POST-MORTEM PULMONARY EDEMA*

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It is especially appropriate that a paper originating from the Medical Division of the Army Chemical Center be included in this anniversary volume of the *Yale Journal of Biology and Medicine*. Dr. Winternitz has been actively connected with chemical warfare medical research from the beginning and particularly during the two wartime periods of greatest activity. During the war against the Central Powers he was concerned with determination of the anatomical changes produced by the various chemical warfare agents. The summary of that work, published in 1920,[1] still serves as a standard description of the lesions. During the recent war, as Chairman of the Committee on Treatment of Gas Casualties of the National Research Council, he supervised the activities of many teams of investigators. It was in great part through his efforts in this capacity that the plans for the Medical Division, Army Chemical Center, were initiated and carried through. The problem of the mechanism of formation of pulmonary edema was acutely brought to the fore by the early studies of chemical warfare agents, particularly chlorine and phosgene. After the first world war Dr. Winternitz made important contributions to the field,[2,3,4,5] and his interest in this ever-present enigmatic subject persists. It is doubly fitting that a paper on the mechanism of formation of pulmonary edema be included in this volume.

In the evaluation of pulmonary edema in experimental animals following exposure to irritant gases, it has been observed that animals examined at intervals after death exhibit more extensive pulmonary edema than similar animals examined immediately. This observation has led to hesitancy in the interpretation of pulmonary findings in animals that succumb when the laboratory is unattended and autopsy is delayed. In sacrificed animals, the effect of the method of sacrifice on the pulmonary findings also required evaluation. The following experiments were performed with the object of determining the influence of the method of sacrifice and of the interval after death on the condition of the lungs at autopsy.

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Methods

Dutch and albino rabbits that had recovered for at least three weeks from skin or eye applications of various toxic chemicals were sacrificed by the following methods:

Air embolism. An intravenous injection of 3 cc. of air per kilogram of body weight was made as rapidly as possible through a #21 gauge needle.

Nembutal. Abbott's Veterinary Nembutal containing 60 mg. per cc. was used for all injections. The injections were made as rapidly as possible through a #21 gauge needle and death occurred promptly. One group of animals were anesthetized slowly with 60 mg. per kg. at the rate of 1 cc. per minute. Respirations ceased in the latter group approximately one minute after injection was completed.

Ether. A cotton pledget soaked with ether was placed in a cone-shaped mask. The animals were anesthetized with open drop ether until respirations ceased. The time of death varied from 5 to 13 minutes.

Electrocution. A 110-volt alternating current was passed through the body with electrodes on the lip and in the rectum. The current was applied for 45 seconds. One group of animals received heparin prior to electrocution.

Exsanguination. In some instances, blood was removed through a needle inserted into the left ventricle; in others, exsanguination was achieved by severing the femoral arteries and veins.

Rabbit punch. The rabbit was suspended by the hind-legs and a sharp blow was administered to the back of the head with the ulnar surface of the hand.

Magnesium sulphate. A saturated solution of magnesium sulphate was administered as rapidly as possible in doses of 1.5 cc. per kg. through a #21 gauge needle.

Procedures. When injections were to be made into the chambers of the heart, the rabbit was tied on its back and the needle introduced into the chest close to the sternum. Blood was withdrawn into the syringe and the position of the needle in the heart was judged by the state of oxygenation of the aspirated blood. Autopsy showed that perforations of the lung were not produced by this technique.

Animals in the groups designated "Immediate" were autopsied as soon as signs of life were no longer present. In the "Delayed" groups, unless otherwise specified, the rabbits (placed on their abdomens with the legs extended) remained at room temperature for the designated time. A few experiments were conducted at 35°F. In some "Delayed" groups the trachea was clamped or a cannula was inserted into the trachea immediately after respiration ceased. The effect of positive air pressure upon the formation of edema was tested by connecting the tracheal cannula to air at 10 cm. of water pressure or at 30 cm. of water pressure for a three-hour period.

In experiments designed to determine the distribution of injected Nembutal within the animal, a small amount of solution containing phosphorous^{32} in the form of sodium phosphate was added to the Nembutal. Samples of blood and tissues taken from various portions of the body after injection were tested for radioactivity by a Beckman MX5 survey meter.

