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THE LOCALIZATION OF THE ACTION OF DRUGS ON THE PULMONARY VESSELS OF DOGS AND CATS.

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Since previous studies of the effect of drugs on the pulmonary vessels have been reviewed by Wiggers [1921], Tigerstedt [1923] and Daly [1932], it is not necessary for us to present a connected historical survey. We will discuss earlier work only in so far as it bears directly on our own experiments, which were devised with the chief object of obtaining information regarding the site of action on the pulmonary circulation of various pharmacologically active substances which are found in tissue extracts.

In early work on this subject the assumption was usually made that any observed effects of drugs on the resistance of the pulmonary vessels to the flow of blood were due to an action on the pulmonary arterioles. The suggestion that changes in the tone of the pulmonary veins might appreciably affect the resistance originated partly from the study of the action of drugs on isolated veins [Inchley, 1923, 1926; Franklin, 1932, who gives other references], and partly from observations of the lung volume when the circulation was intact [Luisada, 1928; Mautner and Pick, 1929]. Mautner and Pick have shown that the blood flow through the portal system of the dog is much influenced by the resistance offered by the hepatic veins under the action of histamine, and they give evidence suggesting that histamine also causes the pulmonary veins to constrict.

The conclusions of Mautner and Pick regarding the portal circulation have been confirmed and extended in an investigation carried out in this laboratory [Bauer, Dale, Poulsson and Richards, 1932]. The present work was undertaken with the object of applying similar methods to the study of the behaviour of the pulmonary vessels. It was hoped that fresh light might be thrown on the importance of the pulmonary veins by

recording the changes in the lung volume as well as other effects during perfusion.

The most widely used method of studying the effect of drugs on the pulmonary vessels is that introduced by Brodie and Dixon [1904], in which the lungs are perfused with blood under constant pressure and the effect on the outflow is recorded. Under these conditions the effect of a vaso-constrictor drug is seen in a diminution of outflow, and some information regarding the main site of the effect may be obtained by recording the volume of the lung. If the effect is predominantly on the inflow to the lung the volume of the lung will be diminished; if it is predominantly on the outflow the volume will be increased. Analogous conclusions may also be drawn regarding the action of vaso-dilator drugs.

In another less commonly used method of perfusing the lungs, the pulmonary artery is connected directly to the perfusion pump, so that the rate of flow is approximately constant and the effects of the drugs are seen in changes in the pressure in the pulmonary artery. If the pump were completely rigid these changes of pressure would not affect its output. Under these conditions, a vaso-motor drug would have practically no effect on the volume of the lung, except in so far as it acted on the veins.

The apparatus which we have used was so devised that the perfusion could be carried out either under conditions of approximately constant pressure, or under conditions of approximately constant flow. In the former case, changes in the volume of the lung were partly due to changes in the resistance to inflow and partly due to changes in the resistance to outflow. In the latter, the effect on the volume of changes in the resistance to inflow was largely eliminated, and effects due to changes in the resistance to outflow were better shown. By changing repeatedly from one method of perfusion to the other, it has been possible further to test the conclusions suggested by the observed effects of drugs. The two methods of perfusion are easily distinguished in the figures, since when the pump is connected directly to the artery the record of the inflow pressure shows large oscillations with the rhythm of the pump, which are absent when the other method of perfusion is used.

The effects shown in our records of lung volume are presumably due to changes in the volume of blood in the capillaries, and it is possible that they are caused in some cases by changes in the tone of these vessels, though we know of no evidence that such changes can occur in the lungs. Constriction of the capillaries would produce an increase in resistance and a decrease in the lung volume, and would thus be indistinguishable in our experiments from constriction of the arterioles, except in so far as the

constriction of capillaries might cause a transient increase in outflow owing to their large blood capacity. When such a combination of effects has occurred, we have been unable to decide whether the effect should be attributed to an action on the arterioles or on the capillaries, and we have been content to consider the two kinds of vessel together and to attribute such effects to an action on the "inflow."

Methods.

The animals (dogs and cats) were anæsthetized with ether and bled through the carotid artery. The blood was either defibrinated or collected in a bowl containing heparine. In experiments with cats, about 50 c.c. of Ringer's solution were usually injected into the jugular vein, so that sufficient blood to fill the apparatus (at least 120 c.c.) was obtained from a single animal. The lungs were then removed from the animal, the arterial cannula was inserted in the pulmonary artery through the wall of the right ventricle, and the venous cannula was inserted through the left auricular appendix as deeply as possible without obstructing the flow, so as to reduce to a minimum possible passive changes in the volume of the auricle. A thread was tied round the heart at the base of the ventricles, in order to prevent blood from escaping from the left auricle through the mitral valve. The bronchial vessels were not perfused.

The lungs were enclosed in the plethysmograph described by Bauer, Dale, Poulsson and Richards [1932], and their volume was recorded by means of a Brodie bellows [1902] attached to this plethysmograph. A tube, which was tied into the trachea, passed to the outside of the plethysmograph and was closed with a clip. It is known that the distension of the lungs by negative pressure applied to the pleural surface decreases the resistance of the pulmonary vascular bed [Daly, 1930], but if the distension is produced by a positive pressure in the trachea the resistance may be increased. As it was desirable to reduce the proportion of the resistance due to simple mechanical factors, the lungs were perfused in an almost completely deflated condition.

