EFFECT OF NICOTIN, CYSTOSIN, LOBELIN, CONIIN, PIPERIDIN AND QUATERNARY AMMONIAS ON ADRENAL SECRETION

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Nicotin is the type of a group of substances, one of whose characteristic properties is the capacity first to stimulate then to paralyze sympathetic ganglion cells (Langley, 1890, 1896, 1919; Langley and Dickinson, 1889, 1890).

The adrenals receive sympathetic innervation by means of the major splanchnics and accessorily through the minor splanchnics. The adrenal medulla has a common embryological origin with the sympathetic ganglia Epinephrin produces sympathico-mimetic effects. All these facts lend a special interest to the study of the influence of nicotin and drugs of similar properties on adrenal secretion.

PREVIOUS WORK. Mansfeld (1908) seems to have first recorded an increased adrenal secretion after nicotin injections. Unhappily we have not been able to obtain his paper.

Direct estimation of epinephrin secreted. Cannon, Aub and Binger (1912) studied the problem in cats. Blood was collected from the vena cava at the level of the adrenals by means of a catheter introduced into the femoral vein. The epinephrin content of this blood was determined by means of the isolated-intestine method. Injections of 3.5 to 7.5 mgm. nicotin considerably increased the epinephrin concentration in 3 to 5 minutes and maintained it for several minutes (in one experiment for 12 minutes). Adrenalectomy suppresses this effect. Stewart and Rogoff (1919) remarked that this increased concentration might be due to a diminished blood flow through the adrenals brought about by the initial bradycardia caused by nicotin. This objection, though plausible, is not valid, as will be seen later; our present experiments clearly show that Cannon's results were caused by an increased epinephrin secretion.

Steward and Rogoff (1919) by means of the "cava pocket" method made quantitative determinations, measuring the amount of epinephrin secreted in a given time (the isolated intestine and uterus being used as tests). Nicotin, after a few seconds, produces in cats a considerable increase in epinephrin output; 15 and 20 times the normal quantity per minute and,

in some cases, high concentrations such as are never seen under any other experimental conditions. This stimulation lasts only a very short time, from 30 to 60 seconds, and is followed by a prolonged period of depression. The diminished epinephrin secretion is the predominant action of nicotin; it is observed after subcutaneous and intravenous injections and is sometimes so marked that no epinephrin is found in the adrenal blood. Gradually the adrenal secretion is restored to normal conditions. These different phases are crudely parallel to the blood-pressure variations and evidently are due to the effects of nicotin on the ganglion cells of the efferent epinephrin secreting paths. In one experiment strychnin was injected after nicotin and the usual adrenal discharge was not observed. The adrenals retain, according to these authors, their normal stock of epinephrin as no difference was found between the epinephrin content of a denervated adrenal and that of the opposite adrenal whose nerves had been kept intact. Later we shall see that nicotin acts directly on the cells of the adrenal medulla.

Eichholtz (1923) used cats anesthetized with urethane to study the effects of several drugs on the epinephrin content of blood taken from the lumbo-adrenal and renal veins (Trendelenburg's surviving frog method as test). Nicotin increases adrenal secretion as much as 0.0005 to 0.009 mgm. per minute per kilo, even when the splanchnic nerve has been previously severed. Similar results were obtained with hordenin bromethylate, tetramethylammonium chloride (in this case on animals injected with atropin) and neurin. Other bases of quaternary ammonias did not increase epinephrin output; e.g., acetylcholin and tetrahydrobetanaphthylamin.

Stroomann (1925) reports an adrenal discharge in man after nicotin administration (Trendelenburg's method being used as test).

Chromaphil reaction and epinephrin content. Elliott (1912) reported the appearance of symptoms denoting sympathetic activity in cats injected with tetrahydrobetanaphthylamin that portray a state of fright, dilatation of the iris, exophthalmos, erection of hairs, etc. The adrenals of these animals contained very little or no epinephrin unless the splanchnics had been previously cut. As we have already mentioned, Stewart and Rogoff (1919) deny adrenal depletion after nicotin injection because a denervated adrenal contains the same quantity of epinephrin as the gland whose nerves are left intact.

Direct effect on a surviving adrenal. By perfusion of the surviving adrenals of cattle with Ringer's solution, at 38°C. it is possible to obtain a fluid that has the properties of epinephrin. There is not only a washing out of epinephrin already present in the gland, but apparently a new formation of this substance or rather of another very similar to it but more stable and resistant to alkali. Adding nicotin to the perfusing fluid increases the

epinephrin output four to six times, although no vascular effect is recorded (Schkawera and Kusmetzow, 1923; Kudrjawzew, 1924). Epinephrin was estimated in these experiments by means of several tests; its action on the blood vessels of the rabbit's ear, human finger, rabbit's kidney, etc. Nikolaeff (1924) also noted an increased epinephrin output, accompanied by a slight vasoconstriction, when coniin was perfused. The effect lasted one hour or more; after two hours a decrease was observed.

Krichel (quoted from Eichholtz, 1923) ascertains that the surviving adrenal vein of a horse is not sensitive to nicotin nor to the bases of quaternary ammonias.

Blood pressure as an internal test. Nicotin (Wertheimer, 1891; Gley, 1914) and anargyrin (Gley, 1892) produce a considerable increase of blood pressure in dogs whose medulla and spinal cord have been destroyed. Adrenalectomy suppresses this effect almost completely. A slight increase may be observed, especially when anargyrin is used, owing to direct action on the blood vessels or on the sympathetic ganglia. Usually they produce vasoconstriction indirectly, discharging epinephrin. Stewart and Rogoff (1919) maintain that Gley should have tested, by means of epinephrin injections, the vasomotor reactions of their animals before and after adrenalectomy, as this operation may alter their ability to respond. Our present experiments will show the exactness of Gley's conclusions.

Langley (1919) obtained an increase of blood pressure after nicotin injection even when the adrenals had been excluded by ligation, a fact that Stewart and Rogoff confirmed. These authors collected the adrenal blood of cats anesthetized by urethane in a "cava pocket" and studied the effects it produced on the blood pressure. After a pronounced but transitory increase of adrenal secretion, they observed, by this method, a marked and prolonged diminuton of the epinephrin output.

The uterus as an internal test. Dale and Laidlaw (1912a) used this method in an important paper describing the effects of several drugs. They used female cats, whose central nervous system had been destroyed, liver excluded from the circulation, and bowels take out. Contractions of the uterus horn were recorded. Nicotin, cystosin and hordenin methyliodid inhibited movements and relaxed the uterus. The isolated uterus generally reacts to these drugs with contractions. Lodal, a derivative of laudanosin, has similar discordant effects *in vivo* and *in vitro*. Stimulation of the major splanchinics produces a relaxation of the uterus, owing to an epinephrin discharge. Lodal does not relax the uterus after adrenalectomy.

The denervated iris as an internal test. In cats nicotin injections dilate the pupil, retract the nictitating membrane and widen the eye fissure. Painting the superior cervical ganglion with the drug produces the same effects, but since they were observed even when the ganglion had been previously taken out, it was supposed that the drug also acted on a peripheral mechanism (Langley and Dickinson, 1890).

Dale and Laidlaw's (1912b) experiments showed that the effects of nicotin on the eye were due to an adrenal discharge. Dilatation of the iris was observed in eyes 13 days after their denervation, that is to say, when all postganglionic fibers had degenerated. Adrenalectomy and temporary closure of the aorta above the adrenal arteries suppressed the results usually observed after injecting nicotin, cystosin and lobelin except for a slight, transitory dilatation. When circulation was reëstablished in the aorta, the eye effects were observed, their intenseness being somewhat diminished but not totally suppressed if the splanchnics had been severed. These drugs have no action on the enucleated eye of the cat. In cats anesthetized with urethane, dilatation of the pupil is seen even after adrenalectomy and extirpation of the superior cervical ganglion. Nicotin and other drugs of similar properties may produce their effects by three different mechanisms: *A*, stimulation of the superior cervical ganglion; *B*, adrenal discharge, and *C*, peripheral sympathico-mimetic action. These same authors report that cystosin dilates the iris of cats whose superior cervical ganglion has been taken out.

In these experiments the ciliary ganglion had not been removed. Langley (1919) remarks that nicotin might have produced these effects by paralysis of this ganglion. Dale and Laidlaw gave cats curare until the cervical sympathetic did not respond to stimulation; in this condition nicotin produces a constriction followed by a dilatation of the pupil, but larger doses are necessary as if it were necessary to overcome a paralysis of the adrenal secreting fibers produced by curare. In one experiment after adrenalectomy a dose of 10 mgm. of nicotin did not dilate the pupil. In two other animals, one curarized and the other injected with brucin, nicotin dilated the pupil but did not affect the nictitating membrane, owing to an adrenal discharge, not to a direct effect on the nerve endings of the iris, since these were paralyzed. When cats treated with ergotoxin and strychnin are injected with nicotin it produces an accelerated heart rate, exophthalmos and dilatation of the pupil.

