ABSTRACT OF THESIS

Name of Candidate Ainsley Iggo

Address 5 Relugas Road, Edinburgh 9.

Degree Doctor of Science *Date* 1963

Title of Thesis Mammalian Afferent Nerve Fibres.

The original research work described in this thesis falls into three categories:- a) a study of mammalian myelinated and non-myelinated afferent nerve fibres and their endings, this forms the principal part, b) an examination of some properties of nerve-cells in the mammalian spinal cord and c) a minor section on the sympathetic nervous system.

a) Afferent nerve fibres. The major advance described is the development of techniques which allow individual active afferent nerve fibres to be identified, and their conduction velocities to be measured, when they are in nerve strands containing many axons. By these means the diameters of the active fibres may be assessed. These techniques, combined with improved dissection methods, have made the non-myelinated axons in peripheral nerves accessible as functional single units. The results of applying the techniques first to visceral nerves, then to cutaneous nerves and finally to somatic muscle nerves, establish that the nerves to the abdominal viscera and to the skin contain an unexpected variety of non-myelinated afferent axons, as judged by the responses of the nerve-endings to quantitative mechanical, thermal and chemical stimuli. In the stomach there were both mechanically-sensitive and pH-sensitive units located in different layers of the stomach wall. In the skin there were mechanoreceptors and thermoreceptors of highly selective sensitivity. Some of these latter nerve-endings and their afferent fibres could function as 'modality-specific' cutaneous transducers, with very considerable and unexplored implications in terms of central nervous function. The muscle afferent C fibres appear to be nociceptors. A detailed examination of single myelinated cutaneous afferent nerve-fibres also supports the thesis that the afferent fibres and their receptors have a selective sensitivity. A relation between the structure of afferent nerve-endings and the selective sensitivity of the afferent unit was clearly established for one particular nerve-ending - a 'cutaneous touch corpuscle' innervated by a thick myelinated axon. The dependence of the structure and sensitivity of the 'touch corpuscle' on its innervation was also established.

b) Spinal cord neurones. The pattern of 'recurrent inhibitory' connections from motor axon collaterals to small interneurones (Renshaw cells) in the ventral horn of the lumbo-sacral spinal cord and from these interneurones to motor-neurones displayed no reflex pattern. In general, there was feedback from motor-axons to adjacent Renshaw cells, which in turn fed back to the motoneuronal nucleus from which the motor-axons arose and also to other adjacent motor-neurones. There was a striking correlation between the intensity of recurrent inhibition and the function of the motor-neurone; those motor-neurones innervating slowly - contracting extensor muscles were most strongly inhibited.

c) Sympathetic nervous system. Depletion of the stores of catecholamines and 5-hydroxytryptamine in the brain of cats by reserpine treatment did not abolish sympathetic preganglionic efferent activity, so that the dramatic depressant action of reserpine on an animal is unlikely to be due to this depletion. The depressant effect of intravenously injected catecholamines on sympathetic preganglionic discharge was shown to be exerted principally, if not entirely, by reflex mechanisms, not by a direct action on the central nervous system.

Use other side if necessary.

MAMMALIAN AFFERENT NERVE FIBRES

Thesis submitted by

A. Iggo, M. Agr. Sc., B.Sc., Ph.D.

for the degree of

D. Sc.

University of Edinburgh

1963

TABLE OF CONTENTS

Afferent Nerve Fibres

10. New specific sensory structures in hairy skin.
Acta Neurovegetativa (1963) 24, 175-180.

11. A cutaneous sense organ in hairy skin. (jointly with A.R. Muir)
XXII Int. Congr. Physiol. Sci. 1962.

12. The structure and function of cutaneous 'touch corpuscles' after nerve crush. (jointly with A.G. Brown)
J. Physiol. (1962) 165, 28-29.

13. The significance of the terminal structure of afferent nerve fibres.
In Olfaction and Taste 1963, 149-150.
Oxford: Pergamon.

14. An electrophysiological analysis of afferent fibres in primate skin.
Acta Neurovegetativa 24, 225-240.

15. Non-myelinated afferent fibres from mammalian skeletal muscle.
J. Physiol. (1960) 155, 52-53.

16. 'Non-myelinated visceral, muscular and cutaneous afferent fibres and pain'
In The Assessment of Pain in Man and Animals 1963, 74-87. Edinburgh: Livingstone.

Spinal Cord Neurones

17. Electrophysiological investigations on Renshaw Cells. (jointly with J.C. Eccles, R.M. Eccles and A. Lundberg)
J. Physiol. (1961) 159, 461-478.

18. Distribution of recurrent inhibition among motoneurones. (jointly with J.C. Eccles, R.M. Eccles and M. Ito)
J. Physiol. (1961) 159, 479-499.

19. The double twitch of the gracilis muscle. (jointly with R.M. Eccles)
J. Physiol. (1961) 159, 500-506.

Sympathetic Nervous System

20. Preganglionic sympathetic activity in normal and in reserpine-treated cats. (jointly with Marthe Vogt)
J. Physiol. (1960) 150, 114-133.

21. The mechanism of adrenaline-induced inhibition of sympathetic preganglionic activity. (jointly with Marthe Vogt)
J. Physiol. (1962) 161, 62-72.

[*Reprinted from the Journal of Physiology*,
1955, Vol. **128**, No. 3, p. 593]
PRINTED IN GREAT BRITAIN

J. Physiol. (1955) 128, 593–607

TENSION RECEPTORS IN THE STOMACH AND THE URINARY BLADDER

BY A. IGGO

From the Physiology Department, University of Edinburgh

(*Received* 21 *January* 1955)

The existence of receptors in the abdominal and pelvic viscera of mammals has long been known from the reflex responses which can be elicited by a variety of experimental procedures (Ranson, 1921; Irving, McSwiney & Suffolk, 1937). Hurst (1911) made an extensive clinical study of the sensibility of the alimentary canal, and concluded that a form of 'muscle-sense' existed in all parts of the alimentary canal, but did not further investigate the receptors.

Afferent impulses from gastric and intestinal receptors of the cat have been recorded in the splanchnic nerves and in fine mesenteric nerve strands by Gernandt & Zotterman (1946) and in the cervical vagus by Paintal (1954). Impulses in hypogastric and pelvic afferent fibres from bladder receptors of the cat have been recorded by Evans (1936) and of the dog by Talaat (1937). It has been customary to regard the sense endings as stretch receptors, but no attempt has been made to establish whether the activity is related to muscle tension and, if so, whether the receptors are 'in parallel' or 'in series' with the muscle fibres.

The problem has been re-investigated by recording from single afferent fibres in the cervical vagus and the pelvic plexus. Particular attention has been directed to the discharge in single fibres during distension and contraction of the viscera and to the size of the afferent fibres. A preliminary account of this work has been published (Iggo, 1954).

METHODS

Gastric afferent fibres. Goats were anaesthetized by an intrajugular injection of pentobarbital, approx. 25 mg/kg. Anaesthesia was maintained for as long as 18 hr by an intravenous pentobarbital drip (5 mg/ml.). The preparations were kept at 37–39° C on a heated table, supplemented with hot-water bags. The single fibre dissection technique was similar to that described by Paintal (1953) and the cervical vagus was immersed in a pool of liquid paraffin B.P. Electrical activity was picked up with Ag/AgCl wire electrodes and was amplified by a conventional resistance-capacitance coupled amplifier, fitted with a loudspeaker. The action potentials were displayed on cathode-ray tubes and recorded photographically on moving bromide paper. The gastric afferent fibres are less than 5 μ diameter and the amplitude of the electrical signal, picked up by external

Ag/AgCl electrodes rarely exceeded 50 μV in single fibre preparations and was less in multifibre preparations. Because the impulses in fibres from lung stretch receptors were large and had a high discharge frequency, they masked the small gastric afferent impulses, even when a loudspeaker was used, and made the isolation of the gastric fibres more tedious.

A gastric balloon was inserted through an incision in the ventral pole of the reticulum or was placed in the reticulum through the rumino-reticular orifice, which was approached from an incision in the left dorsal blind sac of the rumen. The incision and abdominal wound were then closed by sutures. The balloon was connected by a polythene tube to a calibrated pump which delivered known volumes of air. A capacitance manometer using an amplifier based on the circuit of Alexander (1951) was used to record the pressure in the balloon, and was calibrated with a mercury manometer.

Pelvic afferent fibres. Cats, preferably young female animals weighing 2·5–3 kg, were anaesthetized with chloralose, 80 mg/kg, after induction with ethyl chloride and ether.

The pelvic plexus was exposed by a midventral incision, and resection of the ischium pubis. Particular care was taken to avoid exposing the urinary bladder to the air. The urethra, bladder and plexus were immersed in liquid paraffin B.P. at 37–39° C after cannulating the urethra.

Saline (0·9%, w/v, NaCl solution) 39° C, was injected into the bladder from a syringe or more frequently was allowed to flow in from a reservoir at heights of 10–60 cm above the bladder. The intravesical pressure was recorded with a condenser manometer, connected to the bladder by narrow polythene tubing. The bladder volume was recorded by registering the capacitance changes in a narrow vertical sidearm of the reservoir. The reservoir was designed so that a 5 ml. change in volume of the bladder altered the fluid level in the reservoir by 1·3 mm.

Recognition of single fibre units. To ensure that the results presented were from single fibres, the following criteria were adopted:

(*a*) Uniformity of size and shape of the impulse; frequently two units were active in a single filament. Unless the impulses were clearly distinguishable, e.g. Fig. 5, the strand was divided to give single fibres. Two-unit preparations were particularly useful in providing a comparison of the response of individual receptors under identical experimental conditions.

(*b*) Regularity of rhythmic discharge; this was recognizable even in multifibre preparations, and was useful in dissection.

(*c*) Specificity and repeatability of response.

RESULTS

Gastric afferent fibres in the goat

Two types of gastric afferent fibres were distinguished by the discharge during maintained gastric distension.

(*a*) *Sustained discharge.* Eleven single fibres were found in which the response to maintained inflation of the stomach was a slowly adapting or non-adapting discharge of impulses. Two fibres had a resting discharge of 9–14/sec, when the stomach was empty, and a third discharged impulses during the inspiratory phase of respiration. The rest were silent when the stomach was empty. The rate of discharge of three of these fibres had a linear relation to the volume of gastric inflation. At high volumes of inflation, sufficient to cause a marked rise in intragastric pressure, this relation was not found (Fig. 1).

There was no similar proportionality between the discharge of impulses and the intragastric pressure, but the rate of firing rose when the pressure was increased.

The experimental results indicated by the line in the margin were part of the material submitted for my Ph. D.
A.I.

(*b*) *Intermittent discharge.* Eight single fibres were found in which, with the stomach empty, there were infrequent bursts of impulses lasting 1–5 sec, which recurred at intervals up to 30 sec. The discharge was not related to respiratory or cardiovascular activity. With the stomach distended the peak frequency was as high as 40/sec, the duration of the discharge was longer and the bursts of impulses were more frequent. With some fibres the silent intervals were absent at large volumes of inflation. In these conditions the rhythmic character was present as a fluctuation in the rate of firing as illustrated in Fig. 2. There was no linear relation between the rate of discharge of impulses and either intragastric volume or pressure.

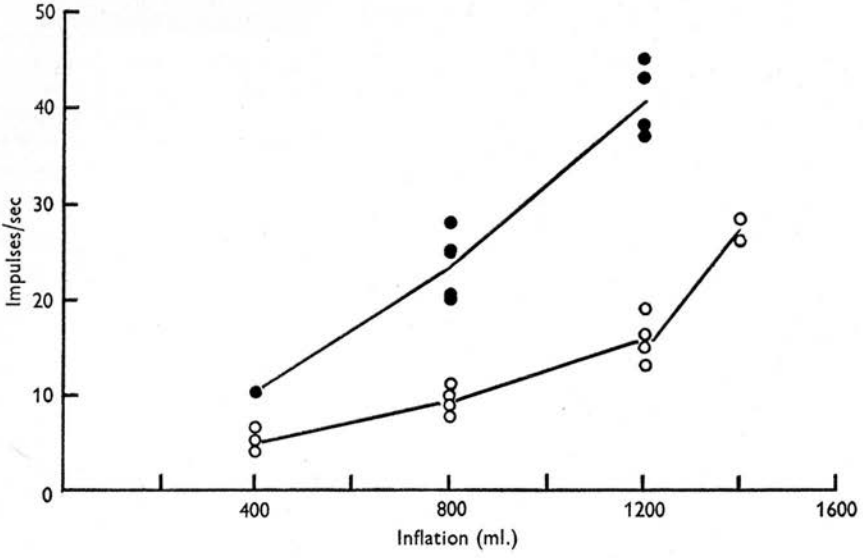


Fig. 1. The rate of discharge of impulses in two slowly adapting gastric afferent fibres at different volumes of sustained gastric distension. The lower curve shows the non-linearity of the response at high volumes. Goat.

On one occasion an unsuccessful attempt was made, by palpating the stomach, to detect localized rhythmic contractions in phase with the bursts of impulses.

Discharge of impulses during gastric contractions

Gastric contractions were produced (*a*) reflexly and (*b*) directly by stimulating gastric efferent fibres in the cervical vagus. These methods are discussed in greater detail elsewhere (Iggo, in preparation).

During reflex isometric contractions, elicited by a 1200 ml. inflation of the stomach of a lightly anaesthetized goat, the intragastric pressure rose in the experiment illustrated from 25 to 40 mm Hg (Fig. 3). The rate of firing in the

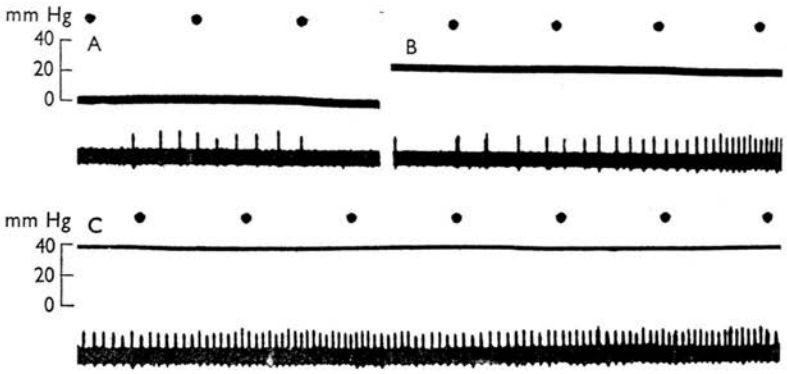


Fig. 2. Impulses in a gastric afferent fibre, in which the discharge was intermittent, during sustained gastric distension. The gastric balloon was empty in A and contained 200 ml. in B and 400 ml. in C. The intermittent character of the discharge seen in A and B is present in C as a variation in the frequency of discharge. In these and subsequent records the upper tracing shows the intragastric pressure, a rise upwards, and the lower tracing shows impulses in the afferent fibre. Goat. Time 1 sec.

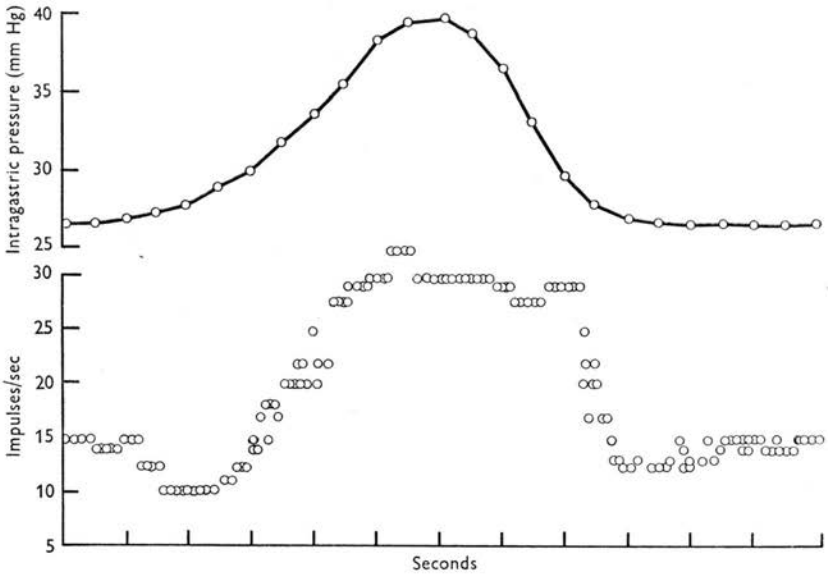


Fig. 3. The discharge of impulses in a slowly adapting fibre during sustained gastric distension of 1200 ml. and a reflex contraction elicited by the distension. Above: intragastric pressure; below: frequency of discharge of impulses in the single fibre. Goat.

single fibre increased from 15/sec before the contraction to 30/sec, reaching a peak value before the contraction was maximal. This type of response was found in the three slowly adapting fibres examined, during each of many reflex gastric contractions.

Gastric contractions produced by electrical stimulation of gastric motor fibres in the cervical vagus initiated or increased the discharge of impulses in six single gastric afferent units. When the stomach was empty a contraction caused the discharge of impulses in some of the fibres. The rate of firing was greater when the stomach was inflated (Fig. 4). In the results illustrated in Fig. 5 there was no discharge at an inflation of 100 ml. There was a brief discharge at 200 ml. inflation. The discharge had a shorter latency, a higher frequency, and a longer duration when the volume was 300 or 400 ml. The peak pressure during the contractions was greater at the higher volumes. During the contractions at the higher volumes of inflation a second fibre became active. The threshold for excitation of this receptor was presumably reached only by a summation of tension due to the contraction and that due to the inflation at volumes of 300 and 400 ml.

The recording system used was inadequate to measure the tension in localized parts of the gastric wall. The experimental results are, therefore, not sufficiently precise to justify an analysis such as that made by Matthews (1933) for the effect of tension on the type B receptors of skeletal muscle in the cat. The gastric receptors, however, did behave in the manner expected of 'in series' tension recorders. Tension arising from passive distension, active contraction or active contractions superimposed on passive distension was an adequate stimulus to the receptors. The rate of firing was greater when the total tension was increased.

Localization of gastric receptors

The location of five gastric receptors was established directly by exploring the serosal surface of the stomach with a probe, by digital compression of the stomach wall, or by pulling on strips of the wall. The abdominal origin of one receptor was confirmed by cutting the right dorsal abdominal vagus at the diaphragm, after which the vagal afferent fibre was silent. The five fibres innervated receptors located in the region of the oesophageal groove, and all the fibres had an intermittent discharge during distension, e.g. Figs. 2 and 4. A fibre with a sustained discharge during distension, was lost before the location of the receptor was definitely established, but the receptor appeared to be situated in the anterior wall of the reticulum. In no instance was the precise situation of a receptor in a particular layer of the wall established.

The response of all these receptors to sustained tension, such as pulling on a strip of the stomach, or sustained digital compression, was a steady discharge of impulses as shown in Fig. 6. This response is in striking contrast to

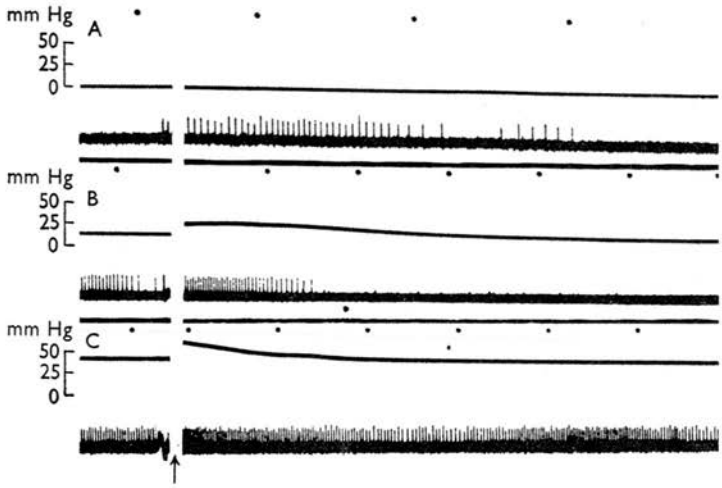


Fig. 4. The effect of gastric contractions produced by stimulating vagal gastric motor fibres. In A the gastric balloon was empty and the contraction occurred in isotonic conditions. In B and C the balloon was inflated with 100 and 300 ml. respectively and the contraction was recorded by an isometric system. The records have been broken at arrow for 3 sec, during which the cervical vagus was stimulated. Goat. Time 1 sec.

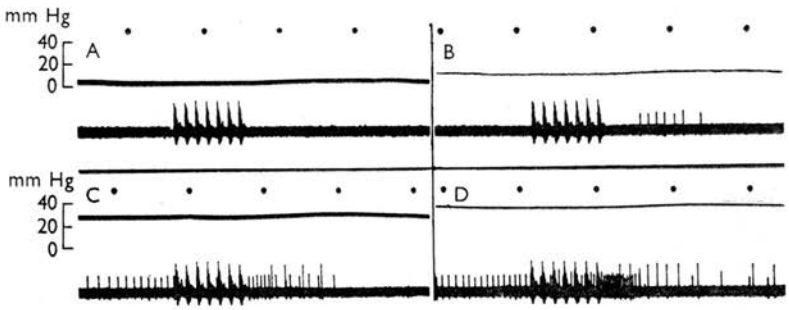


Fig. 5. The effect of gastric distension and of contractions, produced by stimulating gastric motor fibres in the cervical vagus, on the discharge of impulses in gastric afferent fibres. The volume of inflation was 100 ml. in A, 200 ml. in B, 300 ml. in C and 400 ml. in D. The stimulus artifacts can be seen alone in A, in which no impulses were recorded. At the higher volumes of inflation a second fibre was active during the contractions. Goat. Time 1 sec.

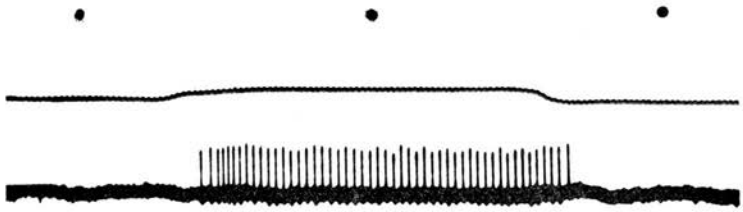


Fig. 6. Impulses in a single gastric afferent fibre when a small region of the stomach near the oesophageal groove was squeezed between finger and thumb. The conduction rate of this fibre, which is also illustrated in Fig. 2, was 6 m/sec. Goat. Time 1 sec.

the intermittent discharge in the afferent fibre when the whole stomach was distended (Fig. 2).

The receptors of the three slowly adapting fibres examined during reflex contractions were not found by these direct means but, from the responses of the fibres during distension and contraction, I consider that they too were located in the gastric wall.

Conduction velocity in gastric afferent nerves

The technique described by Paintal (1953) was used, and the conduction interval (stimulus artifact to action potential) was measured on the screen of the cathode-ray tube. The conduction velocities of the four fibres measured were 12, 6, 5 and 2 m/sec.

Since small fibres are the most difficult to dissect, the range of conduction velocities obtained suggests that the majority of the gastric afferent fibres conduct impulses at very low velocities. This confirms the results obtained by a different method of assessing the conduction velocities of the gastric afferent fibres in the ruminant (Iggo, in preparation). This range of conduction velocities is at the lower limit for the conduction of impulses in mammalian myelinated fibres.

Gastric receptors in the cat

While this paper was in preparation, I received a copy of Dr A. S. Paintal's paper 'A study of gastric stretch receptors', now published. Dr Paintal found that gastric receptors in the cat, in many respects similar to those described in the goat, were unaffected by gastric contractions. This difference was so striking that I have examined the behaviour of gastric receptors during passive distension and active contraction of the stomach in two decerebrated cats under light, 3 mg/kg, pentobarbital anaesthesia and in three cats under chloralose anaesthesia (80 mg/kg).

Fibres were found which behaved in response to distension and compression of the stomach in a manner similar to that reported by Paintal. The stomach did not contract on electrical stimulation of the cervical vagus, and in this respect the cat was in marked contrast to the sheep and the goat. An attempt was made to cause gastric contraction by stimulating the serosal surface of the stomach with square pulses 3–6 c/s for 1–15 sec. In the fundus this stimulation produced at most only small localized contractions, but in the pyloric region it caused a strong contraction which, if the stimulation was continued for 15 sec, spread over the whole pyloric region. This contraction persisted for 10–20 sec after stimulation.

The first two slowly adapting receptors, found by recording from strands of the cervical vagus, were at the cardiac end of the stomach. Under the experimental conditions, I was unable to cause a contraction of this part of the stomach and so the effect of contraction on these fibres was not tested. In

subsequent experiments I located six slowly adapting receptors in the pyloric region of the stomach. The rate of firing of each fibre was greater during pyloric antral contractions than when the stomach was inflated with 50–200 ml. as illustrated in Fig. 7. In two preparations the areas in which receptors had been localized were stimulated for intervals of approx. 2 sec. The contractions

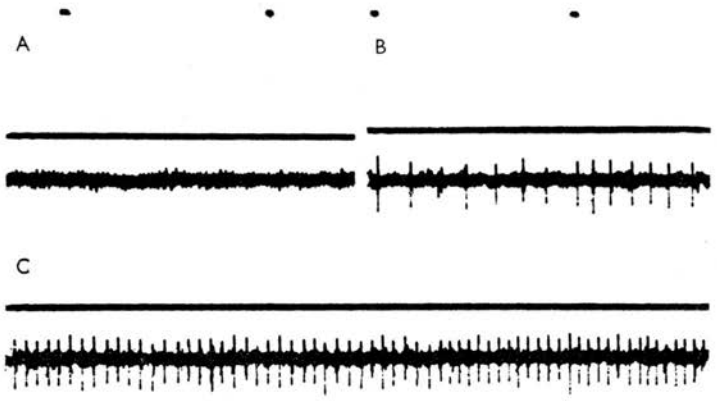


Fig. 7. Impulses in a vagal gastric afferent fibre. The stomach was empty in A and inflated with 100 ml. in B. Direct stimulation of the serosal surface of the pyloric region caused a powerful contraction of the pyloric antrum. Record C was taken after the stimulation was stopped but while the contraction remained. Decerebrated cat. Time 1 sec.

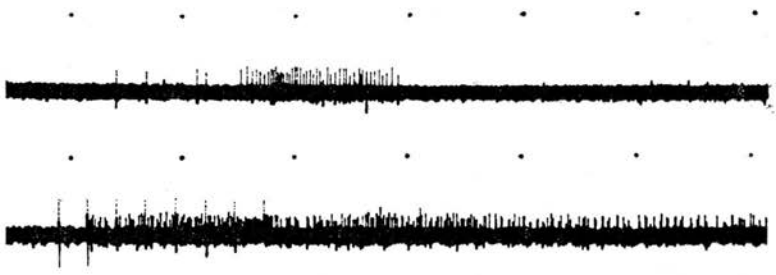


Fig. 8. Impulses in a vagal gastric afferent fibre. The upper record shows the effect of pinching a small region of the pyloric antral wall. The lower record shows the effect of eight electrical stimuli (dotted) applied to the same region. Stimulation caused a localized contraction, observed visually, which persisted for several sec and was associated with a discharge of impulses. Chloralosed cat. Time 1 sec.

produced were small, involving not more than 4 mm^2 of the gastric wall, and were associated with bursts of impulses in the afferent fibres which persisted for as long as the contractions could be seen (Fig. 8). No detectable changes in intragastric pressure were observed. Rhythmic contractions were present in the pyloric antrum of one preparation and a discharge of impulses was

observed during contractions of a small region of the wall containing the receptor, i.e. an intermittent discharge was caused by localized rhythmic gastric contractions.

The gastric receptors of the goat and cat described in this paper behaved in a similar manner when tested under the same conditions. The simplest assumption to make is that they were the same type, i.e. tension receptors 'in series' with the muscle fibres.

Receptors in the bladder of the cat

Impulses in twelve single afferent fibres in the pelvic plexus of cats were recorded during passive distension and active contraction of the bladder.

Afferent discharge during passive distension

When the bladder was empty there was no discharge of impulses. As fluid from the reservoir entered the bladder the intravesical pressure rose and impulses were recorded (Fig. 9). The rate of firing increased until, 5–15 sec after inflow began, a peak frequency not greater than 20–30/sec was reached,

Fig. 9. The discharge of impulses during the inflow of 50 ml. saline into the bladder of a cat in which both pelvic nerves were cut. The onset of inflow is marked by the arrow. Time 1 sec on the intravesical pressure tracing.

which after 1–2 sec fell abruptly to as low as 1/sec; the intravesical pressure meantime continued to rise. After the reservoir was turned off the discharge persisted rhythmically until the bladder was drained. In one experiment the peak discharge in two fibres in the same strand sometimes coincided and at other times did not.

When the intravesical pressure was made to rise more slowly the most consistent change in the firing of impulses was an increased interval from the beginning of distension before the peak frequency of discharge was reached.

The reservoir height never exceeded 60 cm which, according to Mukherjee (personal communication), is insufficient to cause a consistent rise in blood pressure of the chloralosed cat.

The other method used for distending the bladder was more drastic. Fluid was rapidly injected from a hypodermic syringe into the closed bladder. In one experiment the injection of 7·5 ml. into a bladder containing 30 ml. produced an immediate discharge of impulses, as illustrated in Fig. 10, and the abrupt withdrawal of the same volume abolished the activity.

Afferent discharge during bladder contractions

Isometric recording conditions. Rhythmic contractions of the innervated bladder containing 30–60 ml. had a frequency of 4–6/min and the rise in pressure was 5–30 mm Hg. During contractions the discharge of impulses in

Fig. 10. Impulses in pelvic afferent fibres. A: an isometric contraction of the bladder; B: the effect of suddenly raising the intravesical pressure (at arrow) by the injection of 7·5 ml. saline. In each record the upper tracing shows the afferent impulses and the lower tracing shows the intravesical pressure. Cat. Time 1 sec.

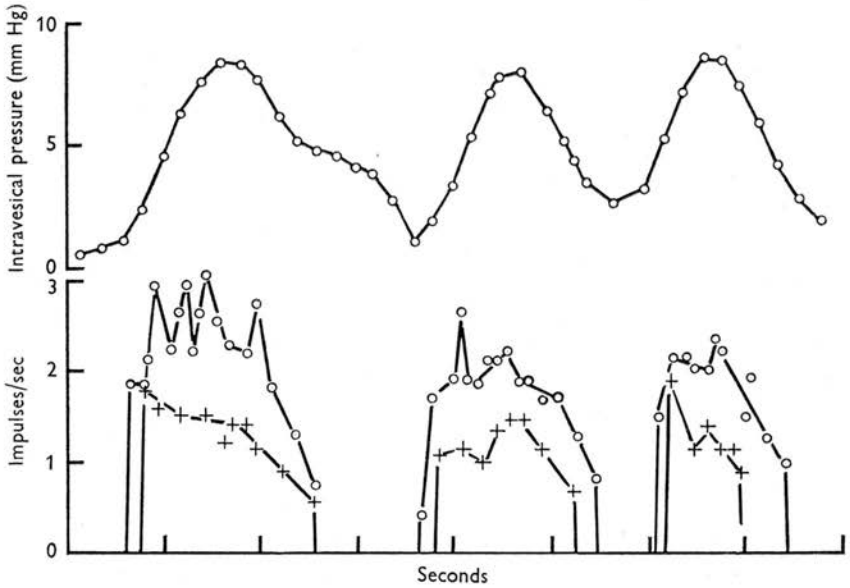


Fig. 11. The discharge of impulses in two pelvic afferent fibres during three successive bladder contractions recorded under isometric conditions. Upper curve: intravesical pressure; lower curves: frequency of discharge in the two fibres graphed separately. Cat.

single afferent fibres reached rates as high as 10/sec as the intravesical pressure was rising, and in some preparations continued after the pressure had begun to fall. There was no discharge between contractions at small volumes of the bladder (Figs. 10, 11).

Isotonic recording conditions. The bladder of the chloralosed cat contracts rhythmically when it is distended by a 5–15 cm head of fluid (Mellanby & Pratt, 1939). Impulses were recorded in single afferent fibres as or just before the bladder volume began to decrease. No further activity was seen until after the bladder had relaxed and the next contraction began. The bladder volume, intravesical pressure and activity in single afferent fibres was recorded simultaneously in the later experiments as illustrated in Fig. 12. At the end of a contraction when fluid was re-entering the bladder a few impulses at low frequency appeared. Next the intravesical pressure, which had been rising very slowly, rose sharply, and simultaneously the impulse frequency rose to 5/sec. The bladder volume and intravesical pressure then began to fall and the discharge of impulses ceased. This behaviour was recorded in five fibres examined.

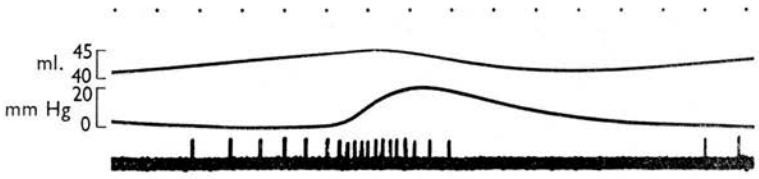


Fig. 12. Impulses in a pelvic afferent fibre during one contraction of an innervated bladder recorded under isotonic condition. The reservoir was 10 cm above the urethra. Upper: bladder volume, a decrease downwards; middle: intravesical pressure, an increase upwards; and lower: impulses in the afferent fibre, retouched for the sake of clarity. Cat. Time 1 sec.

Thus the so-called 'isotonic' recording conditions, while permitting changes in bladder volume, were associated with changes in the intravesical pressure, and the discharge of impulses during the contraction was related to the rise in pressure. Mellanby & Pratt (1939) recorded the pressure changes in a sidearm of the bladder cannula and observed no appreciable change in pressure during contractions recorded under conditions of constant pressure.

Sustained isometric contractions. Mellanby & Pratt (1940) report that when the recording conditions were switched rapidly from isotonic to isometric during the rising phase of an isotonic contraction of the innervated bladder a powerful isometric contraction supervened. This phenomenon was used to test the rate of adaptation of bladder tension receptors. In one experiment such a change in the recording conditions evoked a persistent powerful contraction during which the intravesical pressure exceeded 50 mm Hg. The rate of firing in two fibres, recorded in the same nerve filament, increased from 5/sec before the contraction to a peak of 30–40/sec during the contraction. The rate of firing fell gradually after 2 sec to 20/sec and persisted at this rate for as long as 30 sec until the pressure was released.

The receptors when tested under suitable conditions gave a sustained discharge of impulses, although in other circumstances, e.g. isotonic recording conditions, they gave a brief discharge of impulses.

Localization of bladder receptors

This was a hazardous procedure since the afferent fibres were dissected from the pelvic plexus and manipulation of the bladder frequently dislodged the nerve from the recording electrodes. Two receptors were found near the neck of the bladder. Sustained pull on strips of the bladder wall caused a steady discharge of impulses from the receptors.

DISCUSSION

The results obtained with single unit preparations establish convincingly that the same receptor units, in stomach and bladder, are excited by both passive distension and active contraction of the viscera. The view that the receptors are tension receptors 'in series' with the muscle fibres is based on the following evidence: (1) All the gastric receptors, found by distension of the stomach were, when tested, excited by gastric contractions. The rate of discharge was often greater during isometric contractions than the maximal rate during gastric distension. (2) Bladder receptors, found by using isometric contractions as a stimulus, were excited by passive distension. (3) Passive distension and active contraction when present together were effective in exciting receptors which were not excited by either alone. (4) Contractions of the empty stomach caused a discharge of impulses; in these circumstances the gastric wall shortens. No evidence has been found to support the views either that the receptors are 'in parallel' with the muscle or that they are unaffected by activity of the muscle.

Another feature was that receptors, the discharge from which appeared to have different rates of adaptation, all adapted slowly when excited by direct mechanical means, e.g. digital compression or sustained pull of a small strip of wall. The intermittent discharge of some gastric receptors, which on direct stimulation gave a steady discharge of impulses, was probably due to rhythmic motor activity in restricted parts of the gastric wall. Such a relation was observed in one experiment on a cat. This may be a general experimental limitation to the study of the behaviour of visceral receptors, namely, the discharge of receptors 'in series' with smooth muscle can be influenced by localized changes in motility, which are not revealed when recording total intraluminal pressure.

Talaat (1937) distinguished several types of afferent discharge related to the bladder in the pelvic afferent fibres of the dog. The present results extend his observations to the cat and confirm his findings that passive distension arouses a brief discharge of impulses. The distinction which Talaat based on

a difference in the rate of adaptation between these fibres and those excited by isometric contraction of a strip of the wall is open to doubt. Single fibres were found which responded to passive distension and active contraction, of varying intensities, with discharges of impulses of widely varying durations.

Gernandt & Zotterman (1946) found in the cat that small spikes and slow waves were produced in the splanchnic nerve on manipulation of the small intestine. Because they used multifibre preparations, it is uncertain whether the same receptors were excited by all the stimuli. The results are in accord with the conclusion reached by the single fibre investigation now reported. One striking discrepancy is the maximal rate of discharge from the small fibres which Gernandt & Zotterman (1946) stated was 600/sec, a value 10 times that found in my single fibre studies. I attribute this discrepancy to their use of multifibre preparations in which it is usually impracticable to count impulses in individual nerve fibres.

Paintal (1954), as already mentioned, considers it 'likely that a rise in intragastric pressure unaccompanied by stretching of the stomach wall does not stimulate the receptors'. The above statement certainly did not apply to gastric tension receptors in the cat if the stomach in the region of the receptors was caused to contract. In these circumstances a discharge of impulses resulted during a contraction in which a considerable shortening of the wall occurred, and this in slowly adapting fibres which were excited by inflation of the stomach. An inflow of impulses along the same afferent fibres would therefore be anticipated during both hunger contractions and distension of the stomach by a large meal. On this view Paintal's conclusions about the role of these gastric afferent fibres in the satiation of hunger and thirst are open to doubt.

The tension receptors in the stomach of the goat and the so-called stretch receptors in the stomach of the cat are innervated by fibres with conduction velocities of 2–14 m/sec, that is, by myelinated fibres less than 5μ in diameter. There are several thousand such fibres in the abdominal vagi of sheep and goats (Iggo, in preparation). Gernandt & Zotterman (1946) find large numbers of such fibres in the splanchnic nerves of the cat, and Langley & Anderson (1896) found several thousand in the pelvic nerves of the cat. The widespread distribution of these small myelinated fibres in visceral nerves, and of tension receptors in the viscera, in some cases known to be innervated by fibres of this size, invites the generalization that there are tension receptors innervated by small myelinated fibres at all levels of the alimentary canal.