Conditions resembling more closely those occurring in the intact animal would have been obtained if the lungs had been rhythmically inflated during the experiment. Under these conditions, however, it would have been difficult to distinguish effects on the average volume due to constriction of the bronchi from those due to changes in the blood content of the lungs. We believe that, under the conditions we have chosen, it is unlikely that changes in the tone of the bronchi will affect the record.

In order to convince ourselves that the changes which we were recording did really represent changes in the blood content of the lung, we have performed a control experiment in which we used an elegant method introduced by Daly [1930]. Daly recorded changes in the volume of blood in perfused lungs by recording the corresponding changes in the volume of blood outside the lungs in the apparatus. In most of our experiments we did not use this method because of the difficulty of combining it with a record of the outflow. In our control experiment, the pulmonary artery of a dog was connected directly to the pump and the outflow was not recorded. The plethysmographic record was compared with the record of the volume of blood in the venous reservoir. It was found that occlusion of the inflow or outflow, or the injection of adrenaline histamine or acetylcholine, produced similar but opposite, immediate effects on the two records. The plethysmographic record was slightly less sensitive than the other record and was more liable to show changes unconnected with the injection of drugs, which were probably due to small changes in the temperature of the plethysmograph. We concluded that records obtained in this way would give us a reliable indication of the main site of action of drugs, and enable us to record the rate of outflow from the veins as well as the volume of blood in the lung.

A bath containing water thermostatically kept at 40° was placed about 15–25 cm. above the level of the lungs. This bath contained a spiral glass coil, a pump [Dale and Schuster, 1928] and an arterial reservoir for blood (Fig. 1). Blood from the venous reservoir first passed through the coil and then the pump. After leaving the pump it could be shunted by means of artery forceps applied to rubber connections, so that it either passed directly into the pulmonary artery or else into the arterial reservoir and thence to the pulmonary artery. The level of the blood in the arterial reservoir was kept constant by means of an overflow tube to the venous reservoir. A vertical glass tube was connected to the arterial supply by means of a T-piece. The height to which the blood rose in this tube gave a measure of the arterial pressure, and this was recorded by means of a small Brodie bellows connected to the upper end of the vertical tube. The venous outflow was recorded by the method described by Gaddum [1929], and the blood was collected in a venous reservoir which was open to the air. The outflow recorder was at about the same level as the auricle.

The apparatus was originally designed for use with cats, and our records show that when cats' lungs were perfused the apparatus was effective in maintaining either the perfusion pressure, or the rate of flow,

constant. When the same apparatus was used to perfuse dogs' lungs, it was found to be too small. On the one hand, the pump was not large enough to maintain so great a flow constant against varying pressures, and on the other, the tubing between the arterial reservoir and the cannula was not wide enough to ensure that the pressure in the cannula was quite independent of the rate of flow. Thus, in Fig. 5 it will be seen that hista-

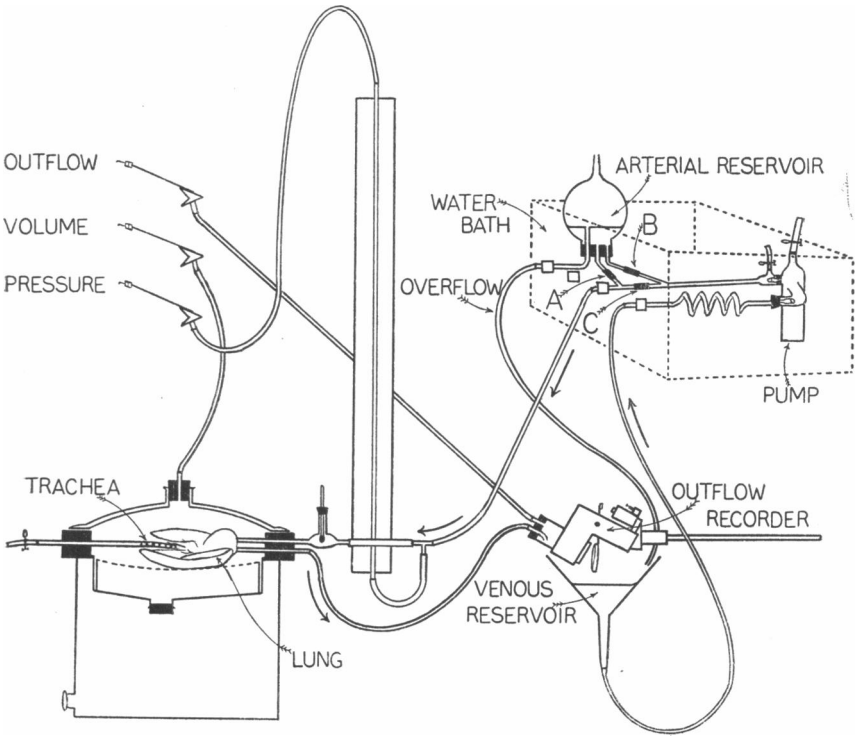

Fig. 1. When *A* and *B* are clamped with artery forceps, and *C* is open, the artery is connected directly to the pump. When *C* is clamped, and *A* and *B* are open, the perfusion pressure is approximately constant.

mine produced a small rise of perfusion pressure and a small fall of outflow even when the blood was so shunted as to maintain these quantities as nearly as possible constant. If a larger apparatus had been used, it would probably have been possible to demonstrate a more striking contrast between the two methods of perfusion in experiments on the dogs' lungs, but since the contrast was already sufficiently definite for our purpose we did not construct another apparatus. In some cases, when the dog was a large one, we have tied off one lung at its hilum and perfused the other.