Stewart and Rogoff (1919) extirpated the superior cervical ganglion of cats and observed, by their "cava pocket" method, a dilatation of the iris after nicotin injections.

Shimidzu (1924) worked on rabbits in which the superior cervical ganglion had been extirpated on one side and injected them with atropin so as to suppress the ocular motor nerve. Nicotin slowly injected (0.027 mgm. per kilogram per minute) did not dilate the pupil. Larger doses (0.05 mgm.) produce an intense and prolonged dilatation (five experiments). Ligature of the adrenals suppresses this effect. In one experiment the larger dose of nicotin produced an adrenal discharge of 0.005 mgm. epinephrin per kilo per minute, estimated by comparing the dilatation produced by the injection of a known dose of epinephrin. Tetrahydrobetanaph-

thylamin dilates the denervated iris in rabbits with or without adrenals.

Blood-platelets. Nicotin reduces the quantity of blood-platelets to a remarkable extent in one or two hours. Later the normal count is restored. In one experiment an increased white count was found two and one-half hours after the injection. These changes in the blood picture might be due to an increased adrenal secretion followed by a prolonged inhibition (Backman, Edström, Grahs and Hultgren, 1925).

EXPERIMENTS. Our experiments were performed on sixty dogs anesthetized with chloralose (0.10 gram per kilogram intravenously injected), with both vagi always cut in the recipients, and in the donors cut only in the experiments performed after May 19. Artificial respiration was given. Tournade and Chabrol's technique was followed so that all the adrenal blood of a donor's left adrenal passed into the circulation (jugular vein) of a recipient. In some cases the right adrenal was used. The first dog generally weighed about 20 kgm., the second, between one-half and two-thirds that weight.

The donor's blood pressure was recorded by a mercury manometer. Artificial respiration prevented possible asphyxia by apnea caused by large doses of the drugs studied. These were usually injected into the jugular vein, though sometimes the external saphenous vein was used.

The recipient's blood pressure was registered with a mercury manometer, the heart rate with an elastic manometer (Straub). Both vagi were cut in the neck and the stellate ganglia extirpated following Carville and Rochefontaine's technique (1874).

The following tests were used to register the effect of adrenal secretion of the donor in the recipient: blood pressure, denervated heart, glycemia, intestinal contractions, and the volume of different viscera. The denervated iris was not used because it was considered superfluous to recur to such a complex test and the uterus was discarded as Dale and Laidlaw's experiments were considered conclusive in this respect. In a few experiments adrenal blood was collected before and after the drugs were injected and the quantity of epinephrin secreted estimated by means of external tests.

To measure the amount discharged by the recipient once an effect was obtained on the donor, known quantities of epinephrin were injected in the donor every six to ten minutes until an identical effect was obtained.

Simple anastomosis. Immediately after the establishment of the anastomosis a slight and sharp increase in blood pressure may be registered in the recipient in some cases, owing to an accumulation of epinephrin whilst the anastomosis is effected. In cases where the recipient has an initial low blood pressure the anastomosis produces a sharp or gradual rise. After a very short time the blood pressure remains constant in all these

cases and in the great majority of the experiments no changes at all were seen.

A rise of blood pressure in the donor, produced by stimulating the right splanchnic, after extirpating the right adrenal, or by injecting epinephrin (0.01 to 0.10 mgm.) does not increase the blood pressure or accelerate the denervated heart of the recipient (fig. 1). In one experiment, where the adrenal circulation was considerably retarded (0.25 cc. per minute), an injection of epinephrin in the donor slightly raised the blood pressure in the recipient. We attribute this to the fact that during the slow circulation the maximum adrenal concentration had been reached, epinephrin improved the circulation and so enabled a larger amount of epinephrin to be put out by the adrenal. Similar situations have already been studied by Stewart and Rogoff.

Fig. 1. 5/4/1925. Upper curve—blood pressure of donor (16.5 kgm.). Lower curve—blood pressure of recipient (8.0 kgm.). *1.* Intravenous injection of 0.01 mgm. epinephrin in donor. *2.* Intravenous injection of 16.0 mgm. cystosin in donor.

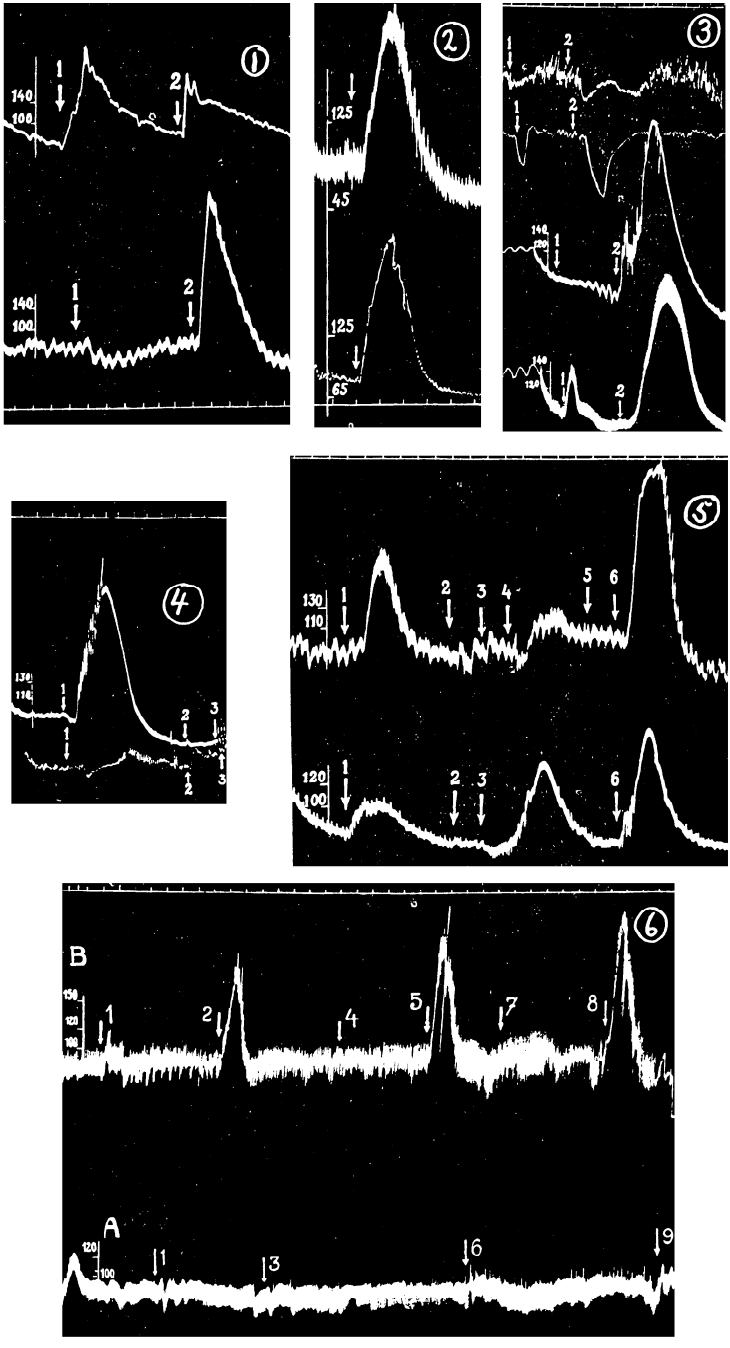
Fig. 2. 7/24/1925. Upper curve—blood pressure of recipient (15.5 kgm.). Lower curve—blood pressure of donor (17.5 kgm.). Time in minutes. Injection 10 mgm. hordenin methyliodid.

Fig. 3. 6/15/1925. Time in minutes. Intestinal movements of recipient (10 kgm.). Volume of spleen of recipient. Blood pressure of recipient. Blood pressure of donor (24 kgm.). *1.* Intravenous injection 0.005 mgm. epinephrin. *2.* Intravenous injection 2 mgm. nicotin.

Fig. 4. 6/22/1925. Dog (18 kgm.) with denervated gut. Upper curve—blood pressure. Lower curve—intestinal movements. *1.* Intravenous injection 1 mgm. nicotin. Adrenals extirpated. *2.* Injection 1 mgm. nicotin. *3.* Injection 2 mgm. nicotin.

Fig. 5. 6/2/1925. Time in minutes. Blood-pressure curve of recipient (8 kgm.). Blood-pressure curve of donor (24 kgm.). *1.* Donor injected intravenously 1 mgm. nicotin. *2.* Transfusion interrupted. *3.* Donor injected intravenously 2 mgm. nicotin. Between 30 and 80 seconds after injection 20 cc. blood drawn from its jugular vein and injected into recipient at *4.* *5.* Transfusion recommenced. *6.* Donor receives 2 mgm. nicotin intravenously.

