

THE DEVELOPMENT OF THE ADRENERGIC FIBRE

BY

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The majority of motor nerves have been shown to release acetylcholine as the transmitter substance. This is true (a) of nerves to skeletal muscle, (b) of parasympathetic nerves, both pre- and postganglionic, and (c) of preganglionic sympathetic nerves. The sympathetic postganglionic fibres, apart from those to the sweat glands, alone have nor-adrenaline as their main transmitter. These fibres are the exception. How has this come about?

That there may have been some evolutionary change is evident from the observations of Young (1936) who found that in two teleost fishes, *Lophius piscatorius* and *Urano-scopus scaber*, the splanchnic innervation of the intestine was motor, and Burnstock (1958) showed that the same was true in the trout, another teleost fish. Because the motor effect was abolished by atropine, he concluded that the innervation was cholinergic.

In this paper, experiments are described in which the periarterial nerves to the intestine of the barn-door fowl have been examined and also found to be principally motor. These observations led to the idea that if in the course of phylogenetic development there has been a change from a motor to an inhibitory function, then because ontogeny recapitulates phylogeny, there should be some indication of this in the newborn rabbit. The periarterial innervation of the intestine of the rabbit in the first few days of life has therefore been studied.

METHODS

All observations have been made by the method described by Finkleman (1930). A loop of intestine about 3 cm long was removed from the freshly killed rabbit together with adjacent mesentery and the artery running in it with its branches supplying the loop. The artery was cut at a distance of 3 or 4 cm from the loop. The contents of the loop were washed out, and the loop was placed in a Petri dish where a silk ligature was tied round the end of the artery. This end was then drawn into a pair of platinum ring electrodes of the pattern described by Burn & Rand (1960a). The loop was then set up in an isolated organ bath of 50 ml. capacity, containing Locke solution at a temperature of 32° C, through which 95% oxygen and 5% carbon dioxide was bubbled. The electrodes were below the surface of the Locke solution. The movements of the gut were recorded by an aluminium lever with a frontal writing point, the movements being magnified about three times.

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Loops were also prepared from chickens aged 5 months or more in the same way, and loops were taken from newborn rabbits between 3 and 8 days old. A loop was prepared from an 8 day old kitten.

RESULTS

Intestine of the chicken

The response of the intestine of the chicken when the periarterial nerves were stimulated is shown in Fig. 1. When stimulation was applied at 3 pulses/sec for 30 sec, or at 5 pulses/sec for 18 sec, there was a large contraction. In the presence of atropine (10^{-6} g/ml.) the same stimulations had almost no effect (Fig. 1c). This observation suggested that the motor response was due to acetylcholine.

