyanosed, the ears and extremities are cold and the peripheral veins are collapsed. The animal, therefore, appears to be in a state of shock.

The levels of plasma protein fall and reach a minimum 24 hr. after exposure. They then gradually rise to normal as the plasma volume increases. The fall in the protein is due to a fall in both the albumin and globulin fractions, as in the

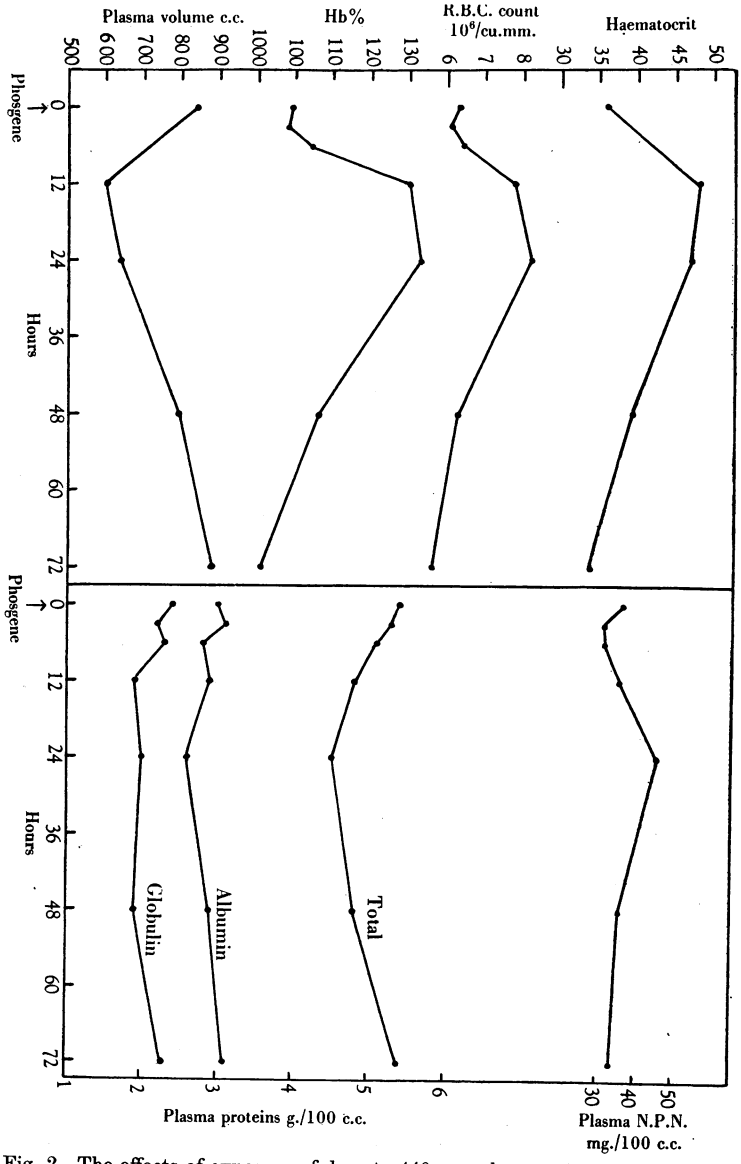

Fig. 2. The effects of exposure of dogs to 440 mg. phosgene/cu.m. for 10 min. Average of nine experiments.

case of the rabbit. The oedema fluid protein at death in fatal cases, 16–24 hr. after exposure, is approximately equal to that in the plasma, Table 4.

Goats. Goats behave in a similar manner to dogs. Pulmonary oedema is relatively gradual in onset, compared with the rapid rate of development in rabbits, and is accompanied by a considerable degree of haemoconcentration with peripheral circulatory collapse. The effects of exposure for 25 min. on two goats are shown in Table 5. A fall in the plasma volume and a concentration of the blood were observed 16 hr. after exposure.

