J. Physiol. (1969), **204**, 51–61
With 4 text-figures
Printed in Great Britain

THE EFFECTS OF ALLOXAN AND HISTAMINE ON THE PERMEABILITY OF THE PULMONARY ALVEOLOCAPILLARY BARRIER TO ALBUMIN

BY BOYD W. GOETZMAN* AND MAURICE B. VISSCHER

From the Department of Physiology, School of Medicine, University of Minnesota, Minneapolis, Minnesota, U.S.A.

(*Received* 1 *January* 1969)

SUMMARY

1. [^{131}I]albumin transport from alveolus to perfusion fluid was evaluated in an isolated dog lung lobe preparation perfused at constant pressure with homologous plasma. The alveoli were filled with a 1:1 mixture of Ringer–Tyrode solution and plasma.

2. For twenty-three lung lobes, an average control permeability coefficient of the alveolocapillary membrane for albumin was found to be $1{\cdot}9 \times 10^{-9}$ cm/sec with a range of $(0{\cdot}3–5{\cdot}5) \times 10^{-9}$ cm/sec.

3. Alloxan in concentrations of 0·22–2·50 mg/ml. perfusate was observed to increase the alveolocapillary membrane permeability to [^{131}I]albumin with a positive correlation between dose and effect. The larger doses of alloxan increased the permeability by a factor of ten or more.

4. Glutathione (GSH), administered intravascularly 2 min before alloxan in an 8:1 molar ratio of GSH:alloxan, delayed the onset of the permeability increase due to alloxan by 40–60 min but did not influence the maximum increase in permeability.

5. Histamine administered intravascularly in concentrations from 0·02 to 0·50 μg/ml. perfusate had no significant effect on the alveolocapillary membrane permeability to [^{131}I]albumin.

INTRODUCTION

The pulmonary vascular effects of alloxan and histamine have been of interest to investigators studying pulmonary oedema (Visscher, Haddy & Stephens, 1956). Alloxan has long been known to produce pulmonary

* Present address: Department of Pediatrics, University Hospitals, University of Minnesota, Minneapolis, Minnesota 55455.

oedema in animals (Peralta, 1945; Houssay, Brignone & Mazzocco, 1946). The mechanism by which alloxan produces pulmonary oedema has been in dispute, with some authors favouring increased pulmonary capillary hydrostatic pressure (Aviado & Schmidt, 1957) and others favouring increased pulmonary capillary permeability to protein (Fejfar, Zajec & Fejfarova, 1959; Staub, Nagano & Pearce, 1967) as the initiating factor. No direct measurement of increased pulmonary capillary permeability in response to alloxan has been reported, however.

The role of histamine in pulmonary oedemogenesis remains uncertain (Visscher *et al.* 1956; Aviado, 1960). In animals other than guinea-pigs, it is difficult to produce acute pulmonary oedema by the administration of histamine (Visscher *et al.* 1956). Past studies on the escape of intravascular dye-protein complexes and oedema formation have established that histamine does increase the vascular permeability in certain regions of the systemic circulation (Spector, 1958). The effect of histamine on the permeability of the pulmonary vasculature has not, to our knowledge, been evaluated directly.

This paper presents experiments that demonstrate a significant increase in pulmonary alveolocapillary membrane permeability in response to intravascular alloxan administration in an isolated perfused dog lung preparation. Similarly, experiments demonstrating a minimal effect on alveolocapillary membrane permeability after the administration of histamine will be presented.

The technique to be described should allow evaluation of other oedemogenic agents.

METHODS

The entire left lung was degassed in healthy, 16–28 kg mongrel dogs anaesthetized with sodium pentobarbitone (30 mg/kg, I.V.) and given sodium heparin (5 mg/kg, I.V.). The left lower lobe was isolated and its artery, vein and bronchus cannulated and connected to the constant pressure perfusion system shown schematically in Fig. 1. Eighteen to twenty minutes of interrupted perfusion was usually required to complete the lung lobe isolation.