Method of examination. At the time of examination, an incision was made into the neck and the trachea was clamped 1 cm. below the thyroid cartilage. The chest plate was removed and the lungs, trachea, bronchi, esophagus, and thymus were removed *en bloc.* The lungs were dissected clean and the trachea was ligated at the level of the carina and severed immediately above the tie. The lungs were examined grossly,

weighed, and immediately submerged in 10% formalin. After hardening for twenty-four hours, thin sections were removed from the right lower lobe and left upper lobe and were fixed in 10% formalin for microscopic sections. The lung weight to body weight ratio was obtained by dividing the lung weight in grams by the body weight in kilograms. The mean and the standard error of the mean for each group were determined. Comparisons between the groups were made by the application of the t test. The P value for such a comparison was taken from standard tables. Significant values for P were .05 or lower.

Results

Immediate examination—gross findings. In animals examined immediately after death the lungs were uniformly light, air containing, and free of gross evidence of edema, regardless of the method of sacrifice. Following exsanguination the lungs were exceedingly pale, while after ether, the pulmonary surfaces appeared pale and cyanotic. Petechial hemorrhages under the pleura and throughout the parenchyma resulted from electrocution. In the groups sacrificed with Nembutal, magnesium sulphate, or the rabbit punch, congestion of the lungs was more marked than in those sacrificed with air. The lung weight to body weight ratios did not differ greatly in any of the groups examined immediately after death (see Table 1, columns 2 and 3). The lowest ratios were obtained in the ether ($3.23 \pm .24$) and the exsanguination groups ($3.57 \pm .21$). Several groups sacrificed with Nembutal had ratios greater than 4.0.

Immediate examination—microscopic findings. The capillaries of the alveolar walls of animals sacrificed by exsanguination or ether were devoid of blood. In contrast, more congestion of the alveolar walls followed Nembutal, magnesium sulphate, and rabbit punch. Small hemorrhages were noted within the alveoli in the electrocuted, air, and rabbit punch groups. Following the rabbit punch, bone marrow emboli were present in the large branches of the pulmonary arteries. In the groups sacrificed by Nembutal and having the highest lung weight to body weight ratio a minute amount of fluid was present in the alveoli in small perivascular foci.

Delayed examination—gross findings. The lungs of all animals except those of the exsanguination group examined three hours after sacrifice were congested and edematous. These findings varied from small patchy areas of edema or generalized increased turgidity in the animals sacrificed with air, electrocution, or the rabbit punch to almost complete involvement of all lobes by dark, currant jelly, boggy consolidation following ether, magnesium sulphate, or Nembutal. The exsanguination group showed no evidence of congestion or edema after three hours. In the other groups the extent of edema paralleled the ratio of the lung weight to body weight (Table 1,

