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No. 59

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By H. H. DALE and P. P. LAIDLAW.

(*From the Wellcome Physiological Research Laboratories, Herne Hill, S.E.*)

Introductory. During the last few years we have come across several instances of alkaloids producing the effects of sympathetic nerve stimulation when injected intravenously, but failing to reproduce these effects when applied to isolated tissues. The particular instance which first attracted our attention was that of the non-pregnant cat's uterus, which is characteristically inhibited in the body by a number of alkaloids which leave it unaffected, or cause it to contract, as an isolated organ. The investigation of this discrepancy formed the starting point of the experiments with which this paper deals, and in the course of which the problem has been extended to the consideration of other organs.

Action on the uterus.

The use of the horn of the cat's or rabbit's uterus, isolated from the body and suspended in warm oxygenated Ringer's solution, as a test-object for drugs, was introduced by Kehrer[1]. Fardon[2] used the same method, repeated Kehrer's observations and tested some additional drugs. Both these observers ascribe to nicotine an inhibitor action on the isolated uterus of the non-pregnant cat. This is hardly in accord with our experience. Of a large number of uteri giving pronounced inhibition with minute doses of adrenine, many have given no response to nicotine; some have acquired an increased tonus; a few have been weakly inhibited by large doses (10 mgms. in 250 c.c. Ringer's solution) such as Fardon used. The same is true of cytisine, the active alkaloid of laburnum, which, as we have shown elsewhere[3], has an action closely

[1] *Arch. f. Gyn.* LXXXI. 1. S. 160. 1907.
[2] *Biochem. Journ.* III. p. 405. 1908.
[3] *Journ. of Pharm. and exper. Therap.* III. p. 205. 1912.

similar to that of nicotine in almost all respects. So weak and infrequent is this inhibitor action, in our experience, that we have been inclined to attribute it to the presence of a few stray ganglion-cells, the horn of the isolated uterus being as a rule presumably ganglion-free[1]. With hordenine-methiodide, which, as one of us has shown[2], also produces effects closely resembling those of nicotine when injected intravenously, we have always observed a rise of tone of the isolated cat's uterus. (See Fig. 1.)

This type of discrepancy is not peculiar to the action of these "nicotine alkaloids." One of us has drawn attention to a very similar contrast in the cases of hydrastinine and of an artificial laudonosine derivative known commercially as "Lodal," both of which were shown to inhibit the uterus of the non-pregnant cat *in situ*, and to excite it to pronounced tonus when isolated[3]. Indolethylamine (de-carboxylated tryptophane) again showed a similar contrast in the experiments of the same observer[4]. Lastly we have the case of pilocarpine. Both Kehrer and Fardon describe this alkaloid as having a motor action on the cat's isolated non-pregnant uterus, and we have repeatedly confirmed this. On the uterus of the virgin guinea-pig,

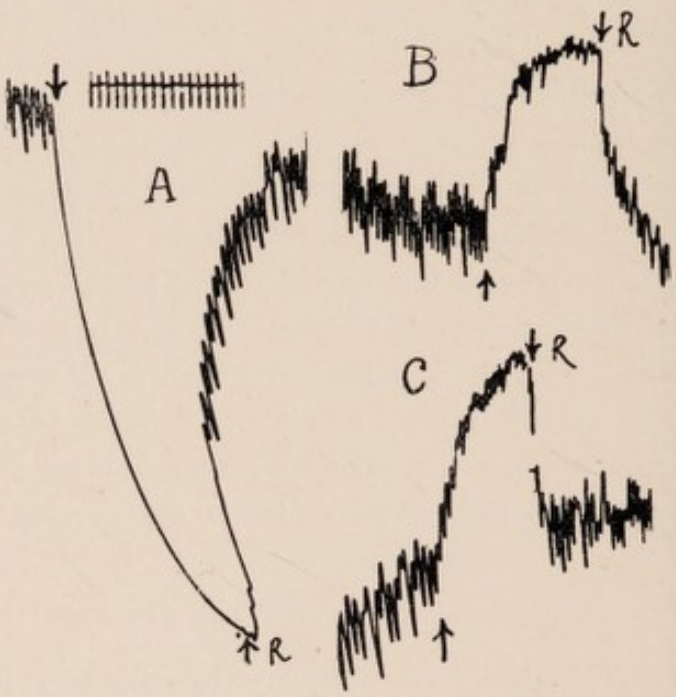

Fig. 1. Isolated uterus of virgin cat in 250 c.c. Ringer. *A*—0·05 mgm. adrenine. *B*—2 mgms. hordenine-methiodide. *C*—2 mgms. pilocarpine. *R*—*R*, fresh Ringer.

[1] The sympathetic ganglia supplying fibres to the cat's uterus are stated to consist of (1) a group in the neighbourhood of the cervices; a few small ganglia from this might be expected in some cases to spread along the uterine artery, though we have never detected them anatomically: (2) the spermatic (ovarian) ganglia situated near the origin of the ovarian artery from the aorta. Filaments from these and the inferior mesenteric ganglia accompany the ovarian vessels. (Langley and Anderson. This *Journal*, xx. p. 372. 1896.)

[2] Barger and Dale. This *Journal*, xli. p. 35. 1910.

[3] Laidlaw. *Biochem. Journ.* v. p. 243. 1911.

[4] Laidlaw. *Ibid.* vi. p. 141. 1911.

which, like that of the cat, is inhibited by stimulating the hypogastric nerves or by adrenine, we find that pilocarpine has a very intense motor action, whether the horn is isolated in Ringer's solution, or observed *in situ* with intravenous injection of the alkaloid. Yet Cushny[1] describes pilocarpine as producing, in the cat, effects on the uterus similar to those of hypogastric nerve-stimulation, of adrenine, or of nicotine; so that its effect on the non-pregnant organ *in situ* is usually inhibition, while its motor effect on the pregnant or recently parturient uterus is reversed by ergotoxine. We have confirmed Cushny's observation as to the inhibitor effect of pilocarpine on the non-pregnant cat's uterus in its natural relations, though the effect is less constant than we had anticipated. Cases have occurred, in our experience, in which a uterus inhibited by adrenine has been stimulated to contraction by pilocarpine even *in situ*. Indeed, such a motor effect of pilocarpine on the virgin cat's uterus, which is always inhibited by adrenine, is described and figured by Cushny[2] also in an earlier paper. But in the main our results agree with his; and what difference there is merely accentuates what is already clear from the effects on the isolated uterus, namely, that the action of pilocarpine on the uterus in the body is a complex of two effects at least: a direct motor action on the uterine muscle, seen still better in the guinea-pig, and an indirect action simulating that of the sympathetic nerve-supply. This indirect action of pilocarpine cannot be detected in the guinea-pig, and is quite possibly absent in that species. It seems more probable, however, that the difference of response in the guinea-pig and cat merely expresses a difference in the relative prominence of the two effects: the direct motor action on uterine muscle being, in the guinea-pig, sufficiently powerful to mask completely the indirect inhibitor action, while, in the cat, the former is comparatively weak and the latter usually predominates.

It may be noted, in passing, that this direct motor action of pilocarpine on uterine muscle cannot be held to correspond with any type of innervation. On the uterus of the guinea-pig, which is so peculiarly responsive to pilocarpine, we find, as Langley and Anderson found in the cat and rabbit, that stimulation of the pelvic nerve is quite without effect. Any doubt as to the vitality of the nerve or efficiency of the stimulus is removed by the powerful contraction of the rectum and bladder. Nor is it possible to suppose that this motor effect of pilocarpine is due to selective action on myoneural junctions corresponding to the motor sympathetic supply, which may be supposed to exist potentially

[1] This *Journal*, XLI. p. 233. 1910.

[2] *Ibid.* XXXV. p. 1. 1906.

even in the virgin; for the pilocarpine effect is absolutely resistant to ergotoxine, though immediately abolished by atropine. (Fig. 2.) The action is, indeed, very similar to that which pilocarpine exerts on the plain muscle of such organs as the bladder or intestine, receiving motor fibres from the oro-anal autonomic system, or the retractor penis of the dog or goat, receiving motor fibres from the true sympathetic. If the uterus had a motor supply from the pelvic nerves, the effect of pilocarpine would be attributed to action on the "nerve-endings" or "myoneural junctions" corresponding thereto, and would furnish another instance of the reproduction of oro-anal autonomic effects by this alkaloid. As it is, no such association can be suggested, unless it be supposed that the uterus has developed a sensitiveness to, or "receptive substance" for pilocarpine, in anticipation of a union with pelvic nerve fibres, which has not, in fact, taken place.

