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NORADRENALINE IN ARTERY WALLS AND ITS DISPERSAL BY RESERPINE

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The presence of noradrenaline in vessel walls was established by the work of Schmitterlw in 1948, but so far there has been nothing to indicate that this noradrenaline plays any part in determining vascular tone. Most workers have continued to believe that this tone is controlled only by the impulses passing along sympathetic nerves and by substances circulating in the blood.

Evidence relating to the noradrenaline in vessel walls has come from a study of the action of nicotine. The pressor effect of nicotine is ordinarily considered to be due to its stimulation of sympathetic ganglia, but nicotine has in addition a direct action on blood vessels. Handovsky and Pick (1913) first described a vasoconstrictor action when they perfused the vessels of the frog's hind legs with a 1% solution; in 1937 Loewi made a similar observation, using a 0.01% solution, and in 1946 Haimovici and Pick obtained vasoconstriction by injecting as little as 5 μ g. of nicotine. Later, Haimovici (1948) showed that continuous perfusion with nicotine, 1 in 50,000, caused initial constriction, but the rate of flow gradually increased and the injection of large doses was then without effect. Haimovici found that the action of nicotine was unaffected by the removal of the sympathetic chains and of the spinal nerves. He concluded that the action was peripheral to the postganglionic fibres and perhaps directly on the blood vessels.

Not only had nicotine an anomalous action on the blood vessels, but so had acetylcholine. The dilator action of small amounts, such as 5 μ g., on the perfused vessels of the isolated rabbit ears was described by Dale in 1914, but in 1918 Reid Hunt stated that large amounts caused vasoconstriction both in the rabbit ear and in the vessels of the muscles of cats and of dogs; in the rabbit ear he injected as much as 5 mg.; he concluded that acetylcholine had a constrictor action on blood vessels which was exerted somewhere "beyond the ganglia cells." In 1932 Hirose observed that when acetylcholine was injected into the femoral artery of a cat the outflow of blood from the leg was diminished.

In 1948 Burn and Dutta observed the constrictor effect of acetylcholine in the perfused rabbit ear, and showed that, like the constrictor effect of adrenaline, it was converted to a dilator effect when tolazoline ("prisco") was added to the perfusion fluid. In 1953 Kottogoda extended this work and showed that the constrictor action of nicotine also was converted to a dilator action when tolazoline was added to the perfusion fluid. He showed that the normal dilator effect of acetylcholine

became a constrictor effect in the presence of atropine, and he noted that both the constrictor effect of nicotine and that of acetylcholine were blocked by hexamethonium. Finally, Kottogoda made the discovery that the site of the constrictor effect of nicotine and acetylcholine was in the skin. When he removed the skin and perfused the network of vessels lying on the cartilage he saw that both substances caused only dilatation. Kottogoda expressed the view that the constrictor action of nicotine and that of acetylcholine was due to the release of an adrenaline-like substance.

Ginzel and Kottogoda (1953) made observations on denervated ears from rabbits in which the stellate ganglion and the superior sympathetic ganglion of one side had been removed three weeks earlier, and also on ears to which the great auricular and great occipital nerves had been cut. They observed that the constrictor effect of nicotine and of acetylcholine was still present, and concluded that this effect did not depend on the presence of sympathetic or sensory nerve fibres. They repeated the opinion that the constrictor action was due to the release of an adrenaline-like substance.

The presence in extracts of various organs of substances having the chemical properties of catechol amines (which might be adrenaline, noradrenaline, and dopamine) was demonstrated by Shaw (1938) and by Raab (1943). Noradrenaline was shown to be present in the heart and in the spleen by von Euler (1946), and noradrenaline was found in the walls of arteries and veins by Schmitterlw (1948).

