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EXAMINATION OF THE PULMONARY CIRCULATION WITH THE MICROSCOPE.

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THE flow of blood in the lung vessels has been observed with the microscope in the entire animal by various workers. Hall [1925] using cats or rabbits anæsthetized with chloretone or urethane, or pithed, studied the circulation at the edge of the lung by transillumination. The chest was opened and the lung suspended by clips to allow the edge to be transilluminated. He noted a distinct slowing and backward movement of the blood stream between the heart beats on stimulation of the vagi and slowing of the heart. He also claims to have seen constriction in the smaller vessels on injection of adrenaline. Wearn, Barr and German [1926] and Wearn, Ernstene and Bromer [1928] observing in cats, under amytal anæsthesia, the vessels at the edge of the lung through a window cut in the thorax with transillumination through a window in the diaphragm from a source of light in the abdomen, noted changes in the number of capillaries with changes in the systemic blood-pressure. They noted that the pressure on the abdominal aorta caused new capillaries and sometimes arterioles to open up, and injection of adrenaline with a rise in systemic blood-pressure to cause an opening up of capillaries.

Olkon and Joannides [1930] examined the capillaries near the lung surface in the dog, using reflected light with cedar oil or glycerine on the pleura. Ether and scopolamine were used. Using artificial respiration they describe changes in the number of capillaries and the flow of blood in them due to alterations in the intrapulmonary pressure. With distension of the lung to a size greater than the normal the single-celled capillaries became fewer, and there was an increase in the flow of blood in the multicellular capillaries surrounding the alveoli. When the intrapulmonary pressure was reduced, the flow in the larger capillaries assumed a to and fro movement, stopping and moving on again.

The present work was undertaken to determine how far alterations in the total pulmonary blood flow and resistance, caused by injection of drugs or by nerve excitations, influenced the smaller blood vessels at the edge of the lung which could be observed with a microscope. To do this the intact animal was first used and the vessels observed by a modification of Hall's method. Later, however, a series of observations were made on isolated lungs perfused with blood at constant pressure, the inflow and outflow of blood being recorded [Gaddum and Holtz, 1933]. By this means it was hoped to correlate the changes in total flow with that taking place locally in blood vessels in the field of the microscope.

Preliminary experiments were undertaken with a view to determining the type of animal most suited for this particular type of examination and any individual variations that might occur in examination of the lungs in different species. At the same time it was hoped to obtain information of the most suitable and simple technique applicable. The animals used were rabbit, cat and dog.

Rabbit. Using Hall's method it was found that vessels containing circulating blood could be seen at the edges of the lungs, but certain disadvantages of this technique became evident. Owing to the delicate texture of the lungs it was difficult even with the most careful manipulation to fix an area for examination without interfering with the circulation at that particular part, moreover when a satisfactory field was under observation the blood circulating in the vessels tended to develop stasis after a short time. Such stasis was apt to occur in vessels at the edges of the lobes after the chest was opened, and, with positive pressure ventilation, even in lobes that had been free from any form of manipulation right up to the time of examination. The lung tissue was apt to tear at the point of attachment of the clips on the slightest traction, and finally failure of the general circulation was much commoner than with the other species of animal experimented on.

Cat. In these animals the preliminary experiments gave much better results. The translucency of the lungs at their borders was such that the blood could be clearly observed circulating in vessels up to the size of 100 microns and, in a few cases, in larger vessels. The lung tissue, whilst being sufficiently translucent, resisted the effects of exposure, and the necessary lung manipulation did not produce stasis in the vessels of the part under examination.

Dog. Two experiments showed that whilst the lung was well suited to resist exposure and manipulation, a good circulation being maintained in the vessels at the very edge of the lobes, the tissue was relatively

opaque so that the vessels and their contents could only be seen indistinctly. Dog lung and rabbit lung were therefore thought less suitable than cat lung for this type of investigation.

Operative technique.

For examination of the vessels with the lungs in situ.