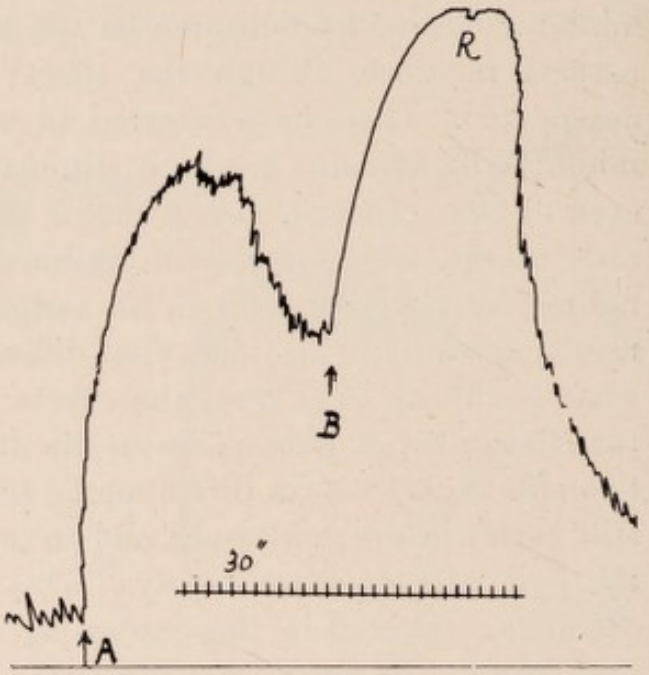

Fig. 2. Isolated uterus of virgin guinea-pig. *A*—2 mgms. ergotoxine. *B*—2 mgms. pilocarpine. *R*—fresh Ringer.

We have, then, in the case of all the alkaloids referred to above, an effect on the cat's uterus resembling that of sympathetic nerve stimulation; but this effect, unlike that of adrenine and the other sympathomimetic amines, does not survive excision of the organ. Several of them have, in addition, a direct motor action on uterine muscle; but the sympathetic effect, when inhibitor, is usually powerful enough to mask this when the uterus is observed *in situ*.

The sympathetic effect appears to be in all cases peripheral in origin, since it is not affected by severing the hypogastric nerves, and thereby interrupting the main nervous path to the uterus. We have not found it practicable to make certain of dividing all the post-ganglionic fibres without interfering with the circulation to the uterine horn; so that the possibility would appear to be open that these alkaloids produce their sympathetic effects by stimulating the peripherally placed ganglia, which are so situated as to be left behind in excising a uterine horn

from the body (*vid. sup.*). It is, indeed, practically certain that the nicotine alkaloids produce some of their sympathetic effect in this manner, and we accepted this action as a provisional explanation of the contrast in previous papers; but it is in more than one respect unsatisfactory. On the one hand it cannot be demonstrated that hydrastinine, or its synthetic analogue, or indolethylamine, has any effect on ganglia. They may be applied to the superior cervical ganglion, in solutions of any strength not sufficient to cause severe osmotic effects, without causing any perceptible movement of the pupil or nictitating membrane. On the other hand, Langley's experiments on the eye, with which we deal in the next section, have made it clear that, in addition to its action on ganglion-cells, nicotine can produce an effect of a more peripheral nature, simulating sympathetic stimulation. We shall show that the same is true of pilocarpine. The existence of such a peripheral sympathetic effect on the uterus cannot be directly proved; on account of the difficulty, mentioned above, of removing the ganglia or dividing the post-ganglionic fibres; but it is not reasonable to suppose that an action so marked on the plain muscle of the eye and orbit is absent in the case of the uterus.

It occurred to us that the anomaly would be explained if it could be shown that the sympathetic effect of these alkaloids on the uterus is in part the indirect result of an accelerating action on the secretion of adrenine. To the latter the non-pregnant cat's uterus is usually extremely sensitive, being perceptibly inhibited by minute injections of the base; so that a comparatively small increase in the rate of its outflow from the supra-renal glands would probably cause a distinct inhibition of uterine tone and rhythm. It is not easy to determine directly, in the case of nicotine and its allies, the extent to which an action of this kind contributes to their effect on the uterus, since their action on the ganglion-cells would remain after removal of the supra-renals. A demonstration that a dose of nicotine, given with the supra-renals intact, produced a more powerful effect than a second dose, given after their removal, would be susceptible of explanation on other grounds.

While our experiments were in progress, however, a paper was published by Cannon, Aub and Binger[1], in which, by examining blood from the vena cava, they directly demonstrated an increased output of adrenine from the supra-renal glands in response to injection of nicotine. On the other hand, the experiments of previous observers have failed to

[1] *Journ. of Pharm. and exp. Therap.* III. p. 379. 1912.

show any action of this kind in the case of pilocarpine. We have been content in the case of the uterus, therefore, to show (1) that uterine inhibition is readily produced in the non-pregnant cat by accelerating the output of adrenine from the supra-renal glands: (2) that, in the absence of the supra-renal glands, the hydrastinine group of alkaloids, having no stimulating effect on ganglion-cells, no longer inhibit the non-pregnant uterus *in situ*: (3) that blood from the cat's supra-renal vein after an injection of pilocarpine has a more powerful inhibitor (adrenine) effect on the isolated non-pregnant cat's uterus than blood obtained from the same source prior to the injection.

(1) It has been shown by Asher[1] that stimulation of the splanchnic nerves, after removal or exclusion from circulation of all the abdominal viscera except the supra-renal capsules, still causes some rise of blood-pressure, which he attributes to accelerated secretion of adrenine. We hear from Dr Elliott that he has confirmed and extended this observation. In our experiment the cat was completely pithed under a volatile anæsthetic, being thereafter kept under artificial respiration. The whole sub-diaphragmatic portion of the alimentary canal was ligatured off and removed, the liver, though left *in situ*, being thus removed from circulation. The splanchnics were dissected in the chest and stimulated together on one pair of electrodes. A string was attached to both horns of the non-pregnant uterus and led over pulleys to a counter-weighted lever, the hinder part of the animal being then immersed in a bath of warm saline. The lever was pulled down by contraction of the uterus, raised by the counter-weight when the uterus relaxed. Fig. 3 shows the effect of stimulating the splanchnic nerves, with the secondary coil at 12 cm. distance and 2 volts in the primary

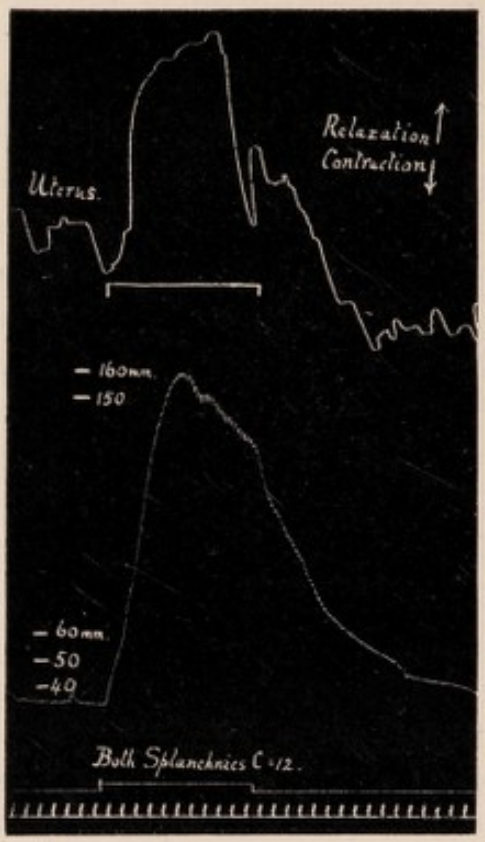

Fig. 3. Effect of stimulation of splanchnics on blood-pressure and uterus of non-pregnant cat (pithed completely and eviscerated).

[1] *Zntrlbl. f. Physiol.* XXIV. p. 10. 1910; and *Ztschr. f. Biol.* LVIII. p. 274. 1912.

circuit. It will be seen that a rise of about 120 mm. of blood-pressure occurs, accompanied by marked relaxation of uterine tone.

(2) The uterus of a non-pregnant cat was arranged to record as above. The spinal cord had been previously cut at the second vertebra under ether, the brain completely destroyed and artificial respiration applied. Fig. 4 shows the effect of injecting 10 mgms. of "Lodal." It will be seen that this causes a small rise of blood-pressure accompanied by pronounced inhibition of the uterus. The supra-renal glands were then excised, and the uterus promptly acquired an intense and persistent tonus, similar to that produced by the hydrastinine group of alkaloids on the isolated uterus. Further injections of 10 and 20 mgms.