The functional significance of the visceral tension receptors is a field for future investigation. There is strong evidence that some of the gastric receptors in the sheep and the goat are concerned in regulating gastric motility in these animals (Iggo, in preparation). Hurst (1911), on the basis of a wealth of clinical observation, in which he found, for example, that patients were unable to discriminate between an atonic dilatation of the stomach and a small

hypertonic stomach, concluded 'that ill-defined sensations, which occur in all parts of the alimentary canal and which may be grouped together as "the sensation of fullness" are due to stretching of its muscle coat and constitute a form of muscle sense'. His deductions and the experimental findings now reported are complementary.

Denny-Brown & Robertson (1933) have made an interesting study of the sensations aroused by distension of the urinary bladder in conscious human subjects and reported that several qualities of sensation culminating in an irrepressible desire to micturate ensued before a sensation of pain was experienced. The results can be interpreted to indicate that the afferent discharge recorded in pelvic afferent fibres is concerned with functions in addition to pain, e.g. reflex micturition. The observation made by Hurst that distension of the alimentary canal gave rise to a sensation of fullness which graded imperceptibly into pain can also be quoted to support the conclusion that not all afferent inflow from the gut is necessarily painful.

SUMMARY

1. Methods are described for isolating and recording from single fibres from receptors in the stomach of the goat and cat and from urinary vesical receptors in the cat.

2. The gastric receptors were excited by passive distension and active contraction of the stomach and adapted slowly to sustained digital compression.

3. Gastric receptors were found in the oesophageal groove region in the goat and in the cardiac and pyloric regions of the stomach in the cat.

4. The conduction velocities in some of the gastric afferent fibres in the goat were 2–12 m/sec.

5. Impulses were recorded in single afferent fibres in the pelvic plexus during passive distension and active contraction of the urinary bladder.

6. The discharge under isotonic and isometric recording conditions was related to the intravesical pressure.

7. The receptors in the bladder were slowly adapting.

8. It was concluded that the gastric and bladder receptors were tension-recorders 'in series' with the muscle fibres.

I wish to express my gratitude to Prof. D. Whitteridge, F.R.S., for his stimulating interest in this problem. I am indebted to W. T. S. Austin, Esq., for valuable advice on electronic problems.

REFERENCES

ALEXANDER, W. (1951). An electron ultramicrometer. *Electron. Engng*, **23**, 479–450.

DENNY-BROWN, D. & ROBERTSON, E. G. (1933). On the physiology of micturition. *Brain*, **56**, 149–190.

EVANS, J. P. (1936). Observations on the nerves of supply to the bladder and urethra of the cat, with a study of their action potentials. *J. Physiol.* **86**, 396–414.

GERNANDT, B. & ZOTTERMAN, Y. (1946). Intestinal pain: an electrophysiological investigation on mesenteric nerves. *Acta physiol. scand.* **12**, 56–72.

HURST, A. F. (1911). *The Sensibility of the Alimentary Canal.* London: Oxford University Press.

IGGO, A. (1954). Receptors in the stomach and bladder. *J. Physiol.* **126**, 29–30*P*.

IRVING, J. T., MCSWINEY, B. A. & SUFFOLK, S. F. (1937). Afferent fibres from the stomach and small intestine. *J. Physiol.* **89**, 407–420.

LANGLEY, J. N. & ANDERSON, H. K. (1896). The innervation of the pelvic and adjoining viscera. *J. Physiol.* **19**, 372–384.

MATTHEWS, B. H. C. (1933). Nerve endings in mammalian muscle. *J. Physiol.* **78**, 1–53.

MELLANBY, J. & PRATT, C. L. G. (1939). The reactions of the urinary bladder of the cat under conditions of constant pressure. *Proc. Roy. Soc.* B, **127**, 307–322.

MELLANBY, J. & PRATT, C. L. G. (1940). The reactions of the urinary bladder under conditions of constant volume. *Proc. Roy. Soc.* B, **128**, 186–201.

PAINTAL, A. S. (1953). The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.* **121**, 341–359.

PAINTAL, A. S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *J. Physiol.* **126**, 255–270.

RANSON, S. W. (1921). Afferent paths for visceral reflexes. *Physiol. Rev.* **1**, 477–522.

TALAAT, M. (1937). Afferent impulses in the nerves supplying the urinary bladder. *J. Physiol.* **89**, 1–13.

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. XLII, 1957.

GASTRO-INTESTINAL TENSION RECEPTORS WITH UNMYELINATED AFFERENT FIBRES IN THE VAGUS OF THE CAT.

By A. IGGO. From the Department of Physiology, University of Edinburgh.

(*Received for publication 3rd October* 1956)

MOST of the axons in the abdominal visceral nerves are unmyelinated [Chase and Ranson, 1914] and afferent [Evans and Murray, 1954], and although there is abundant evidence in the physiological literature that the abdominal viscera have a sensory innervation [see Whitteridge, 1956], there have been few attempts to record electrophysiologically from the primary afferent fibres.

Gernandt and Zotterman [1946], who recorded electrical activity in the splanchnic nerves and in fine mesenteric nerve strands of the cat, concluded, from multifibre preparations, that much of the activity set up by intestinal changes was in C fibres. More recently the single fibre technique, which although tedious has the advantage of precision, was used to record activity in vagal afferent fibres from abdominal receptors of the cat by Paintal [1954 *a*, *b*] and of the cat and goat by Iggo [1955]. With this technique, accurate measurements of the conduction velocity in centripetal fibres from specified receptors could be made. No single fibres with conduction velocities less than 2 m./sec. in the cervical vagus were reported. The lack of information about fibres with conduction velocities less than 2 m./sec., *i.e.* the numerically preponderant unmyelinated (C) fibres, is due to the technical difficulty of isolating them as single units.

In the present investigation an attempt has therefore been made to record electrophysiologically from single vagal afferent C fibres with receptors in the stomach and intestines. This paper will describe the activity in afferent units sensitive to distension, while the measurement of the conduction velocity in the fibres will be described elsewhere [Iggo, 1957 *a*].

METHODS

Cats starved overnight were used, preferably young animals weighing 2–3 kg. They were anæsthetized with chloralose (80 mg./kg.) after induction with ethyl chloride and ether. Body temperature was kept at 37°–39° C. by placing the cat on a table with thermostatic heat control.

Dissection of Single Fibres.—The method was similar to that

previously described [Iggo, 1955]. Particular care was taken to keep the blood supply to the vagus undisturbed; the vagus and sympathetic nerve were left in their common sheath, and as short a length of the nerve as possible dissected away from the connective tissue attachments to the common carotid artery. When removing the sheath from the vagus large blood vessels were avoided and not more than 2 cm. of the sheath was removed. Streaming of red blood cells could be seen during the experiment in the vessels on the surface and in the depths of the vagus. The dissection was done on a black perspex platform with a sharp needle and fine watchmaker's forceps under a binocular microscope (× 12–36, rarely × 48).

Recording Electrodes.—These were Ag wires coated electrolytically with AgCl, and were 0·5 mm. dia. and 1 mm. apart. The electrodes were mounted permanently in resin, and the holder was rigidly attached to a micro-manipulator with controlled movement in three planes. This device was a particular benefit when handling the finest vagal strands, because the electrodes could be placed in any position without the back-lash which was so troublesome a feature of the older method. This matter of rigid mounting is of importance, because any movement of the recording system produced large artefacts with the very fine vagal strands used. To avoid artefacts all mechanical fittings were secured as rigidly as possible.

Stimulating Electrodes.—These were Ag wires and were 1 cm. apart. One pair of stimulating electrodes was placed under the intact cervical vagus and in isolation from other tissues about 30–40 cm. below the recording electrodes. Another pair of stimulating electrodes was placed on the branch of the abdominal vagus containing the afferent fibre dissected from the cervical vagus. The conduction velocity of the afferent fibres could therefore be computed by measuring the time taken for an impulse set up either in the cervical vagus or in the abdominal vagus to reach the recording electrodes. The distance from each position of the stimulating electrodes to the recording electrodes was measured directly after the experiment was finished.

Distension of the Gut.—The abdomen was opened by incising the linea alba from the xiphisternum to within 5–10 cm. of the symphysis pubis. The skin flaps and the margins of the abdominal wound were tied to a circular frame, so as to form a pool which was filled with liquid paraffin (B.P.) to prevent drying and loss of heat from the abdominal viscera.

To prevent the regurgitation of fluid used to distend the stomach a ligature was tied round the cardiac sphincter. This operation had to be done carefully to avoid ligation of the gastric vagi. Another ligature was placed around the pyloric sphincter after the polythene tube from the 50 ml. hypodermic syringe used for distension and the polythene tube leading to the condenser manometer had been passed from the

opened duodenum into the stomach. If the stomach contained any material, usually hair, it was removed before fastening the ligature.

When a search was made for intestinal receptors, a ligature was tied round the pyloric sphincter and a second ligature was tied round the intestine 30 to 50 cm. below the first ligature. The inflow tube and the pressure recording tube were passed through the lower ligature. In some experiments an intestinal receptor, after being located by distension and by manipulation of the gut, was isolated in a short length of intestine by tying ligatures on either side of the receptor. Pieces of intestine as short as 2 cm. were sometimes isolated by this means. Distension with known volumes of warm saline [0·9 per cent (w/v) NaCl] was ensured by using a hypodermic syringe.

Intravenous injections were given through an indwelling cannula in the right femoral vein.

Results

The activity in 29 afferent units dissected from the right cervical vagi of 15 cats has been examined. In a few instances there was only one live fibre in the nerve strand on the recording electrode. However, such occasions were rare. The strands more often contained only one active unit together with several viable but inactive axons. A few of the results were obtained with multi-unit strands, *i.e.* strands containing several active units and many viable axons. In these last preparations, the unit under examination had distinctive action potentials. In this investigation, the conduction velocities of all except one of the centripetal fibres were 1·3 m./sec. or less.

The Adequate Stimulus: Distension

All the afferent units were stimulated by distension of the œsophagus, stomach or the small intestine. This use of an adequate stimulus is highly selective and easy to use, but it does not sample all types of receptors in the gut. Paintal [1954 *b*] has shown that phenyl-di-guanide excites a curious variety of endings, including some visceral endings not sensitive to distension. Other receptors have been found by tactile stimulation of the mucosa [Iggo, 1957 *b*]. After the receptor had been localized by manipulation of the gut, ligatures were tied to occlude the lumen on each side of the receptor. Distension of the isolated part then gave a discharge in the fibre whereas distension of adjacent regions did not.

In all these experiments great care was taken to avoid over-distension. The volumes used for gastric distension were rarely greater than 150 ml. and were usually between 50 and 100 ml. The intragastric pressure seldom rose above 25 mm. Hg. When short lengths of the intestine

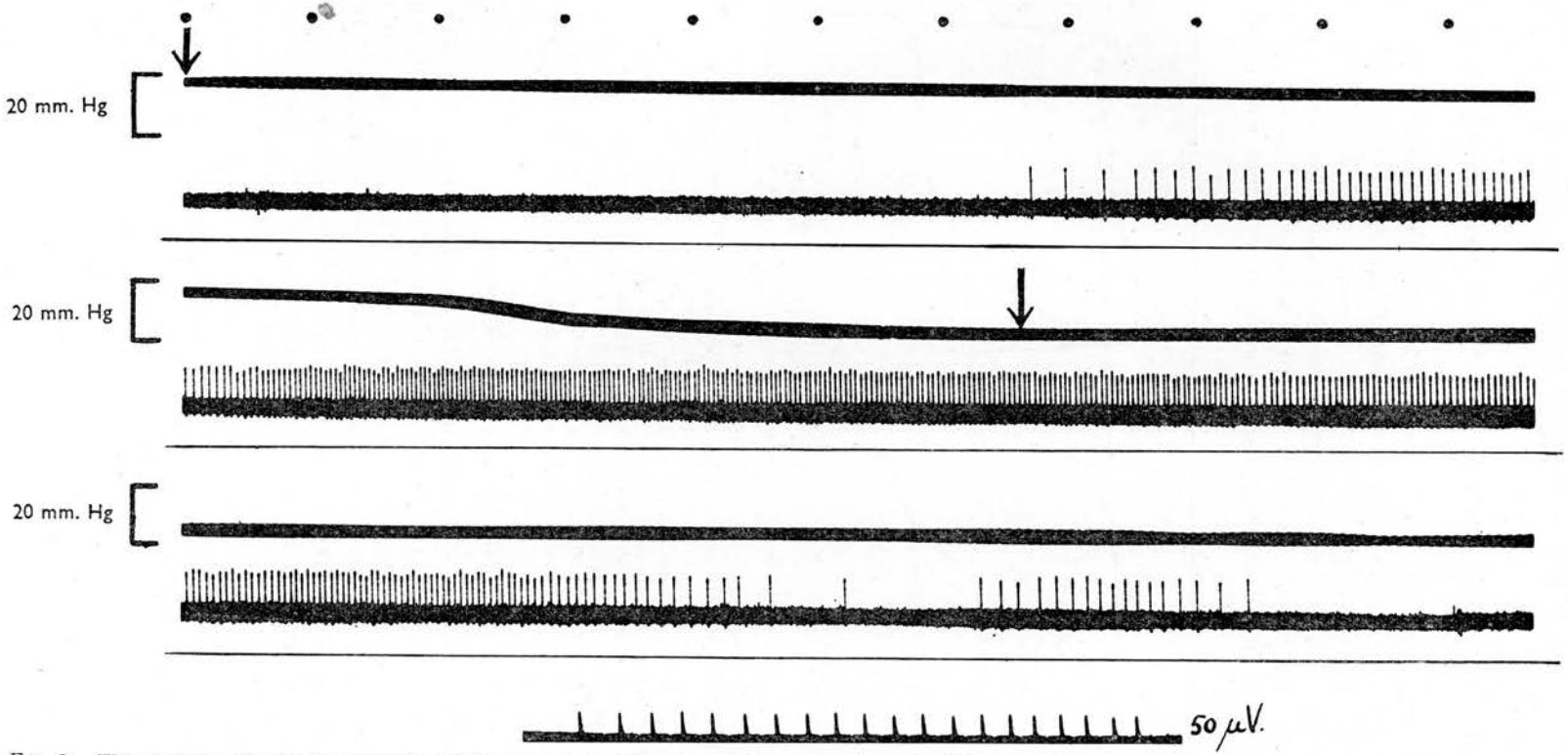


FIG. 1.—The response of a receptor in the pyloric antrum to distension of the whole stomach. The records are continuous. Inflow of 50 ml. of saline began at the arrow at top L.H. corner and finished at the arrow in the second row. The fluid remained in the stomach for the rest of the record. Time in 1 sec. intervals.

were used, volumes as small as 0·2 ml. were sufficient to excite the receptor.

As the fluid entered the viscus the intraluminal pressure rose and impulses appeared in the fibre or the resting discharge increased. The most sensitive units were stimulated by pressures as low as 2 mm. Hg. The impulses disappeared as soon as the fluid was withdrawn. If distension was sustained the frequency of discharge fell slowly (fig. 3). Sometimes the impulses disappeared and at other times the discharge became rhythmical (fig. 1).

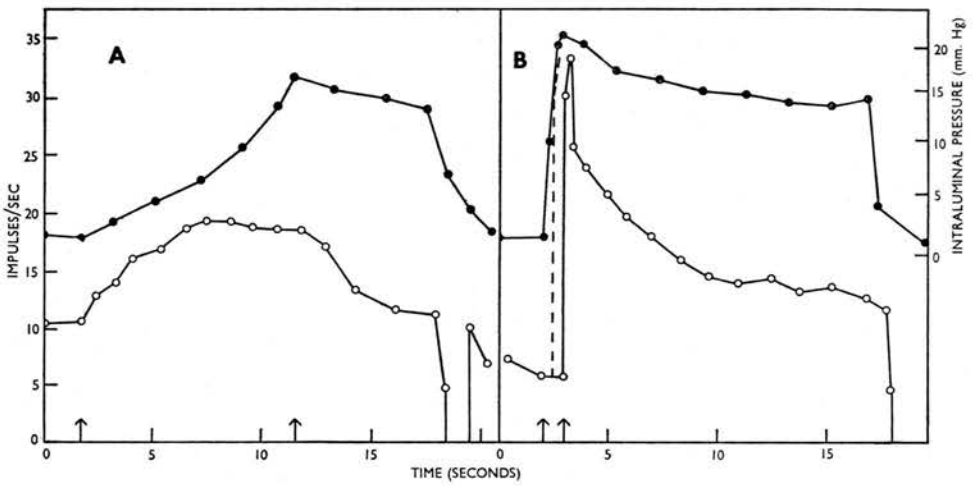


FIG. 2.—The effect of the rate of distension on the discharge of a fibre from a receptor isolated in a short length of the jejunum.

●——● intraluminal pressure; O——O discharge in the afferent fibre—each point is the average frequency of 10 impulses; — — — time at which the impulses left the jejunum, computed by making an allowance for the conduction time.

A. Slow inflow of saline at the rate of 5 ml. in 10 sec. between the arrows.
B. Rapid inflow of saline at the rate of 5 ml. in 1 sec. between the arrows.
The saline was withdrawn at 17–18 sec. in both A and B.

The Latency of the Response

When the viscus was distended rapidly the discharge in the afferent fibre (recorded high in the neck) did not appear for 0·2 sec. or longer after the onset of distension. Paintal (1954 *a*) has also noticed this delay and ascribed it to his methods of recording the pressure. However, in all the gastro-intestinal fibres reported in this paper, the time taken for the impulse to travel from the receptor to the recording electrodes was at least 200 msec and often 500 msec. When allowance has been made for this time, the interval between the onset of the stimulus and the first impulse is very short (figs. 2 and 3). The discharge began almost at peak frequency long before all the fluid was injected and often had fallen to a lower frequency before distension was complete (fig. 2).

The Rate of Distension

The response of gastric, duodenal and jejunal receptors to both slow and rapid distension was examined. Fig. 2 shows the response of a receptor, isolated in a 2 cm. length of jejunum, to the inflow of saline at rates of 5 ml./10 sec. and 5 ml./1 sec. At the slower rate of inflow the frequency of discharge gradually rose to 20 impulses/sec. and reached this level before the inflow of fluid was complete. After the inflow had ceased the frequency fell rapidly and at the same time there was a slow fall in intraluminal pressure.

At the more rapid rate of saline inflow the frequency of discharge of impulses reached a peak frequency of 35 impulses/sec. for a period of less than 1 second. This was almost twice the frequency reached when the same volume of saline was injected at the slower rate. After this brief burst of activity the frequency of discharge fell abruptly to 26 impulses/sec. and then to 13 impulses/sec. during the next 13 seconds. The intraluminal pressure rose to a higher level when distension was rapid. The slow fall in pressure after the inflow of fluid ceased, which was observed with both slow and rapid distension, was probably due to relaxation of the smooth muscle.

It has previously been shown that distension-sensitive gastric receptors are "in series" with the smooth muscle [Iggo, 1955]. If the afferent units described above are also "in series" with the smooth muscle, then part of the variation in response to different rates of distension was due to tension changes in the muscle.

The Effect of Visceral Contraction on the Afferent Units

(*a*) Contractions of the viscera caused by faradic stimulation of the cervical vagi were clear-cut only in the œsophagus. The œsophageal unit illustrated in fig. 4 responded with a burst of impulses 2–3 sec. after the vagal stimulus when the œsophagus was empty. After the œsophagus was distended the response began within 0·8 sec. of the shock but the peak frequency occurred late in the contraction, at the same time as it occurred when the œsophagus was empty. The conduction velocity of this fibre was 7·5 m./sec., and therefore conduction time contributed little to the latency of the response. The receptor was probably at the lower end of the œsophagus.

(*b*) Localized contractions of the pyloric antrum and the small intestine were readily evoked by faradic stimulation of the serous surface of the viscus. Whenever the visceral wall containing a receptor contracted, there was a burst of impulses which persisted as long as the contraction lasted (fig. 3). This response was still present after the viscus had been opened along its free margin and a contraction was set

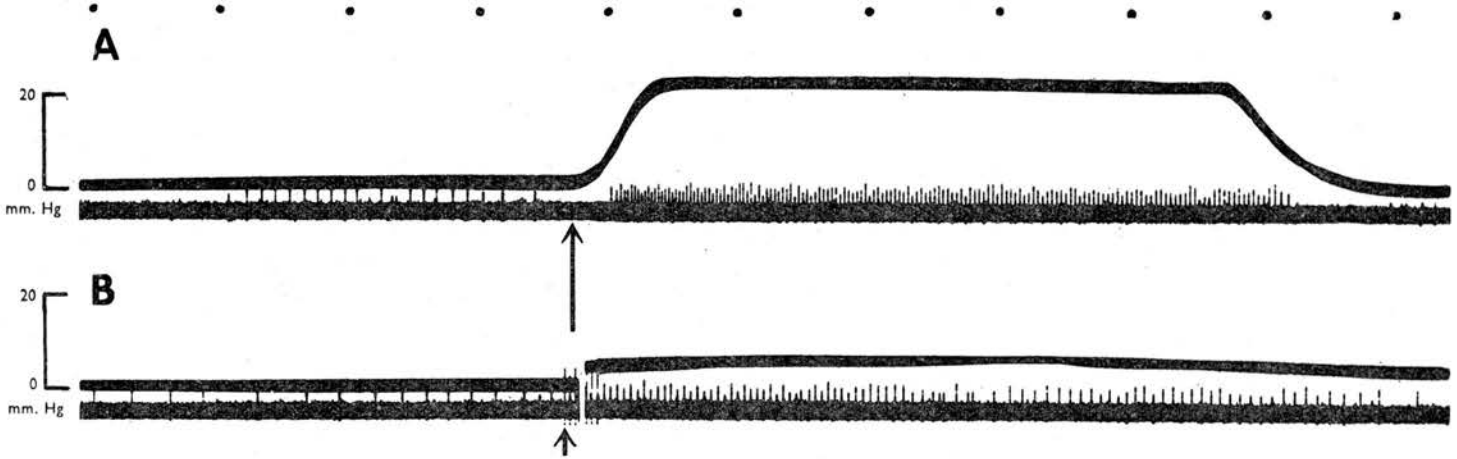


FIG. 3.—The response of a duodenal receptor to distension and contraction. The upper tracing in each record is the pressure recorded in a 2 cm. length of the duodenum, and the lower tracing shows impulses recorded in an afferent fibre dissected from the cervical vagus.

A. Five ml. of saline were injected rapidly into the lumen. The arrow below the tracing shows the time at which the first impulse left the duodenum; its position was fixed by making an allowance for the conduction time from the duodenum to the recording electrode.

B. The serosa was stimulated faradically at the arrow and part (10 sec.) of the record has been cut out. The contraction caused by the stimulation has excited the receptor. Time 1 sec.

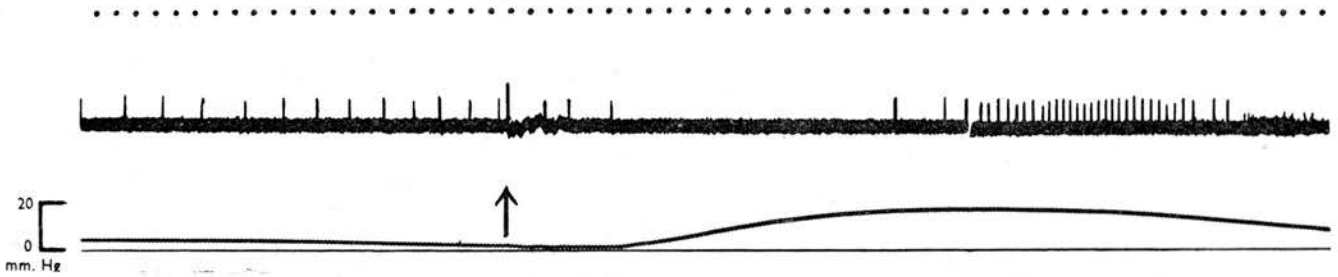


FIG. 4.—The discharge in a fibre from a receptor in the lower œsophagus. The conduction velocity of this fibre was 7·5 m./sec. The œsophagus was partly distended and the discharge of impulses during inspiration can be seen at the left. At the arrow a single shock was given to the cervical vagus, and the œsophageal contraction is recorded by the manometer (lower tracing with the baseline drawn in). The contraction excited the receptor. Time in 1/10 sec. intervals.

up in the flap so formed. The response did not therefore depend on a rise in intraluminal pressure although in the intact viscus this has been observed.

(*c*) The response of receptors during rhythmical contractions has been examined using short lengths of the intestine. Isolated jejunal segments sometimes showed rhythmical movements at a frequency of about 12/min. Such movements were especially useful since they provided changes in tension in the smooth muscle in the absence of artificial stimulation, *i.e.* they were "natural" contractions. Furthermore, these contractions enabled the response of the receptors to be examined during small localized changes in motility such as cannot always be revealed when recording from the whole organ. Fig. 5

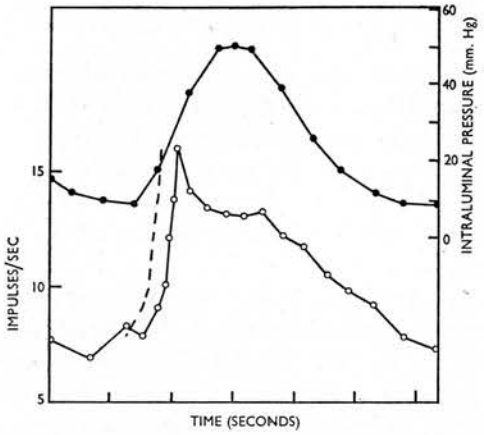


FIG. 5.—The discharge of impulses in an afferent fibre from a receptor in a 2 cm. length of the jejunum during one of a series of rhythmical isometric jejunal contractions. This is not the unit illustrated in fig. 3. ●——● intraluminal pressure; O——O discharge of impulses in the fibre; - - - time at which impulses left the jejunum, computed by making an allowance for conduction time.

illustrates the response of a jejunal unit during one such contraction. The contraction clearly stimulated the receptor. The early onset of the response was revealed when allowance was made for the conduction time.

Whatever method was used to elicit a contraction of the smooth muscle, the distension-sensitive endings were always stimulated by a contraction at the site of the receptor, *i.e.* they were "in series".

Localization of the Receptors

If the afferent unit was still alive after all the necessary measurements had been made, an attempt was made to see if the tension receptors

were restricted to a particular layer of the visceral wall. The viscus was opened along its free margin, *i.e.* away from the larger blood vessels, and the mucosa was scraped off. All the tension endings tested were unaffected by removal of the mucosa and submucosa. When the nerve trunk containing the axon was pinched there was no burst of impulses, so it may be assumed that the response to pinching the viscus was due to stimulation of the receptor. Attempts to distinguish between the serosa and external muscle as the site of the receptors were unsuccessful. It is concluded therefore that tension receptors are present in the serosa or the muscularis externa of the stomach and intestines. Whether they are completely absent from the mucosa and submucosa is not known, but so far no tension receptors have been found in these regions.

The Action of Chemicals

(a) *Phenyl-di-guanide.*—In order to see whether the tension receptors could be differentiated from the receptors described by Paintal [1954 *b*] the action of phenyl-di-guanide was tested. With a single exception all the tension receptors which were tested were stimulated by an intravenous injection of phenyl-di-guanide (Table I). Their behaviour towards this drug was therefore similar to that of the receptors described by Paintal [1954 *b*].

(b) *Adrenaline.*—An intravenous injection of 50 μg./kg. adrenaline aroused a discharge of impulses which persisted for several minutes, whereas a dose 10–20 μg./kg. failed to excite the tension receptors on which this dose was tried (Table I). It may be doubted whether the massive doses needed to excite the endings can confer any physiological significance on this action of adrenaline.

(c) *Acetylcholine.*—Gernandt and Zotterman [1946] noticed that a 1 mg. per cent (w/v) solution of ACh when put on the surface of the intestine caused a strong contraction and set up small impulses in the mesenteric nerve strands. A similar action has been found during the present study. ACh on the opened intestine evoked a persistent discharge in the afferent units and a prolonged contraction of the muscle. Since the receptors are "in series" with the smooth muscle, it is not possible to distinguish a direct action by stimulation of the receptor from an indirect action through contraction of the muscle.

(d) 0·1*N HCl.*—When a solution of 0·1N HCl was poured over the mucosa or serosa it failed to affect any of the tension receptors on which it was tried (Table I).

Discussion

The distension-sensitive receptors with centripetal C fibres are "in series" tension receptors, and are similar in their responses to the same type of ending in the goat with centripetal myelinated fibres [Iggo,

TABLE I.—THE RESPONSE OF GASTROINTESTINAL RECEPTORS WITH UNMYELINATED CENTRIPETAL VAGAL FIBRES TO MECHANICAL AND CHEMICAL STIMULI

Site of receptor	Stimulus: Pinch	Distension	Contraction	Phenyl-diguanide i.v. μg./kg.	Adrenalin i.v. μg./kg.	0·1N HCl
Cardia	+	+	..	50 +	..	..
,,	+	+	..	..	..	..
Fundus	+	+	..	40 +	..	..
,,	+	+	..	80 +	..	..
,,	+	+	..	..	..	..
,, *	+	+	+F V	..	..	..
,, *	+	+	+F V	..	..	..
,,	+	+	..	..	..	..
,,	+	+	+F	55 +	..	..
Pyloric antrum	+	+	+F	40 +	10 −	−
	+	+	+F	130 −	20 −	−
,,	+	+	..	..	..	..
,,	+	+	+F	..	..	..
,,	+	+	..	..	..	..
,, *	+	+	..	..	..	−
,,	+	+	..	..	..	..
Duodenum	+	+	+FR	50 +	..	..
,,	+	+	+F	100 +	..	..
,,	+	+	..	55 +	..	..
,,	+	+	+FR	80 +	..	..
,, *	+	+	+F	..	..	−
Jejunum	+	+	+FR	150 +	50 +	..
,,	+	+	..	..	..	..
,, *	+	+	+F	..	..	..
,,	+	+	+R	..	..	..
,,	+	+	+R	..	..	..
,,	+	+	..	..	..	..

+ stimulation; − no action; .. not tested.

* Removal of the mucosa and submucosa did not abolish the response.

F: Contraction caused by faradic stimulation of the serosa.
V: Contraction caused by faradic stimulation of the cervical vagus.
R: Rhythmical movements of the intestine at the site of the receptor.

1955]. This discovery that C fibres can be isolated as single units weakens the assumption, based on indirect evidence, that all the gastric tension receptors previously described in the cat [Iggo, 1955] had small myelinated centripetal fibres. However, there are visceral tension receptors with small myelinated vagal centripetal fibres; the only example found in the present study was at the lower end of the œsophagus, and the conduction velocity of the fibre was 7·5 m./sec.

Since the receptors are "in series" with the contractile elements of smooth muscle it was predicted that smooth muscle tone would modify the response of the endings to distension. This was so. Rapid distension caused a higher level of intraluminal pressure than did slow distension with the *same volume of fluid,* and the rate of discharge

was much higher with rapid distension. The slow fall in frequency of discharge when distension is sustained, sometimes loosely called adaptation, can also be related to a slow relaxation of the smooth muscle as in figs. 2 and 3. Both these records are from short lengths of intestine in which the pressure recorded was more closely related to the tension in the wall than it was in fig. 1, where the pressure was recorded from the whole stomach. These results are inconsistent with the suggestion that the receptors act as accurate volume-signalling devices.

Downman, McSwiney and Vass [1948] reported that the majority of stimuli to the small intestine of the cat, such as pinching and squeezing, gave reflex responses when applied to the intestinal wall after removal of the mucosa and when spread of the stimulus to the adjoining mesentery was prevented. The intestinal tension receptors responded to similar stimuli after removal of the mucosa and submucosa and so may contribute to the reflex effects described by Downman *et al.* [1948]. The effect of contraction on the tension receptors is not unexpected if the endings are in the muscularis externa. There are other sense-endings in addition to these tension receptors. Paintal [1954 *b*] has reported that receptors in the mesentery adjacent to the intestines are unaffected by intestinal distension, and gastric mucosal endings also unaffected by distension have been found [Iggo, 1957 *b*].

There have been few reports on the detailed terminations of the sensory fibres in the abdominal viscera. Langworthy and Ortega [1943] saw spindle-shaped endings parallel with the muscle fibres in methylene-blue-stained preparations of the stomach of the cat. This report was not confirmed by Ohi [1954 *a*, *b*] or Utsushi [1954] who, in silver-stained preparations of the adult rat and human embryo stomach and duodenum, found only simple branched or unbranched free terminals of small fibres in all layers of the visceral wall. These free terminals were distinguished by the authors from autonomic efferent fibres and classed as cerebrospinal afferent fibres.

The suggestion of Gernandt and Zotterman [1946] that fine mesenteric nerve strands and the splanchnic nerves contain afferent C fibres with endings in the intestine is interesting. The published records of activity in very small fibres are all from fine mesenteric nerve strands, and there is no reason to suppose that all the fibres from which they recorded were splanchnic in origin. In the present work, when a short length of the jejunum containing a tension receptor with a vagal afferent fibre was isolated by tight ligatures from the rest of the intestine the activity of the afferent unit was unimpaired, so that the centripetal axon must have passed inward along the mesenteric plexus. The activity which Gernandt and Zotterman [1946] recorded, and which may have arisen in vagal as well as splanchnic fibres, was evoked by pinching the intestine, peristaltic intestinal contractions and intense contractions caused by putting 1 mg. per cent (w/v) acetylcholine on the intestine. This

behaviour is identical with that recorded in single vagal afferent fibres in the present work. The conduction velocity in these fibres was never greater than 1·3 m./sec., so that the conclusion of Gernandt and Zotterman [1946] that the activity which they recorded was in C fibres is confirmed.

With the knowledge that unmyelinated fibres innervate "in series" tension receptors, it is possible to reassess the mechanisms of visceral sensation. Pangs of hunger are associated with powerful gastric contractions [Cannon and Washburn, 1912] which were usually well advanced before any sensation was perceived. Part of the delay can be attributed to the building up of tension in the empty stomach and to events in the central nervous system. However, at least 0·5 to 1 sec., if not longer, would be required for the impulses set up in C fibres to travel from the gastric tension receptors to the medulla.

The transient sensation of fullness which follows the very rapid ingestion of food can also be attributed to the "in series" receptors. After rapid distension there is a relaxation of the gastric smooth muscle, and when this occurs the discharge from the previously active receptors would be diminished and so the sensation aroused by their activity would disappear.

Pain can arise from changes in the viscera. Stürup [1940] demonstrated that visceral contractions are a particularly effective source of pain. His conclusion is supported by the spasmodic occurrence of pain in intestinal colic. This pain can be relieved by atropine, which abolishes the myenteric reflex [Bozler, 1949] and so suppresses reflex contractions. Payne and Poulton [1927] found that isometric œsophageal contractions, *i.e.* contractions against an incompressible balloon, were intensely painful, whereas the normal œsophageal contractions in swallowing were not noticed. The intraluminal pressure was much higher during these isometric contractions. Visceral pain may also be caused by very rapid gastric distension, whereas very slow distension may pass unnoticed [Hurst, 1911]. The factor common to these different situations is the development of a high tension in the visceral wall which, as the present work shows, is an "adequate" stimulus for the distension-sensitive receptors.

As this discussion shows, there are a variety of dissimilar sensations which can be ascribed to the same receptors, and Whitteridge [1956] has described other sensations as well as a variety of vascular reflexes. Before information about the C fibres was available, there was some difficulty in reconciling these conflicting sensations and reflexes with the small number of afferent fibres. Now that we know that the abundant C fibres include axons from tension receptors, it is at least easier to postulate different central destinations for fibres from the same or different regions of the viscera to account for the diverse roles of the one type of receptor.

SUMMARY

1. Electrical activity was recorded in single afferent C fibres dissected from the cervical vagus of the cat. The receptors were distension-sensitive endings in the œsophagus, stomach or small intestines. The latency of the response to distension was largely due to the time taken for the impulses to travel from the receptor to the recording electrodes.

2. The rate of discharge of impulses was much higher when distension with the same volume of fluid was rapid than when distension was slow. This was seen most clearly when only short lengths of the intestine were distended. It is suggested that the effect is partly due to smooth muscle tone.

3. Contraction of smooth muscle at the site of the receptor set up a discharge of impulses in the afferent fibre, and this response was not dependent on changes in intraluminal pressure. It was concluded that the receptors were tension-signalling devices "in series" with the contractile elements in the smooth muscle.

4. The receptors were unaffected by removal of the mucosa and submucosa, and probably lay in the muscularis externa of the stomach or intestines.

5. The receptors were excited by intravenous injection of phenyl-diguanide and adrenaline and by acetylcholine poured over the opened viscus. They were unaffected by 0·1N HCl. The difficulty of interpreting the action of ACh was pointed out.

REFERENCES

BOZLER, E. (1949). "Myenteric reflex", *Amer. J. Physiol.* **157,** 329–337.

CANNON, W. B. and WASHBURN, A. L. (1912). "An explanation of hunger", *Amer. J. Physiol.* **29,** 441–454.

CHASE, M. R. and RANSON, S. W. (1914). "The structure of the roots, trunk and branches of the vagus nerve", *J. comp. Neurol.* **24,** 31–60.

DOWNMAN, C. B. B., MCSWINEY, B. A. and VASS, C. C. N. (1948). "Sensitivity of the small intestine", *J. Physiol.* **107,** 97–106.

EVANS, D. H. L. and MURRAY, J. G. (1954). "Histological and functional studies on the fibre composition of the vagus nerve of the rabbit", *J. Anat. Lond.* **88,** 320–337.

GERNANDT, B. and ZOTTERMAN, Y. (1946). "Intestinal pain: an electrophysiological investigation on mesenteric nerves", *Acta physiol. scand.* **12,** 56–72.

HURST, A. F. (1911). The Sensibility of the Alimentary Canal. London: Oxford University Press.