Fig. 6. 6/6/1925. Blood-pressure of test dogs A and B. *1.* Intravenous injection of 5 cc. "normal" adrenal blood given by donor during 1 minute before having received nicotin. *2.* Intravenous injection of 8 cc. adrenal blood taken from donor during first minute after injecting 2 mgm. nicotin. *3.* Intravenous injection of 15 cc. adrenal blood taken from donor during second minute after injecting 2 mgm. nicotin. *4.* Intravenous injection of 7.5 cc. adrenal blood taken from donor during fifth minute after injecting 2 mgm. nicotin. *5.* Intravenous injection of 7 cc. adrenal blood taken from donor during first minute after injecting 1 mgm. cystosin. *6.* Intravenous injection of 15 cc. adrenal blood taken from donor during second minute after injecting 1 mgm. cystosin. *7.* Intravenous injection of 8.5 cc. adrenal blood taken from donor during fifth minute after injecting 1 mgm. cystosin. *8.* Intravenous injection of 7 cc. adrenal blood taken from donor during first minute after injecting 1 mgm. lobelin. *9.* Intravenous injection of 16 cc. adrenal blood taken from donor during second minute after injecting 1 mgm. lobelin.


①
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⑥
A
B

Effects on the blood pressure and heart rate. Nicotin injections in the donor produce different effects on the recipient according to the doses used; 0.1 mgm. given to a dog weighing 20 kgm. generally has no effect or a very slight one on the donor, 1 mgm. considerably raises the blood pressure in both dogs, and 5, 10, 15 and 18 mgm. doses increase this effect. The donor shows a slowing of the heart beat, sometimes a complete stop of a few seconds, followed by slow beats that afterwards increase in frequency, so that 1 or 2 minutes later a moderate tachycardia is seen, with occasional spurts. When the vagi are cut, the initial slowing is less marked. Blood pressure, of course, drops when the slowing of the heart beat is marked, but rapidly increases whether the heart rate is slow or fast, though in the latter case it goes up higher. Afterwards it gradually returns to the previous level. The period of increase lasts from 3 to 5 minutes.

The recipient shows no effects until after 30 to 60 seconds. Then an increased heart rate and a sharp rise of blood pressure are registered. The maximum heart rate is attained between 30 and 90 seconds after the initial rise and the peak of the blood pressure is always reached within 2 minutes and lasts from 3 to 8 minutes. This rise in blood pressure is more marked than that registered by the donor. Perhaps nicotin diminishes the animal's sensitiveness to adrenalin. The variation of blood pressure lies between 170 and 206 mm. Hg and the increase in heart rate is about 100 beats per minute.

Cystosin and lobelin produce the same results as nicotin under these experimental conditions, but weight for weight they are more active, especially lobelin.

Several quaternary ammonias are also active: tetramethylammonium chloride, bromide and iodide and hordenin iodinmethylate. This last drug gives a marked effect, yet curiously enough an equal dose of hordenin sulphate is inactive. One experiment with tetrapropylammonium iodide gave negative results, as did coniin (5, 10 and 20 mgm.), piperidin (141 mgm.), and tetrahydrobetanaphthylamin (68 mgm.) (table 1).

Effects on intestinal contractions. Nicotin paralyzes intestinal contractions (Bayliss and Starling, 1899). Langley and Magnus (1905) localized the inhibitory effect and ascribed it to a peripheral mechanism because inhibition was observed when all post-ganglionic fibers going to the jejunum had been severed. The isolated intestinal strip is only inhibited by very large doses (Magnus, 1905), so probably "*in vivo*" the effect is due principally to an adrenal discharge. We have already mentioned that Cannon, Aub and Binger (1912) obtained inhibitory effects in cats with cava blood taken after nicotin injection only when the adrenals were present.

A donor (24 kgm.) received 2 mgm. nicotin. An adrenal discharge was

produced and registered by inhibition of the recipient's (10 kgm.) intestine. The blood pressure rose 198 mm. Hg. To obtain this rise it was necessary to inject 0.075 mgm. epinephrin. When small discharges were obtained,

TABLE 1

DATE, 1925	WEIGHT		DRUG INJECTED	DOSE	BLOOD PRESSURE VARIATION		INCREASE IN HEART RATE OF RECIPIENT
	Donor	Recipient			Donor	Recipient	
	kgm.	*kgm.*		*mgm.*	*mm. Hg*	*mm. Hg*	
4/4	19.0	9.0	Nicotin	19.0		+206	88
5/12	21.5	11.0	Nicotin	5.0	−40 +204	+174	112
5/15	25.2	11.0	Nicotin	5.0	+140	+200	106
5/16	23.5	16.0	Nicotin	2.5	+50	+190	66
5/26	15.0	10.0	Nicotin	1.0	+64	+170	76
5/27	17.5	9.0	Nicotin	0.01	0	0	0
5/27	17.5	9.0	Nicotin	0.1	+20	0	0
5/29	15.0	11.0	Nicotin	0.09	+30	0	0
5/30	19.0	8.5	Nicotin	1.0	−40 +200	+180	130
5/20	17.0	7.5	Cystosin	0.6	+40	+150	90
5/20	17.0	7.5	Cystosin	0.6	+48	+190	92
5/5	25.2	11.0	Lobelin	4.0	+170	+112	94
5/6	22.0	8.5	Lobelin	1.0	−70 +150	+140	84
5/18	18.0	8.0	Lobelin	2.0	−10 +130	+314	130
5/23	18.0	11.5	Lobelin	0.5	+130	+130	80
5/8	18.0	12.0	Coniin	5.0	+10	0	0
5/18	18.0	8.0	Coniin	10.0	−28	0	0
6/30	31.0	29.0	Coniin	20.0	+16	0	2
7/21	19.5	11.5	Tetramethylammonium chloride	34.0	−70 +44	+160	80
6/30	31.0	29.0	Tetramethylammonium chloride	33.0	0	+174	70
7/20	23.5	11.0	Tetramethylammonium bromide	10.0	0	0	0
7/21	19.5	7.0	Tetramethylammonium bromide	12.0	+56	+126	68
6/30	31.0	29.0	Tetramethylammonium iodid	40.0	0	+174	70
7/20	23.5	11.0	Tetrapropylammonium iodid	18.0	0	0	0
7/21	19.5	7.0	Hordenin sulphate	10.0	+22	0	0
7/21	19.5	7.0	Hordenin methyliodate	11.0	+186	+178	112
7/20	23.5	11.0	Piperidin	141	0	0	0
8/18	17.0	8.5	Tetrahydrobetanaphthylamin	68	+120	0	0

intestinal movements might be stimulated. Intestinal movements were registered by means of a small rubber bag filled with water, which was put into the upper part of the ileum, and communicated with a burette partially

filled with water. The changes in pressure were registered by a Marey tambour. The abdomen was left open so that contraction of the diaphragm and abdominal wall would not be a disturbing factor (fig. 3).

The inhibition observed cannot be attributed to nicotin, as the amount passing from the donor to the recipient is too small to produce any effect. Injection of a quantity of epinephrin equal to the amount discharged produces inhibition.

Cystosin inhibits intestinal contractions (Dale and Laidlaw, 1912). The amount of epinephrin discharged under the influence of this drug is capable of inhibiting the intestine. Nicotin does not have any inhibitory effect on the intestine of adrenalectomized animals. A dog (18 kgm.), whose intestine had been denervated by extirpation of the celiac plexus and section of the nerves accompanying the superior mesenteric artery, was given 1 mgm. nicotin. A rise in blood pressure was observed, accompanied by inhibition of peristalsis (fig. 4). Increased intestinal contractions were registered three and a half minutes later. After taking out the adrenals, 1 mgm., and a few minutes later, 2 mgm. nicotin were injected and no inhibition followed; on the contrary, an increase in tone and strong

TABLE 2

Glucemia per cent (Folin and Wu)

Donor	0.0694	0.0689	0.099	0.08	0.0694	0.0606
Recipient	0.0892	0.0869	0.103	0.121	0.1105	0.0819
Minutes after injection		15	30	45	60	75

contractions were observed. There were no variations in the blood pressure record.

Both these experiments show that the dose of nicotin injected produced effects on the intestine by means of an adrenal discharge. We have not tried larger doses which might inhibit the denervated intestine of adrenalectomized dogs.

Volume of the spleen. A marked contraction is registered in the spleen of the recipient during the adrenal discharge produced by nicotin injected in the donor (fig. 3).