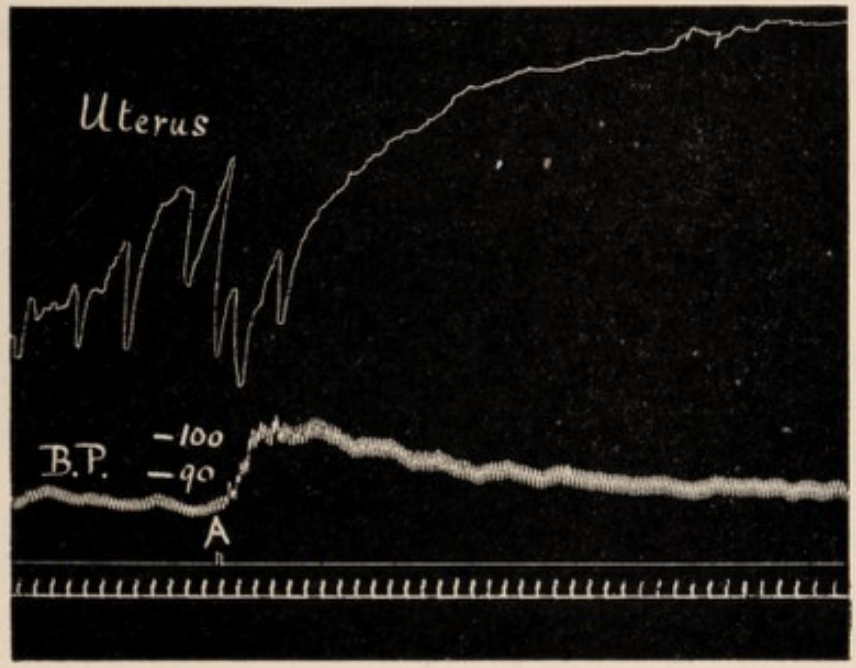

Fig. 4. Effect of 10 mgms. "Lodal" on blood-pressure and uterus of virgin cat.

of "Lodal" had then no trace of inhibitor action (Fig. 5), whereas the inhibitor effect of this alkaloid or hydrastinine can usually be reproduced by repeated injections, if the supra-renal glands are left *in situ*. A subsequent injection of adrenine produced the usual inhibition. It should be noted that the distance of the uterine tracing from the base-line in Fig. 5 is due to adjustment of the lever. The uterus was much more tonic at this stage than at the stage represented in Fig. 4.

(3) The blood was collected from both supra-renal veins of a cat under A.C.E. mixture by the method used by Biedl[1], Ehrmann, and others. The abdomen being opened, the whole alimentary canal, from the cardiac orifice of the stomach to the lower part of the rectum, was

[1] *Pflüger's Arch.* LXVII. p. 443. 1897.

removed, after ligature of all the vessels. The aorta and inferior vena cava were then tied at as short a distance as possible below the origin of the renal vessels. These latter were tied close to the hilus of the kidney on both sides. Cannulæ were inserted in the headward end of one external jugular vein, for collection of blood, and the heartward end of the other for injections. Through the latter, 50 mgms. of hirudin per kilogram of body weight were injected. A loose ligature was placed round the vena cava just above the point of entry of the right supra-renal vein, and small veins, entering the segment of vena cava between this and the permanent occlusion below the renal veins, were sought for and ligatured off. A clamp was applied a short distance above the lower ligature on the cava and a cannula for collecting blood inserted just below the clamp. By releasing the clamp and tightening the upper ligature, the blood from both supra-renal veins was made to flow out through the cannula. The rate of normal flow varied between very wide limits in different experiments; this, however, was explained by the discovery of small tributary veins from the lumbar muscles, left unligatured, whenever a rapid flow was observed. This necessarily diluted the supra-renal blood considerably, but even from such animals the results have comparative value, since the normal supra-renal blood (pure or thus mixed) obtained before pilocarpine, was in each experiment compared with blood collected under the same conditions as soon as possible after an injection of pilocarpine had been made. Before describing our own results we should note that neither Ehrmann[1] nor Tscheboksaroff[2] observed any change in the rate of output of

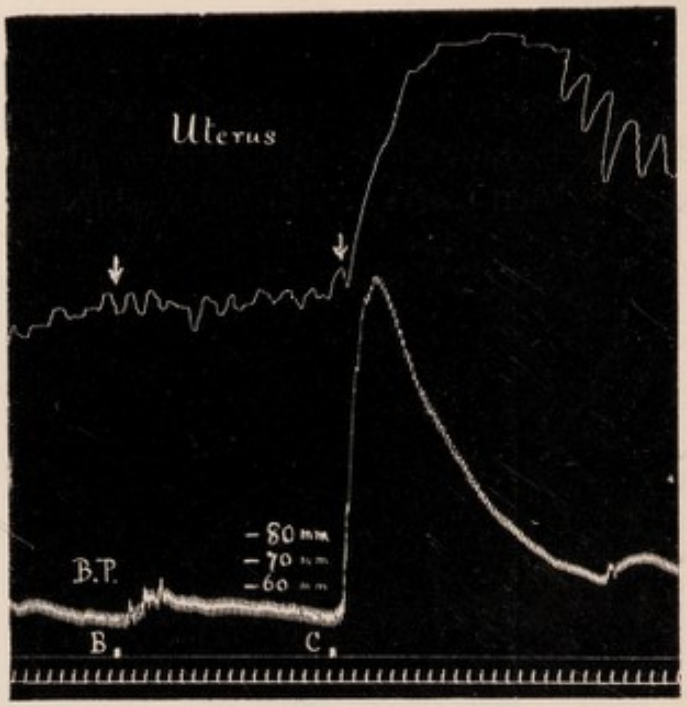

Fig. 5. Same experiment as in Fig. 4, but with supra-renal capsules removed. At *B*—20 mgms. "Lodal": at *C*—0·05 mgm. adrenine.

[1] *Arch. f. exper. Path. und Pharm.* LV. p. 44. 1906.

[2] *Pflüger's Arch.* CXXXVII. p. 59. 1911.

adrenine as the result of injecting pilocarpine. But Ehrmann[1] estimated the adrenine by use of excised frog's eyes, in which the specificity of the response is open to question and would, in any case, presumably be complicated by the presence of pilocarpine. Moreover, he used the rabbit, in which the action of pilocarpine on the supra-renal glands may be comparatively slight. Tscheboksaroff used the dog, draining one gland only, and estimated the adrenine by injecting the serum into a second dog, and observing the effect on the blood-pressure. His failure to obtain evidence of any increased output after giving pilocarpine is the more significant in that he observed a definite increase with physostigmine. It should be noted, however, that he cut the splanchnic nerves in all his observations. Elliott[2] observed no exhaustion of the supra-renal medulla in the cat when the splanchnics were cut and pilocarpine injected.

The chief object of our experiments being to ascertain whether pilocarpine injections do, in fact, increase the rate of adrenine secretion in the cat, rather than to elucidate the mechanism of such action if it occurs, we left the splanchnics intact, except in one experiment, in which they were cut at a later stage. The use of hirudin we consider important. In its absence there is recurrent difficulty from formation of clots in the cannula, which not only interrupts the experiments, but may seriously vitiate the result by producing a temporary venous congestion in the supra-renal glands. The use of hirudin is, further, important from another point of view. O'Connor[3] has pointed out that the physiological action of fresh serum, quite apart from adrenine content, destroys the value of physiological estimations of adrenine in serum by methods depending on motor effects on plain muscle, such as those of Fraenkel[4] with the rabbit's uterus, of O. B. Meyer[5] with isolated arterial segments, of Laewen[6] and of Trendelenburg[7] with perfusion of the frog's arterial system. We entirely agree with O'Connor's criticism. The physiological action of fresh serum on plain muscle, being, as O'Connor points out, of very similar type to that of β-iminazolylethylamine, is a serious complication even in methods of adrenine estimation depending on inhibitor effects, such as

[1] *Arch. f. exper. Path. und Pharm.* LIII. p. 97. 1905.
[2] This *Journal*, XLIII. *Proc. Phys. Soc.* p. xxxii. 1911.
[3] *Arch. f. exper. Path. und Pharm.* LXVII. p. 195. 1912.
[4] *Ibid.* LX. p. 395. 1909.
[5] *Zeit. f. Biol.* XLVIII. p. 353. 1906.
[6] *Arch. f. exper. Path. und Pharm.* LI. p. 415. 1904.
[7] *Ibid.* LXIII. p. 161. 1910.

the methods of Cannon and de la Paz[1], and of Hoskins[2], with isolated portions of rabbit's intestine, or our own method with the isolated uterus of the non-pregnant cat. Hirudin blood has, indeed, a small degree of this motor action on plain muscle, but far less than fresh serum.