The cats were anæsthetized with ether and medinal (0·42 g. per kg. body weight) and the chest opened under artificial respiration. Several methods were used for exposing the lungs and these can be classified as follows:

(*a*) Extensive removal of the chest wall, the intercostal vessels being ligated. The edges of the lobe to be examined were supported by rubber covered clips. The lobe being thus suspended at a slightly higher level than the others, light could be transmitted through it from a carbon arc lamp, and the lung examined by a microscope fixed horizontally as in the method used by Hall [1925].

(*b*) Using the same apparatus but with a modified operative technique. It was found that if the lowest intercostal space above the insertion of the diaphragm was opened on each side as far as the xiphisternum, the whole chest wall could be lifted upwards and forwards towards the head, the ribs assuming the more horizontal position of deep inspiration. If the pericardium was slung up behind the xiphisternum the heart was kept well in the chest, whilst the lower borders of the lower lobes could project through the space formed and could be transilluminated and examined. This method involved less shock to the preparation. With both these preparations the lungs were kept distended with a continuous insufflation of oxygen [Meltzer, 1909]. The state of distension of the lungs being adjusted by the resistance of the outflow; active positive pressure respiration was given between the observations to prevent any excess of CO_2.

(*c*) The chest was opened and one side partially removed. The bronchus was tied on the open side without involving the pulmonary vascular supply to the lung, air being kept in the lung to maintain the lobes partially distended. The edges of the lobe could now be examined either with a microscope fixed horizontally, or with their edges over the stage of a vertical microscope by suitable arrangement of the preparation. This was found to be useful in the case of the smaller sized animals. In these cases observations could be made following injection of drugs into the circulation but not following nerve stimulation, as by this

method the nerve supply was included in the ligature. The drugs were injected either into a cannula in the saphenous or jugular veins or directly into the right heart. The results from all these experiments are included in Table I.

TABLE I. Cat, entire animal with open thorax.

Vagal stimulation.

Date	Blood vessel under observation: Size of vessel in μ	Calibre	Flow	Rate and force of heart beat
24. iv. 31	86	N.C.	Slow and rev.	N.C.
11. v. 31*	25	N.C.	Back flow	Heart stopped
18. v. 31	76	N.C.	Stopped, back and forward with each beat	Slowed
18. v. 31	57	N.C.	,,	,,
18. v. 31	76	N.C.	,,	,,
18. v. 31	38	N.C.	,,	,,
18. v. 31	27	N.C.	Stasis	,,
21. v. 31	57	N.C.	Slow and rev.	,,
17. vi. 31	20	N.C.	Slow and back flow between beats	,,

Adrenaline.

Date	Dose γ	Size of vessel in μ	Effect upon: Calibre	Effect upon: Flow	Rate and force of heart beat
24. iv. 31	100	86	N.C.	Stop and rev.	? N.C.
18. v. 31	100	76	N.C.	+pulse	+force
18. v. 31	100	57	N.C.	+pulse	+force
18. v. 31	100	76	N.C.	+pulse	+force
18. v. 31	100	38	N.C.	+pulse	+force
21. v. 31	100	57	N.C.	+pulse	+rate
21. v. 31	100	Cap.	Opening	+pulse	+rate
17. vi. 31	100	177	Apparent contraction ($177 \rightarrow 90\,\mu$)		
30. vi. 31	3	60	N.C.	Back flow	Slowing
30. vi. 31	10	60	? dilatation	Stop followed by great increase	
5. iii. 33*	1000	60	N.C.	+rate	+rate +force

Cap. = capillary; N.C. = no change; + = increased; − = decreased; Sl. = slight; rev. = reversed; P.A. = pulmonary artery. In every experiment in which the blood inflow increased the outflow also increased; also, with an inflow decrease the outflow always decreased.

* Observation repeated three times.

For examination of the vessels in the isolated perfused lung.

The cat was anæsthetized with ether and bled from the carotid, the blood being defibrinated and placed in a Dale and Schuster [1928] perfusion apparatus. A cannula was placed in the pulmonary artery and another in the left auricle, and the lungs removed from the chest.