Volume of the denervated limb. Only two experiments of this type were performed. In one Anrep's technique was followed. One milligram of nicotin given to a dog weighing 10 kgm. produced a marked rise of blood pressure and a short dilatation followed by a constriction of the denervated limb (Dale and Richards' plethysmograph).

A donor (18 kgm.) was given 1 mgm. nicotin, the blood pressure rising 70 mm. Hg. The recipient (9 kgm.) had a rise in blood pressure of 160 mm. Hg, the denervated heart increased in rate 70 beats per minute, the denervated limb, after a short dilatation, contracted markedly.

Effect on the blood sugar. On previous occasions we have shown that the simple anastomosis does not modify the blood sugar in either the donor or the recipient. But when nicotin is given to the donor, the blood sugar rises in both animals.

Protocol—Donor (19 kgm.). 1 mgm. nicotin injected intravenously. Recipient 9 kgm. Anesthesia: chloralose, 1 hour before injection of nicotin. Before transfusion the adrenal vein gave 11.4 cc. per minute. When transfusion ceased, it gave 1 cc. per minute. Blood pressure rose 210 mm. Hg in the recipient, the heart rate increased 88 beats per minute.

The effects observed are not due to nicotin passing from the donor to the recipient. Nicotin produces different effects in the donor and in the recipient. The donor has a less considerable increase in blood pressure and an initial slowing, followed by an irregular increase in the heart rate. The greater rise in blood pressure of the recipient is accompanied by an increased heart rate. But this is not sufficient evidence that both effects are not produced directly by nicotin, arriving in the recipient with the blood transfused.

To clear up all doubts in this respect, 1 to 2 mgm. nicotin were injected in the donor and the rise in blood pressure registered in the recipient. Transfusion was then interrupted and a second dose of nicotin given; 20 cc. of blood were taken from an artery a few seconds later and rapidly injected into the jugular vein of the dog which had recently been the recipient. No effects were seen on the blood pressure or the heart rate. When 2 mgm. were given, a slight effect was observed. Twenty cubic centimeters represent about twice the amount of blood that usually passes from one dog to another. If now the anastomosis is once more established, nicotin again produces a discharge in the donor registered by the recipient.

Protocols.

6/2/1925. Donor (24 kgm.). Recipient (8 kgm.). Anastomosis and 1 mgm. nicotin injected in the donor. Blood pressure in donor rose from 80 to 112 mm. Hg; in recipient from 90 to 180 mm. Hg. Heart rate increased from 90 to 108. Transfusion was interrupted and 6 minutes later 2 mgm. nicotin were injected in the donor; between 30 and 80 seconds after 20 cc. of arterial blood were collected and rapidly injected into the jugular of the recipient. Blood pressure of the donor rose from 80 to 170 mm. Hg and from 90 to 122 mm. Hg in the recipient; the heart rate increased from 88 to 100 in the latter. Transfusion was again established and 9 minutes later 2 mgm. nicotin given intravenously to the donor. The blood pressure rose from 80 to 100 mm. Hg in the donor and from 90 to 260 mm. Hg in the recipient, whose heart rate rose from 88 to 192 (fig. 5).

6/5/1925. Donor (23 kgm.). Recipient (9.3 kgm.). Transfusion interrupted and 1 mgm. nicotin injected intravenously in donor. Between 25 and 85 seconds after 20 cc. arterial blood collected and rapidly injected into the recipient's jugular. Blood pressure rose from 90 to 140 mm. Hg in the donor and remained 160 mm. Hg in the recipient. The heart rate was 116 before and 118 after the injection. Transfusion was again established and 1 mgm. nicotin given intravenously to the donor.

Blood pressure rose from 90 to 148 mm. Hg in the donor and from 160 to 280 mm. Hg in the recipient; the heart rate increased from 114 to 154.

These protocols show that when greater doses of nicotin are given to the donor, the amount passing to the recipient may be sufficiently large to produce slight effects concurring with the more marked effects occasioned by adrenal discharge. A simple calculation shows that the sharp increase in blood pressure is seen in the recipient when from 4 to 15 cc. only of the donor's blood has entered its circulation, that is to say, a dose of nicotin so small as to be ineffective.

Amount and duration of the discharge. The anastomosis method has two definite drawbacks for pharmacodynamic studies. The substance injected passes continuously in small amounts from one animal to the other and may produce direct effects on the recipient or reacting dog. Secondly, it

TABLE 3

DATE	WEIGHT	DRUG INJECTED INTRAVENOUSLY	MAXIMUM EPINEPHRIN SECRETION PER MINUTE AFTER INJECTION	EPINEPHRIN SECRETED PER KILOGRAM PER MINUTE
	kgm.	*mgm.*	*mgm.*	*mgm.*
6/6/1925	19		0.007	0.000368
		Nicotin 2	0.030	0.00157
		Cystosin 1	0.050	0.0026
		Lobelin 1	0.070	0.00368
6/8/1925	16		0.001	0.000062
		Nicotin 2	0.100	0.0062
		Lobelin 1	0.050	0.0031
6/9/1925	20		0.001	0.00005
		Nicotin 5	0.100	0.0050

gives no precise knowledge as to the quantitative variations occurring each minute in the amount of epinephrin secreted. Further experiments were necessary to fill this gap.

A dog was prepared in the same way as the donors of our previous experiments, but the anastomosis was not established. The adrenal blood was collected in graduated tubes containing 1 cc. of a 5 per cent sodium citrate solution and its adrenal content estimated on two dogs which had previously had their vagi cut and their stellate ganglia extirpated while under chloralose anesthesia and artificial respiration. The weight of the recipients was always from one-half to two-thirds that of the donor. Blood pressure and heart rate were recorded. Before injecting the adrenal blood their reaction to an 1:1,000,000 solution of epinephrin was tested and at the end of the experiment a second test was made with 1:100,000 solution and the blood-pressure raising effect of the different samples of blood com-

pared with that of the standard solution. Two dogs were used so as to give a sufficient interval between injections.

Nicotin (2 to 5 mgm.), cystosin (1 mgm.) and lobelin (1 mgm.) produced marked discharges for 1 to 2 minutes after the injections. After 2 or 3 minutes the normal level of secretion is restored; sometimes a period of diminished secretion follows the discharge. A second injection of the same or another epinephrin-secreting drug produces a second discharge. The amount secreted is considerable, 0.04 to 0.10 mgm. per minute for the left adrenal. This represents an increase of from four to one hundred times the normal quantity (table 3). The concentration also increased considerably—in one case, to 1:40,000.

TABLE 4

6/6/1925

	2 MGM. NICOTIN INTRAVENOUSLY					1 MGM. CYSTOSIN INTRAVENOUSLY				1 MGM. LOBELIN INTRAVENOUSLY			
Time after first injection, minutes	—	1	2	3	4	5	16	17	18	25	26	27	28
Adrenal blood collected, cc	5	18	15	8	7	7	17	15	8.5	18	16	6	1
Epinephrin, mgm.	0.007	0.03	Trace			Trace	0.05	0.005	0.005	0.07	0.004		
Dog A, 14 kgm., rise in blood pressure, mm. Hg			0					18			7		
Increase in heart rate per minute			0					8			0		
Dog B, 15 kgm., rise in blood pressure, mm. Hg	22	98				0	142		4	140			
Increase in heart rate per minute	4	52				0	62		0	98			

Protocols.

6/6/1925. Dog (19 kgm.). Vagi cut in neck. Blood from left adrenal contained 0.004 to 0.0075 mgm. epinephrin per minute. After intravenous injection of 2 mgm. nicotin the epinephrin secreted in the first minute was 0.03 mgm.; from the second to the fifth minute the quantity of epinephrin in the samples was so small that it was impossible to estimate it with our method. Fifteen minutes after the nicotin injection 1 mgm. cystosin was given intravenously and 0.05 mgm. epinephrin was secreted during the first minute, 0.005 mgm. during the second minute, and 0.006 mgm. during the fifth minute. Nine minutes after the second injection, 1 mgm. lobelin was given intravenously, and 0.07 mgm. epinephrin was secreted during the first minute and 0.004 mgm. during the second minute (table 4, fig. 6).

6/8/1925. Dog (16 kgm.). Artificial respiration. Vagi not cut. Blood from left adrenal contained less than 0.001 mgm. epinephrin per minute. Intravenous injection of 2 mgm. nicotin gave 0.1 mgm. epinephrin during the first minute, and 0.045 mgm. during the fifth, eighth and tenth minutes. Lobelin (1 mgm.) was then

injected and again the secretion increased to 0.005 mgm. during the first minute, 0.05 during the second minute, and less than 0.001 during the sixth minute (table 5, fig. 7).