We find the non-pregnant cat's isolated uterus a highly sensitive indicator of adrenine action. The effect, being inhibition, cannot be confused with that of other substances in the blood or serum which increase the tone of plain muscle. It shares this advantage with the rabbit's intestine, used by Cannon and his co-workers and by Hoskins. It has, in addition, the advantage, that varying submaximal doses of adrenine cause relaxation of varying rate and completeness; so that by finding a dose of adrenine which produces, on a given uterus, relaxation of the same speed and depth as a certain dose of blood, one can form a tolerably accurate estimate of the proportion of adrenine in the blood. As a general method it has the disadvantage that non-pregnant female cats are at times difficult to obtain, and that not all of them have uteri of the desirable high sensitiveness to adrenine. For our present investigation, however, it was the obvious choice.

We suspended a horn of the uterus in warm oxygenated Ringer's solution in the usual manner, using a small cylindrical bath holding 20 c.c., with the usual arrangements for changing the Ringer's solution without disturbing the organ or interrupting the record seriously. Addition to the bath of jugular blood, in any volume, if it produced any effect at all, caused only a small increase of tone. Normal blood from the supra-renal veins, on the other hand, always showed evidence of containing adrenine. The proportion present varied with the rate of outflow, being considerably greater when the flow was slow (pure supra-renal blood) than when it was rapid (small tributary veins discovered post-mortem). We performed five experiments in all, using a loop of rabbit's intestine as indicator in one. In every experiment there was a markedly supernormal proportion of adrenine in blood from the supra-renal vein collected after an injection of pilocarpine. In most cases, however, the rate of flow was much slower, owing to the cardiac inhibition. Allowance had to be made for this; for, if it were assumed that the supra-renal glands formed and secreted adrenine at constant rate, the mere retardation of the circulation would increase the content of the venous blood in inverse proportion to the rate of outflow. It was

[1] *Amer. Journ. of Physiol.* XXVIII. p. 64. 1911.

[2] *Journ. of Pharm. and exper. Therap.* III. p. 93. 1911.

necessary to show, therefore, that the increase of adrenine content was relatively greater than the decrease in rate of flow. The following are the details of the experiments. It will be seen that an accurate standardisation of the adrenine content was made in one case only. In others it was considered sufficient to show that the increase of adrenine was more than could be accounted for by diminished perfusion.

Exp. 1. Rate of flow from the vena cava slow. (Unmixed supra-renal blood.)

(*A*) Normal sample—3 c.c. collected in 45″.

(*B*) Sample 1′ after 4 mgms. pilocarpine—1·5 c.c. collected in 128″.

$$\frac{\text{Normal rate}}{\text{Pilocarpine rate}} = \frac{128 \times 2}{45} = 5{\cdot}7.$$

The samples were tested on an isolated non-pregnant uterus in 20 c.c. of Ringer's solution.

0·1 c.c. of *B* produced a relaxation of the uterus approximately equal to that produced by 0·1 c.c. of 1 : 100,000 adrenine. (Dilution in bath 1 in 20 millions.)

0·1 c.c. of *A* caused a scarcely perceptible relaxation.

0·5 c.c. of *A* produced a moderate effect.

0·05 c.c. of *B* produced a deeper relaxation than that caused by 0·5 c.c. of *A*, but less than that produced by 0·75 c.c. of *A*. (Fig. 6.)

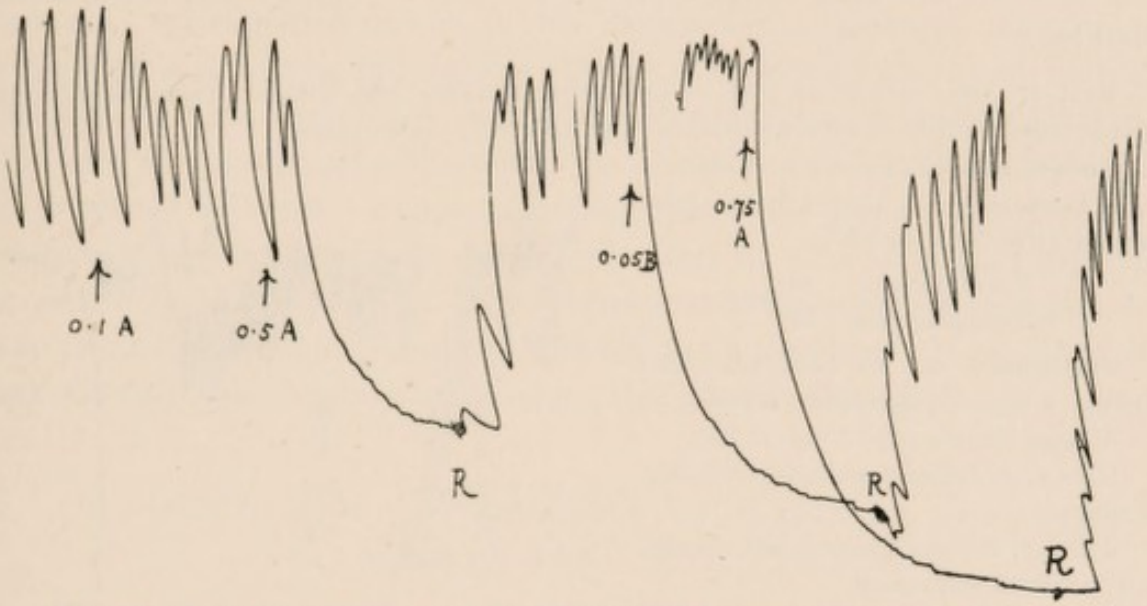

Fig. 6. Testing of supra-renal blood on isolated non-pregnant cat's uterus. See text (Exp. 1).

The adrenine content of *B* was, therefore, more than 10 times, but less than 15 times that of *A*—say 12 times. We have, then, rates of flow as 5·7 : 1,

adrenine content as 1 : 12.

Clearly the rate of output of adrenine has been at least doubled by the pilocarpine injection. During the effect of pilocarpine the blood from the supra-renal vein, entering the general circulation at the rate of about 1 c.c. in 85″, contains about 0·01 mgm. adrenine per c.c.

Exp. 2. Rate of flow slow. (Unmixed supra-renal blood.)
Normal samples (*A*)—3·5 c.c. in 91″.
Sample taken 1′ after 2 mgms. of pilocarpine (*B*)—3·5 c.c. in 242″.

$$\frac{\text{Normal rate}}{\text{Pilocarpine rate}} = \frac{242}{91} = 2{\cdot}8.$$

In this case a dose of pilocarpine blood caused only slightly greater inhibition of the isolated uterus than a dose of normal blood three times as large. The adrenine output per unit time was, therefore, not decidedly increased.

Exp. 3. The samples were tested on an isolated loop of rabbit's jejunum, atropinised to exclude pilocarpine action. A small tributary vein was left untied, so that a rapid flow of blood, comparatively poor in adrenine, was obtained.
Normal sample (*A*)—5 c.c. in 25″.
Sample taken 1′ after 4 mgms. pilocarpine (*B*)—5 c.c. in 26″.
Rates therefore equal.
1 c.c. *B* caused pronounced inhibition.
1 c.c. *A* caused much weaker inhibition.
2 c.c. *A* caused weaker inhibition than 1 c.c. *B*.

Rate of adrenine output per unit time is, apparently, more than doubled.

Exp. 4. One very small vein from lumbar muscles left untied. Flow accordingly rather rapid and adrenine content somewhat lowered by admixture.
Normal sample (*A*)—5 c.c. in 39″.
Sample taken 2′ after 4 mgms. pilocarpine (*B*)—5 c.c. in 83″.

$$\frac{\text{Normal rate}}{\text{Pilocarpine rate}} = \frac{83}{39} = 2{\cdot}1.$$

Splanchnics cut on both sides and further 4 mgms. pilocarpine injected.
3′ later sample (*C*)—5 c.c. in 99″.
0·5 c.c. *B* causes maximal relaxation of uterus.
0·25 c.c. *B* causes slower but eventually maximal relaxation.
1 c.c. *A* causes a scarcely perceptible inhibition (see Fig. 7); therefore adrenine strength of *B* is much more than four times that of *A*, whereas the rates of flow were as 1 : 2.

