Pathogenesis of Pulmonary Edema by Alloxan

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Alloxan causes pulmonary edema in anesthetized dogs by a local action in the lungs. Measurements of blood pressures, flow, vascular resistance and blood volume of intact and perfused lungs suggest that the initiation of edema is due to capillary congestion brought about by constriction of pulmonary veins.

Chemical substances have been widely used as experimental tools in investigations of the pathogenesis of pulmonary edema. It is reasonable to expect chemicals to increase capillary permeability, and phosgene is a good example. This agent has been shown not to affect pulmonary arterial pressure, so that the pulmonary edema is best explained by increased capillary permeability. Surprisingly enough, this now appears to be the only chemical that causes pulmonary edema by increasing capillary permeability in the lungs. All other compounds that have been properly investigated produce sufficient hydrostatic changes in the pulmonary vessels to account for the pulmonary edema. Thus intramyocardial injections of solutions of silver nitrate, zinc hydroxide and alcohol cause failure of the left ventricle and increased pulmonary capillary hydrostatic pressure. Intravenous injection of epinephrine and intracranial injections of ammonium salts and fibrin cause intense constriction of systemic vessels resulting in the shifting of blood from the periphery to the lungs. (See references cited in the review articles of Altschule, Luisada and Cardi, Visscher and co-workers and Henneman.) All of these examples of chemically-induced pulmonary edema demonstrate the importance of pulmonary congestion (accompanied by increased capillary hydrostatic pressure) resulting from a primary action of the foreign substance outside of the lungs vessels.

Other compounds that are known to produce pulmonary edema have not been sufficiently investigated to differentiate between the relative roles of increased hydrostatic pressure and increased capillary permeability. Peralta showed that alloxan (in doses larger than 150 mg./Kg.) produced acute pulmonary edema in cats. Gruhzit, Peralta and Moe found that it caused an increase in pulmonary arterial pressure (in dogs) but felt that this effect played no obvious contributory role in the production of pulmonary edema. In our earlier experiments on alloxan edema, we were able to demonstrate two types of pulmonary vasoconstrictor action of alloxan. One type is accompanied by a decrease, the other by an increase in the volume of blood in the lungs. The latter, which plays an important role in the appearance of edema, appeared to be due to constriction of the pulmonary veins. This action of alloxan, although unfavorable in nature, deserves some attention, because it demonstrates a possible lead for improved therapy of poisoning by such agents. We therefore, decided to investigate it further. The results are reported below.

METHODS

All the experiments were performed on dogs under anesthesia by morphine (2 mg./Kg. subcutaneously) and chloralose (70 mg./Kg. intravenously). A tracheal cannula was inserted and the following measurements were made: (a) Respiratory movements by a pneumograph; (b) heart rate by a direct writing electrocardiograph; (c) carotid blood pressure by the usual mercury manometer; and (d) analysis of arterial blood for oxygen and carbon dioxide content by the method of the Van Slyke and Neil. The special technics involving the pulmonary circulation have been described in earlier articles and are as follows: (e) Pulmonary arterial and left auricular pressures; (f) pulmonary venous outflow; (g) vascular resistance of the perfused lobe in situ; (h) vascular resistance and blood volume of perfused excised lungs; (i) radioactive measurements of erythrocytes (tagged with phosphorus) in the lung capillaries; and (j) radioactive...
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FIG. 1. Effects of alloxan (50 mg./Kg. intravenously) on respiration, carotid blood pressure and heart rate. Note the appearance of fluid in tracheal cannula 20 min. after injection. Dog died 1 hour later of pulmonary edema.

measurements of plasma albumin (tagged with iodine131) content in the lungs. Each of these methods is briefly described below in the appropriate sections.

RESULTS

Alloxan Edema in the Intact Dog

The intravenous injection of alloxan monohydrate in 30 dogs in doses of 100 mg./Kg. was invariably followed by death from pulmonary edema. Froth in the tracheal cannula appeared within 1/2-1 hour after injection. Respiration and circulation were studied in hopes not only of arriving at a better understanding of the causation of the edema, but also of selecting a dependable sign of edema formation before the gross appearance of fluid in the respiratory passages.

Respiration. The injection of alloxan immediately caused a brief period of apnea lasting for about 1/2 min. (fig. 1). After bilateral vagotomy this apnea was totally absent. It therefore appears to resemble the apnea produced by veratrum alkaloids, i.e., it is attributable to stimulation of pulmonary receptors. The more important respiratory effect of alloxan began about 10 to 30 min. after the injection, and consisted of progressively increasing polypnea. This polypnea occurred in spite of cervical vagotomy (in 3 dogs) but was eliminated by bilateral upper thoracic sympathectomy (in 3 other dogs). It therefore appears also to be a reflex response, arising from receptors whose afferent connections lie in the thoracic sympathetic. The nature and function of these receptors are not yet clear.

