CONTRIBUTIONS TO THE PHYSIOLOGY OF THE LUNGS. PART II. On the Innervation of the Pulmonary Blood Vessels; and some Observations on the Action of Suprarenal Extract.

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IN the course of our experiments upon the lungs we have spent much time in studying the question of the innervation of the pulmonary vessels, and as the final result of this work have devised a method which we bring forward as a general test for the existence of a nerve supply to any set of blood vessels.

The investigation arose from the results we obtained when repeating those experiments of Bradford and Dean and of François Franck from which they concluded that the pulmonary blood vessels were supplied with vaso-constrictor fibres. Although we were able to confirm most of the experimental results they had recorded, we were unable to agree with them in concluding that the effects they observed could only be explained on the supposition that the pulmonary vessels were supplied with constrictor fibres and were therefore under nervous control. We obtained evidence that in all the methods they adopted cardiac effects had not been entirely eliminated, and that while in most cases cardiac acceleration had been excluded, cardiac augmentation was still present[1]. In order to solve the problem it therefore became necessary to devise some method which would entirely exclude all possibility of a cardiac change. We first studied the rate of flow of blood through these vessels during an artificial perfusion with blood at constant pressure, in the hope that the nerves, if present, would remain excitable for some time after death. We showed that by taking a set of vessels known to possess

[1] We propose to deal fully with these experiments in a future communication.

vaso-constrictor nerves excitation of those nerves, during an artificial perfusion, still led to constriction. When, however, similar experiments were extended to the lung vessels absolutely negative results were obtained, thus indicating the absence of vaso-constrictor nerves. Further evidence of a more positive nature was gained by studying the action of suprarenal extract and other drugs upon the pulmonary vessels, and when we found that adrenalin produced no constriction we obtained the basis of the method we propose as a general test for the presence of constrictor nerves to any set of vessels. The method essentially depends on proving that adrenalin, in its typical action on peripheral vessels, produces its effect by exciting the nerve endings—not by exciting the muscle fibres. The chief experiments detailed in this paper therefore adduce evidence to prove this point in the physiology of the action of adrenalin[1].

Method. The perfusion of the different isolated organs was effected by slight alterations in the apparatus already described and figured by one of us[2]. Such modifications as were required are indicated in Fig. 1. The pressure of perfusion was maintained by connecting the main blood receiver to a large can of compressed air. The air was delivered from this can through a tube ending in a pin-hole orifice, so that a slow stream was forced into the main blood receiver, the excess above that required for maintaining the pressure at a constant height escaping through the mercury valve. The pressure used for perfusion varied for different organs, from 25 to 150 mm. Hg, and was registered by a manometer connected with the blood receiver.

The rate of outflow of blood from the vein was recorded in the following way:—A small glass receiver, provided with a single outflow tube from the bottom, was closed above by a rubber cork pierced by two holes; through one of these passed a tube opening about a quarter of the way down the receiver and connected above to the vein. The orifice for the exit of the venous blood was thus kept at a constant level and was adjusted to lie about 2 cms. below the level of the vein. The bottom outlet from the receiver was connected to the pump of the perfusion apparatus, and the blood was thus withdrawn and returned to the main blood receiver. Through the second hole in the cork passed a

[1] In our first experiments the solution of suprarenal extract employed was one prepared from the tabloids of Burroughs and Welcome. In the later experiments the 1 in 1000 solution of adrenalin of Parke Davis and Co. was used. The results were of a much more uniform character when this latter preparation was taken.

[2] Brodie. This *Journal*, xxix. p. 266. 1903.

glass tube opening just below the cork, so that the air space in the receiver could, at any time, be connected to a bellows-recorder, and in that way the volume of blood flowing in or withdrawn could be continuously recorded. The general arrangement of the apparatus is figured in the accompanying sketch (Fig. 1).

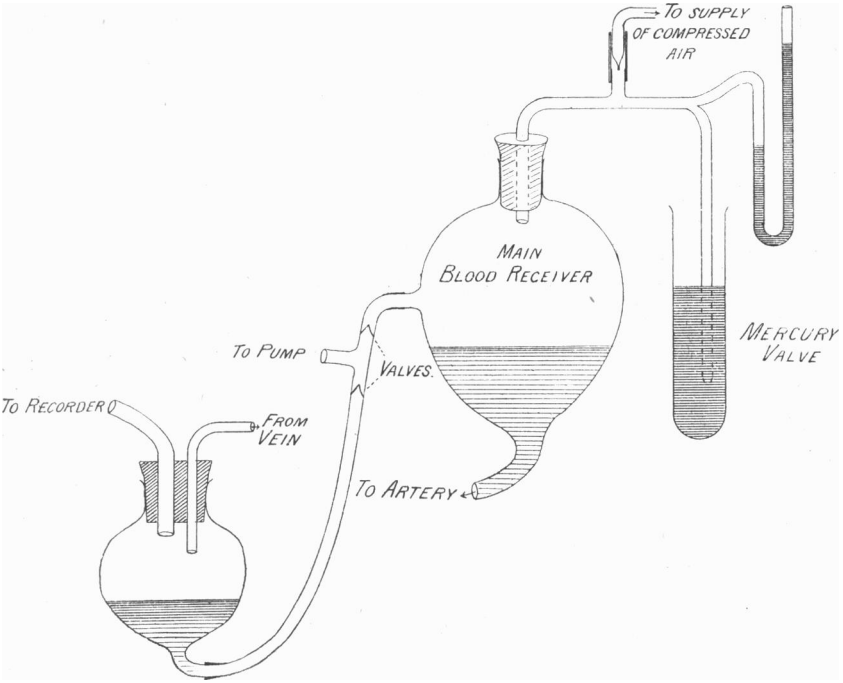

Fig. 1.

The pump used in these experiments is represented diagrammatically in Fig. 2. It consists of a crank driven by a coned-pulley. This gives a vertical movement to a metal rod, which movement is in turn communicated to the piston of a hypodermic syringe. The extent of the vertical movement can be roughly adjusted by varying the point of attachment of the rod to the crank by the clamping screw *B*. On the vertical rod is interposed the mechanism *A*. This consists of a cylinder through the upper orifice of which the vertical rod passes. The end of the rod is provided with a collar which fits loosely inside the cylinder, and the upper orifice permits the rod to pass freely, but when the collar reaches the top of the cylinder the latter is moved upwards with it. The lower end of the cylinder is provided with a screw of fine pitch, the

outer end of which is attached to the syringe, while the end within the cylinder faces the lower end of the rod. Thus, in the position figured in the diagram, the downward thrust of the rod will not be communicated to the piston until the end (*d*) of the rod reaches the upper end (*e*) of the screw. Similarly, on the up-stroke, the piston is not moved until the collar (*d*) reaches the top of the cylinder. Hence by screwing the cylinder downwards more movement will be communicated to the piston and conversely, so that this mechanism serves as a fine adjustment for regulating the thrust of the piston, *i.e.* the volume of liquid extracted per revolution[1]. In an experiment, the pump is first adjusted roughly by the screw *B* until the quantity withdrawn is nearly equal to that entering the small receiver, and then by the fine adjustment until the two volumes are exactly equalised. The graphic record thus obtained is seen in the first part of Fig. 4, in which the mean level of the tracing remains horizontal. If now the volume of blood discharged by the vein increases, the inflow is in excess of the withdrawal, so that the record rises, and conversely. The method is extremely sensitive and shows quite minute changes in the rate of flow of the blood.