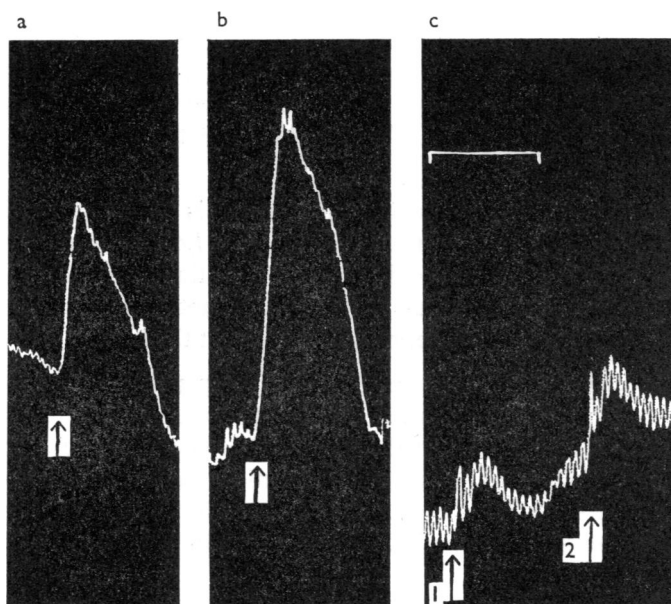


Fig. 1. Responses of the intestine of a chicken, 5 months old, to stimulation of the periarterial nerves in the mesentery. (a) Motor response to stimulation at 3 pulses/sec for 30 sec; (b) motor response to stimulation at 5 pulses/sec for 18 sec; (c) greatly diminished responses to the same stimulations (1 \uparrow , 3 pulses/sec for 30 sec; 2 \uparrow 5 pulses/sec for 18 sec) in the presence of atropine 10^{-6} g/ml. Time marker, 5 min.

The effects of acetylcholine, of 5-hydroxytryptamine and of noradrenaline are shown in Fig. 2. The contraction caused by 5-hydroxytryptamine (10^{-7} g/ml.) was much less than that caused by acetylcholine (0.2×10^{-7} g/ml.); noradrenaline caused relaxation in concentrations of 2×10^{-8} g/ml. and of 2×10^{-7} g/ml.

When the muscle was left in the bath for 3 to 4 hr, it developed some degree of spasm, and then stimulation caused a small relaxation at a frequency of 3 pulses/sec for 8 sec, or a relaxation preceding contraction as seen in Fig. 3. It was concluded that while the main transmitter released was acetylcholine, there was evidence of the release of a small amount of noradrenaline preceding the release of acetylcholine.

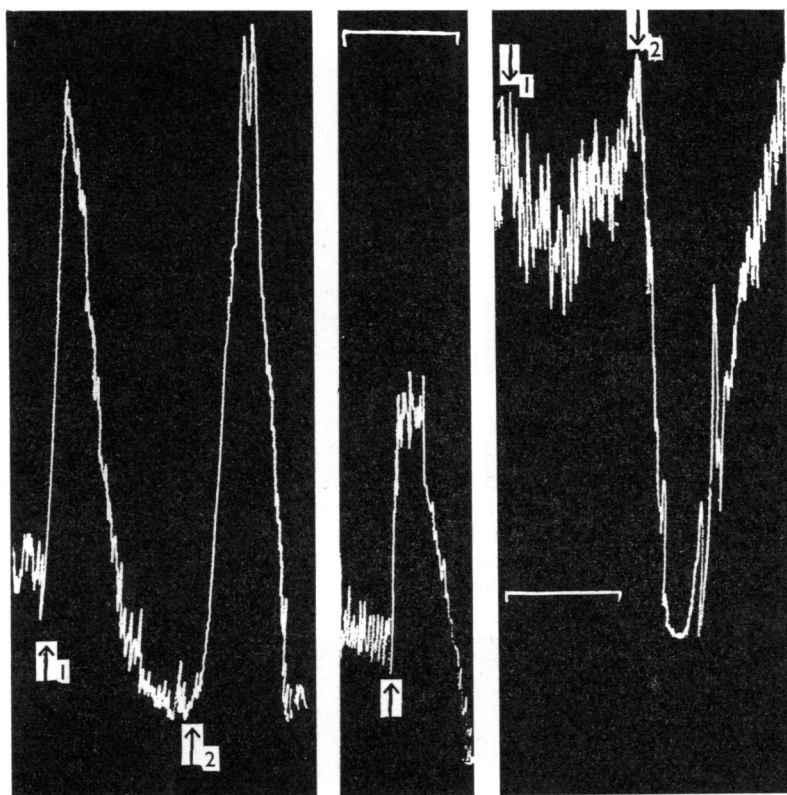


Fig. 2. In the left hand panel are the motor responses produced in a loop of intestine of a chicken by periarterial stimulation at 5/sec for 30 sec (\uparrow_1), and by addition of acetylcholine $1 \mu\text{g}$ to the bath to make a concentration of $2 \times 10^{-8} \text{ g/ml}$. (\uparrow_2). In the middle panel is the motor response to the addition of 5-hydroxytryptamine $5 \mu\text{g}$ (\uparrow) to make a concentration of 10^{-7} g/ml . In the right hand panel are shown the inhibitions produced in another loop by noradrenaline $1 \mu\text{g}$ and $9 \mu\text{g}$ added to make concentrations of $2 \times 10^{-8} \text{ g/ml}$. (\downarrow_1) and $2 \times 10^{-7} \text{ g/ml}$. (\downarrow_2). Time marker, 5 min.