The constant pressure perfusion system (*Fig.* 1). The lung reservoir, H, contained 0·9 % NaCl (w/v) at 22–24° C. Perfusion fluid was pumped continuously from the venous reservoir, E, to the arterial reservoir, F. The arterial pressure was 'set' by the fluid level in the arterial reservoir. The venous pressure was 'set' by the level of the venous cannula, B, emptying into the venous reservoir, E. The top of the lung reservoir, H, was the zero pressure reference.

The perfusion fluid was heparinized, homologous plasma continuously aerated with a 95 % O_2–5 % CO_2 gas mixture. Because the lung lobe contained some residual blood, the perfusate had a haematocrit of 1–5 %. The pH values of the perfusion fluid in control studies were found to be between 7·38 and 7·48. The flow was measured at the beginning of each experiment and periodically thereafter by collecting the venous outflow. Flow rates were approximately 2 ml./min per gram lung.

Alveolobronchial system. Five to ten minutes after perfusion had begun, the alveolobronchial compartment of the degassed isolated lung lobe was filled with a 1:1 mixture of homologous plasma and Ringer–Tyrode solution at pH 7·40 and room temperature. The lung reservoir was then completely filled and sealed. The pressures inside the closed lung reservoir and left lower lobe bronchus were continuously monitored with cannulas connected to strain-gauge pressure transducers.

Tracer preparation, sampling and analysis. [^{131}I]albumin was used as the test substance. The commercial product was dialysed against 0·45 % NaCl (w/v) until less than 0·01 % of the total remaining radioactivity was dialysable. Albumin permeability could then be evaluated without interference from free iodide. Alveolobronchial fluid radioactivity was made about 1×10^6 c.p.m./ml. by using 100 μc of

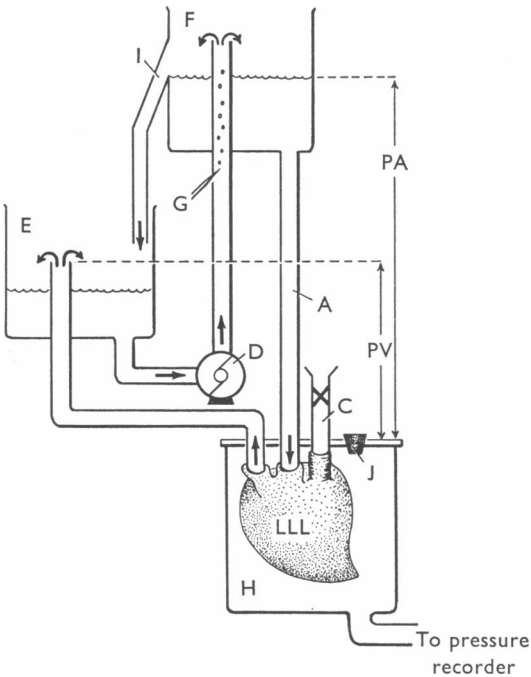

Fig. 1. Scheme of perfusion system.

[^{131}I]albumin for each experiment. The appearance of [^{131}I]albumin in the vascular compartment was followed by periodic sampling from the venous reservoir. Alveolobronchial fluid was sampled at the beginning and end of each experiment. Radioactivity was determined in a well-type scintillation counter.

Alloxan. Previous investigators (Aviado & Schmidt, 1957; Staub *et al.* 1967) have used alloxan (50–150 mg/kg) intravenously to produce acute pulmonary oedema in dogs. Corresponding plasma concentrations would be 1·2–3·5 mg/ml. plasma. Alloxan is unstable in solution and was therefore dissolved immediately before administration into the venous reservoir.

Lazarow (1946) reported that a 6:1 molar ratio of glutathione, a sulphydryl group contributor, to alloxan completely protected rats against alloxan-induced diabetes mellitus. Smaller ratios were ineffective. A series of experiments utilizing an 8:1

molar ratio of GSH:alloxan was performed. Glutathione solution, neutralized to a pH of 7·0, was administered 2 min before alloxan in these experiments.