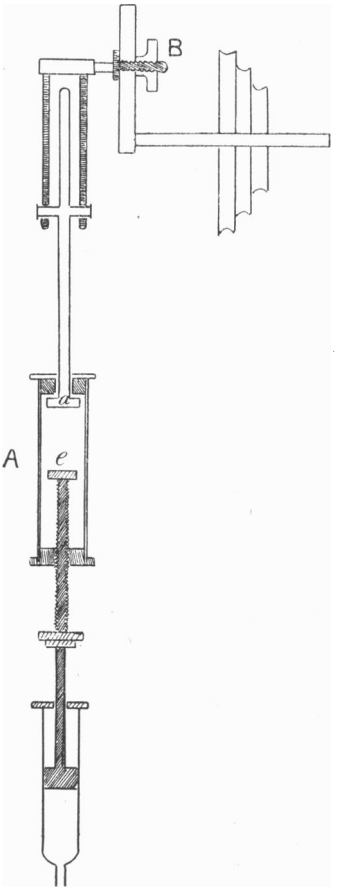

Fig. 2.

In constructing the apparatus, we have particularly aimed at devising one of such dimensions that the blood derived from the animal itself may suffice for the perfusion. Thus, the tubing conveying the blood to and from the organ is kept as short as possible and is of small diameter. The capacity of the valves is also reduced to a minimum. By carefully attending to such points as these we have found that the blood collected from a cat is amply sufficient for perfusion of the limbs,

[1] The pump was made for us by the Cambridge Scientific Instrument Company.

intestines, lungs, or even for the liver. A disadvantage of the method is that a drug once introduced continues to circulate through the organ, though, of course, it is much more diluted after its first passage through the vessels. We have been surprised to find how very little, in most instances, the previous injection of one drug interferes with the typical course of reaction of a second drug subsequently injected. We have been careful, in all cases, to control the action of a drug by studying it in an experiment in which it was injected first.

It is convenient to interpose a 3-way tap between the pump and the valves, so that by a simple movement the pump may be connected either to the receivers or to a graduated tube. By turning the pump on to the graduated tube the volume of fluid extracted per thrust can be measured, or, in an instant, the withdrawal of blood can be stopped without stopping the pump. This is often a great convenience. In many instances the rate of flow is very greatly altered by the injection of a drug. Thus, the flow may become so rapid that the pump is quite unable to cope with it. In such cases in our earlier experiments, we either stopped the flow to the artery for a time, or diminished the pressure of perfusion, or increased the thrust of the pump. These procedures all spoilt the course of the record because we could no longer follow with accuracy any change in rate as compared to that before injection. The final plan we adopted was to connect a syringe by means of a T-piece inserted in the tubing leading to the pump, so that by it any volume of blood might be withdrawn from the small receiver and returned to the main receiver. Thus in Fig. 7 the long verticals indicate the times at which successive amounts of 6 c.c. of blood were thus withdrawn. This procedure also serves as a measure of the increase of flow through the organ. By a similar arrangement, known volumes of blood were withdrawn from the main receiver and injected into the small one when a marked constriction had been produced (see Fig. 6).

The organs we have perfused for these experiments are the hind-limbs, kidney, intestines, and lungs. In the case of the limbs the cat was the animal usually employed, either one or both limbs being perfused. In the former case the cannulæ were placed high up in the femoral vessels, and in the latter as low down as possible in the abdominal aorta and inferior vena cava respectively.

The course of our experiment consisted in first anæsthetising the animal with ether and then cutting across the carotids and collecting every available drop of blood. In some experiments the animals were killed by pithing and the blood rapidly collected. This avoidance of all

anæsthetic in no case made the least difference in the results. The blood was kept warm, defibrinated, filtered through glass-wool and used directly for the perfusion. In a few instances, when a considerable amount of fluid was required, the blood was diluted with an equal volume of warm Ringer's solution, but as a rule this dilution was avoided. It is quite inadmissible when the lungs are to be perfused, for with diluted blood a great amount of œdema is speedily produced. Citrated blood was used in a few instances, but so far as the limbs are concerned it possesses no advantage over defibrinated blood, and has the disadvantage that if the perfusion is prolonged (1 to 2 hours) it tends to clot. In our first experiments great care was taken to keep the blood and organ at body temperature. Later, we found that when studying the action of a drug upon the blood vessels the reactions were precisely the same even though the temperature was allowed to fall several degrees.

The introduction of drugs in solution to the blood flowing to the perfused organ requires considerable care. In the earlier experiments we were content to inject the drug, dissolved in saline solution, by means of a fine hypodermic needle piercing the tubing leading directly to the artery. Here two sources of error are introduced. In the first place, unless great care is observed the injection leads to a transient rise of pressure in the artery and therefore the outflow temporarily increases; in the second place, the admixture with a saline solution diminishes the viscosity of the blood and for this reason the velocity of the flow is again temporarily increased. The latter error is the more important one since in most of the experiments the former was eliminated by the use of a system of tubes indicated in Fig. 3, in which the clear part represents glass-tubing and the shaded rubber. The fluid from the main receiver reaches a glass Y-piece and can then traverse either limb *C* or limb *D*, the resistance of either path being the same. The two tubes are again

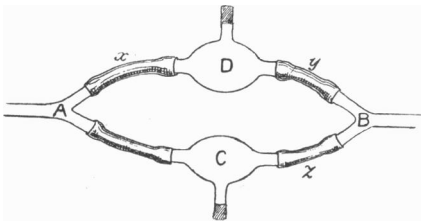

Fig. 3.

united by a second Y-piece at *B*. The whole apparatus is filled with the perfusing fluid, but at one side—*e.g.* at *x* and *y*—clips are placed on

the rubber tubing. When a drug is to be injected, the cork is removed from the lateral tube of the bulb *D*, the drug to be tested is carefully introduced and the cork replaced. The clamp on *x* is now removed and at the required instant that on *y* is also taken off and a second later is placed on *z*. In this way no alteration in pressure is set up, and as soon as one drug has been introduced the second bulb is ready for a further injection. In later experiments we avoided producing a rise of pressure by injecting the drug at some point between the main receiver and a small glass-wool filter always interposed between the receiver and the artery. This filter consists of a short piece of glass tubing, of larger diameter than the rest, which is loosely plugged with glass-wool and is of the greatest importance in that it holds back any particles which may have gained admittance to the main receiver.

While we thus avoided all changes of pressure, our tracings show that the second factor still remains, and to obviate it, it was necessary to dissolve the drug to be injected in the same solution as was being used for the perfusion. Though we found that the admixture of a small amount of saline solution to the blood caused an acceleration in the flow we always employed this method in our later experiments because of its greater convenience. This was done because we found that the effect of the dilution was quite transitory and was usually over before the action effected by the drug commenced. The small increase in rate of flow seen in many of our tracings directly after the injection is due to this cause and must be allowed for (see Fig. 7, p. 488).

To control the results obtained on limbs, some experiments were performed on the intestines. The general method was the same as before, the cat and dog being again the animals employed. The unsatisfactory part of an intestinal perfusion when studying alterations in the rate of flow, is the frequent occurrence of muscular movement which follows the administration of many drugs. It is quite impossible to eliminate this entirely; thus, on perfusing with barium-chloride, violent contractions are produced, whereas with suprarenal extract the normal movements are, for a time, completely inhibited; either change produces a slight effect upon the rate of flow of blood.