TABLE 4. The proteins, g./100 c.c., of the plasma and of the oedema fluid in the lungs of dogs and goats 16–24 hr. after exposure to phosgene. Means and S.E. of means of seven experiments with dogs and of five with goats

	Dogs		Goats	
	Before exposure	After exposure	Before exposure	After exposure
Plasma proteins	5·74±0·17	4·51±0·19	7·38±0·32	5·27±0·41
Plasma albumin	3·23±0·12	2·27±0·09	3·13±0·14	2·17±0·18
Plasma globulin	2·51±0·08	2·24±0·12	4·25±0·36	3·10±0·28
Oedema fluid proteins	—	5·15±0·38	—	5·04±0·39
Oedema fluid albumin	—	3·08±0·33	—	2·49±0·25
Oedema fluid globulin	—	2·07±0·29	—	2·55±0·25

TABLE 5. Effects of exposure of goats to phosgene

Goat No.	Hb %	Haematocrit reading % cells	Plasma vol. (c.c.)	Total blood vol. (c.c.)
251: Before phosgene	96	31·6	1635	2390
16 hr. after phosgene	129	38·6	1185	1930
252: Before phosgene	66	20·6	3380	4260
16 hr. after phosgene	88	28·9	2240	3150

The oedema fluid proteins closely resemble the final plasma proteins just before death which occurred about 16 hr. after exposure in the experiments represented in Table 4.

The lymph drainage of the lung after exposure to phosgene

The experiments already described show that phosgene probably damages the lung capillaries, allowing the escape of a fluid rich in proteins and closely resembling plasma. The drainage of this fluid will be by way of the lymphatics, as the disturbance of the osmotic balance will prevent the reabsorption of the fluid directly into the blood stream.

Experiments were therefore carried out to ascertain the lymph drainage of the lung before and after exposure to phosgene in dogs anaesthetized with 'Nembutal'. The right lymph duct was cannulated in eight dogs and the lymph collected. The dogs were then exposed to phosgene and the experiment continued until the death of the animal. Death occurred in the eight animals from $5\frac{1}{2}$ to $12\frac{1}{2}$ hr. after exposure. The exposure was purposely arranged to produce severe pulmonary oedema fairly quickly. The results in all cases were similar, and a typical experiment is represented in Fig. 3.

The lymph flow. The normal lymph flow from the right lymph duct in a series of seventeen dogs, of an average weight of 21·9 kg., varied from 0·1 to

8·2 c.c./hr. with an average of 2·6 c.c./hr. Considering the amount of blood passing through the lungs and the rich plexuses of lymph capillaries, this flow is not great.

After exposure to phosgene, the rate of flow remained approximately the same for 2–5 hr., varying slightly in different animals. In some cases there was a slight increase and in others a slight decrease during this first stage. In the next stage, 2–5 hr. after exposure until the death of the animal, the rate of

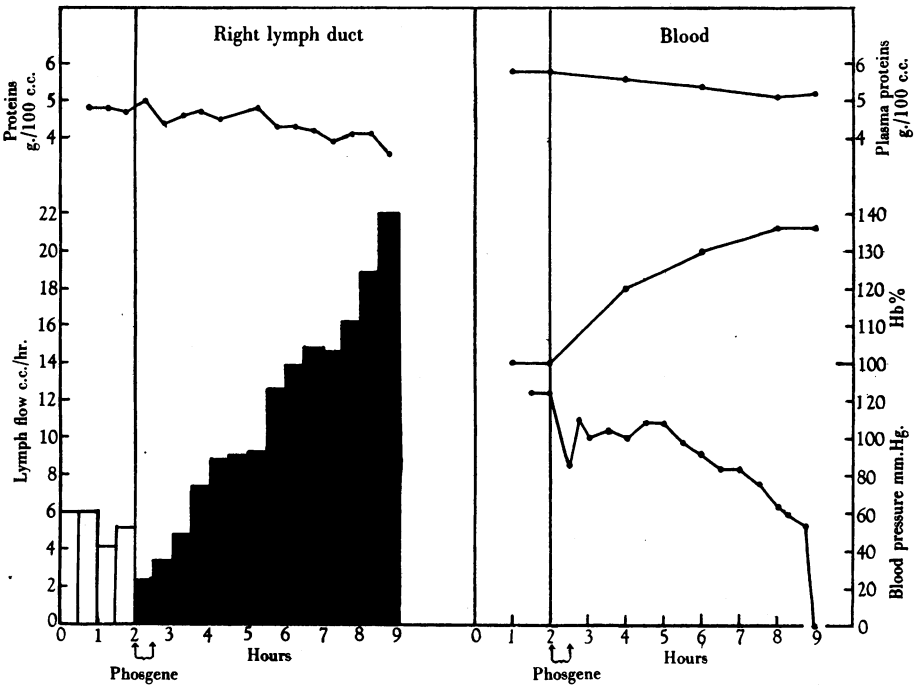

Fig. 3. The effects of exposure of an anaesthetized dog to phosgene.

lymph flow rose fairly steeply to reach a considerable level. This increase lasted as a rule until death, except in a few cases where there was a terminal falling off in the rate of flow due to exhaustion and decrease in respiration.