IGGO, A. (1955). "Tension receptors in the stomach and the urinary bladder", *J. Physiol.* **128,** 593–607.

IGGO, A. (1957 *a*). "Conduction velocity in vagal afferent fibres." [In preparation.]

IGGO, A. (1957 *b*). "pH receptors in the gastric mucosa of the cat." [In preparation.]

LANGWORTHY, O. R. and ORTEGA, L. (1943). "Sensory endings on gastric muscle", *J. comp. Neurol.* **79,** 425–430.

Ohi, K. (1954 *a*). "Innervation, especially sensory innervation, of stomach in white rat", *Tohoku J. exp. Med.* **60,** 289–296.

Ohi, K. (1954 *b*). "Innervation, especially sensory innervation, of duodenum in white rat", *Tohoku J. exp. Med.* **60,** 297–306.

Paintal, A. S. (1954 *a*). "A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst", *J. Physiol.* **126,** 255–270.

Paintal, A. S. (1954 *b*). "The response of gastric stretch receptors and certain other abdominal and thoracic vagal receptors to some drugs", *J. Physiol.* **126,** 271–285.

Payne, W. W. and Poulton, E. P. (1927). "Experiments on visceral sensation. Pt. I. The relation of pain to activity of the human œsophagus", *J. Physiol.* **63** 217–241.

Stürup, G. (1940). Visceral Pain. Copenhagen: Nyt Nordisk Forlag.

Utsushi, S. (1954). "Innervation of duodenum and ductus choledocus in human embryo", *Tohoku J. exp. Med.* **60,** 311–321.

Whitteridge, D. (1956). "The effects of distension of viscera", *in* Lectures on the Scientific Basis of Medicine, Vol. IV, 305–310. University of London: The Athlone Press.

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. XLII, 1957.

GASTRIC MUCOSAL CHEMORECEPTORS WITH VAGAL AFFERENT FIBRES IN THE CAT.

By A. IGGO. From the Department of Physiology, University of Edinburgh.

(*Received for publication* 14*th May* 1957)

Electrical activity was recorded in 19 single fibres, with very slow conduction velocities, dissected from the cervical vagi of 10 cats. The nerve endings of these fibres were in the gastric mucosa and were destroyed when the mucosa was scraped away. A brief burst of impulses was recorded when the mucosa was stroked firmly with a smooth rod but there was no response when the stomach was made to contract, when it was compressed firmly between finger and thumb, or when it was lightly distended. Over-distension excited 3 of the mucosal nerve endings. The mucosal nerve endings were specifically sensitive to either acid or alkaline solutions on the mucosa. The discharge of impulses in the afferent fibre persisted for as long as the stronger solutions used (0·1 N-NaOH or 0·1 N-HCl) remained on the mucosa. The threshold of the acid receptors was pH 3 or lower and of the alkali receptors was pH 8 or above. The endings were not affected by hypotonic or hypertonic solutions or by any other chemicals that were tried. A clear distinction is made between the gastric mucosal nerve endings described in the paper and the tension and "stretch" receptors which are found in the deeper layers of the gastric wall. It is concluded that the mucosal nerve endings probably function as slowly-adapting chemoreceptors. A low-threshold gastric "stretch" receptor is described.

Both mechanical and chemical stimulation of the gastric mucosa elicit a variety of sensations and reflex responses. Carlson and Braafladt [1915] and Wolf and Wolff [1947] found that there was a sensation of pressure or an undifferentiated awareness when the normal gastric mucosa of their subjects was rubbed or squeezed; touch, however, was not perceived. Gastric movements in sheep and goats may be enhanced by acidification of the gastric contents [Titchen, 1953] or by mechanical stimulation of the gastric mucosa [see Habel, 1956, for review]. There does not seem to have been any previous electrophysiological account of afferent fibres with endings in the mucosa. The gastric afferent fibres already described came from tension and "stretch" receptors [Paintal, 1954 *a*; Iggo, 1955, 1957 *a*]. The endings of these fibres were in the outer layers of the gastric wall.

The present study is part of a wider investigation of small afferent fibres. It was begun when a gastric afferent fibre was found which did not fit into the category described previously. The ending of the fibre was destroyed when the gastric mucosa was removed. This paper describes the response of similar fibres to mechanical and chemical stimulation. A preliminary account of this work has already appeared [Iggo, 1957 *b*].

Methods

The manner in which the cats were prepared for the dissection of single fibres and the electrical recording methods have already been described [Iggo, 1955, 1957 *a*]. Endings in the mucosa were found by rubbing a smooth probe, introduced into the stomach through a small hole, over the surface of the mucosa and listening to the amplified action potentials picked up from the strand of the cervical vagus. Once a mucosal unit was found, the stomach was cut open so as to expose the appropriate part of the mucosa. The mucosa exposed in this way tended to become colder than the rest of the cat. To prevent this the fluids used to irrigate it were kept at 39–40° C. and warm pads of cotton wool were put on to the mucosa whenever convenient.

Histological Examination of the Mucosa.—This was done in three units to see how much of the mucosa had been removed by scraping. The appropriate part of the gastric wall was cut out and fixed in formalin (10 per cent v/v). Sections were cut and stained with hæmotoxylin-eosin.

Chemical.—The following solutions were used to test the pH sensitivity of the mucosal nerve-endings: 0·1 N-NaOH, diluted as required; 0·05 M-borax, pH 9·3; 0·2 N-HCl diluted as required; mixtures of 0·2 M-Na_2HPO_2 and 0·1 M-citric acid to give solutions of different pH, according to McIlvaine [1921].

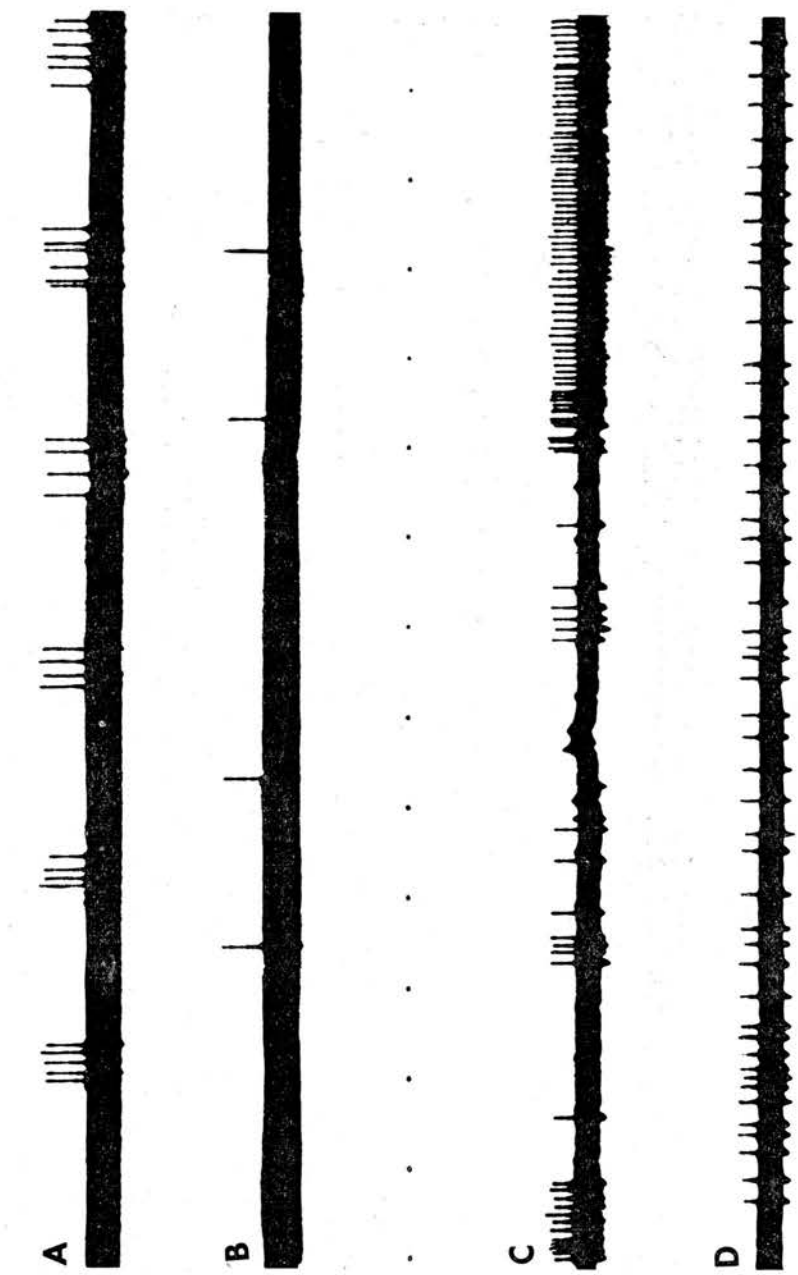
The pH of the solutions used was measured with a glass electrode. The pH of the solutions in actual contact with the mucosa was not measured.

Results

The 19 vagal fibres described in this paper were isolated as single units from the right cervical vagi of 10 cats. The conduction velocities of the fibres ranged from 1 to 5 m./sec. [Iggo, 1957 *c*].

Location of the Receptors.—The receptors were found in all parts of the stomach. Because of its accessibility the pyloric antral mucosa was explored most thoroughly and the majority of the receptors described were in that region. There were no systematic differences in the properties of receptors in different parts of the stomach.

If the afferent unit was still alive after the tests, to be described, were made and the conduction velocity of the centripetal fibre had been measured, an attempt was then made to find the layer of the gastric wall in which the fibre ended. This was done by gradually scraping away the mucosa with a scalpel blade. When the most sensitive spot of the gastric mucosa was scraped there was a brief burst of impulses in the single fibre (fig. 1*a*) Vigorous scraping gradually removed the mucosa and at some stage in this denudation the characteristic response to scraping became shorter until only a single impulse was set-up in the fibre (fig. 1*b*). Further scraping abolished even this response but electrical stimulation still excited the fibre. There was no response to a previously effective chemical stimulus when a mechanical stimulus had become ineffective so that it is clear that the chemicals were acting on the nerve ending and not on the axon. Removal of one-third or less of the mucosa was sufficient to destroy the endings of 4 fibres; with 3 units it was necessary to expose the muscularis mucosa before the response



FIGS. 1*a*, *b*, *c* and *d*.—The discharge in centripetal fibres from gastric receptors in response to mechanical stimulation. (*a*) and (*b*) Fibre 40, mucosal receptor. Each group of impulses was caused by stroking the mucosa with a smooth probe, (*a*) before and (*b*) after the superficial one-third of the mucosa had been scraped off. (*c*) Fibre 41, tension receptor. The first 3 groups of impulses were elicited by stroking the mucosa and the last group by light pressure on the mucosa. (*d*) Fibre 52, mucosal unit. The discharge was aroused by a very firm stretch of the mucosa.

Time marks, 1 sec.

was lost. With 1 unit the response to mechanical stimulation was absent after the mucosa had been removed by splitting the gastric wall through the submucosa.

It was noticed several times that removal of the mucosa at the centre of the most sensitive area did not prevent a response to mechanical stimulation of the adjacent intact mucosa. Histological examination revealed that the muscularis mucosa was exposed at the centre, the spot which no longer responded to stimulation, and that the surrounding mucosa was undamaged. These results suggest that a single axon innervates an area perhaps as large as 5 cm.^{2} and does this by sending collateral fibres into the superficial mucosa from a main trunk which ramifies in the deeper layers of the mucosa.

TABLE I.—THE RESPONSE OF GASTRIC MUCOSAL NERVE ENDINGS TO MECHANICAL AND CHEMICAL STIMULATION

Fibre No.	Stroke	Stretch	Contraction	Compression	Acid	Alkali	Ringer-Locke	Threshold
15 m	+	+E	−	−	−	..	−	..
27 m	+	−	..	−	..	..	..	..
28	+	−	..	−	..	..	−	..
29 m	+	−	..	..	−	+	−	..
36	+	−	..	..	..	..	−	..
37 m	+	−	..	−	..	..	..	..
40 m	+	−	..	−	−	+	−	0·01 N-NaOH
46	+	−	−	−	−	+	−	pH 8·0
48	+	−	..	..	+	−	−	..
49	+	−	..	..	−	+	..	pH 8·0
52 m	+	+E	..	..	+	−	..	pH 2·2
53 m	+	+E	−	..	−	+	−	pH 9·0
55 m	+	−	−	..	−	+	−	pH 9·3
56 m	+	..	−	..	−	+	−	..
58 m	..	..	..	..	−	+	−	pH 9·0
59 m	+	..	..	..	+	−	−	..
60 m	+	..	..	..	−	+	−	0·01 N-NaOH
61 m	+	..	..	..	+	−	−	pH 3·0
62	..	..	..	..	+	..	−	pH 1·5

+ =response; − =no response; E =over-distension or firm stretch of the mucosa; m =mucosal origin confirmed by scraping off the mucosa; .. = not tested.

The Response to Mechanical Stimulation.—Light touch or digital compression failed to excite any of the mucosal nerve endings. A burst of impulses was recorded in each of the fibres when a small area of the mucosa was stroked with a smooth probe or cotton wool (fig. 1*a*), but not when more distant parts of the mucosa were stroked. A sustained irregular stream of impulses was recorded in 3 of the units when the mucosa was very firmly stretched and when the stomach was over-distended with saline or with an air-filled balloon. The peak frequency of discharge was low; 10/sec. in Fibre 52, fig. 1*d*. Gastric contractions caused by faradic stimulation of the serosa or the mucosa did not excite the mucosal nerve endings (Table I).

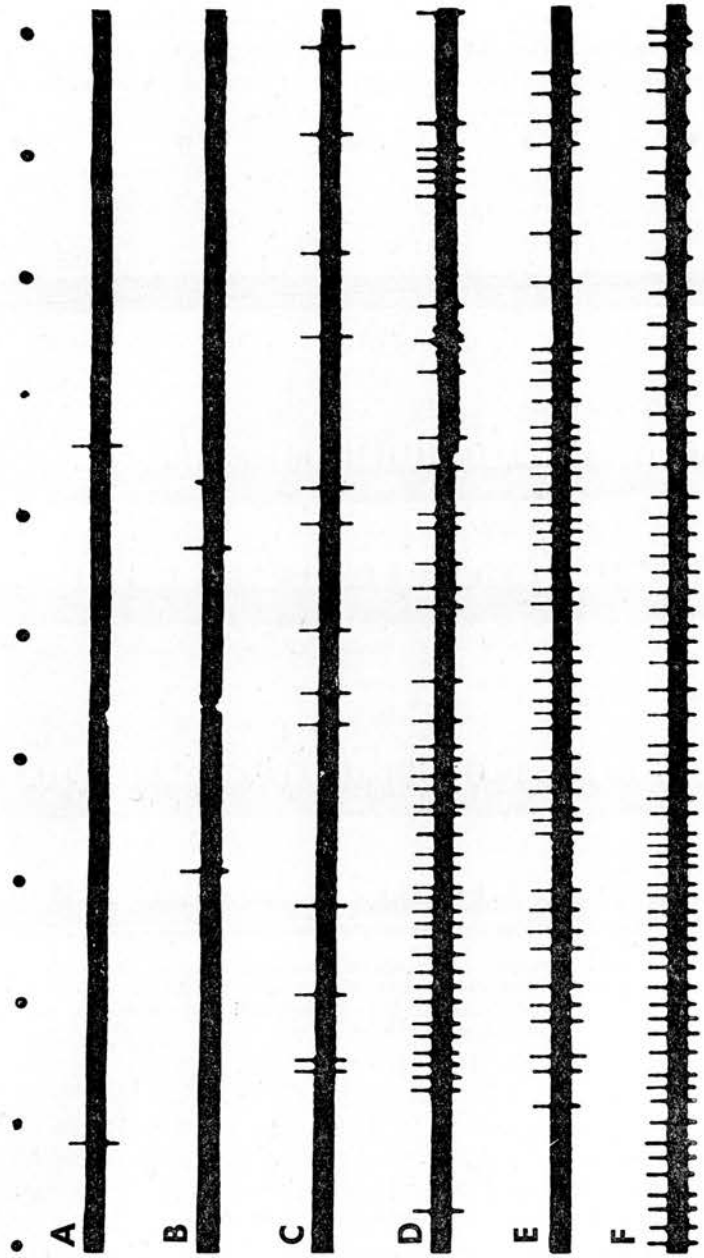
Gastric tension receptors, which adapt slowly to light gastric distension, gastric contractions and digital compression of the gastric wall [Iggo, 1955, 1957 *a*], were also excited when the mucosa was stroked with a smooth rod (fig. 1*c*). The response was not so localized as with the mucosal nerve endings and the discharge was easily prolonged by steady, light pressure on the mucosa. These differences in the response to mechanical stimulation made it possible to distinguish the centripetal fibres of the mucosal endings from those of tension receptors.

The Action of Chemicals.—When it became clear that there were vagal afferent units with endings in the gastric mucosa an attempt was made to see if they were sensitive to chemicals in solution. The following fluids, when tried, all failed to excite the afferent units; distilled water, Ringer-Locke solution, 0·15 M-NaCl solution, 0·6M-NaCl solution, peptone solution, oleic acid, ethanol (30–70 per cent), glucose or sucrose solution, bile from the gall bladder, culinary mustard suspension (30 per cent w/v). The 4 units tested were insensitive to phenyl diguanide (40–80 μg/kg. intravenously). The mucosal endings were, however, excited by solutions of either high or low H-ion concentration and could be divided into 2 classes on this basis.

Acid Sensitive Receptors.—(a) Exposed mucosa. Five units out of 14 units tested were excited by acid solutions. The threshold was about pH 3 for the most excitable units (Table I), but at this pH the discharge was erratic and lasted only a short time. The discharge was persistent and the peak frequency was higher when more acidic test solutions were used (fig. 2). In 2 instances, repeated application of strong solutions (0·1 N or 0·2 N-HCl) damaged the mucosa after several hours and destroyed the receptors. No precise measurement of the frequency of discharge at different acidities was made.

(b) Intact stomach. The receptors described in (a) were found by mechanical stimulation of the mucosa. This method of detection may have altered the reaction of the endings to chemical stimuli and so 4 experiments were done to see if acid-sensitive units could be found by perfusing the intact stomach with acidic solutions. Only 1 unit (Fibre 62) was isolated in this way. This low yield of only 1 unit in 4 experiments is attributed to technical difficulties rather than to the sparsity of receptors. The unit was not excited by Ringer–Locke solution or by 0·03 N-HCl (pH 1·5). It was excited by 0·1 N-HCl and the discharge was more prolonged, but the peak frequency was only a little higher with 0·2 N-HCl (fig. 3). This unit, therefore, had a higher threshold than the units described in (a), but whether this difference was due to the isolation procedure is not known.

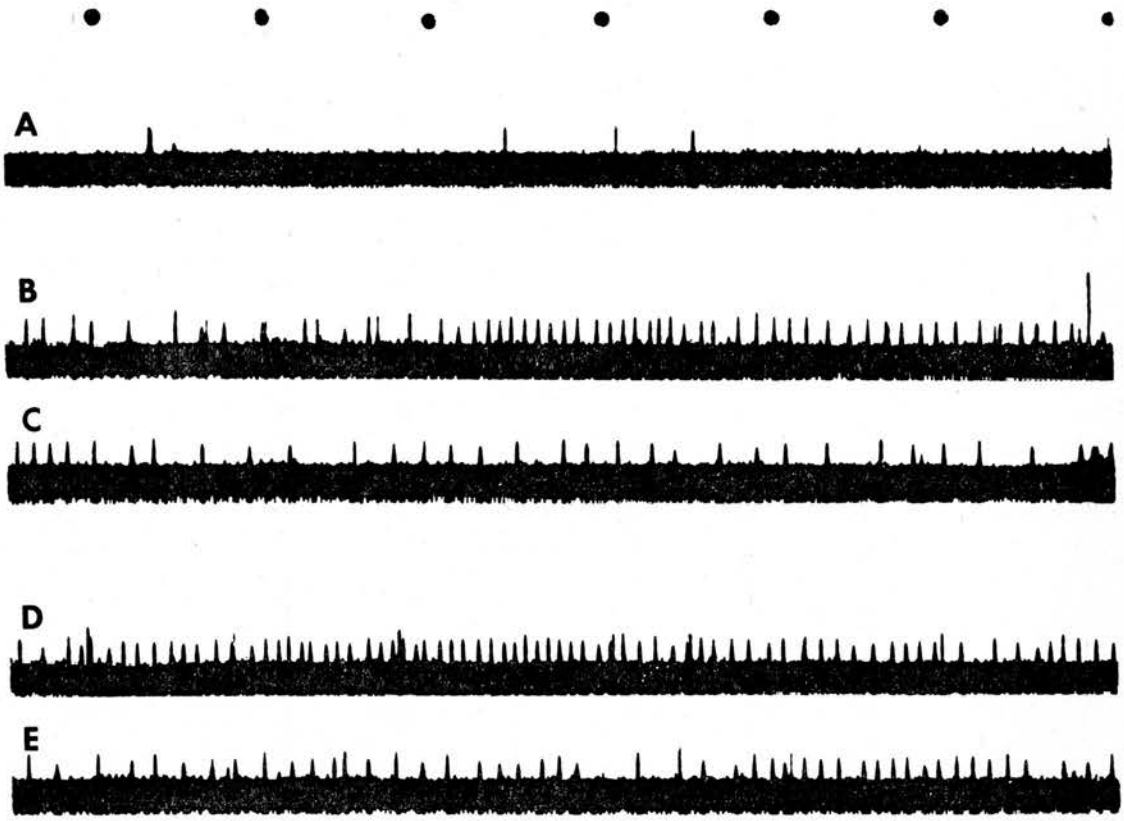
Alkali Sensitive Receptors.—Exposed mucosa. Nine of the 13 units tested with both acid and alkaline solutions gave a sustained discharge of impulses when 0·1 N-NaOH was put on the exposed mucosa (fig. 4*b* and Table I). The peak frequency of the discharge was 30–50 impulses/sec. When a few drops of solution were used the discharge was brief, but when several ml. were used it persisted for as long as the solution remained on the mucosa. Repeated applications of 0·1 N-NaOH were made, on occasion



FIGS. 2*a*, *b*, *c*, *d*, *e* and *f*.—The response of an acid-sensitive mucosal unit to various fluids on the exposed mucosa, Fibre 52. (*a*) Distilled water; (*b*) buffer solution at pH 5; (*c*) at pH 3; (*d*) at pH 2; (*e*) and (*f*) are continuous, 0·1 N-HCl.
Time marks, 1 sec.

for 3 hr., and the receptors continued to respond in a consistent way although the peak frequency of the discharge varied. This treatment did not impair the response of the receptors to less alkaline solutions or to mechanical stimulation.

Attempts to establish the threshold of the receptors to alkaline solutions were unsatisfactory because of random fluctuations in sensitivity. The

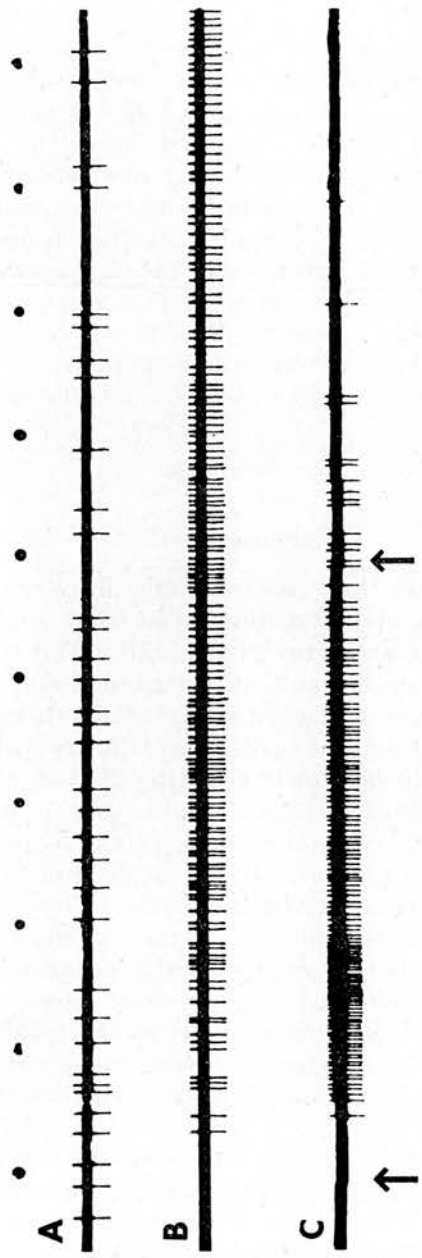


FIGS. 3*a*, *b*, *c*, *d* and *e*.—The response of an acid-sensitive receptor in the intact stomach to solutions put into the stomach through the cardiac sphincter, Fibre 62. (*a*) 0·03 N-HCl, pH 1·5; (*b*) 0·1 N-HCl; (*c*) the same unit 30 sec. later with the acid still in the stomach; (*d*) 0·2 N-HCl; and (*e*) 30 sec. later as in (*c*).

Time marks, 1 sec.

results are given in Table I and, as is shown, the most excitable units were excited at pH 8. At this level the discharge was unpredictable but, when present, was of low frequency and short duration. The frequency and persistence of the discharge was greater with more alkaline solutions. A layer of mucous which prevented alkalis from exciting 1 receptor did not block mechanical stimulation.

Specificity of Reaction.—The 2 classes of receptor described were quite distinct and, as is illustrated in fig. 4*c*, the discharge in an alkali-sensitive



FIGS. 4*a*, *b* and *c*.—The response of an alkali-sensitive mucosal unit to fluids on the exposed mucosa, Fibre 49. (*a*) Borate buffer, pH 9·3; (*b*) 0·1 N-NaOH; (*c*) 0·1 N-NaOH was put on the mucosa at the first arrow and while the unit was still active 0·1 N-HCl was added at the second arrow. This abolished the activity.

Time marks, 1 sec.

unit could be abolished promptly when acid was put on the mucosa; acid alone did not excite the receptor. The converse held for the acid-sensitive receptors. These results emphasize the need to isolate single units in an analysis of this kind.

Gastric "Stretch" Receptor.—The existence of volume receptors in the gastric wall, described by Paintal [1954 *a*], has been questioned [Iggo, 1955, 1957 *a*]. Some gastric mucosal endings gave a slowly adapting discharge of impulses when the stomach was over-distended (Table I). Apart from these endings which could be mistaken for "stretch" receptors, 1 solitary example of a low-threshold "stretch receptor was found. The unit was excited by distension, stretch and digital compression of the stomach but was not excited by gastric contractions nor by acid or alkaline solutions. It was, therefore, quite distinct from the gastric tension receptors which were all excited by gastric contractions. Careful dissection showed that the receptor was on the greater curvature of the stomach, probably in the serosa or omentum. The unit was not excited by massive intravenous doses of phenyl diguanide (150 mg./kg.).

Discussion

The present work shows that vagal afferent fibres with endings in the gastric mucosa can be very clearly distinguished from similar afferent fibres with endings in the outer layers of the gastric wall. The distinction is based on the response of the receptors to both mechanical and chemical stimuli. The mucosal receptors were not excited by moderate gastric distension (< 20 mm. Hg), gastric contractions or digital compression of the gastric wall, all of which evoked a persistent slowly adapting discharge of impulses from tension receptors [Iggo, 1955, 1957 *a*]. Some of the mucosal receptors yielded a slowly adapting train of impulses to excessive gastric distension or to a powerful stretch of the mucosa. In the absence of further information about the location of the endings or their reaction to chemicals these results could lead to the mistaken conclusion that the gastric wall contains large numbers of "stretch" receptors, *i.e.*, receptors which could function unambiguously as volume-signalling devices. Low-threshold gastric stretch receptors do exist but they are much less numerous than the tension receptors and mucosal receptors; the solitary example found in the current series of 51 single units was in the serosa or omentum on the greater curvature of the stomach.

Receptors of each of the above classes were excited when the gastric mucosa was stroked firmly with a smooth glass rod, but whereas the mucosal units responded with a brief burst of 1–10 impulses the tension and stretch receptors responded with a burst of impulses which could easily be prolonged by keeping up a light pressure on the mucosa. This reaction of receptors in the deeper layers of the gastric wall to stimulation of the mucosa shows that caution should be exercised in attributing reflex responses elicited by mucosal

stimulation to the exclusive excitation of mucosal nerve endings. Receptors in the deeper layers of the stomach appear to be at least as sensitive as mucosal endings to certain forms of mechanical stimulation.

Another striking difference between the mucosal receptors and those in the deeper layers of the gastric wall was in the response of receptors to chemicals on the mucosa. The mucosal receptors displayed a characteristic sensitivity to the pH of solutions in contact with the mucosa whereas the tension receptors were unaffected unless gastric movements were aroused. This sensitivity of the mucosal receptors to the pH of fluids on the mucosa is, perhaps, the most interesting finding in the present investigation. The response is not an injury discharge. Repeated stimulation for several hours with 0·1 N-NaOH or 0·1 N-HCl gave consistent results and did not interfere with the response of the endings to weaker solutions. The reaction was a property of the nerve endings; when the mucosal surface was scraped off, a previously effective stimulus no longer caused a discharge of impulses even though electrical stimulation was effective. It is clear that the solutions were not exciting the axons directly.

The response to acids or alkalis was specific. This is well shown in fig. 4 where the discharge in an alkali-sensitive unit evoked by 0·1 N-NaOH disappeared when 0·1 N-HCl was added. Activity in an acid-sensitive unit could be abolished by alkali. If the discharge was caused by injury then both the acid and the alkali might be expected to arouse a similar response from all the vagal endings in the mucosa.

Acid receptors are well known in the sense of taste but there is no agreement on the specificity of response. The single unit studies of Pfaffmcn [1941, 1955] show that some acid receptors can be excited by non-acidic solutions whereas Andersson and Zotterman [1950] found fibres in the frog which were excited by acid but not by water and hypertonic salines on the tongue. The gastric acid receptors isolated as single unit preparations in the present work were specifically sensitive to acid; they were not excited by hypertonic or hypotonic solutions or by Ringer-Locke solution. This specificity suggests that they are concerned with signalling the pH of the gastric contents.

Gastric motility is enhanced by low gastric acidity in sheep [Titchen, 1953]. The gastric acid receptors described in this paper may initiate these reflex responses. There is convincing evidence that acid in the duodenum reflexly depresses gastric motility and gastric acid secretion via vagal paths [Thomas, Crider and Mogan, 1934; Pincus, Friedman, Thomas and Rehfuss, 1944; Code and Watkinson, 1955; Sircus, personal communication]. The threshold for all these reflex responses was pH 2·5 or less; this is very similar to the threshold for the gastric mucosal acid receptors. If there are similar acid receptors in the duodenal mucosa they may be the afferent limb of the reflexes from the duodenum. It is concluded that the acid-sensitive mucosal endings are slowly-adapting chemoreceptors and, as suggested in the preliminary report [Iggo, 1957 *b*], that they function as pH receptors.

The function of the alkali-sensitive mucosal receptors is unknown and,

indeed, they are unlikely ever to be excited by alkali in the stomach since the gastric pH rarely rises above 7 [James, 1957].

The histological evidence for afferent nerve endings in the mucosa of the glandular stomach has recently been strengthened by Schofield [personal communication] who found vagal fibres which passed through the enteric plexuses and ramified in the mucosa to send branches to end close to the gastric mucosal surface in the rat. The present results on the location of the mucosal receptors are complementary for they show that the receptors can be destroyed by scraping off the outer layers of the mucosa. Moreover, with some individual afferent units, if the centre of the sensitive area was removed down to the muscularis mucosa, the sensitivity of the adjacent mucosa did not change.

Through the kindness of Dr. A. S. Paintal, I have read the manuscript of a paper in which he describes intestinal receptors which he believes to lie in the mucosa. Paintal was unable to alter the activity of one of these endings when he put "dilute acid or a strong solution of soda-lime" on the mucosa. This fact, together with other differences, makes it unlikely that these intestinal receptors are the same as the mucosal chemoreceptors described in this paper.

Several types of receptor in the gastric and intestinal wall have now been identified by recording from single afferent fibres dissected from the cervical vagus. One significant feature of the various investigations is that each class of receptor was found by the use of a particular stimulus so that there was an arbitrary, though sometimes intentional, selection of the units examined. The stimuli used to make the initial isolation of the afferent units were: (*a*) phenyl diguanide and distension [Paintal, 1954 *a*, *b*]; (*b*) mild distension [Iggo, 1955, 1957 *a*]; (*c*) rubbing the mucosa combined with insensitivity to distension [this paper]; (*d*) irrigation of the stomach with solutions of different pH [this paper]; and (*e*) phenyl diguanide coupled with insensitivity to distension [Paintal, 1957]. The receptors isolated by these means have had some properties in common but there have also been clear-cut differences so that 4 types of behaviour can be recognized, although definite histological structures cannot be assigned to them. They are: (1) tension receptors in series with the contractile elements in the outer muscular layers of the stomach and intestines [Iggo, 1955, 1957 *a*]; (2) distension-sensitive receptors in the outer layers of the gastric wall which are not excited by gastric contractions or by intravenous phenyl diguanide [this paper]; (3) chemoreceptors in the gastric mucosa [this paper]; and (4) distension-insensitive intestinal receptors [Paintal, 1957] believed to be in the mucosa.

REFERENCES

ANDERSSON, B. and ZOTTERMAN, Y. (1950). "The water taste in the frog", *Acta physiol. scand.* **20,** 95–100.

CARLSON, A. J. and BRAAFLADT, L. H. (1915). "Contributions to the physiology of the stomach. XVIII. On the sensibility of the gastric mucosa", *Amer. J. Physiol.* **36,** 153–170.

CODE, C. F. and WATKINSON, G. (1955). "Importance of vagal innervation in the regulatory effect of acid in the duodenum on gastric acid secretion", *J. Physiol.* **130,** 233–252.

HABEL, R. E. (1956). "A study of the innervation of the ruminant stomach", *Cornell Vet.* **46,** 555–628.

IGGO, A. (1955). "Tension receptors in the stomach and the urinary bladder", *J. Physiol.* **128,** 593–607.

IGGO, A. (1957 *a*). "Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat", *Quart. J. exp. Physiol.* **42,** 130–143.

IGGO, A. (1957 *b*). "Gastric pH receptors with slowly conducting afferent fibres", *J. Physiol.* **137,** 17–18 *P*.

IGGO. A, (1957 *c*). "Conduction velocity in vagal afferent fibres", *J. Physiol.* [In the press.]

JAMES, A. H. (1957). The Physiology of Gastric Digestion. London: Edward Arnold.

MCILVAINE, T. C. (1921). "A buffer solution for colorimetric comparison", *J. biol. Chem.* **49,** 183–186.

PAINTAL, A. S. (1954 *a*). "A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst", *J. Physiol.* **126,** 255–270.

PAINTAL, A. S. (1954 *b*). "The response of gastric stretch receptors and certain other abdominal and thoracic receptors to some drugs", *J. Physiol.* **126,** 271–285.

PAINTAL, A. S. (1957). "Responses from mucosal mechanoreceptors in the small intestine of the cat", *J. Physiol.* [In the press.]

PFAFFMAN, C. (1941). "Gustatory afferent impulses", *J. cell. comp. Physiol.* **17,** 243–258.

PFAFFMAN, C. (1955). "Gustatory nerve impulses in rat, cat and rabbit", *J. Neurophysiol.* **28,** 429–440.

PINCUS, I. J., FRIEDMAN, N. H. F., THOMAS, J. E. and REHFUSS, M. E. (1944). "A quantitative study of the inhibitory effect of acid in the intestines on gastric secretion", *Amer. J. dig. Dis.* **11,** 205–208.

THOMAS, J. E., CRIDER, J. O. and MOGAN, C. J. (1934). "A study of reflexes involving the pyloric sphincter and antrum and their role in gastric evacuation", *Amer. J. Physiol.* **108,** 683–700.

TITCHEN, D. A. (1953). "Reflex contractions of the reticulum", *J. Physiol.* **122,** 32 *P*.

WOLF, S. and WOLFF, H. G. (1947). Human Gastric Function. London: Oxford University Press.

[*Reprinted from the Journal of Physiology*,
1958, Vol. **142**, No. 1, p. 110.]
PRINTED IN GREAT BRITAIN

J. Physiol. (1958) 142, 110–126

THE ELECTROPHYSIOLOGICAL IDENTIFICATION OF SINGLE NERVE FIBRES, WITH PARTICULAR REFERENCE TO THE SLOWEST-CONDUCTING VAGAL AFFERENT FIBRES IN THE CAT

BY A. IGGO

From the Department of Physiology, University of Edinburgh

(*Received* 17 *November* 1957)

Although there are many unmyelinated afferent axons in the peripheral nerves of mammals there is very little known either about their behaviour or about the receptors with which they are connected. Myelinated fibres, on the other hand, have frequently been studied as single units. This situation is due to the difficulty of isolating the unmyelinated fibres as single units for electrophysiological recording. Activity in unmyelinated afferent fibres has been recorded in multifibre preparations, notably by Zotterman (1939) and Maruhashi, Mizuguchi & Tasaki (1952). Only occasionally did these authors succeed in analysing activity in individual fibres. Douglas & Ritchie (1957*a*, *b*) have recently succeeded in detecting C fibre activity in whole nerves.

Because of this lack of information about individual unmyelinated fibres it was decided to follow up the observation (Iggo, 1956*b*) that afferent fibres with conduction velocities as low as 1·3 m/sec could be isolated as single units from the cervical vagus of the goat. In addition to measurements of conduction velocity other tests were used in an attempt to decide whether the fibres were unmyelinated. New methods for identifying single fibres in a multifibre strand are described. With the aid of these methods and by refinements of the usual dissection methods very slowly conducting fibres have now been isolated in the cat. Evidence is presented for the conclusion that most of these fibres were probably unmyelinated. Preliminary reports have already appeared (Iggo, 1956*b*, 1957*c*). The gastric and intestinal receptors for which these figures were the centripetal axons are described elsewhere (Iggo, 1957*a*, *b*).

METHODS

The methods used to prepare the cats, to dissect single fibres from the cervical vagus and for electrical recording have been fully described in previous papers (Iggo, 1955, 1957*a*). The general arrangement of the stimulating and recording electrodes is shown in Fig. 1. All the stimuli, S_1,

S_2 and S_3, were locked to the time base of the oscillograph and could be pre-set in any desired position. The electronic circuits which provided 'locked' stimuli and 'expanded' time-base facilities have been described by Dickinson (1950) and Paintal (1953).

