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The effects of "autonomic drugs" on villous movement in the small intestine of the pigeon

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Summary

1. The responses of pigeon duodenal villi to intravenous injection or local application of "autonomic drugs" were studied and compared with those reported in dogs by other workers.

2. Choline esters, anticholinesterases, noradrenaline, adrenaline and nicotine all stimulated villous movement in the pigeon. Similar responses to these drugs have been reported in the dog. The effects of these drugs on villous activity could be inhibited by pretreatment of the bird with suitable antagonists, although hexamethonium was ineffective in preventing the effects of nicotine. Some of the antagonists also stimulated the villi.

3. Isoprenaline caused inhibition of villous movements, which could be prevented by pretreatment of the bird with propranolol. It appears that in pigeons the villi have both α-adrenoceptors, stimulation of which increases villous activity, and β-adrenoceptors, stimulation of which depresses villous movements.

Introduction

Intestinal villi can be divided into two main groups. They may be either flattened, leaf-like and incapable of movement or long, finger-like structures which can contract and relax. Among animals possessing the latter type are the dog, fox, cat, eagle, chicken and pigeon (Kokas, 1930, 1932). Most of the previous work on villous movement has been done on dogs and it therefore seemed of interest to study villous activity in another species. The pigeon was selected and villous movement was observed in the duodenum, where the villi are said to be the largest and most active of those in the pigeon intestine (Verzar & McDougall, 1936).

Methods

Pigeons of either sex weighing 300–500 g were used. They were allowed free access to food and water before the experiment. Anaesthesia was induced by intramuscular injection of urethane (1·5 g/kg). A cannula was then inserted into the brachial vein and surgical anaesthesia produced by intravenous injections of urethane (400–700 mg in divided doses). The temperature of the bird was maintained at 39°–40° C throughout the experiment with a homeothermic blanket placed beneath it.

The duodenal loop was exposed through a midline abdominal incision. A 2–3 cm incision was made round the tip of the loop along the opposite side to the mesenteric attachment, thus exposing the mucosa. Ligatures were tied round the duodenum on either side of this incision to prevent contamination with intestinal contents of the mucosa under observation. Care was taken to avoid damage to the pancreas, mesenteric vessels or nerves.

The exposed mucosa was pinned out in a modified Petri dish, diameter 7 cm, as shown in Fig. 1. Hot water circulating through the rubber tubing kept the temperature of the Ringer-Locke solution in the dish constant at 38°–39° C. The dish was supported on a small board placed over the lower part of the bird. After the preparation had been washed by gentle irrigation with warm Ringer-Locke solution it was left undisturbed for 10–15 min. The solution in the dish was then replaced with fresh Ringer-Locke solution prewarmed to approximately 38° C.

The villi were observed at $\times 25$ magnification with a stereoscopic microscope, the field being illuminated by a small lamp attached to the microscope. An eyepiece graticule divided the field into four 5 mm squares, each of which contained 30–70 villi at this magnification. Only the villi in one square were observed and each definite retraction of a villus was counted as one movement. Counts were done over a number of consecutive 1 min periods, control counts being made at the beginning of each experiment for a period of 5 min. Counting was never started for at least 3 min after fresh Ringer-Locke solution had been added to the dish.

Drugs were administered either by intravenous injection through the cannula in the brachial vein or by adding them to the Ringer-Locke solution bathing the mucosa. Not more than 0·4 ml drug solution was added to the Ringer-Locke solution in the dish, which was then gently stirred for about 10 s. The dish had a volume of approximately 20 ml.