6/9/1925. Dog (20.5 kgm.). Vagi cut in neck. Artificial respiration. Epinephrin secreted by left adrenal was not more than 0.001 mgm. per minute. Nicotin (5 mgm.) intravenously injected increased the secretion to 0.07 mgm. during the first minute, 0.01 mgm. during the second minute; by the fourth minute the previous rate of secretion was again restored and at the seventeenth minute the amount secreted was a little less than before injecting the drug (table 6, fig. 8).

A great increase was observed in the adrenal circulation, which may be due to the general rise of blood pressure or to local vasomotor effects; this last cause has to be determined by other methods than the one we used. In some cases an increased adrenal secretion was recorded, although the circulation did not increase but actually diminished, therefore, it cannot be attributed to a better adrenal circulation.

Repeated injections. As has already been said, nicotin produces an adrenal discharge followed by a short period of diminished secretion. A second injection of 1 or 2 mgm. of the drug has the same effect when given 10 to 15 minutes after the first dose, and the observation can be repeated any number of times. If the interval between the injections is shortened, the discharge becomes gradually smaller, this being more marked the larger the doses and the shorter the intervals between them.

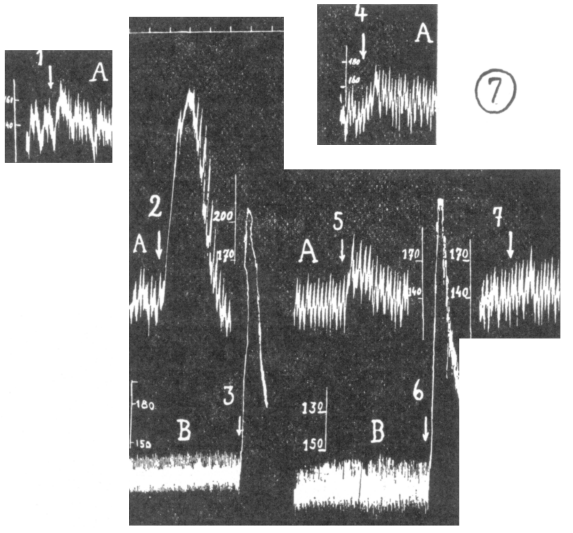
Protocol.

6/30/1925. Donor (19 kgm.). Recipient (8.5 kgm.). A preliminary stimulation of the left splanchnic produced an adrenal discharge. Fifteen nicotin injections were then given, as is seen in table 7 (fig. 9).

Fig. 7. 6/8/1925. Blood-pressure curves of test dogs A and B. *1.* Intravenous injection 8 cc. adrenal blood given by donor during 1 minute before injecting drugs. *2.* Intravenous injection 4 cc. adrenal blood given by donor during first minute after injecting 2 mgm. nicotin. *3.* Intravenous injection 10 cc. adrenal blood given by donor during second minute after injecting 2 mgm. nicotin. *4.* Intravenous injection 7 cc. adrenal blood given by donor during fifth minute after injecting 2 mgm. nicotin. *5.* Intravenous injection 2.5 cc. adrenal blood given by donor during the first minute after injecting 1 mgm. lobelin. *6.* Intravenous injection 8.2 cc. adrenal blood given by donor during second minute after injecting 1 mgm. lobelin. *7.* Intravenous injection of adrenal blood given by donor during sixth minute after injecting 1 mgm. lobelin.

Fig. 8. 6/9/1925. Blood-pressure curves of test dogs A and B. Small records. *1.* Injection of 0.003 mgm. epinephrin. *2.* Injection of 0.005 mgm. epinephrin. *3.* Injection of 11 cc. adrenal blood given by donor during 1 minute. Large record: *1.* Injection of 16 cc. adrenal blood given by donor during first minute after injection of 5 mgm. nicotin. *2.* Injection of 27 cc. adrenal blood given by donor during second minute after injection of nicotin. *3.* Injection of 7.4 cc. adrenal blood given by donor during fourth minute.

Fig. 9. 6/30/1925. Time in minutes. Blood-pressure curve of recipient (8.5 kgm.). Blood-pressure curve of donor (19 kgm.). At each arrow 1 mgm. nicotin injected intravenously into donor.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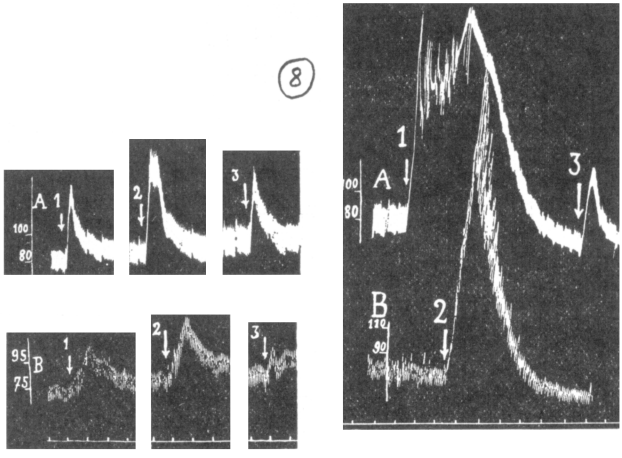
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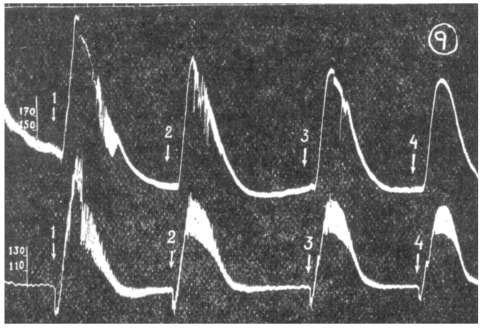
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TABLE 5

6/8/1925

	TIME, MINUTES																							
	—	2½	6	8	12	14	18	20½	30	32	45	53	54	55	56	57	58	63	64	67	102	108	116	122	130
Epinephrin, mgm.	0.003	0.003	0.005	0.005	0 008	0.008	0.012	0 012													0.05	0.10	0.02	0.075	0.1
Adrenal blood collected, cc.									8	8	7	5.5	4	10	9.8	7	4.2	25	9.2	5					
Epinephrin content, mgm.									<0.001	<0.001	0.001	—	0.1	0.045	—	0.002	0.002	0 005	0.05	<0.001					
Dog A, 9 kgm., rise in blood pressure. mm. Hg.	23		30		70		90		8				150			22		22		17	120		98	144	150
Increase in heart rate per minute	4		8		18		30		1				90			2		12		2	90		52	100	100
Dog B, 9 kgm., rise in blood pressure mm. Hg.		4		8		18		22		2	6			206			6		215			270			
Increase in heart rate per minute		6		12		16		26		2	6			110			0		104			124			

Blood pressure of A when experiment started 140 mm. Hg.
Blood pressure of B when experiment started 125 mm. Hg.
Pulse rate of A when experiment started 114 beats per minute.
Pulse rate of B when experiment started 98 beats per minute.
At 54 minutes 2 mgm. nicotin intravenously. At 63 minutes 1 mgm. lobelin intravenously injected.

TABLE 6

6/9/1925

	TIME IN MINUTES										
	0	2	6	9	15	17	1	2	3	4	14
							5 mgm. nicotin intravenously				
Epinephrin, mgm	0.003	0.003	0.005	0.005							
Adrenal blood collected, cc					11	11	16	27	13	7.4	7
Epinephrin content, mgm					0.001	0.001	0.07	0.1		0.001	0.001
Dog A, rise in blood pressure, mm. Hg	52			74	48		160			56	14
Increase in heart rate per minute	4			6	2		72				2
Dog B, rise in blood pressure, mm. Hg	30			40	20		250				
Increase in heart rate per minute	8			20	2		74				

Blood pressure of A at commencement 80 mm. Hg.
Blood pressure of B at commencement 75 mm. Hg.
Heart rate of A at commencement 76 beats per minute.
Heart rate of B at commencement 124 beats per minute.

TABLE 7

TIME	NICOTIN	BLOOD PRESSURE RISE	INCREASE IN HEART RATE BEATS PER MINUTE	EPINEPHRIN SECRETION
minutes	*mgm.*	*mm. Hg*		*mgm.*
0	1	180	134	0.01
9	1	175	94	0.01
19	1	160	74	0.09
27	1	145	94	0.07
34	1	150	14	0.01
39	1	154	84	0.08
44	1	120	40	0.04
49	1	120	50	0.04
56	1	120	60	0.04
61	2	145	78	0.06
66	2	65	26	0.01
71	2	78	36	0.02
76	2	120	58	0.04
84	5	110	60	0.03
89	5	20	8	0.01
Total				0.74

Four minutes after the last dose was given, strong stimulation of the left splanchnic of the donor produced only a slight discharge that gave a rise of blood pressure in the recipient of 20 mm. Hg. Nicotin (0.1 mgm. in 0.1 cc. water) was injected into the donor's left adrenal and a rise of blood pressure of 22 mm. Hg was registered in the recipient. The donor's left splanchnic was again stimulated and a marked rise of blood pressure (100 mm. Hg) was seen in the recipient. The donor's adrenal weighed 1 gm. and contained 0.33 mgm. epinephrin. The recipient had a rise of blood pressure of 165 mm. Hg and the heart rate was accelerated 84 beats per minute when 0.1 mgm. epinephrin was injected. A dose of 0.05 mgm. gave a rise of blood pressure of 135 mm. Hg and an increase in heart rate of 76 beats per minute. This experiment shows how difficult it is to suppress the effects of splanchnic stimulation and to exhaust the epinephrin content of the adrenals in the dog.