The maximal changes in lymph flow in the eight dogs were as follows: 1·6–10·6, 0·5–18·0, 0·8–8·4, 5·0–22·0, 0·3–4·2, 5·2–22·0, 0·1–2·4 and 1·2–3·7 c.c./hr. Though the lymph flow before exposure to phosgene varied greatly in different dogs, there was always a considerable increase in flow as the lungs became more oedematous.

The rate of removal of oedema fluid from the lungs can be estimated from the lymph flow. The production of oedema fluid is not so easy to determine, but the measurement of the lung/heart weight ratio will give an approximate indication of the excess amount of fluid in the lungs at the end of the experi-

ment. Calculations have been based on normal lung/heart weight ratios of 1·0 for greyhounds and 1·3 for other dogs. In Table 6 are shown the amounts of oedema fluid in the lungs at death, the time of survival after phosgene and the ratio between the lymph volume and the total volume of oedema fluid plus

TABLE 6. Volume of oedema fluid formed and of lymph drained away, after exposure of dogs to phosgene

Dog	Weight (kg.)	Lungs g./ heart g.	Oedema fluid (c.c.)	Hours of survival	Total lymph (c.c.)	$\frac{\text{Lymph}}{\text{Oedema + lymph}} \times 100$
Greyhound	29	760/300	460	9	41·4	8·3
,,	23	570/270	300	12½	55·3	15·6
Mongrel	20	470/142	285	6½	28·9	9·7
,,	29	700/210	426	10	95·3	18·3
,,	15	351/139	170	5½	8·7	4·9
,,	20	605/151	409	7	78·9	16·2
,,	16	427/144	240	10¼	19·9	7·7
Greyhound	26	770/290	480	10	4·4	1·0
					Mean	10·2

lymph. Part of the excess weight of the lungs is no doubt due to an increase in the blood in the pulmonary vessels, so these figures can be approximations only. However, it seems that the lymphatics have drained away on the average about 10% of the fluid formed in these acute experiments.

The protein content of the lymph. In a series of seventeen normal dogs, the mean protein concentration of the lymph from the right lymph duct was 3·6% compared with a mean plasma protein concentration of 5·2%. The protein content of the lymph draining from the normal lung is, therefore, high. Warren & Drinker (1942) found a mean of 3·66% protein in the lung lymph of a series of eighteen dogs.

When pulmonary oedema developed and the lymph flow increased, the protein concentration of the lymph fell slightly as the plasma-protein concentration fell. This occurred in all cases. Average figures of eight experiments showed that the plasma-protein concentration fell from 5·2 to 4·1% just before death, the lymph-protein level fell from 4·0 to 3·3% while the oedema-fluid-protein concentration at death was 5·4%.

Blood changes. During the breathing of phosgene the blood pressure fell, probably due to a vagal reflex slowing the heart. After exposure to phosgene the blood pressure generally rose again somewhat, and then fell as haemoconcentration and anoxia progressed. The haemoconcentration and plasma protein changes in these acute experiments were similar to those already described in unanaesthetized dogs.

DISCUSSION

The primary disturbance in phosgene poisoning appears to be damage to the lung capillaries, causing an increase in their permeability to the plasma

proteins. This causes an upset of the osmotic effect of these proteins, and oedema results, as in the case of a thermal burn. If the animal survives, oedema progresses for 24–48 hr. after which the disturbance is righted, and then the excess fluid is gradually drained away.