Holzbauer and Vogt (1956) and Carlsson and Hillarp (1956) observed that the alkaloid reserpine diminished the amount of adrenaline and noradrenaline in the adrenal medulla. Bertler, Carlsson, and Rosengren (1956) stated that the catechol amines present in the heart of the rabbit disappeared when an intravenous injection of reserpine (5 mg./kg.) was given to the rabbit 16 hours previously. We took hearts from rabbits treated in this way and demonstrated that, when the atria were suspended in a bath containing atropine, the addition of nicotine to the bath no longer caused stimulation (Burn and Rand, 1958). Our results indicated that the acceleration of the rate and increase of the amplitude caused by nicotine were due to the release of noradrenaline and adrenaline present in the heart. As a consequence we were led to ask whether the action of nicotine on the vessels of the rabbit ear would also be modified by administration of reserpine, and we now describe experiments which have been performed to test this possibility, and others arising from them.

Methods

In treating rabbits with reserpine we have injected a 0.1% solution prepared in 20% ascorbic acid. We have given an intraperitoneal injection on the first day and an intravenous injection on the second day. The rabbit was killed on the third day. From the time the rabbit was injected it was kept in a warm room at 27° C. We prepared both ears for perfusion, using the apparatus for recording the outflow described by Stephenson (1948), and arranging the perfusion as described by Burn (1952). The perfusion was carried out at room temperature (20° C.) with a solution containing NaCl 9 g., KCl 0.42 g., CaCl₂ 0.24 g., NaHCO₃ 0.5 g., dextrose 1 g., distilled water 1,000 ml. This solution was filtered and aerated with oxygen before use.

The catechol amine content of the skin of the rabbit ear was estimated. A depilatory cream was applied to the outer surface of the ear usually two days in advance and the hair and cream were carefully removed after 10 minutes. When the rabbit was killed all the skin of the dorsal surface was taken, the subcutaneous tissue near the base was trimmed off, and the skin was weighed. It was chopped finely by a McIlwain chopper and transferred to an ice-cold mortar, in which it was ground with a little sand. Cold acid saline (pH 4.5–5.5) was added, and the tissue was again ground. The whole was transferred to a centrifuge tube and was spun at 12,000 per minute at 0° C. The supernatant was used directly for estimation on the rat's blood pressure, or it was stored at –10° C.

The noradrenaline present in the aorta was determined by removing the aorta from the arch down to the origin of the superior mesenteric artery or sometimes lower; care was taken to ensure that no adrenal tissue was included; loose tissue was cut away, the aorta was opened, washed with saline, dried between blotting paper, and then weighed. It was placed in the deep freeze for at least 30 minutes and cut up with fine scissors in an ice-cold mortar. From this point the same procedure was followed as for the skin of the ear.

The estimation on the rat blood pressure was performed as described by Muscholl and Vogt (1957), in which the brain and spinal cord of the rat are destroyed by pithing (Shipley and Tilden, 1947), and the rat receives 1–2 mg. of atropine.

Observations on strips of rabbit aorta were made by taking the thoracic aorta and cutting it into a spiral. This was facilitated by passing a rod through the aorta and fixing the rod in a horizontal position. When the end of the aorta was held in forceps and the spiral cut was begun, the cut piece could be gently pulled away from the rod, the aorta slipping round the rod. The spiral strip was suspended in an 8-ml. bath, the upper end being attached to a lever and suitably weighted.

Perfusion of Isolated Rabbit Ears

The first observations were made to ensure that the effect of nicotine, and that of acetylcholine in the presence of atropine, were consistent effects to be observed in the ears of all rabbits. We perfused both ears from nine rabbits and found that injections of nicotine acid tartrate from 5 to 20 µg. always caused vasoconstriction; we found also that, when 8 µg. of atropine was first injected, injections of acetylcholine from 20 to 40 µg. also caused vasoconstriction.