TABLE 1

EFFECT OF METHOD OF SACRIFICE ON LUNG WEIGHT

Method of sacrifice and treatment	Immediate autopsy: No. of animals	Immediate autopsy: Lung weight per kilo ± S.E. mean (Grams)	Autopsy after 3 hours: No. of animals	Autopsy after 3 hours: Lung weight per kilo ± S.E. mean (Grams)	t value	P
Exsanguination	5	3.57 ± .21	6	3.62 ± .13	2.138	>.05
Open drop ether	5	3.23 ± .24	10	7.42 ± .73	3.918	.01
Air (3 cc./kg.) i.v.	5	3.65 ± .18	5	4.81 ± .31	3.213	.01
Air (3 cc./kg.) i.v.—open cannula	..	.....	5	5.14 ± .61	2.330	.05
Air (3 cc./kg.) i.v.—trachea clamped	..	.....	5	5.51 ± .33	4.950	.001
Electrocution	5	3.91 ± .45	5	5.80 ± .34	3.323	.01
Heparin, then electrocution	..	.....	5	5.24 ± .28	....	....
Rabbit punch	10	3.85 ± .20	11	6.68 ± .42	5.855	.001
$MgSO_4$ (1.5 cc./kg.) R. ventricle	5	3.24 ± .31	5	9.77 ± .46	11.736	.001
$MgSO_4$ (1.5 cc./kg.) L. ventricle	5	3.86 ± .25	5	8.21 ± .95	4.425	.01
Nembutal (100 mg./kg.) i.p.	5	3.69 ± .25	5	5.98 ± .60	3.491	.01
Nembutal (60 mg./kg.) i.v.—slow	5	4.04 ± .11	5	5.33 ± .84	1.532	>.05
Nembutal (60 mg./kg.) i.v.—rapid	5	4.27 ± .38	5	6.04 ± .51	2.776	.05
Nembutal (100 mg./kg.) i.v.	5	3.83 ± .27	5	9.47 ± .74	7.116	.001
Nembutal (100 mg./kg.) i.v.—trachea clamped	..	.....	6	9.35 ± .77	6.246	.001
Nembutal (100 mg./kg.) i.v.—open cannula	..	.....	19	9.46 ± .62	4.617	.001
Nembutal (100 mg./kg.) i.v.—kept at 35°F.	..	.....	15	6.96 ± .62	2.821	.01
Nembutal (100 mg./kg.) i.v.—10 cm. water*	..	.....	6	7.43 ± .49	6.121	.001
Nembutal (100 mg./kg.) i.v.—30 cm. water*	..	.....	6	4.82 ± .23	2.835	.05
Nembutal (200 mg./kg.) i.v.—open cannula	..	.....	8	13.54 ± 1.26	....	....
Nembutal (100 mg./kg.) L. ventricle	5	4.01 ± .16	10	8.80 ± .75	4.426	.001
Nembutal (100 mg./kg.) R. ventricle	5	4.57 ± .37	10	12.14 ± 1.06	4.893	.001

* Positive air pressure applied through tracheal cannula.

columns 4 and 5). The highest values were obtained with Nembutal or magnesium sulphate, particularly when introduced into the right ventricle. The degree of edema following Nembutal varied directly with the quantity injected. There was no significant difference in the appearance of the lungs or in the lung weights in the groups given 100 mg. per kilogram of Nembutal or air injected intravenously whether the trachea was clamped, opened to the atmosphere by insertion of a cannula, or untouched during the three-hour post-mortem period. Application of positive tracheal pressure suppressed the formation of edema; the lung weights varied inversely with the magnitude of the pressure. Maintenance of Nembutal-sacrificed animals at 35°F. during the three-hour period diminished the amount of edema formed. Administration of heparin prior to electrocution did not change the lung weight appreciably. Intravenous administration of 100 mg. per kilogram of Nembutal produced greater pulmonary edema than intraperitoneal or left ventricular injection but less change than injection into the right ventricle.

Delayed examination—microscopic findings. In the exsanguination group there was no evidence of change after three hours (Fig. 1, a and b). Capillary congestion was not prominent in the animals sacrificed with air, but there were patchy areas of edema involving alveoli and bronchioles (Fig. 2, a and b). Following the other methods of sacrifice, congestion and edema were more extensive and the degree of involvement paralleled the ratio of the lung weight to the body weight (Fig. 3, a and b, and Fig. 4, a and b). In the most severe instances almost all alveolar space was filled with homogeneous pink-staining material (Fig. 5, a and b).

Comparison of immediate and delayed groups. The results of comparison of the lung weights of the immediate and the delayed groups sacrificed by the various methods are presented in Table 1, columns 6 and 7. Where special procedures were performed during the interval after death (tracheal clamp, positive pressure, etc.), the comparison was made with the group of animals sacrificed by the same method and examined immediately. In two instances a statistical analysis was not made because suitable controls were lacking. The lung weights after three hours were significantly increased over the corresponding immediate controls in all instances except following exsanguination and after slow injection of 60 mg. per kilogram of Nembutal.

Effect of the interval after death. A few experiments were performed to determine the effect of the length of the post-mortem interval upon the

quantity of edema. The results of these experiments are presented in Table 2. The lung weight reached a maximum before four hours after sacrifice.