Newborn rabbit

The response of the intestine of the newborn rabbit to periarterial nerve stimulation showed much variation. Thus the top panels of Fig. 4 show that the stimulation caused a contraction at four frequencies from 3/sec to 20/sec. The loop of intestine which was used was taken from a 3 day old rabbit. The lower panels of Fig. 4, also taken from a 3 day old rabbit of a different litter, however, show that while stimulation caused a contraction at the frequencies of 3/sec and 5/sec, it caused a slight inhibition at the higher frequencies of 10/sec and 20/sec. When stimulation was applied in the presence of hyoscine at a frequency of 3/sec, there was no effect.

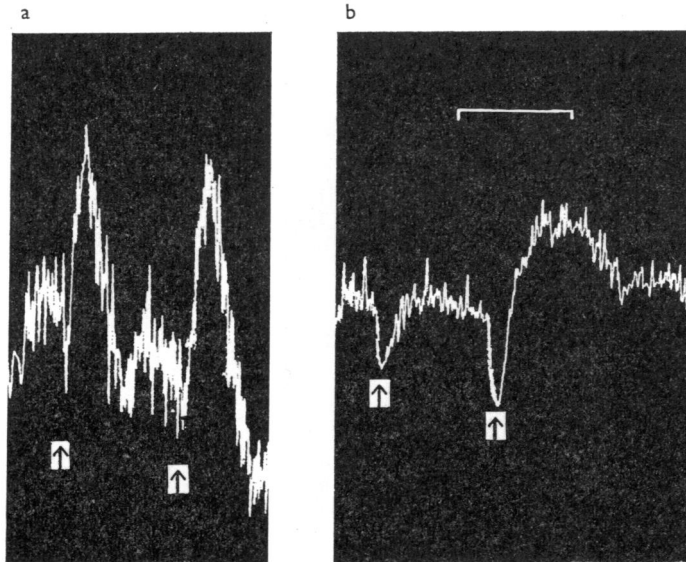


Fig. 3. Responses of chicken intestine to periarterial stimulation (a) 3/sec for 45 sec and 5/sec for 60 sec when the preparation had been set up 10 min previously and (b) 3/sec for 8 sec and 3/sec for 30 sec when the preparation had been in the bath for 5 hr. Note the relaxation at the beginning of stimulation in (b). Time marker, 5 min.