The next observations were made on the ears from rabbits treated on the two previous days with reserpine. They received 1.5 mg./kg. by intraperitoneal injection on the first day, and 5 mg./kg. by intravenous injection on the second. We perfused the ears of eight rabbits and observed that, in the ears of seven, neither nicotine nor acetylcholine (in the presence of atropine) caused vasoconstriction, no matter how large the amount injected. In one rabbit, however, constrictor effects were seen. The difference between ears from normal and from reserpine-treated rabbits is shown in Fig. 1.

We perfused the ears of 10 other rabbits treated on two days with smaller total amounts of reserpine. In three of these neither nicotine nor acetylcholine caused vasoconstriction; in three these substances caused constriction; and in four they caused a slight constrictor response followed by dilatation.

We tested other constrictor substances to determine their effect on the ears of rabbits treated with reserpine. We

FIG. 1.—Perfused rabbit ears. Outflow record. Upward=increased outflow=vasodilatation. (a) and (b) Normal ear; 10 µg. ACh and then 8 µg. atropine followed by 20 µg. ACh. Then 10 µg. nicotine acid tartrate. (c) Ear from rabbit given reserpine; 10 µg. nicotine acid tartrate; then 8 µg. atropine followed by 20 µg. ACh.

found that hydroxytryptamine caused constriction as in normal rabbits; its effect was unchanged by reserpine treatment. We also tested tetraethylammonium; its constrictor effect was unchanged. We examined piperidine, but found that it was without vasoconstrictor action in a dose of 0.1 mg., although 5 µg. of nicotine was effective.

Presence of Noradrenaline in the Skin

These observations gave support to the indication in Kottagoda's work that nicotine caused vasoconstriction by releasing an adrenaline-like substance. Since he had also shown that the constrictor action disappeared when the skin was removed, we proceeded to extract the skin in the hope of detecting the presence of an adrenaline-like substance in it. The extracts were tested in comparison with noradrenaline on the blood pressure of the rat. We extracted the skin from the ears of normal and of reserpine-treated rabbits and obtained the results given in Table I. These showed that

TABLE I.—Active Substance Estimated as Noradrenaline (µg./g.) in the Skin of Rabbit Ears

Control Rabbits	Reserpine-treated Rabbits
0.10	0.03
0.06	0.04
0.11	0.03
0.20	0.03
0.07	0.03
0.05	0.005
Mean 0.1	0.03

measurable amounts of a substance similar in behaviour on the blood pressure to noradrenaline, and estimated in terms of it, were present in the skin of the normal ears but absent from the skin of ears from rabbits treated with reserpine.

The mean amount was $0.1 \mu\text{g./g.}$ in the normal ears and $0.03 \mu\text{g./g.}$ in the ears from treated rabbits.

Chromaffin Cells in the Skin

Adams-Ray and Nordenstam (1956) have described a system of chromaffin cells in human skin assembled round nerves and blood vessels, and containing granules of the same type as those in the adrenal medulla. Since we were able to extract an adrenaline-like substance from the skin of the ears we consulted Mr. E. H. Leach, of the University Department of Physiology, and he kindly agreed to make a histological examination. He reported as follows.

The ears of four rabbits were removed after death and fixed in Orth's fluid. Paraffin sections were cut at 8μ and stained by the modified Giemsa method described by Nordenstam and Adams-Ray (1957). In ears from two normal rabbits, cells similar to those described by them were seen in the dermal and sub-dermal tissues. They were usually in the neighbourhood of blood vessels. Occasionally cells forming part of the endothelial lining were found to contain similar granules. There was no difficulty in distinguishing these cells from mast cells, as the latter were more spherical in shape and contained larger granules. Control sections stained with azul II confirmed the distinction; the mast-cell granules stained metachromatically, whereas the fine chromaffin granules did not stain. The chromaffin granules did not give a positive reaction with Schmorl's ferriferrocyanide reagent, although melanin and premelanin granules did. This was a finding which differed from that of Nordenstam and Adams-Ray.