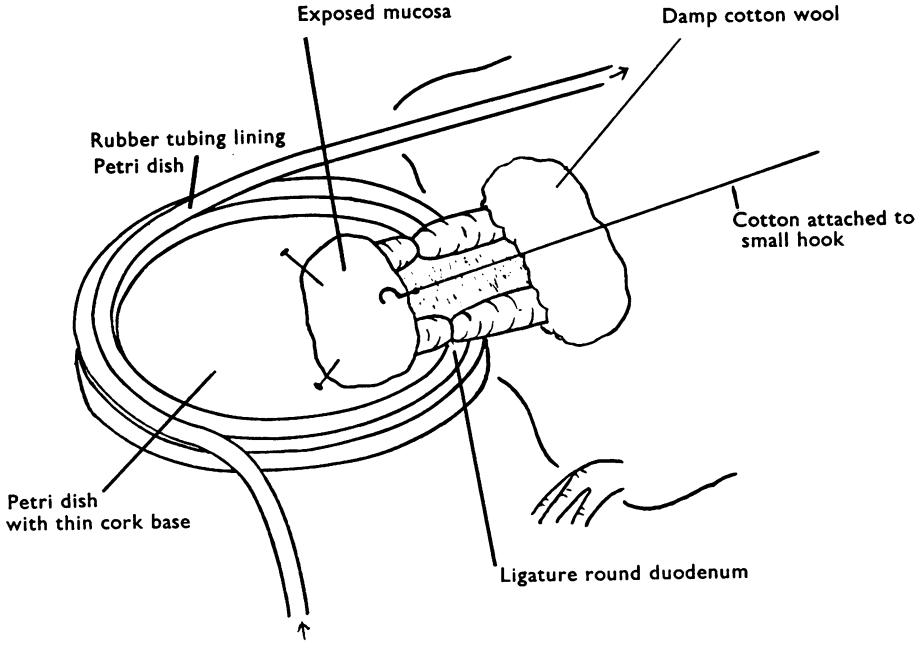

FIG. 1. Diagram of duodenum prepared for observation of villous movement.

The drugs used were acetylcholine bromide, methacholine chloride, carbachol (carbamylcholine chloride), physostigmine sulphate, neostigmine methylsulphate, atropine sulphate, noradrenaline acid tartrate, adrenaline tartrate, isoprenaline sulphate, phenoxybenzamine hydrochloride, tolazoline hydrochloride, propranolol hydrochloride, nicotine hydrogen tartrate, hexamethonium bromide, pempidine tartrate and pentolinium tartrate. Fresh solutions were prepared daily from stock solutions by dilution with 0·9% saline. Concentrations of drugs are expressed as weights of the salts except in the case of acetylcholine, noradrenaline and adrenaline, where they are expressed as weights of the bases.

Each experiment was repeated on at least four separate occasions.

Results

Movements of the villi generally consisted of rapid shortening (approximately 1 s) and slower lengthening (3–6 s). Occasional lashing movements were also seen, but these were disregarded in this investigation. The movements of any one villus occurred at irregular intervals and did not seem to be influenced by the activity of neighbouring villi.

In all experiments qualitatively consistent results were obtained when the same drug and route of administration were used. However, both the control counts and those obtained after drug administration varied from animal to animal. Examples of the range of variation which occurred are given in Tables 1 and 2, where the results of eight experiments with intravenous acetylcholine and ten experiments with intravenous isoprenaline respectively are listed. The effects of other drugs are illustrated by data obtained from one representative experiment in each case.

Effect of choline esters

Intravenous injection of acetylcholine (4 μg/kg) stimulated villous activity for 30–45 s. This increase began a few seconds after the injection. Similar stimulation was obtained after intravenous injection of 4 μg/kg methacholine or carbachol, although with both these drugs the increased activity lasted for up to 3 min. Movements of the mucosa and outer musculature of the intestine were observed for several minutes and mucus secretion also occurred. Addition of acetylcholine or methacholine to the Ringer-Locke solution bathing the mucosa to give a concentration of 10 μg/ml caused increased villous activity for 2–4 min after acetylcholine and for 5–10 min after methacholine. Carbachol had an effect similar to that of methacholine, but in lower concentrations (2·5–5 μg/ml). Typical responses to these drugs are shown in Table 1.

The responses of the villi to intravenous injection or local application of any of the three choline esters were completely inhibited by intravenous injection of atropine (1 mg/kg) 5 min previously.