A large number of perfusions were carried out on the lungs of cats, dogs, and rabbits. The effects of the various drugs investigated were invariably the same in all three animals. The animals were anæsthetised and the blood collected. The thorax was then opened and a cannula tied in the pulmonary artery. A strong ligature was now passed round the heart and tied round the ventricles. A second ligature was next

tied round the root of one lung, thus limiting the perfusion to the opposite lung. A wide-necked cannula was then tied in the left auricular appendix, and perfusion commenced, the lungs remaining *in situ*. If the experiment was to be continued for a considerable time, the lungs were sometimes removed and enclosed in a warm chamber, but in short experiments no advantage was gained by thus removing them. In about half the perfusions, a cannula was placed in the trachea and the lungs rhythmically inflated by warm air to about their normal extent. In the greater number of experiments, artificial respiration was omitted, and it may be stated at once that no essential difference was in any case obtained as a result of the artificial ventilation, all the drugs which we have tested producing the same effects, whatever the condition of the lungs in this respect may have been.

VERIFICATION OF THE METHOD UPON ORGANS KNOWN TO BE SUPPLIED BY CONSTRICTOR NERVES.

The organs we chiefly used for this purpose were the limbs or small intestines. In both instances, excitation of the vaso-motor nerves during the course of a perfusion, causes considerable constriction of the vessels. We found it to be much easier to obtain a good reaction from organs taken from dogs than from those taken from cats. Moreover, the effect was much more readily obtained on the intestine than on the limbs. Thus, Fig. 4 gives the result of a short excitation of the nerves running with the intestinal vessels. After a short latency the tracing falls rapidly, showing that the outflow from the vein has considerably diminished. This persists some time (30 secs.) after the excitation has ceased, but gradually recovers and the tracing once more becomes horizontal (100 secs.), showing that the outflow is again the same as before excitation. The change in the rate of flow from the vein, as well as being indicated by the general fall of the tracing, can also be estimated by the rate of rise of the lever in each upward stroke:—the downward stroke of course indicating the withdrawal of blood by the pump. This reaction may be repeated many times, indeed we found that the nerves remained excitable, in many experiments, for from one to two hours after the removal of the intestines from the body, and as similar results can be obtained with limb or kidney vessels, we conclude that the method is a suitable one for the purpose in view.

The next effect to which we must refer is that produced by injecting small doses of adrenalin into the circulating blood. Schäfer and

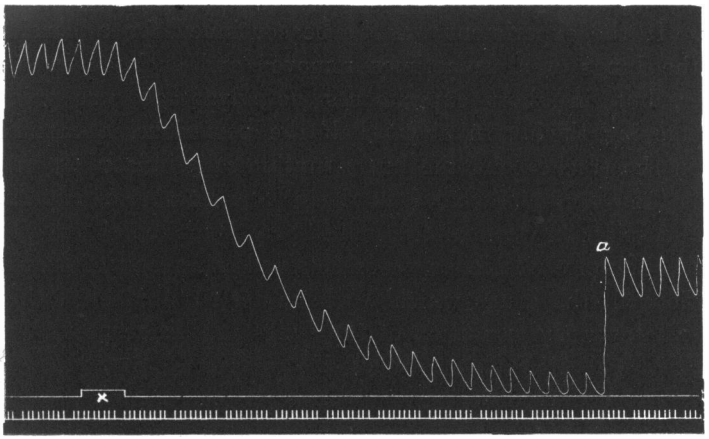

Fig. 4[1]. $\times \frac{1}{2}$ linear. Cat. Perfusion of intestines. Excitation of the nerves accompanying the superior mesenteric artery. Coil at 5 cms. Single Leclanché cell. Time record gives seconds. Pressure of perfusion 90 mm. Hg. At the point marked 'a' the pump was stopped for a few seconds to allow the tracing to be written at a higher level.

Oliver[2], in their paper upon the action of suprarenal extract, showed that perfusion of the extract through the vessels of pithed frogs greatly diminished the flow or even cut it off altogether. They also gave abundant evidence proving that, in the mammal, the extract caused constriction of arterioles, and that the main action of the drug was a direct one upon the vessels peripherally. Biedl[3] showed that, in the perfusion of surviving organs, suprarenal extract produced contraction of arterioles, and the same has been shown for the kidney vessels by Gottlieb[4]. These results we have abundantly confirmed, and in Fig. 5 *A* we give the effect of the injection of a very minute quantity of adrenalin, the organ being the intestine. On examining the tracing, it is seen that the first effect is produced after a latency of eleven seconds, and then very marked constriction develops. Part of this

[1] All tracings are to be read from left to right. An upward movement of the lever indicates increased volume of blood in the small receiver.

[2] Schäfer and Oliver. This *Journal*, XVIII. p. 230. 1895.

[3] Biedl. Preliminary communication in *Aeusser. Erkrankungen d. Nebennieren.* Wien, 1897, p. 26.

[4] Gottlieb. *Schmiedeberg's Archiv*, XLIII. p. 286. 1899.

latency is due to the time occupied by the drug in reaching the vessels. After a reaction such as this the vessels do not relax for a considerable

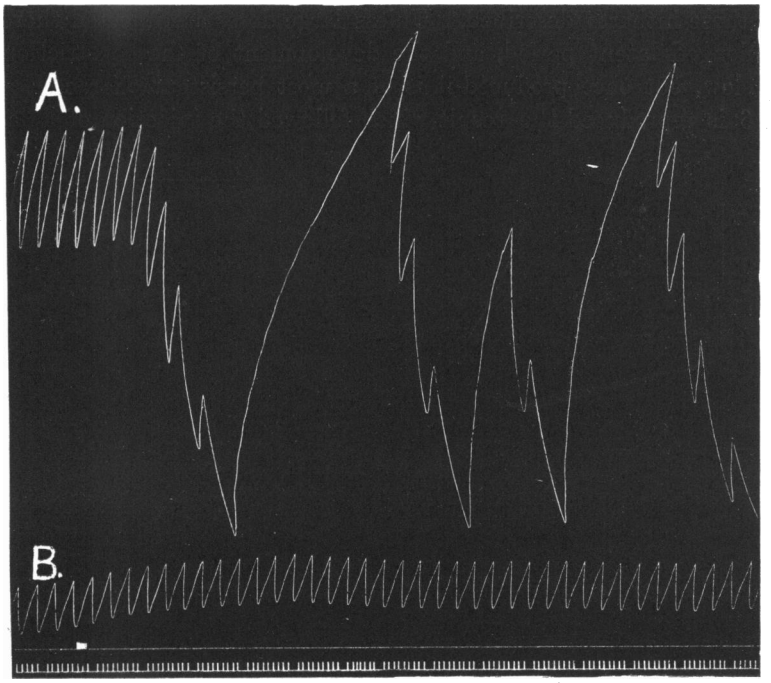

Fig. 5. × ½. Cat. Perfusion of small intestines. Pressure of perfusion 100 mg. Hg.

A. Result of injecting 0·5 c.c. adrenalin solution 1 in 20,000.

B. Effect of a similar injection of the same adrenalin solution after the administration of 50 mgrms. of apocodeine.

The difference in the heights of the two tracings is due to the substitution of a less sensitive recorder before the second record was written.

time, but ultimately they completely return to their original condition. A fresh injection then produces an identical result and small doses can be injected many times in succession, each injection producing the same marked constriction.