In the ears of the two rabbits treated with reserpine very few chromaffin granules could be identified. In some sections not a single granule was observed. If present in a cell they were few in number and light in staining affinity. The mast-cell granules were not affected.

Noradrenaline in the Aorta

Having discovered that a substance like noradrenaline which was present in the skin was caused to disappear by injections of reserpine, we then decided to examine the arteries in order to see if their content of noradrenaline had also diminished. For this purpose we used the thoracic aorta, extracting it as we extracted the skin of the ears. The extracts were tested as before on the blood pressure of the rat, and a record of an experiment is given in Fig. 2, which showed that 0.4 ml. of an extract prepared from the aorta of a normal rabbit had a pressor effect like that of $0.02 \mu\text{g.}$ of noradrenaline, while the same volume of an extract from the aorta of a rabbit treated with reserpine had no pressor effect. The estimates of a series of

Fig. 2.—Blood pressure of pithed rat given atropine. Injection at 20 of $0.02 \mu\text{g.}$ noradrenaline. Injection at A of 0.4 ml. aorta extract from rabbit treated with reserpine. Injection at B of 0.4 ml. aorta extract from normal rabbit.

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TABLE II.—Active Substance Estimated as Noradrenaline ($\mu\text{g./g.}$) in Rabbit Aorta

Control Rabbits	Reserpine-treated Rabbits
0.34	0.16
0.42	0.15
0.25	Less than 0.04
0.27	" " 0.11
0.50	" " 0.14
0.53	" " 0.07
0.73	" " 0.06
0.42	" " 0.14
0.21	
0.71	
0.75	
Mean 0.47	0.11

experiments are given in Table II. This shows that all values for reserpine-treated rabbits were lower than the lowest value for control rabbits, and that the noradrenaline present was reduced to about one-fifth or less by treatment with reserpine.

Perfusion of the Dog's Hind Leg

We examined the effect of nicotine in the vessels of the dog's hind leg perfused with blood, using a Dale-Schuster pump. We recorded the arterial resistance and the venous outflow as shown in Fig. 3. When 0.1 mg. of nicotine (base) was injected we observed an increase in resistance and a decrease in outflow as shown in Fig. 3a. We then infused

Fig. 3.—Perfused hind leg of dog. Upper record of venous outflow. Lower record of arterial resistance. (a) Shows increase of arterial resistance when 0.1 mg. nicotine (base) was injected into arterial cannula. A slow infusion of acetylcholine (2 mg. in all) was then made, and the injection of 0.1 mg. nicotine then had no effect. (b) A similar injection in a different experiment in which the sciatic nerve had been cut 11 days previously. Nicotine caused vasodilatation.

acetylcholine into the blood entering the leg at a rate approximately $1 \mu\text{g./second}$ for 30 minutes. The injection of 0.1 mg. of nicotine had then almost no effect as shown. We carried out another perfusion in a dog in which the left sciatic nerve was cut 11 days previously. In this experiment the injection of 0.1 mg. of nicotine caused the fall of resistance and increase of outflow shown in Fig. 3b. This fall of resistance was also seen in the perfused leg of a dog which had been given reserpine, 0.5 mg./kg. , on two successive days before the experiment.

We concluded that the vasodilator action was the direct effect of nicotine seen when the vessel walls contained little noradrenaline. We perfused the hind leg of three other dogs. In one 0.1 mg. of nicotine increased the resistance slightly, but a large rise, greater than that in Fig. 3a, was seen when another nicotine-like substance D.M.P.P. (dimethyl-phenyl-piperazinium) was injected in a dose of 0.5 mg. In the other two there was little or no rise in arterial resistance with nicotine, but there was probably some release of noradrenaline, or the fall shown in Fig. 3b would have occurred. The general conclusion was that in the vessels of the dog's hind leg, nicotine and substances acting like it released noradrenaline, but that the effect was not regularly demonstrable as in the perfused rabbit ear.