Extent of circulation after injection of Nembutal into left ventricle. Because of the unexpected severity of the pulmonary edema following introduction of 100 mg. per kilogram of Nembutal into the left ventricle, it became of interest to determine the extent of the circulation after such an injection. Death ensues within a few seconds; rarely is there any respiratory effort, and heart action ceases promptly. The following experiment was made to

TABLE 2

EFFECT OF INTERVAL AFTER SACRIFICE BY NEMBUTAL (100 MG./KG.) ON LUNG WEIGHT

Interval after sacrifice	*Treatment*	*Number of animals*	*Lung weight per kilo* ± *S.E.* mean
			Grams
Immediate		5	3.83 ± .27
1 hours	Cannula in trachea	5	5.42 ± .58
2 hours	Cannula in trachea	5	7.09 ± 1.39
3 hours	Cannula in trachea	19	9.46 ± .62
4 hours	Cannula in trachea	5	10.88 ± 1.53
6 hours	Cannula in trachea	5	10.95 ± .74

determine whether under such circumstances the injected material reached the lung.

A rabbit was sacrificed by left ventricular injection of Nembutal (100 mg. per kilogram) in 6.8 cc. of solution containing phosphorous[32] in the form of sodium phosphate (total activity of injected material: 23.8 m. rep.). Death was instantaneous. Clamps were immediately placed on the carotid arteries, trachea, the great vessels at the base of the heart, the hilum of each lung, and the aorta at the level of the diaphragm. Samples of blood and tissue from various sites were removed and tested for radioactivity. Only background activity was recorded from blood removed from the right ventricle, cerebral cortex, liver, and intestine. Radioactivity in excess of background was obtained from blood from the left ventricle, the thoracic aorta, the abdominal aorta, spleen, kidney, and all lobes of the lungs. Greater activity was present in the lower than in the upper lobes. It was concluded that despite the rapidity of death following introduction of Nembutal into the left ventricle, the injected material had reached the lungs.

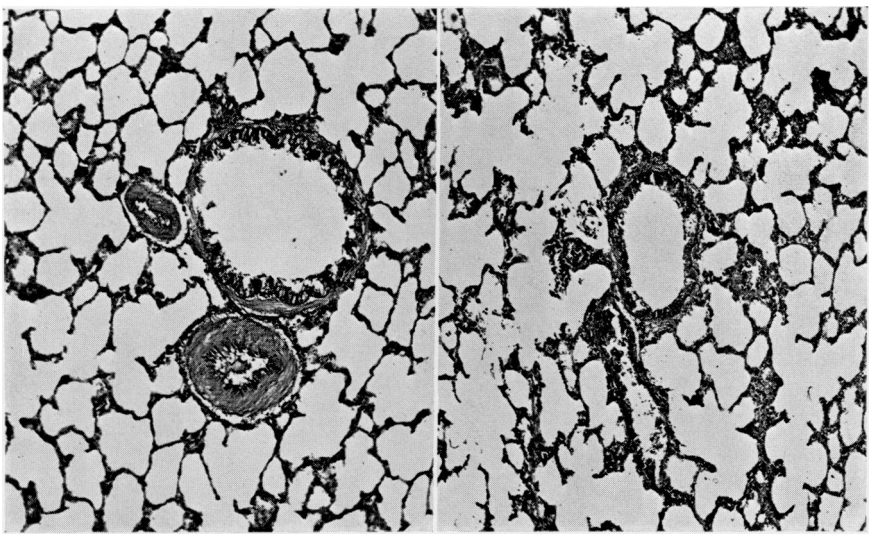

FIG. 1. Lungs of rabbits sacrificed by exsanguination: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