The lungs were washed through with defibrinated blood, especial care being taken to exclude any air bubbles. They were then attached to the perfusion pump and perfused with defibrinated blood. The lungs lay on a glass stage covering the microscope stage, and the microscope was

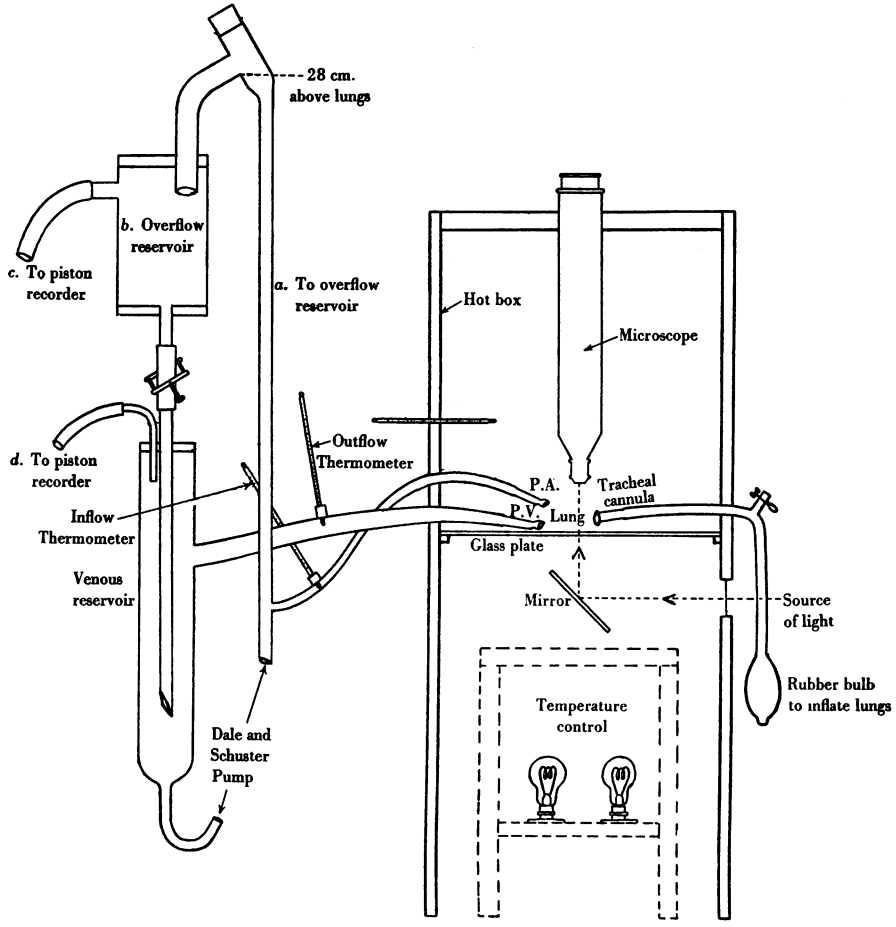

Fig. 1. Diagram of apparatus—see text.

enclosed in a hot box at 38° C. (Fig. 1). By this means the edges of all the lobes could be examined. The lungs themselves were covered in gauze moistened with warm saline, only the edge to be examined being exposed. In one series of experiments the lungs were perfused with a constant blood inflow and the pulmonary arterial pressure recorded (Table II). In a second series, the lungs were perfused at a constant pulmonary

TABLE II. Isolated cat lung perfused at constant blood inflow.

		Blood vessel under observation			
		Size of vessel	Effect upon		
Date	Dose γ	in μ	Calibre	Flow	P.A. pressure
			Acetyl-choline.		
15. iv. 32	1000	40	N.C.	+ +	Rise
15. iv. 32	1000	40	N.C.	+	Rise
			Adrenaline.		
9. iii. 32	1000	100	N.C.	− and rev.	N.C.
15. iv. 32	100	40	N.C.	+	Rise
22. iii. 32	200	80	N.C.	− and rev.	Not taken
17. v. 32	100	50	N.C.	− and rev.	Rise 3 cm. H_2O Fall 4 cm. H_2O
			Histamine.		
9. iii. 32	500	100	N.C.	+ and rev.	Rise 5 mm. Hg
18. iv. 32	500	30	? +	+ +	Rise
15. iv. 32	500	40	N.C.	+ +	Rise
15. iv. 32	100	40	N.C.	+	Rise
15. iv. 32	400	40	N.C.	+	Rise
19. iv. 32	500	40	N.C.	Sl. +	Rise 5 cm. H_2O
17. v. 32	100	40	N.C.	N.C.	Rise 5 cm. H_2O
17. v. 32	100	50	N.C.	− and stop	Rise 1 cm. H_2O Fall 3 cm. H_2O