Noradrenaline in Dog Aorta

We extracted portions of the aorta of two normal dogs, and when the extracts were tested on the rat blood pressure we found that the amounts of noradrenaline present were respectively 1.1 and $0.8 \mu\text{g./g.}$ The aorta of a dog treated with reserpine on two successive days (0.5 mg./kg. intraperitoneally) contained less than $0.03 \mu\text{g./g.}$

Results with Aorta Strips

A series of observations were made on strips of rabbit aorta cut in a spiral and suspended in an isolated organ bath of 8 ml. As shown in Fig. 4a such strips responded with regularity when 0.1 mg. of nicotine (base) was added to the bath

FIG. 4.—Isolated aorta strip. (a) Contractions of strip from normal rabbit when 0.3 mg. nicotine acid tartrate was added to 8-ml. bath. (b) Contractions of strip from rabbit treated with reserpine. 0.5 mg. nicotine acid tartrate first caused a small contraction, but the next addition caused relaxation. Between the two the addition of 0.005 µg. of adrenaline caused contraction.

so that the concentration was 1.25×10^{-5} g./ml. When a strip was prepared from a rabbit treated with reserpine the effect of nicotine was variable; thus in the strip of which a record is given in Fig. 4b the first addition of nicotine to the bath caused a contraction, but the muscle began to relax before the bath fluid was changed, and when the second addition of nicotine was made the muscle relaxed. Out of a total of 10 strips prepared from rabbits treated with reserpine, nicotine caused only relaxation in six. In the others it appeared that sufficient noradrenaline was still present for nicotine to cause a contraction.

We tested substances which inhibited the action of nicotine in aorta strips from normal rabbits. The most active substance was hexamethonium, which depressed the contraction in a concentration of 1.25×10^{-7} g./ml. as shown in Fig. 5, but it was not always as active as this, and usually larger concentrations were needed. Pentobarbitone depressed the action of nicotine as shown in Fig. 6. This effect was due to depression of the release of noradrenaline, for the pentobarbitone did not diminish the action of noradrenaline itself. Because of a statement in the paper by Haimovici and Pick (1946) we also tested thiamine, and observed that it also depressed the action of nicotine.

FIG. 5.—Isolated aorta strip. Contractions due to addition of 0.3 mg. nicotine acid tartrate to 8-ml. bath. The second contraction was in the presence of 1 µg. hexamethonium, which reduced its height.

FIG. 6.—Isolated aorta strip. (a) Contractions due to the addition of 0.3 mg. nicotine acid tartrate to bath of 8 ml. The second contraction was preceded by the addition of 0.2 mg. pentobarbitone to the bath. (b) Contractions due to the addition of 0.09 µg. noradrenaline to the bath. These were not diminished in the presence of pentobarbitone.

Sensitivity to Noradrenaline

We observed that aorta strips taken from rabbits treated with reserpine were much more sensitive to noradrenaline and adrenaline than were strips taken from normal rabbits. This can be seen from the records in Fig. 4b and Fig. 6b. In Fig. 4b the aorta strip was from a reserpine-treated rabbit and the contraction was caused by 0.005 µg. of adrenaline. In Fig. 6b the strip was from a normal rabbit and the contraction was caused by 0.09 µg. of noradrenaline. Aortic strips from normal rabbits often failed to respond at all to 0.02 µg. of noradrenaline when this dose was first applied, but they became more sensitive after noradrenaline had been applied several times. Aortic strips from reserpine-treated rabbits were highly sensitive to the first application of noradrenaline, after which the sensitivity declined; later on it increased again as in normal rabbits. In Table III is

TABLE III.—Height of Contractions (mm.) of Isolated Aortic Strips when Noradrenaline was First Applied in an 8-ml. Bath

From Normal Rabbits		From Rabbits Treated with Reserpine	
Dose	Contraction	Dose	Contraction
0.02 µg.	0	0.02 µg.	75
0.08 „	8	0.02 „	94
0.02 „	2	0.001 „	26
0.02 „	10	0.005 „	51
0.02 „	9	0.005 „	10
Mean 0.032 „	6	0.01 „	51

given the height of the contraction in response to an initial application of noradrenaline for “normal” and for “reserpine-treated” strips. The mean contraction for reserpine-treated strips was more than eight times that for normal strips, although the mean dose was less than one-third as great. In other words, the “reserpine” strips showed a hypersensitivity which is a well-known feature of denervated tissues.