Two types of difficulty were encountered in experiments with cats. In the first place, it sometimes happened that the vessels rapidly acquired such a marked tone that the blood almost ceased to flow. In such cases, the addition of adrenaline to the blood was found to relax the tone and to allow the experiment to be completed [cf. Löhr, 1923]. In the second place, the plethysmographic record frequently showed a steady increase in lung volume, which was so marked as to prevent records being taken until more than an hour after the perfusion started. This change was apparently due to the use of defibrinated blood. When heparine was used, the lung reached a steady volume much more rapidly. These difficulties were not encountered in experiments on dogs' lungs, and in this case no difference was noticed between the results obtained with defibrinated and heparinized blood.

The drugs were injected into the rubber tubing leading to the arterial cannula. The volume of fluid injected was between 0·05 and 0·2 c.c. The solution of adrenaline used in most of the experiments contained chloretone, but a few control experiments showed that all the effects recorded were also produced by a sample of the base prepared synthetically and dissolved by the aid of HCl. Histamine was used in the form of the acid phosphate, but the doses are given in terms of the base. The doses of all other substances are given in terms of the total weight of the salt used to prepare the solutions for injection.

We wish to express our thanks to Dr K. Lohmann who very kindly supplied us with the specimens of muscle adenylic acid and adenylpyrophosphate which we have used. The latter was in the form of the barium salt. This was dissolved with the aid of a small quantity of HCl. An excess of sodium sulphate was added to precipitate the barium and the solution was then centrifuged. The supernatant fluid was neutralized before use with NaOH.

Results.

Adrenaline.

Dogs. In confirmation of much previous work, we find that adrenaline normally increases the resistance of the dog's pulmonary vessels to the flow of the blood. This effect is accompanied, as shown in Fig. 2 *a*, by a fall in the lung volume, and is thus mainly due to the constriction of the inflow. This conclusion is confirmed by the fact, shown in Fig. 2 *b*, that when the rate of inflow is made more nearly constant by connecting the pulmonary artery directly to the pump the effect on the volume is diminished. In two experiments out of ten, the substitution of a direct

perfusion by the pump for the perfusion under constant pressure actually caused a reversal of the effect on the volume, which was now increased under the action of adrenaline (cf. Fig. 5). Though the main action of adrenaline in this experiment was on the inflow, the drug appeared therefore to have also a constrictor action on the outflow.

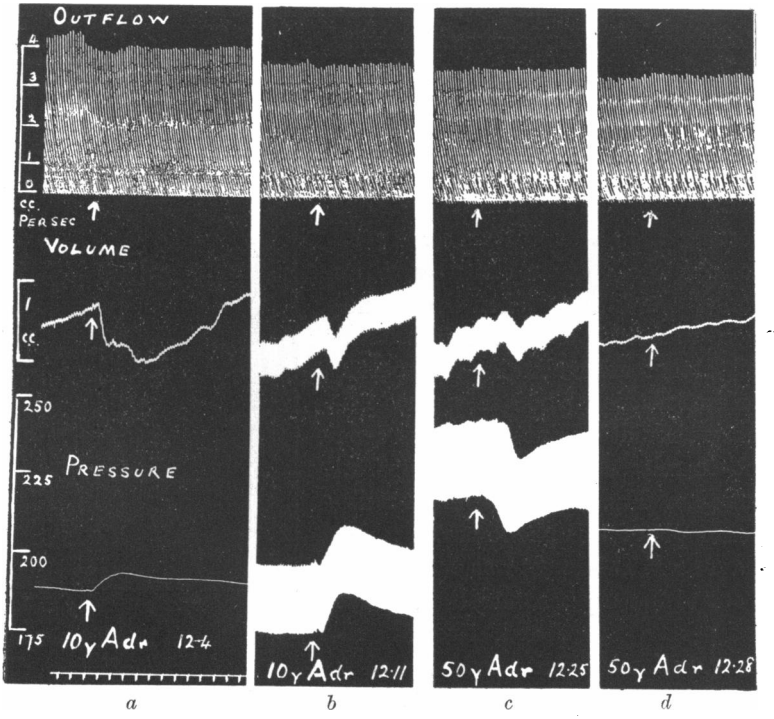

Fig. 2. Dog. Adrenaline (+chloretone). Outflow. Lung volume. Perfusion pressure (mm. of blood). At 12.16, 1 mg. of ergotoxine ethane sulphonate was added to the blood in the venous reservoir.

Fig. 3 shows another effect which adrenaline sometimes produced. In this record, the second injection of 25γ of adrenaline is seen to cause a slight rise in the perfusion pressure. This would not be sufficient appreciably to affect the output of the pump, but the effect, if any, would be to cause a diminution of the rate of flow of blood into the lungs. Nevertheless, the rate of outflow was definitely increased. Under these circumstances the effect on the volume of the lung was inevitably a fall. This increase of outflow was clearly shown when the pump was connected

directly to the pulmonary artery (Fig. 3 *b*), but not when the lungs were perfused at constant pressure (Fig. 3 *a*). This was presumably because the rate of inflow fell in the latter case, owing to increase of the inflow resistance, while it was maintained practically constant in the former case. Adrenaline certainly produced a restriction of the inflow in this experiment, but the record of the rate of outflow shows that it had other effects as well. We do not know whether this increase of outflow was due to dilatation of the pulmonary veins letting the blood flow out, or to constriction of the capillaries forcing it out.