arterial pressure with measurement of the inflow and outflow of blood. A modified form of the method used by Gaddum and Holtz was adopted in that an overflow side tube (Fig. 1, *a*) was attached to the inflow tube at a height of 28 cm. above the pulmonary artery. The pump was adjusted so that blood just trickled over into this side tube, the pressure therefore being maintained at 28 cm. of blood. The side tube was connected to a reservoir (*b*), the interior of which was in communication with a piston recorder (*c*); this recorder therefore registered a slowly rising line on the smoked paper. A diminution in inflow to the lungs caused an increase in the reservoir inflow, thus augmenting the upward slope of the volume recorder tracing; an increase in lung inflow caused a corresponding diminution in the slope of the recorder tracing. The bore of the side tube was wide to prevent any significant change in pulmonary arterial pressure with an alteration in blood flow. This method has been used by Berry and Daly [1931]. The venous outflow was recorded by connecting a piston recorder (*d*) to the venous reservoir. The total pulmonary changes were recorded simultaneously with observations on the vessel in the field of the microscope. Tracings were taken in every experiment given in Table III and those performed on November 30, 1932, and March 27, 1933, are shown in Figs. 2 and 3. The blood perfused consisted of the blood of the animal from which the lungs were

TABLE III. Isolated cat lung perfused at constant pressure.

Date	Dose γ	Blood vessel under observation: Size of vessel in μ	Effect upon: Calibre	Effect upon: Flow	Total pulmonary outflow
			Acetyl-choline.		
26. vii. 32	4	50	? +	N.C.	N.C.
26. vii. 32	4	Cap.	+	N.C.	+
16. xi. 32	10	62	N.C.	+ +	+
16. xi. 32	50	62	N.C.	+	+
16. xi. 32	100	62	N.C.	+	+
23. xi. 32	100	50	N.C.	+ to −	+ to −
23. xi. 32	100	50	N.C.	Immed. stop and rev.	+
25. xi. 32	5	50	? +	+ +	Sl. +
25. xi. 32	50	50	N.C.	Stop	− −
25. xi. 32	50	50	? −	Slow − rev.	−
26. xi. 32	50	10	N.C.	? +	−
26. xi. 32	5	50	N.C.	+ to −	+ to −
26. xi. 32	50	50	N.C.	N.C.	−
26. xi. 32	50	50	N.C.	+	−
26. xi. 32	50	50	N.C.	+ to −	+ to −
26. xi. 32	1	50	N.C.	+	+ to −
26. xi. 32	5	10	? +	+ to −	Sl. + to −
26. xi. 32	5	50	N.C.	N.C.	+ to −
30. xi. 32	100	50	N.C.	+	+
30. xi. 32	2 mg.	50	N.C.	+	+
30. xi. 32	3 mg.	42	N.C.	+ +	+
30. xi. 32	50	50	N.C.	+	+
30. xi. 32	50	50	N.C.	+	+
30. xi. 32	50	50	N.C.	+	+
30. xi. 32	5	50	? +	+	+
27. iii. 33	100	50	N.C.	N.C.	− −
27. iii. 33	100	50	N.C.	N.C.	−
			Adrenaline.		
26. vii. 32	5	Cap.	N.C.	N.C.	+
23. xi. 32	50	50	N.C.	N.C.	+
23. xi. 32	100	50	N.C.	Sl. −	Sl. −
30. xi. 32	12	50	N.C.	−	−
27. iii. 33	100	50	N.C.	−	N.C.
27. iii. 33	500	50	N.C.	N.C.	Sl. +
			Histamine.		
6. vii. 32	100	80	N.C.	−	−