Compounds Tested for Reserpine-like Action

We have made some tests of other substances chemically related to reserpine to see if they removed noradrenaline from the rabbit heart and vessels. These substances were serpentine, serpentinine, ajmaline, and yohimbine. When the rabbits injected with these substances were killed the ears were perfused, the atria were isolated and set up in an organ bath, and an extract was prepared from the aorta.

We found that the constrictor effect of nicotine in the perfused rabbit ear was abolished by a total amount of 6.5 mg. of reserpine per kg. We injected three rabbits with serpentine nitrate, one with a total dose of 6.5 mg./kg., a second with 15 mg./kg., and a third with 40 mg./kg. One ear of the rabbit injected with 15 mg./kg. gave a dilator response to nicotine. The isolated atria of the rabbit which received the low dose were not accelerated by nicotine, nor were those of that which received the high dose. The aortic extracts of all rabbits gave an atypical effect on the blood pressure, not resembling the effect of noradrenaline. One rabbit was injected with 15 mg. of serpentine hydrochloride per kg. Its tissues behaved in every way like those of a normal rabbit. One rabbit injected with 10 mg. of ajmaline per kg. was normal in the response of its ears and atria to nicotine, but the effect of the aortic extract on the blood pressure was again atypical. Two rabbits were injected with yohimbine hydrochloride, the second with a total of 40 mg./kg. in five doses on five successive days. Nicotine caused constriction of the perfused ears, though it did not cause much acceleration of the isolated atria. The atria, however, did not respond to adrenaline. The aortic extract was pressor, but the effect was unlike that of noradrenaline.

Discussion

The origin of the noradrenaline which is present in the artery walls is not known for certain. Schmiterlw (1948) found that the amount was greatest in the outer part of the wall where the nerve fibres entered. Noradrenaline has, however, also been found in other organs, and Goodall (1951) made the observation that the amount present in the heart declined after degeneration of the sympathetic nerves; in the sheep's heart the greatest fall occurred after removal of the right stellate ganglion. Von Euler and Purkhold (1951) observed a similar fall in the spleen, the kidney, the liver, and the salivary glands. In the spleen the normal amount of noradrenaline was 3-4 $\mu\text{g./g.}$, but 5-15 days after section of the sympathetic fibres it had fallen to the level of 0.005-0.05 $\mu\text{g./g.}$ The amount present rose again when sufficient time had passed for regeneration. We know that the antidiuretic hormone is formed in the supraoptic nucleus and then passed along the nerve fibres to the posterior lobe of the pituitary gland, where it accumulates. It is therefore a reasonable assumption that the noradrenaline present in the artery walls comes steadily out from the sympathetic nerve fibres, which were shown by von Euler (1946) to contain it, and that it collects in the wall somewhere near the nerve endings. Fatt and Katz (1950) have shown that there is a continuous small release of acetylcholine at motor nerve endings in skeletal muscle.

The evidence given in this paper shows that the accumulation of noradrenaline can be dispersed by the action of reserpine in the course of several hours, and that when it is gone certain substances, among which are nicotine and acetylcholine, lose a vasoconstrictor action which they previously possessed. Nicotine and acetylcholine thus appear as substances able to release noradrenaline from this store. It is of interest to recall that patients who have attacks of thrombo-angiitis obliterans find that their attacks are precipitated by smoking (McDevitt and Wright, 1955). It may be such a release of noradrenaline by nicotine which brings on the attacks.