Histamine. Histamine acid phosphate was administered into the venous reservoir in 0·02, 0·1 and 0·5 μg/ml. perfusate. These are concentrations shown to have significant haemodynamic effects in isolated lungs by other authors (Gaddum & Holtz, 1933; Gilbert, Hinshaw, Kuida & Visscher, 1958).

Permeability calculations. The system schematized in Fig. 1 was considered to represent a case of 'uptake from a constant source' (Solomon, 1953) since only 1–2 % of the [^{131}I]albumin added to the alveolobronchial fluid crossed the alveolocapillary membrane to the vascular fluid during the time of observation. The perfusate volume was shown to change by 1 % or less after 2 hr of perfusion by following the haematocrit of whole blood. Therefore, compartment volumes were assumed to be constant. Fick's first law was applied to the system to obtain the following equation: $P = V_B\dot{C}_B/C_A A_M$; where P = the permeability coefficient of the alveolocapillary barrier in cm/sec; V_B = volume of vascular compartment, ml.; $\dot{C}_B$ = rate of change of [^{131}I]albumin concentration in vascular compartment, c.p.m./ml. sec; C_A = [^{131}I]albumin concentration in alveolar compartment, c.p.m./ml., and assumed to be constant; and A_M = area of alveolocapillary membrane, cm^2.

In each experiment, $\dot{C}_B$ was evaluated by sampling from the vascular compartment. Several samples were taken during a control period and an average control permeability coefficient, P_C, calculated for each experiment. Permeability changes during an experiment were calculated as percents of the control permeability coefficient, $\%P_C$, as indicated by the simplified formula: $\%P_C = (\dot{C}_B$, at time $t/\dot{C}_B$, control) $\times 100$.

In calculating the permeability coefficient, P, an estimate of the surface area of the alveolocapillary membrane based on alveolar fluid volume was used. Dead space determinations similar to Taylor & Guyton's (1965) showed that about 85 % of the alveolobronchial fluid added entered the alveoli. Assuming that alveoli were either filled or empty and using an alveolar radius of 90 micra for spherical alveoli one arrives at the following equation for the surface area of the alveoli filled: $A_M = 273V_A$ where V_A is the volume of the alveolobronchial fluid used in ml. and A_M is the area in cm^2.

The perfusate volume, V_B, was the sum of the plasma volume added to the system and the whole blood present in the pulmonary vessels of the lung lobe. The latter was calculated from the measured values of heart blood haematocrit and perfusate haematocrit utilizing the dilution principle.

The wet weight of the left lower lobe, W, was obtained by subtracting the weight of the excised bronchus at the end of the experiment from the initial weight of the lobe and bronchus combined.

RESULTS

The compartmental volumes, alveolocapillary surface area estimates, perfusion pressures, alloxan, glutathione and histamine concentrations, and control permeability coefficients for the experiments reported appear in Tables 1 and 2. The maximum % increase in the permeability coefficient for the alloxan experiments and the % change in the permeability coefficient for the histamine experiments appear in the final column for comparative purposes.

The average control permeability coefficient, P_C, for all twenty-three experiments reported is $1{\cdot}9 \times 10^{-9}$ cm/sec with a range $(0{\cdot}3\text{–}5{\cdot}5) \times 10^{-9}$ cm/sec.