removed, in some cases with gum saline added, and in a few experiments mixed with blood from another animal. Great variations were shown in different preparations in the time taken before the onset of œdema. In all experiments comparisons were only made in cases where the vessel could be seen clearly and the blood could be observed flowing through it at a good rate. Of the fields examined, any in which the blood stream was slow or stagnant were discarded. There could therefore be no doubt, whatever the state of other areas in the lung, that the one under obser-

vation had an active circulation. The arterioles were recognized by the relative thickness of their walls, together with the observation of the blood flowing from the larger into the smaller vessels.

The examination of most of the fields was made with a magnification of approximately 80 diameters. In certain experiments all lobes were tied off except the one under observation in an attempt to localize the action of the drug.

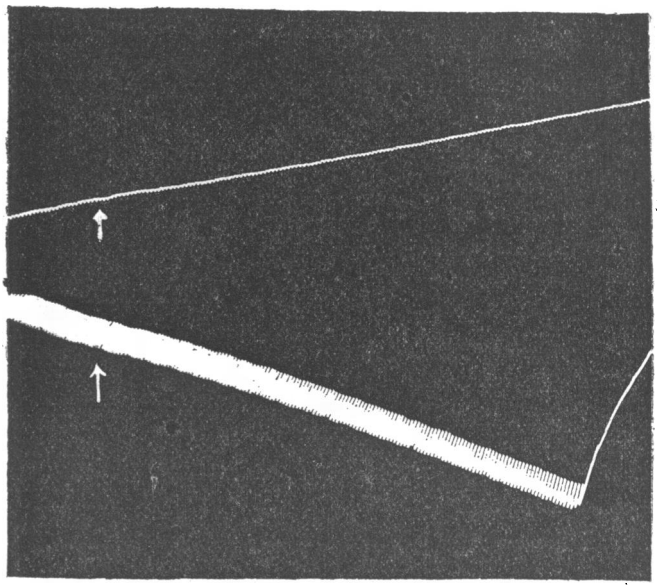

Fig. 2. (27. iii. 33.) Isolated perfused lungs at constant perfusion pressure. For interpretation of tracings see text. In this figure and in Fig. 3 read from left to right; the upper tracing = overflow reservoir, and lower tracing = venous reservoir. The tracings were taken simultaneously with the observation of the blood vessel under the microscope. At arrow adrenaline (1000γ) was injected. No change is shown on the tracings of the inflow or outflow; at the same time there was very marked slowing of the blood stream in the vessel under observation.

Discussion of results.

On the calibre of the vessels observed.

In twenty experiments with the lungs *in situ*, shown in Table I, where there was evidence of a vascular change taking place in the lung, either as a result of nerve stimulation or injection of a drug, there was no evidence of any alteration in calibre of a vessel observed at the edge of

the lung, except in four cases, two of which were doubtful. In the case where there appeared to be constriction on the injection of adrenaline, the vessel under observation was definitely larger than the average vessel which can be seen microscopically with any degree of clearness near the edge of the cat lung. Evidence is therefore against vessels of a calibre up to 100 μ being affected in calibre to any degree which is visible with the microscope during a vascular change in the lungs. That the change