A certain leakage of noradrenaline from the store perhaps occurs normally. In other organs after section of the sympathetic nerves the store disappears, which suggests that it has to be replenished in order to maintain it, and that some noradrenaline continually leaks away. If there is a similar situation in the blood vessels, the leak from the store may play some part in determining the vascular tone.

We already have given evidence that this happens in the heart (Burn and Rand, 1958). We set up a series of isolated atria from normal rabbit hearts and found that the mean rate of contraction at 29° C. was 146 a minute. We also set up atria from rabbits treated with reserpine from which

the noradrenaline and adrenaline had gone, and found that the mean rate of contraction was 112 a minute. The difference between the two figures was highly significant. Thus the noradrenaline and adrenaline present in the heart were shown to play a part in determining the rate.

The clinical conditions of Raynaud's disease and of Buerger's disease seem to be due to a factor causing excessive tone, and on the basis of the experiments we have described might be benefited by treatment with reserpine. Scalfi, Jacono, and Iuliani (1956) have indeed given reserpine to 13 cases of arterial disease recording vasodilatation by "photoplethysmography." They found that when 1 mg. of reserpine was injected intramuscularly there was prolonged vasodilatation in eight of the cases. Three were cases of Raynaud's disease, and dilatation occurred in two; in the other case lesions of scleroderma were present.

Whether there are substances normally present in the body which release noradrenaline from the store in the vessel walls we do not know. Piperidine was shown by von Euler (1945) to be present in human urine; it has a weak nicotine-like action, about 1/20 of that of nicotine. We tested its action on the perfused rabbit ear and failed to observe any vasoconstrictor effect. We also considered hydroxytryptamine, and made the observation that its accelerating action on isolated atria was absent in atria from rabbits treated with reserpine; however, its constrictor action on ear vessels was not affected.

The release of noradrenaline by nicotine was powerfully inhibited by hexamethonium; hence if noradrenaline, released from the artery wall by any similar agent, is a factor of importance in hypertension or peripheral vascular disease, hexamethonium will diminish the release in addition to blocking the ganglia. We also observed that pentobarbitone had some effect in preventing the release of noradrenaline by nicotine; such an action might conceivably contribute to the fall in blood pressure in patients with hypertension who are given rest in bed and barbiturates.

The increased sensitivity of reserpine-treated cats to the pressor action of adrenaline was first described by Bein, Gross, Tripod, and Meier (1953), and we have now observed that aorta strips from rabbits treated with reserpine were much more sensitive to noradrenaline and adrenaline than were normal strips. Thus the store of noradrenaline in normal aorta strips must keep the response to added noradrenaline small, and presumably in the body the store keeps the response to sympathetic impulses small. The effect of reserpine on the vascular tone is thus not simple. As Bein (1955) has shown, it diminishes the stream of impulses from the sympathetic centres, but this is not its only effect. In addition it removes the store of noradrenaline from the artery walls, and in doing so makes them more responsive to such impulses as still arrive. Thus the fall of blood pressure due to the diminution in the central outflow is less than it would be. In other words, under the influence of reserpine the blood pressure falls, but not greatly, because the fewer impulses act on a more sensitive vascular bed containing less noradrenaline.

The action of hexamethonium in patients with hypertension was shown by Harington (1956) to be greater when they had received 3 mg. of reserpine intravenously three to four hours before. Smirk (1956) has stated that the effect of hexamethonium is greater in sympathectomized patients. The common feature of giving reserpine and sympathectomy is that the patients may have less noradrenaline in the artery wall, and it is possible that the greater effect of hexamethonium is because of this.