The results of experiments appear in graphical form in Figs 2–4 as % control permeability versus time (min). Alloxan or histamine was administered at zero time. The graphs *a* to *d* in Fig. 2 show an increasing effect on the permeability coefficient for [^{131}I]albumin as the dose of alloxan was increased from 0·22 to 2·50 mg/ml. perfusate. The larger doses of alloxan increased the permeability to [^{131}I]albumin by a factor of ten or more. The onset of the permeability effect was a few minutes after the administration

TABLE 1. Data tabulation for alloxan experiments. V_A = alveolobronchial fluid volume, V_B = perfusion volume, W = wet wt. of lung lobe, A_M = area estimate of alveolocapillary membrane, P_A and P_V = pulmonary artery and vein pressures respectively, P_C = control permeability coefficient, and % P_C = per cent increase of permeability coefficient after indicated dose of alloxan

No.	V_A (ml.)	V_B (ml.)	W (g)	A_M (10^4 cm^2)	P_A (cm H_2O)	P_V (cm H_2O)	Alloxan GSH mg/ml.)	GSH (mg)	P_C (10^{-9} cm/ sec)	Max. % P_C
1	100	115	56	2·7	20	12	0·22	0	1·3	196
2	100	105	51	2·7	27	16	0·24	0	1·1	250
3	104	125	52	2·8	21	11	0·40	0	1·6	170
4	100	117	33	2·7	23	13	0·43	0	3·9	250
5*	110	117	57	3·0	23	15	0·86	0	5·5	430
6	100	112	47	2·7	19	13	0·89	0	0·8	396
7	100	104	53	2·7	19	12	0·96	0	0·6	280
8*	110	110	67	3·0	27	17	1·13	0	3·6	424
9	110	113	57	3·0	14	8	1·33	0	1·2	672
10	120	107	40	3·3	23	13	1·86	0	0·5	1660
11	142	82	35	3·9	23	13	2·50	0	1·0	1060
12	120	126	53	3·3	18	8	0	1200	0·3	198
13	120	113	68	3·3	21	14	0·71	0	0·8	1060
14	100	108	37	2·7	21	13	0·74	1200	2·6	1080
15	100	113	56	2·7	20	14	0·71	1200	0·8	1060

* Dog 5 had evidence of pneumonic consolidation in two lobes and dog 8 had a suspected early pneumonia.

TABLE 2. Data tabulation for histamine experiments

No.	V_A (ml.)	V_B (ml.)	W (g)	A_M (10^4 cm^2)	P_A (cm H_2O)	P_V (cm H_2O)	Histamine (μg/cm^3)	P_C (10^{-9} cm/sec)	Max. Δ % P_C
1	100	110	45	2·7	26	17	0·02	2·2	+55
2	100	133	52	2·7	22	16	0·02	1·1	−21
3	100	228	65	2·7	14	7	0·10	1·5	−30
4	100	257	54	2·7	18	12	0·10	2·1	−37
5	100	258	50	2·7	24	20	0·10	4·1	+20
6	100	258	54	2·7	16	10	0·50	2·1	−35
7	100	258	54	2·7	24	19	0·50	1·7	−35
8	100	116	55	2·7	25	16	0·50	1·2	−62

of alloxan with the maximum increase in permeability appearing 20–40 min later. In a few of the longer experiments, e.g. 1 and 13, it appeared as if the alloxan effect may be reversible.