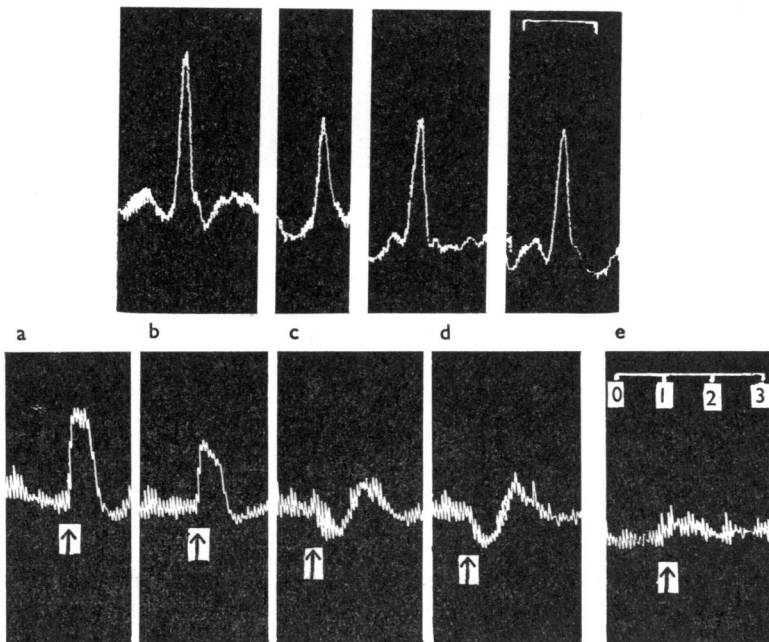


Fig. 4. Loops of intestine from two 3 day old rabbits. The upper panels show that in the first loop the responses to all frequencies of stimulation (3/sec, 5/sec, 10/sec and 20/sec) were motor. Time marker, 3 min. The lower panels show that in the second loop from a different rabbit of a different litter, the responses were motor to frequencies of stimulation of 3/sec (a) and of 5/sec (b), but were inhibitor to frequencies of 10/sec (c) and 20/sec (d); (e) shows the absence of the motor response to the frequency of 3/sec in the presence of hyoscine (10^{-6} g/ml.).

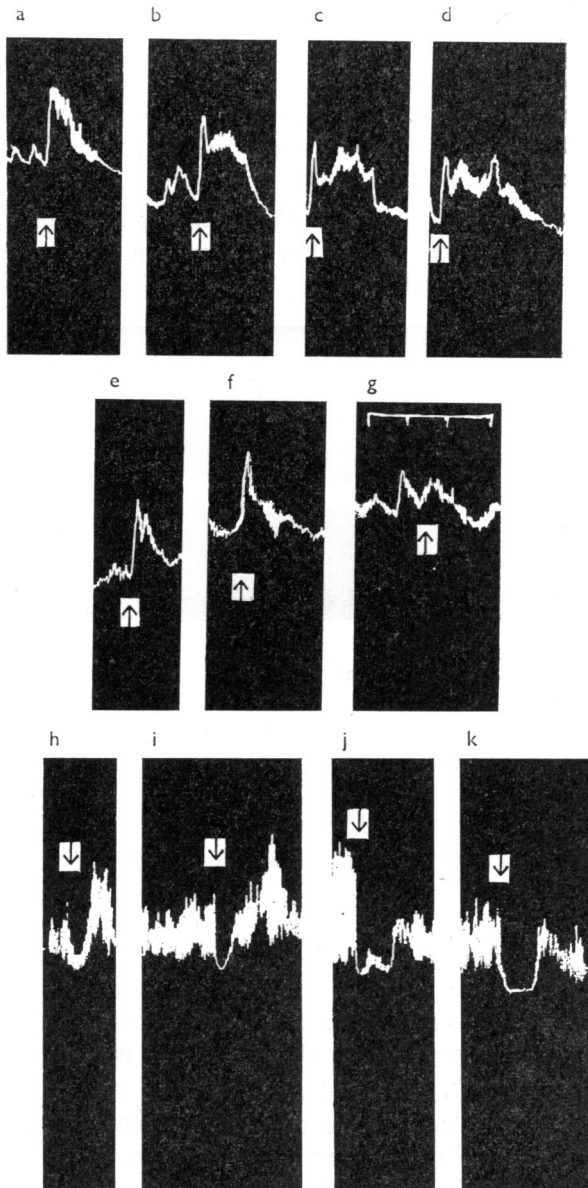


Fig. 5. (a)–(g) show the responses to periaarterial stimulation of two loops of intestine taken from the same 8 day old rabbit. (a) 3/sec for 30 sec; (b) 3/sec for 45 sec; (c) 5/sec for 90 sec; (d) 10/sec for 90 sec; (e) 3/sec for 60 sec; (f) 3/sec for 60 sec; (g) 3/sec for 60 sec. All responses were motor except (g) where, in the presence of hyoscine, there was no response. Time marker, 3 min. (h)–(k) were from a different rabbit of the same litter, which was also 8 days old. At each frequency (3/sec, 5/sec, 10/sec and 20/sec, respectively, for 60 sec) the response to stimulation was inhibition.