In addition to adrenalin, pilocarpine, muscarine, barium-chloride, and veratrine all produce constriction of intestinal or limb vessels in perfusion experiments. We desire especially to draw attention to the effect produced by pilocarpine or muscarine, since these drugs are generally considered to act by stimulating nerve endings. Their action is practically identical with that of adrenalin, so that it is not necessary

to reproduce tracings. The only important difference is that much larger doses are required.

The reaction obtained by injecting a soluble barium salt is different from those hitherto described. Unless large doses are injected there is a prolonged latent period, and the development of the constriction is very slow, but once produced it is of a most persistent character. In Fig. 6 is reproduced the result which followed the injection of a large

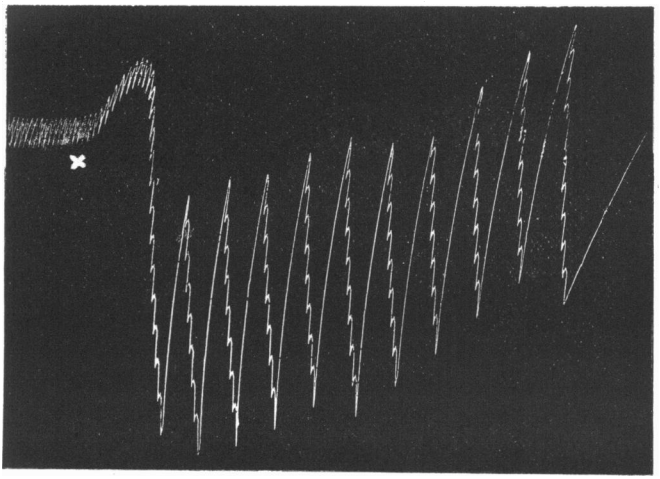

Fig. 6. Cat. Perfusion of the hind limbs. Result of injecting 2 c.c. of a 2·4 % solution of barium-chloride. Pressure of perfusion 90 mm. Hg. The ten verticals indicate the times at which successive amounts of 6 c.c. each were injected into the small receiver. The total duration of the tracing was 5 mins. 40 secs.

dose of barium-chloride. The first effect was a dilatation of the vessels, which is greater in amount than can be accounted for by the injection of the saline solution. Then follows a rapid constriction, which is seen to be very persistent. This tracing is introduced for comparison with a similar one obtained from the lung (Fig. 10). As the dose is a large one the typical slow onset of a barium constriction is not seen. Exactly similar results follow the injection of a dose of veratrine.

Though there are several drugs which cause constriction when administered during a perfusion, adrenalin is by far the most active. One 10,000,000th of a gram of adrenalin will produce a constriction of as great intensity as that caused by one 100th of a gram of barium-chloride, and unless minute doses are injected the vessels constrict so powerfully that the flow entirely ceases.

EXTENSION OF THE EXPERIMENTS TO THE PULMONARY VESSELS.

Having thus shown that in perfusion we possess a very sensitive and direct method of studying the changes in calibre of blood vessels, and having found that the vaso-constrictor fibres retain their excitability under these conditions for a considerable time after death, we next extended our observations to the pulmonary vessels. Here we obtained results differing most markedly from any of those yielded by any of the organs on the systemic circulation.

Excitation of any of those nerves which Bradford and Dean, or François Franck have described as containing vaso-constrictor fibres for the lung vessels, invariably gave us negative results. We never obtained the least effect upon the outflow of blood on exciting either the spinal cord, the white rami communicantes from the upper thoracic spinal nerves, the sympathetic chain between the successive ganglia, the ganglion stellatum, the loops of the annulus of Vieussens, or the inferior cervical ganglion. Stimulation of the fibres in the root of the lung was equally ineffective. The results were invariably the same in the dog, cat, or rabbit. The only effect ever observed was a slight variation in level in the tracing, which quickly readjusted itself. This only occurred in a few of the experiments, and was frequently absent on repeating the stimulation, and we satisfied ourselves that it was due to disturbance of the lung owing to the necessary manipulations, or to an escape of current producing some contraction of the thoracic muscles. From these results we must conclude, either that there is no vaso-constrictor supply to the pulmonary arterioles in these nerves, or that the nerves are present but become inexcitable very soon after the death of the animal. The latter possibility does not seem to be very probable in view of the fact that vaso-constrictor nerves in other positions have been frequently found by us to remain excitable for more than two hours after death, but we have further controlled the result by diminishing the time which elapsed between the death of the animal and the stimulation of the nerve to the shortest possible limit. This we effected by preparing the nerves and vessels before bleeding the animal. Sufficient blood for the perfusion was then withdrawn, defibrinated and placed in the perfusion-apparatus. The animal was next bled and the cannulæ rapidly inserted. In this way only two minutes were lost between the bleeding of the animal and the excitation of the prepared nerve. The result was just the same as before. We therefore conclude that the first explanation given is the correct one, and that no constrictor fibres for the lung vessels are con-

tained in the upper thoracic sympathetic system. Stimulation of the vagus is equally without effect, nor on the other hand have we been able to discover vaso-dilator fibres for the lungs in any of the nerves investigated.

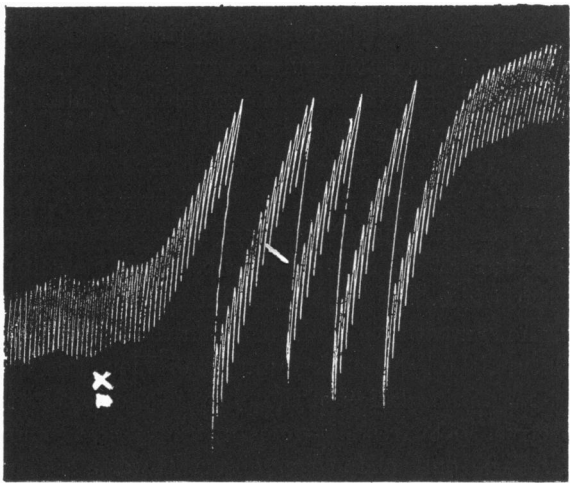

Fig. 7. Cat. Perfusion of right lung. Result of an injection of 1 c.c. of a saline solution of adrenalin (1 in 20,000). Pressure of perfusion, 30 mm. Hg. Four successive amounts of 6 c.c. each were withdrawn during the dilatation. At the end the thrust of the pump was increased. The total duration of the tracing was 5 mins. 4 secs.