Effect of anticholinesterases

Intravenous injection of 50 μg/kg physostigmine or 20 μg/kg neostigmine had a stimulating effect on villous activity which reached a peak at the third–sixth minute after injection (Table 1). Mucosal movements were also stimulated. Pretreatment of the pigeon with atropine (1 mg/kg) injected intravenously prevented the response of the villi to either of these drugs.

TABLE 1. *Effect of intravenous injection or local application of choline esters and anticholinesterases on villous movement*

Drug	Route	Dose	Control period (counts/min)					After drug (counts/min)							
			1	2	3	4	5	1	2	3	4	5	6	7	8
Choline esters															
Acetylcholine	i.v.	4 μg/kg	3	0	0	2	1	58	1	0	2	3			
	i.v.	4 μg/kg	0	0	1	0	0	46	0	0	1	1			
	i.v.	4 μg/kg	0	0	0	1	0	21	0	0	0	0			
	i.v.	4 μg/kg	4	5	5	3	3	49	2	0	2	3			
	i.v.	4 μg/kg	4	1	5	4	5	59	4	2	4	3			
	i.v.	4 μg/kg	0	0	1	1	1	57	2	1	1	0			
	i.v.	4 μg/kg	4	3	3	4	2	37	2	1	3	4			
	i.v.	4 μg/kg	0	0	0	1	1	62	2	1	0	0			
Methacholine	i.v.	4 μg/kg	6	7	12	12	10	110	38	15	12	9			
Carbachol	i.v.	4 μg/kg	3	2	3	1	3	114	30	5	4	2			
Acetylcholine	local	10 μg/ml	3	1	1	1	2	61	55	9	1	0			
Methacholine	local	10 μg/ml	1	1	0	0	0	65	95	51	74	2			
Carbachol	local	2·5 μg/ml	0	1	0	0	0	56	61	37	6	12			
Anticholinesterases															
Physostigmine	i.v.	50 μg/kg	2	7	5	4	5	8	19	61	60	27	15	7	6
Neostigmine	i.v.	20 μg/kg	2	0	2	3	2	2	4	24	35	14	11	5	2

TABLE 2. *Effect of intravenous injection or local application of catecholamines on villous movement*

Drug	Route	Dose	Control period (counts/min)					After drug (counts/min)							
			1	2	3	4	5	1	2	3	4	5	6	7	8
Noradrenaline	i.v.	4 μg/kg	3	1	3	2	2	35	3	2	2	2			
Adrenaline	i.v.	4 μg/kg	3	2	1	3	0	41	3	2	3	2			
Isoprenaline	i.v.	4 μg/kg	25	26	21	23	22	1	16	25	20	21			
	i.v.	4 μg/kg	24	28	24	23	25	0	7	19	28	25			
	i.v.	4 μg/kg	12	16	8	8	10	0	11	13	15	14			
	i.v.	4 μg/kg	22	28	33	25	26	5	21	20	23	29			
	i.v.	4 μg/kg	5	7	4	5	4	1	2	3	6	4			
	i.v.	4 μg/kg	7	5	4	5	4	0	2	6	4	5			
	i.v.	4 μg/kg	3	2	2	3	4	0	1	3	3	2			
	i.v.	4 μg/kg	8	4	3	5	4	0	7	8	3	3			
	i.v.	4 μg/kg	9	7	12	10	11	2	10	6	9	10			
	i.v.	4 μg/kg	20	8	18	15	23	1	23	18	11	11			
Noradrenaline	local	10 μg/ml	3	3	0	1	0	26	77	8	0	1	0	0	2
Adrenaline	local	5 μg/ml	3	3	5	3	1	14	20	10	7	3	0	3	4
Isoprenaline	local	10 μg/ml	9	7	0	7	8	5	3	2	1	2	2	1	0

Effect of atropine

Neither intravenous injection of atropine (1–2 mg/kg) nor addition of atropine to the Ringer-Locke solution covering the mucosa to give a concentration of 30–40 μg/ml had any effect on villous movement. This concentration of atropine completely inhibited the response of the villi to acetylcholine (4 μg/kg) injected intravenously after the mucosa had been in contact with atropine for 10 min.