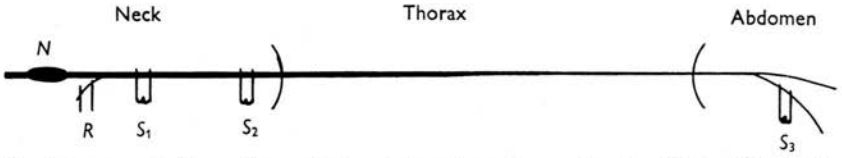


Fig. 1. Arrangement of recording and stimulating electrodes used to identify individual afferent fibres in multifibre strands and to measure conduction velocities in different parts of the vagus. *R*, recording electrodes under a fine strand dissected from the upper cervical vagus; S_1, S_2 and S_3 positions of the stimulating electrodes; *N*, nodose ganglion.

RESULTS

Identification of individual fibres in a multifibre strand

Attempts to measure the conduction velocity in selected axons have previously been hindered by the need to isolate single live fibres. Single fibres have now been identified and examined in strands containing several live axons. Two methods which have made this possible are the 'collision' technique and the 'peripheral stimulus' technique.

The 'collision' technique

With this technique it was possible to identify in a compound action potential, generated by as many as fifteen fibres, the contribution of a single fibre which could be activated independently of the rest of the fibres. The conduction velocity in the single fibre could then be assessed in the manner to be described.

Since two impulses travelling towards each other in an axon are extinguished when they collide, it is possible to block an impulse coming up from the receptor (orthodromic impulse) by sending an impulse down the fibre (antidromic impulse) at the right time (Fig. 2).

Orthodromic impulses. To recognize that an impulse had been lost in this way it was necessary to have a well defined pattern of activity. Two sources of orthodromic impulses were used: (*a*) excitation of the receptor by a natural stimulus to give a regular series of impulses in the afferent fibre (Fig. 3); and (*b*) electrical stimulation of the nerve near the receptor (Fig. 4*A*). This method was used when there was only a brief irregular burst of impulses in response to a natural stimulus or when the conduction velocities in the proximal and distal parts of the axon were to be compared.

Antidromic impulses. The cervical vagus was stimulated electrically (S_2 in Fig. 1), at various intensities, some distance below the recording electrodes (*R* in Fig. 1). In this way a controlled source of antidromic impulses in some or all of the fibres in the strand dissected from the vagus was available. The

impulses which travelled up the vagus from the stimulating electrodes were recorded as a compound action potential and used to indicate which of the fibres in the strand were excited by the shock.

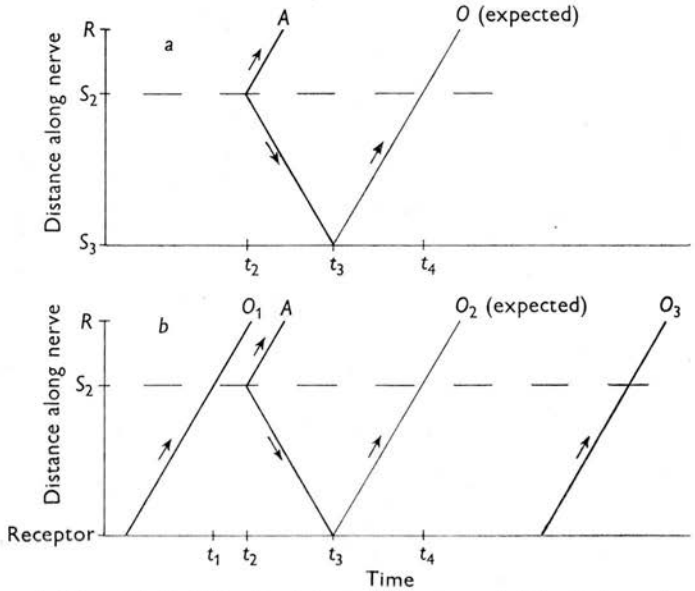


Fig. 2. The principle of the 'collision' technique. In both *a* and *b* the distance along the nerve is shown on the ordinate, with the same abbreviations as are used in Fig. 1, and the time is shown on the abscissa; arrows show the direction in which impulses travel.

a: Both the antidromic (*A*) and orthodromic (*O*) impulses are initiated by electrical stimuli. When the nerve is stimulated through S_2 at time t_2 the antidromic impulse reaches the lower electrodes just as the orthodromic impulse initiated there begins to ascend. The impulses collide and the orthodromic impulse is blocked. Any antidromic impulse initiated later than t_2 will collide in this way.

b: The antidromic impulse is initiated electrically and the orthodromic impulses (O_1, O_2 and O_3) are part of a regular series of impulses coming from the receptor. Any antidromic impulse initiated between t_2 and t_4 will collide with and block O_2. Antidromic impulses initiated between t_1 and t_2 will reach the receptor before O_2 starts, leading to a delay in the initiation of O_2 and a 're-setting' of the natural rhythm.

The principle of the 'collision' method is illustrated in Fig. 2 and an example is shown in Fig. 3. Collision can occur only if the antidromic impulse does not reach the lower stimulating electrodes (S_3) in Fig. 2*a*, or the nerve ending in Fig. 2*b*, before the orthodromic impulse has started to ascend. If there is to be a collision the longest time by which the shock through S_2 can precede the expected arrival of the orthodromic impulse at S_2 is given by (t_4-t_2) in Fig. 2*a*. This interval is twice the time taken for an impulse to travel from S_2 to S_3 in Fig. 2*a*.

i.e. $$(t_4-t_2)=\frac{2d}{v} \text{ sec},$$

where d is the distance from S_2 to S_3 and v is the conduction velocity in the fibre.

The lowest frequency of orthodromic impulses at which a random antidromic impulse will always collide with an orthodromic impulse is given, so long as an appropriate adjustment is made for the effect of the refractory period, by the equation

$$f=\frac{1}{(t_4-t_2)}=\frac{v}{2d}\ \text{sec}^{-1}.$$

This relation has been used by Douglas & Ritchie (1957*a*) to make an estimate of the frequency of firing in individual fibres in whole nerve trunks.

Antidromic impulses which start earlier than t_2 in Fig. 2 will have effects which depend on the origin of the orthodromic impulses. If they are initiated by electrical stimulation of the nerve trunk (S_3) then the early antidromic impulses will not interfere with the passage of orthodromic impulses except for a slight effect on the conduction time and an effect on the threshold for electrical stimulation (S_3) by those impulses which pass S_3 just before t_3, i.e. during their relative refractory period. The situation is quite different if the orthodromic impulses come from the receptor and form a regular series (Fig. 3). Those antidromic impulses which are too early to collide will cause a 're-setting' of the rhythm. Matthews (1931) has previously described this effect. The rhythm is 're-set' by a single antidromic impulse in the sense that the next orthodromic impulse after the antidromic impulse is delayed beyond the time it was expected. This delay arises because the antidromic impulse has interfered with events at the receptor. The antidromic impulse takes d/v sec to travel from S_2 to the receptor and has an effect on the rhythm identical with that of an hypothetical orthodromic impulse which left the receptor when the antidromic impulse arrived. The hypothetical impulse would take d/v sec to reach S_2. The next actual orthodromic impulse at S_2 would be expected at the normal interval after this hypothetical impulse. Thus the delay caused by interference at the receptor would be $(t_2-t_1)+2d/v$ sec, where t_1 is the time at which the preceding orthodromic impulse reached S_2, t_2 is the time at which the antidromic impulse is initiated, d is the distance from the receptor to S_2 and v is the conduction velocity in the fibre.

Both collision and interference at the receptor will cause a gap in the regular series of orthodromic impulses. The gap caused by collision is due to the failure of an orthodromic impulse to reach the recording electrodes and always equals twice the interval between impulses, i.e. $2/f$ sec (Fig. 3*B*). It is therefore easy to recognize. The gap caused by interference at the receptor equals the 're-setting' delay plus the normal impulse interval; it is $(t_2-t_1)+2d/v+1/f$. This gap may, with very early antidromic impulses, and especially if $1/f \gg 2d/v$, be difficult to distinguish from normal fluctuations in impulse interval. Because of this it is preferable to send in the antidromic impulses at a time which will

ensure that collision is possible (see (3) below). 'Re-setting' was not noticed with the gastro-intestinal fibres, which were slowly conducting and for which $f > v/2d$. Both collision and 're-setting' were recorded with a pulmonary stretch fibre, $v = 36$ m/sec, where $f < v/2d$ at frequencies about 40/sec.

The following procedure was found to be the most convenient way of using the collision technique:

(1) A single active unit in a strand of the cervical vagus was isolated without attempting to carry the dissection to the point of having only a single live fibre left.

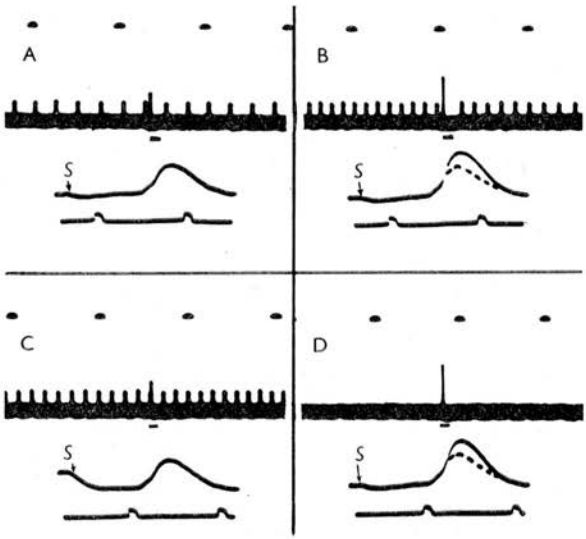


Fig. 3. Identification of an active afferent fibre in a multifibre strand by the collision of antidromic and orthodromic impulses. In each record the upper time marks, at 0·1 sec intervals, refer to the upper tracing which shows the activity in a pulmonary stretch fibre recorded with a stationary cathode-ray beam; the lower time marks, at 1 msec intervals, refer to the lower tracing which shows the compound action potential set up by a stimulus through S_2, recorded with a moving spot. The lower tracing corresponds to that part of the upper tracing which is underlined.

A, weak stimulus causes no change in stretch fibre activity but provokes a compound action potential, showing the existence of viable but quiescent fibres in the strand. *B*, *C* and *D*, the stimulus intensity was raised above that in *A* and kept at this new value in each record. *B*, the cervical shock, S_2, is followed by a gap in the train of impulses. *C*, when a natural impulse travelled past the electrodes S_2 about 1 msec before the shock S_2 was sent in, the compound action potential was identical with that in *A* and there was no gap in the train of impulses; i.e. the centripetal axon from the pulmonary stretch fibre was refractory. *D*, the full compound action potential recorded when the nerve was silent, and used for conduction velocity measurements. This ensured that there was no chance for slowing in the relative refractory period to give spuriously low conduction velocities.

The area of the potential above the dotted line in 3 *B* and *D* is contributed by a single fibre, the fibre carrying impulses from the pulmonary stretch receptor.

The stimulus artifact is marked, *S*, in this and in subsequent figures.

(2) If the orthodromic impulses were initiated by S_3 the time base was triggered manually and the velocity of the time base was adjusted until the orthodromic impulse was at the right-hand side of the screen of the cathode-ray tube (Fig. 4). If a natural series of orthodromic impulses were used the time base was triggered by an orthodromic impulse and the velocity of the time base adjusted until the next orthodromic impulse was at the right-hand side of the screen.

(3) The shock, S_2, was sent in soon after the start of the sweep and the stimulus intensity was found which just caused the orthodromic impulse to disappear. With fast fibres it was necessary to move S_2 close to the second orthodromic impulse to obtain collision, i.e. for fibres in which $f \ll v/2d$,

(4) The compound action potential was examined at the critical S_2 size. When a correspondence was observed between the absence of the orthodromic impulse and the presence of a fixed increment on the compound action potential (and vice versa), then this increment was taken as being generated in the fibre from the active receptor (Fig. 3).

(5) With most fibres, records were taken of the effect of sending antidromic impulses in at random after the fibre had been identified (Fig. 3).

The 'collision' method proved to be relatively easy to use. It did not require the accurate prediction of the time at which orthodromic impulses would be expected to pass the S_2 electrodes. The two events to be watched took place at different parts of the screen, so that confusion was avoided. Paintal (1953) has used the refractory period of orthodromic impulses to identify which fibre in a multifibre strand came from a particular receptor. Paintal's method cannot be used satisfactorily with strand containing more than three or four fibres. It has been used in Fig. 3*C* to test the collision technique. In this record the antidromic stimulus (S_2) happened to fall just after an orthodromic impulse has passed the stimulating electrodes. As a result the axon was in the relative refractory state and was not excited by the shock. The increment on the compound action potential above the dotted line in Fig. 3*D* was absent in Fig. 3*C*; moreover, there was no gap in the regular series of impulses in Fig. 3*C*. It was apparent that this increment on the compound action potential arose from an impulse in the centripetal fibre from the active receptor, a conclusion already reached from Fig. 3*A*, *B*.

The 'collision' technique is of particular value where difficulty is found in isolating single live fibres, that is, small fibres, because it reduces the amount of dissection needed. With very fast fibres, especially when $f \ll v/2d$, it is less useful; these are the circumstances in which it is relatively easy to isolate single live fibres.

The 'peripheral stimulus' technique

A single unit was isolated in a strand containing several live fibres and the stimulating electrodes (S_3 in Fig. 1) were placed as close as possible to the receptor. A shock through S_3 usually excited only one of the fibres in the strand on the recording electrodes. The method is illustrated in Fig. 4 with a gastric mucosal fibre. In this experiment the strand contained two live fibres as shown in Fig. 4*D*; a shock through S_2 on the cervical vagus initiated the impulses marked *X* and *Y*. However, only one of these fibres was excited by a shock through S_3. The impulse initiated by S_3 reached the recording electrodes

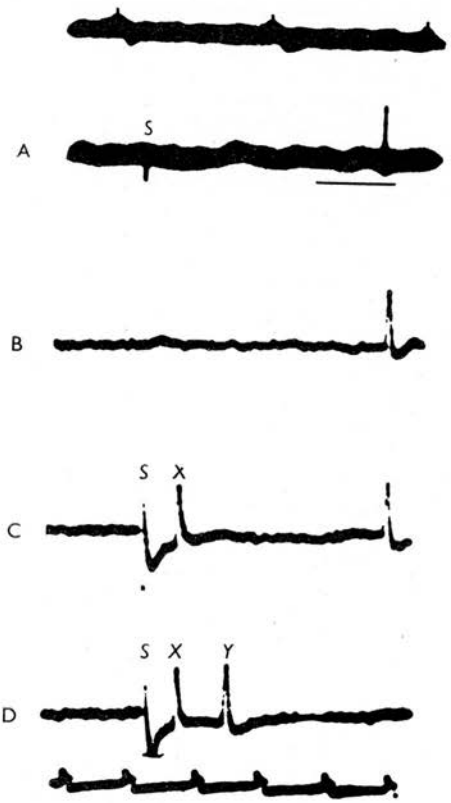


Fig. 4. The identification, in a strand dissected from the cervical vagus, of the centripetal fibre from a gastric mucosal receptor. Fibre no. 46. Stimulating electrodes S_2 and S_3 (Fig. 1) were used. The tracing *A* was recorded with a slow sweep velocity and shows the S_3 artifact and the impulse initiated by the shock. Tracings *B*, *C* and *D* correspond to the underlined part of tracing *A*; in these records the sweep velocity was higher. Tracings *C* and *D* show also the S_2 artifact and impulses initiated by this shock, which was stronger in *D* than in *C*.

The conduction velocity of the fibre *Y* was 5 m/sec in the cervical vagus and 1·7 m/sec in the abdomen and thorax. The upper time marks, 0·1 sec intervals, refer to *A* and the lower time marks, 10 msec intervals, refer to *B*, *C* and *D*.

when the faster-conducting fibre, X, was excited at S_2 (Fig. 4C) but failed to do so when the slower-conducting fibre, Y, was also excited at S_2 (Fig. 4D). This failure of the impulse initiated at S_3 to reach the recording electrodes in the latter instance was due to its collision with an antidromic impulse, initiated at S_2, in the fibre Y. The probable explanation for this result is that in a random sample of a few fibres from the cervical vagus (say 10 from more than 30,000), such as are taken when a fine strand is dissected, there is likely to be only one which enters the vagus in a fine peripheral branch in the abdomen.

The identity of the fibre could be checked by comparing the size and shape of the action potentials initiated by electrical stimulation of the peripheral vagus and by normal excitation of the receptor.

Tests of the single unit response

The most convincing evidence that the electrical activity, which formed the basis of the conduction velocity measurements, came from single nerve fibres is that the response to electrical stimulation was all-or-none and that if an electrical shock fell in the absolute or early relative refractory period of a natural impulse the spike disappeared as a unit leaving the rest of any C wave in the compound action potential unchanged. At no time was any fractionation of the action potential spike seen, such as would be expected if the activity arose in several fibres.

Accuracy of the conduction velocity measurements

The method of Blair & Erlanger (1933), as developed by Paintal (1953), was used to measure the conduction velocity in single fibres. The velocity was calculated from the time taken by an impulse to travel the known distance from the stimulating cathode (S_2 in Fig. 1) to the recording electrodes (R in Fig. 1, see also Fig. 4). The use of a single conduction distance may introduce errors due to the variable shock-response delay (Blair & Erlanger, 1933)—this can be minimized by using shocks well above threshold—and to the impulse not arising precisely at the cathode (Rushton, 1949). To estimate the size of the cumulative error the conduction velocity in single axons was measured from two positions of the stimulating electrodes on the cervical vagus (S_1 and S_2 in Fig. 1). The time taken for an impulse to travel from S_2 to R was longer than the time taken to travel from S_1 to R by the conduction time between S_2 and S_1. The difference was used to compute a third value for the conduction velocity. The three values are compared in Table 1 for ten fibres of widely differing conduction velocities. In a uniform fibre the most accurate estimate of velocity is probably that derived from the conduction time between the stimulating electrodes (S_1 and S_2). On this assumption the use of a single conduction distance led to a consistent over-estimate of the conduction velocity. The error

was greatest when the conduction distances were short. The conduction velocities were so much higher from the proximal than from the distal electrodes for three fibres (45, 50 and 55) that a real difference in conduction velocity seems to be the most reasonable explanation. Evidence is presented later to show that this does occur with some fibres. This change in conduction velocity could be held to account for the isolation of single slowly-conducting fibres. Thus the fibres could be myelinated at the recording electrodes, lose their myelin sheaths distal to the electrodes but still have conduction velocities in the C range (see section on Type of Fibre, p. 120). However, for each of the fibres 47, 51 and 52 the conduction velocities derived from the three measurements agree closely.

TABLE 1. A comparison of three estimates of the conduction velocity in ten single vagal fibres. The velocities were computed from $v = d/t$; in columns I and II, d is the distance from S to R, and t is the time interval between the stimulus artifact and the arrival of the impulse at R; in column III, d is the distance between S_1 and S_2, and t is the difference between the conduction times in columns I and II. The arrangement of the electrodes is shown in Fig. 1

		Conduction velocity (m/sec)		
Fibre no.	Receptor	I, short distance $S_1 - R$ (19–25 mm)	II, long distance $S_2 - R$ (47–55 mm)	III, by difference
44	Pulmonary inflation	34	33·5	32·4
43	Pulmonary inflation	19·6	19·2	18·5
54	Pulmonary inflation	17·6	16·9	16·5
53	Gastric mucosal	2·8	2·6	2·4
50	Unidentified	2·0	1·5	1·2
55	Gastric mucosal	1·8	1·5	1·3
45	Oesophageal tension	1·4	1·1	1·0
47	Gastric tension	1·3	1·2	1·1
52	Gastric mucosal	1·2	1·1	1·0
51	Gastric tension	1·1	1·05	1·0

Hence, it may be concluded that for these fibres there was no abrupt change in conduction velocity, i.e. they were uniform along their length from S_2 to R. Because of their low conduction velocities these fibres were almost certainly unmyelinated. Thus it follows that they were unmyelinated *at the recording electrodes*. Fibre 50, on the other hand, was so much faster from S_1 to R than from S_2 to S_1 that the possibility that it was myelinated at R must be considered, even though the velocity between S_1 and S_2 was only 1·2 m/sec.

A further source of error is slowing in the relative refractory period. This could occur if the shock to the vagus followed immediately after an impulse had passed the stimulating electrodes and could be avoided quite simply by not using such records for the conduction velocity measurements. In this paper only big differences of conduction velocity are considered relevant; errors of 10%, such as might arise when single conduction distances are used, do not invalidate any of the conclusions.

The conduction velocity in different groups of vagal fibres

The fibres, which are grouped according to the type of receptor, were dissected from the right cervical vagi of twenty-five cats.

Respiratory and cardiovascular afferent fibres. Several fibres from respiratory and cardiovascular receptors were isolated in order to test the methods used to investigate gastric and intestinal afferent fibres. The conduction velocities fell within those reported by Paintal (1953), that is, pulmonary inflation receptors (slowly adapting) 19–50 m/sec, atrial receptors 10 and 15 m/sec, systemic baroceptor 13 m/sec.

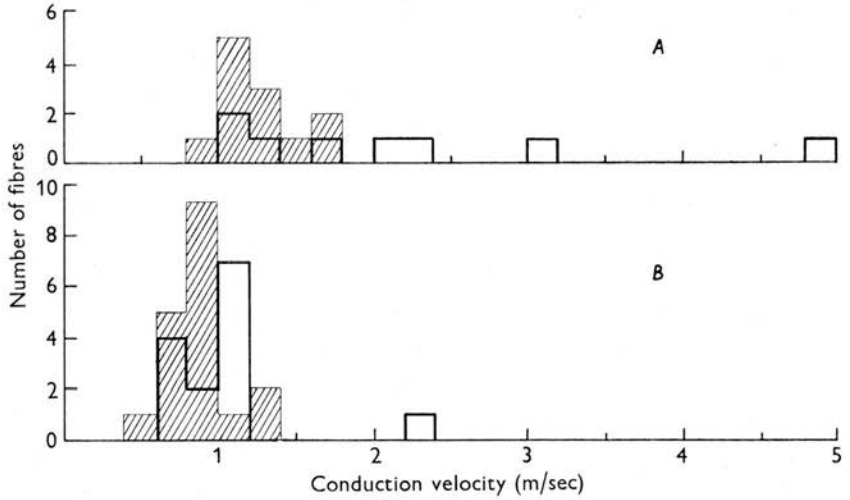


Fig. 5. Conduction velocities in single fibres dissected from the cervical vagus of the cat. *A*, centripetal fibres from gastric mucosal chemoreceptors; *B*, centripetal fibres from gastric and intestinal 'in series' tension receptors. The cross-hatched areas represent conduction velocities in the abdomen and thorax (S_3 to S_2 in Fig. 1); and the areas bounded by thick lines the conduction velocities in the cervical vagus (S_2 to R in Fig. 1). With two exceptions, both mucosal units, the conduction velocities were below 2·5 m/sec.

Fibres from mechanoreceptors in the stomach and intestines. These include the slowest single mammalian fibres in which conduction velocities were measured. The results are given in Fig. 5*B*. There were no gastric or intestinal 'in series' tension receptors with centripetal fibres faster than 2·5 m/sec. One faster fibre (7·5 m/sec) came from an 'in series' tension receptor at the lower end of the oesophagus. Andrew (1957) has found similar receptors in the upper oesophagus and their conduction velocities were probably even higher. The only gastric 'stretch' receptor found in the present investigation had a centripetal fibre in which the conduction velocity was 1·0 m/sec (Fibre 38). The receptors are described in other papers (Iggo, 1957*a*, *b*).

Fibres from chemoreceptors in the gastric mucosa. Two classes of receptors, distinguished by their sensitivity to the pH of solutions bathing the gastric mucosa, have been described (Iggo, 1957*b*). The conduction velocities in the centripetal fibres from two acid-sensitive receptors were 1 m/sec (Fig. 5*A*) which is the same as for the tension receptors. The other results shown in Fig. 5*A* are from alkali-sensitive receptors. Five of the fibres with conduction velocities in the range 1–1·2 m/sec in the abdomen and thorax had conduction velocities in the range 1·3–5 m/sec in the cervical vagus.

Conduction velocity in different parts of individual axons

An impulse may travel more quickly in the proximal than in the distal part of the vagus. A particularly striking example is shown in Fig. 4. The fibre generating the impulse *Y* had a conduction velocity of 5 m/sec in the neck but a velocity of only 1·7 m/sec in the thorax and abdomen. The figure also shows how the 'collision' technique was used to ensure that the two measurements were made on the same fibre. A comparison of the conduction velocity in the distal vagus with the velocity in the proximal vagus for nineteen axons is made in Fig. 6. An increase in velocity of the impulses as they travelled headwards was most common in the more rapid fibres of the slow fibres; faster, that is, than 1 m/sec. Most of these particular fibres came from alkali-sensitive receptors in the gastric mucosa.

These results suggest that the diameter of some fibres is greater in the proximal than in the distal vagus, and that some small myelinated fibres lose their myelin sheaths on their way to the periphery. The large myelinated fibres examined had uniform conduction velocities in the cervical vagus (Table 1).

Type of fibre

The conduction velocity of impulses in peripheral nerves is related to axon diameter and is commonly used as such an index in electrophysiological studies. Most of the slow fibres described in this paper had conduction velocities below 2·5 m/sec. This is the maximal velocity of the C wave of the compound action potential in the cat (Gasser, 1950) and for this reason 48 of the 51 fibres from the stomach and intestines would be regarded as unmyelinated. Several other properties related to fibre diameter were examined to test this hypothesis.

Duration of the action potential spike. Valid measurements of the duration of the action potential spike can be made either if monophasic action potentials are recorded or if the recording electrodes are far enough apart to prevent interaction of the potentials recorded at each electrode as the impulse travels past them. Attempts to make the action potential monophasic by blocking the nerve at the second recording electrode with 50 mM-$MgCl_2$ in Ringer–Locke solution failed. A drop of 1% (w/v) KCl solution on the second electrode blocked the fast fibres but not the slow fibres. Some results for fast fibres

(> 10 m/sec) are plotted in Fig. 7. The difficulty in blocking conduction is attributed to the liquid paraffin (B.P.) around the nerve preventing access of solutions to fibres in the strand. The second way to measure the duration of the action potential in slow fibres could not be used. On the basis of previous

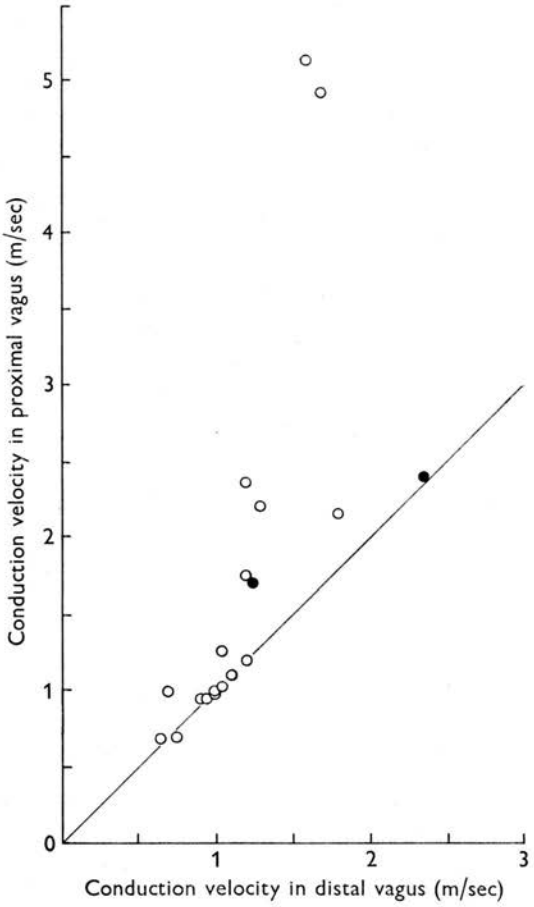


Fig. 6. Conduction velocity of single fibres in the proximal part of the vagus plotted against the velocity of the same fibres in the peripheral part of the vagus. The diagonal line indicates position of points when velocities were equal. ○ = in these fibres the velocities compared are for the distance S_2 to R (proximal) and S_3 to S_2 (distal); ● = the proximal vagus is S_1 to R and the distal vagus is S_2 to S_1.

estimates of the spike duration in C fibres (e.g. Gasser, 1950) a distance of only 4·5 mm between the recording electrodes would be sufficient to separate the two components of the diphasic action potential in the slow fibres. However, by the time that a single slow fibre had been isolated only a fragile short length of nerve was left and such an inter-electrode distance was impracticable. The

durations of the action potential spikes of slow fibres plotted in Fig. 7 are minimal values obtained from spikes which were monophasic because the impulse did not reach the second recording electrode, presumably because the fibre was damaged.

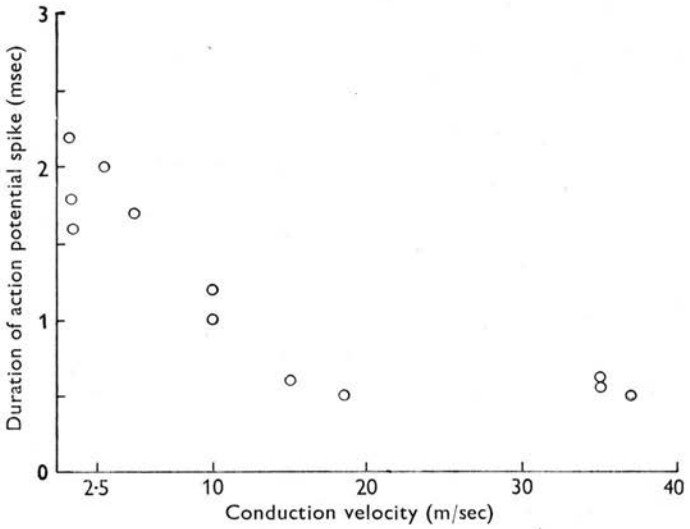


Fig. 7. The duration of the action potential spike in single fibres dissected from the cervical vagus: each point represents one fibre.

The results plotted in Fig. 7 show that there was a gradual increase in the duration of the spike for fibres with conduction velocities below 10–15 m/sec, with no inflexion at 2·5 m/sec. An inflexion would be expected if the action potentials of the unmyelinated fibres were distinctly longer than those of the smallest myelinated fibres. In previous work on slow fibres (summarized by Grundfest, 1940), in which whole nerve trunks were used, it was possible to examine only the fastest fibres of any type so that this gradual increase in the duration of the action potential of the slow fibres was not noticed.

Duration of the local excitatory state. This was measured in three slow fibres (less than 2·5 m/sec) and one fast fibre (35 m/sec). The slow fibres could be excited by a second, just subthreshold, shock as late 0·8–1·1 msec after the conditioning, just subthreshold, shock. This was much longer than in the fast fibre, where the local excitatory state lasted only about 0·2 msec, and is less than that found by Grundfest (1939) for the C wave of the compound action potential. No fibres with intermediate conduction velocities were examined.

Threshold for electrical stimulation. Accurate measurements of threshold were not made. Without exception, however, the voltage of the stimulus needed to excite the very slow fibres was at least twenty times larger than that needed to excite the fastest fibres.

Amplitude of the action potential spikes. This was often unexpectedly large from the slow fibres, being as much as 100 μV. It is not at all unusual for fast fibres (>20 m/sec), even in the same strand, to have spike amplitudes less than this. The amplitude depends so much on the recording conditions that estimates of fibre diameter based on a comparison of the spike heights of fibres in different strands are unreliable. The observation recorded here emphasizes that conclusions reached from such comparisons, even for fibres in the same strand, need not be valid.

None of these tests was sufficient to distinguish between the thinnest myelinated fibres and the unmyelinated fibres.

DISCUSSION

The general conclusion to be drawn from the results described in this paper is that both small myelinated fibres and unmyelinated fibres from specified receptors can be isolated *in vivo* as single units for electrophysiological recording. As dissected out, strands from the cervical vagus usually contained a number of live fibres. Only on a few occasions was a strand containing only one C fibre isolated. The identification of a single active unit within strands containing several fibres was made possible by the 'collision' technique and the 'peripheral stimulus' technique and after these techniques were developed no attempt was made to continue the dissection until the strand contained only one live fibre.

The conclusion that the slowest vagal fibres were unmyelinated rests on the conduction velocity measurements. It lacks direct histological proof. The attempt to settle the question by other electrophysiological tests has done no more than confirm that the fibres behaved as slow fibres both in the undissected part of the vagus and where they were dissected out in a fine filament. As Erlanger (1937) has said 'conduction rate is used as the base line for comparison' in the study of single fibres because anatomical units cannot be identified with the properties observed. Even the elegant single fibre technique of Tasaki (1953) fails with unmyelinated mammalian fibres (Maruhashi *et al.* 1952).

It may be asked how it is possible to dissect out single units if the unmyelinated fibres run together as bundles in a Schwann sheath (Gasser, 1955). The answer perhaps lies in Gasser's demonstration that there is a constant interchange of fibres between bundles. Longitudinal dissection of a fine strand into finer filaments would break many of the fibres as they passed from bundle to bundle. It would then be a matter of chance whether, in the filaments obtained by subdividing a long strand, there would be any unbroken C fibres.

Gasser (1955) presents evidence to show that C fibres conform to the rule of isolated conduction and that there is no significant cross-excitation. The present work supports his conclusion. For example, action potential spikes had the same amplitude and configuration at the recording electrodes no

matter where along the axon they were initiated. If there were cross-excitation then a more distant origin of the impulse should give a greater opportunity for interaction between fibres and consequently a larger action potential, since more fibres would be carrying impulses. Again, vagal strands were sometimes isolated which contained several C fibres from gastric receptors. It was possible to excite any one of the fibres by local stimulation of its receptor without at the same time initiating impulses in the other fibres by interaction in the nerve trunk. When several fibres were active at the same time the impulses behaved independently of each other.

A loss of myelin from vagal fibres was suggested by Gaskell (1886), Langley (1900) and Chase & Ranson (1914) to account for the sparsity of myelinated fibres in the abdominal vagi compared with their abundance in the upper cervical vagus. This may have been, in part, due to the myelinated fibres leaving the vagus in its upper thoracic branches, as Gaskell (1886) first noted. Some of the electrophysiological results, however, suggest that small myelinated fibres may lose their myelin sheaths. Higher conduction velocities in the proximal than in the distal vagus, first seen in the goat (Iggo, 1956*b*) were also found in the cat for both myelinated and unmyelinated fibres in the cervical vagus. None of the myelinated fibres isolated in the cervical vagus had myelin sheaths in the abdomen, as judged by their conduction velocities. There is no evidence that the change in conduction velocity was due to branching, for it occurred far away from the endings in the alimentary tract and yet for each fibre there was only one spot in the gut from which impulses could be initiated by the application of the specific stimulus. The simplest explanation for this change in conduction velocity is that the fibres became smaller as they travelled to the periphery and that, as Langley suggested in 1900, some of the small myelinated afferent fibres lose their myelin sheaths a long way from their endings.

Douglas & Ritchie (1957*a*) have just published a technique, also based on the collision of orthodromic and antidromic impulses, which allows them to identify the type of fibre active in whole nerve trunks. The accurate localization of the receptors, the rate of discharge in individual fibres and the specificity of response for individual receptors elude analysis by their method. The ingenious technique of these authors may be useful where the more direct approach, described in this paper, cannot be made. Their method allows a preliminary survey to be made quickly and may yield an estimate of the relative number of fibres from different classes of receptor in a whole nerve.

The fibres examined in detail came from receptors, described elsewhere (Iggo, 1957*a*, *b*), in the oesophagus, stomach and intestines. The conduction velocities were measured in a much larger sample than in previous work, which was based on a few single fibres (Paintal, 1954; Iggo, 1955) or on the compound action potential generated by fibres which, on electrical stimulation of the

cervical vagus, reflexly enhanced gastric movements (Iggo, 1956*a*). The velocities of four single fibres from 'in series' tension receptors in the goat, 2–12 m/sec (Iggo, 1955), were similar to the velocities derived from the compound action potentials in the sheep (Iggo, 1956*a*). They were, however, higher than those in the corresponding fibres in the cat as now reported. The conclusion (Iggo, 1955) that gastric tension receptors in the cat have myelinated afferent fibres is not supported by the present, direct, evidence. There are species differences in the number of myelinated fibres in the abdominal vagi; they are abundant in the sheep and goat (Iggo, 1956*a*) but are less than 2% of the fibres in the cat (Agostini, Chinnock, Daly & Murray, 1957) so that myelinated gastric afferent fibres are more likely to be isolated in the ruminant. This may not be the reason for the failure to find fast fibres from tension receptors in the cat because faster gastric afferent fibres were found in the present work. They came, not from tension receptors, but from mucosal chemoreceptors (Fig. 5) and included some small myelinated fibres. Paintal (1954) reported that the mean velocity in the fibres from the gastric mechanoreceptors which he isolated in the cat 'would be about 9 m/sec'. He measured the velocity in one single fibre and in several fibres in a strand which 'apparently consisted exclusively' of gastric afferent fibres. The velocities he gives are outside the range for the gastric afferent fibres reported here, although a careful search was made for fast fibres from gastric tension receptors. Indeed, many tension receptors were discarded when a preliminary test showed that they had very slowly conducting afferent fibres. Support for the conclusion that tension receptors have unmyelinated afferent fibres comes from the work of Douglas & Ritchie (1957*b*). They found that phenyldiguanide, which excites the tension receptors (Iggo, 1955, 1957*a*), caused a discharge of impulses in C fibres in the abdominal vagus.

SUMMARY

1. Two methods used to identify individual active units in a strand dissected from the cervical vagus and containing several live fibres are described.

2. Single afferent fibres with conduction velocities as low as 0·5 m/sec were identified in strands dissected from the cervical vagi of twenty-five cats; 48 of the 51 fibres had velocities below 2·5 m/sec. The receptors were in the oesophagus, stomach and intestines.