The effects on the blood volume and circulation are secondary to the local loss of fluid. In the rabbit, the fluid loss into the lungs is rapid; but the withdrawal of fluid from undamaged tissues into the blood is also rapid, with the result that the plasma volume usually falls but little. In the dog and goat, however, the plasma loss is more rapid than the withdrawal of fluid from the tissues, so that the plasma volume falls considerably. The compensatory mechanism for rapidly restoring the plasma volume in rabbits appears to be detrimental to the life of the animal, for the more rapidly the plasma volume is restored, the more rapidly the oedema progresses. By partially dehydrating rabbits beforehand, the mortality rate can be decreased and haemoconcentration occurs. These observations would indicate that, in cases of haemoconcentration and fall in plasma volume due to a loss of fluid into the lungs, it would be better not to try to increase the plasma volume by transfusions, for anoxia, due to pulmonary oedema, is a more important factor in causing death than haemoconcentration and decreased blood volume.

As the oedema fluid has approximately the same protein content as plasma, the drainage of this fluid will be by way of the lymphatics. Histologically, the lymphatics are widely dilated in pulmonary oedema, an observation which once suggested that there might be a blockage preventing the removal of the oedema fluid (Vedder, 1925). The experiments described above show that the lymph flow from the lungs of dogs is greatly increased as oedema develops. For the first 2–5 hr. after exposure to phosgene there is little change in lymph flow although interstitial oedema, as seen histologically, usually begins within the first half-hour. The sponginess of the lung may account for this first phase. As the lungs become more oedematous and expiration more forceful, the lymph flow increases rapidly. It has been calculated that the lymphatics removed about 10% of the oedema fluid formed in the experiments described, where death occurred 6–12 hr. after exposure.

After 24 hr., the production of oedema fluid seems to lessen and the animal recovers. If, however, dogs be killed several days later, when haemoconcentration has disappeared, the lungs may still be oedematous. No experiments have been performed on the lymph drainage in this late period, but it appears that, after the acute increase in lymph flow with the rapid onset of oedema, the flow decreases and the excess fluid in the alveoli is gradually drained away in the course of several days. Experiments dealing with the drainage of fluids from the lungs will be described in a subsequent article (Courtice & Phipps, 1946).

It is difficult to explain the behaviour of the lymph proteins after phosgene poisoning. It was expected that the concentration would not fall but rise, as it

does in pulmonary oedema resulting from lewisite poisoning (Cameron, Courtice & Short, 1946). Post-mortem examinations were made in all cases and the cannulated duct was traced down to the right tracheo-bronchial node into which flowed the afferents coming from the hilum of the lung. In some cases 1 c.c. of 4% Evans Blue dye was inserted by catheter, passed along the trachea, into the lung and dye appeared in the lymph in increasing concentration. In one case 2 c.c. of 4% dye were injected into the right lung about 30 min. before death when it was seen (from the blood pressure and general condition) that the animal would not live much longer. Dye immediately appeared in the tracheal cannula (the animal had been expelling frothy fluid in the tracheal cannula for about 30 min. beforehand), and in 10 min. dye appeared in the lymph and continued to increase in concentration until death. On the other hand, the blood plasma taken at death contained very little blue dye. There seems little doubt, therefore, that the cannulated duct always drained the lung.

SUMMARY

1. Phosgene poisoning has been investigated in rabbits, dogs, and goats in order to obtain further information regarding the process of oedema formation.

2. In rabbits, pulmonary oedema is often rapid in onset, but there is little or no haemoconcentration or change in plasma volume. If the rabbit lives on a drier diet than normal for a few days before exposure to phosgene, the loss of fluid into the lungs causes a considerable degree of haemoconcentration and the risk of death is lessened.

3. The oedema fluid has about the same protein concentration as the plasma, suggesting an increase in capillary permeability. The loss of protein from the blood is approximately the same as the gain in protein in the oedema fluid in the lungs. This confirms that there is only a local loss of fluid in phosgene poisoning.

4. In dogs and goats exposed to phosgene, considerable haemoconcentration occurs as fluid is lost into the lungs. This is accompanied by a fall in plasma protein concentration, both albumin and globulin being affected. As in the rabbit, the oedema fluid closely resembles plasma in its protein composition.

5. The lymph drainage of the lung in anaesthetized dogs has been investigated before and after exposure to phosgene. As the oedema develops, the lymph flow increases rapidly and reaches a level many times the normal. In spite of this great increase in lymph flow, the lymph collected in these acute experiments, where death occurred 6–12 hr. after exposure, amounted on the average to only 10% of the total oedema fluid in the lungs.

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