The results obtained by the action of drugs upon perfused pulmonary vessels gave us valuable confirmatory evidence of this conclusion. This is especially the case with adrenalin, the drug which in our perfusion experiments upon other organs proved extraordinarily powerful in effecting constriction. When a dose of adrenalin is injected into the blood perfusing the lung vessels either no change occurs or dilatation is produced as in the result reproduced in Fig. 7. The tracing shows that not only was no constriction produced but on the other hand an actually opposite result was obtained,—an increase in the rate of flow, and this too with a dose of the extract twice as great as that which was shown to produce an enormous constriction of the intestinal vessels. A repetition of the injection is followed, as a rule, by less dilatation than that first recorded, and this is also the case even though a much greater dose of the extract be given. We have often injected a dose 20 times greater than one which is sufficient to stop the flow through the intestinal vessels almost completely, without any alteration in the result. Although

distinct dilatation is the common result which follows an injection of adrenalin into the pulmonary vessels, we have on many occasions observed it to produce but little if any effect. Fig. 8 shows one of these tracings, where only a slight amount of dilatation is produced. The

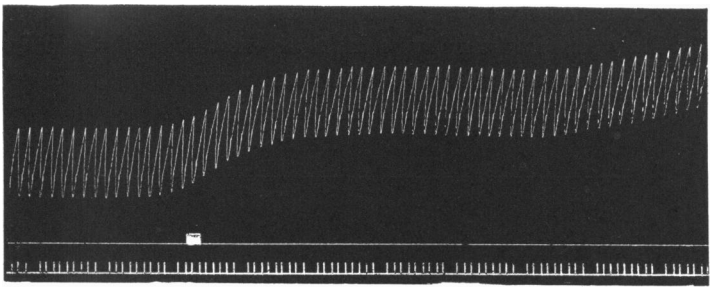

Fig. 8. $\times \frac{1}{3}$. Dog. Perfusion of right lung. Injection of 5 c.c. of a saline solution of adrenalin (1 in 50,000). Pressure of perfusion 30 mm. Hg.

dose given in this experiment is twice that given in the preceding tracing, but we have found that this makes no difference in the result. The animal also was different, but again we have not been able to make out any constant variations depending upon the animal employed. The previous state of the vessels as regards constriction or dilatation is of importance, but we have often observed considerable dilatation in vessels which were apparently well dilated before the injection was made. We may point out that the evidence we adduce that adrenalin does not cause contraction of the pulmonary arterioles, confirms the results of other observers (Gerhardt, Biedl and others) who have studied the question by recording the pulmonary blood-pressure. Similar experiments by ourselves gave the same result.

The explanation of this striking difference in the action of adrenalin appears to us to lie in one of two directions. Either (1) the observed dilatation is the expression of the direct action of adrenalin upon smooth muscle fibres, or (2) it is due to an excitation of the terminals of vaso-dilator fibres. Against this latter hypothesis is the fact that during perfusions with excitation of nerves we have not been able to obtain the least evidence of the existence of dilator fibres any more than we have been able to prove the presence of constrictor fibres. On the other hand, the first explanation suggested implies, firstly, that the pulmonary vessels are not supplied with constrictor fibres, and secondly, that adrenalin, when it causes constriction excites nerve-terminals and not

muscle-fibre. In our opinion, these are the correct conclusions to be drawn, and further confirmatory evidence is given in the following section of this paper.

In addition to the marked divergence of the pulmonary as compared with the systemic vessels in their reaction to adrenalin, there are further differences with other drugs. Thus, muscarine or pilocarpine, which constrict limb and intestinal vessels, cause dilatation of the pulmonary vessels when perfused through them. An instance of this is given in Fig. 9, where a dose of pilocarpine was administered.

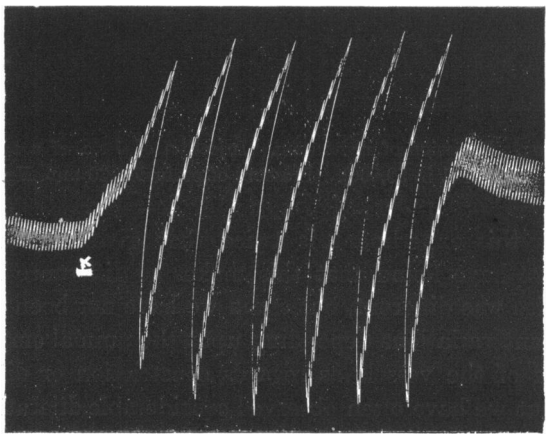

Fig. 9. Cat. Perfusion of lung. Injection of 2 c.c. of a 1 % solution of pilocarpine nitrate. Pressure of perfusion 30 mm. Hg. The long verticals indicate the withdrawal of 12 c.c. of blood in each instance. The thrust of the pump was increased at the end of the tracing. The total duration of the tracing was 7 mins. 2 secs.

When, however, barium-chloride is injected the result is the same as in the case of limb or intestinal vessels. One must, of course, make allowance for the relatively greater flow through the pulmonary vessels, since through it the concentrated dose of the salt acts for a shorter time upon these vessels. Thus Fig. 10 shows the great diminution in the rate of flow through the lung vessels which followed an injection of barium-chloride. This should be compared with Fig. 6 (p. 486), when it is seen that the two reactions are practically identical. The preliminary increase in rate of flow seen in Fig. 10 is again greater than can be explained by the dilution of the blood with the saline solution. The strength of the solution injected was so chosen as to be isotonic with

serum. In these experiments with barium the maximum contraction is often only attained after some two to three minutes.

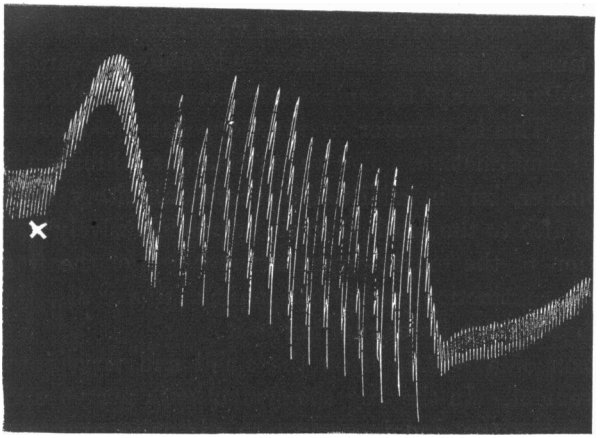

Fig. 10. Cat. Perfusion of the right pulmonary vessels. Injection of 2 c.c. of a 2·4 % solution of barium-chloride. Pressure of perfusion 33 mm. Hg. 14 successive amounts of blood each of 6 c.c. were injected and at the end of the tracing the thrust of the pump was readjusted. Total duration of the tracing 6 mins. 56 secs.

WHICH TISSUE DOES ADRENALIN EXCITE? MUSCLE OR NERVE-ENDING?

The results we have thus far discussed raise the debated question as to whether adrenalin acts on muscle or on nerve-endings. In our opinion the latter view is the correct one, and we will discuss the evidence which leads us to that conclusion under the following sections.

A. *The action of adrenalin upon any tissue is invariably that which follows excitation of the sympathetic nerves supplying the tissue.* It will not be necessary for us to describe in detail the action of suprarenal extract upon the different tissues of the body. It is sufficient to point out what those actions are, and only to treat of them more fully where the result appears to require some further explanation. We may refer to the full description which is given by Langley[1] in a recent volume of the *Journal.*

Circulatory system. The action of adrenalin upon the heart is to produce acceleration and augmentation. Upon the frog's heart but slight acceleration is produced and commonly no augmentation. After prolonged administration of minute doses dropped ventricular beats may

[1] Langley. This *Journal*, XXVII. p. 237. 1901.

occur, but the whole action requires further investigation. The action upon systemic blood vessels is always to excite constriction.

The neuro-muscular mechanisms of the alimentary tract. Adrenalin inhibits any movements of the stomach which may have been occurring before the time of injection and likewise inhibits the cardiac sphincter. According to Page May[1] the splanchnics contain no inhibitory fibres for the stomach. This is, however, in direct opposition to all other observers and requires confirmation. The vagus contains inhibitory fibres to the cardiac sphincter, but in addition to these, inhibitory fibres are also found in the fifth to the ninth thoracic nerves inclusively[2].