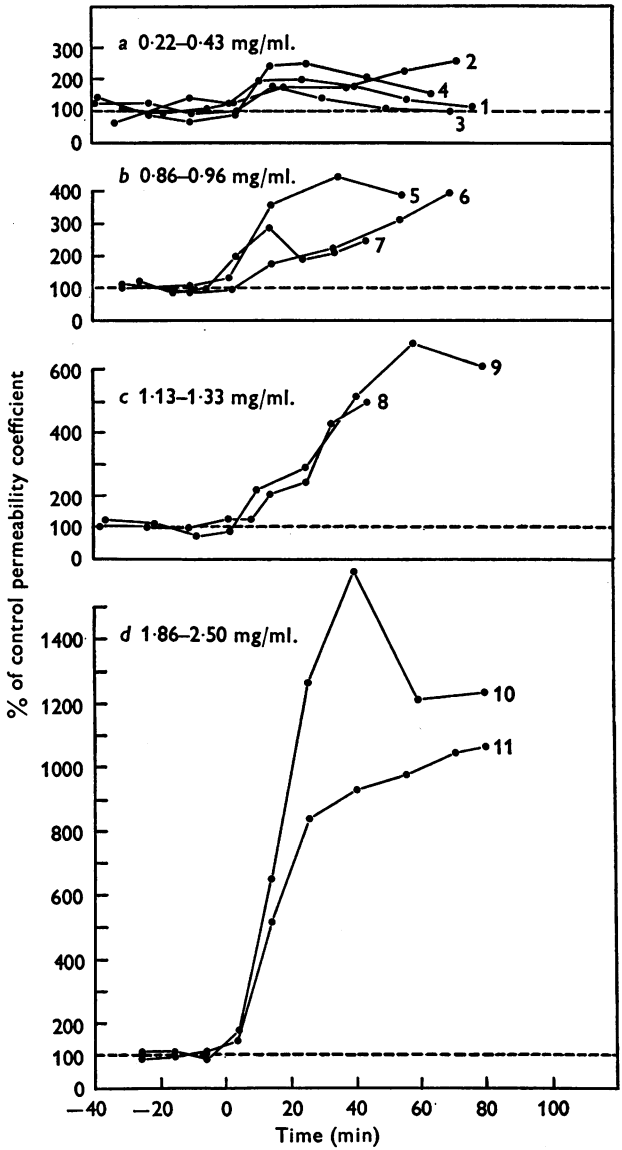

Fig. 2. The effect of alloxan, in various dosages, on the permeability of the alveolocapillary membrane to [^{131}I]albumin. Numbers in parentheses refer to individual experiments in Table 1. Alloxan administered at zero time. Concentrations given refer to alloxan concentration in perfusate.

The effect of glutathione on the permeability effect of alloxan is shown in Fig. 3. In experiments 14 and 15, GSH was administered in an 8:1 molar ratio of GSH:alloxan 2 min before the alloxan. It is seen that the maximal increase of permeability due to alloxan was not affected by GSH in this concentration, however, the onset of the permeability change was delayed by 40–60 min in these experiments. Six experiments with smaller GSH:alloxan molar ratios (2:1 and 4:1) showed no significant effect on the

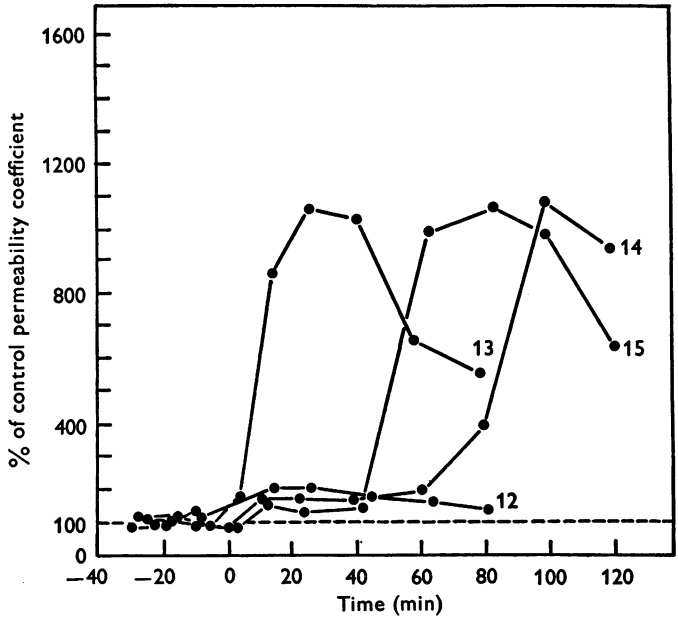

Fig 3. The effect of pre-administration of glutathione on the response to alloxan. Graph 12 indicates 1200 mg GSH at zero time; 13, 80 mg alloxan at zero time; 14, 80 mg alloxan at zero time + 1200 mg GSH at −2 min; and 15, 80 mg alloxan at zero time + 1200 mg GSH at −2 min.

permeability change induced by alloxan and are not reported here. GSH in the large dose used is seen to have some effect on the permeability of the alveolocapillary membrane to [^{131}I]albumin in experiment 12. This may well have been a pH effect since the perfusate pH fell by 0·25 units after this dose of GSH. Control experiments on the effect of pH on [^{131}I]-albumin permeability in the system used have not been performed to date.