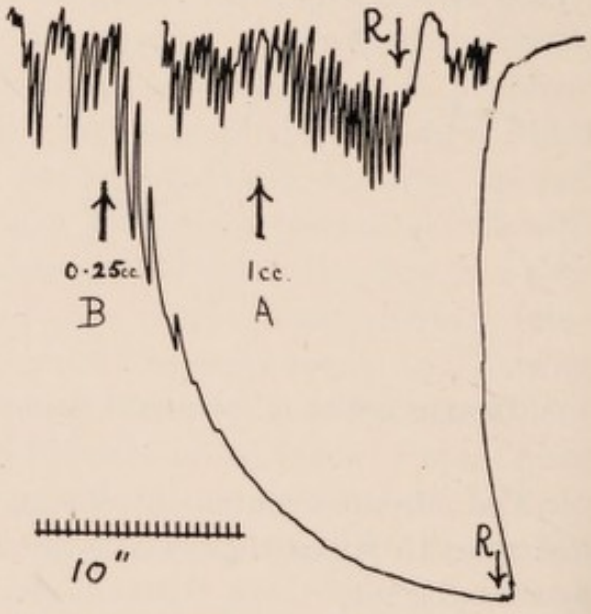

Fig. 7. Similar to Fig. 6. See text (Exp. 4).

Pilocarpine has, therefore, more than doubled the rate of adrenine secretion.

Later, the uterus having become less sensitive, so that 0·25 c.c. *B* produced an incomplete relaxation, 0·5 c.c. *C* produced full relaxation. Apparently, therefore, section of the splanchnics has not materially

reduced the rate of output of adrenine under pilocarpine, when once started. Tested against a solution of adrenine, made up by diluting a 1 in 10,000 solution with jugular blood, 0·5 c.c. *B* gave a relaxation practically identical with that produced by 0·25 c.c. 1 in 100,000 adrenine. The concentration in *B* is therefore approximately 1 in 200,000. Blood containing this concentration of adrenine is entering the general circulation, after injection of 4 mgms. pilocarpine, at the rate of 5 c.c. in 83″, or 1 c.c. in 17″. 0·01 mgm. of adrenine therefore enters the circulation in 34″.

The cat used in this experiment was much larger and in better condition than that used in Experiment 1, in which, after the same dose of pilocarpine, 0·01 mgm. of adrenine was secreted in 85″.

Exp. 5. Normal sample (*A*)—3 c.c. in 3′.
Sample after 3 mgms. pilocarpine (*B*)—1 c.c. in 2′.

$$\frac{\text{Normal rate}}{\text{Pilocarpine rate}} = 2.$$

The quantities collected being small, it was merely ascertained that, on a not very sensitive uterus, 0·4 c.c. *B* caused a pronounced relaxation, while 0·8 c.c. *A* had no preceptible effect. Pilocarpine, therefore, halved the rate of flow, but much more than doubled the adrenine content. Out of five experiments, therefore, we have obtained definite evidence of increased adrenine output from the supra-renal glands, as the result of giving pilocarpine, in all except one (Exp. 2). In one case (Exp. 1), in which the rate of output was determined with moderate accuracy, it was found to be approximately doubled by pilocarpine: in another it was shown to be certainly much more than doubled (Exp. 4).

Effects of the nicotine group of alkaloids and of pilocarpine on the eye of the cat.

Effects on the normal eye. Langley and Dickinson[1] showed that the effects of nicotine on the pupil and nictitating membrane were the resultant of two opposed actions, referable to the cranial autonomic and the sympathetic nerve supplies. In the cat, effects referable to the sympathetic were usually predominant, so that the first effect of injecting nicotine intravenously was usually dilatation of the pupil, retraction of the nictitating membrane, widening of the palpebral fissure. These effects could be produced in uncomplicated form by painting nicotine on the superior cervical ganglion: but Langley and Dickinson

[1] This *Journal*, xi. p. 265. 1890.

found that, when one superior cervical ganglion was excised, intravenous injection of nicotine produced this complex of cervical sympathetic action nearly as well in the deganglionated as in the normal eye, though they noted that the effect usually began some five seconds later in the former. From this observation it would appear that, in addition to its characteristic stimulation of autonomic ganglion-cells, nicotine has a more peripheral action. A similarly peripheral action of nicotine on the movements of the small intestine was described by Langley and Magnus[1], who found that nicotine caused inhibition of these movements in a loop of cat's jejunum after section of all post-ganglionic nerve-fibres running to it, and that this inhibition was "not one whit less than normal" even after degeneration of practically all such post-ganglionic fibres.

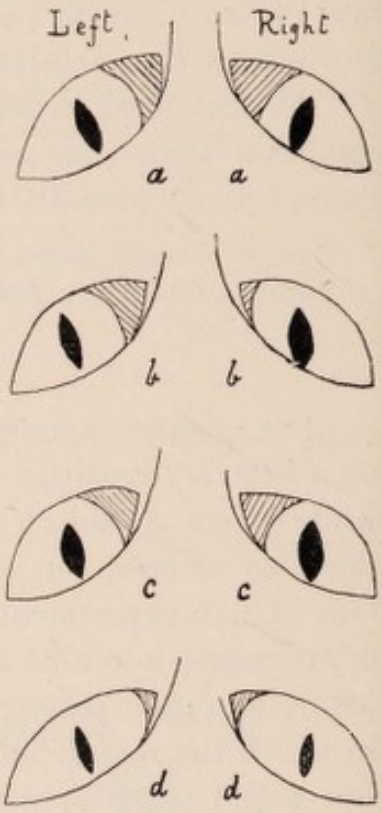

Fig. 8. Line drawings from photographs of eyes of cat under A.C.E. *a*, Normal. *b*, 2 % pilocarpine applied to right superior cervical ganglion. *c*, After excision of this ganglion. *d*, After subsequent injection of 2 mgms. pilocarpine intravenously.

We have shown elsewhere that, in their action on the cat's eye, as in practically all other respects, cytisine and hordenine-methiodide closely resemble nicotine.

In the case of pilocarpine the most marked action on the eye is the intense constriction of the pupil, which, as Anderson has shown, is not impaired by complete denervation. This is sufficient under ordinary conditions to mask completely any sympathetic effect of pilocarpine on the dilator of the pupil. There is another action of pilocarpine, however, to which we have found no previous reference, in which it reproduces the effect of the sympathetic nerve-supply to the orbit and not of that from the third cranial nerve. We refer to the quite obvious retraction of the nictitating membrane and the less marked, but definite widening of the palpebral fissure which follow the intravenous injection of pilocarpine. In producing these effects the action of pilocarpine is closely parallel to that of nicotine and the other alkaloids above mentioned, except that it is slower and much more persistent. If the superior cervical ganglion be exposed and a 1 to 2 % solution of

[1] This *Journal*, XXXIII. p. 34. 1905.

pilocarpine nitrate applied to it carefully with a soft brush, as in Langley's nicotine experiments, the effects of sympathetic stimulation are visible in the corresponding eye. The dilatation of the pupil is small and comparatively brief, for, as traces of pilocarpine become absorbed into the circulation, the effect is quickly annulled by the much more powerful stimulation of the sphincter. The retraction of the nictitating membrane, on the other hand, persists. Fig. 8 *b* illustrates the contrast between the two eyes in this respect[1]. It represents a photographic record from a cat under A.C.E. mixture. Both cervical sympathetic nerves have been cut and 1 % pilocarpine applied to the right superior cervical ganglion.

The fact that the effect is due to stimulation of the ganglion-cells is confirmed by its rapid disappearance when the ganglion is excised (Fig. 8 *c*). Pilocarpine has therefore a stimulating action on sympathetic ganglion-cells, similar to though weaker than that of nicotine, in addition to its more striking peripheral effects. But again, as in the case of nicotine, pilocarpine produces these effects of sympathetic stimulation after the ganglion has been removed or the post-ganglionic fibres cut. Fig. 8 *d* shows the effect of injecting 2 mgms. of pilocarpine intravenously after excision of the right superior cervical ganglion. It will be seen that, in addition to constriction of the pupil, retraction of the nictitating membrane occurs equally on the two sides.

Effects after degeneration of peripheral sympathetic neurones. These peripheral sympathetic effects of nicotine and pilocarpine survive the degeneration of the post-ganglionic fibres arising in the superior cervical ganglion. In the experiment which we made on this point, degeneration was allowed for 13 days after excision of one ganglion with aseptic precautions. For testing the effect of degeneration the animal was again anæsthetised with chloroform and A.C.E. mixture. The pupil on the operated side was throughout markedly hypersensitive to sympathetic effects, showing the paradoxical dilatation as compared with the normal eye[2], even before anæsthesia was complete. When the animal was fully under the anæsthetic 2 mgms. of pilocarpine caused complete retraction of both nictitating membranes and dilatation of both pupils. In the normal eye this was succeeded by a phase of constriction. In the eye

[1] In preparing all the figures of this kind the same process has been followed. Instantaneous photographs were taken, and enlarged outline drawings of the important features made from these with a pantograph. It should be added that the width and shape of the palpebral fissure are not in all cases significant, since the eyelids were, in some instances, held gently apart, to facilitate the photographing of the pupil.