The action of the extract upon the stomach of the frog we found was to excite contraction, and in this animal this is also the action of the sympathetic nerve fibres running to it.

The action of adrenalin upon the small and large intestine is to inhibit movement. In the frog the sympathetic nerves excite contraction of the intestines, and in them also adrenalin excites contraction. "In the rabbit the extract causes inhibition of the internal anal sphincter; in the cat and dog the extract causes slight contraction of the sphincter. The effect in the several animals resembles in kind that produced by stimulation of sympathetic nerve fibres; but in the rabbit the inhibitory action is rather more marked, in the cat and dog considerably less." (Langley.)

The action of the extract upon the spleen is to excite a powerful contraction.

Salivary glands. Adrenalin excites secretion, but this effect is not of much value for the argument one way or the other, because of the double secreto-motor nerve supply to these glands. We return to these experiments again when we consider the effect of adrenalin upon tissues whose post-ganglionic sympathetic fibres have been allowed to degenerate (p. 499).

Respiratory tract. Adrenalin has no action on the muscles of the bronchioles, and the motor and inhibitory nerves of these muscles are all contained within the vagus.

Urinary tract. Adrenalin excites a powerful contraction of the ureter. Injected intravenously, it inhibits the bladder in the mammal. When applied locally to the bladder, the part to which it is applied first contracts, but in a minute or so the local contraction gives way to local inhibition. On the other hand, we found in the frog, that adrenalin

[1] Page May. *British Med. Journ.* Sept. 13th, 1902.

[2] Langley. *Schäfer's Textbook*, II. p. 695.

causes contraction of the bladder, and this again is the effect following excitation of the sympathetic nerves to this viscus.

Internal generative organs. In the male, the extract causes contraction of the vas deferens and seminal vesicles. In the female, of the uterus and vagina.

External generative organs. The extract causes contraction of the external generative organs, both in the rabbit, the cat, and the dog. All the effects are less strong than those caused by sympathetic stimulation. (Langley.)

Erector muscles of the hair. Adrenalin causes erection of the hairs in the cat. (Lewandowsky.) Langley confirmed this, but found that the action was much weaker than had been stated by Lewandowsky. He states that the effect is never comparable to that produced by stimulating the nerves to these muscles.

The eye. In the cat, adrenalin causes dilatation of the pupil, withdrawal of the nictitating membrane, separation of the eyelids and protrusion of the eyeball: *i.e.* exactly those effects which follow excitation of the cervical sympathetic. The effects are less obvious in the rabbit, although in this animal also the dilatation of the pupil is easily seen. In the dog, the eyelids and the nictitating membrane are little if at all affected, and the pupils slowly and somewhat feebly contract instead of dilating. When large doses were given, Langley obtained evidence of slight dilatation of the pupil and the other eye symptoms in this animal. In the frog the extract causes dilatation of the pupil.

We see then that an examination of the action of adrenalin upon the various tissues of the body shows that in every tissue the effect produced is exactly that which follows excitation of its sympathetic nerve supply. It is true, that in a few instances the action is distinctly less than that produced by nerve excitation, though it is the same in kind; but this is the only limitation which it is necessary to note. As the actions in the cases of different tissues are of very varied characters, and in the case of some organs is of an exactly opposite character to that observed in the case of others, the most reasonable supposition to make in explanation of the results is to conclude that adrenalin works by throwing into activity the one common tissue present in all instances, namely, the sympathetic nerve terminals. This gives a simple explanation, while the supposition that it acts directly upon the tissue elements themselves, *e.g.* the smooth muscle fibres, requires the assumption that it can produce exactly opposite effects upon muscular coats of different organs.

Though pointing out how closely the actions of suprarenal extract upon the different tissues corresponded with the effects following excitation of their nerve-supply, Langley came to the conclusion that the extract excited the tissue cells themselves and not the nerve terminals. In forming this conclusion he was chiefly guided by the results obtained upon the tissues supplied by the cervical sympathetic after the superior cervical ganglion had been extirpated and the post-ganglionic segments allowed to degenerate.

B. *The striking difference in the behaviour of the pulmonary vessels as compared to the systemic, receives a simple explanation if the extract acts upon sympathetic nerve-endings only*[1]. The main evidence from which we conclude that the pulmonary vessels are not innervated has already been given, and we need only recapitulate it here. It consists of the following facts:—

(1) Nerve stimulation during the course of a perfusion experiment gives absolutely negative results, although positive results are to be obtained easily in similar experiments upon innervated organs.

(2) Similar negative results have been obtained by us when investigating the question by recording the pressures in the pulmonary artery, pulmonary vein, and right auricle and aorta; or by recording the blood-volume changes in the lung. In the majority of cases other observers have arrived at the same conclusion. Where positive results have been obtained they have always been slight in amount, and there is evidence that the observers in question have not eliminated all the factors which may influence the result.

(3) Histologically, we have completely failed to demonstrate the presence of nerve terminals in the walls of the pulmonary arterioles, though this negative evidence is not of any decisive value.

Hence, if adrenalin does not act upon the nerve-endings when exciting contraction in systemic vessels, we shall have to assume that when acting upon the muscle tissue of systemic vessels it makes it

[1] In this connection it is necessary for us to define clearly what we understand by the expression nerve-ending. By it we mean the connecting link between the nerve fibre and the muscle fibre. It is not necessarily a constituent part of the muscle fibre nor yet of the nerve fibre. There is plenty of evidence in the action of drugs of the existence of such a connecting link. It can only exist where nerve fibre joins on to muscle fibre, but it does not necessarily follow that it should degenerate when the nerve fibre which terminates in it degenerates. If our conception of this neuro-muscular junctional tissue is correct the name nerve-ending is obviously a misnomer. We have, however, retained it, since we think that it is commonly regarded as something distinct from the nerve fibre itself.

contract, while when acting upon the similar tissue of the pulmonary vessels it makes it relax.

(4) Lastly, pilocarpine and muscarine, drugs which are universally regarded as acting upon nerve terminals and not upon muscle fibres, excite contraction of systemic vessels but relaxation of pulmonary vessels.

C. *The action of adrenalin upon the systemic vessels is in proportion to the innervation of those vessels.* The chief evidence we have in this connection is from some experiments by Ferrier and Brodie upon the innervation of the cerebral vessels. A detailed account of these experiments has not yet been published[1], but we may state that they prove that adrenalin can constrict the cerebral vessels, though a large dose of the extract is required. Similar evidence of the action of suprarenal extract upon the cerebral vessels has been obtained by Biedl and Reiner[2]. All researches upon the innervation of these vessels prove that the vaso-constrictor supply is very scanty in amount.

In the last place, Langley[3] has pointed out, that adrenalin has a very unequal action on the blood vessels of different parts of the body. "Thus, injection of suprarenal extract causes great pallor of the uterus, and but little in the bladder. It has a strong action on all skin arteries and so far as I have seen on all medium sized arteries in the body. In the abdominal viscera its effect is great on the main branches of the cœliac and superior mesenteric arteries; the effect on the easily visible, though small, branches of these arteries in the stomach and intestines appears to me to be less than that on either the medium sized arteries or on the arterioles. The veins in these organs are little if at all affected."