3. Several of the fibres had conduction velocities which were higher in the proximal than in the distal part of the axon. This was especially noted in centripetal fibres from alkali-sensitive receptors in the gastric mucosa.

4. To test the classification of the fibres based on the conduction velocity measurements, the duration of the action potential spike, the persistence of the local excitatory state and the threshold for electrical stimulation were measured. While the results confirmed that the fibres were small, they were

not sufficient to distinguish the smallest myelinated from the unmyelinated fibres. The inadequacy of the relative amplitude of the action potential spikes as a reliable index of fibre diameter is pointed out.

5. It is concluded that unmyelinated mammalian afferent fibres had been isolated as single units for electrophysiological recording.

REFERENCES

AGOSTINI, E., CHINNOCK, J. E., DALY, M. DE B. & MURRAY, J. G. (1957). Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat. *J. Physiol.* **135**, 182–205.

ANDREW, B. L. (1957). Activity in afferent nerve fibres from the cervical oesophagus. *J. Physiol.* **135**, 54–55*P*.

BLAIR, E. A. & ERLANGER, J. (1933). A comparison of the characteristics of axons through their individual electrical responses. *Amer. J. Physiol.* **106**, 524–564.

CHASE, M. R. & RANSON, S. W. (1914). The structure of the roots, trunk and branches of the vagus nerve. *J. comp. Neurol.* **24**, 31–60.

DICKINSON, C. J. (1950). *Electrophysiological Technique.* London: Electronic Engineering.

DOUGLAS, W. W. & RITCHIE, J. M. (1957*a*). A technique for recording functional activity in specific groups of medullated and non-medullated fibres in whole nerve trunks. *J. Physiol.* **138**, 19–30.

DOUGLAS, W. W. & RITCHIE, J. M. (1957*b*). On excitation of non-medullated afferent fibres in the vagus and aortic nerves by pharmacological agents. *J. Physiol.* **138**, 31–43.

ERLANGER, J. (1937). In ERLANGER, J. & GASSER, H. S. (1937). *Electrical Signs of Nervous Activity.* Philadelphia: University of Pennsylvania Press.

GASKELL, W. H. (1886). On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. *J. Physiol.* **7**, 1–80.

GASSER, H. S. (1950). Unmedullated fibres originating in dorsal root ganglia. *J. gen. Physiol.* **33**, 651–690.

GASSER, H. S. (1955). Properties of dorsal root unmedullated fibres on the two sides of the ganglion. *J. gen. Physiol.* **38**, 709–728.

GRUNDFEST, H. (1939). The properties of mammalian B fibres. *Amer. J. Physiol.* **127**, 252–262.

GRUNDFEST, H. (1940). Bioelectrical potentials. *Ann. Rev. Physiol.* **2**, 213–242.

IGGO, A. (1955). Tension receptors in the stomach and the urinary bladder. *J. Physiol.* **128**, 593–607.

IGGO, A. (1956*a*). Central nervous control of gastric movements in sheep and goats. *J. Physiol.* **131**, 248–256.

IGGO, A. (1956*b*). Afferent fibres from the viscera. *XX int. physiol. Congr.* 458–459.

IGGO, A. (1957*a*). Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. *Quart. J. exp. Physiol.* **42**, 130–143.

IGGO, A. (1957*b*). Gastric mucosal chemoreceptors with vagal afferent fibres in the cat. *Quart. J. exp. Physiol.* **42**, 398–409.

IGGO, A. (1957*c*). Conduction velocity in vagal afferent fibres. *J. Physiol.* **138**, 21*P*.

LANGLEY, J. N. (1900). In E. SCHÄFER, *Textbook of Physiology*, vol. II, p. 605. Edinburgh: Pentland.

MARUHASHI, J., MIZUGUCHI, K. & TASAKI, I. (1952). Action currents in single afferent fibres elicited by stimulation of the skin of the toad and the cat. *J. Physiol.* **117**, 129–151.

MATTHEWS, B. H. C. (1931). The response of a muscle spindle during active contraction of a muscle. *J. Physiol.* **72**, 153–174.

PAINTAL, A. S. (1953). The conduction velocities of respiratory and cardiovascular afferent fibres in the vagus nerve. *J. Physiol.* **121**, 341–359.

PAINTAL, A. S. (1954). A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation of hunger and thirst. *J. Physiol.* **126**, 255–270.

RUSHTON, W. A. H. (1949). The site of excitation in the nerve trunk of the frog. *J. Physiol.* **109**, 314–326.

TASAKI, I. (1953). *Nervous Transmission.* Illinois: Thomas.

ZOTTERMAN, Y. (1939). Touch, pain and tickling: an electrophysiological investigation on cutaneous sensory nerves. *J. Physiol.* **95**, 1–28.

Reprinted from the QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY,
VOL. XLIV, 1959.

CUTANEOUS HEAT AND COLD RECEPTORS WITH SLOWLY CONDUCTING (C) AFFERENT FIBRES.

By A. IGGO. From the Department of Physiology, University of Edinburgh.

(*Received for publication 5th June* 1959)

The sensitivity of cutaneous receptors in the cat was examined by recording electrically from thin strands dissected from the saphenous nerve and applying a variety of thermal and mechanical stimuli to the skin. Responses from receptors sensitive to heating the skin by 10 + ° C. and other receptors excited by cooling the skin by a similar amount are described. The receptors adapted slowly to thermal stimuli and were not readily excited by mechanical stimuli. The conduction velocities in the afferent fibres were, with one exception, in the range 0·5 to 1·2 m./sec. and the fibres were presumably unmyelinated. It was concluded that two new types of thermoreceptor had been revealed.

CUTANEOUS thermal sensations and pain caused by heating or cooling the skin in man may be carried, in part, by unmyelinated afferent fibres [Lewis and Pochin, 1937 *a* and *b*; Zotterman, 1933 and 1939; Wolf and Hardy, 1941; Gasser, 1943]. This conclusion, which is based on the use of differential blocking of cutaneous nerves and the nerve-endings, is supported by the electrophysiological work of Zotterman [1933 and 1939], Maruhashi, Mizuguchi and Tasaki [1952] and Dodt [1955] which has shown that heating the skin and tongue of the frog, toad and cat may excite small afferent fibres. Because there had been no single unit studies of the receptors it was not known, however, whether the response was a general property of the small afferent fibres or whether there were specific differences among the receptors. Methods were recently described [Iggo, 1958 *a*] which make such an analysis possible and the present work was started to examine the thermal sensitivity of cutaneous receptors with C afferent fibres in the cat. Brief reports of the results, which reveal the existence of thermoreceptors with unmyelinated afferent fibres, have been published [Iggo, 1958 *b* and 1959].

METHODS

The fifteen cats used were anæsthetized with chloralose (80 mg./kg. i.v.) after induction of anæsthesia with ethyl chloride and ether. Drill pins inserted through each end of the tibia of the right leg were then fixed to a supporting frame so as to keep the limb fixed rigidly in one place. The pins were inserted from the lateral aspect of the leg to avoid damage to the skin innervated by the saphenous nerve. The skin over the saphenous nerve in the thigh was incised, reflected and the cut edges tied to a metal ring fixed above the leg. The trough formed was filled with paraffin (B.P.) after the nerve had been prepared for the final stages of dissection. To do this the saphenous nerve was ligated high in the thigh, so as to block the central passage of any impulses set up by electrical stimulation of the nerve later in the experiment, and then dissected free from connective tissue, with as little disturbance

to the blood supply as possible, at two places in the thigh. A black perspex plate, on which the fine dissection of the nerve for recording purposes was to be done, was put under the proximal dissected part of the nerve and a pair of silver stimulating electrodes placed under the distal part. A silver earth electrode was inserted into the quadriceps muscle between these two points.

Methods for recording electrical activity in the fine strands dissected from the nerve have been described, as have the methods for identifying single afferent fibres and assessing their conduction velocities [Iggo, 1958 *a*].

Cutaneous Stimulation.—The thermal stimulators used were (*a*) brass and copper rods, 10 mm. diameter, heated to the desired temperature in a water bath or Dewar flask and pressed lightly on the skin; (*b*) radiators, made from nichrome wire heated electrically to a dull red or metal plates at a black heat, held not closer than 2 mm. to the skin; (*c*) ice or water run on to the skin and (*d*) thermodes 10 mm. diameter, circulated with hot or cold ethanol.

The mechanical stimuli are described in detail elsewhere, as is the method used to measure the pressure exerted on the skin [Iggo, in preparation].

Temperature Recording.—Thermistors (Stantel Type F2311/300) were used to register the temperature. The resistance of the thermistor, which varies with the temperature, was measured by recording the change in voltage across a fixed resistance in series with the thermistor and a 1·5 V dry battery. The voltage was amplified with a D.C. amplifier and displayed on a cathode ray screen. The system was calibrated by immersing the thermistor in water at temperatures in the range to be recorded. Surface temperatures were recorded by pressing the thermistor gently on the skin close to the receptor under examination. The temperature of the under-side of the skin was examined by first making a small hole in the skin, about 10 mm. away from the receptor, and then by blunt dissection freeing the skin from the underlying connective tissue. The thermistor was inserted into the pocket so formed and pressed gently against the under-side of the skin just beneath the receptor, and arranged so that it was not touching the deeper tissues.

Results

The receptors whose behaviour is described in this paper were in the skin on the antero-medial aspect of the lower thigh, the leg and the foot.

Conduction Velocities of the Afferent Fibres.—These were measured in ten of the seventeen fibres and ranged from 0·5 to 17 m./sec. (Table I). Apart from Fibre No. 5 the conduction velocities were less than 1·2 m./sec. and the assumption is made that the fibres, except for No. 5, were unmyelinated. The relation of conduction velocity to fibre diameter is dealt with more fully in another paper [Iggo, 1958 *a*]. The results described below, with the one exception stated, refer to the slowly conducting fibres.

Size of Receptive Fields.—Both thermal and mechanical stimuli were used in making an assessment of the size of the receptive fields, which were taken to be that part of the skin from which a discharge of impulses could be aroused with the weakest effective stimulus. The receptive fields were small, not more than 5 × 5 mm., and the fields for thermal and mechanical stimuli were coincident. The thermoreceptors thus have receptive fields similar in size to the mechanoreceptors, which have been examined more thoroughly in this respect [Iggo, in preparation].

Sensitivity to Mechanical Stimulation.—All the receptors could be excited by mechanical stimulation but the intensity of stimulation required was

TABLE I.— THE RESPONSES OF CUTANEOUS THERMORECEPTORS TO THERMAL AND MECHANICAL STIMULATION OF THE SKIN, WITH THE CONDUCTION VELOCITIES OF THE AFFERENT FIBRES.

Fibre No.	Conduction velocity m./sec.	Stimulus		
		Heat	Cold	Mechanical threshold
1	0·66	+	−	High
2	1·0	″ 55° C.	+	High
3	1·0	″ 48° C.	−	5 g.
4	..	+	..	High
5	17	+	..	1·5 g.
6	..	″ 52° C., * 46° C.	..	3 g.
7	..	″ 50° C.	..	4·5 g.
8	..	″ 55° C.	..	High
9	0·7	+	..	High
10	0·8	* 44° C.	−	..
11	0·7	+	−	..
12	..	−	+	..
13	1·0	−	+	High
14	1·15	..	+	5 g.
15	..	−	* 17° C.	5 g.
16	..	..	* 13° C.	..
17	0·5	..	* 14° C.	5 g.

The figures in columns under stimuli give the threshold value of the stimulus.

″ Temperature of metal rod. * Surface temperature of skin.
+ Receptor excited by the stimulus. − Receptor not excited by the stimulus.
.. Indicates that the stimulus was not tried.
High = the receptors were more difficult to excite mechanically than most of the C mechanoreceptors.

always high: the most sensitive unit required a pressure of 3 g. before it discharged an impulse. Measurements of the thresholds were made for five receptors (Table I). Several other receptors could only be excited by squeezing the skin with forceps, by pulling hairs or by pressing sharp metal pins into the skin. The receptors were not always easy to find when mechanical stimuli were used, *e.g.* the ending of Fibre No. 14 could not be localized by mechanical stimuli until after the receptive field for thermal stimulation was established.

The sensitivity of the receptors to mechanical stimulation was not constant, but increased after repeated heating and cooling, *e.g.* Fibre No. 3 after 2 hr. repeated thermal stimulation was more readily excited by pressure than initially. Despite these variations in threshold the heat receptors were always much less sensitive to pressure than the most excitable known C mechanoreceptors (threshold 50 mg.) but overlapped in sensitivity the high threshold C mechanoreceptors. They differed from the latter in having a well-defined response to thermal stimulation.

The effect of cutaneous temperature on the response to mechanical stimulation was examined for only one heat receptor (No. 5, an Aδ fibre). The receptor discharged at a higher frequency, in response to a standard pressure, when the skin was hot than when it was normal; thus at 53°–59° C. the adapted response to steady pressure was 25–27 impulses/sec. but at 33° C. it was only 15–17 impulses/sec.

Response to Thermal Stimuli.—Two main types of response were found (Table I). (A) units which were excited by heating the skin, using stimulators at 48° C. or hotter (eleven fibres), and (B) units which were excited by cooling the skin with stimulators at 10° C. or colder (six fibres). In addition two

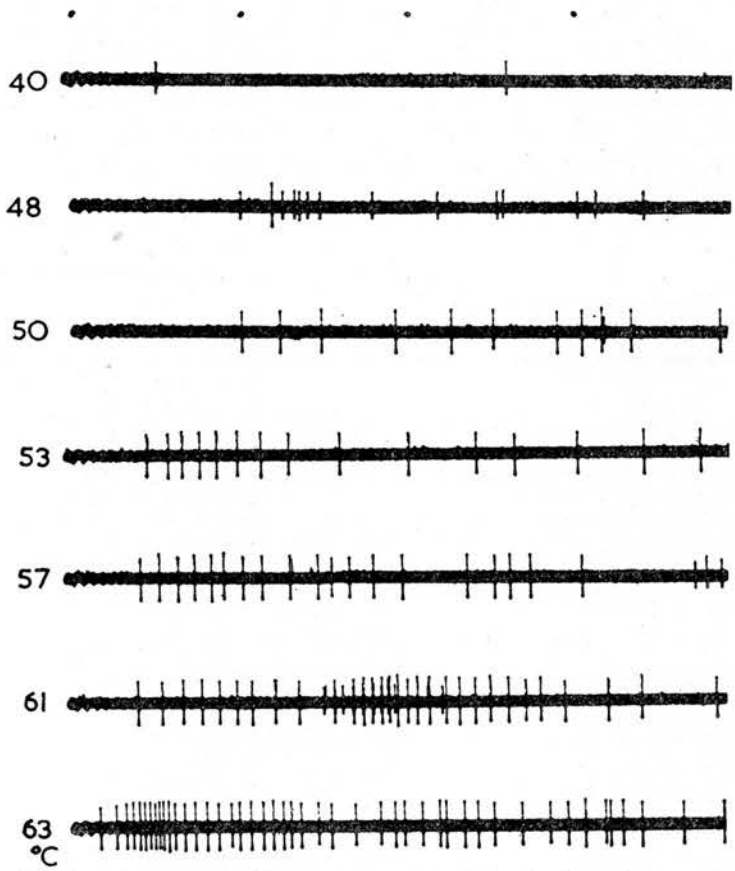


FIG. 1.—The response of heat receptor (Fibre No. 3) when a brass rod (10 mm. diameter) at the temperatures indicated, was put on to the skin and left there for the duration of the record. The small impulses seen in the upper two tracings are from a mechanoreceptor, 10 mm. away from heat receptor, and were evoked by the mechanical effect of putting the hot rod on the skin. The heat receptor on the other hand, was not excited by such a mechanical stimulus. Time in seconds at the top of the figure.

other fibres with much more sensitive receptors were found, one was excited by cooling and the other by warming the skin by less than 2° C. This paper is not concerned with the behaviour of these more sensitive thermoreceptors, which will be described by Hensel, Iggo and Witt [in preparation].

Thermoreceptors Excited by Heating the Skin.—The most sensitive of these heat receptors was excited by placing a metal rod at 48° C. on the skin (at 28°–33° C.) and leaving it there for several seconds. The response had a latency of 1·2 sec. and consisted of one impulse (fig. 1). At higher rod

temperatures the discharge appeared with a shorter latency, lasted longer and was at a higher frequency (fig. 1). The maximal frequency of response in this and other fibres was 100 impulses/sec. but this frequency was never maintained for more than a few impulses. A discharge of 50 impulses/sec. might last for 1 sec. but thereafter the frequency fell and the discharge often became irregular. Whether this effect was due to a fall in the temperature of the stimulator is not known since its temperature was not recorded. Stimulus-response curves for the fibre illustrated in fig. 1 were measured with an interval of 1·5 hr. between the two sets of measurements. The curves were similar and presumably represented the normal response of the receptor to thermal stimulation.

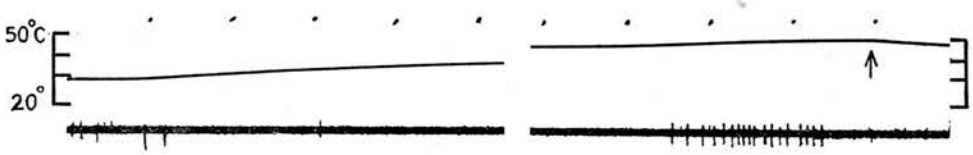


FIG. 2.—The response of a heat receptor (Fibre No. 10) to radiant heat. The upper tracing shows the surface temperature of the skin, recorded by a thermistor on the surface, and the lower tracing shows the impulses in the afferent fibre. The response was brief and did not continue even though the cutaneous temperature was still increasing. The peak temperature is marked by the arrow. The second part of the record was taken 10 sec. after the first, but the heating was continuous, and during the interval there was no discharge of impulses in the fibre. Time in seconds.

Radiant heat was frequently used to test for the presence of heat-sensitive fibres in strands dissected from the saphenous nerve. The discharge of impulses was usually brief when a glowing radiator was used, even though the skin temperature continued to rise after the discharge had stopped (fig. 2). A more persistent discharge could sometimes be obtained by using a radiator at black-heat or by preventing the skin temperature from rising too high.

The threshold skin temperature for excitation of heat receptors was measured using radiant heat for Fibres Nos. 6 and 10 (fig. 2). The surface temperature was 44°–46° C. before impulses appeared in the fibre. The under-surface temperature at which impulses first appeared was also measured for Fibre No. 10. It was 33° C. at one trial and 40° C. at the next trial: the initial temperature of the under-surface was higher on the latter occasion.

The receptors adapted relatively slowly to continued stimulation, but accurate measurements could not be made because the stimulator temperature was not constant. The receptors were still firing after 10-sec. stimulation. Radiant heat, arranged so that the surface temperature of the skin did not exceed 40° C., continued to excite the receptor of Fibre No. 6 for 50 sec., at which time the stimulus was removed. Frequent repetition of thermal stimulation at the higher temperatures led to an insensitivity of the receptor, so that at a constant temperature of stimulation the response became smaller. When an interval of 3 min. was left between each test with a rod the response to each test was more uniform.

In six experiments cooling the skin did not excite the heat receptors.

The odd fibre (No. 2 in Table I) had a low frequency, 5/sec., irregular discharge of impulses when the skin was cooled.

Thermoreceptors Excited by Cooling the Skin.—Cutaneous temperatures had to reach low levels before these cold receptors were excited. The threshold temperatures were measured for three units (Nos. 15, 16, 17 in Table I) and were 21°, 13° and 14° C. The rate of cooling was slow when ice was put on the skin and the impulses appeared after a latency of 2–3 sec. at a gradually increasing frequency (fig. 3). The maximal frequency of discharge from the cold receptors was 10/sec. Different steady frequencies of discharge were found but no relation between cutaneous temperature and rate of firing was

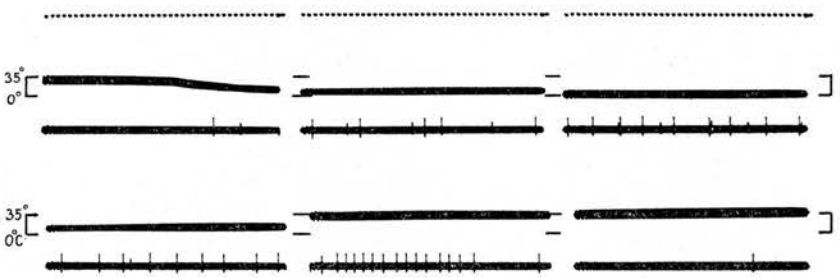


FIG. 3.—The response of a cold receptor (Fibre No. 15) and another fibre to cooling the skin with ice and then re-warming it. In each record the upper tracing is the surface temperature recorded by a thermistor and the lower tracing shows the impulses in two afferent fibres. The records were taken at the following times: (A) When ice was put on the skin, (B) 15 sec. later, (C) 35 sec. later, (D) 60 sec. later, just after the ice had been removed, (E) 75 sec., just after a pad of cotton-wool soaked in water at 55° C. was put on the skin, this record shows part of the re-warming burst of impulses and (F) 82 sec. The fibre giving the smaller impulse was not excited by re-warming the skin. Time at 1/10 sec. intervals.

established. All the receptors would fire at steady frequencies of 2–4 impulses/sec. when the skin was at 3° C. If a fibre was firing steadily, then lowering the skin temperature still further in the range used in these experiments (not lower than 1° C.) increased the rate of discharge. The receptors adapted slowly to a constant stimulus, *e.g.* Fibre No. 16 discharged steadily at 2- 3/sec. for 3 min.

Removal of the cold stimulus and rewarming the skin stopped the discharge of impulses in four of the fibres, the frequency falling as the temperature rose. The other two fibres had a different response. In these two the rate of discharge increased when the cutaneous temperature began to rise. The frequency of discharge was higher during rewarming than at any stage of cooling for Fibre No. 15. The discharge was brief (fig. 3) and never lasted longer than 10 sec.

None of the cold receptors was excited by heating the skin from 30° C. to a higher temperature.

Thermal Gradients in the Skin.—These were assessed in two experiments by recording the temperature of the surface of the skin during thermal stimulation and then repeating the test while recording from the under-side of the skin. The results are clearest for the cold receptors. The thermal gradient across the skin was no greater when they were firing than when the skin was

first cooled, at which time they were silent. The fibres were firing steadily at a time when the thermal gradient across the skin was less than 1° C. and the skin temperature was low, *e.g.* 6° C. in fig. 4. These cold receptors are thus similar to the cold receptors in the cat's tongue [Hensel and Zotterman, 1951] in so far as they discharge steadily when the thermal gradient is very small. They may not fire at all when the gradient is steep.

The Effect of Heating and Cooling the Saphenous Nerve.—Axons in peripheral nerves can be excited by sudden changes in the temperature of the

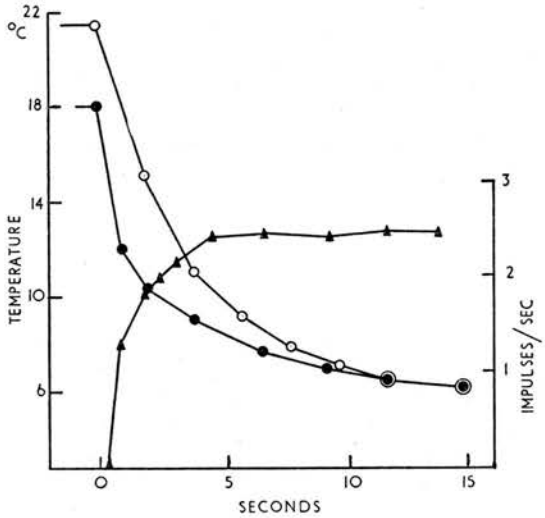


FIG. 4.—The relation between cutaneous temperature and the discharge of impulses (▲–▲) in Fibre No. 17. The filled circles show the temperature of the thermistor when it was on the surface of the skin and the open circles show the temperature of the subcutaneous thermistor taken during repetition of the cooling. Ice was put on the skin at zero time, and as can be seen the temperature gradient through the skin was negligible after about 12 sec. The discharge of impulses in the fibre is not influenced by the gradient.

nerve [Bernhard and Granit, 1946; von Euler, 1947; Dodt, 1953]. The effects described above might therefore be due to such a non-specific effect, rather than to the excitation of specialized receptors. This is unlikely since the mechanoreceptors, even in adjacent skin and exposed simultaneously to the same thermal stimuli, were not excited by thermal changes sufficient to elicit a high frequency response from the thermoreceptors. Two experiments were done in which the saphenous nerve between the skin field and the recording electrodes was dissected out and placed on or under a thermode and then heated by an amount sufficient to excite the thermoreceptors when the stimulus was applied to the skin. In most instances the axons in the nerve were not excited by thermal changes which evokes a vigorous response when they were produced in the skin. Cooling the skin may arouse a response from mechanoreceptors but the frequency of discharge is low and the response not always sustained [Iggo, 1959].

Discussion

The results described provide clear evidence that the nerve-endings of the unmyelinated cutaneous fibres do not respond in the same way to all forms of cutaneous stimulation. Receptors sensitive to mechanical stimulation (threshold as low as 50 mg.) will be reported elsewhere [Iggo, in preparation] and the present results show that there are C afferent fibres whose endings combine an insensitivity to mechanical stimuli with a sensitivity to either high or low cutaneous temperature. The effective stimuli caused large changes ($\pm 10°$ C.) in cutaneous temperature. Although no histological difference can be seen in the afferent plexuses in hairy skin which could account for the differential sensitivity of the nerve-endings [Weddell, Palmer and Pallie, 1955] there can be no doubt that the endings of the unmyelinated afferent axons do differ in their sensitivity to stimuli which in man give rise to distinctive sensations.

Douglas and Ritchie [1957] and Douglas, Ritchie and Straub [1959] have recently been examining the responses in C fibres in the saphenous nerves of cats and have reported the absence of any specific thermal receptors. Their results are not supported by the more direct methods of analysing cutaneous receptors which were used in the present investigation.

Small changes in cutaneous temperature ($\pm 1°$ C.) can be detected in man and these responses could clearly not be served by the receptors described in this paper. More extreme thermal changes may arouse different sensations which eventually merge into pain. The pain threshold in man and the threshold of the heat and cold receptors now described are similar. Thus Lewis and Love [1926] found that pain appeared in the human forearm when the surface temperature was 43° C. This is similar to the cutaneous temperature at which the C heat receptors are first excited. Skouby [1952] has examined the threshold for human pain using heated thermodes. Pain first appeared at thermode temperature of $48° \pm 1°$ C.; this is similar to the threshold temperature for the heat-sensitive fibres. Thermal stimuli can cause pain in the ischæmic forearm [Lewis and Pochin, 1937 *a*] at a time when first pain can no longer be produced. Although there is still debate about the mechanism of the dual pain response [Bishop and Landau, 1958; Jones, 1958] the evidence that it is due to impulses in two sets of afferent fibres and that the second pain is mediated by unmyelinated afferent fibres is very strong. However, sensations of warmth and heat can also be evoked from the ischæmic arm after the first pain response has gone so that although the C heat-sensitive endings may mediate pain provoked by heating the skin they could also signal heat, or some may be concerned with pain and others with heat. At present the question can only be posed. The cold receptors described in this paper may likewise be associated with the pain evoked by cooling the skin to 17° C. or below [Wolf and Hardy, 1941]. This cold pain can also be evoked in ischæmic skin after first pain is absent [Wolf and Hardy, 1941; Iggo, unpublished experiments] so that unmyelinated fibres mediate at least part of it. The problem of pain fibres is still however unresolved.

An attempt to identify the pain fibres by making close-arterial injections of pain-producing chemicals was inconclusive [Fjällbrant and Iggo, 1958].

ACKNOWLEDGMENT

I wish to thank Miss Wilma Sandie for her willing assistance in these experiments.

REFERENCES

BERNHARD, C. G. and GRANIT, R. (1946). "Nerve as model temperature end organ", *J. gen. Physiol.* **29,** 257–265.

BISHOP, G. H. and LANDAU, W. M. (1958). "Evidence for a double peripheral pathway for pain", *Science,* **128,** 712–713.

DODT, E. (1953). "Differential thermosensitivity of mammalian A-fibres", *Acta physiol. scand.* **29,** 91–108.

DODT, E. (1955). "Aktivierung markhaltiger und markloser Schmerzfasern bei temperaturreizen", *Pflügers Arch.* **260,** 225–238.

DOUGLAS, W. W. and RITCHIE, J. M. (1957). "Non-medullated fibres in the saphenous nerve which signal touch", *J. Physiol.* **139,** 385–399.

DOUGLAS, W. W., RITCHIE, J. M. and STRAUB, R. W. (1959). "Discharges in non-myelinated (C) fibres in the cat's saphenous nerve in response to changing the temperature of the skin", *J. Physiol.* **146,** 47–48P.

EULER, C. VON (1947). "Selective responses to thermal stimulation of mammalian nerve fibres", *Acta physiol. scand.* **14,** Supp. 45.

FJÄLLBRANT, N. and IGGO, A. (1958). "Cutaneous afferent fibres and pain-producing chemicals", *J. Physiol.* **145,** 28–29P.

GASSER, H. (1943). "Pain-producing impulses in peripheral nerves", *Res. Publ. Ass. nerv. ment. dis.* **23,** 44–62.

HENSEL, H. and ZOTTERMAN, Y. (1951). "Action potentials of cold fibres and intracutaneous temperature gradient", *J. Neurophysiol.* **14,** 377–385.

IGGO, A. (1958 *a*). "The electrophysiological identification of single nerve fibres, with particular reference to the slowest-conducting vagal afferent fibres in the cat", *J. Physiol.* **142,** 110–126.

IGGO, A. (1958 *b*). "Single C fibres from cutaneous receptors", *J. Physiol.* **143,** 47–48P.

IGGO, A. (1959). "A single unit analysis of cutaneous receptors with C afferent fibres", *Ciba Foundation Study Group. The Nervous Mechanism of Pain and Itch.* London: Churchill.

JONES, M. H. (1958). "Evidence for a double peripheral pathway for pain", *Science,* **128,** 713–714.

LEWIS, T. and LOVE, W. S. (1926). "Vascular reactions of the skin to injury. Part III. Some effects of freezing, of cooling and of warming", *Heart,* **13,** 27–60.

LEWIS, T. and POCHIN, E. E. (1937 *a*). "The double pain response of the human skin to a single stimulus", *Clin. Sci.* **3,** 67–76.

LEWIS, T. and POCHIN, E. E. (1937 *b*). "Effects of asphyxia and pressure on sensory nerves of man", *Clin. Sci.* **3,** 141–155.

MARUHASHI, J., MIZUGUCHI, K. and TASAKI, I. (1952). "Action currents in single afferent nerve fibres elicited by stimulation of the skin of the toad and the cat", *J. Physiol.* **117,** 129–151.

SKOUBY, A. P. (1952). "Sensitization of pain receptors by cholinergic substances", *Acta physiol. scand.* **24,** 174–191.

WEDDELL, G., PALMER, E. and PALLIE, W. (1955). "Nerve endings in mammalian skin", *Biol. Rev.* **30,** 159–195.

WOLF, S. and HARDY, J. D. (1941). "Studies on pain. Observations on pain due to local cooling and on factors involved in the 'cold pressor' effect", *J. clin. Invest.* **20,** 521–533.

ZOTTERMAN, Y. (1933). "Studies in the peripheral mechanism of pain", *Acta med.scand.* **80,** 185–242.

ZOTTERMAN, Y. (1939). "Touch, pain and tickling: an electrophysiological investigation on cutaneous sensory nerves", *J. Physiol.* **95,** 1–28.

J. Physiol. (1960), **152**, *pp.* 337–353
With 8 *text-figures*
Printed in Great Britain

CUTANEOUS MECHANORECEPTORS WITH AFFERENT C FIBRES

By A. IGGO
From the Department of Physiology, University of Edinburgh

(*Received* 5 *February* 1960)

The analysis of cutaneous afferent mechanisms by recording electrically from the afferent nerve fibres has been pursued vigorously since Adrian (1926) first developed suitable techniques. As a result there is now an extensive literature on, and general agreement concerning, the larger myelinated afferent fibres. However, the non-myelinated sensory fibres, because they are difficult to isolate as single units, have not been intensively studied, even though they are 4–5 times more numerous than the myelinated fibres in cutaneous nerves (Gasser, 1955). Adrian (1931) in the earliest work on the fine fibres had concluded that pain was carried by the smallest fibres because the stimuli required to excite a discharge of low-amplitude impulses from frog's skin were either strong pressure or chemicals which caused 'defensive movements' in spinal frogs. Zotterman in 1939, using improved techniques, recorded impulses of small amplitude from cutaneous nerves of the cat and he argued that they were carried by very small axons which were probably non-myelinated. Weak mechanical stimuli, such as lightly stroking the hairs, were sufficient to arouse a discharge of small impulses at low frequency, with a persistent after-discharge. Stronger mechanical stimuli and severe thermal stimuli caused a more abundant discharge of the small impulses in the multifibre strands which were used. Maruhashi, Mizuguchi & Tasaki (1952) were able, in toads, to prepare fine nerve strands containing only a few non-myelinated axons, and substantiated Zotterman's conclusion that some C fibres could be excited by lightly touching the skin. In addition, they found that there were other C fibres which were excited by severe thermal but not by mechanical stimuli. In the cat, although they could not keep the C fibres alive unless there were also A fibres in the strands, they recorded responses evoked by stroking the skin and when 'noxious and heat stimuli' were used. More recently, Douglas & Ritchie (1957*a*) recording from intact cutaneous nerves in the cat, have also confirmed Zotterman's observation by showing that C fibres were excited by stroking the skin. As many as 50 % of the C fibres examined by their technique could be excited by mechanical stimuli. They did not find any fibres excited by heating the skin, even when

their stimuli were sufficient to burn the fur. Many of the fibres excited by mechanical stimuli also showed a short-lasting response when the skin was cooled (Douglas, Ritchie & Straub, 1959).

Convincing comparisons of the activity in different sensory units cannot be made when the afferent impulses are recorded in multifibre preparations. Exact information about the properties of receptors can, however, be obtained by examining activity in single afferent fibres. Techniques for recording the activity in single afferent C fibres which were developed during work on the visceral afferent innervation (Iggo, 1957*a*, *b*; 1958*a*) have now been used, together with quantitative mechanical and thermal stimulation of the skin, to analyse the cutaneous afferent C fibres. This paper deals with the properties of the mechanically sensitive C sensory units; the thermoreceptive units are reported elsewhere (Iggo, 1959*b*; Hensel, Iggo & Witt, 1960). Preliminary accounts of the results have been published (Iggo, 1958*b*, 1959*a*).

METHODS

The cats used were anaesthetized with chloralose (80 mg/kg, I.V.) after induction of anaesthesia with ethyl chloride and ether. The right leg was abducted and fixed by two drill pins, one in each end of the tibia, so that the medial surface lay uppermost. The pins were inserted from the lateral side of the leg and care was taken to avoid any damage to the medial skin which is innervated by the saphenous nerve. The saphenous nerve was ligated in the groin, dissected free in the upper thigh, and covered with liquid paraffin B.P. which filled a trough formed by tying the edges of the skin wound to a metal ring. A black Perspex plate was placed under the nerve and the fine dissection (at $\times 40$) carried out with sharp needles and knives. Stimulating electrodes were placed under the saphenous nerve 20–30 mm distal to the recording electrodes. Further details of the recording and stimulating techniques and the methods used to identify single afferent fibres have been described (Iggo, 1958*a*). The thermal stimuli used were provided by metal rods (10 mm diam.) at the desired temperature, by small electrical radiators, and in the later experiments by thermodes with which the temperature could be altered as required without changing the cutaneous mechanical stimulus. The cutaneous temperatures were measured with thermistors (Stantel, Type F2311/300).

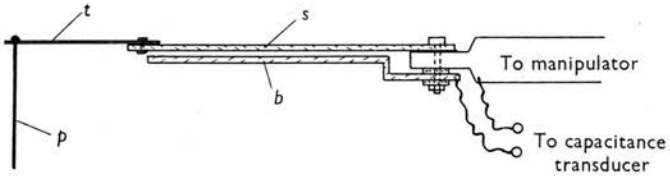


Fig. 1. Diagram to illustrate the mechanotransducer used to stimulate the cutaneous receptors. *s*, flat steel spring; *b*, flat brass plate of dimensions similar to *s*; *t*, tufnol rod screwed on to *s*; *n*, stiff nylon thread, 0·25 mm² tip area, attached to *t*. The unit was attached to a micromanipulator.

Mechanical stimuli. The mechano-transducer used is illustrated in Fig. 1. The nylon probe *p* (tip area 0·25 mm²) was attached to a rigid light rod of tufnol *t*, which was mounted on a flat steel spring *s*. The spring was fixed above, and insulated from, a flat brass plate of

similar dimensions, which in turn was fastened securely to a 10 mm diameter brass tube. The tube was attached to a micromanipulator which had calibrated movements in two directions at right angles to each other and parallel to the skin surface. In use, the probe was lowered on to the skin; as the downward movement continued after contact was made, the spring was deflected away from its resting position, so applying a force to the skin and altering the capacitance of the unit. The resistance of the spring to deformation and thus the sensitivity of the unit could be varied by using different springs. Calibration of the unit with an air-damped balance allowed the force exerted on the skin to be calculated. The voltage output of the condensor transducer (Alexander, 1951) was displayed on cathode-ray tubes for visual control and photography as required.

RESULTS

The receptors described in this paper were in the skin on the antero-medial surface of the lower thigh, the leg and the foot. The afferent fibres were dissected from the saphenous nerve in the upper thigh. There was a distance of at least 100 mm between the recording electrodes and the endings of the afferent fibre. Care was taken to avoid working on receptors in skin close to the wound in order to remove any risk of altered sensibility of the endings, such as the hyperalgesia reported by Lewis (1942). For the same reason, frequent exposure of the skin to noxious stimuli was also avoided. In some early experiments, the use of sharp pins for exploring the skin abolished the responses of single units.

The sensory units to be described were all excited by mechanical stimuli and were either not excited at all by thermal stimuli or gave a brief discharge of impulses when the skin was heated or cooled. They are classed as mechanoreceptors because of this differential sensitivity.