Effect of catecholamines

Both noradrenaline and adrenaline stimulated villous activity for approximately 1 min after intravenous injection (4 μg/kg). Transient blanching of the mucosa occurred immediately after the injection and in the case of adrenaline this was accompanied by retraction of all the villi for a few seconds. After an intravenous injection of isoprenaline (4 μg/kg), however, villous movements were almost completely inhibited for between 30 s and 3 min, after which normal activity was resumed. Addition of noradrenaline or adrenaline to the Ringer-Locke solution covering the mucosa to give a concentration of 5–10 μg/ml resulted in progressively increasing pallor of the mucosa. Villous movements were stimulated for the first 2–5 min, after which activity returned to the control level or occasionally slightly below it. Movements of the mucosa and outer musculature of the intestine decreased. When this experiment was repeated with isoprenaline at a final concentration of 10 μg/ml a gradual decrease in the amount of villous activity was observed. These results are illustrated in Table 2.

If phenoxybenzamine (1 mg/kg) was injected intravenously 1 h beforehand, the villi were not stimulated by a subsequent intravenous injection or local application of noradrenaline or adrenaline. The initial retraction of the villi after adrenaline still occurred however and the blanching of the mucosa after either drug was reduced but not abolished. Similar results were obtained after pretreatment of the bird with tolazoline (1–2 mg/kg) injected intravenously 5–15 min beforehand (Figs. 2 and 3), although the response of the villi to local application of either drug was only partially inhibited. It was also noticed that after tolazoline there was sometimes a decrease in the number of villous movements compared with control values in the first minute after an intravenous injection of adrenaline, as illustrated in Fig. 3. The response of the villi to isoprenaline was not affected by phenoxybenzamine or tolazoline.

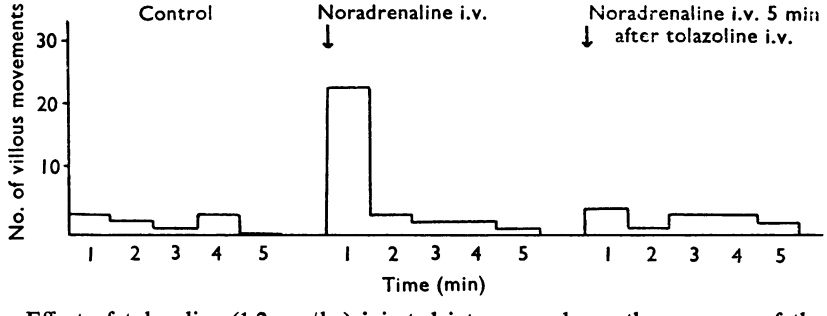

FIG. 2. Effect of tolazoline (1·2 mg/kg) injected intravenously on the response of the villi to an intravenous injection of noradrenaline (4 μg/kg).

An intravenous injection of propranolol (500–600 μg/kg) 5–10 min earlier completely prevented the response of the villi to isoprenaline given either by intravenous injection (Fig. 4) or by application to the mucosa. This dose of propranolol did not reduce the response of the villi to an intravenous injection of noradrenaline or adrenaline and the response to adrenaline was in fact sometimes potentiated, as shown in Fig. 4.