Continuous injection. By means of a Woodyatt pump, nicotin was injected continuously into a donor's jugular vein.

6/15/1925. Donor (24 kgm.). Recipient (10 kgm.). During 14 minutes 20 mgm. nicotin were injected in 200 cc. normal saline solution, that is to say, 1.428 mgm. per minute or 0.0595 mgm. per kilo per minute. Three minutes after the commencement of injection the donor's blood pressure rose suddenly, 80 mm. Hg in 1 minute.

Fig. 10. 6/15/1925. Time in minutes. Blood-pressure curve of recipient (10 kgm.). Blood-pressure curve of donor (24 kgm.). From *1* to *2* donor receives 1.428 mgm. nicotin per minute intravenously. From *2* to *5*, 4.28 mgm. nicotin per minute. *3*. Intense stimulation of left splanchnic during 10 seconds. *4*. Intense stimulation of left splanchnic during 20 seconds.

Fig. 11. 6/17/1925. Time in minutes. Blood pressure of donor (30 kgm.). Blood pressure of recipient (11 kgm.). From the arrow onwards donor receives during 10 minutes 12 mgm. nicotin in 150 cc. normal saline.

Fig. 12. 6/19/1925. Time in minutes. Blood pressure of recipient (9.5 kgm.). Blood pressure of donor (30 kgm.). From *1* to *4* donor received in 20 minutes 10 mgm. nicotin in 250 cc. normal saline; from *4* to *5*, 10 mgm. nicotin in 10 minutes; from *5* onwards 9.5 mgm. nicotin in 15 minutes.

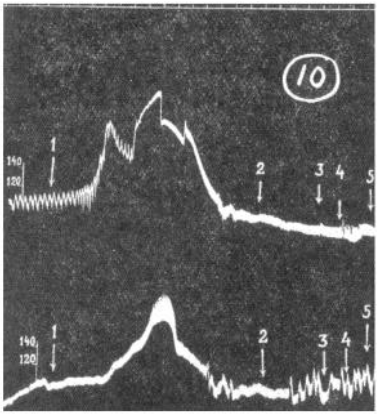
Fig. 13. 6/2/1925. Time in minutes. Blood-pressure curve of recipient (5 kgm.). Blood-pressure curve of donor (24 kgm.) with left adrenal denervated. At the arrow 1 mgm. lobelin intravenously injected into donor.

Fig. 14. 7/21/1925. Blood-pressure curve of recipient (11.5 kgm.). Blood-pressure curve of donor (19.5 kgm.) with left adrenal denervated. Time in minutes. At the arrow intravenous injection to donor of 12 mgm. tetramethylammonium bromide hydrate.

Fig. 15. 6/5/1925. Time in minutes. Blood-pressure curve of recipient (9.3 kgm.). Blood-pressure curve of donor (23 kgm.). *1*. Injection of 0.1 cc. nomal saline into donor's adrenal. *2*. Injection of 0.15 mgm. nicotin in 0.1 cc. normal saline into donor's adrenal.

Fig. 16. 7/20/1925. Blood-pressure curve of recipient (11.0 kgm.). Blood-pressure curve of donor (23.5 kgm.). Time in minutes. *1*. Injection of 0.0001 mgm. nicotin into donor's adrenal. *2*. Injection of 0.00001 mgm. nicotin into donor's adrenal.

Fig. 17. 6/5/1925. Time in minutes. Blood-pressure curve of recipient (9.3 kgm.). Blood-pressure curve of donor (23 kgm.). Donor's semilunar ganglion painted with a 1 per cent solution of nicotin.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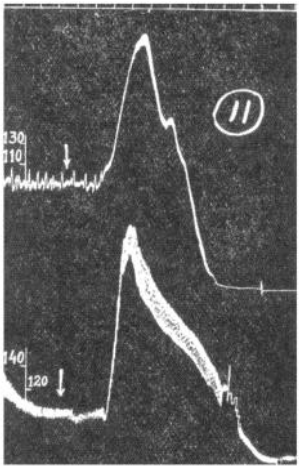
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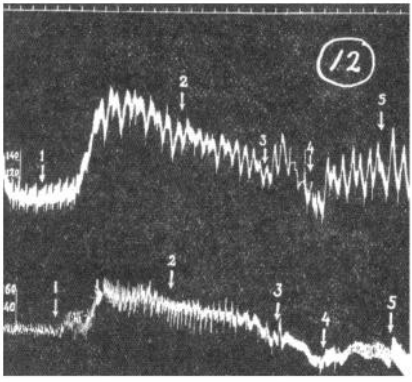
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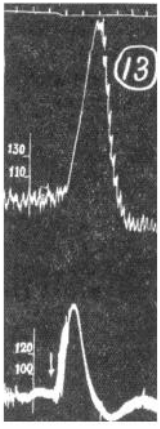
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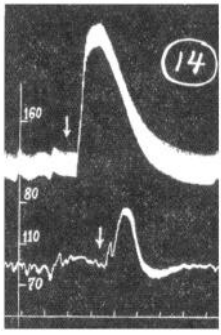
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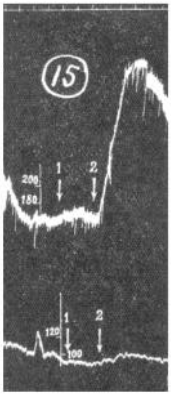
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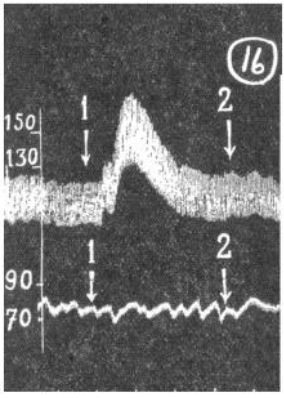
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then, after a slight fall, a second rise of 110 mm. Hg was recorded; after that the blood pressure fell and a third short rise was followed by a rapid fall. The blood pressure remained above the normal level for 9 minutes, a duration never obtained with single injections. The recipient's blood pressure rose gradually 3 minutes after the commencement of injection and reached a first peak in the fourth minute; after a slight fall in the sixth minute, a second peak was attained in the eighth minute (110 mm. Hg); the blood pressure then gradually fell, returning to the original level in the sixteenth minute. During the fourteenth minute a second solution of greater strength was injected, so that 30 mgm. nicotin in 200 cc. saline were given in 7 minutes, that is to say, 4.28 mgm. per minute or 0.178 mgm. per kilo per minute. No effect was registered in donor or recipient. Four minutes after the second injection was started, the donor's left splanchnic was strongly stimulated for 10 seconds and 2 minutes later for 30 seconds. No effect was observed in either of the dogs (fig. 10).

6/17/1925. Donor (30 kgm.). Recipient (11 kgm.). In 10 minutes, 12 mgm. nicotin in 150 cc. normal saline (1.20 mgm. per min. or 0.040 mgm. per kgm. per min.) were injected in the donor's jugular vein. Two minutes and 45 seconds after starting the injection a sharp rise of blood pressure was observed in the donor; the peak (170 mm. Hg) was reached in 3 minutes; then the blood pressure fell rapidly reaching 0 in the eighth minute and the animal died during the tenth minute after injection. The recipient's blood pressure rose rapidly 45 seconds after the donor's had started to rise and reached a peak of 180 mm. Hg. Then it fell slowly, returning to its original level in the tenth minute. A maximum increase in heart rate of 78 beats per minute was registered during the first six minutes of the rise of blood pressure (fig. 11).