D. *When the vaso-constrictor fibres of the systemic blood vessels are paralysed adrenalin no longer produces constriction.* One of the best methods of testing the truth of our hypothesis would be, to paralyse the nerve-terminals of the systemic vessels and then see whether such vessels behave to the various drugs in the same way as the pulmonary vessels. We have attempted to produce this condition in three ways:—

I. By trying to find a drug which, while exerting no harmful effect upon the muscle fibres, will paralyse the nerve-endings.

II. By experimenting upon organs removed some time after the

[1] A statement of the results obtained is given in Prof. Ferrier's *Harveian Oration* (London, Bale and Sons. 1902).

[2] Biedl and Reiner. *Pflüger's Archiv*, LXXIX. p. 158. 1900.

[3] Langley. *Loc. cit.* p. 248.

death of an animal, acting on the supposition that the nerve-terminals die earlier than the muscle fibres.

III. By experimenting upon organs in which the vaso-constrictor fibres had been made to degenerate by dividing the post-ganglionic segment at some previous date.

Of these methods, the first has given us the most decisive results. We have experimented with several drugs with this object in view, but need only consider three of them, viz. cocaine, curare, and apocodeine. Cocaine hydrochloride, which, at first sight, appeared to be the most likely drug to produce the result we aimed at, did not prove very satisfactory. In an experiment upon the hind limbs in which the total perfusing fluid amounted to about 300 c.c. the gradual introduction of 1·3 grams of the drug did not abolish the constrictor action of a dose of adrenalin subsequently injected. The first effect of the introduction of the cocaine was to produce some constriction. This quickly passed off, and subsequent additions produced less and less effect, the final state of the vessels being a full dilatation. The adrenalin was now injected, when a distinct constriction followed, though the effect was not so marked as in the control injection made before the introduction of the cocaine.

In later experiments still larger amounts were added but without completely abolishing the adrenalin action. Moreover, we found that the cocaine when used in large doses injured the muscle fibres as well as the nerves, so that no decisive distinction between an action on nerve as distinct from muscle could be expected. Thus, in one experiment solid cocaine was added until it amounted to 2 % of the perfusing fluid. In this case the vessels were widely dilated, but neither adrenalin nor barium produced any constriction, so that it was obvious that both muscle and nerve had been paralysed.

A further attempt was made in experiments conducted in the following way:—A 5 % solution of the alkaloid in normal salt solution was injected into the vessels so as to fill them completely, and was left there for ten, fifteen, or twenty minutes. The cocaine was next washed out by a little saline solution, and the perfusion with blood then commenced. In all these experiments the results were again unsatisfactory.

Though we failed to produce paralysis of the nerve-terminals without injury of the muscle fibres by the use of cocaine, when experimenting upon blood vessels, we obtained a positive result by the application of this drug to the frog's stomach. Suprarenal extract when applied to the frog's stomach excites contraction. If, however, the stomach be

painted with a 0·05 % solution of cocaine, the whole of the nerves are paralysed, and, if adrenalin be now applied, the muscle instead of contracting relaxes to a slight degree.

The next drug we employed was curare. For these experiments we used a 1 % extract of an active sample of curare (Merck) made in normal saline solution. This extract first excites contraction, but after about three small injections no further constriction is observed, and later injections produced dilatation alone. We only obtained partial success by using these curarised vessels. Thus a moderate dose of adrenalin entirely failed to excite constriction, but if one about four times larger than that which, before curare, produced a decided effect, was injected, a constriction was produced, though it was much slower in its onset and course, and decidedly less extensive than the typical normal reaction. The action of barium was still perfectly retained. We thus obtained some evidence of a direct antagonistic action between curare and adrenalin, though only to a partial degree. We must point out that though the administration of very large doses of curare weakens, it does not entirely abolish the constriction following electrical excitation of vaso-constrictor nerves.

The drug which we finally found satisfactory for our purpose was apocodeine. One of us (W. E. D.)[1], has shown that this alkaloid acts more particularly upon nervous structures. In moderate doses it paralyses sympathetic ganglia, while if still larger doses are injected into an animal it produces paralysis of many nerve-endings. Among these are the nerve-terminals of the vaso-constrictor nerves. The first effect of an injection of apocodeine, during the course of a perfusion through the limbs, kidney, or intestines, is to produce constriction. This quickly passes off, and subsequent injections produce less and less effect, until after the injection of a few c.c. of a 1 % solution no constriction is produced, and in most cases a dilatation takes its place. It is now found that the nerve-endings are paralysed. Stimulation of the vaso-constrictor fibres is entirely without effect. Further, the injection of adrenalin, pilocarpine, or muscarine fails to produce constriction (Fig. 5, *B*, p. 485). If excessive doses of the drugs be subsequently administered, as a rule some constriction is produced. This is notably the case with very large doses of adrenalin, but the difference in the response of the blood vessels before and after the treatment with apocodeine is of the most striking character. Thus in the experiment from which Fig. 5, *B*, was taken an

[1] Dixon. This *Journal*, xxx. p. 97. 1903.

injection of 10 c.c. of the adrenalin solution produced a slight but persistent constriction after a latent period of 45 secs. In the last place, the injection of 1 to 2 c.c. of an isotonic solution of barium-chloride through these apocodeinised vessels produces a perfectly typical constriction, one running a course in all respects identical to that produced in an unpoisoned set of vessels. This is evidence, therefore, that the muscles have not been injured by the treatment with apocodeine, a conclusion which is confirmed by the fact that they also react to changes in the perfusion pressure in a perfectly typical manner.

We may now sum up the evidence we gain from this series of experiments. Cocaine, curare, and apocodeine, three drugs which are known to act chiefly, if not exclusively, upon nerve-terminals, all modify or abolish the action of adrenalin. Cocaine gives no positive evidence when the experiments are conducted upon blood vessels, but does so in the case of the frog's stomach. Curare partially paralyses vaso-constrictor nerve-endings, and also partially abolishes the adrenalin action upon them. Lastly, apocodeine paralyses these nerve-endings, and also abolishes the constrictor action of adrenalin unless very excessive doses are given. In many instances, moreover, the action of adrenalin after apocodeine is to produce relaxation. Thus the systemic vessels may become exactly comparable to the pulmonary, after apocodeine has produced its full effect. In these experiments with apocodeine and curare the muscular tissue of the vessels is apparently uninjured. It will react by contracting if the pressure of perfusion be suddenly raised, and it will react normally to barium-chloride. We conclude, therefore, that adrenalin acts upon the part paralysed by these drugs, *i.e.* upon the nerve-endings.

II. A second method which we adopted, was to perfuse the limbs or intestines of animals several hours after death, hoping to find a stage in which adrenalin produced no constriction while barium-chloride still produced its typical effect. If this occurred, it would prove that the part excited by adrenalin died before that excited by barium-chloride, and in such a case the part dying first would, in all probability, be the nerve-endings. Taken by themselves the experiments would not be conclusive, but could only be taken as part of the general evidence, since they would be quite open to the interpretation that barium-chloride was a far more powerful excitant than adrenalin, and could cause a contraction of fibres which were so far dead as to be unable to respond to adrenalin. If, on the other hand, we were to find that the excitability to both drugs was lost simultaneously, the experiments could decide nothing one way or the other.