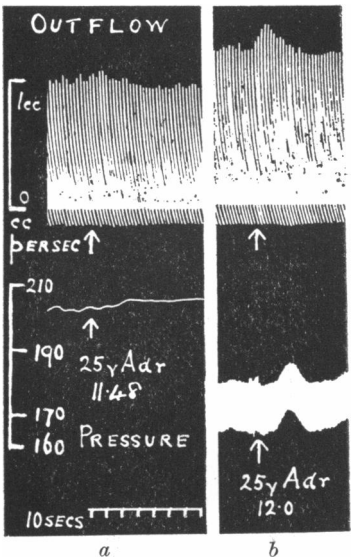

Fig. 3. Dog. Adrenaline (chloretone-free). Outflow. Perfusion pressure (mm. of blood).

The effect which has just been described is the only evidence we have obtained which might be interpreted as indicating that a drug was having an action on the outflow contrary to its action on the inflow. When this phenomenon was first observed, it seemed possible that adrenaline had a specific acion in releasing blood from the pulmonary circulation, just as it has been found to release blood from the portal circulation. Fig. 3 shows the most striking example of this phenomenon which we have seen. This effect was only seen in five out of ten experiments, and, as has already been mentioned, two experiments provided definite evidence that adrenaline was constricting the pulmonary veins. We have not been able to determine what factors govern the direction of the action of adrenaline on the pulmonary veins.

The constrictor effect of adrenaline is reversed by ergotoxine. In the experiment shown in Fig. 2, 1 mg. of ergotoxine ethane sulphonate was added at 12.16 to the blood in the venous reservoir. This produced a reversal of the effect of adrenaline on the perfusion pressure, while the direction of the effect on the volume was unaltered. The main effect of this dose of adrenaline was now dilator, and, since it still produced a fall in the lung volume, the effect was mainly on the outflow. This conclusion is supported by the fact that, after ergotoxine, the effect on the volume was best shown when the lungs were connected directly to the pump, so that the rate of flow of blood into the lungs was kept nearly constant.

When the lung was perfused at constant pressure, the increase in the rate of outflow, which was slight but just visible on the outflow record, was compensated by an increased rate of inflow, so that no effect on the volume was produced. When the lungs were connected directly to the pump, this increase in the rate of inflow could not occur.

In another experiment the vaso-dilator action of adrenaline after ergotoxine was accompanied by an increase in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure. In this experiment the dilatation must have been mostly due to an effect on the inflow.

Cats. It is well known that adrenaline may, under different conditions, produce either constriction or dilatation of the pulmonary vessels of the cat. The factors controlling the direction of the effect have been studied by Tribe [1914], and we are able, in general, to confirm her conclusions. In the early part of the experiments, the injection of small doses of adrenaline has invariably caused a large and prolonged vaso-dilatation. This effect is shown in Fig. 4. When the lungs were perfused in this experiment at an approximately constant pressure (Fig. 4 *a*), the injection of 2γ of chloretone-free adrenaline caused an increase in the rate of outflow and no effect on the volume of the lung. When the artery was connected directly to the pump (Fig. 4 *b*), the same injection caused a fall in the perfusion pressure and a small fall in lung volume. The record of outflow shows that the change in the perfusion pressure was not sufficient appreciably to affect the output of the pump. It is clear from the effect of this second injection that adrenaline may produce dilatation of the pulmonary venules in the cat. The fact that adrenaline had no effect on the volume when the perfusion was carried out at constant pressure shows that the pulmonary arterioles were also dilated, and that the increase in the rate of outflow from the lungs was in that case exactly compensated by an increase in the rate of inflow. When the artery was connected directly to the pump, no such increase in the rate of inflow could occur, and the volume of the lungs accordingly fell. Results similar to those shown in Fig. 4 have usually been obtained, but, in some experiments in which adrenaline produced similar evidence of vaso-dilatation by both methods of perfusion, it had no definite effect on the volume of the lung, even when the artery was connected directly to the pump.

In our first experiments, carried out in October, the vaso-dilator action of adrenaline was only seen in the early part of the experiment. The injection of a large dose of adrenaline at this stage caused a very large dilatation. In one experiment, for example, the injection of 0·1 mg. of

adrenaline increased the flow to four or five times its original value, and the vessels never recovered their original tone. In these first experiments, about an hour after the perfusion had started, the injection of small doses of adrenaline ceased to have any effect, and the injection of larger doses (20γ–200γ) caused vaso-constriction. In later experiments, carried out in March, the vaso-dilator action was much more persistent, and, in order to obtain vaso-constriction, it was necessary to use chloretone-free adrenaline,

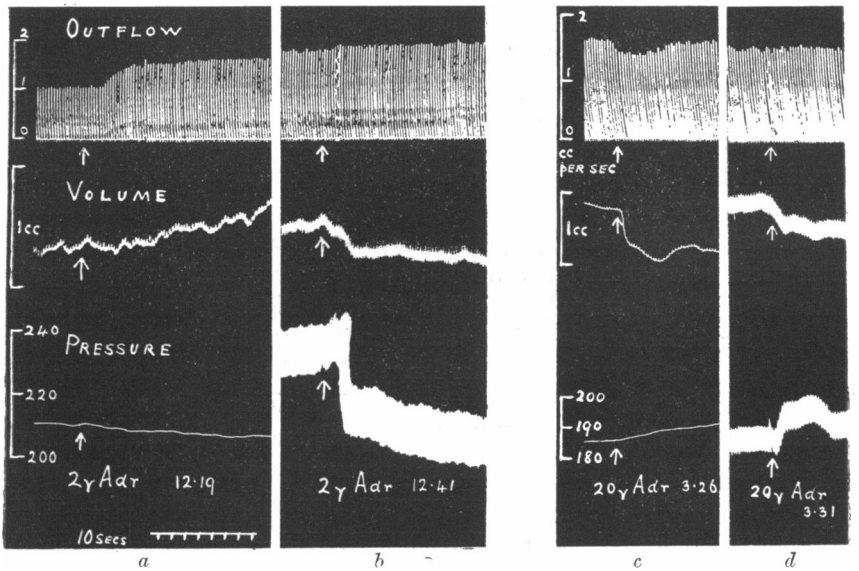

Fig. 4. Cat. Adrenaline (chloretone-free) in two different experiments. Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