An opportunity was taken to examine loops of intestine from two rabbits of the same litter, each 8 days old. In the top row of panels shown in Fig. 5, it is seen that in the first rabbit stimulation caused contraction at frequencies of 3/sec, 5/sec and 10/sec. The middle row of panels shows the effect of stimulation at 3 pulses/sec in a second strip of intestine from the same rabbit. There was again contraction except when hyoscine was present in the bath. The bottom row of panels shows the effect of stimulation in a strip taken from a different rabbit of the same litter, also 8 days old. In this the effect of stimulation was inhibition at all frequencies.

An examination was made of the effect of stimulating the sympathetic fibres to the intestine of an 8 day old kitten. The result is shown in Fig. 6, in which stimulation produced inhibition at all frequencies.

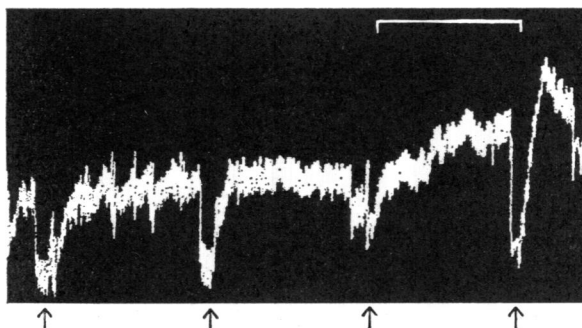


Fig. 6. Record from the loop of intestine of an 8 day old kitten. The responses to stimulation of the periaarterial nerves were recorded, and at all frequencies (5/sec, 3/sec, 1/sec and 10/sec respectively) the responses were inhibitions. The first three stimulations were applied for 30 sec, and the last for 15 sec. Time marker, 5 min.

DISCUSSION

To the evidence of Young (1936) and Burnstock (1958) that the splanchnic innervation of the intestine of teleost fishes is not inhibitory, and that its main effect is motor, caused by the release of acetylcholine, can now be added similar evidence for the 5 month old chicken. This finding supports the idea that a change from the cholinergic innervation of the intestine of the trout and the chicken to the adrenergic innervation of the intestine of the rabbit has taken place in the course of evolution. It is therefore not surprising that a similar change has been observed in the newborn rabbit.

Thus it has been found that in the first 8 days of life (or longer) the periaarterial nerves to the rabbit intestine may be motor, and that the contraction is caused by the release of acetylcholine. At some point, however, which may be as early as the third day or later than the eighth day, the innervation begins to change from motor to inhibitor and, as we know, the inhibition is caused by the release of noradrenaline.

It is of great interest that a similar change has been observed by Boatman, Shaffer, Dixon & Brody (1965) in the newborn dog. They perfused the hindleg vessels and stimulated the lumbar sympathetic chain, and found that in the period from day 1 to day 14, stimulation caused a fall of perfusion pressure as a result of vasodilatation, despite the

facts that the blood pressure was very low—being at day 1 only 30 mm Hg—and that the injection of adrenaline caused a rise in perfusion pressure. Since the fall in perfusion pressure caused by stimulation was abolished by atropine, the authors concluded that the fall was the result of the release of acetylcholine. After 2 weeks the effect of stimulation was to cause vasoconstriction.

In 1933 Burn perfused the hindlegs of the adult dog, after leaving them without a circulation for 40 min. When the perfusion began, he added adrenaline drop by drop to the blood reservoir to raise the tone in the vessels. When he stimulated the lumbar sympathetic chain, he observed vasodilatation which was later (Bülbring & Burn, 1935) shown to be caused by release of acetylcholine. When the addition of adrenaline to the blood reservoir had continued for more than 1 hr he observed that sympathetic stimulation caused vasoconstriction. He concluded that the change in the response to sympathetic stimulation from vasodilatation to vasoconstriction was caused by uptake of adrenaline from the blood by sympathetic nerve endings.