In these experiments we found that the excitability of the vaso-constrictor nerve-trunks to electrical stimuli was lost in from 2 to 3 hours after death, but that up to 6 hours after death the reactions to adrenalin, pilocarpine, and barium were precisely similar to those obtained in experiments in which the perfusion was started without any delay. Further experiments were tried in which the animals were kept 12, 16, and 20 hours before being perfused. The results were again the same, the only difference being that the contraction of the arterioles was found to be much greater at the start and that the reactions were not so marked. One experiment performed 24 hours after death was especially interesting. The adrenalin reaction had almost entirely disappeared, only a small reaction being obtained with the first injection, and nothing with a second and third. These injections were followed by one of barium, and the effect was also found to be very much weakened, though not to the degree seen with adrenalin. The experiment, however, was in no way decisive.

We would point out, that if our contention that adrenalin stimulates nerve-endings and not muscle fibres is true, these results prove that the nerve-endings are much more resistant than the nerve-trunks. The former are still slightly excitable 24 hours after death, whereas the latter have lost their excitability in about 3 hours after death. This again is evidence that the nerve-ending is something distinct from the nerve fibre. (See footnote, p. 494.)

III. The third method we adopted was that of degeneration. This method has already been tested by several other observers (Lewandowsky, Langley, Schultze, and others). For our experiments we chose the vessels of one of the hind limbs. In the first series we divided the sciatic only, removing about 2 cms. of the nerve. The animals were kept alive for 4, 7, and 14 days, were then killed and the hind limb, whose nerve was divided, perfused in the ordinary way. In all cases the reactions were just as active as in normal animals. In two further animals we therefore divided the anterior crural nerves as well as the sciatic, and kept them for 2 and 3 months respectively before perfusing them. Again the reactions were just the same as in normal animals. The place of division of the nerves was certainly incorrect, but we did not pursue the investigation further by this method because, with one exception, all observers are agreed that tissues react to drugs usually regarded as exciting nerve-endings in the normal manner, even after the post-ganglionic segments have been allowed to degenerate. Thus, in the particular instance of adrenalin,

we have the following evidence. Lewandowsky[1] found that the various eye effects of suprarenal extract were still produced after degeneration of the post-ganglionic segments of the superior cervical sympathetic ganglion. This has also been confirmed by Langley[2]. Again, Langley showed that suprarenal extract excited a flow of saliva, in a typical manner, in a cat in which the superior cervical ganglion had been removed 10 days previously. In this same experiment Langley also observed the effect of the extract upon the blood vessels of the submaxillary gland, and upon the erector muscles of the hair in the face area, and found that the effects were the same as those produced normally, though the vascular effect was rather less distinct. In our previous paper upon the bronchial muscles[3], we describe an experiment in which we divided the vagus in a cat, and 56 days later tested the nerve and the reaction of the bronchial muscles to pilocarpine. We found the nerve trunk completely inexcitable, and pilocarpine produced absolutely no constriction of the bronchial muscles on that side on which the nerve had been divided, though it produced a typical result on the other side. In the light of our subsequent experience we are inclined to doubt the conclusion we then drew, viz. that the bronchoconstrictor nerve endings had completely degenerated. We think that it is possible that had we injected a larger dose of pilocarpine we might have observed a constriction. In any case, however, there is no question but that the nerve endings had become much less excitable. We think it important to test this experiment still further, especially as it stands as an isolated instance.

These degeneration experiments are of the greatest importance in connection with the view we hold that adrenalin acts upon nerve endings and not upon muscle fibre. If the nerve endings have really degenerated and adrenalin still produces its typical result our contention falls to the ground. It does not necessarily follow that because the excitability of the nerve trunk has completely disappeared that therefore the nerve-endings have also degenerated, especially when we remember that there is much pharmacological evidence that the nerve-ending is to be considered as something *sui generis* and not merely a terminal portion of the nerve fibre. We would further pass the criticism upon the two degeneration experiments of Lewandowsky and of Langley, that they had not allowed a sufficient time to elapse between the section of the

[1] Lewandowsky. *Arch. f.* (*Anat. u.*) *Phys.* 1899, p. 360.
[2] Langley. *Loc. cit.* p. 244.
[3] Dixon and Brodie. This *Journal*, XXIX. p. 141. 1903.

nerve and the testing of the nerve-ending by the adrenalin. We think that the evidence we adduce in this paper, apart from the degeneration experiments, conclusively proves that adrenalin acts on nerve-endings. Consequently we think that the fact that the extract still produces an effect, which be it noted, was weakened in the case of the submaxillary blood vessels, is evidence that the nerve-endings had not completely degenerated.

The view that suprarenal extract exerts its action directly upon muscle substance derives its origin from Oliver and Schäfer[1]. Their main reason for this conclusion was, that the muscle twitch given by a frog's somatic muscle which had been treated with suprarenal extract somewhat resembled that of a veratrinised muscle. We do not think that this fact affords any cogent reason for their supposition. If this form of reasoning is valid then surely the extract should be expected to act always in the same manner upon all smooth muscle, especially as the tissue is one of the least differentiated of the body. It must also be remembered how easy it is to obtain a slightly prolonged muscle twitch; even normal saline, made up without a calcium salt, will do this whilst other bodies which certainly act on nerve will also produce the same result. Of such substances oxycolchicine may be taken as an example. This drug, as we have satisfied ourselves, stimulates the same nerve-endings as pilocarpine, and its action is completely antagonised by a small dose of atropine, nevertheless it prolongs the muscle twitch markedly.

We have performed a few experiments on the eyes of anæsthetised cats, and have found, like Wessely[2], that subconjunctival injections of adrenalin produce decided dilatation of the pupil, which commences near the seat of injection and reaches its maximum in about fifteen minutes. If, in a similar manner, a small dose of barium-chloride be injected, a well-marked constriction develops, starting also near the seat of injection. Here then we have an instance of a drug which acts directly on muscle, producing contraction of the pupil when acting locally. So, too, if suprarenal extract acted on muscle directly the more powerful constrictor should again overcome the dilator muscle. The fact that it does not do so can only mean, either that the drug acts on sympathetic nerve-endings and not on cranial autonomic fibres, or that the two sets of similarly developed muscle fibres possess fundamentally different properties.

[1] Oliver and Schäfer. This *Journal*, XVIII. p. 230. 1895.

[2] Wessely. *Ber. üb. d. 28 Versamml. d. Ophth. Ges. Heidelberg*, 1900, p. 69.

Conclusions.

1. A method is described by which variations in the calibre of arterioles are determined by recording variations in the rate of flow of blood through the vessels when perfused at constant pressure.

2. Stimulation of the vaso-constrictor nerve supply to the vessels of the intestines or limbs gives a positive effect more than two hours after the death of the animal.

3. Adrenalin, pilocarpine, muscarine and barium-chloride all cause constriction when added to the blood perfused through the intestines or limbs.

4. In no case does excitation of any sympathetic or vagal nerve fibres passing to the lungs produce any effect upon the rate of flow of blood through the pulmonary vessels.

5. While barium-chloride produces a constriction similar to that observed in the systemic vessels, adrenalin, pilocarpine and muscarine all produce dilatation.

6. If the vaso-constrictor nerve-endings of the limbs or intestines be paralysed by apocodeine or curare, the constrictions usually produced by adrenalin, pilocarpine and muscarine are abolished or may even become converted into dilatations; *i.e.* these vessels then behave like the pulmonary vessels.

7. The pulmonary arterioles possess no vaso-motor nerve supply.

8. Barium-chloride acts directly upon muscle fibre. Adrenalin, pilocarpine and muscarine excite constriction by acting on nerve endings.

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