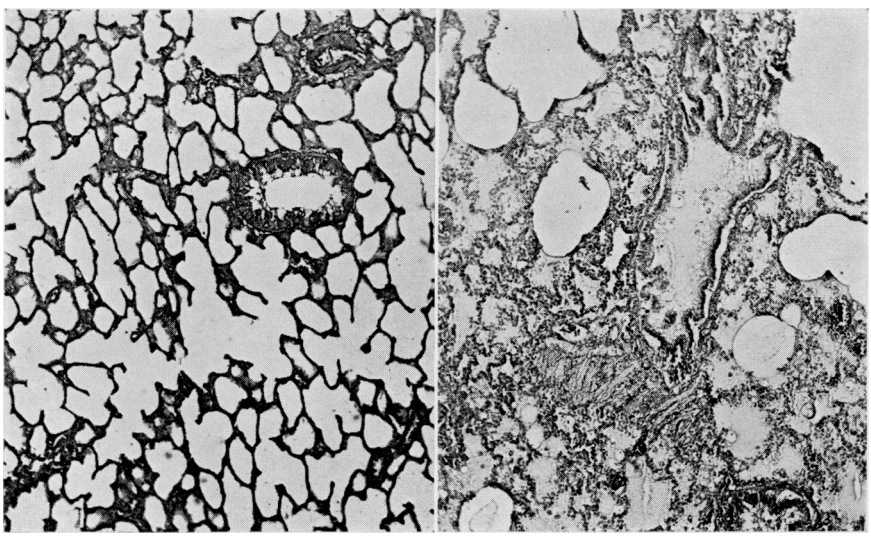

FIG. 2. Lungs of rabbits sacrificed by intravenous injection of air: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

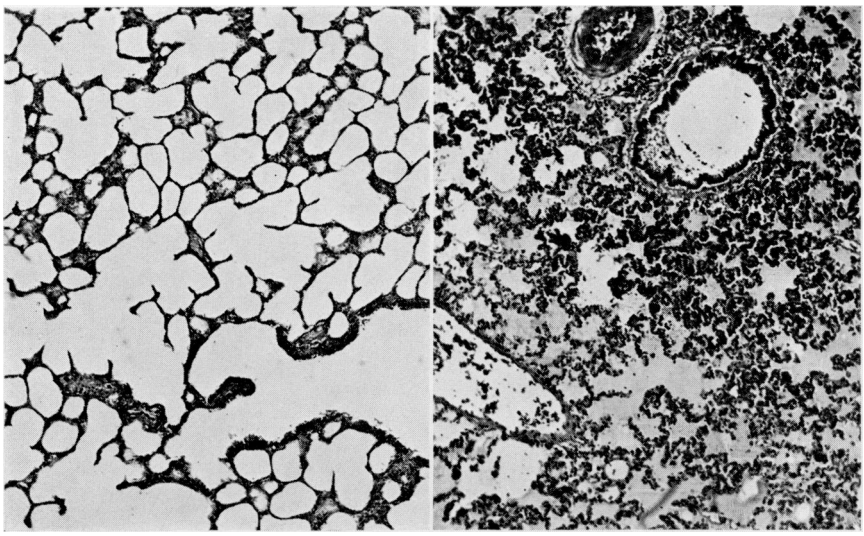

FIG. 3. Lungs of rabbits sacrificed by rabbit punch: a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

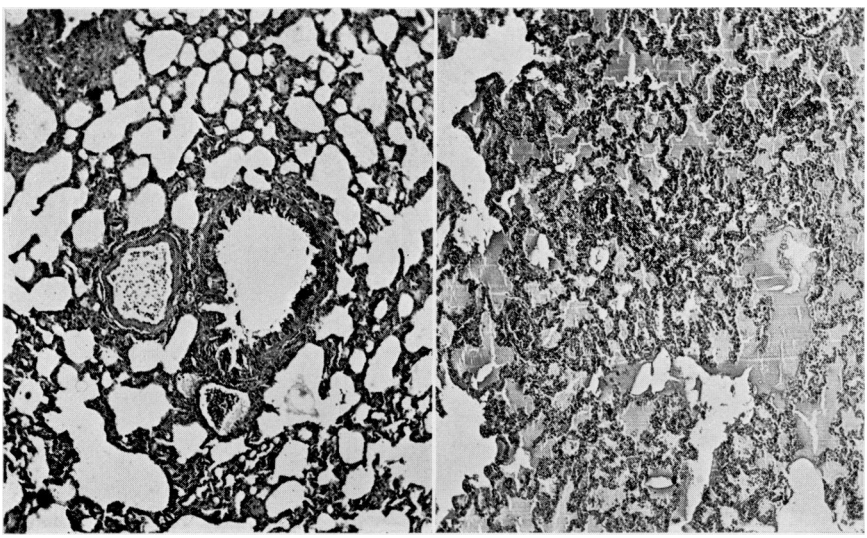

FIG. 4. Lungs of rabbits sacrificed by intravenous injection of Nembutal (100 mg. per kilogram): a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