[2] Cf. Anderson. This *Journal*, xxx. p. 290. 1903.

on the operated side the pupil became maximal and remained so for the rest of the experiment, in spite of vigorous artificial respiration, to exclude the possibility of an asphyxial effect, and a further injection of 4 mgms. of pilocarpine. In the normal eye a secondary dilatation followed the constrictor phase, but the pupil became smaller again under the further dose of pilocarpine. The pupil on the operated side being already maximal, after the first dose of pilocarpine, no further dilatation with nicotine was possible. After the immediate effects of the first injection of nicotine had passed off, however, the nictitating membranes came somewhat forward, as normally happens. It was then possible to observe, with a further injection of nicotine (3 mgms.), that the nictitating membrane was retracted more rapidly and fully on the operated than on the normal side. Although, therefore, the experiment was not in all respects satisfactory, clear information was obtained on the main point under investigation: it was proved that the sympathetic effects of pilocarpine and nicotine on the eye, already known to be produced in the absence of the superior cervical ganglion, are still produced after degeneration of the post-ganglionic fibres, and produced apparently with supernormal intensity. In other words, the effect resembles that of adrenine, and one can so far sum up the sympathetic effects of this group of alkaloids on the eye as a combination of action on the cells of the superior cervical ganglion with a peripheral effect of the type which one of us has elsewhere termed "sympathomimetic."

Effects on the isolated iris. Again, however, as in the case of the uterus, these effects of the nicotine alkaloids are not seen on the isolated cat's iris. The preparation is made as follows.

A cat is killed, by pithing the brain through the foramen magnum and the eyes are excised as quickly as possible. The cornea and sclerotic are cut through close to the ciliary region. The vitreous humour and lens are removed, leaving only a ring of sclerotic with the ciliary body and iris. The preparation is dropped into a beaker of Ringer's solution kept at 37°—39° C., through which oxygen is bubbled. The beaker may conveniently be stood in a white enamelled vessel also containing Ringer's solution, kept at the same temperature by a small flame underneath. The preparation is arranged with the anterior surface of the iris uppermost. The drug to be tested is added to the beaker, due precautions being taken not to alter the temperature of the contents, and, when its effect is complete, the preparation can be washed in the solution in the outer vessel, while that in the beaker is changed.

Under such conditions of isolation the pupil rapidly constricts to a small slit. All the true sympathomimetic amines which we have tested promptly dilate it, the effect attaining its maximum in 1—2 minutes. In clean Ringer the pupil constricts again and the preparation retains its sensitiveness for hours. Nicotine, cytisine and hordenine-methiodide produced no perceptible effect in doses up to 10 mgms. in 75 c.c. of Ringer's solution. We have not tested larger doses, as we considered that effects, if any were obtained, with higher concentrations would be of doubtful significance, while it was already clear that these alkaloids, in concentrations many times as great as that produced in the blood of the animal by intravenous injection of an effective dose, had no effect on the isolated iris. Fig. 9 *a* shows a photograph of the isolated iris before

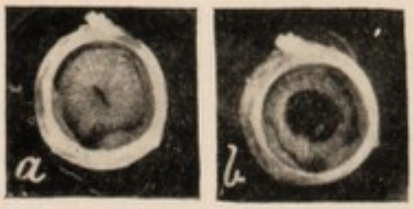

Fig. 9. Isolated iris of cat. *a*, Normal. *b*, 5 mgms. of p.-hydroxyphenylethylamine added to the bath (75 c.c.).

addition of any drug to the bath. It would also serve, without modification, to represent the appearance after addition of one of the nicotine alkaloids. Fig. 9 *b* is a photograph of the same preparation taken 2 minutes after adding 5 mgms. of p.-hydroxyphenylethylamine to the bath. Similar effects were produced by 1 mgm. of methylaminoethylcatechol and by 0·1 mgm. of adrenine.

Significance of the supra-renal capsules. In investigating the part played by supra-renal secretion on the peripheral effects of these alkaloids we proceeded as follows. In cats under A.C.E. or ether we extirpated the superior cervical ganglion on one side and cut the cervical sympathetic nerve on the other, thereby excluding the interference of reflex or central effects. In the first experiment the supra-renal capsules were also removed completely. Intravenous injection of 2 mgms. of pilocarpine nitrate then caused retraction of the nictitating membrane only on the side retaining the ganglion, that on the extirpated side remaining perfectly stationary. Both pupils became intensely constricted. Intravenous injection of nicotine, hordenine-methiodide, or cytisine (2—3 mgms. of each) caused a rather weak and evanescent dilatation of the pupil and retraction of the nictitating membrane on the side retaining the

ganglion only. On the extirpated side the only perceptible effect was a forward movement of the nictitating membrane so as to cover the pupil almost completely. Enough was visible, however, to allow the observation that no dilatation occurred. Clearly, then, under these conditions, the sympathetic effects of all these alkaloids were reduced to a direct action on the cells of the superior cervical ganglion, which, moreover, was weak and evanescent as compared with the effect seen in the animal possessing intact supra-renal capsules.

We next sought a method of excluding the supra-renal capsules temporarily from circulation. This was accomplished by passing a thin string round the aorta just above the origin of the cœliac axis. The aorta is found at this point by dividing the peritoneum and dissecting through the lower fibres of the left pillar of the diaphragm. By traction on the loop the aorta is occluded completely at this point, so that the body tailwards of the block, including the supra-renal capsules, receives only such blood as can reach it by anastomoses, which, during a temporary occlusion of about 1 minute, must be negligible in amount. It was necessary, in the first instance, to control the effect of simple occlusion and release of the aorta, without injection of any drug[1]. In the cat, under A.C.E. mixture or chloroform, occlusion up to 2 minutes and release usually produced no perceptible effect on the pupil or nictitating membrane. In one cat under A.C.E., while occlusion for 1′ produced no effect, occlusion for 2′ was followed, a few seconds after release, by a slight, rapidly evanescent dilatation of the pupil, without perceptible movement of the nictitating membrane. Since, in the experiments with drugs, the maximum period of occlusion was 1′ and the effects on release very marked, this trifling effect after 2′ closure cannot be regarded as invalidating the results. Under ether the effect of closure and release, without injected drugs, is more marked, a very definite, though still small dilatation occurring after occlusion for only 1′, and a very small effect even after 30″. This is in accordance with Elliott's[2] observation that ether is itself a stimulant of adrenine secretion, prolonged etherisation leading to exhaustion of the capsular medulla. But under ether the after-effects of the alkaloids are also proportionately increased, so that again the control does not in any way invalidate the results. Nevertheless we made only one experiment under pure ether, in order to avoid this complication.

The animal being deeply under the anæsthetic, so that automatic

[1] Cf. Popielski. *Pflüger's Archiv*, CXXXIX. p. 571. 1911.

[2] This *Journal*, XLIII. *Proc. Phys. Soc.* p. xxxii. 1911.

fluctuations of the pupil and movements of the nictitating membrane were quite absent, the aorta was occluded, a cannula in the femoral artery connected to a mercury manometer allowing the completeness of the occlusion to be verified. The alkaloid to be tested was then injected by the jugular vein. Under such conditions 2—4 mgms. of pilocarpine produced fairly rapid retraction of the nictitating membrane on the side retaining the ganglion (Fig. 10 *b*). Typically the membrane on the extirpated side was unaffected, but in several instances it was also retracted to a small extent. Possibly this indicates a true peripheral action of pilocarpine on Müller's muscle, but we shall discuss this point later. Both pupils became intensely constricted. When the effect was complete, *i.e.* in 1′ or less, the aorta was released. A very considerable relaxation of the now intense constriction of the pupils occurred, lasting for several seconds: the nictitating membrane on the side retaining the ganglion, if not already retracted completely, became so, whilst that on the deganglionated side, which in many cases had not moved up to this point, also retracted more or less completely (Fig. 10 *c*). The dilatation of the pupil, being antagonised by the direct action on the sphincter, was evanescent: the retraction of the nictitating membranes, on the other hand, was long persistent, like that produced by pilocarpine in an animal with normal supra-renal circulation. We had, in fact, simply divided this normal effect into two stages, a first due to action on the ganglion-cells and a second due to accelerated secretion of adrenine and appearing, in this experiment, only on release of the aorta. After a large dose of pilocarpine the dilator effect on the pupil could be repeatedly evoked by occluding the aorta for 30″ to 1′ and releasing it again. As the effect of pilocarpine waned, as shown by escape of the heart from its inhibitor action, the after-effect of such periods of aortic occlusion likewise diminished.