The change observed by Boatman *et al.* (1965) in the newborn dog in the first 2 weeks of life may have the same explanation. Indeed the uptake process, which has been extensively studied since 1961, has been shown by Glowinski, Axelrod, Kopin & Wurtman (1964) to develop in the rat heart between the seventh and the fourteenth day of life. Thus the most likely explanation of the change in the effect of sympathetic stimulation in the rabbit and the dog at this time is that it is the result of the development of the uptake process.

The question then arises what substance is taken up, for it is unlikely that noradrenaline is circulating in the blood. Burn & Rand (1960b) studied the rise of blood pressure caused in the rat by the injection of tyramine. After treatment of the rat with reserpine, which removed noradrenaline from sympathetic nerves, tyramine had almost no pressor action. If, however, an infusion of noradrenaline was given, tyramine had a much greater effect. An infusion of the precursors of noradrenaline was also effective in restoring the pressor action of tyramine. An infusion of dopamine or of L-DOPA restored the pressor action for a much longer time than an infusion of noradrenaline. It was specially interesting that an infusion of phenylalanine was also effective in restoring the pressor action of tyramine. It therefore seems reasonable to suggest that the uptake process which reverses the sympathetic response is the uptake of phenylalanine into the sympathetic fibre, which then converts this substance into noradrenaline.

Attention is drawn to the observations shown in the lower panels of Fig. 4, where in a 3 day old rabbit, stimulation of the periarterial nerves caused contraction at the frequencies of 3/sec and 5/sec, but caused inhibition at the frequencies of 10/sec and 20/sec. It is clear that at the lower frequencies acetylcholine was released. Presumably it was also released at the higher frequencies, and because the frequencies were higher, reached a higher concentration. Evidently some noradrenaline was there, and it may be that the higher concentration of acetylcholine was able to release it. A similar change in response was observed by Burn (1932, Fig. 3) when stimulation of the lumbar sympathetic with an induction coil for 3 sec caused vasodilatation, but stimulation for 30 sec caused vasoconstriction. Day & Rand (1961) studied the response of the rabbit ileum to periarterial stimulation in rabbits from the same litter at 12, 27, 44, and 61 days of age. They applied shocks at the high rate of 50/sec and observed inhibition in all preparations.

When guanethidine was added to the bath to prevent the release of noradrenaline they observed a motor response, which was large in the 12 day old rabbit, but diminished as the age increased. This diminution was, however, probably caused by the increasing size of the pendular movements with age. Certainly Burn (1932) found that acetylcholine was always released by stimulation of the sympathetic in the vessels of adult dogs when the noradrenaline had disappeared as a result of anoxia.

The sympathetic fibres to the intestine in the rabbit and to the vessels in the dog are cholinergic at birth, but when the uptake process begins they become adrenergic. A reversion to the condition at birth can be effected either by a period of anoxia or by injecting the animal with reserpine. The adrenergic fibres then become cholinergic once more. Thus the relation of noradrenaline to the sympathetic postganglionic fibre resembles the relation of the bullets to a pistol. The pistol can be loaded or it can be unloaded. But the acetylcholine is there all the time and seems to be part of the pistol.

SUMMARY

1. The sympathetic innervation of the intestine of the 5 month old chicken is motor as it is in the trout and other teleost fish; it is not inhibitor as in the rabbit. The motor innervation is cholinergic.

2. The sympathetic innervation of the intestine of the newborn rabbit is also cholinergic, but it changes to adrenergic at a time which may be as early as the third day of life, or may be later than the eighth day. The time when the change occurs varies even in animals of the same litter.

3. The change seems to occur when the uptake process begins and may be caused by the uptake of phenylalanine into the nerve fibre, where it is converted to noradrenaline.

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