so that the constrictor effect of the adrenaline should not be masked by the feeble dilator effect of the chloretone. Fig. 4 *c*, *d* show that this action of adrenaline was mostly due to constriction of the inflow. It was accompanied by a fall in the volume of the lung, which was most marked when the perfusion was carried out at constant pressure (Fig. 4 *c*). When the artery was connected directly to the pump the rate of inflow was artificially maintained almost constant and comparatively little change in lung volume occurred (Fig. 4 *d*). It is possible that the small decrease in volume which did occur under these conditions was partly due to dilatation of the venules.

Histamine.

In their original description of the effect of histamine in increasing the resistance of the pulmonary vessels, Dale and Laidlaw [1910] assumed that the effect was due to an action of the drug on the pulmonary arterioles. Inchley [1923], however, devised a method of perfusing arteries and veins separately with Ringer's solution, and showed that they were both constricted by histamine. Later [Inchley, 1926] he found that isolated

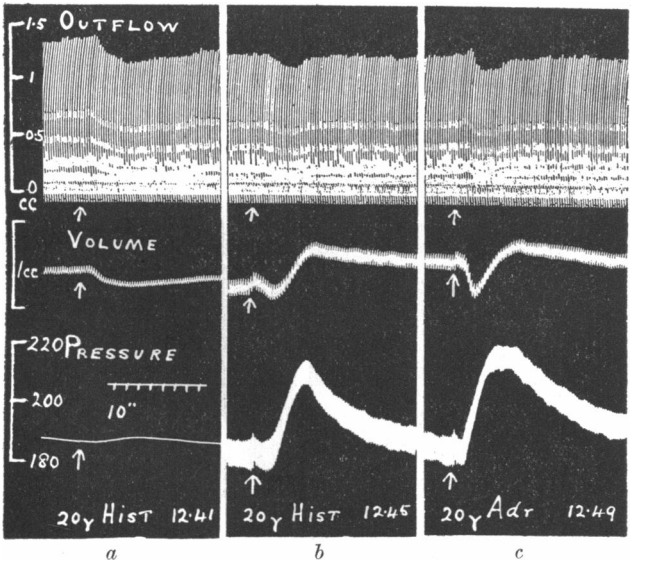

Fig. 5. Dog. Histamine and adrenaline (chloretone-free). Outflow (c.c. per sec.). Lung volume. Perfusion pressure (mm. of blood).

veins in general, and the pulmonary veins in particular, were more sensitive to histamine than isolated arteries, and concluded that the effects of histamine were mainly due to an action on the veins. This view was also held by Mautner [1923] and was supported by experiments in which the lung volume of dogs with intact circulation was recorded, together with the pressures in the pulmonary artery and in the left auricle [Luisada, 1928; Mautner and Pick, 1929].

In our experiments, histamine has invariably produced vaso-constriction, both in cats and dogs. When the perfusion was carried out at constant pressure, the fall in blood flow was usually accompanied by a fall in lung volume, and was thus mainly due to constriction of the inflow.

When the artery was connected directly to the pump, this fall in lung volume was followed by a rise which was probably due to the constriction of the outflow. These effects are shown in Fig. 5, taken from an experiment on a dog, and in Fig. 6, taken from an experiment on a cat. In some experiments, larger doses of histamine produced a very large and prolonged rise in lung volume. This effect was not reversible and was probably due to œdema. In the experiments from which tracings are given, the volume

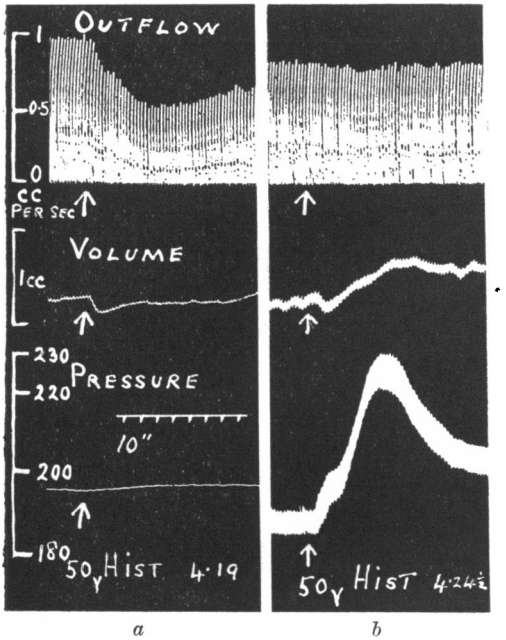

Fig. 6. Cat. Histamine. Outflow. Lung volume. Perfusion pressure (mm. of blood).

showed a tendency to return to its original level, and, though it is possible that histamine caused some œdema in these experiments, the rise in volume cannot have been entirely due to this, and must have been caused, at least in part, by some more easily reversible change.