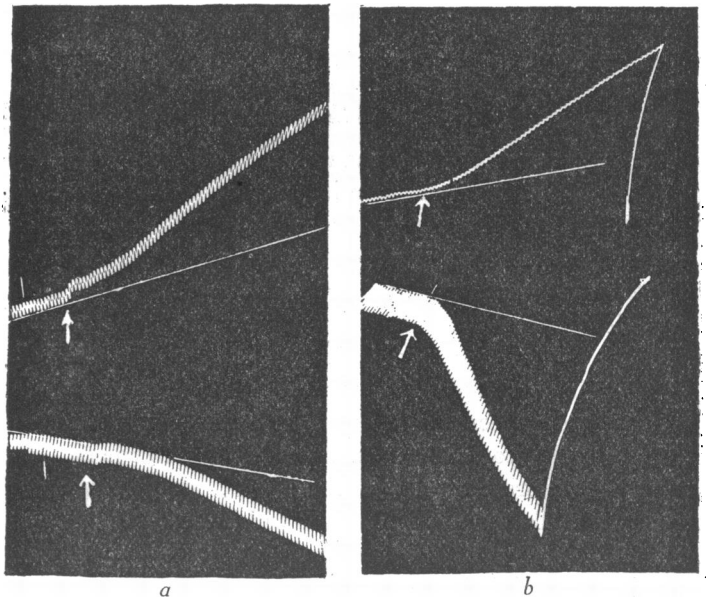

Fig. 3. Isolated perfused lungs at constant perfusion pressure. (*a*) (30. xi. 33.) At arrow adrenaline (12γ) was injected. Decrease in total pulmonary inflow and outflow with slowing of the blood stream in the vessel under observation. (*b*) (27. iii. 33.) At arrow acetyl-choline (50γ) was injected. Marked decrease in total pulmonary inflow and outflow with no change in flow in the vessel under observation.

takes place in larger and more central vessels which cannot be observed with the microscope is indicated, although not proved, by this absence of change in calibre in these vessels up to 100 μ, and by the calibre alteration taking place in the case of the one larger vessel observed. That a change too small to be detected may be taking place in the vessels under observation is a possibility that has to be considered.

In the isolated perfused lung (Tables II and III) the same results were found. Vessels of approximately the same calibre being chosen in each case. Whereas a marked change is shown to be taking place in the

vascular bed of the lung as indicated by the tracings and the alteration of flow in vessels under observation, there is no change in calibre seen except in five doubtful cases which could not be measured definitely on the micrometer scale. These findings fail to confirm the results of Hall [1925], who found a definite constriction of the vessels at the edge of the lung to take place after injection of adrenaline in rabbits and cats. In the intact preparation in the greater number of cases medinal was used after ether induction, and this might account for a difference found in the results [Daly, 1933], but in the isolated lung preparation no anæsthetic other than ether was used.

On the blood flow in the vessels observed.

In the entire animals marked changes in the rate, and in some cases the direction of blood flow were seen in the vessel under observation, on nerve stimulation or drug injection. On stimulation of the vagus (Table I) a slowing and, in some cases, a definite reversal of flow is found to be taking place in the vessel under observation. In the capillaries a cessation of flow and a breaking-up of the continuity of the cells were observed. This confirms the observations made by Hall [1925].

With adrenaline, changes in flow were also noticeable in the vessel under observation, a decrease or even a complete reversal being seen. Since it was desirable to eliminate the changes due to the cardiac factor the isolated lungs were perfused by the method already described with the results seen in Tables II and III. It will be seen that this affords a means of observing the total change taking place in the pulmonary vascular bed, so that it can be correlated with the changes noted in the vessel under observation. Were the same change to take place equally in all areas of the lung on injection of a drug so as to cause either an increase or a decrease in the blood flow through the lung vessels, the change observed locally by the microscope should agree with the general change recorded by the inflow and outflow of the lung vessels. Thus, on an increase in total inflow and outflow, *i.e.* on increased flow of blood through the pulmonary vascular system, an increase in the rate of flow would be expected in the vessel observed locally, and with a decreased flow a decrease in rate would be expected in the vessel observed. An examination of the results of experiments on the isolated lung summarized in Tables II and III shows that whilst in most cases the local change is similar to the general one, there are several examples of a general alteration in flow with no local one and of a general alteration of flow with the reverse effect taking place locally.