The polypnea could not be entirely accounted for by changes in oxygen and carbon dioxide content of the arterial blood. Anoxemia usually appeared after the appearance of tracheal froth whereas the onset of polypnea preceded both. We have not yet succeeded in showing that the early onset of edema is directly responsible for the polypnea. It cannot be regarded as an unequivocal sign of pulmonary edema.

Heart Rate and Carotid Blood Pressure. These were variable and did not behave in a characteristic pattern. The most frequent response (seen in 21 out of 30 dogs) was a rise in carotid blood pressure by about 10 to 50 mm. Hg. This rise lasted only for about 2 min. and was shorter in duration than the effects of alloxan on the pulmonary circulation, which are described in the remaining paragraphs.

Pulmonary Arterial and Left Atrial Pressures. The most consistent circulatory response to alloxan was pulmonary arterial hypertension. The rise began immediately, coincidently with the onset of apnea, but it lasted for several minutes (fig. 2). In each of 10 dogs, the pulmonary arterial pressure rose by about 50 per cent of the control value. Simultaneous recording of left atrial pressure revealed no change; pulmonary hypertension on the basis of left ventricular failure can be dismissed.

Pulmonary Venous Outflow. The vein of the left lower lobe was cannulated for collecting the
Fig. 2. Effects of alloxan (100 mg./Kg. intravenously) on pulmonary arterial pressure (top), left atrial pressure (middle), and carotid blood pressure (bottom). Chest was opened by an incision through the left fourth intercostal space, and cannulas were inserted into the vessels of the left upper lobe. Note that the rise in pulmonary arterial pressure lasted longer than the rise in carotid pressure, whereas the left atrial pressure was unchanged.

Fig. 3. Effects of alloxan (100 mg./Kg. intravenously) on lung capillary blood volume, pulmonary arterial pressure, carotid blood pressure and respiratory rate. The time scale which starts with 110 min. represents time after injection of 10 ml. of dog’s own blood tagged with 0.1 μc. of phosphorus32. Note that the appearance of fluid in respiratory passages is preceded by a rise in radioactive counts.

Fig. 4. Effects of alloxan on capillary blood volume and plasma albumin content of the lungs in two separate dogs. One dog (upper half) received 5 ml. of its own blood tagged with 0.1 μc. of phosphorus32 and the other (lower half) received 1 μc. of human serum albumin tagged with iodine131. Note that the rise in radioactive counts happened before the appearance of fluid in tracheal cannula.

outflow of blood; the corresponding artery was intact. The outflow was directly measured in an open reservoir from which it was returned by a Dale-Schuster pump directly into the left atrium. In such preparations, the pulmonary venous outflow showed a definite increase following the intravenous injection of alloxan. The increase in flow was not greater than 30 per cent and it lasted for about 2 min. These changes could not entirely explain the accompanying pulmonary hypertension, which amounted to about 50 per cent and lasted for at least 5 min. All these observations suggest that the pulmonary vascular constriction is the more important cause for the hypertension and this is confirmed by the perfusion experiments reported below.

Radioactive Measurements of Erythrocytes and Plasma Albumin in the Lungs. The typical effects observed in 10 dogs (5 using erythrocytes tagged with phosphorus32 and 5 using albumin tagged with iodine131) are depicted in figures 3 and 4. The radioactivity from the lungs was recorded by means of a Geiger counter applied to the surface of the lung inside an air-tight tube, around which the chest was tightly closed. The immediate response to intravenous injection of alloxan was a reduction in phosphorus32 counts, indicating a reduction in volume of erythrocytes in the lung capillaries. The onset of this change coincided with pulmonary arterial hypertension in the same dog, but radioactive counts of plasma albumin in the lungs (measured in other dogs) were unaltered at this time. It is noteworthy
that the severity of pulmonary hypertension did not coincide with the severity of reduction in radioactive erythrocytes; the peak of hypertension occurred within 1 min. after injection, whereas the peak in reduction of capillary blood volume occurred 3 to 10 min. later. This is another indication that blood pressure and blood volume of the lungs (measured by the radioactive method) can be affected by blood flow and vascular resistance in opposite directions. Although increased blood flow (described above) might have contributed partially to the pulmonary hypertension, the coincidental pulmonary vascular constriction apparently was so intense that the blood volume in the capillaries was actually reduced.