In Fig. 6 *b* the conclusion that histamine produced constriction of two separate structures is confirmed by the double nature of the rise in perfusion pressure. The first effect of the drug was constriction of the inflow, which caused a rise of perfusion pressure and a small fall of volume. This was rapidly followed by constriction of the outflow, which caused a further rise of perfusion pressure and a simultaneous rise in lung volume. In the

same way the record in Fig. 6 *a* which shows the rate of outflow when the lung was perfused at constant pressure also gives an indication that the drug was acting on two separate structures.

Our results, which were based on experimental procedures which were, in some respects, less drastic than Inchley's, confirm Inchley's main conclusion that histamine constricts both inflow and outflow. These two actions tend to produce contrary effects on the volume and the observed changes in volume are, therefore, small. Under the conditions of our experiments there was no marked and constant difference between the sensitivity of the two kinds of vessels, though in some experiments the arteries appeared to be rather more sensitive than the veins. In this respect our findings differ from those of Inchley.

Acetylcholine.

Acetylcholine has been found to produce vaso-constriction in the pulmonary vessels of rabbits [Antoniazzi, 1931; Euler, 1932] and cats [Hirose, 1932]. In their experiments on dogs Daly and Euler [1932] found that acetylcholine sometimes produced vaso-constriction and sometimes vaso-dilatation. Franklin [1932] found that acetylcholine dilated the dog's pulmonary arteries and constricted the veins. We have found that cats are more sensitive to acetylcholine than dogs.

Cats. The result of a typical experiment is shown in Fig. 7. The outflow records are not included in this figure or in Fig. 8 because they showed no changes. It will be seen that small doses (1–3γ) produced vaso-dilatation, and had no definite effect on the lung volume. After a larger dose (20γ) the initial vaso-dilatation was followed by vaso-constriction and a rise in lung volume. This shows that the pulmonary veins were constricted. In another experiment, the result of which has not been reproduced and in which, when the pump was connected directly to the arterial cannula, acetylcholine (10γ) caused a rise of perfusion pressure and lung volume rather larger than that shown in Fig. 6, it was found that when the perfusion was carried out at constant pressure the same dose caused a diminution of flow and a small and indefinite fall in the volume of the lung. From these results we concluded that these doses of acetylcholine produced constriction of both inflow and outflow in the cat. When the inflow was kept practically constant by the pump, constriction of the outflow caused the rise in lung volume shown in Fig. 7, but, when the perfusion was carried out at constant pressure, the rates of inflow and outflow were both diminished and there was practically no effect on the volume. These effects were all abolished by atropine.

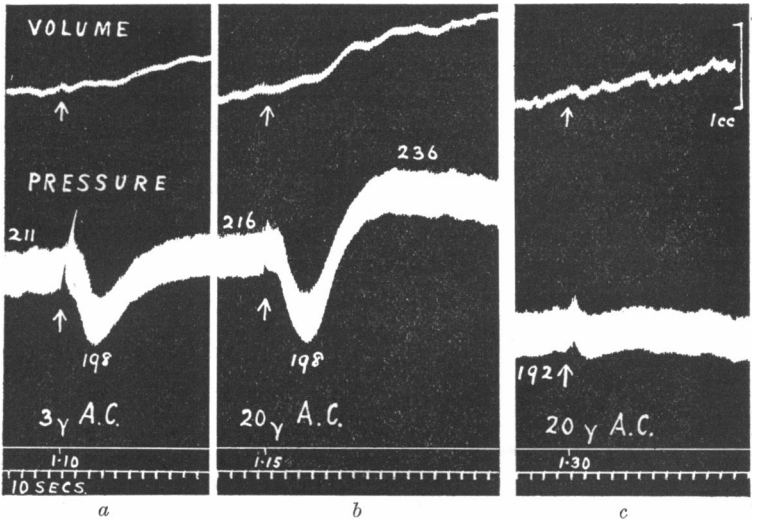

Fig. 7. Cat. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood). At 1.22 1 mg. of atropine sulphate was added to the blood in the venous reservoir.

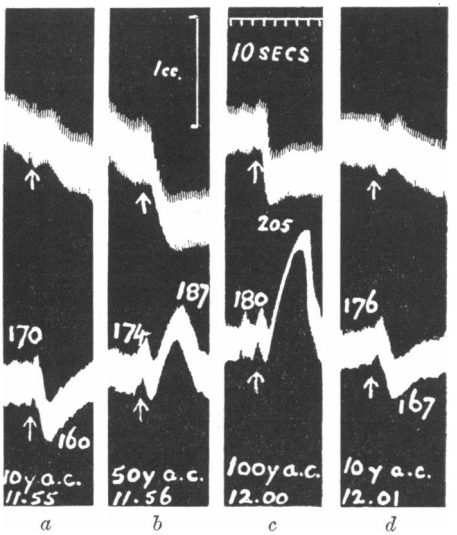

Fig. 8. Dog. Acetylcholine. Lung volume. Perfusion pressure (mm. of blood).

Dogs. In the experiment shown in Fig. 8 the effects of acetylcholine on the perfusion pressure in a dog's pulmonary artery were similar to the effects seen in experiments with cats, though the doses used were larger. A small dose (10γ, Fig. 8 *a*) caused vaso-dilatation. After larger doses the initial dilatation was followed by vaso-constriction. The dilator action of small doses was not seen in some experiments, but the constrictor action of large doses was always present. The vaso-dilatation was accompanied by a very small increase in lung volume, but, in contrast to the effects seen in cats, the vaso-constriction was always accompanied by a fall in lung volume, even when the pump was connected directly to the pulmonary artery as in the experiment shown in Fig. 8. These effects were increased by eserine and abolished by atropine. In one experiment, eserine converted a vaso-dilator effect into a vaso-constrictor one. This action was presumably due to an increase of the effective concentration of acetylcholine.