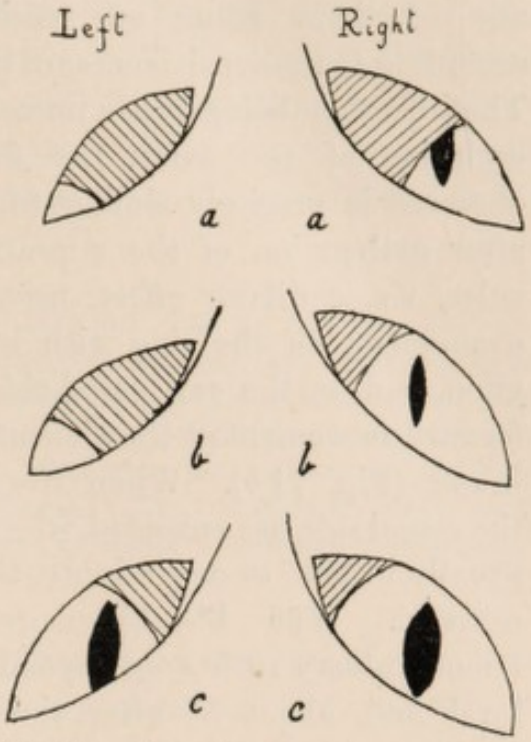

Fig. 10. Eyes of cat under A.C.E. Left superior cervical ganglion excised. *a*, Normal. *b*, 2 mgms. pilocarpine intravenously with aorta occluded. *c*, Aorta released.

With nicotine, cytisine, and hordenine-methiodide the effects were considerably more striking, and were of the same nature in the case of each of these alkaloids, 1 to 3 mgms. of any of them being an efficient dose, according to the sensitiveness of the animal. The injection being made immediately on occlusion of the aorta, the first result observed is precisely similar to that seen after extirpation of the supra-renal capsules, viz. a dilator effect, usually rather evanescent, on the side with intact ganglion, and on the extirpated side a simple forward movement of the nictitating membrane (Fig. 11 *b*). When the effect on the sound side has subsided, which happens usually in half to one minute, the aorta is released. The blood-pressure in the femoral artery rushes up beyond its initial level and, about 5″ after the release, a secondary full dilatation of both pupils and retraction of both nictitating membranes occur (Fig. 11 *c*). The extent and persistence of this vary somewhat widely in different cats, but it has been, in our experiments, almost invariably more extensive and persistent than the primary ganglionic effect. So that there is no room for doubt that the larger factor in the sympathetic effects of these alkaloids on the eye, as seen in the normal cat under A.C.E. or ether, is not their direct action on the ganglion-cells, but the action of adrenine, poured into the circulation in response to their injection.

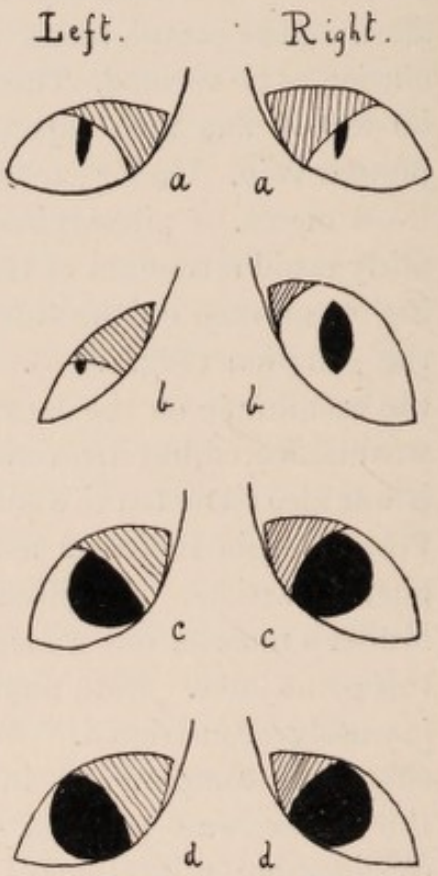

Fig. 11. As in Fig. 10. *a*, Normal. *b*, 3 mgms. nicotine intravenously with aorta occluded. *c*, Aorta released. *d*, 3 mgms. nicotine intravenously without occlusion of aorta.

Effect of section of the splanchnics. The mechanism of this supra-renal stimulation is not quite clear. Elliott has shown that almost any influence which disturbs the normal blood-pressure, either by causing a prolonged rise or a prolonged fall, tends to exhaustion of the supra-renal medulla, provided the splanchnics are intact. It seemed probable, therefore, that part of the effect might be of central origin, and experiments made with the splanchnics cut on both sides confirmed this suggestion. The section of the splanchnics did not abolish the

secondary effect of the nicotine group following release of the aorta, but it was markedly weaker than in the animal with intact splanchnics. So weak was it, indeed, in one experiment under A.C.E. mixture, and one under paraldehyde, that the effect of nicotine on the normal eye, with intact ganglion and with the supra-renal circulation free from interference, was obviously diphasic, the primary ganglionic effect almost subsiding before the weak secondary effect due to supra-renal secretion appeared. The following extract from an experiment under paraldehyde illustrates this point.

The cervical ganglion was extirpated on the left side. The cat had received 1 c.c. paraldehyde per kilo by rectal injection, which sufficed to maintain deep anæsthesia after the initial dissection, during which a little chloroform was given in addition. The splanchnics were divided on both sides. 11.15 a.m. 2 mgms. of nicotine injected into the jugular vein, the aorta being patent throughout. The right pupil dilates and the right nictitating membrane retracts. The left moves forward. The first effect on the right eye passes off. Then, about 10—15″ after the injection, a secondary dilatation of both pupils and retraction of both nictitating membranes occur.

Whereas, therefore, in the normal animal the direct (ganglionic) and indirect (supra-renal) actions merge into a continuous effect, section of the splanchnics produces a separation similar to that caused by temporary occlusion of the aorta. This may be taken to indicate that the supra-renal component of the normal effect is partly central, partly peripheral in its origin. In the case of pilocarpine, the evidence from experiments of this kind appears to indicate that the effect on the supra-renal glands is mainly central. In an experiment under A.C.E. with cut splanchnics, we observed no retraction of the nictitating membrane on the deganglionated side following injection of pilocarpine, either on release of the aorta after temporary occlusion, or when it was left patent throughout.

We have given in a previous section evidence obtained by direct examination of blood leaving the supra-renals, according to which the flow of adrenine, evoked by pilocarpine, is not noticeably reduced by section of the splanchnics when it has once become established. It seems probable, then, that pilocarpine, like the nicotine alkaloids, has some effect of a more peripheral nature, but the relative importance of central and peripheral action would need further and more complicated experiment for its determination. Our main concern has been to establish the importance of an action on the supra-renal glands, whether through the central nervous system, or through some more peripheral mechanism, such as the ganglion-cells.

Effects under urethane and on the pithed animal. If our experiments had been limited to animals under the anæsthetics mentioned above, we should have regarded the peripheral effect of the nicotine alkaloids on the pupil etc. in the absence of the superior cervical ganglion, as entirely due to accelerated adrenine secretion. In the case of pilocarpine we have seen that, even under these anæsthetics, some retraction of the nictitating membrane may be produced after removal of the supra-renals and the ganglion; but this could reasonably be referred to the stimulating effect of this alkaloid on plain muscle in general, and apparently apart from innervation, to which we have referred in a previous section. A third type of effect, however, bearing at least a strong resemblance to sympathetic stimulation, but persisting after the supra-renals and the ganglion have been removed, can be observed with nicotine and its pharmacological analogues also, if the animal be anæsthetised either with urethane or by destroying the brain and cord under a volatile anæsthetic, subsequently removed by artificial respiration with pure air. Under such conditions the typical effect of the nicotine alkaloids, on the eye deprived of the superior cervical ganglion, is forward movement of the nictitating membrane and dilatation of the pupil. On the side retaining the ganglion a first injection of 2 mgms. of any of these alkaloids causes the typical ganglionic effect, both dilatation of the pupil and retraction of the nictitating membrane being present. But the ganglion-cells, in an animal deprived of the supra-renals, seem very susceptible to the paralytic action of nicotine etc., possibly because the blood-pressure is low and the circulation feeble. Subsequent injections accordingly produce an effect of the same type on both sides, viz. slow dilatation of the pupil, without movement of the nictitating membrane. This dilatation can hardly be due to direct action on plain muscle, since it fails on the excised iris. Nor is it due to rise of intraocular tension, caused by rise of general blood-pressure (cf. Henderson and Starling[1]); for it is still produced by injections of the nicotine alkaloids, when, on account of ganglionic paralysis, they no longer produce any blood-pressure effect.