About 15 to 30 min. after the injection of alloxan, the radioactive counts of phosphorus and iodine started to increase gradually, reaching a maximum peak of about 25 per cent above the control counts. Since both isotopes were used separately, it was not possible to compare the latent period for the rise in count of each following the injection of alloxan. However, when compared with the first appearance of grossly visible froth in the tracheal cannula, the rise in phosphorus counts happened less than 20 min. before, whereas the rise in iodine counts occurred more than 20 min. earlier, both signs were either preceded or followed by polypnea.

Death of the animal from pulmonary edema was preceded by a fall in phosphorus counts but there was no concomitant change in iodine counts. This is another demonstration that the former is more sensitive than the latter to alterations in pulmonary blood flow. Gross, histologic and chemical examination of the excised lungs after death confirmed the extensive edema brought about by alloxan.

**Alloxan Edema of the Perfused Lungs**

**Perfused Lobe in situ.** The local effects of alloxan on pulmonary vascular resistance were investigated in a lobe perfused at a constant flow. A Dale-Schuster pump was used to suck venous blood continuously from the right atrium of a donor dog (via a metal cannula inserted into one external jugular vein). The outflow of the same pump supplied the left lower lobe of a recipient dog whereas a second pump returned the pulmonary venous outflow back to the donor dog. The injection of alloxan into the perfused lobe caused a rise in perfusion pressure indicating local vasoconstriction. This was consistently seen in 4 cross-circulation experiments and a typical response is depicted in figure 5. When alloxan was injected intravenously to the recipient dog, the perfused lung was unaffected but the pulmonary arterial pressure (of intact lobes that were supplied by the dog's own heart) and carotid blood pressure increased as expected.

**Perfused Isolated Lungs.** The lungs of a dog killed by bleeding were suspended in a lucite box under negative pressure ventilation. The collected blood was stored in a heated reservoir (at 40 C.) and used to perfuse the lungs. Continuous measurements of perfusion pressure and volume of blood in this reservoir were utilized to measure changes in vascular resistance and volume of blood in the lungs brought
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FIG. 6. Effects of alloxan on excised lungs perfused at a rate of 390 ml./min. Note the persistent pulmonary arterial hypertension and the initial increase followed by decrease of volume of blood in the reservoir.

about by alloxan. In 5 such preparations, alloxan added to the perfusing blood caused an immediate rise of arterial perfusion pressure and an increase in the amount of blood in the reservoir (fig. 6). Since inflow was kept constant, these observations mean that alloxan caused pulmonary vasoconstriction and reduction of volume of blood in the lungs. After about 3 min., the blood distribution (between the lungs and the reservoir) returned to the control level but the rise in arterial pressure persisted. The reason why, at this point, the remaining vascular constriction was reflected by the pressure rise but not by blood distribution is not known; it probably means a diminution in intensity or site of constriction as compared to the earlier phase, or both. This suggests that alloxan caused an immediate pulmonary vasoconstriction intense enough to expel measurable amounts of blood into the perfusion reservoir.

The next and final change brought about by alloxan in the isolated lungs was reflected as a further rise in pulmonary arterial pressure associated with a decrease in volume of blood in the reservoir. There is, therefore, increased volume of blood in the lungs which is later accompanied by the appearance of fluid in the tracheal cannula. The explanation for the various changes in blood volume in the lungs will be discussed below.

Once edema appeared in the tracheal cannula of the perfused lung, the loss of fluid was rapid. It continued even when the following additional procedures were performed: positive pressure ventilation with 100 per cent oxygen, further reduction of perfusion flow and pressure and use of fresh blood without alloxan. The histologic picture of the lung resembled that of the intact dog dying of alloxan edema. Capillary engorgement and presence of alveolar fluid were outstanding. Three other lungs perfused similarly but without alloxan added did not develop edema after at least 4 hours of perfusion.

DISCUSSION

The fact that alloxan edema can be produced in the excised perfused lungs means that its causation lies in the lung parenchyma. These results derived from the intact dog and the perfused lung preparation can be discussed together to arrive at an hypothesis for the pathogenesis of edema. The effects of alloxan can be divided into the following three stages:

Pulmonary Hypertension with Reduction of Pulmonary Blood Volume. The immediate rise in pulmonary arterial pressure in the intact dog is accompanied by increased pulmonary blood flow. The most important cause for the hypertension is generalized pulmonary vasoconstriction which is based on the observed rise in arterial pressure of one lobe (in situ) or of all lobes (isolated) perfused at a constant flow. The volume of blood in the perfused lungs (measured directly) and in the intact lungs (measured indirectly by the radioactive phosphorus method) uniformly decreased during the peak of pulmonary arterial hypertension. This initial stage is probably not as important as the third stage in the causation of edema.