It is doubtful whether significance should be attached to the very small rise in lung volume shown in Fig. 8 *a*, *d* after the injection of 10γ of acetylcholine. The direction of this change would indicate that the dilator action of the drug was on the inflow. We can, however, be more confident that large doses of acetylcholine constrict the inflow and have no significant action on the outflow.

Franklin [1932] has recently published an account of the action of acetylcholine on the dog's pulmonary vessels. He found that this drug invariably relaxed isolated rings of pulmonary artery and constricted isolated rings of pulmonary vein, and deduced that it would produce a rise of lung volume. The small dilatation of the lung which followed the injection of 10γ of acetylcholine in the experiment shown in Fig. 8 *a*, *d* is probably the effect which Franklin predicted. It is not difficult to suggest possible explanations of the fact that he did not observe the other more marked effect of larger doses. Franklin's experiments were confined to the trunks of the vessels outside the lungs, and it is improbable that changes in the tone of these large vessels can have much influence on the blood flow. It is possible that acetylcholine has an effect on the small vessels which does not occur in the large ones. Our own failure to obtain evidence of a constrictor action of acetylcholine on the veins was probably due partly to the fact that the effects of such an action would be masked in our experiments by the increase of the inflow resistance, and partly to the fact that the acetylcholine would be mostly destroyed before it reached the veins. In any case acetylcholine was the only drug which never produced any considerable rise in the dog's lung volume in our

experiments, and we consider that it is improbable that the action of acetylcholine on the pulmonary vessels can cause the lungs to dilate appreciably in the whole dog.

Adenosine and its compounds.

Adenosine and its compounds have been shown to be present in a large number of tissues. Some of their pharmacological effects have been studied by Drury and Szent-Györgyi [1929], Bennet and Drury [1931], Zipf [1931], Ostern and Parnas [1932], Deuticke [1932] and Drury [1932]. Zipf has drawn attention to the fact that they are probably responsible for those effects of defibrinated blood which Freund [1920] attributed to the "Frühgift."

We have studied the effects on the pulmonary vessels of adenosine, muscle adenylic acid and adenylpyrophosphate. The latter substance was first isolated from voluntary muscle [Lohmann, 1931]. The effects of all these three substances in our experiments were qualitatively the same, and were in many ways similar to those of acetylcholine. The cat's vessels were much more sensitive than the dog's vessels.

In experiments with cats small doses produced vaso-dilatation, and when the dose was increased the initial dilatation was followed by vaso-constriction. The dilatation was not accompanied by any change in the volume of the lung or outflow and its site could, therefore, not be identified (Fig. 9 *a*, *b*). Fig. 9 *c* shows that, when the lung was perfused at approximately constant pressure, the injection of 0·25 mg. of adenylpyrophosphate caused vaso-constriction, accompanied by a fall in lung volume. This effect must have been due to an action mainly on the inflow. On the other hand, when the pump was connected directly to the artery the initial fall in lung volume was followed by a large and irreversible increase. This second effect may have been due either to prolonged constriction of the outflow or to œdema.

The effects of muscle adenylic acid and of adenosine were indistinguishable from one another when the substances were given in equimolecular doses. Both these substances were, however, much weaker in their action than adenylpyrophosphate. In Fig. 9 *a*, *b* it will be seen that 0·005 mg. of adenosine produced less dilator effect than a dose of adenylpyrophosphate corresponding to 0·005 mg. of its barium salt. Since the ratio of the molecular weights of these substances is 3·3, this result indicates that the addition of the extra phosphate groups to the molecule of adenosine increased its activity more than 3·3 times. In another experiment the constrictor effects of larger doses were compared,

and it was found that each molecule of adenylpyrophosphate was about equivalent in this action to fifteen molecules of adenosine. Since adenylic acid is not more active than adenosine, it is the addition of the pyrophosphate group which leads to the increase of activity. It was, however, found in one experiment that sodium pyrophosphate itself had no action