The results of experiments with histamine appear in graphic form in Fig. 4. There appeared to be no significant effect on the alveolocapillary membrane permeability to [^{131}I]albumin in the dose range of 0·02–0·50 μg histamine/ml. perfusate. The slight fall in permeability observed when the larger amount of histamine was used could possibly be due to a decreased membrane area secondary to venular constriction.

Haemodynamic changes

The perfusion flow rate during the control periods was usually 100–140 ml./min or 2–3 ml./min per gram tissue. After the administration of alloxan, the flow typically decreased by 10–30% but returned to or exceeded the control period flow within 5–10 min. Several experiments were terminated when the flow began to decrease 40–60 min after the

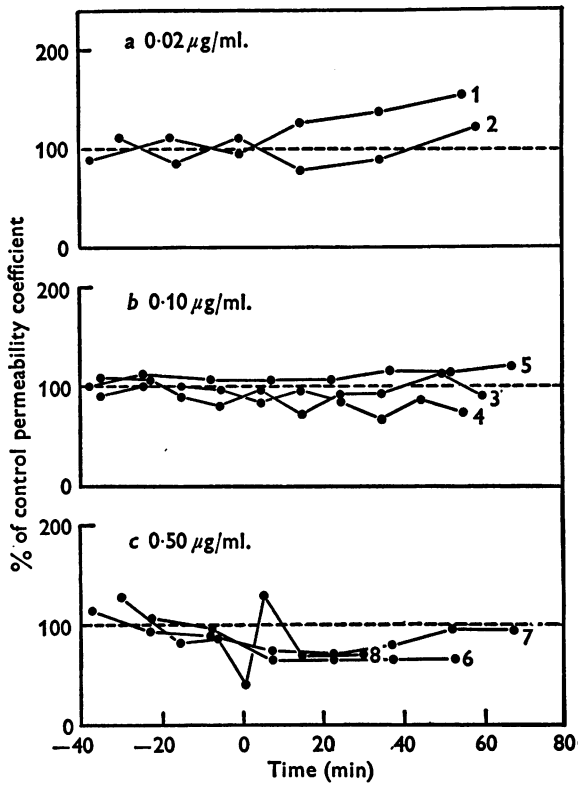

Fig. 4. The effect of histamine on the permeability of the alveolocapillary membrane to [^{131}]albumin. Numbers in parentheses refer to experiment numbers in Table 2. Histamine administered at zero time. Histamine concentrations given refer to concentration in perfusate.

alloxan was given. Air embolism could produce such changes. This was carefully watched for, but tiny bubbles could be missed.

There was a more sustained decrease in flow of 10–40%, depending on the dose, after the administration of histamine. Twenty to thirty minutes were required for return to control flow values. During this time, the pulmonary vessels appeared to be refractory to additional histamine.

DISCUSSION

The experimental data reported here demonstrate directly that alloxan administered intravascularly in dosages of 0·22–2·50 mg/ml. perfusate led to increased permeability of the alveolocapillary membrane to [^{131}I]-albumin. Haemodynamic effects were minimized by utilizing a constant pressure perfusion system. Pulmonary arterial pressures never exceeded 20 mm Hg and venous pressures were less than 13 mm Hg. Thus, it was impossible to reach the pulmonary capillary pressure of 24 mm Hg found by Guyton & Lindsey (1959) to be necessary to produce pulmonary oedema on a haemodynamic basis.