Under similar conditions pilocarpine causes maximal retraction of both nictitating membranes, the only difference being that retraction on the side retaining the superior cervical ganglion begins earlier and proceeds more rapidly. This action is possibly referable to direct action

[1] This *Journal*, xxxi. p. 305. 1904.

on the plain muscle of the floor of the orbit. We have not found it possible to treat this as an isolated organ.

It remained to be tested whether these peripheral effects, seen under urethane, or without chemical anæsthetics, and independent alike of ganglion-cells and of supra-renal secretion, survived the degeneration of the peripheral sympathetic neurones.

The left sup. cerv. ganglion was removed from a cat under ether, and the animal kept for ten days. The cat was then anæsthetised with urethane (1½ grms. per kilo) given hypodermically. An hour after this had been injected a little chloroform was given on a mask to deepen the anæsthesia. Both vagi and cervical sympathetics were cut, both supra-renal capsules removed, and cannulæ put in the external jugular vein, for injections, and the femoral artery, for record of the blood-pressure. Thereafter chloroform was discontinued, deep anæsthesia being maintained by the urethane. The following extracts from the experimental record show the effects of nicotine and hordenine-methiodide.

11.50. Both pupils small and nictitating membranes partly forward: left pupil somewhat larger and n. m. less forward than right. (Slight paradox.) Inject 3 mgms. nicotine. The right eye shows an immediate "ganglionic" effect—rapid dilatation of the pupil (rather small) and full retraction of the n. m. This lasts only a few seconds, the pupil constricting again, and the n. m. moving forward beyond its initial position. Meanwhile the n. m. on the left side moves forward from the first. A secondary dilatation of the pupil then begins on the left side, soon followed by the right. This proceeds till the left pupil is maximal, the right ¾ dilated. The left n. m. then slowly retracts till only a small portion is visible, the right remaining forward. The dilatation of the right pupil passes off while the left continues maximal, with ¾ retracted n. m. and wide palpebral fissure. The effect does not subside fully for about 15 mins.

12.10. Aorta ligatured just below the diaphragm.

12.15. 4 mgms. hordenine-methiodide injected. The effect was substantially similar to that of nicotine. The secondary dilatation of the right pupil was comparatively small and evanescent. On the left side the n. m. retracted more completely, the pupil dilated less fully than after nicotine; but the effect was a similarly prolonged one.

We see, then, that the peripheral effect of these alkaloids, as observed under urethane after removal of the supra-renals and the superior cervical ganglion, not merely persists after degeneration of the post-ganglionic sympathetic fibres, *but is greatly exaggerated by such degeneration.* The plain muscle of the eye and orbit acquires, under these conditions, an abnormal sensitiveness to this peripheral action, precisely similar to that which it acquires to the action of adrenine (cf. Meltzer and Auer[1], Elliott[2]).

It remained to be seen whether this abnormal sensitiveness was retained by the isolated iris. The cat was killed, and the eyes removed and the irides isolated in 100 c.c. of oxygenated Ringer's solution as described earlier in this section. Nicotine in doses of 1 to 10 mgms. produced no trace of effect on the pupil of the left

[1] *Amer. Journ. of Physiol.* xi. p. 28. 1904. [2] This *Journal*, xxxii. p. 401. 1905.

(deganglionated) eye; the latter was as completely refractory as the normal isolated iris. Ten mgms. of hordenine-methiodide, after washing of the iris and transference to fresh Ringer's solution, merely intensified the constriction of the pupil. But this left iris, transferred again to fresh Ringer's solution, showed a very abnormally high sensitiveness to adrenine. 1/50 mgm. (*i.e.* one in five millions), which had no perceptible effect on the right (normal) isolated iris, caused a prompt and very rapid dilatation of the left pupil. Later, in fresh Ringer, a very distinct effect was obtained with 1/250 mgm. (*i.e.* 1 in 25 millions).

We have, then, this suggestive series of facts. The cat's eye rendered supersensitive to adrenine by degeneration of its sympathetic nerve-supply, shows a similar supersensitiveness to a peripheral sympathetic effect which follows injection of the nicotine alkaloids, and which, in an animal under urethane, is not abolished by removal of the supra-renals or exclusion of all abdominal viscera from circulation. But the iris, so supersensitive to this effect in the body, is as indifferent to the nicotine alkaloids as the normal iris when isolated. We have seen that the peripheral effect of these alkaloids in the cat under chloroform or ether is definitely traceable to increased output of adrenine from the supra-renal glands. The suggestion is almost irresistible, that the remarkably similar effect, which, under urethane, persists after decapsulation, is also due to the production, in response to the injection, and from some other source, of a substance having, at least in this direction, an adrenine-like effect. No other conception of this action which has yet presented itself to us accords so well with its time relations and with its total disappearance when the responsive tissue is treated as an isolated organ. We are not, however, in a position to present it as anything more than an attractive speculation. We hope by further experiment to attain direct evidence for or against it. At present we can only settle one point, namely, that the "other source" of such a hypothetical substance cannot be identified with the minor deposits of chromaffin tissue scattered over the sympathetic system, nor with sympathetic ganglia proper. If it were so, ligature of the aorta at the diaphragm, excluding from circulation all but a small portion of the sympathetic system, should assuredly reduce the effect to a minimal trace. We have seen, on the contrary, that this procedure makes little, if any difference in its intensity.

Summary and discussion.

Our main results may be summarised as follows.

(1) Certain alkaloids, which produce the effect of sympathetic nerve-stimulation on the cat's uterus *in situ*, fail to do so when the organ is isolated from the body. The same is true of the action of some of them on the cat's pupil.

(2) The factors contributing to this sympathetic effect are, (*a*) a stimulating action on sympathetic ganglion-cells, seen in the cases of nicotine and its allies, and of pilocarpine: (*b*) acceleration of the rate of adrenine secretion from the supra-renal glands: (*c*) a peripheral sympathomimetic effect, seen in the animal under urethane, or anæsthetised by destroying the brain, and independent of the ganglia and the supra-renal glands. This effect, which we have only observed certainly in the case of the nicotine alkaloids, is greatly increased, like that of adrenine, by degeneration of the peripheral sympathetic neurones. The nature of this effect, whether direct or indirect, will be further investigated.

The importance of these different factors varies with the different alkaloids and with different methods of anæsthesia. In the cases of the hydrastinine group, and of indolethylamine, there is no evidence of a ganglionic effect, nor of any peripheral sympathomimetic action in the absence of the supra-renal glands. Probably the sympathetic effects produced by these alkaloids on the uterus *in situ* are to be referred entirely to accelerated output of adrenine. The nicotine alkaloids and pilocarpine have a marked stimulant action on sympathetic (among other) ganglion-cells; but in the animal under chloroform or ether, with well-stored supra-renal medulla, this action on the ganglia is almost overshadowed by the large effect of the outpouring of adrenine which results from the injection of one of these alkaloids. Thus in Langley and Dickinson's experiments, made on the cat under chloroform or ether, the effect of nicotine on the eye deprived of the superior cervical ganglion, which they found to be practically equal to that on the intact side, was presumably not a direct nicotine effect, but the result of the adrenine secretion which the injection of nicotine evoked. By analogy it is probable that the main factor in the sympathetic action of these alkaloids on the uterus is accelerated adrenine secretion, though stimulation of the ganglia doubtless plays some part. We have mentioned

the fact that the sympathetic effect of pilocarpine on the cat's uterus, as described by Cushny, is, in our experience, a somewhat uncertain effect. This becomes readily intelligible when it is realised that the effect is, in any case, the algebraic sum of three factors, namely, stimulation of sympathetic ganglia and acceleration of adrenine secretion, both producing inhibition, with direct motor action on the uterine muscle. It may well be that partial exhaustion of the suprarenals, or any influence depressing their response to pilocarpine, would be sufficient to turn the balance of effect over from inhibition to predominance of the direct motor effect on the muscle.

These observations appear to us to emphasise the importance of controlling the effects of drugs on the whole animal, and especially effects of a sympathomimetic nature, by a study of their action on isolated organs. It may be the case that the organ, isolated in Ringer's solution, is abnormal in its response to certain drugs, and that certain types of receptive mechanism, not separable from the organ anatomically, do not survive such conditions of experiment. On the other hand it is clear that, if the action as seen in the whole animal is alone considered, effects may be attributed to a drug, and special receptive mechanisms postulated for their explanation, though they are really secondary to effects in other parts of the body. It is to be expected that effects secondary to adrenine secretion, such as we have demonstrated in the case of a few alkaloids, will prove to be an important factor in the action of others. The possibility of their presence ought, at any rate, to be considered in the case of any drug which appears to produce effects of the sympathomimetic type, especially when these form only one feature of a more complex action.