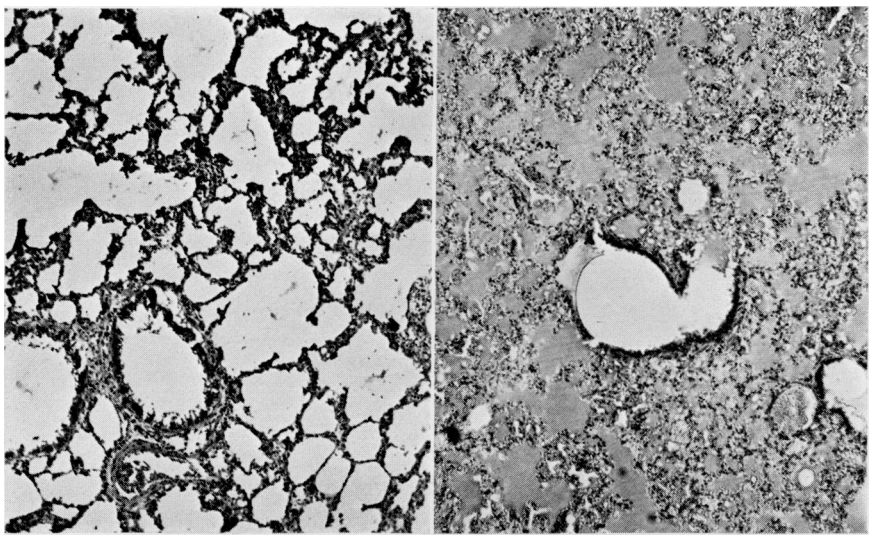

FIG. 5. Lungs of rabbits sacrificed by right ventricular injection of Nembutal (100 mg. per kilogram) : a. Immediate autopsy. x 114; b. Autopsy delayed 3 hours. x 114.

Discussion

Pulmonary edema that cannot be differentiated by its gross or microscopic appearance from that produced by many noxious agents develops in animals during the post-mortem period. It is not possible to evaluate the lung findings in experimental animals unless examination is performed immediately after death. Autopsy in humans is usually delayed several hours and pulmonary edema and congestion are almost constant findings. The experiments reported here suggest that in many instances such pulmonary findings in human cases may result from post-mortem change.

Two of the factors responsible for the formation of pulmonary edema in the post-mortem period and brought out by these experiments are a pressure gradient between pulmonary vasculature and the alveolar spaces and an alteration of capillary permeability. It is apparent that a gradient of pressure must exist between the pulmonary vasculature and the alveolar spaces for edema to occur. Exsanguination with its concomitant lowering of intravascular pressure prevents formation of edema. Positive air pressure of 10 cm. of water applied to the alveoli diminishes the amount of edema. A pressure of 30 cm. of water effectively prevented edema.

The quantity of edema varies with the dose and route of administration of the Nembutal. It appears that the amount of edema parallels the concentration of Nembutal in the pulmonary capillaries and that Nembutal has a direct action on the permeability of these structures. Intravenous administration of 100 mg. per kilogram of Nembutal results in more edema than intraperitoneal injection of the same quantity but causes less edema than the introduction of the same amount of material into the right ventricle. Left ventricular administration causes less edema than intravenous or right ventricular injection. Experiments with radioactive material injected into the left ventricle indicate that some injected material reaches the lungs via the bronchial arteries or by reverse flow through the pulmonary veins or both.

Summary and conclusion

Pulmonary congestion and edema develop during the first few hours of the post-mortem period in rabbits sacrificed by a variety of methods including: Nembutal, magnesium sulphate, intravenous air, electrocution, rabbit punch, and the inhalation of ether. Following exsanguination post-mortem pulmonary edema does not occur. The quantity of edema present three hours after death varies with the method of sacrifice, the route of administration of the lethal agent, the temperature, and the intratracheal

pressure. Evaluation of pulmonary edema and congestion should not be made in experimental animals unless examination is made immediately after death.

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