This work substantiates the conclusions from haemodynamic studies of Fejfar *et al.* (1959) and Staub *et al.* (1967) that the primary oedemogenic action of alloxan is increased pulmonary capillary permeability to protein. These authors found little or no elevation of left atrial or pulmonary venous capillary pressure at the time of oedema fluid accumulation in the lungs of living dogs. Aviado & Schmidt (1957) interpreted their original data on pulmonary arterial and left atrial pressures in conjunction with the relative radioactivity concentration of ^{32}P labelled red cells and [^{131}I]-albumin in the lung after alloxan administration to indicate primarily a haemodynamic basis for alloxan-induced pulmonary oedema. Aviado (1965) now appears to favour a combination of increased capillary permeability and haemodynamic factors in alloxan-induced pulmonary oedema.

The onset of increased alveolocapillary membrane permeability to [^{131}I]albumin occurred within a few minutes after the administration of alloxan. However, the maximum effect occurred 20–40 min later. This time roughly corresponds to the delay in onset of pulmonary oedema when alloxan is administered to living intact animals (Fejfar *et al.* 1959; Staub *et al.* 1967).

The mechanism by which alloxan produces increased permeability of the alveolocapillary membrane is unknown. However, since alloxan is known to react with sulphhydryl groups of proteins, it would be expected that sulphhydryl enzymes might be inactivated by alloxan (Lazarow, 1946). Sulphhydryl enzyme systems may be necessary for maintaining the integrity of the pulmonary alveolocapillary membrane and when inactivated by alloxan an increase in capillary permeability occurs.

The possibility exists that isolated perfused lungs are more susceptible to alloxan injury than are the lungs of living intact animals. One might hypothesize a decrease in tissue glutathione, GSH, during the perfusion. We are not aware of any data relating to this point, however. Red blood cells contain considerable GSH (Jocelyn, 1959) which might exert a pro-

tective effect against alloxan. Our perfusates contained small red cell masses. This was not felt to be significant since a perfusate of whole blood in our system would have contained only an estimated 20–30 mg GSH (Jocelyn, 1959) and the administration of 1200 mg GSH did not permanently protect the lungs against alloxan.

The average alveolocapillary membrane permeability coefficient of $1{\cdot}9 \times 10^{-9}$ cm/sec is in reasonably good agreement with the average value of $2{\cdot}8 \times 10^{-9}$ cm/sec found by R. L. Goodale in an as yet unpublished study from this laboratory, considering the fact that different methods for calculating area were employed in the two studies. No other measurements of this permeability coefficient for the pulmonary alveolocapillary membrane to albumin are available, however Landis & Pappenheimer (1963) reported the muscle capillary permeability coefficient for [^{131}I]albumin as 10×10^{-9} cm/sec. Thus, it appears that for large molecules the pulmonary alveolocapillary membrane resembles capillaries in muscles whereas Taylor & Guyton (1965) found this membrane to be more like a cellular membrane for small solutes such as glucose, urea, and ^{24}Na. That is, they found permeability coefficients for these substances to be much smaller in the lung capillaries than in muscle capillaries.

The histamine experiments did not show any significant effect of histamine on the alveolocapillary membrane permeability to [^{131}I]albumin in the dosages used. This observation is in agreement with the findings of Rowley (1964) and Haddy (1960) that in the rat hind limb and dog hind limb, respectively, no increase in oedemogenesis with histamine was observed when hydrostatic pressure effects were prevented. Also, Chambers & Zweifach (1947) found no increase in vascular permeability in the rat mesentery after local or intravenous administration of histamine at the dosage levels used. Eiseman, Bryant & Waltuck (1964) showed that histamine is only slowly metabolized by the isolated lung, 80 % of an injected histamine load being present 1 hr after administration. Therefore, in our experiments, the lung vessels were exposed to relatively constant histamine levels.

This work was aided by USPHS Grants nos. 03212 and 05138.