Intermediate Stage. This consists of diminished pulmonary hypertension but the blood volume changes obtained from the perfused lung preparation and the radioactive intact lung preparation are contradictory. The former method shows no change while the latter shows a decreased volume of blood. The explanation for this disagreement probably lies simply in the differences in the state of the lungs, i.e., the isolated lungs have lost some nervous regulatory mechanism as a result of death of the animal, perfusion, vessel cannulation, etc. In any event, this stage is a transition between the first stage of decreased volume of blood and the next stage of increased volume.
Stage of Pulmonary Hypertension, Congestion, and Edema. The pulmonary arterial hypertension (seen in the intact and perfused lungs) and pulmonary vasoconstriction (in the perfused lungs) continued during this last phase. A concurrent increase in blood volume is seen in both the perfused lungs and the intact lungs (by radioactive phosphorus method). The best explanation to account for all these results is that after a latent period (of about 15 to 30 min.), the constriction is localized in the post-capillary or venous side of the lung vessels. A simple dilatation of capillaries is not likely since vascular resistance is still high. A precapillary constriction combined with capillary dilatation is acceptable but is a more complex explanation. The congestion of pulmonary capillaries is either accompanied or followed by an increase in plasma albumin in the lungs (tagged by radioactive iodine), and by polypnea which precedes the appearance of froth in the respiratory passages.

This theory of causation of congestion and edema by pulmonary venous constriction is new for chemical agents but has been previously postulated for edema following the inhalation of steam. Direct proof for such a mechanism is being sought by direct visualization of the lung vessels. Its significance in clinical forms of edema and in the normal state, if any, is even more remote.

The observation that congestion accompanies alloxan edema raises a number of questions. Is the edema entirely arising from the pulmonary hypertension and congestion? Or does alloxan also increase capillary permeability? If permeability is altered, the problem remains of deciding whether alloxan increases permeability by a direct action, or by an indirect action resulting from the congested and distended capillaries. If the latter is true, then the relief of congestion would correct the defect in permeability and would reverse the edema. Since pulmonary congestion is a common accompaniment of most forms of clinical pulmonary edema, all these problems are not entirely theoretic, but are also of therapeutic importance.

The experimental use of alloxan as a diabetogenic agent is now extended to include the production of pulmonary edema. It is particularly useful in dogs because of its simplicity and dependability, as compared to more complex methods (saline infusion, intramedullary injections, intramyocardial injection). With the radioactive methods now available for continuous detection of congestion and edema, it is possible to test objectively various therapeutic attempts such as positive pressure breathing, oxygen inhalation, alcohol inhalation, ganglion blocking agents, etc. This new method in dogs will supplement the available tests, using mice, guinea pigs, rats, and rabbits in which survival, lung weight and lung moisture content are observed.

Summary

Alloxan causes pulmonary hypertension, congestion and edema in anesthetized dogs. All of these effects can be elicited in the perfused excised lungs, and are brought about by two types of vascular actions, an immediate vasoconstriction accompanied by reduction in blood volume in the lungs and a delayed vasoconstriction accompanied by increased blood volume. It is postulated that the second effect of alloxan is caused by a local constriction of pulmonary veins and is the most important cause of edema. The additional role of increased capillary permeability has not been excluded.

The appearance of edema fluid in the respiratory passages in the intact dogs is preceded by three signs, polypnea, increased volume of blood in the lung capillaries and increased amount of plasma albumin in the lung parenchyma. The last two signs, measured by radioactive methods (phosphorus for erythrocytes and iodine for albumin), are suitable for testing the progress of pulmonary edema before and after therapy.

Summario in Interlingua

Alloxano causa hypertension pulmonar, congestion, e edema in canes anesthesiati. Omne iste effectos pote esser evocate in pulmones excitidate perfundite. Illos resulta de duo typos de action vascular: Un vasoconstriction immediate accompaniate per reduction del volumine de sanguine in le pulmones e un vasoconstric-
tion retardate accompaniate per augmentation del volumine de sanguine. Es postulate que le secunde efecto de alloxano es causate per un constriction local del venas pulmonar e es le plus importante causa de edema. Le possibilitate que etiam le augmentate permeabilitate capillare ha un rolo contributori non es excludite.

Le apparition de fluido edemic in le passages respiratori de canes intacte es precedite per tres signos: polypnea, augmento del volumine de sanguine in le capillares pulmonar, e augmento de albumina plasmatic in le parenchyma pulmonar. Le secunde e le tertie de iste signos, mesurate per methodos radioactive (phosphoro$^{32}$ pro erythrocytos e iodo$^{131}$ pro albumina), se presta al uso in tests del progresso de edema pulmonar ante e post le therapia.

REFERENCES


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