Conduction velocity of the afferent fibres

The conduction velocities of twenty-seven of the fifty-eight afferent fibres reported in this paper were measured by techniques already described (Iggo, 1958*a*). For nine of the fibres, two measurements of the velocity were made; one in the nerve trunk in the thigh and the second for conduction from the cutaneous endings of the fibre to the recording electrodes. For the latter measurement the stimulating electrodes were placed on the skin and the action potential identified by its similarities to action potentials evoked by natural stimuli. With the very fine nerve strands used, which contained only a few live fibres, this procedure was exact. Conduction velocities computed by the latter measurement were invariably slower than by direct measurement in the main trunk of the nerve. The average ratio for the nine fibres was 1·16:1. A similar phenomenon was reported for visceral afferent C fibres in the vagus (Iggo, 1958*a*) and in cutaneous myelinated fibres by Maruhashi *et al.* (1952). The remaining eight conduction velocities in Fig. 2*a* were measured from the skin to the recording electrodes. All the velocities were in the range

0·55–1·25 m/sec for mechanoreceptors; most of the velocities lay between 0·65 and 1·05 m/sec. If an adjustment is made for the slower conduction velocity in the distal part of the nerve these figures become 0·6 and 1·4 m/sec for conduction in the main saphenous nerve. These values are within the limits of the velocities of the slowest and fastest waves of the compound action potential of C fibres in the saphenous nerve reported by Gasser

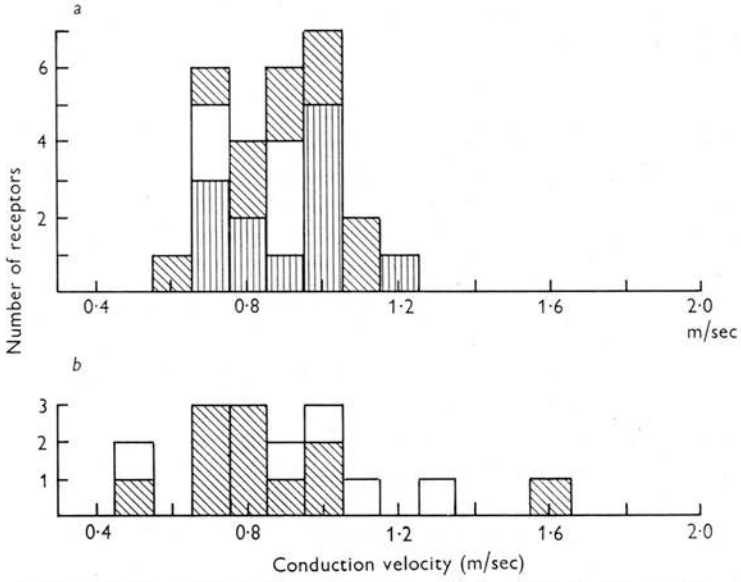


Fig. 2. The relation between the conduction velocity of single afferent fibres and the sensitivity of their endings in the skin. (*a*) Mechanically-sensitive afferent units; these are the units described in this paper. The units are divided into three groups, of low ▥, medium □ and high ▧ sensitivity, as described in the text. There is no obvious segregation according to mechanical sensitivity of the endings. (*b*) Temperature-sensitive units; ▧ heat-sensitive, □ cold-sensitive. These results are taken from Iggo (1959) and Hensel *et al.* (1960). The conduction velocities in this figure are for conduction from the endings in the skin to the recording electrodes.

(1950). One fibre with a conduction velocity of 2·6 m/sec was recorded in the present work but the receptor was not identified; this velocity is at the upper limit for conduction in C fibres according to Gasser's results.

There was no apparent relation between the mechanical sensitivity of the mechanoreceptors and the conduction velocity of the afferent fibres. Figure 2*b* shows that the same conclusion holds for the thermoreceptors; there is no preferred velocity for the thermoreceptors and, as with the mechanoreceptors, the majority lie between 0·65 and 1·05 m/sec. There does not seem to be any segregation according to the thermal sensitivity of the units either.

The conduction velocity of the remainder of the units in this paper were assessed either from less accurate measurements of the conduction time (six fibres) or from the general features of the response to stimulation of the receptors (twenty-six fibres). Three useful guides to the size of the fibres were: (*a*) the interval between stimulation of the receptor and the arrival of the first impulse at the recording electrodes, a few milliseconds for myelinated fibres and more than 100 msec for the C fibres; (*b*) the configuration of the impulse and in particular the sound produced when the amplified impulses were played through a loudspeaker; the C spikes were longer than the A spikes, and less sharply inflected; and (*c*) the presence of an after-discharge. The last was the least reliable guide, since although the afferent units with myelinated fibres conducting impulses as slowly as 9 m/sec did not exhibit an after-discharge, neither did all the C fibres. Assessments of conduction velocity based on these indirect measures are, of course, less reliable than the direct measurements, and the results from such units were used only to supplement the data obtained from twenty-seven units of known conduction velocity. Whenever a unit with a new type of response to natural stimulation was isolated, the conduction velocity of the afferent fibre was one of the first measurements made.

The response to mechanical stimulation of the skin

The standard stimulus used in the present work was pressure on the skin with a vertically applied nylon thread (cross-sectional area 0·25 mm^2). The load (a force of 5 g wt. in Fig. 3) reached a steady value within 100–500 msec of its application and the probe was kept at the final position until it was removed after some seconds (Fig. 3). The rate of application was not constant from trial to trial since the probe was lowered on the skin by manually turning the vertical movement of the micromanipulator on which the probe was mounted. The discharge of impulses in the afferent fibres reached its peak frequency within 100 msec of the first impulse and remained at or near the peak value for as long as the load was approaching the final value and for a short time after the steady value was reached. In the example shown (Fig. 3) there was an initial burst (peak frequency 60/sec) lasting 500 msec, after which the frequency of discharge fell quickly at first and then more slowly. In this figure the upper record has been moved to the left by a distance which equals the conduction time from the nerve endings to the recording electrodes. As a result, the relation between stimulus and response of the nerve-endings is shown more clearly. A similar response is shown in Fig. 5. The most persistent discharge of impulses was obtained when the probe was on the centre of the receptive field. At one trial the peak discharge was 50/sec, this fell to 16/sec after 1 sec, and 10 sec later it was 4/sec. With some units,

the discharge persisted at frequencies as high as 20/sec for 30 sec. This pattern of response was characteristic of the C fibres, an initial high-frequency burst of impulses during and immediately after application of the stimulus followed by a variable persistence of the discharge at a

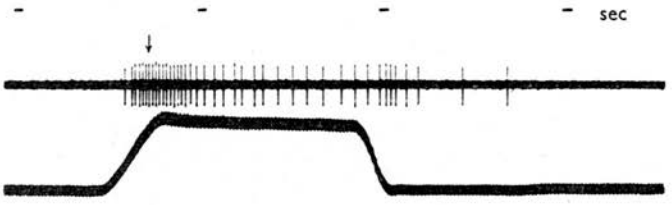


Fig. 3. The response of a C mechanoreceptor to mechanical stimulation of the skin. Initially the probe was stationary just above the skin and was then lowered and reached its final position in 350 msec. This position was maintained for 1 sec and the probe was then lifted clear of the skin. The initial burst of impulses reached a peak frequency of 60/sec as the load was being applied, after 1 sec the frequency had fallen to 12/sec. There was an off-burst as the probe was removed from the skin and a brief after-discharge.

In this figure the upper trace, which shows the impulses in a single C fibre, has been moved to the left by a distance which corresponds exactly with the conduction time from the nerve endings to the recording electrodes. The arrow indicates the *original* position of the first impulse. This has been done to show more exactly the temporal relation between stimulus and response. It has not been done for any other records in this paper.

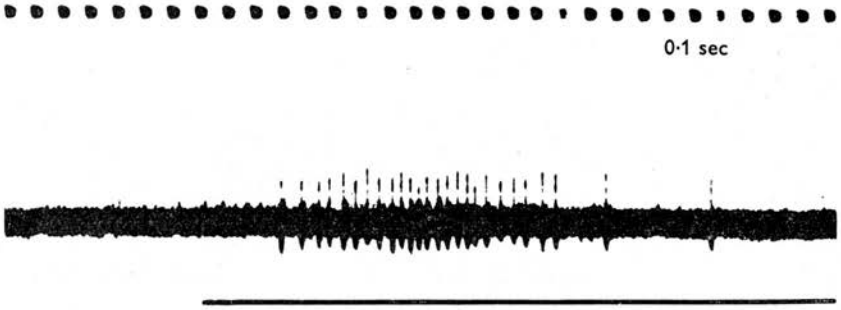


Fig. 4. The brief response of a low-sensitivity C mechanoreceptor to squeezing a fold of the skin with forceps. The stimulus indicated by the lower line was continued throughout the latter part of the record, at which time the unit was not responding.

gradually falling frequency during the steady maintenance of the stimulus. An example of a brief response is given in Fig. 4 which shows the discharge of impulses in the fibre from an insensitive mechanoreceptor in response to squeezing a fold of the skin with forceps.

Frequency of discharge in single C fibres

The peak frequency of discharge in response to natural stimulation was 100 impulses/sec. This high frequency, evoked only by high-intensity stimulation, was rarely maintained for more than a few impulses even with very rapidly applied loads. The peak frequencies in a sample of nineteen fibres ranged from 20 to 65 impulses/sec (mean 44). The sample included examples of all the categories enumerated in Fig. 7. There was no attempt to use maximal stimuli, nor were fibres firing at high frequencies selected. A more significant index of the activity in the C fibres is probably the total number of impulses carried in 1 sec. This ranged from 17 to 48 impulses (mean 24) for the same group of fibres, in the first second of stimulation. In some fibres the discharge persisted at 30 impulses/sec for several seconds during stimulation but more often the rate of firing had fallen to 5–10/sec, or had stopped, within a few seconds. Examples of single units are illustrated in Figs. 3, 4 and 5. Similar rates of firing were found for the thermoreceptors with afferent C fibres (Iggo, 1959*b*; Hensel *et al.* 1960) and for the visceral afferent C fibres (Iggo, 1957*a*, *b*).

Repetition of brief mechanical stimulation sets up an irregular discharge of impulses. For example, stroking the skin 4 times/sec gave frequencies in a single fibre fluctuating between 5 and 50 impulses/sec.

The rate of discharge in small myelinated fibres has also been recorded. With small A fibres (e.g. an A fibre with a conduction velocity of 9 m/sec) the frequency exceeded 200 impulses in the first second of stimulation. As with the C fibres there was an intermittent discharge when the skin was stroked repetitively; the bursts of impulses were, however, even more distinct than with the C fibres.

Size of the receptive field

The receptive field in these experiments is defined as that area of skin from which a steady discharge of impulses could be evoked in the afferent fibre by the weakest stimulus to the skin. The fields as measured probably over-estimate the area of skin in which the sensitive nerve endings were present, because the spread of the stimulus through the skin was unavoidable.

The field was measured in ten fibres by mapping the response of the individual unit to punctate stimulation with the 0·25 mm^2 probe, mounted on a micromanipulator. The pressure was monitored in order to produce a standard stimulus. Typical results are illustrated in Fig. 5. At the most sensitive spot on the skin the discharge appeared with the shortest latency, the highest frequency and the slowest adaptation. When the probe was moved away from such a spot there was a smaller response

until with the probe 3 mm away from the centre of the field, the unit failed to fire unless the stimulus intensity was increased. The influence of the position of the stimulus on the latency of the response is shown in Fig. 5*b*. At the centre of the field the latency was 145–165 msec, and if the conduction time (130 msec) is subtracted, this is reduced to 15–35 msec. The latency 2 mm away had increased to more than 400 msec.

A flat surface was necessary for accurate mapping and the most successful experiments were those in which the receptor was in the medial skin of the thigh or leg. For the ten carefully mapped units the fields measured from 2×2 to not more than 5×5 mm^2. In seven other fibres the fields were mapped less accurately, but similar dimensions were found. No unit was found with a larger receptive field. However, for the hair receptors and the most sensitive mechanoreceptors, the apparent field was sometimes as large as 50×50 mm^2. The size depended on the intensity of stimulation. Such apparently large fields resulted from the use of unmonitored stimuli. Slight movements caused by exploring the skin and hair were sufficient, with the most sensitive units, to excite endings in skin several centimetres away from the probe. Progressive reduction of the stimulus led eventually to the accurate location of the usual small field.

There was never more than one small part of the skin surface from which a discharge of impulses could be evoked by a stimulus about twice threshold. The single C fibres presumably, therefore, terminate in a small area of skin and any branching must occur close to the terminations.

Rate of adaptation

The discharge of impulses provoked by pressing steadily on the centre of the receptive field of a C mechanoreceptor did not persist indefinitely even when the stimulus was well above threshold. The following result is typical of many of the more sensitive units. There was an initial burst of impulses at 60–70/sec as the load was being applied. After 1 sec the frequency had fallen to 25/sec. This quick phase of adaptation was succeeded by a more gradual fall in the frequency. At 1·5 sec, the rate was 16/sec and after 10 sec it was 4/sec. Other units adapted more slowly: 20 sec after the load was applied one unit was firing at 30/sec. Still other units adapted more quickly, e.g. Fig. 4 shows a response to squeezing the skin which lasted only 1·5 sec even though the stimulus was maintained.

The A hair receptors and the A touch receptors adapted much more quickly than the comparable C fibres. The slowly-adapting A pressure receptors in the cat and rabbit, on the other hand, have a very much more persistent discharge and were still firing after 5 min of steady stimulation (Frankenhaeuser, 1949; N. Fjällbrant & A. Iggo, unpublished experiments).

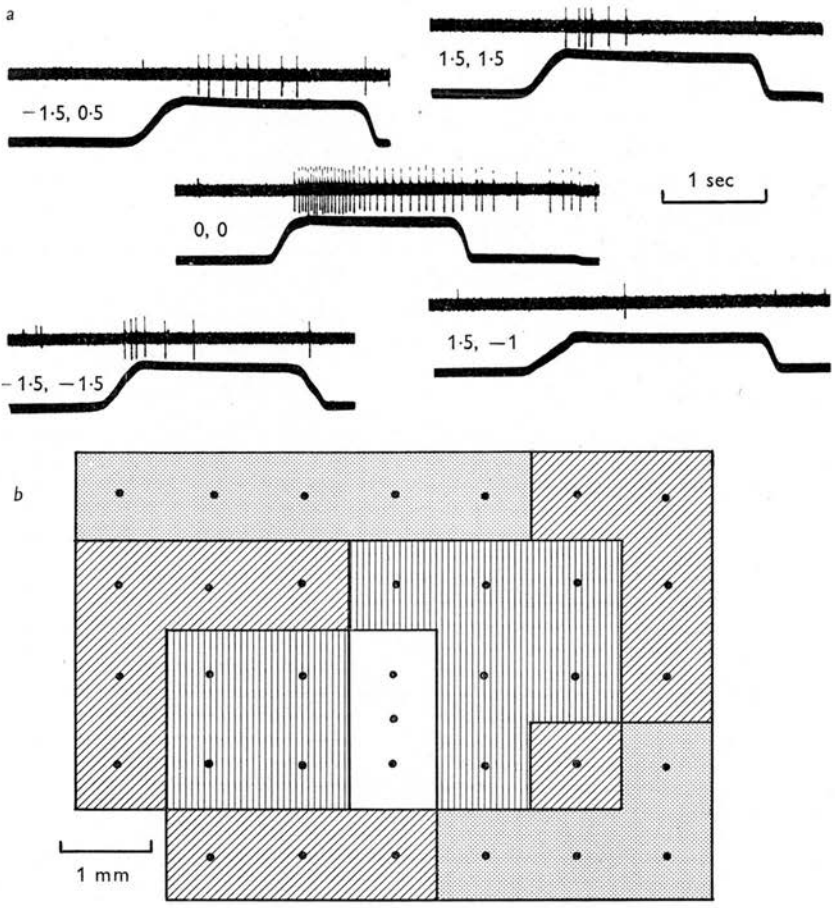


Fig. 5. The receptive field of a C mechanoreceptor. (*a*) shows five records obtained when different spots on the skin were touched with the probe. The final load is nearly the same (2 g wt.) for all positions. The distances of the probe from the most sensitive central position are indicated in millimetres. (*b*) is a diagram summarizing all the results for the same unit. The skin was stimulated on a 1 mm grid and each spot is indicated by a filled circle in the diagram. The latency of the first impulse at each position is shown by the shading. At the centre of the field the latency was least, the rate of firing was highest and the persistence of the discharge was longest. The following meaning is given to the shading:

	Latency (msec)	Impulses in first 1·5 sec
□	145–165	35
▥	200–350	22
▨	440–750	6
▩	—	0

After-discharge

A characteristic feature of the C mechanoreceptors, especially the more sensitive examples, was the persistence of the discharge of impulses for as long as 10 sec following a brief stimulation of the skin. It was especially prominent when the skin was stroked (Fig. 6). In this figure, the peak frequency of discharge was similar when the skin was stroked and when it was pressed. The after-discharge, however, was present only after stroking. Another example is shown in Fig. 8*b*; there was a persistent after-discharge in C fibres when the tips of the cat's fur were stroked. The rate of firing

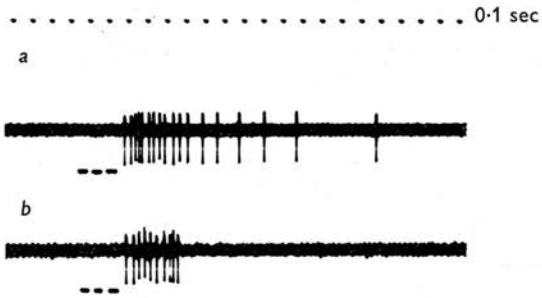


Fig. 6. The response of a C mechanoreceptor (threshold, 50 mg wt.) to (*a*) stroking the skin and (*b*) pressing briefly on the skin with the nylon probe. The dashes indicate the approximate duration of the stimuli.

during the after-discharge was always low. The peak frequency could reach 100/sec when the load was being applied. After removal of the load the frequency of the after-discharge was about 5/sec initially and as it was disappearing the rate would fall to less than 0·5/sec. An especially pronounced after-discharge was found when the skin had been left undisturbed for as long as 30 min. At the same time there was an enhanced excitability of the endings to mechanical stimulation.

Inexcitability as a consequence of repeated stimulation

When a mechanical stimulus was repeated frequently at short intervals, the response to successive stimuli was progressively reduced. This effect was more evident when the stimulus was the nylon probe than when the skin was stroked. In a typical example the receptor was stimulated by pressing on the skin 10 times in 13 sec. The number of impulses evoked by the successive stimuli were 7, 2, 3, 3, 1, 2, 1, 0, 0, 0. One minute later the unit could be excited again by a similar stimulus. Similar stimulation of another unit for 25 sec abolished the response to mechanical stimulation for 4 min, after which there was a gradual recovery of the initial sensitivity. At 15 min the after-discharge reappeared and the response was almost

restored to its original value. Stroking the skin for 30 sec did not alter the immediate response of this same unit to stroking the skin, but did lessen the after-discharge. Rubbing the skin abolished the response to mechanical stimulation.

When an interval of at least 30 sec was left between successive stimuli, the response to a standard stimulus was uniform. More frequent stimulation led to a progressive fall in the response.

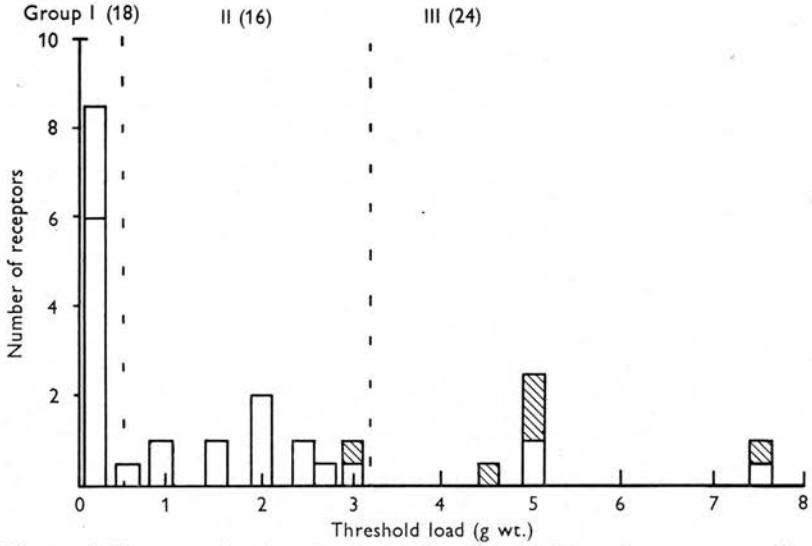


Fig. 7. A histogram showing the threshold values for 24 mechanoreceptors. The hatched areas ▨ refer to 6 thermoreceptors (data from Iggo, 1959*b*). The interrupted vertical lines at 0·5 and 3·1 g wt. indicate the boundaries chosen in the classification of the mechanoreceptors into three groups, I, II, and III according to sensitivity (see text). The numerals in parentheses give the total number of units of the appropriate sensitivity which were found. They include, in addition to the units the sensitivity of which was measured, receptors whose sensitivity was assessed from less accurate tests.

Sensitivity of the mechanoreceptors

There was a wide range in the sensitivity of the mechanoreceptors to mechanical stimulation, as is indicated by the thresholds which ranged from 25 mg wt. to 5 g wt., a ratio of more than 200:1. Figure 7 is a histogram showing the frequency distribution of all the units for which there were threshold measurements. It probably does not represent a fair sample of the mechanoreceptors because the most sensitive endings were the easiest to find. An alternative way to describe the sensitivity of the receptors is in terms of their response to the mechanical stimuli to which the skin is normally exposed and which are also commonly employed in the experimental analysis of reflexes initiated from the skin. These are

touching, stroking, rubbing, pinching, etc. The following division into three categories, although arbitrary, is useful in indicating the wide range in sensitivity of the C mechanoreceptors.

1. *Highly sensitive endings* (18 *fibres*). This group includes the receptors to the left in the histogram in Fig. 7 and a boundary at 500 mg wt. has been chosen. The most sensitive of these units had a threshold of 25 mg wt. Some of these very sensitive units could be excited by touching the tips of the hairs, whereas other equally, or even more, sensitive to direct pressure on the skin could not be excited by similar movements of the hairs. The C hair receptors were excited by moving only the tips of hairs, and the rate of discharge in response to such mild stimulation was surprisingly high; e.g. for one unit the discharge was at 25/sec when the tips of the hairs were brushed and was not more than doubled by a firm stroke of the skin. Another unit gave a burst of impulses lasting 1·5 sec, with a peak frequency of 32/sec, when the hairs were bent backwards. This persistent discharge from the C hair receptors when they were moved from rest to a new position and held there is quite characteristic and very different from the brief burst of impulses at high frequency from the A hair receptors (Fig. 8).

2. *Medium sensitivity* (20 *fibres*). Units excited by stimuli between 0·5 and 3 g wt. are placed in this group. These units were not excited by lightly touching the fur or lightly stroking the skin. A firmer stroke of the skin or light pressure was required.

3. *Low sensitivity* (24 *fibres*), threshold greater than 3 g wt. These units were not excited easily by a firm stroke along the skin with a smooth rod. Firm pressure with the points of forceps or a sharp pin or squeezing a fold of the skin, or pulling the hairs was required. The usual response was a burst of impulses lasting 1–2 sec, e.g. Fig. 4.

Comparison with A mechanoreceptors

The cutaneous receptors innervated by myelinated (A) and by non-myelinated (C) fibres have overlapping thresholds for mechanical stimulation. The thresholds of the most sensitive A touch and A hair receptors were too low to be measured with the transducer used to excite the C mechanoreceptors; they are therefore more sensitive than the most sensitive C mechanoreceptor yet found. Many A pressure receptors had thresholds of more than 1 g wt., i.e. higher than the most sensitive mechanoreceptors, and Frankenhaeuser (1949) has reported similar thresholds for slowly-adapting pressure receptors with afferent fibres in the rabbit's sural nerve. There are still other A pressure receptors which were not excited by loads of less than 5 g wt. (A. Iggo & N. Fjällbrant unpublished observations). Some of these differences are illustrated in

Fig. 8, which shows the activity in a multifibre strand of the saphenous nerve. Very weak stimuli excited only A hair fibres, stronger stimuli brought in C hair fibres and pulling the hairs added one A fibre (with the largest spike in the tracing, an A pressure receptor) together with several other unidentified units. The A pressure receptor could also be excited by pressing on the skin.

The effect of thermal stimuli

If the mechanoreceptors were excited at all by heating the skin, they required much higher temperatures of the stimulating rod than did the

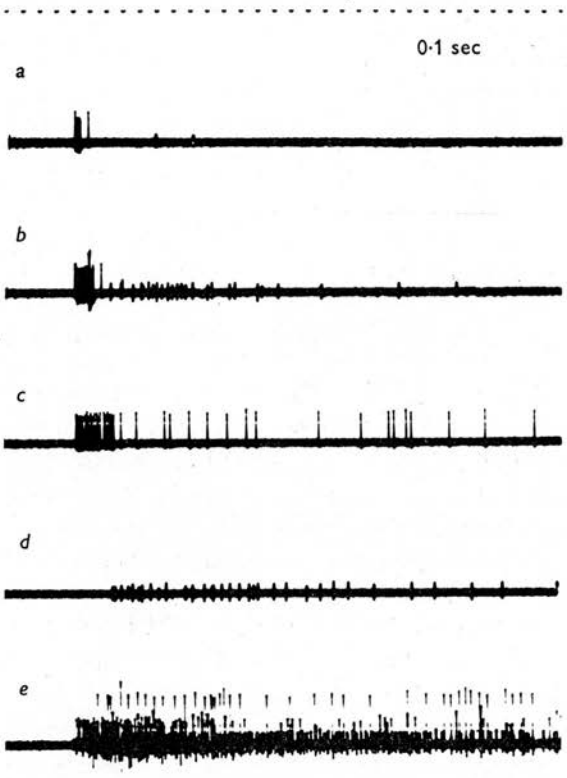


Fig. 8. Impulses in a multifibre strand of the saphenous nerve. *a* and *b* show the response to stroking the tips of the hairs, lightly in *a* and more firmly in *b*. In *a* there is a brief high-frequency burst of impulses in a myelinated fibre; this burst is longer in *b* and in addition there is a later more prolonged burst of impulses in at least two C fibres. These records illustrate the greater sensitivity of the A hair receptors. *c* and *d* show the response to bending the hairs and holding them in their new positions; *c* for the A hair fibre and *d* for the C hair fibres. The discharge in the non-myelinated fibres persists at a relatively higher frequency than in the A fibre. *e* illustrates the effect of pulling on the hairs. Several additional units are now firing, including an A pressure receptor (largest spike).

heat receptors (Iggo, 1959*b*). There was a brief discharge of impulses lasting perhaps 1 sec when rods at 80–100° C touched the skin. The units thereafter failed to respond to previously effective mechanical stimuli and also failed to be excited by the heat. Presumably the nerve endings had been damaged by the high temperatures needed to provoke a response. The heat receptors on the other hand gave consistent responses with much milder thermal stimuli, and continued to respond to appropriate stimulation for at least 3 hr (Iggo, 1959*b*). Zotterman (1936) has also recorded this effect of high temperature in abolishing the response of A mechanoreceptors to mechanical stimulation at the same time as it provoked a discharge of small impulses in other fibres.

TABLE 1. The relative proportions (%) of different types of cutaneous receptors innervated by C fibres

	Total sample of C fibres (97 fibres)						
Fibres ...	Mechanoreceptors 58			Thermoreceptors 39			
				Heating skin		Cooling skin	
Sensitivity ...	High	Medium	Low	High	Low	High	Low
	31	28	41	8	60	16	16

(Data from Iggo, 1959*b*; Hensel *et al.* 1960; and the present paper.)

Cooling the skin may excite C fibres which can also be stimulated by mild mechanical stimulation (Douglas *et al.* 1959). When single C mechanoreceptors were examined, the discharge of impulses excited by cooling the skin was brief and at a low frequency. In order to demonstrate the effect of cooling, it was necessary to ensure that the thermal stimulus was not contaminated by any mechanical component, otherwise the much more effective mechanical stimulus masked the response. Thermodes were used in the most successful experiments. The rate of firing in response to sudden cooling of more than 4° C was less than 5/sec, compared with more than 50/sec in response to mechanical stimulation (see also Hensel *et al.* 1960). In contrast, the C cold fibres responded with a regular discharge of impulses which continued at least 30 min (Hensel *et al.* 1960).

The thermoreceptors had very high thresholds for mechanical stimulation; they fell into category III of the mechanoreceptors, but could be distinguished from the latter by their responses to thermal stimuli.

DISCUSSION

The single fibre results reported in this paper permit unambiguous statements to be made about some of the cutaneous receptors innervated by non-myelinated fibres. The receptors are not uniformly sensitive to all

cutaneous stimuli nor are they excited only by noxious or injurious stimuli. The results are in agreement with those of Zotterman (1939), Maruhashi *et al.* (1952) and Douglas & Ritchie (1957*a*), in showing that some of the C receptors have a high mechanical sensitivity. They go further, however, in establishing a wide range in the sensitivity of the individual units.

Douglas *et al.* (1959) have re-opened the question of the specificity of the afferent nerve endings by their suggestion that the excitation of the same C fibres by touch and by cooling is an example of dual specificity. Their methods, however, do not allow the responses of individual units to be followed. When single C fibres were examined the mechanoreceptors and the thermoreceptors exhibited significant differences in their responses to the same stimuli, particularly with respect to thresholds, rates of firing and the persistence of discharge during constant stimulation.

Table 1 gives a summary of all the results with single afferent C fibres which have been obtained in the recent series of investigations. The sample is biased, first, because among the mechanoreceptors the most sensitive were the easiest to find, and secondly, in the work on thermoreceptors reported by Hensel *et al.* (1960) the thermoreceptors were selected and the mechanoreceptors were discarded. It is evident, however, that the mechanoreceptors, as Douglas & Ritchie (1957*a*) have suggested, are the predominant type. There are also numerous thermoreceptors with C afferent fibres.

There was no apparent relation between the conduction velocity of the afferent fibres and either the degree or kind of sensitivity of the nerve endings to natural stimuli (Fig. 2). The scarcity of fibres of the slowest conduction velocity in the sample may reflect either the greater difficulty of isolating them as single units, or a real difference in the specificity of the endings. Douglas & Ritchie (1957*a*) also had difficulty in exciting the slowest fibres with mechanical stimuli.

The rate of firing in individual C fibres is shown by the present work to be lower than in the myelinated cutaneous afferent fibres, where it may exceed 800 impulses/sec in response to natural stimulation of the receptors. Direct recording of the impulses in single C fibres during natural stimulation of both visceral (Iggo, 1957*a*, *b*) and cutaneous (Iggo, 1959*b*; Hensel *et al.* 1960; this paper) afferent fibres has revealed rates of firing up to 100 impulses/sec. Douglas & Ritchie (1957*b*) using an indirect method estimated that the 'maximum rate at which mammalian non-medullated cutaneous afferent fibres fired in response to touch was low'—not faster than 10 impulses/sec. This very low estimate may have been due to the population of C fibres failing to respond in the uniform manner assumed in their analysis. The stimuli which they used would be likely to produce

widely fluctuating rates of discharge in an inconstant sample of the population which they were testing.

Previous estimates of the size of the receptive fields for C fibres have relied on extrapolation from work on myelinated afferent fibres (Tower, 1940; Bishop, 1946), and led to the expectation that they would be large. The present results show that individual C fibres have quite limited fields. The mechanisms of cutaneous localization may therefore be simpler than has hitherto been assumed, since there is probably much less overlap of receptive fields than was expected.

The non-myelinated cutaneous fibres can no longer be regarded as exclusively nociceptive in function, since the most sensitive C mechanoreceptors and C thermoreceptors are comparable in sensitivity with touch and temperature sense in man. There is, however, a wide range in the thresholds for natural stimulation within the group of non-myelinated cutaneous afferent fibres. An attempt to identify the nociceptors by making close-arterial injections of allegedly pain-producing chemicals into the skin was inconclusive (Fjällbrant & Iggo, 1959).

SUMMARY

1. Electrical activity was recorded in single afferent fibres, with conduction velocities ranging from 0·55 to 1·25 m/sec, dissected from the saphenous nerves of cats anaesthetized with chloralose.

2. The cutaneous receptors were excited by mechanical stimuli, and the threshold loads ranged from 25 mg wt. to 7·5 g wt. The receptors were insensitive to heat and gave a brief discharge when the skin was cooled.

3. The discharge in individual fibres reached peak frequencies of 100/sec and persisted at rates as high as 50/sec for 1 sec. This discharge had an initial quick phase of adaptation, lasting less than 1 sec followed by a slow phase which, exceptionally, lasted for 30 sec. Frequent repetition of stimulation led to a temporary inexcitability of the ending.

4. The receptive fields of the receptors were small, ranging from 2×2 mm^2, to not more than 5×5 mm^2.

I wish to thank Mrs N. Fjällbrant for her help with some of the experiments and Miss W. Sandie for her willing assistance throughout.

REFERENCES

ADRIAN, E. D. (1926). The impulses produced by sensory nerve endings. *J. Physiol.* **61**, 49–72.

ADRIAN, E. D. (1931). The messages in sensory nerve fibres and their interpretation. *Proc. Roy. Soc.* B, **109**, 1–18.

ALEXANDER, W. (1951). An electron ultramicrometer. *Electron. Engng*, **23**, 479–480.

BISHOP, G. H. (1946). Neural mechanisms of cutaneous sense. *Physiol. Rev.* **26**, 78–102.

DOUGLAS, W. W. & RITCHIE, J. M. (1957*a*). Non-medullated fibres in the saphenous nerve which signal touch. *J. Physiol.* **139**, 385–399.

DOUGLAS, W. W. & RITCHIE, J. M. (1957*b*). On the frequency of firing of mammalian non-medullated fibres. *J. Physiol.* **139**, 400–407.

DOUGLAS, W. W., RITCHIE, J. M. & STRAUB, R. W. (1959). Discharges in non-myelinated (C) fibres in the cat's saphenous nerve in response to changing the temperature of the skin. *J. Physiol.* **146**, 47–48*P*.

FJÄLLBRANT, N. & IGGO, A. (1959). Cutaneous afferent fibres and pain-producing chemicals. *J. Physiol.* **145**, 28–29*P*.

FRANKENHAEUSER, B. (1949). Impulses from a cutaneous receptor with slow adaptation and low mechanical threshold. *Acta physiol. scand.* **18**, 68–74.

GASSER, H. S. (1950). Unmedullated fibres originating in dorsal root ganglia. *J. gen. Physiol.* **33**, 651–690.

GASSER, H. S. (1955). Properties of dorsal root unmedullated fibres on two sides of the ganglion. *J. gen. Physiol.* **38**, 709–728.

HENSEL, H., IGGO, A. & WITT, I. (1960). A quantitative study of sensitive thermoreceptors with C afferent fibres. *J. Physiol.* (in the Press).

IGGO, A. (1957*a*). Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. *Quart. J. exp. Physiol.* **42**, 130–143.

IGGO, A. (1957*b*). Gastric mucosal chemoreceptors with vagal afferent fibres in the cat. *Quart. J. exp. Physiol.* **42**, 398–409.

IGGO, A. (1958*a*). The electrophysiological identification of single nerve fibres, with particular reference to the slowest conducting vagal afferent fibres in the cat. *J. Physiol.* **142**, 110–126.

IGGO, A. (1958*b*). Single C fibres from cutaneous receptors. *J. Physiol.* **143**, 47–48*P*.

IGGO, A. (1959*a*). A single unit analysis of cutaneous receptors with C afferent fibres, in *Ciba Foundation Study Group. Pain and Itch*, pp. 41–49. London: Churchill.

IGGO, A. (1959*b*). Cutaneous heat and cold receptors with C afferent fibres. *Quart. J. exp. Physiol.* **44**, 362–370.

LEWIS, T. (1942). *Pain*. New York: Macmillan.

MARUHASHI, J., MIZUGUCHI, K. & TASAKI, I. (1952). Action currents in single afferent nerve fibres elicited by stimulation of the skin of the toad and cat. *J. Physiol.* **138**, 80–97.

TOWER, S. (1940). Units for sensory reception in the cornea: with notes on nerve impulses from sclera, iris and lens. *J. Neurophysiol.* **3**, 486–500.

ZOTTERMAN, Y. (1936). Specific action potentials in the lingual nerve of cat. *Skand. Arch. Physiol.* **75**, 105–120.

ZOTTERMAN, Y. (1939). Touch, pain and tickling: an electrophysiological investigation on sensory nerves. *J. Physiol.* **95**, 1–28.

J. Physiol. (1960), **153**, *pp.* 113–126
With 9 text-figures
Printed in Great Britain

A QUANTITATIVE STUDY OF SENSITIVE CUTANEOUS THERMORECEPTORS WITH C AFFERENT FIBRES

By H. HENSEL, A. IGGO* and INGRID WITT
From the Department of Physiology, Marburg/Lahn, Germany

(*Received* 16 *March* 1960)

It has been demonstrated that heating or cooling the cat's skin can elicit a discharge of impulses in slowly-conducting (C) afferent fibres (Iggo, 1958*b*, 1959*a*, *b*; Douglas, Ritchie & Straub, 1959; Douglas & Ritchie, 1959). However, it was not clear whether the C group contained fibres from 'specific' thermoreceptors comparable in sensitivity with those in the cat's tongue (Hensel & Zotterman, 1951*a*; Dodt & Zotterman, 1952). Most of the C fibre thermoreceptors hitherto found responded only to rather extreme heating or cooling (Iggo, 1959*a*, *b*) and therefore were possibly concerned with pain. They were relatively insensitive to mechanical stimulation. There are also numerous C fibres which respond both to mild mechanical stimulation and to sudden cooling of the skin (Douglas *et al.* 1959; Iggo, 1959*a*). They have been classified as mechanoreceptors by Iggo (1960).

In the present investigation sensitive specific cold and warm receptors with afferent C fibres have been found and their behaviour has been studied quantitatively. The opportunity has also been taken to examine quantitatively the responses of C heat receptors (Iggo, 1959*b*) and of a few C mechanoreceptors to thermal stimulation. A preliminary account of these results has been published (Hensel, Iggo & Witt, 1959).