REFERENCES

AVIADO, D. M. (1960). The pharmacology of the pulmonary circulation. *Pharmac. Rev.* **12**, 159–239.

AVIADO, D. M. (1965). *The Lung Circulation*, vol. 2, pp. 878–886. New York: Pergamon Press.

AVIADO, D. M. & SCHMIDT, C. F. (1957). Pathogenesis of pulmonary edema by alloxan. *Circulation Res.* **5**, 180–186.

CHAMBERS, R. & ZWEIFACH, B. (1947). Intercellular cement and capillary permeability. *Physiol. Rev.* **27**, 436–463.

EISEMAN, B., BRYANT, L. & WALTUCK, T. (1964). Metabolism of vasomotor agents by the isolated perfused lung. *J. thoracic cardiovasc. Surg.* **48**, 798–806.

FEJFAR, Z., ZAJEC, F. & FEJFAROVA, M. (1959). Alloxan induced experimental pulmonary edema. I. Hemodynamic alterations. *Cor Vasa* **1**, 56–72.

GADDUM, J. H. & HOLTZ, P. (1933). The localization of the action of drugs on the pulmonary vessels of dogs and cats. *J. Physiol.* **77**, 139–158.

GILBERT, R. P., HINSHAW, L. B., KUIDA, H. & VISSCHER, M. B. (1958). Effects of histamine, 5-hydroxytryptamine and epinephrine on pulmonary hemodynamics with particular reference to arterial and venous segment resistances. *Am. J. Physiol.* **194**, 165–170.

GUYTON, A. C. & LINDSEY, A. W. (1959). Effect of elevated left atrial pressure and decreased plasma protein concentration on the development of pulmonary edema. *Circulation Res.* **7**, 649–657.

HADDY, F. J. (1960). Effect of histamine on small and large vessel pressures in the dog foreleg. *Am. J. Physiol.* **198**, 161–168.

HOUSSAY, B. A., BRIGNONE, R. F. & MAZZOCCO, P. (1946). Diabetes metaaloxanica en el perro. *Soc. Arg. biol. Rev.* **22**, 195–231.

JOCELYN, P. C. (1959). Glutathione. *Biochem. Soc. Symp.* **17**, 43–56.

LANDIS, E. & PAPPENHEIMER, J. (1963). Exchange of substances through capillary walls. In *Handbook of Physiology*, vol. 2, chap. 29, pp. 961–1034. Washington, D.C.: American Physiological Society.

LAZAROW, A. (1946). Protective effect of glutathione and cysteine against alloxan diabetes in the rat. *Proc. Soc. exp. Biol. Med.* **61**, 441–447.

PERALTA, B. R. (1945). Mecanismo de la fase inicial hyperglycemia de la aloxanaca en el gato. *Rev. Inst. Salubridad y Enferm. Trop* **6**, 117–122.

ROWLEY, D. A. (1964). Venous constriction as the cause of increased vascular permeability produced by 5-hydroxytryptamine, histamine, bradykinin and 48/80 in the rat. *Br. J. exp. Path.* **45**, 56–67.

SOLOMON, A. K. (1953). The kinetics of biological processes: special problems connected with the use of tracers. *Adv. biol. med. Phys.* **3**, 65–97.

SPECTOR, W. G. (1958). Substances which affect capillary permeability. *Pharmac. Rev.* **10**, 475–505.

STAUB, N. D., NAGANO, H. & PEARCE, M. L. (1967). Pulmonary edema in dogs, especially the sequence of fluid accumulation in lungs. *J. appl. Physiol.* **22**, 227–240.

TAYLOR, A. E. & GUYTON, A. C. (1965). Permeability of the alveolar membrane to solutes. *Circulation Res.* **16**, 353–362.

VISSCHER, M. B., HADDY, F. J. & STEPHENS, G. (1956). The physiology and pharmacology of lung edema. *Pharmac. Rev.* **8**, 389–434.