On the question whether noradrenaline is normally released from the store in the arteries and is a factor in determining vascular tone, there is at present only circumstantial evidence. Hitherto the recovery of tone which occurs in sympathectomized limbs has not been explained. It may be due to the reappearance of noradrenaline in the vessel wall, though if there is such a reappearance the noradrenaline presumably does not reach the vessel by way of sympathetic nerves. Ginzel and Kottgoda (1953) observed

that the constrictor action of nicotine was present in the rabbit ear three weeks after sympathectomy, and this action was blocked by hexamethonium. This might suggest the existence of peripherally situated ganglia which were unaffected by "sympathectomy." Then chromaffin tissue was found in the rabbit ear as it was found in human skin. It is clear that we have as yet incomplete knowledge of sources of noradrenaline and adrenaline which may affect vascular tone. It seems, however, that they can all be dispersed by the action of reserpine.

The use of reserpine for peripheral conditions is, however, limited by its effect on the central nervous system. What is needed is a quaternary compound which will not penetrate the blood-brain barrier. Unfortunately neither serpentine nor serpentine, which are quaternary, has the same effect as reserpine. It may be that either choline 2:6-xylylether bromide, synthesized by Hey and Willey (1954) and investigated by Exley (1957), or a quaternary form of one of the benzoquinolizine derivatives described by Pletscher, Besendorf, and Bächtold (1958), or a quaternary form of reserpine itself would have the action desired.

Summary

The work of Schmitterlów showed that noradrenaline is present in artery walls, but hitherto it has not been considered to affect vascular tone. Certain substances such as nicotine, and (in the presence of atropine) acetylcholine, cause vasoconstriction in the vessels of the rabbit ear, and the mechanism of their action has not been understood hitherto. This vasoconstriction has now been shown to be due to the release of noradrenaline from the artery wall, since the vasoconstriction was absent in the ears from rabbits treated with reserpine.

When the skin of rabbit ears was extracted it was found to contain a substance like noradrenaline, but ears from rabbits treated with reserpine did not contain it. Mr. E. H. Leach demonstrated the presence of chromaffin cells in the ears of normal rabbits. These cells were absent in the ears of rabbits treated with reserpine.

Strips of aorta from normal rabbits, when suspended in a bath, contracted on addition of nicotine. They were found to contain noradrenaline in a mean amount of 0.5 $\mu\text{g./g.}$ Strips of aorta from rabbits treated with reserpine did not contract on addition of nicotine. They contained only traces of noradrenaline.

Nicotine had a constrictor action on the perfused vessels of the dog's hind leg, though not in all preparations.

Strips of aorta from rabbits treated with reserpine were highly sensitive to noradrenaline. From this it appeared that the store of noradrenaline in the vessel wall must normally reduce the effect of noradrenaline, and probably the effect of sympathetic impulses also.

Conditions such as Raynaud's disease may be due to a release of noradrenaline from the store in the artery wall, and might therefore benefit from the treatment with reserpine, which would reduce the store.

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SOME ASPECTS OF THE RECENT EPIDEMIC OF INFLUENZA IN DUNDEE

BY

A COMBINED STUDY GROUP*

The need for epidemiological intelligence reports from representative places in different countries on the progress and varying behaviour in influenza during an epidemic or pandemic has long been felt and sometimes stressed. The present communication from Dundee is intended as a contribution to the further development of such an intelligence service in the United Kingdom. Dundee appears to have suffered relatively late in the recent epidemic, and if, as some authorities fear, this epidemic is the first wave of a series our observations may be of special interest.

In assessing the time when the influenza reached epidemic proportions in Dundee the following three sets of weekly returns were useful: first certificates received by the Ministry of National Insurance; records of absenteeism kindly provided by various large industrial establishments; and returns of weekly percentage absence of pupils in local authority schools.

Sporadic cases of influenza were reported from housing districts as early as mid-August, and there was serological evidence of influenza A in a few cases dating from this time, but the disease did not reach epidemic proportions until the beginning of October; this was possibly about a fortnight later than in Edinburgh and Glasgow but about the same time as in Aberdeen.

The maximum figure for the weekly returns of "first certificates" received by the Ministry of National Insurance

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