An analysis of the series of readings in Table III where a vascular change has been induced by injection of acetyl-choline, adrenaline or histamine shows the following variations:

Change in total pulmonary circulation—blood flow	Rate of flow in observed vessel	No. of observations recorded
Increase	Increase	17
Increase	No change	4
Increase	Decrease	1
Decrease	Decrease	7
Decrease	No change	4
Decrease	Increase	2

In both the intact and isolated lungs there are examples of complete reversal of flow sometimes lasting for over a minute after the injection, and whilst this reversal of flow usually takes place with a slowing of the

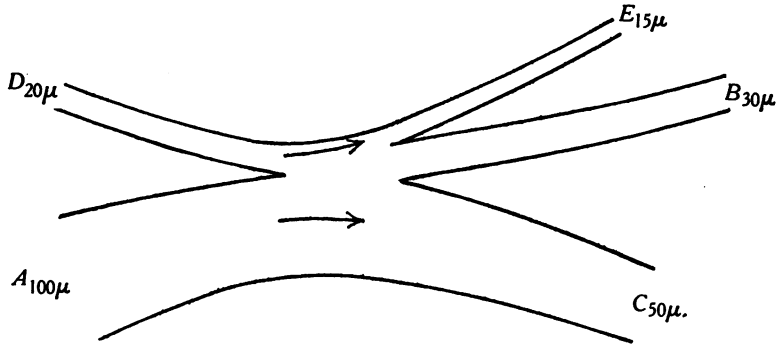

Fig. 4. Diagram of blood vessels seen under the microscope; the arrows indicate the direction of blood flow at the commencement of the observation. The effect of an adrenaline injection on the flow is described in the text.

rate of flow in the vessel, there is at least one observation of a sudden reversal of flow at a marked increased rate following injection of histamine in the intact animal. This anomaly between the local and general flow (Figs. 2, 3) shows that, whatever the alteration in the general vascular flow in the lung may be, it is not referred to all parts of the vascular bed, and in some areas the reverse effect to the general one is taking place. The underlying causes, both physical and physiological, are too numerous for any conclusions to be made at this stage of the work; moreover, the changes in blood flow in the vessels under observation are sometimes extremely complex. An example of this is shown in an experiment (Table II, 17. v. 32), where the following changes were observed taking place. Half a minute after the injection of adrenaline (100γ) the blood-pressure rose 3 cm. (H_2O) followed by a gradual fall, which

continued until 2 min. after the injection, when it had fallen 4 cm. (H_2O) below the initial value. Observations on the blood vessels in the area during this time showed a marked slowing of the stream flowing from *A* to *C*, which had been flowing rapidly at the commencement of the observation, although pulsation was still present in the vessel walls. Complete cessation of flow from *A* to *C* took place 3 min. after the injection, and as soon as this happened the flow, which had continued from *D* to *E*, suddenly reversed its direction so as to flow from *E* to *D*. The blood in *B* gave only oscillatory movements during the whole of the observation. After some time the blood flow recommenced from *A* to *C* and the direction in *D* and *E* returned to normal. These changes are no doubt due to more than one mechanism, but it is worthy of note that the possibility of a Venturi action being responsible has already been considered [Swindle, 1930; Berry and Daly, 1932]. This also raises the question of the drug not reaching all areas with equal facility and its action on different parts being unequal owing to a difference in initial state of tone of the vessels in different areas. This is borne out in the work of Cohnheim and Litten [1875] and later by Toyama [1925] who found a variation in the degree of ease with which dyes injected into the pulmonary vessels penetrated different vascular areas of the lung. In an isolated experiment (January 5, 1933) on the entire animal where the lung capillaries were examined beneath a high power, it was found on injection of adrenaline (2 mg.) that there was an appreciable increase in the number of white cells passing through the capillaries.

The effects on the total pulmonary flow from the drugs used are shown by the tracings of the inflow and outflow in the isolated lungs: those due to adrenaline show dilatation or constriction not related to the dose. Histamine causes constriction, and in any given experiment acetyl choline shows dilatation with small doses but with larger doses constriction alone, or dilatation followed by constriction. This confirms the work of Gaddum and Holtz [1933].

Summary.

The vessels at the edge of the lung were studied beneath the microscope, using transillumination both in the animal with the chest open and the isolated perfused lungs. No appreciable change in calibre of arterioles of a diameter up to 100 μ was observed on injection of adrenaline, acetyl-choline or histamine, or by nerve stimulation, although these procedures frequently brought about an appreciable alteration in the total pulmonary blood inflow and outflow.

A definite alteration in rate, and in some cases in direction of blood flow, was noted and their significance in their correlation with the changes in the total vascular bed is discussed.

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