6/19/1925. Donor (30 kgm.). Recipient (9.5 kgm.). In 20 minutes 10 mgm. nicotin in 250 cc. normal saline (0.5 mgm. per min. or 0.0167 mgm. per kgm. per min.) were injected into the donor's jugular. The donor's blood pressure started to rise 45 seconds after commencement of injection and reached a peak of 70 mm. Hg in three and one-half minutes. Then it fell slowly and reached the previous level in the eighteenth minute after injection. The recipient's blood pressure started to rise the third minute and reached a peak of 120 mm. Hg between the fourth and fifth minutes. A gradual fall brought the pressure to the previous level twenty minutes after injection. The rapidity of the injection was now doubled so that 10 mgm. nicotin in 250 cc. normal saline were given to the donor in 10 minutes. The donor's blood pressure rose only 10 mm. Hg; the recipient's was maintained between 40 and 80 mm. Hg above the base line. A third injection of 9.5 mgm. nicotin in 115 cc. normal saline given in 15 minutes produced no results in either dog; neither did a fourth injection of 40 mgm. nicotin in 40 cc. normal saline given in 4 minutes alter the blood pressure of donor or recipient. The prolonged rise when nicotin was injected slowly is remarkable, since we had never before observed it to last so long.

Before the first injection was started, the donor's left splanchnic was stimulated and a rise of blood pressure of 60 mm. Hg was registered in the recipient. During the injections the splanchnic was again stimulated 19, 23, 28, 31, 34 and 38 minutes after the first injection. A rise of blood pressure always resulted in both a donor and the recipient. The last stimulation gave a rise of 30 to 40 mm. Hg in the recipient. Here again it was impossible to paralyze the splanchnic (fig. 12).

The following conclusions can be drawn from the three experiments just described: *A*, nicotin produces no effect until a certain quantity has entered the circulation; *B*, once this minimal quantity has arrived, the

rise of blood pressure is sharp in both animals; *C*, although a continuous stream of nicotin enters the circulation, it is effective for only 8 or 10 minutes in either dog; *D*, the vasomotor and adrenal secreting effects of splanchnic stimulation can only be suppressed with great difficulty.

Adrenal extirpation. Adrenalectomized dogs show the same rise of blood pressure when nicotin is injected as normal animals. A dog weighing 23 kgm., and which had had both adrenals extirpated, gave a rise of blood pressure of 206 mm. Hg after the injection of 10 mgm. nicotin. A second adrenalectomized animal (20 kgm.) showed a rise of blood pressure of 190 mm. after injection of 5 mgm. nicotin. This does not mean that epinephrin plays no part in the rise in blood pressure, but simply that it is not the sole factor in its production. In an intact animal a vasomotor and an adrenal mechanism work together; when the adrenals are suppressed, only the vasomotor factor is present and in transfusion experiments only the adrenal mechanism is active in the recipient.

It is remarkable that the rise of blood pressure is generally greater in the recipient that receives the discharge of one adrenal only, than in the donor that has both the other adrenal and the vasomotor mechanism active. A possible explanation of this is that nicotin diminishes the response of the muscles of the blood vessels.

The importance of the adrenal factor is seen in the following experiment. The celiac plexus of an animal is taken out. The injection of 1 mgm. nicotin produces a rise of blood pressure of 180 to 200 mm. Hg. Then the adrenals are extirpated and injections of 1, 2 and 5 mgm. nicotin give no rise in blood pressure. Either the nervous mechanism (celiac plexus) or the humoral mechanism (adrenals) must be present to obtain a rise in blood pressure after nicotin injection. If only one of them is suppressed, the other acts vicariously; when both are destroyed, no effect is observed.

INFLUENCE OF NERVOUS SYSTEM. It is a common observation that destruction of the central nervous system does not suppress the rise in blood pressure produced by nicotin.

Section of the splanchnics and adrenal denervation. The section of the major splanchnic does not suppress the discharge in the homolateral adrenal (experiment of 6/15/1925). Bilateral section of the major and minor splanchnics, extirpation of the left abdominal sympathetic chain and the right adrenal does not suppress the discharge of the left adrenal after nicotin (experiments of 4/17/1925 and 4/18/1925). The same is true when the right adrenal is extirpated and the left denervated (experiment of 4/20/1925). The extirpation of the left adrenal and the semilunar ganglion does not suppress the discharge in the right adrenal when cystosin is given (experiment of 5/4/1925). Complete denervation of the left adrenal does not suppress its discharge after injections of nicotin, cystosin, lobelin (fig. 13), hordenin methylicdate and tetramethylammnoium iodid. In

these experiments to be absolutely sure that no nervous connections with the adrenals remained, the celiac ganglion was removed and all the nerve structures around the hepatic, superior mesenteric and renal arteries were destroyed. The adrenal was dissected so that it was only attached by the lumbo-adrenal vein and a few small arteries. This diminished the adrenal circulation somewhat—0.5 to 1 cc. blood per minute flowing from the lumbo-adrenal vein. Epinephrin injections in the donor sometimes caused a slight rise of blood pressure in the recipient, especially when the adrenal

TABLE 8

Adrenals denervated

DATE, 1925	WEIGHT		DRUG INJECTED		RISE OF BLOOD PRESSURE		INCREASE IN HEART RATE RECIPIENT BEATS PER MINUTE	TOTAL EPINEPHRIN DISCHARGED
	Donor	Recipient			Donor	Recipient		
	kgm.	*kgm.*		*mgm.*	*mm. Hg*	*mm. Hg*		*mgm.*
4/15	17.5	9.0	Nicotin	17.5	124	200	130	0.2
4/17	18.0	7.0	Nicotin	18.0	90	40	42	0.1
4/18	19.0	10.0	Nicotin	11.0	90	190	122	0.1
4/20	19.0	5.3	Nicotin	10.0	210	170	88	0.06
6/1	15.5	12.5	Nicotin	2.0	94	160	34	
6/2	24.0	5.0	Nicotin	2.0	108	166	84	0.04
6/2	24.0	5.0	Nicotin	1.0	26	90	18	0.03
5/4	16.5	8.0	Cystosin	16.5	60	140	122	0.075
6/1	15.5	12.5	Cystosin	1.0	68	130	44	
6/1	15.5	12.5	Lobelin	0.5	60	124	38	
6/2	24.0	5.0	Lobelin	1.0	88	172	92	0.04
7/24	17.5	15.5	Hordenin sulphate	10.0	20	0	0	
7/24	17.5	15.5	Hordenin sulphate	20.0	24	0	0	
7/24	17.5	15.5	Hordenin methyliodid	10.0	140	132	60	
7/24	17.5	15.5	Tetramethylammonium iodid	10.0	50	50	18	
7/24	17.5	15.5	Hordenin sulphate	10.0	20	0	0	
7/24	17.5	15.5	Hordenin sulphate	20.0	24	0	0	
7/24	17.5	15.5	Hordenin methyliodid	10.0	140	132	60	
7/24	17.5	15.5	Tetramethylammonium iodid	10.0	50	50	18	
7/24	17.5	15.5	Tetrabetahydronaphthylamin	20.0	0	0	0	

circulation was very slow (0.25 cc. per minute), but never produced the considerable increases seen after nicotin, cystosin or lobelin. Asphyxia, stimulation of the medulla, etc., produced no rise of blood pressure in the recipient. It can, therefore, definitely be stated that nicotin and the other drugs studied produce their effects through a real increase in the epinephrin secretion and not by a simple alteration of the circulation.

Ergotoxin. As this drug paralyzes the endings of the sympathetic and suppresses or inverts the effects of its stimulation or those of epinephrin

injection we thought it would be of interest to study the effects on the nervous mechanism of adrenal secretion.

Protocol.

5/16/1925. Donor (23.5 kgm.). Recipient (16 kgm.). Epinephrin (0.05 mgm.) was injected into the donor, whose blood pressure rose 170 mm. Hg. No effect was observed on the recipient. Three injections of 25 mgm. ergotoxin were made with 5 and 3 minute intervals between them. The same dose of epinephrin now gave a rise of only 70 mm. After 40 mgm. more of ergotoxin, epinephrin still gave a rise of 30 mm. Hg. With 2 to 3 minute intervals 4 injections of 25 mgm. ergotoxin were given and epinephrin still produced a rise of 30 mm. The donor's left splanchnic was stimulated and its blood pressure rose only 20 mm. whereas the recipient's showed a considerable increase (190 mm.); proof that an adrenal discharge had been produced. Five subsequent doses of 25 mgm. ergotoxin suppressed all rise of blood pressure in the donor after splanchnic stimulation, but did not interfere with the adrenal discharge, as a rise of 60 mm. was recorded in the recipient. Nicotin (5.2 mgm.) was then injected into the donor, whose blood pressure rose 40 mm.; the recipient's blood pressure went up 190 mm. owing to an adrenal discharge.

TABLE 9

DATE, 1925	WEIGHT		NICOTIN INJECTED INTO THE ADRENAL	BLOOD-PRESSURE VARIATION		INCREASE IN HEART RATE OF RECIPIENT
	Donor	Recipient		Donor	Recipient	
	kgm.	*kgm.*	*mgm.*	*mm. Hg*	*mm. Hg*	
6/ 5	23.0	9.3	0.15	0	180	66
6/ 5	23.0	9.3	0.15	0	90	58
7/20	23.5	11.0	0.0001	0	50	40
7/20	23.5	11.0	0.00001	0	0	0

This experiment shows us that ergotoxin does not interfere with the action of nicotin on adrenal secretion. It also seems to indicate that the nerve endings of the splanchnics in the adrenals are not paralyzed by ergotoxin when the vaso-constrictor fibers have already been blocked.