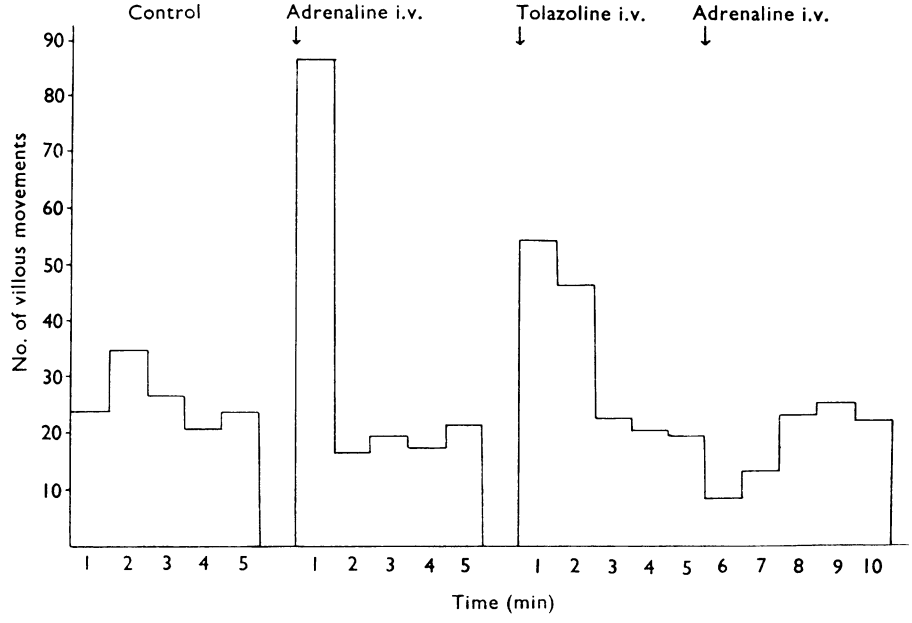

FIG. 3. Effect of tolazoline (1·0 mg/kg) injected intravenously on the response of the villi to an intravenous injection of adrenaline (4 μg/kg).

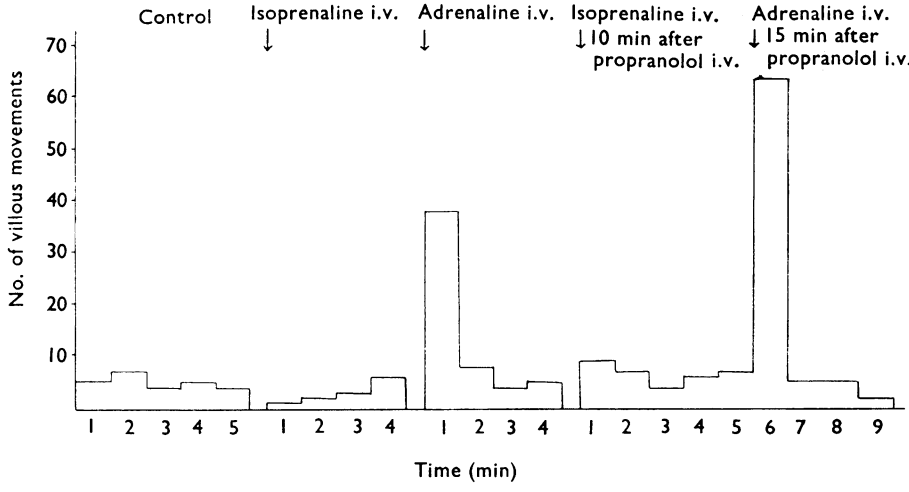

FIG. 4. Effect of propranolol (500 μg/kg) injected intravenously on the response of the villi to intravenous injections of isoprenaline (4 μg/kg) and adrenaline (4 μg/kg).

Effect of α- and β-adrenoceptor blocking agents

It is well known that the full effect of phenoxybenzamine is slow to develop. It was therefore not possible to do a control count before injecting it, as the mucosa could only be used for approximately 1 h after opening the duodenum. However, there was no evidence that phenoxybenzamine inhibited villous movements after intravenous injection (1 mg/kg). Tolazoline (1–2 mg/kg), as shown in Fig. 3, or propranolol (0·5–1 mg/kg) injected intravenously usually had a stimulating effect on villous activity for the first few minutes after injection. Addition of either of these drugs to the Ringer-Locke solution covering the mucosa to give concentrations of 40–50 μg/ml did not, however, affect the activity of the villi. These concentrations were sufficient to inhibit the response of the villi to intravenous injections of noradrenaline (4 μg/kg) and isoprenaline (4 μg/kg) respectively given 10 min later.