METHODS

The cats used were anaesthetized with chloralose (60 mg/kg intramuscular) and urethane (250 mg/kg intramuscular). After depilation the leg was fixed by drill pins inserted through each end of the tibia. The skin over the saphenous nerve was incised, the nerve was ligated proximally and dissected free from connective tissue. The cut edges of the skin were tied to a metal ring and the trough formed was filled with liquid paraffin B.P. Fine strands of the nerve were dissected on a black Perspex plate and laid on silver electrodes. A pair of stimulating electrodes was placed under the nerve about 3 cm distal to the recording electrodes and an earthed plate was placed between the two pairs of electrodes. The amplified impulses in the fine nerve strand were recorded by means of two separate systems, each consisting of a cathode-ray oscilloscope and a recording camera. The first system recorded the impulses in the usual way with a stationary cathode-ray beam and moving bromide paper (Fig. 1*A*). The expanded sweep of the second oscilloscope was triggered by the square-wave pulse

* On leave from Physiology Department, University of Edinburgh.

generator used for electrical stimulation of the nerve, and allowed an exact measurement of conduction velocity. Photographic records of the moving beam were taken on slowly moving bromide paper (Fig. 1*B*). In order to test mechanical sensitivity, the skin was stroked or pressed with a wooden pin or touched with cotton wool. Calibrated hairs of various stiffness were used for more quantitative stimulation.

Quantitative thermal stimulation was accomplished by a water-circulated thermode of 2 cm diam., which was placed on the receptive field of the afferent fibre. The water was supplied by 4 ultra-thermostats set at various constant temperatures ($\pm 0{\cdot}02°$ C). By means of a special switch each thermostat could be connected with the thermode, and constant temperatures or rapid thermal changes could thus be applied to the skin. The thermode allowed thermal changes to be imposed without any alteration in the mechanical stimulus. A fine thermocouple was placed on the receptive field and the temperature was recorded synchronously with the deflexions of the first oscilloscope with a special recording camera (Hensel, 1953). In some experiments thermal radiation was also used for heating the skin.

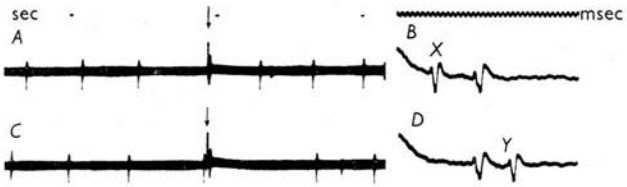


Fig. 1. The identification of the active cold fibre in a multi-fibre strand of the saphenous nerve.

A, Spontaneous discharge of a single C cold fibre and effect of electrical stimulation of the saphenous nerve (fibre No. 1). Arrows mark stimulus artifacts in *A* and *C*. *B*, Record with expanded sweep corresponding to that part of *A* marked by arrow. The first impulse (marked *X*) is the spontaneous cold fibre discharge, the second is from another C fibre excited by electrical stimulation. *C*, The same discharge as in *A*, but with stronger electrical stimulation. There is now a gap in the series of impulses. *D*, Expanded sweep. Both impulses were caused by electrical stimulation, the first is identical with the second in the upper record, the second is in the cold fibre. The conduction velocity was 1·1 m/sec. (See text for further explanation.)

RESULTS

Identification of single C fibres

This was based on the 'collision' technique (Iggo, 1958*a*), which makes it possible to measure conduction velocity in the one active fibre of a strand containing other fibres capable of conducting but not active. Figure 1 shows a record, taken with two oscilloscopes, of the impulses in a thin strand of the saphenous nerve. The steady discharge of a single cold fibre (fibre No. 1) in Fig. 1*A* and *C* was caused by a constant skin temperature of 25° C. During the natural discharge two electric shocks were sent into the saphenous nerve. The upper pair of tracings (Fig. 1*A* and *B*) shows a failure of the electrically produced impulse to interfere with the train of impulses from the receptor; the expanded sweep, Fig. 1*B*, shows one of the ascending impulses first (marked *X* in the figure) followed by an impulse in a different fibre caused by the shock to the nerve.

The lower pair of tracings shows in Fig. 1*C* a gap in the train of impulses. This appears immediately after the shock artifact and was caused by collision of the electrically produced antidromic impulse, corresponding to *Y*, with the natural impulse from the receptor. The expanded sweep, Fig. 1*D*, shows two impulses; the first is identical with the second impulse in the upper record and the second, *Y*, is an impulse in the cold fibre caused by the shock to the saphenous nerve. The similarity of this impulse to the first impulse in the upper tracing is further evidence that the second impulse in the lower tracing is in the cold fibre. The time between electrical stimulus and recorded impulse was 28 msec and the conduction distance was 31 mm, so that the conduction velocity turned out to be 1·1 m/sec.

In some experiments the slowly conducting fibres were identified only by the shape and duration of the C impulse and the typical sound in the loudspeaker.

Receptors excited by cooling the skin

Several C fibres responded to moderate cooling of the skin with an increase in frequency, and to heating with a decrease or complete inhibition, of the discharge. Within a certain range of constant temperatures a steady discharge was observed. These cold fibres were not excited by the mechanical stimuli used. In some examples the size of the receptive field was assessed by stimulation with small thermodes. The receptive fields were not larger than a few square millimetres.

The impulses in a single C fibre when the skin was cooled are shown in Fig. 2. A steady discharge can be seen at the constant initial temperatures (Fig. 2*C*, *D*, *E*). During cooling the impulse frequency rose rapidly, reached a maximum within the first second and then declined gradually. In the records *B*–*E* a slight transient temperature rise of about 1° C just before cooling led to a complete inhibition of the steady discharge.

The discharge frequency of another single C fibre (fibre No. 3) on cooling and rewarming the skin is shown in Fig. 3. The initial temperatures between 31 and 33·5° C corresponded to an indifferent temperature sensation in man. Even very slight cooling from 33 to 32·3° C increased the frequency from 1 to 22 impulses/sec, whereas warming by about a tenth of a degree caused the discharge to stop immediately. The 'dynamic' sensitivity of this cold receptor was 30 impulses/sec. ° C. This means that sudden cooling of 1° C would raise the impulse frequency by 30 impulses/sec. At indifferent temperatures a discharge was elicited by cooling of only 0·2° C. This was the final value of the external temperature change; the actual cooling when the first impulse was recorded was smaller than 0·1° C.

At constant temperatures the cold receptors exhibited a steady discharge, the frequency being a function of absolute temperature. The steady

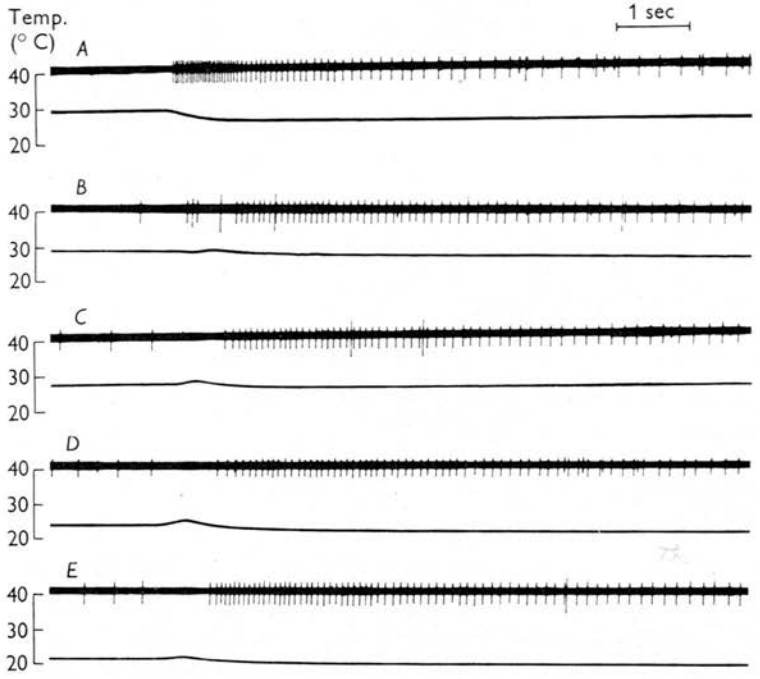


Fig. 2. Afferent impulses of a single C fibre and skin temperature when cooling the skin (fibre No. 1). Conduction velocity 1·1 m/sec. *A*, Cooling from 29 to 25·5° C; *B*, 29·3–28° C; *C*, 28·0–26·5° C; *D*, 24·5–22·5° C; *E*, 22–20·5° C.

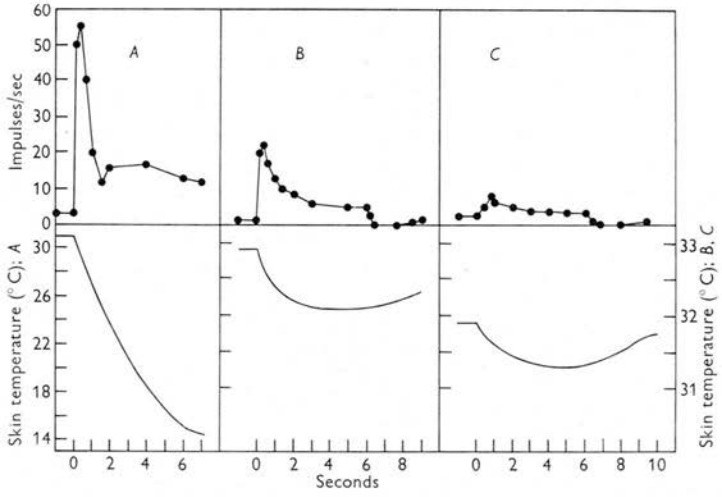


Fig. 3. Impulse frequency of a single cold fibre when cooling and warming the skin (fibre No. 3). The conduction velocity of the fibre was 1·5 m/sec. The left-hand temperature scale refers to *A*, and the right-hand scale to *B* and *C*.

discharge of the cold fibre No. 3 at constant temperatures between 28·8 and 14·2° C is shown in Fig. 4, and the steady discharge frequency is plotted against temperature in Fig. 5. Curve 1 was obtained by lowering the constant temperature stepwise from 37 to 14° C, keeping the temperature constant for 3 min after each step. Curve 2 was the result of the reverse procedure, starting at the lowest temperature. As the two curves are not identical it is probable that the adaptation time of 3 min was not

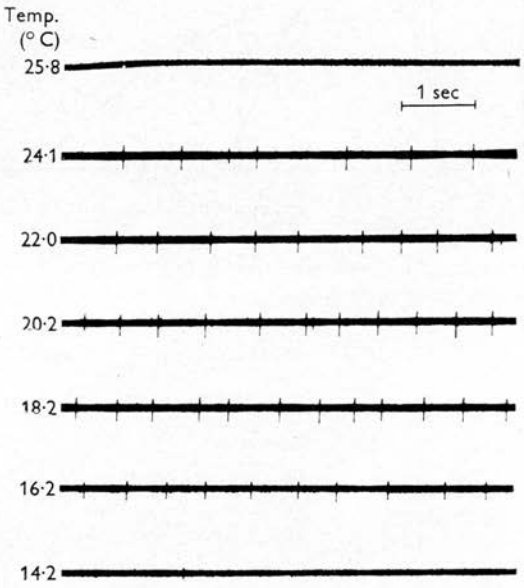


Fig. 4. Steady discharge of impulses in the single *C* fibre shown in Fig. 3 (fibre No. 3) at various constant temperatures of the skin.

quite sufficient to reach the final value of the steady discharge. The maximum frequency was reached at temperatures of 16·5 and 19·5° C, respectively. The width of the maximum varies considerably for different fibres (Fig. 6). Whereas fibre No. 3 has a well defined maximum, the cold fibre No. 1 exhibits practically the same impulse frequency over a temperature range of more than 10° C.

Receptors excited by heating the skin

C fibres have been found which could be excited by an external warming of only 0·3° C. This was the final value of temperature rise, the actual value at the first impulse being less than 0·1° C (Fig. 7*A*). Cooling by the same amount (Fig. 7*B*) inhibited the discharge completely. Most of these warm fibres were not excited by the mechanical stimuli used; one fibre had a

mechanical threshold of 2 g when touching the receptive field with a von Frey hair. There was no reaction of the warm fibres on sudden cooling from 30–33° C to low temperatures (10° C).

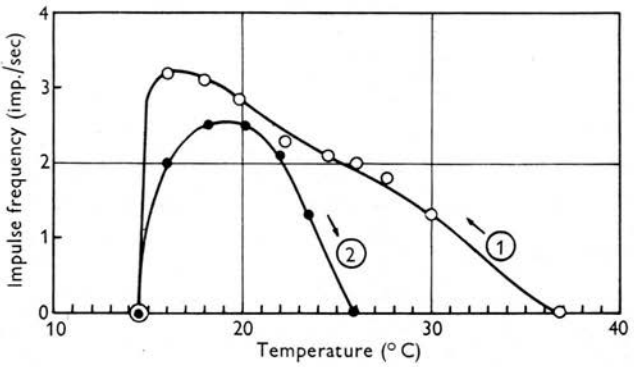


Fig. 5. Steady discharge frequency of a single C fibre (fibre No. 3), excited by cooling, at various constant temperatures. 1, Starting from high temperatures; 2, starting from low temperatures.

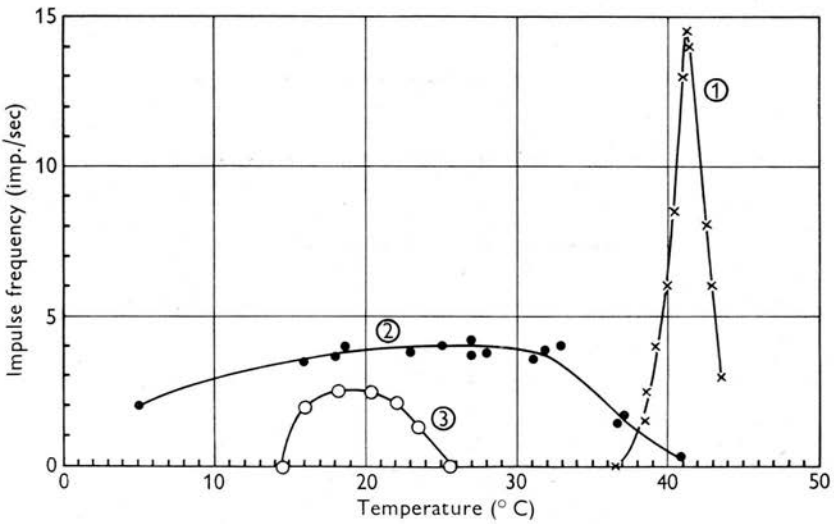


Fig. 6. Steady discharge frequency of three different single C fibres as a function of constant skin temperature. 1, Fibre excited by warming, fibre No. 8; 2, fibre excited by cooling, fibre No. 1; 3, fibre excited by cooling, fibre No. 3.

The frequency of discharge in a single warm fibre during various temperature changes, starting from indifferent initial temperatures between 31 and 33·5° C, is shown in Fig. 8. At a constant temperature of 40° C (last curve) a steady discharge is seen. Slight cooling of a few tenths of a degree stopped the discharge; slight warming by the same amount made the

discharge reappear. The dynamic sensitivity of this warm receptor was +33 impulses/sec.° C, which is of the same order as that of the C cold receptor. The maximum frequency of discharge and the time course of adaptation was also similar to that of the cold fibres (cf. Fig. 3).

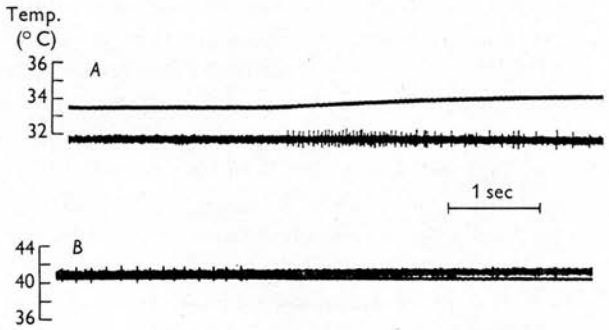


Fig. 7. Afferent impulses recorded in a single warm fibre (fibre No. 7) and the skin temperature, during slight warming and cooling of the skin.

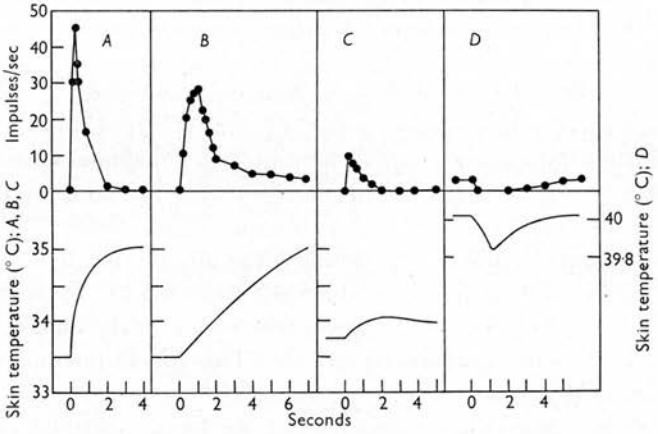


Fig. 8. Frequency of the discharge of impulses in a single C fibre (fibre No. 7), from a warm receptor, during cutaneous temperature changes. The left-hand temperature scale applies to *A*, *B* and *C* and the right-hand scale to *D*.

The warm fibre with the highest sensitivity exhibited a steady discharge at constant temperatures between 38·5 and 43° C. It fired a regular sequence of impulses, and showed a very sharp maximum at 41·2° C (Fig. 6, curve 1).

A number of non-myelinated fibres were found which could only be excited by heating the skin from indifferent temperatures (30–33° C) to more than 37° C. These probably correspond to the C heat receptors described by Iggo (1959*b*). In some cases, only a slow phasic discharge occurred during the temperature rise; other fibres also fired at high constant

temperatures, some with periodical bursts of impulses. One fibre with a high maximum frequency of 80 impulses/sec was silent at temperatures above 58° C; the inhibition was reversible. No other fibres were tested at these high cutaneous temperatures.

In order to prevent damage of the skin by the high temperatures, the thermal stimuli were maintained for not longer than about 30 sec. Under these conditions the threshold temperatures for a steady discharge of at least 30 sec duration varied from 38·5 to above 48° C in various fibres. Since the constant temperatures were maintained only for a short period of time, the values obtained may possibly not represent the true thresholds for the steady discharge.

Two of these less sensitive warm fibres were excited by the mechanical stimuli used. One fibre reacted to pressing the finger against the skin with a maximum discharge of 35 impulses/sec; on heating, the same fibre reached a maximum frequency of 44 impulses/sec.

Some features of 18 non-myelinated cold and heat fibres are summarized in Table 1. The temperature ranges for the steady discharge of the warm fibres 8 to 18 may not be correct, because the adaptation time was only 30 sec.

Receptors excited by mechanical stimulation

Cutaneous mechanoreceptors with afferent C fibres are more easily found than thermoreceptors, since the number of mechanosensitive nerve endings seems to exceed that of thermoreceptors. The sensitivity of these C mechanoreceptors varies within a wide range (Iggo, 1960). The threshold of the most sensitive units (hair 20 mg) was similar to that of the most sensitive A mechanoreceptors in the cat's skin (Witt & Hensel, 1959). A typical feature of the C mechanoreceptors was a marked after-discharge, as described first by Zotterman (1939). This phenomenon was never observed in the A fibres.

In the present investigation impulses of mechanosensitive C fibres have not been studied systematically. We have confirmed previous findings (Iggo, 1959*a*, *b*, 1960), that C mechanoreceptors were excited by cooling the skin. In our occasional observations the maximum frequencies of three different C fibres amounted to only 17, 11 and 5 impulses/sec on sudden severe cooling from 32 to 8° C, whereas mechanical stimulation caused a discharge frequency as high as 100 impulses/sec. The lowest threshold for a phasic discharge of a C mechanoreceptor was cooling by 2° C. The discharge of two C fibres is shown in Fig. 9; one was excited by heating the skin (a warm fibre) and the other (spikes downward) reacting to light mechanical vibration of the thermode due to turbulence caused by switching on the hot and cold water supply. The latter fibre was excited briefly during cooling of the skin, whereas the warm fibre was not.

TABLE 1. Responses of single thermosensitive C fibres

Fibre no.	Conduction velocity (m/sec)	Response to thermal stimulation†		Max. frequency (imp./sec)	Threshold temp. change (° C) (initial temp. *ca.* 32° C)	Temp. for steady discharge (° C)		Response to mechanical stimulation‡
		Cooling	Warming			Lowest	Highest	
1	1·1	+	−	38	< −1 (*x*)	14	26 (> 30)	0
2	*C**	+	−	45	< −4	.	< 28	0
3	1·5	+	−	55	−0·2	< 6·5	41	0
4	.	+	−	.	.	.	*ca.* 32	0
5	0·6	+	−	40	.	5	32	0
6	*C*	−	+	55	+0·3	> 36	.	Threshold 2 g
7	.	−	+	65	+0·3	38·5	43	0
8	.	.	+	.	39 (*xx*)	> 48	.	0
9	.	.	+	.	42	45	.	0
10	*C*	−	+	80§	41	41	57	0
11	.	.	+	60	.	*ca.* 40	.	0
12	.	.	+	44	44	46	.	Firm pressure
13	.	.	+	.	41	.	.	0
14	.	−	+	50§	37	38·5	.	0
15	0·85	.	+	.	43	.	.	High threshold
16	.	.	+	.	< 47	> 48	.	0
17	*C*	−	+	38§	38	42	.	0
18	.	−	+	.	38·5	.	.	0

(*x*) The final values of the external temperature change are given.

(*xx*) For fibres 8–18 the absolute values of the final temperature are given.

* *C* indicates that the conduction velocity was less than 2 m/sec, but was not measured accurately.

† +, Excitation; −, inhibition. The effect depended on the initial temperature; when this was in the range where the fibre was excited then the activity was inhibited, otherwise there was no response. ‡ 0 = no response. § Bursts.

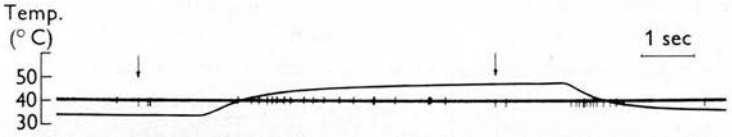


Fig. 9. Afferent impulses of two C fibres and the skin temperature when heating and cooling the skin. Arrows mark the slight mechanical vibration of the thermode caused by changing the water flow. There are two active fibres in this strand. The diphasic impulses are from a unit excited by heating the skin. This unit was not excited by the light mechanical stimuli which excited the other fibre. This latter fibre was excited by suddenly cooling the skin.

DISCUSSION

The old and controversial problem of 'specificity' of cutaneous sensory mechanisms has been carried to a new stage by recent electrophysiological and histological investigations. It should be kept in mind that the concept

of 'specific' sensory fibres or receptors has two quite different aspects: (1) the specific *sensation* aroused by stimulation of a receptor ('specific energy' in the sense of Müller (1844) and (2) the response of a *receptor* to a specific stimulus. Of course, only the second problem can be adequately solved by animal experiments, and even then it remains uncertain whether the results can be properly applied to man. Since certain cutaneous and other receptors in the cat respond to both mechanical and thermal stimulation (Hensel & Zotterman, 1951*b*; Hensel, 1952; Witt & Hensel, 1959; Iggo, 1959*a*, *b*, 1960; Douglas & Ritchie, 1959; Lele & Weddell, 1959), the classification of cutaneous receptors as 'mechanoreceptors' or 'thermoreceptors' may seem to be arbitrary. However, *quantitative* measurements of the sensitivity of some individual receptors to both kinds of stimuli have revealed systematic differences. *Qualitative* experiments alone are not sufficient and may sometimes be misleading. Until the properties of an individual unit have been examined quantitatively it would perhaps be better not to use the terms 'specific' and 'non-specific' and it would certainly be premature to argue about peripheral 'specificity'. At the least it is necessary to define exactly the properties of the 'specific' receptor being dealt with. For the cold (warm) receptors the following definition is proposed with respect to the discharge in the afferent fibre: (1) Frequency rise (fall) on sudden cooling, (2) no response on sudden warming (cooling), if the fibre is silent, or an inhibition of a resting discharge, (3) a steady discharge dependent on temperature, (4) no response to non-painful mechanical stimulation (or at least a considerably higher threshold than the most sensitive mechanoreceptors, cf. Fig. 7, Iggo, 1960), (5) thermal sensitivity comparable with temperature sense in man.

The results described in this paper demonstrate clearly the existence of cutaneous sensory C fibres whose endings are sensitive either to warming or to cooling, whereas they are not excited by mild or non-painful mechanical stimulation. Unlike the heat and cold fibres described by Iggo (1959*b*), which may be concerned with pain, several C fibres found in the present investigation were stimulated by temperatures which in man cause no pain at all. The thresholds of these cold and warm receptors, amounting to only a few tenths of a degree from an indifferent temperature (30–33° C), correspond well with the thresholds of cutaneous cold and warm sensation in man (Hensel, 1952). On our proposed definition they are thermoreceptors.

The general behaviour of the cutaneous C thermoreceptors is comparable with that of the thermoreceptors in the cat's tongue. The values for the dynamic and static responses of the non-myelinated cold and warm fibres (Table 2) are of the same order as those found for the specific cold receptors in the tongue (Hensel & Zotterman, 1951*a*). However, there are some dif-

ferences between the C thermoreceptors in the skin and the thermoreceptors in the tongue. The lingual nerve of the cat apparently contains no C fibres sensitive to mild thermal stimuli (Zotterman, personal communication); in this nerve there are A fibres sensitive to gentle warming or cooling. The cutaneous warm receptors with C fibres are more sensitive than the lingual warm fibres and show no paradoxical discharge (Dodt & Zotterman, 1952) during cooling. Further, the C cold fibres from the skin seem to be active at lower constant temperatures and to have a maximum discharge at a lower temperature than the cold fibres in the tongue.

TABLE 2. Maximum sensitivity (impulses/sec.° C) of single temperature fibres in the cat

		Cutaneous warm receptor with C fibre	Cutaneous cold receptor with C fibre	Lingual cold receptor with A fibre*
Dynamic sensitivity		+33	−30	−30
Static sensitivity	Positive range	+ 8	+ 3	+ 1
	Negative range	− 5	− 0·7	− 2·5

* From Hensel & Zotterman (1951*a*).

A number of A fibres which responded to thermal as well as light mechanical stimulation were found by Witt & Hensel (1959) in the saphenous nerve of the cat. No fibres were detected in the A group which were stimulated by temperature alone. These findings support the view that cutaneous temperature impulses in the cat may be carried mainly in non-myelinated fibres. We do not know the cat's temperature sensation, but the existence of thermal receptors is indicated by vasomotor reflexes in the ear to moderate heating or cooling of the legs in the unanaesthetized cat. These reactions were not caused by changes in blood temperature, which were actually the reverse of those necessary to give the observed changes in ear blood flow. The recorded hypothalamic and rectal temperatures rose during cooling and fell during heating of the extremities (Kundt, Brück & Hensel, 1957).

There are several reports dealing with the excitation of cutaneous fibres by both mild mechanical stimuli and cooling. Some cutaneous A fibres (Witt & Hensel, 1959) and C fibres (Douglas *et al.* 1959; Iggo, 1959*a*, 1960; this paper) in the cat are excited by non-injurious pressure and by cooling. The A fibres sometimes gave a steady discharge with constant temperatures whereas the C fibres were excited only when the cutaneous temperature was falling. The results have been interpreted in different ways. Iggo (1960) has suggested that the C fibres innervate mechanoreceptors which, in common with other mechanoreceptors, are excited by cooling. Douglas *et al.* (1959) consider that the response may indicate dual specificity of the

endings. The present results, which show clearly that there are cutaneous C thermoreceptors which are much more sensitive to thermal changes than the C mechanoreceptors, provide a more convincing explanation for cutaneous thermosensitive mechanisms. If the A and C fibres described do indeed innervate mechanoreceptors, the results may account for the old observation (Weber's illusion, 1846) that weights feel heavier when they are cold (Hensel & Zotterman, 1951*b*; Douglas *et al.* 1959; Witt & Hensel, 1959).

In the last few years, in what has aptly been referred to as 'the tide of recent work', the classical doctrine of specific cutaneous receptors has been severely attacked on the basis of new histological findings and physiological investigations (Hagen, Knoche, Sinclair & Weddell, 1953; Weddell, Palmer & Pallie, 1955; Sinclair, 1955; Lele & Weddell, 1956, 1959; Oppenheimer, Palmer & Weddell, 1958; Weddell, Palmer & Taylor, 1959). Weddell *et al.* (1955) confirmed the original observation of Adrian (1931) that touching hairs sets up impulses in rapidly-conducting cutaneous fibres. Thermal stimuli failed to do so. Since the hair follicles have a distinctive innervation by large nerve fibres, Weddell (1960) now considers that their nerve endings may be specifically excited by light mechanical stimuli. To this extent he thus revives von Frey's (1895) suggestion that different qualities of cutaneous sensation are served by distinct histological structures. The other major type of afferent innervation of hairy skin is the diffuse arborization of 'free' nerve endings (Weddell *et al.* 1959). These presumably serve the other modalities (Weddell, 1960) even though there are apparently no histological differences to be seen among them. However, as Sinclair (1955) admits, it remains possible that specialized endings exist, but that the differences are too subtle to be revealed by present histological methods. This latter view is supported by the present work on C fibres innervating receptors in hairy skin and by recent investigations on the neurohistology of the cat's tongue, where Kantner (1957) has found only a network of 'free' nerve endings, although specific mechanoreceptors and thermoreceptors are present in the area. On the other hand there are reports of cutaneous nerve endings which, it is claimed, are not highly specific. In some regions, such as the cornea (Lele & Weddell, 1959) the non-specific fibres are thought to predominate. These conclusions rely on experiments with multi-fibre techniques for recording from the cutaneous nerves. Such techniques give results which are notoriously difficult to interpret. In addition, an important assumption in Lele & Weddell's argument appears to be that specific thermoreceptors are absent from the cornea. The present work makes this assumption less convincing. Lele & Weddell may have missed the thermoreceptors because the afferent fibres were non-myelinated. They recorded impulses in the myelinated fibres only.

SUMMARY

1. Impulses in slowly-conducting (C) afferent fibres were recorded in fine strands dissected from the saphenous nerves of cats anaesthetized with chloralose. The conduction velocities in some of the fibres ranged from 0·6 to 1·5 m/sec.

2. In response to well defined thermal stimuli the fibres responded either to warming or to cooling of the skin. The thresholds for mechanical stimulation were high.

3. Changes in skin temperature of 0·2° C or less were sufficient to excite the most sensitive cold and warm fibres.

4. The dynamic sensitivity of some fibres was 30 impulses/sec. ° C, which is as great as both the sensitivity of the most sensitive cold fibres in the cat's tongue and the temperature sense in man.

5. Constant temperatures led to a steady discharge of impulses, with a maximum between 16 and 27° C for the cold fibres and above 41° C for the warm fibres.

The participation of A.I. in the experiments described in this paper was made possible by a grant from the 'Deutsche Forschungsgemeinschaft'.

REFERENCES

ADRIAN, E. D. (1931). The messages in sensory nerve fibres and their interpretation. *Proc. Roy. Soc.* B, **109**, 1–18.

DODT, E. & ZOTTERMAN, Y. (1952). Mode of action of warm receptors. *Acta physiol. scand.* **26**, 345–357.

DOUGLAS, W. W. & RITCHIE, J. M. (1959). The sensory functions of the non-myelinated afferent nerve fibres from the skin. *Ciba Foundation Study Group, No.* 1, *Pain and Itch*, pp. 26–40. London: Churchill.

DOUGLAS, W. W., RITCHIE, J. M. & STRAUB, R. W. (1959). Discharges in non-myelinated (C) fibres in the cat's saphenous nerve in response to changing the temperature of the skin. *J. Physiol.* **146**, 47–48*P*.

HAGEN, E., KNOCHE, H., SINCLAIR, D. C. & WEDDELL, G. (1953). The role of specialized nerve terminals in cutaneous sensibility. *Proc. Roy. Soc.* B, **141**, 279–287.

HENSEL, H. (1952). Physiologie der Thermoreception. *Ergebn. Physiol.* **47**, 166–368.

HENSEL, H. (1953). Ein Photokymographion für kombinierte Registrierung von Kathodenstrahloszillogrammen und Lichtzeigerausschlägen. *Pflüg. Arch. ges. Physiol.* **256**, 37–42.

HENSEL, H., IGGO, A. & WITT, I. (1959). Cutane Thermoreceptoren mit marklosen afferenten Fasern. *Pflüg. Arch. ges. Physiol.* **270**, 82–83.

HENSEL, H. & ZOTTERMAN, Y. (1951*a*). Quantitative Beziehungen zwischen der Entladung einzelner Kältefasern und der Temperatur. *Acta physiol. scand.* **23**, 291–319.

HENSEL, H. & ZOTTERMAN, Y. (1951*b*). The response of mechanoreceptors to thermal stimulation. *J. Physiol.* **115**, 16–24.

IGGO, A. (1958*a*). The electrophysiological identification of single nerve fibres, with particular reference to the slowest-conducting vagal afferent fibres in the cat. *J. Physiol.* **142**, 110–126.

IGGO, A. (1958*b*). Single C fibres from cutaneous receptors. *J. Physiol.* **143**, 47–48*P*.

IGGO, A. (1959*a*). A single unit analysis of cutaneous receptors with C afferent fibres. *Ciba Foundation Study Group, No.* 1, *Pain and Itch*. pp. 41–56. London: Churchill.

IGGO, A. (1959*b*). Cutaneous heat and cold receptors with slowly-conducting (C) afferent fibres. *Quart. J. exp. Physiol.* **44**, 362–370.

IGGO, A. (1960). Cutaneous mechanoreceptors with afferent C fibres. *J. Physiol.* **152**, 337–353.

KANTNER, M. (1957). Die Sensibilität der Katzenzunge. *Acta neuroveg., Wien*, **15**, 223–234.

KUNDT, H. W., BRÜCK, K. & HENSEL, H. (1957). Hypothalamustemperatur und Hautdurchblutung der nichtnarkotisierten Katze. *Pflüg. Arch. ges. Physiol.* **264**, 97–106.

LELE, P. P. & WEDDELL, G. (1956). The relationship between neurohistology and cutaneous sensibility. *Brain*, **79**, 119–154.

LELE, P. P. & WEDDELL, G. (1959). Sensory nerves in the cornea and cutaneous sensibility. *Exp. Neurol.* **1**, 334–359.

MÜLLER, J. (1844). *Handbuch der Physiologie des Menschen.* Vol. 1, p. 667ff. Coblenz: Hölscher.

OPPENHEIMER, D. R., PALMER, E. & WEDDELL, G. (1958). Nerve endings in the conjunctiva. *J. Anat., Lond.*, **92**, 321–352.

SINCLAIR, D. C. (1955). Cutaneous sensation and the doctrine of specific energy. *Brain*, **78**, 584–614.

VON FREY, M. (1895). Beiträge zur Sinnesphysiologie der Haut, III. *Ber. sächs. Ges. (Akad.) Wiss*, **47**, 166–184.

WEBER, E. H. (1846). Der Tastsinn und das Gemeingefühl. In Wagner's *Handwörterbuch der Physiologie*, Vol. III/2, pp. 481–588. Braunschweig: Vieweg.

WEDDELL, G. (1960). The structure of the skin in relation to its innervation. In *Progress in the Biological Sciences in Relation to Dermatology*, pp. 55–63. London: Cambridge University Press.

WEDDELL, G., PALMER, E. & PALLIE, W. (1955). Nerve endings in mammalian skin. *Biol. Rev.* **30**, 159–195.

WEDDELL, G., PALMER, E. & TAYLOR, D. (1959). The significance of the peripheral anatomical arrangements of the nerves which serve pain and itch. *Ciba Foundation Study Group, No.* 1, *Pain and Itch*, pp. 3–10. London: Churchill.

WITT, I. & HENSEL, H. (1959). Afferente Impulse aus der Extremitätenhaut der Katze bei thermischer und mechanischer Reizung. *Pflüg. Arch. ges. Physiol.* **268**, 582–596.

ZOTTERMAN, Y. (1939). Touch, pain and tickling: an electrophysiological investigation on cutaneous sensory nerves. *J. Physiol.* **95**, 1–28.

Note added in proof

I. Iriuchijima & Y. Zotterman (*Acta physiol. scand.*, in the Press) have also recorded from cutaneous thermal fibres similar to those described in this paper.

J. Physiol. (1961), **156**, *pp.* 578–590
With 4 *text-figures*
Printed in Great Britain

THE EFFECT OF HISTAMINE, 5-HYDROXYTRYPTAMINE AND ACETYLCHOLINE ON CUTANEOUS AFFERENT FIBRES

By NANCY FJÄLLBRANT and A. IGGO
From the Physiology Department, University of Edinburgh

(*Received* 5 *December* 1960)

The cutaneous nociceptors have thin myelinated and non-myelinated afferent fibres (see Zotterman, 1959 for a review), but there is little information about the sensitivity of the endings of individual fibres to cutaneous stimuli. This arises because the small diameters of their afferent fibres have delayed an exact analysis with single-fibre techniques and also because stimuli sufficient to excite the nociceptors will, in addition, not only excite the receptors highly sensitive to the particular stimulus but also excite receptors which are highly sensitive to other forms of energy, i.e. the specificity of the nerve endings is not absolute. Identification of the nociceptors is thus made more difficult. All the non-myelinated fibres, in particular, have come to be regarded as pain fibres, even though Zotterman (1939) had described a discharge of small impulses, which were presumed to be in non-myelinated cutaneous fibres, when only mild mechanical stimuli were used. More recently Douglas & Ritchie (1957*b*) recording from intact nerves, and Iggo (1960) using single-fibre techniques proved that many C fibres could be excited by mild mechanical stimulation of the skin. A quantitative examination of single units established that the mechanically sensitive C fibres had a wide range of thresholds, from 20 mg to more than 7·5 g. Other C fibres were specifically sensitive either to heating or to cooling of the skin (Iggo, 1959*b*; Hensel, Iggo & Witt, 1960; Iriuchijima & Zotterman, 1960). No units could accurately be identified as nociceptors, although the thresholds to all stimuli of the least sensitive were so high that the effective stimuli could be regarded as noxious. For this reason additional more specific tests are required in order to identify the nociceptors.