Injection into the adrenal gland. The experiments described seemed to show that nicotin, cystosin and lobelin have a directly stimulating action on the adrenal medulla.

Injection of saline solution (0.1 to 0.15 cc.) directly into the adrenal medulla of a donor produced no effects in the recipient (six experiments). When 0.1 or 0.15 cc. of a 0.1 per cent nicotin solution is injected in exactly the same conditions, a marked adrenal discharge is immediately observed (two experiments).

Protocol.

6/5/1925. (Fig. 15) Donor (23 kgm.). Recipient (9.3 kgm.). An injection of 0.15 cc. of an 0.9 per cent sodium chloride solution was made into the donor's left adrenal. No change in blood pressure or pulse rate was registered in either dog. Three minutes later 0.15 cc. of a 0.1 per cent solution of nicotin in normal saline

was injected in the same way. The donor's blood pressure rose 6 mm. Hg; the recipient's pulse rate increased 64 beats per minute and its blood pressure rose 120 mm. After an interval of 42 minutes the injection of normal saline again produced no change. A second injection of nicotin, given as before, did not alter the donor's blood pressure or pulse rate, but raised the recipient's blood pressure 80 mm. Hg and increased the pulse 58 beats per minute.

The minimal effective dose of nicotin that produces an adrenal discharge when injected into the adrenal medulla is very small. We obtained a discharge with 0.0001 mgm. and not with 0.00001 mgm. (see fig. 16). Many other drugs have been studied but no one was nearly as potent as nicotin in this respect. (These experiments will be referred to in a later paper.)

Action on the semilunar ganglion. Nicotin produces its effects in a complex way. It acts on the central nervous system, on the sympathetic ganglia and directly on some tissues. It has been maintained that the nerve fibers that stimulate adrenal secretion arrive at the medulla without a relay in the ganglia. Elliott (1913) is of this opinion, having observed that section of the splanchnics is followed by degeneration of medullated fibers in the adrenal medulla, where nerve cells are very rarely found.

The semilunar ganglion was carefully isolated from the surrounding tissue by means of rubber membranes and then painted with a 1 per cent solution of nicotin. The donor's blood pressure rose only 3 mm., but the recipient's rose 76 mm. and the heart rate increased 66 beats per minute. Both responses lasted for 4 minutes (fig. 17).

Nicotin, therefore, stimulates the cells of the semilunar ganglion and apparently a relay does exist here in the adrenal secretory path. Only two sources of error are possible in our experiment; first, the nicotin might have been absorbed and thus have entered the adrenal by way of the blood or lymph; second, the diffusion of the drug would lead to the direct stimulation of the adrenal. Precautions were taken to avoid diffusion, so this second source of error can be practically disregarded.

We have confirmed Langley's observation on the difficulty of paralyzing the dog's sympathetic by means of nicotin.

Localization of the action of nicotin on adrenal secretion. Probably nicotin starts the secreting impulse in the central nervous system, the semilunar ganglion and the adrenal medulla.

The stimulation of the adrenal centers may be admitted, since nicotin has a diffuse stimulating effect on the central nervous system. In the present paper no proof has been given that this is so. It would be necessary to perfuse the head with nicotin solutions or to inject them into the carotid arteries. Under any other experimental conditions, peripheral effects come into play.

The stimulation of the celiac ganglion apparently occurs as the experi-

ments of painting it with nicotin show. This fact has some importance as a proof that at least some of the splanchnic fibers have a relay station in the ganglion before passing to the adrenal. Elliott's (1913) contention that all the fibers go directly to the medulla cells without a peripheral synapse cannot be maintained. At the most some fibers may follow an uninterrupted course, the others are relayed. Stewart and Rogoff had admitted *a priori* the direct action of nicotin on the celiac ganglion, but they offered no proof of this assertion.

The direct action of nicotin on the adrenal medulla, a tissue closely related embryologically with the sympathetic ganglia, had been suspected by various authors. Eichholtz (1923) showed that an increased adrenal secretion was present after nicotin injections in animals whose splanchnics had been severed. Our experiments prove that not only nicotin, but cystosin and lobelin produce an adrenal discharge when the gland is completely denervated. Eichholtz did not extirpate the celiac ganglion so that the results of his experiments may be attributed to a direct effect on the ganglion and not on the adrenal. A complementary proof is given by the experiments in which an adrenal discharge is obtained by injecting the drug directly into the gland.

SUMMARY

1. Using Tournade and Chabrol's method of suprareno-jugular anastomosis, it has been observed that nicotin, cystosin, lobelin, hordenin methyliodid and quaternary ammonias produce a marked adrenal discharge, generally lasting a short time.

2. The discharge is not due to the increase of blood pressure observed in the donor.

3. The effects observed are not due to the passage of the drug through the anastomosis from the donor to the recipient.

4. The adrenal discharge produces the following effects in the recipient: marked increase in blood pressure, great acceleration of the heart rate, inhibition of the intestine, contraction of the spleen or the denervated limb, and hyperglycemia.

5. The peak in the discharge occurs between the first and third minute, and can be as much as 0.10 mgm. per minute for one gland. Three to five minutes later the normal level or a lower one is established.

6. Repeated injections produce repeated discharges but when the injections are made at short intervals and the dose is large, the effects diminish in intensity. Continuous injections produce a continuous discharge, but of decreasing amounts.

7. The discharge is produced after the central nervous system has been destroyed, the splanchnics cut or the gland has been thoroughly denervated.

8. A discharge is obtained when the celiac ganglion is painted with nicotin.

9. Nicotin, cystosin, lobelin and quaternary ammonias stimulate the adrenal medulla directly.

10. Nicotin produces its characteristic effects, rise of blood pressure and retarding of heart rate, in adrenalectomized dogs.

BIBLIOGRAPHY

BACKMAN, E.-L., G. EDSTRÖM, E. GRAHS AND G. HULTGREN. 1925. C. R. Soc. Biol., xciii, 186.

BAYLISS, W. M. AND E. H. STARLING. 1899. Journ. Physiol., xxiv, 99.

CAMUS, L. 1906. C. R. Acad. Sciences, cxlii, 237.

CANNON, W. B., J. C. AUB AND C. A. L. BINGER. 1912. Journ. Pharm. Exper. Therap., 1912, iii, 379.

CARVILLE AND ROCHEFONTAINE. 1874. C. R. Soc. Biol., xxvi, 140.

DALE, H. H. AND P. P. LAIDLAW. 1911–1912a. Journ. Pharm. Exper. Therap., iii, 205.

1912b. Journ. Physiol., xlv, 1.

DIXON, W. E. 1924. Heffter-Handb. d. Exper. Pharm. Julius Springer, Berlin.

EICHHOLTZ, F. 1923. Arch. f. Exper. Pathol. u. Pharm., xcix, 172.

ELLIOTT, T. R. 1913. Journ. Physiol., xlvi, 285.

GLEY, E. 1889. C. R. Soc. Biol., xli, 110.

1892. C. R. Soc. Biol., xliv, 680.

1914. C. R. Acad. Sciences, clviii, 2008.

KUDRJAWZEW, N. N. 1924. Zeitschr. f. d. gesammt. exper. Med., xli, 114.

LANGLEY, J. N. AND W. L. DICKINSON. 1889. Proc. Roy. Soc., xlvi, 423.

1890. Proc. Roy. Soc., xlvii, 379.

1890. Journ. Physiol., xi, 509.

LANGLEY, J. N. 1890. Journ. Physiol., xi, 123.

1896. Journ. Physiol., xx, 223.

1919. Journ. Physiol., liii, 147.

LANGLEY, J. N. AND R. MAGNUS. 1905. Journ. Physiol., xxxiii, 34.

MAGNUS, R. 1905. Pflüger's Arch., cviii, 1.

MANSFELD, G. 1908. Maly's Jahresberichte, xxxviii, 1250, cit. Backman.

NIKOLAEFF, M. P. 1924. Zeitschr. f. d. gesammt. exper. Med., xlii, 213.

SCHKAWERA, G. L. AND A. I. KUSMETZOW. 1923. Zeitschr. f. d. gesammt. exper. Med., xxxviii, 37.

SHIMIDZU, K. 1924. Arch. exper. Path. u. Pharm., ciii, 52.

STEWART, G. N. AND J. M. ROGOFF. 1919. Journ. Pharm. Exper. Therap., xiii, 183.

STROOMANN. 1925. Klin. Wochenschr., iv, 1186.