Effect of nicotine

Intravenous injection of nicotine (200–800 μg/kg) caused blanching of the mucosa and simultaneous retraction of all the villi. After a few seconds the mucosa became hyperaemic and the villi showed increased activity for approximately 1 min (Fig. 5). Movements of the mucosa and outer musculature of the duodenum were increased for a period of 1–2 min. Addition of nicotine to the Ringer-Locke solution bathing the mucosa to give a concentration of 200 μg/ml stimulated villous movements for about 2 min.

Hexamethonium, injected intravenously in doses of up to 4 mg/kg 5–10 min before an intravenous injection or local application of nicotine, had little effect on the response of the villi to nicotine. However, pretreatment of the pigeon with pempidine (0·6–1 mg/kg) injected intravenously 5–10 min beforehand blocked the response of the villi to nicotine as shown in Fig. 5, although it did not affect the initial blanching of the mucosa occurring after an intravenous injection of nicotine. Similar results were obtained after pretreatment of the bird with pentolinium (600 μg/kg) injected intravenously 10 min beforehand. An intravenous injection of atropine (1 mg/kg) also inhibited the response of the villi to nicotine injected intravenously 5 min later and the blanching of the mucosa was reduced. Neither phenoxybenzamine (1 mg/kg) nor tolazoline (1 mg/kg) on intravenous injection prevented the villi responding to intravenous injections of nicotine respectively 1 h and 10 min later. Blanching of the mucosa after nicotine was unaffected by pretreatment with either of these drugs.

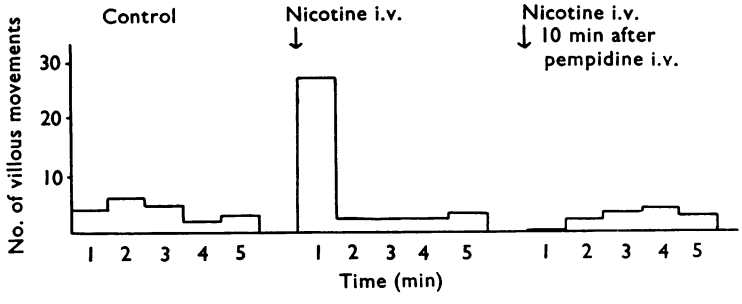

FIG. 5. Effect of pempidine (600 μg/kg) injected intravenously on the response of the villi to an intravenous injection of nicotine (200 μg/kg).

Effect of ganglion blocking agents

Intravenous injection of hexamethonium (0·5–4 mg/kg) sometimes stimulated villous activity slightly for the first few minutes after injection. There was never any inhibition of villous movements. Pempidine (1 mg/kg) had no effect on villous activity after intravenous injection but after local application to give a concentration of 500 μg/ml it had a slight initial stimulating effect. No depression of villous movements was observed. This concentration of pempidine was sufficient to inhibit the response of the villi to nicotine (200 μg/kg) injected intravenously after the mucosa had been in contact with pempidine for 10 min. Pentolinium (600 μg/kg) also did not affect villous activity after intravenous injection.

Discussion

The general appearance of villous movement in pigeons seemed similar to that described in dogs by other workers, but the pigeon villi were usually much less active than dog villi. There were also a number of similarities in the responses to drugs. For example, choline esters stimulated villous activity in both species, the results in dogs having been reported by Ludany, Obal & Santha (1950). Verzar & Kokas (1927) found that physostigmine strongly stimulated villous movements in dogs and this was confirmed in pigeons in the present work. Neostigmine had a similar effect in pigeons. The response to intravenous injection of either anticholinesterase was much slower in onset than that to intravenous injection of a choline ester. This presumably reflects the gradual build-up of acetylcholine due to inhibition of the cholinesterases.