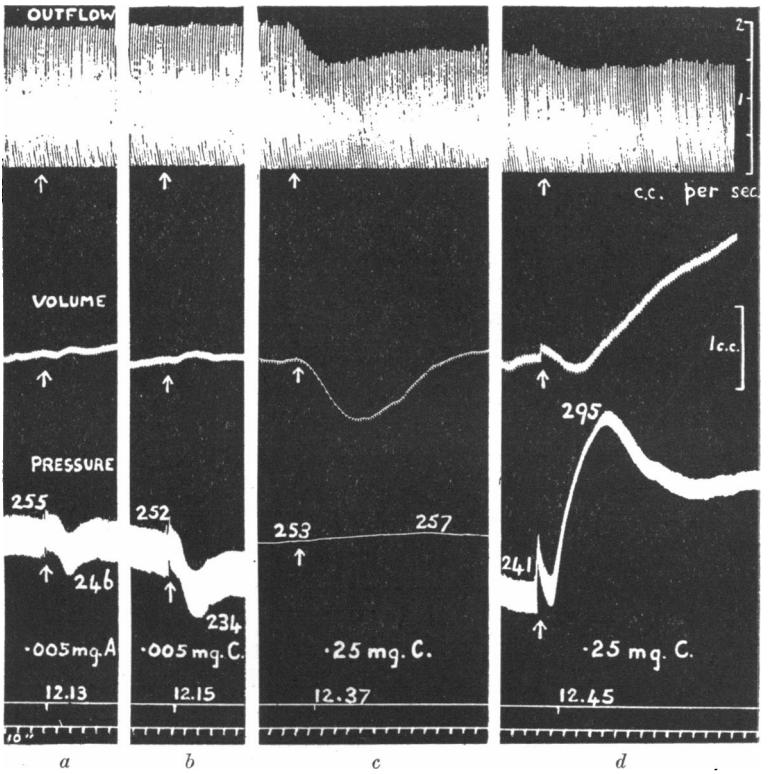

Fig. 9. Cat. *A.* Adenosine. *C.* Adenylpyrophosphate (doses in terms of weight of the barium salt). Outflow. Lung volume. Perfusion pressure (mm. of blood).

when given in comparable doses. In preparing the barium adenylpyrophosphate for injection, an excess of sodium sulphate had been added to ensure complete precipitation of the barium. A control experiment showed that quantities of sodium sulphate, considerably larger than those present in the doses used, had no action in these experiments.

The pulmonary vessels of dogs were much less sensitive, and it was not found possible to inject sufficient of these substances to compare their actions satisfactorily. In one experiment, however, it was found that a

dose corresponding to 0·5 mg. of barium adenylpyrophosphate produced a small dilatation, followed by constriction of a dog's vessels, while equivalent doses of adenosine and muscle adenylic acid had no action at all.

Discussion.

The actions of the different drugs are summarized below.

Adrenaline. In dogs its chief action was constriction of the inflow. Its effect on the outflow varied. Its dilator action after ergotoxine was sometimes mostly on the inflow and sometimes on the outflow as well. In cats its chief action was dilatation of both inflow and outflow. This action was not abolished by ergotoxine. When constriction occurred, it was mostly due to an action on the inflow.

Histamine. Constricted both inflow and outflow in cats and dogs.

Acetylcholine. Small doses produced dilatation in cats and sometimes also in dogs. Larger doses produced constriction. In cats this occurred both on the inflow and outflow, but in dogs the action was mostly on the inflow.

Adenosine compounds. Small doses produced dilatation and larger doses constriction. In cats the constrictor effects of large doses were mostly due to an action on the inflow. Adenylpyrophosphate produced both effects much more actively than either adenosine or muscle adenylic acid.

Though we have recorded changes in the volume of blood in the lungs, our discussion has hitherto been confined to the consideration of the contribution of the different parts of the vascular system to the total resistance to the flow of blood. Most of the effects observed have been mainly due to vaso-motor changes in the inflow, but evidence has been presented which indicates that the action of histamine and adrenaline (see Figs. 2–6), and possibly also acetylcholine and adenosine, on the tone of the pulmonary veins may have an appreciable effect on the total resistance of the lungs to the flow of blood.

We must now consider what direct significance our results may have in regard to the effect of the drugs we have used on the blood content of the lungs in the intact animal. This is of importance from two points of view. In the first place, the lungs form one of the most important potential reservoirs for blood in the body [Magnus, 1930]. In the second place, the blood content of the capillaries is one of the factors which influence the length of time during which blood is exposed to the air in the alveoli. One of the factors controlling the blood content of the lungs is the hydro-

static pressure in their capillaries. This pressure must always be less than that in the pulmonary artery and greater than that in the left auricle. The mean of these two pressures is sometimes taken as an estimate of the capillary pressure [Magnus, 1930]. It is possible, however, that the stimulation of nerves or the application of drugs might produce changes in the capillary pressure, by acting selectively either on the arteries or the veins, so that the capillary pressure approached either that in the pulmonary artery or to that in the left auricle. The changes which we have recorded in the lung volume have been attributed to such a selective action, but these changes in volume were very small. It is just possible that vaso-motor actions might produce much larger changes under physiological conditions when the lungs are being normally ventilated, but it is improbable that this action is of much importance. The result shown in Fig. 3 may be taken as a demonstration that a drug may have an action on the outflow contrary to its action on the inflow, and that such an action can be detected by the methods we have used. This result was of doubtful interpretation, exceptional in nature and inconstant in appearance. In every other case in which an action on the pulmonary veins has been demonstrated or suspected this action has been in the same direction as the action which the drug had on the arteries in the same experiment. The pulmonary veins have, in fact, responded in such a way as to neutralize the changes in lung volume which would have occurred if the action of the drug had been solely on the inflow. There is thus no reason for supposing that the pulmonary veins play a part in the control of the blood content of the pulmonary circulation which is in any way comparable with the part which the hepatic veins have been found to play in the control of the blood content of the portal circulation.

Summary.

1. The lungs of dogs and cats were perfused with blood in such a way that either the perfusion pressure or the rate of flow could be maintained approximately constant. The blood content of the lungs was recorded by means of a plethysmograph.

2. The details of the effects seen are summarized above at the beginning of the discussion.

3. The plethysmographic record indicated that the most striking effects of adrenaline, histamine, acetylcholine and adenosine compounds were mainly due to an action on the pulmonary arteries, but evidence was obtained that changes in the tone of the pulmonary veins might also

have an appreciable effect on the total resistance of the pulmonary vessels to the flow of blood.

4. In almost every case where definite evidence was obtained that a drug was acting on the pulmonary veins, this action was such as to neutralize the change in lung volume which would have occurred if the drug had acted solely on the inflow.

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