The effects of noradrenaline and adrenaline on villous activity in pigeons also appeared to be very similar to those reported in dogs by other workers (King & Arnold, 1922 ; King & Robinson, 1945 ; Gati, Ludany & Santha, 1958). Both these substances stimulated villous movements, whilst isoprenaline, which has apparently never been tested in dogs, had an inhibitory effect on villous activity. From the results obtained with these catecholamines and their antagonists it is apparent that both α- and β-adrenoceptors are present in pigeon villi. Stimulation of the α-receptors resulted in increased villous activity, whilst stimulation of the β-receptors produced inhibition of villous movements. Both α- and β-adrenoceptors are known to be present in the intestine of the dog (Ahlquist & Levy, 1959), the guinea-pig (Brody & Diamond, 1967) and man (Bucknell & Whitney, 1964), but in the case of the outer musculature relaxation occurs when either type of receptor is stimulated. It was also noted that adrenaline, but not noradrenaline, sometimes had a brief depressant effect on villous activity on intravenous injection after pretreatment of the bird with tolazoline. This was presumably due to the effect of adrenaline on the β-adrenoceptors appearing after blockade of the α-receptors. Similarly, the potentiation of the response of the villi to an intravenous injection of adrenaline which was sometimes seen after propranolol had been injected can be attributed to the absence of any inhibitory effects of adrenaline on villous activity while the β-adrenoceptors were blocked.

Although the effects of autonomic nerve stimulation on villous activity in the pigeon have not been studied, the stimulating effect of both choline esters and catecholamines (with the exception of isoprenaline) on villous movement suggests that in pigeons parasympathetic and sympathetic nerve stimulation can both result

in increased villous activity. This agrees with the results in dogs reported by Ludany & Jourdan (1935) and Kokas & Ludany (1938). The muscle fibres of the villi originate in the muscularis mucosae and various workers (for example King & Robinson, 1945 ; King, Glass & Townsend, 1947) have reported that contraction of this muscle layer is also initiated by both parasympathetic and sympathetic stimulation.

Nicotine stimulated villous activity in pigeons and a similar effect was observed in dogs by Hambleton (1914) and Ludany, Gati, Rausch & Hideg (1960). In dogs villous movements were said to be inhibited for some minutes after the initial stimulation by nicotine, but this effect was not observed in pigeons. When the bird was pretreated with ganglion-blocking agents it was found that hexamethonium had little effect, although pempidine and pentolinium both inhibited the response of the villi to nicotine, and it appears that pigeons are particularly resistant to the effects of hexamethonium. This phenomenon cannot be explained. It has been suggested that nicotine, in addition to its ganglion stimulating effect, can also have a direct action on smooth muscle, partly through an effect on the acetylcholine receptors and, to a lesser extent, on receptors sensitive to phenoxybenzamine (Day & Vane, 1963). In addition, it may liberate noradrenaline from peripheral stores (Burn, Leach, Rand & Thompson, 1959). Noradrenaline release by nicotine or an action of nicotine on phenoxybenzamine-sensitive receptors did not seem to play a significant part in the present experiments as neither phenoxybenzamine nor tolazoline affected the response of the villi to nicotine. Although atropine inhibited the response of the villi to nicotine it is unlikely that this was due to blockade of the direct effects of nicotine on peripheral cholinergic receptors, since nicotine did not affect villous activity in a pigeon injected with pempidine or pentolinium and was therefore not exerting a peripheral effect. These results also suggest that the main action of nicotine on the villi is through parasympathetic, rather than sympathetic ganglia, although stimulation of either should theoretically cause increased villous activity.

Most of the drugs tested had similar effects after either intravenous injection or local application to the mucosa, which suggests that these were direct effects on villous activity rather than a response to systemic changes. It is, however, possible that local variations in the mucosal circulation may sometimes have an effect on villous movements, as for instance in the case of the intense blanching of the mucosa occurring after local application of noradrenaline or adrenaline. Apart from this there was little indication that transient blanching or flushing of the mucosa in themselves either increased or decreased villous activity.

From the results described in this paper it is clear that there are many similarities between the responses of dog and pigeon villi to autonomic drugs. Alpha and β-adrenoceptors have, however, so far not been differentiated in the intestinal villi of the dog. In this paper the presence of both types of adrenoceptor has been clearly demonstrated in the intestinal villi of the pigeon. Their stimulation causes respectively an increase or decrease of villous activity.

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