If all cutaneous nociceptors were excited by all noxious agents, as is generally assumed, then the injection into the skin of pain-producing chemicals might provide such an additional test. Histamine, acetylcholine, 5-hydroxytryptamine (5-HT) and mixtures of histamine and acetylcholine will in low concentrations (10^{-3}–10^{-8} g/ml.), cause pain when put on blister bases in man (Armstrong, Dry, Keele & Markham, 1953), as will histamine when it is injected into the superficial layer of the skin

(Rosenthal, 1950) and a mixture of histamine and acetylcholine when it is pricked into the skin (Emmelin & Feldberg, 1947; Skouby 1953). Histamine when put on frog skin may provoke a discharge of impulses in afferent fibres (Habgood, 1950; Dodt, 1955; Arthur & Shelley, 1959) as will acetylcholine in the appropriate conditions (Jarrett, 1956). In mammals, on the other hand, Brown & Gray (1948), Douglas & Gray (1953), Witzleb (1959) and Douglas & Ritchie (1960) excited some of the fibres in multi-fibre strands of cutaneous nerves with the close-arterial injection of acetylcholine, but not with histamine.

The present paper describes an attempt to identify cutaneous nociceptors by making close-arterial injections of histamine, acetylcholine, histamine and acetylcholine and 5-hydroxytryptamine into the skin while recording from single-unit preparations. Preliminary accounts of the results have been published (Fjällbrant & Iggo, 1959; Iggo, 1959*a*).

METHODS

The eleven cats used were anaesthetized with chloralose, 80 mg/kg I.V., after induction of anaesthesia with ethyl chloride and ether. A skin incision was made in the mid thigh above the saphenous nerve. The femoral artery was then dissected in the thigh and the muscular branches to a point about 2 cm distal to the origin of the saphenous artery were ligated. A fine polythene cannula was inserted in a retrograde direction into the femoral artery in Hunter's canal so that its tip was just distal to the orifice of the saphenous artery. The femoral artery was occluded centrally during the injection, which was washed in with Ringer–Locke solution. The thoroughness of the ligation of the muscular branches was checked in some experiments by injecting Indian ink through the cannula at the end of the experiment.

The fixation of the leg with metal pins through each end of the tibia, the dissection of the single units, the methods used to provide quantitative mechanical and thermal stimulation of the skin and the recording methods were similar to those described previously (Iggo, 1959*b*, 1960).

Chemicals. The chemicals were stored dry and were dissolved in Ringer–Locke solution as required during each experiment: histamine acid phosphate (Burroughs Wellcome), acetylcholine chloride (Roche Bros. Ltd), 5-hydroxytryptamine creatinine sulphate (Abbott Laboratories) and impure bradykinin (a gift from Dr E. W. Horton). The amounts injected are expressed as the base, with the exception of bradykinin.

Method of injection. The close-arterial injections were made into the femoral artery at the orifice of the saphenous artery. The latter supplies blood to a large area of the skin on the medial aspect of the thigh, leg and foot. This procedure was adopted because the single fibres were dissected from the nerve in the mid thigh and the position of the receptive fields could not, therefore, be predicted. As a result, however, the conditions of the experiment differ from the similar investigations of Brown & Gray (1948), Douglas & Gray (1953) and Douglas & Ritchie (1960) in that the chemicals were put into the blood stream at a greater distance from the receptors in the present work and the blood flow was not restricted to the skin. The chemicals thus probably had, in the present work, a more gradually rising concentration at the nerve endings and some of the acetylcholine might also have been destroyed in the blood.

RESULTS

Multi-fibre strands of the saphenous nerve

Multi-fibre strands were used in the initial experiments because the actions of the injected chemicals could be tested on a large sample of afferent fibres. The identification of a particular active fibre was, however, usually unreliable when there were numerous fibres in a strand.

Histamine. The quantities used ranged from 6·6 to 230 μg. The largest doses were little more effective than 60 μg; in most experiments the doses ranged from 10 to 20 μg. These amounts are less than the quantities of histamine released rapidly in the skin by drugs such as compound 48/80 (Feldberg & Paton, 1951), and may therefore be regarded as within physiological limits.

The first reaction to an injection of histamine appeared within 5 sec with the largest doses and the peak of activity occurred at 1–3 min. The maximum frequency of discharge was low, never greater than 10/sec, and the impulses were often grouped in bursts. The activity did not persist beyond 6 min. Only a small proportion of the fibres in a multi-fibre strand were excited by the histamine.

Acetylcholine (Ach) was tested, in doses ranging from 20 to 60 μg, on six strands. Any discharge of impulses appeared soon after the injection, as reported by Brown & Gray (1948), Douglas & Gray (1953) and Douglas & Ritchie (1960). With one strand, containing both myelinated and non-myelinated fibres, there was a burst of activity, in a few of the fibres, which began before the injection was completed, reached a peak 20 sec after the start of the injection, fell quickly to low level at 35 sec and then persisted until 110 sec. On another occasion impulses which appeared to be in a single A fibre were recorded; the frequency was not greater than 16 sec at the peak of excitation and bursts of impulses were present for 4 min after the injection.

Histamine and acetylcholine. Mixtures of these chemicals when pricked into human skin were reported by Emmelin & Feldberg (1947) and Skouby (1953) to cause stinging pain. For this reason the mixtures were injected in the present work.

The combined injections were more potent than the injection of either chemical alone. The discharge was maximal 1–3 min after the injection (Fig. 1) and bursts of impulses continued for as long as 10 min. The maximal frequency of discharge was not very high, and when it could be recognized as arising in particular fibres it was never more than 5/sec.

5-*hydroxytryptamine.* Douglas & Ritchie (1957*a*) found that 5 hydroxytryptamine (5-HT) was very potent in exciting the cutaneous afferent

fibres. This is confirmed by the present work. Injection of 4–40 μg of 5-HT aroused a discharge which began within 20 sec, reached its peak at 1–3 min and persisted on one occasion for as long as 13 min. The discharge was, as with the other chemicals, intermittent; groups of impulses were separated by longer intervals of silence.

The characteristic response to injection of all the above chemicals was,

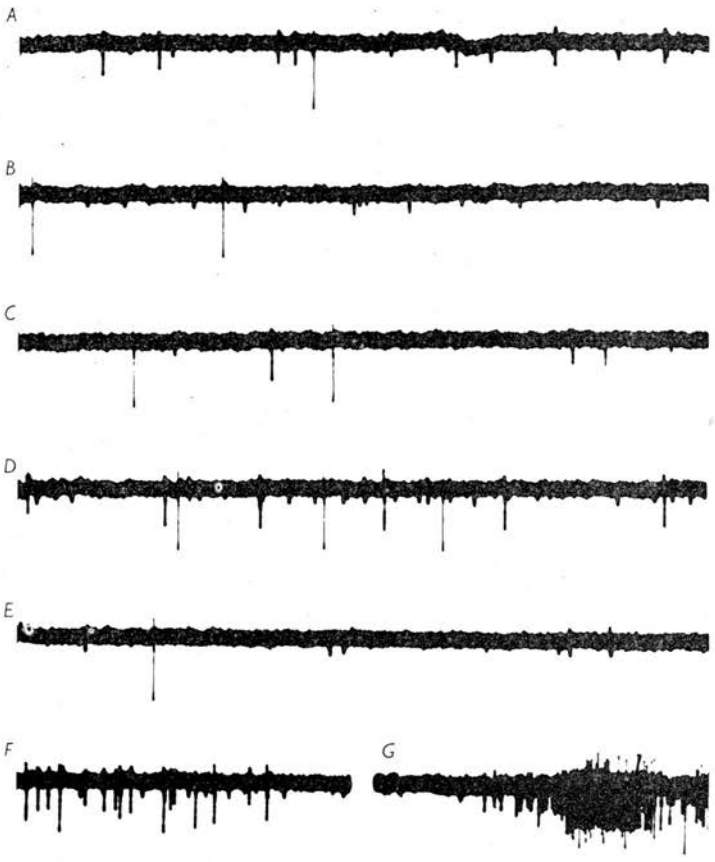


Fig. 1. The effect of an injection of a mixture of histamine (20 μg) and acetylcholine (40μg) on a multi-fibre strand of the saphenous nerve. The base line before the injection, not shown, was silent. The records from above down were taken at 15 sec, 30 sec, 45 sec, 1 min 45 sec and 3 min 45 sec after the injection. The rate of firing in individual fibres did not exceed 5/sec and only a small proportion of all the fibres in the strand were excited. An indication of the number of fibres in the strand can be gauged from the response to (*F*) heating the skin and (*G*) stroking the skin firmly. Time marker, 0·1 sec, applies to all tracings.

therefore, a low-frequency sporadic discharge of impulses in only a few of the fibres in multi-fibre strands of the saphenous nerve.

Action on single afferent fibres

An important advantage gained by the use of single units, combined with quantitative natural stimulation of the receptors, is that not only can any excitatory action of the chemicals be measured accurately but also any depression of excitability can be detected and measured. In addition the conduction velocities of the afferent fibres can be measured and thus give an indication of the types of fibres studied.

Cutaneous myelinated afferent fibres

Hair receptors, touch receptors, slowly-adapting pressure receptors and insensitive pressure receptors were examined. Only one group of these receptors was found to be affected, the slowly-adapting pressure receptors.

Slowly-adapting pressure receptors

These may be the same as the pressure receptors in the rabbit's skin described by Frankenhaeuser (1949). The thresholds for mechanical stimulation of the receptors were about 1 g wt. and the conduction velocities ranged from 20 to 55 m/sec. A conspicuous feature of some of these receptors was a very steady resting discharge of impulses at frequencies of 3–15 impulses/sec, similar to the discharge in some corneal afferent fibres (Tower, 1940) and in cutaneous fibres (Witt & Hensel, 1959; Hunt & McIntyre, 1960). The resting discharge could be altered by mechanical stimulation of the skin, increasing smoothly in frequency when pressure was applied. The resting discharge was depressed for a few seconds after strong mechanical stimulation of the skin, and returned gradually during a few seconds to the resting level. If a unit was silent it could sometimes be aroused to a persistent steady discharge by vigorous mechanical stimulation. The persistent regular discharge of these units was particularly useful since the receptors were influenced by the chemicals used in this work.

Histamine. The action of histamine was consistent. It was recorded 19 times in 21 trials using 11 fibres, with doses ranging from 6·6 to 66 μg. In the later experiments in the series 10 μg was invariably effective. The effect on the resting discharge of one fibre is illustrated in Fig. 2 (open circles). Within 20 sec of the injection the resting discharge began to increase and was maximal at 1–3 min. The discharge then stopped, more rapidly than it had increased. It was absent for 2 min. The silence in other fibres lasted from 10 sec to 12 min. Recovery to the original value was gradual, taking as long as 30 min. In one experiment the resting

discharge was permanently enhanced after repeated injections. If a fibre was silent before the injection a discharge of impulses could be evoked, with a time course similar to that for the active fibres.

The response of the same unit to an intermittently applied mechanical stimulus passed through a similar cycle of enhancement, depression and recovery when histamine was injected. The initial enhancement was most obvious at the time when the resting discharge was failing. The recovery from the depression was also earlier for the response to mechanical stimulus than for the resting discharge, and there was a period of enhancement during the recovery (Fig. 2, filled circles). Two interesting features of the

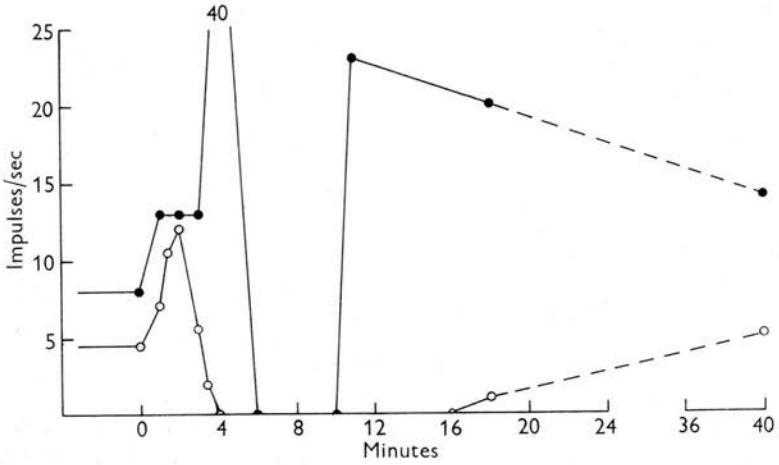


Fig. 2. The effect on an A pressure receptor (threshold, 1·25 g wt. conduction velocity, 35 m/sec) of an injection of 66 μg histamine. ○—○ resting discharge, ●—● the response to intermittent pressure with a small probe. The stimulus intensity chosen was less than twice threshold intensity. The maximal response to mechanical stimulation coincided with the failure of the spontaneous discharge, but, as described in the text, the discharge during mechanical stimulation was not as persistent during this phase as it was normally.

enhancement were, (1) the units did not continue to discharge throughout the period of mechanical stimulation as they did normally, there was an apparent acceleration of adaptation, and (2) the firing became intermittent, with gaps that initially were a multiple of the pulse interval. These gaps are unlikely to have been due to a failure of the impulses to leave the nerve terminals, since, initially, the normal interval was just doubled. This response implies that the usual sequence of events at the nerve ending leading to and following the discharge of an impulse had taken place, but that the impulse did not travel to the recording electrodes.

The response to a second injection of histamine during the depression associated with an initial dose was not examined.

Acetylcholine. Only two units were tested. One was unaffected by doses of 30 and 60 μg; the other was excited by 30 μg. Within 30 sec of the start of injection the frequency of the resting discharge was enhanced and it reached a maximum at 3–4 min (Fig. 8 in Iggo, 1959*a*). Twelve minutes later the discharge had fallen to its original value. This is a very much more prolonged action than was reported for ACh by Brown & Gray(1948) and Douglas & Gray (1960). The persistence of the effect suggests that it might have been due to a secondary action of the ACh.

Histamine and acetylcholine. The combination of ACh with histamine tended to intensify the action of histamine (Fig. 8 in Iggo, 1959*a*). In this figure the typical response to histamine, which was absent when only 20 μg of histamine was injected, was present when half that quantity of histamine was injected together with 24 μg of ACh.

5-hydroxytryptamine. The effects of 5-HT (1–20 μg) on both the resting discharge and the response of the pressure receptors to mechanical stimulation were similar to those caused by histamine. During the recovery from 5-HT the resting discharge was enhanced. On one occasion there were two phases of excitation of a pressure receptor with no resting discharge. A second phase of enhancement of a steady discharge was never seen with histamine or with mixtures of histamine and ACh.

Hair receptors, insensitive mechanoreceptors

Although many nerve strands contained examples of these types of unit there was no occasion when the injection of any of the chemicals aroused a discharge of impulses in the fibres. Five hair receptors and two insensitive mechanoreceptors with myelinated afferent fibres, isolated as single units, were tested with histamine and with histamine and ACh. No change in the response to quantitative mechanical stimulation was found.

Cutaneous non-myelinated afferent fibres

Mechanoreceptors

Ten single mechanoreceptors with non-myelinated afferent fibres were examined and included examples of all the Groups I, II and III (Iggo, 1960).

Histamine. Only two of the ten units were excited by the injection of histamine (10–160 μg). The largest response was a few impulses, at less than 5/sec, within 30 sec of the injection. An immediate short-lived enhancement of the response to mechanical stimulation may have been missed because the units were not tested at frequent intervals. In order to obtain a steady base line the mechanical stimuli could not be repeated more frequently than once every 30 sec, because more frequent stimulation leads to an inexcitability of the nerve endings (Iggo, 1960). The two units

tested most thoroughly both revealed a persistent depression after histamine (Fig. 3); one unit did not recover its original excitability for 1 hr. The other four units were apparently unaffected by histamine in the quantities injected.

Acetylcholine. Only one of the four units examined was excited by 30 μg of ACh; other unidentified units in the same strand fired intermittently, at frequencies not greater than 6/sec, for 5–30 sec after the injection. With one of the units there was an apparent twofold enhancement of the response to mechanical stimulation 30 sec after the injection.

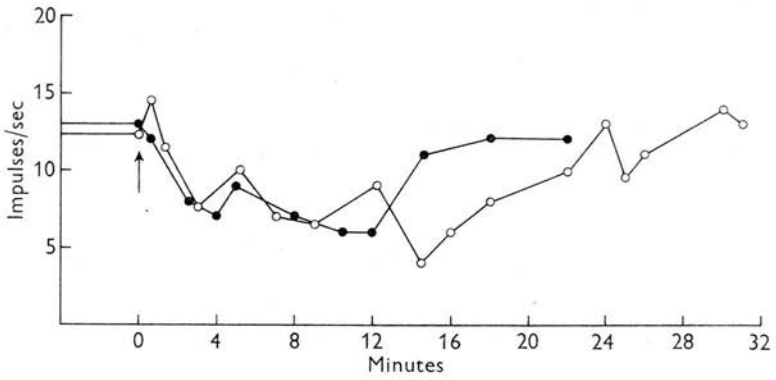


Fig. 3. The effect of histamine 10 μg ○—○ and of a mixture of histamine, (10 μg) and acetylcholine (24 μg) ●—● on the response of a C mechanoreceptor to pressure on the skin with a nylon probe, tip diameter 0·25 mm. The threshold of the unit was 1·5 g wt. At no time was there a discharge of impulses in the absence of a stimulus.

Histamine and acetylcholine. The mixture, which was tested on seven mechanoreceptors with non-myelinated afferent fibres, was more effective than the injection of either chemical alone. A low frequency discharge at 4–5 imp/sec was the largest response, but brief bursts of impulses did persist for as long as 14 min. The response to monitored mechanical stimulation was enhanced briefly by as much as 50 % with three of the seven units. A later depression was evident with six of the seven units and lasted for as long as 30 min (Fig. 3).

5-hydroxytryptamine. One of the two units tested was excited by 2 μg of 5-HT. The discharge was weak, about 1/sec. The response to mechanical stimulation was simultaneously enhanced and during the depression which followed the enhancement it fell to 25 % of the control value.

Thermoreceptors

Only the less sensitive thermoreceptors with non-myelinated fibres (Iggo, 1959*b*) were tested.

Histamine. The one *heat receptor* tested was excited by histamine, and 6 min after the injection of 8·3 μg of histamine there were still bursts of impulses. The rate of firing was, however, low, less than 5/sec. The mechanical sensitivity of this unit showed a brief but pronounced enhancement 15 min after the injection of 66 μg of histamine. A brief stroking of the skin, which initially was insufficient to excite the unit, evoked a discharge of impulses at 14/sec which persisted at a slowly declining frequency for at least 10 sec. This response was most unusual. Later testing with the same mechanical stimulus again failed to excite the unit.

The one *cold unit* tested was depressed by the injection of 100 μg of histamine; the steady discharge of impulses produced by ice on the skin ceased 20 sec after the injection and returned 73 sec later. This unit gave a very conspicuous burst of impulses when the skin was heated after being kept at a low temperature (the 'rewarming burst', Iggo, 1959*b*). This burst of impulses was prolonged after the histamine injection, and lasted nearly 60 sec compared with the usual 15 sec for this unit.

Acetylcholine was not tested on the thermoreceptors.

Histamine and acctylcholine. One *heat receptor* was tested. Injections of 10 μg histamine and 20 μg ACh evoked an intermittent discharge of impulses (Fig. 4), each burst lasting not longer than 2 sec and recurring at variable intervals for as long as 14 min. The maximal frequency was 5/sec. The response to thermal stimulation was depressed after the injection (Fig. 4) even when there were intermittent bursts of impulses, with recovery after about 10 min.

5-hydroxytryptamine. The *heat receptor* tested was excited by 2 μg of 5-HT. Bursts of impulses at low frequency were present for 4 min. The response to thermal stimuli, tested at 3 min intervals, was depressed in much the same way as by histamine and ACh.

Intradermal injections of histamine and of 5-hydroxytryptamine. Histamine was pricked or rubbed into the skin in the receptive fields of two slowly-adapting pressure receptors with myelinated afferent fibres. On each occasion there was an enhancement of the resting discharge, twofold with one unit and fourfold with the other. The peak frequency fell gradually to the resting level after 38 and 8 min, respectively. 5-HT was pricked into the skin in the receptive fields of two mechanoreceptors with non-myelinated afferent fibres and each time the units became unresponsive to mechanical stimulation.

Other observations

Bradykinin (impure) was injected in doses of 300–400 μg in three cats. The eight slowly-adapting pressure receptors with myelinated afferent fibres which were tested were all excited by the injection. A steady resting

discharge of 7·5/sec in one fibre rose after 25 sec to 20/sec, remained at this level for 6 min, and slowly fell to the original value after 30 min. Non-myelinated fibres in the same strand were excited only very feebly if at all.

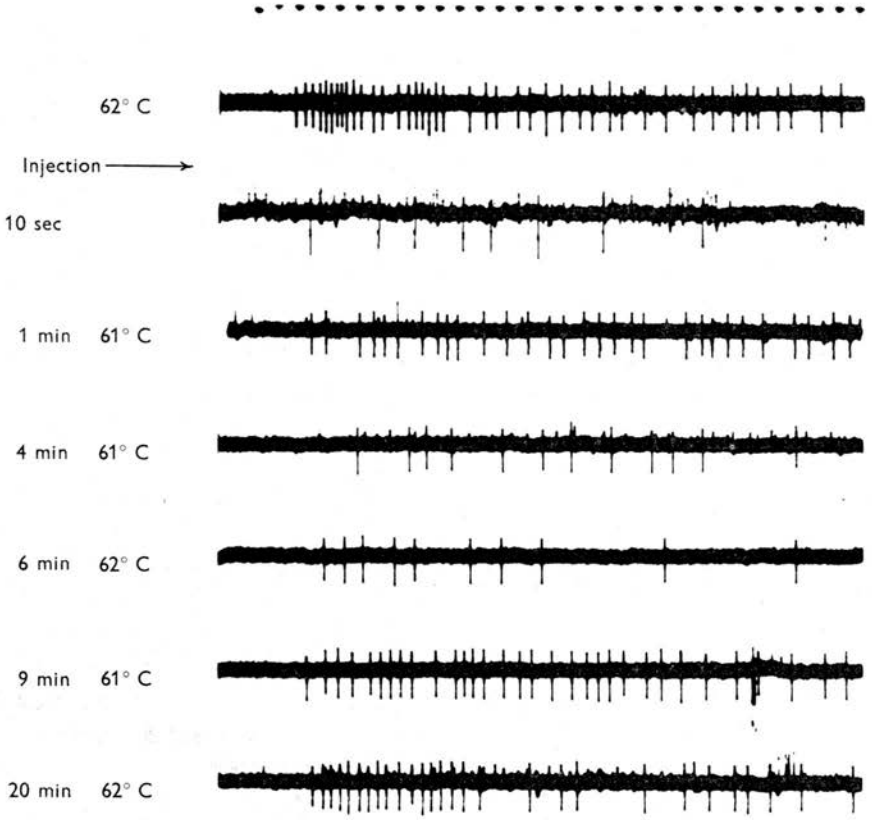


Fig. 4. The effect of an injection of histamine, (10 μg) and acetylcholine, (20 μg) on a C heat receptor. Before the injection there was a brisk response when a brass rod, 10 mm diameter, at 62° C was placed on the skin. After the injection this response was progressively diminished and was minimal at 4–6 min. Twenty minutes later the thermal response was almost normal. Ten seconds after the injection there was a burst of impulses in the fibre in the absence of heat (the record was taken with twice the gain of the other records in order to reveal the activity aroused in other fibres by the injection). The irregular discharge at very low frequencies recurred for 6 min and was present even when the sensitivity to the thermal stimulus was low. The temperature of the rod, which was placed on the skin near the start of each record except the second and left on for the rest of the trace, and the times of application, are indicated at the left. The injection was made at zero time. Spikes retouched. Time marker, 0·1 sec.

DISCUSSION

The various cutaneous afferent fibres were not uniformly sensitive to the intra-arterial histamine, histamine and acetylcholine, and 5-HT. There was a definite excitatory action on the slowly-adapting pressure receptors with myelinated afferent fibres and on the two heat receptors with non-myelinated afferent fibres tested. There were other unidentified responses. The pressure receptors displayed a phase of increased resting discharge and enhanced responsiveness to natural stimulation, followed by a deep depression. This enhancement of the response to natural stimulation was greatest as the resting discharge was falling after its initial rise (Fig. 2). The excitation of the heat receptor was associated with a diminished response to natural stimulation. This unit also displayed a much enhanced response to mechanical stimulation at the time it was recovering its sensitivity to thermal stimuli. The responses of other mechanoreceptors and thermoreceptors with non-myelinated afferent fibres to natural stimulation were all depressed by histamine and 5-HT, even though a weak discharge of impulses might be provoked by the injection. Hair receptors with myelinated afferent fibres were apparently unaffected by the chemicals. Any action of acetylcholine was excitatory, but the quantities injected were apparently inadequate to reveal the strong excitatory actions found in other experiments.

The primary aim in the present work was to establish whether histamine and 5-HT would be satisfactory test agents in identifying nociceptors. The actions of both chemicals in exciting the pressure receptors with myelinated afferent fibres is an indication that they are not. Preliminary experiments with impure bradykinin led to the same conclusion. The unsuitability of acetylcholine for this purpose is evident from the very generalized action of this chemical in exciting most afferent fibres if a sufficient quantity is used (see Paintal, 1956, for a review). Douglas & Ritchie (1960) have recently described an excitant action of acetylcholine on cutaneous non-myelinated fibres. The present experiments in which there was no conspicuous discharge of impulses following acetylcholine, but in which histamine and acetylcholine were effective, are more comparable with the sensory studies of Emmelin & Feldberg (1947) and Skouby (1953), since these investigators made intracutaneous injections of acetylcholine which by itself had no sensory effect, yet caused a change in the response to histamine when both chemicals were injected together.

Until more is known of the properties of the least sensitive cutaneous receptors it is premature to claim that the nociceptors have been satisfactorily identified. Certainly no fibres with a more or less uniform sensitivity to all noxious stimuli, including the pain-producing chemicals, have

yet been found. Instead, there are indications that the nociceptors may be modality-specific, since insensitive cold receptors, heat receptors and mechanoreceptors with non-myelinated afferent fibres have been described (Iggo, 1959*b*, 1960). The idea that the chemicals we have used could pick out all the nociceptors may thus be ill-founded.

SUMMARY

1. Close-arterial injections into the saphenous artery were made while electrical activity was recorded in cutaneous afferent fibres dissected from the saphenous nerves in cats anaesthetized with chloralose.

2. Histamine (6·6–230 μg), 5-hydroxytryptamine (4–40 μg), acetylcholine (20–60 μg) and histamine and acetylcholine excited briefly some of the fibres, both large and small, in multi-fibre strands. Identification of the active fibres was not usually possible.

3. The only single myelinated afferent fibres which were affected were those from slowly-adapting mechanoreceptors. After injections of histamine (6·6–66 μg), 5-hydroxytryptamine (1–20 μg) and of histamine and acetylcholine there was an initial enhancement, lasting 2–4 min, of both the resting discharge and the response to pressure, followed by a depression of sensitivity, with recovery after 10–60 min.

4. The non-myelinated fibres examined as single units included both mechanoreceptors and thermoreceptors. Some heat and mechanoreceptors were excited by the injection of histamine and acetylcholine, histamine and 5-hydroxytryptamine, but the rate of firing was never greater than 5/sec and was sporadic. The sensitivity of the endings to natural stimuli was depressed, even in those fibres which were excited by the injections.

5. Impure bradykinin enhanced the steady resting discharge of slowly-adapting A pressure receptors but had only a very weak action on C fibres.

REFERENCES

Armstrong, D. R. M. L., Keele, C. A. & Markham, J. W. (1953). Observations on chemical excitants of cutaneous pain in man. *J. Physiol.* **120**, 326–351.

Arthur, R. P. & Shelley, W. B. (1959). The peripheral mechanism of itch in man; in *Ciba Foundation Study Group No.* 1. *Pain and Itch. Nervous Mechanisms*, pp. 84–95. London: Churchill.

Brown, G. L. & Gray, J. A. B. (1948). Some effects of nicotine-like substances and their relation to sensory nerve-endings. *J. Physiol.* **107**, 306–317.

Dodt, E. (1955). Aktivierung markhaltiger und markloser Schmerzfasern bei Temperaturreizen. *Pflüg. Arch. ges. Physiol.* **260**, 225–238.

Douglas, W. W. & Gray, J. A. B. (1953). The excitant action of acetylcholine and other substances on cutaneous sensory pathways and its prevention by hexamethonium and D-tubocurarine. *J. Physiol.* **119**, 118–128.

Douglas, W. W. & Ritchie, J. M. (1957*a*). Discharges on non-myelinated afferent fibres in the cat's saphenous nerve in response to touch and to drugs. *J. Physiol.* **139**, 9–10*P*.

Douglas, W. W. & Ritchie, J. M. (1957*b*). Non-medullated fibres in the saphenous nerve which signal touch. *J. Physiol.* **139**, 385–399.

DOUGLAS, W. W. & RITCHIE, J. M. (1960). The excitatory action of acetylcholine on cutaneous non-myelinated fibres. *J. Physiol.* **150**, 501–514.

EMMELIN, N. & FELDBERG, W. (1947). The mechanism of the sting of the common nettle (*Urtica urens*). *J. Physiol.* **106**, 440–455.

FELDBERG, W. & PATON, W. D. M. (1951). Release of histamine from skin and muscle in the cat by opium alkaloids and other histamine liberators. *J. Physiol.* **114**, 490–509.

FJÄLLBRANT, N. & IGGO, A. (1959). Cutaneous afferent fibres and pain-producing chemicals. *J. Physiol.* **145**, 28–29*P*.

FRANKENHAEUSER, B. (1949). Impulses from a cutaneous receptor with slow adaptation and low mechanical threshold. *Acta physiol. scand.* **18**, 68–74.

HABGOOD, J. S. (1950). Sensitization of sensory receptors in the frog's skin. *J. Physiol.* **111**, 195–213.

HENSEL, H., IGGO, A. & WITT, I. (1960). A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. *J. Physiol.* **153**, 113–126.

HUNT, C. C. & MCINTYRE, A. K. (1960). Properties of cutaneous touch receptors in cat. *J. Physiol.* **153**, 88–98.

IGGO, A. (1959*a*). A single unit analysis of cutaneous receptors with C afferent fibres; in *Ciba Foundation Study Group No.* 1. *Pain and Itch. Nervous Mechanisms*, pp. 41–56. London: Churchill.

IGGO, A. (1959*b*). Cutaneous heat and cold receptors with slowly-conducting (C) afferent fibres. *Quart. J. exp. Physiol.* **44**, 362–370.

IGGO, A. (1960). Cutaneous mechanoreceptors with afferent C fibres. *J. Physiol.* **152**, 337–353.

IRIUCHIJIMA, J. & ZOTTERMAN, Y. (1960). The specificity of afferent cutaneous C fibres in mammals. *Acta physiol. scand.* **49**, 267–278.

JARRETT, A. S. (1956). The effect of acetylcholine on touch receptors in frog's skin. *J. Physiol.* **133**, 243–254.

PAINTAL, A. S. (1956). Excitation of sensory receptors in thoracic and abdominal viscera. *XX int. physiol. Congr. Rev.* 78–89.

ROSENTHAL, S. R. (1950). Histamine as possible chemical mediator for cutaneous pain. Dual pain response to histamine. *Proc. Soc. exp. Biol., N.Y.*, **74**, 167–170.

SKOUBY, A. P. (1953). The influence of acetylcholine, curarine and related substances on the threshold for chemical pain stimuli. *Acta physiol. scand.* **29**, 340–352.

TOWER, S. S. (1940). Units for sensory reception in the cornea; with notes on nerve impulses from sclera, iris and lens. *J. Neurophysiol.* **3**, 486–500.

WITT, I. & HENSEL, H. (1959). Afferente Impulse aus der Extremitätenhaut der Katze bei thermischer und mechanischer Reizung. *Pflüg. Arch. ges. Physiol.* **268**, 582–596.

WITZLEB, E. (1959). Zur Frage von cholinergischen Mechanismen bei der Erregung von afferenten Systemen. *Pflüg. Arch. ges. Physiol.* **269**, 439–470.

ZOTTERMAN, Y. (1939). Touch, pain and tickling: An electrophysiological investigation on cutaneous sensory nerves. *J. Physiol.* **95**, 1–28.

ZOTTERMAN, Y. (1959). The peripheral nervous mechanism of pain: a brief review; in *Ciba Foundation Study Group No.* 1. *Pain and Itch. Nervous mechanisms*, pp. 13–34. London: Churchill.

A SINGLE UNIT ANALYSIS OF CUTANEOUS RECEPTORS WITH C AFFERENT FIBRES

A. IGGO

Department of Physiology, University of Edinburgh

KNOWLEDGE of the function of the very numerous unmyelinated afferent fibres has been slow to accumulate because the small diameter of the fibres has delayed exact analysis by single fibre techniques. There is now clear evidence that the C fibres in man mediate "second" pain (Zotterman, 1933, 1939*a*), and Gasser (1943*b*) comments on the probable existence of thermal sensations mediated by C fibres. The C fibres in animals also presumably carry painful sensations (see Clark, Hughes and Gasser, 1935). However there is now evidence that in the cat and rabbit the C fibres can be excited by mild mechanical stimulation of the skin (Douglas and Ritchie, 1957*b*).

Recently, in work on visceral innervation, methods were developed which allowed activity in single afferent C fibres to be recorded (Iggo, 1958*a*). These techniques have now been used to start an analysis of the cutaneous afferent C fibres. The two developments which have made this analysis of activity in single mammalian C fibres possible are (*a*) refinements of the technique for dissecting fine strands from peripheral nerves and (*b*) methods which allowed active fibres in multi-fibre strands to be identified and their diameter assessed. In (*a*) the most important changes are rigid fixation of the nerve with respect to the recording electrodes; dissection at high magnification ($\times$ 40 for the later stages); minimal handling of the fine strand, achieved by mounting the recording electrodes on a micromanipulator so that the electrodes could be moved into position without any backlash. In (*b*) a technique based on the collision of descending and ascending impulses has been worked out so that stimulation of the active axon at a point between the receptor and the recording electrodes leads to a change in the pattern of impulses coming from the receptor. After identification the

In CIBA Foundation Study Group No. 1. Pain and itch ...

conduction velocity of the active fibre can be measured in the usual manner and on this basis its diameter assessed. The method is illustrated in Fig. 1 and is described fully by Iggo (1958*a*). C fibres can be isolated as single units, but the methods just described make this unnecessary; this in turn leads to less handling and consequent longer survival of the fibres, in some instances up to seven hours.

The conclusion that impulses in single axons were being recorded is based on the following considerations. (1) The action potential spike was an indivisible unit. The wave contributed to the compound action potential by the active fibre was identical

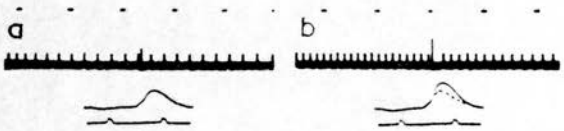


FIG. 1. Identification of the active fibre in a nerve strand by collision of descending and ascending impulses. The upper tracing shows a train of impulses in a single afferent fibre, and the lower tracing, on a faster-moving record, the compound action potential set up by stimulating the nerve trunk between the receptor and the recording electrodes. In (a) the interpolated nerve shock has not disturbed the train of impulses, whereas in (b) it is followed by a gap in the train. The nerve shock in (b) was stronger and the compound action potential has an extra component above the dotted line. This new wave is generated by an impulse in active fibre (from Iggo, 1958*a*). Time marks at 0·1 sec. intervals for upper trace and 1 msec. intervals for lower trace.

with the action potential set up by sensory stimulation. The spike behaved as a unit and was "all-or-none" whether the nerve was stimulated close to or far from the recording electrodes. (2) Individual units had small receptive fields and a characteristic pattern of response to different forms of stimulation. If there were two units in the same strand with overlapping receptive fields, they still responded independently when electrical or "adequate" stimuli were applied, e.g. Fig. 4. This result, and the specifying of response, taken together with Gasser's (1955) elegant demonstration that unmyelinated axons pass frequently from one Schwann sheath system to another in the peripheral nerve trunk, is difficult to explain unless the afferent units are

private lines and there is no supra-threshold interaction between individual axons.

Several categories of response have now been distinguished among the afferent C units and on this basis the receptors divided into several classes. Since the distinction depends on the thoroughness with which the receptors were tested, the types of stimuli used will be described.

Mechanical stimuli. These were graded from movement of single hairs, through light stroking of the fur, various intensities of stroking of the skin, pulling of hairs, pinching folds of the skin, to pressing pins into the skin. To measure the differences in sensitivity a blunt nylon thread, mounted on a steel spring which formed one plate of a condenser manometer input, was pressed on to the most sensitive spot on the skin. An advantage of this device was that the pressure applied to the skin was measured instead of simply the displacement of the stimulating probe.

Thermal stimuli. It was clear early in this investigation that receptors specifically sensitive to small differences in cutaneous temperature were either rare or non-existent. Accordingly, more severe thermal stimuli were used. Brass or copper rods, 1 cm. diameter, cooled to less than 10° or heated above 50°, were effective stimuli for some receptors. Radiant heat, either from heated metal plates or from an electrically heated coil of wire, was also used, in particular as a probe to test for the presence of heat receptors. Cold stimuli also included ice blocks. Most recently thermodes, heated or cooled by circulating ethanol at the appropriate temperature through them, have been used in order to prevent any change in mechanical conditions as the temperature was altered.

Chemical stimuli. The application of chemicals to the skin has rarely been used, since the mammalian skin is resistant to chemical action. Concentrated NaCl solution (30 per cent, as used by Gessler, 1957) was tried on one occasion, after first making superficial cuts in the epidermis. This was such an unsatisfactory and non-specific stimulus that it was not repeated. The close-arterial injection of pain-producing chemicals has also been tried in a series of experiments in an attempt to identify the pain fibres.

The afferent units with centripetal C fibres had several similar properties. The evidence for specific differences between the afferent units will be given later.

Size of receptive field. This was always small, less than 5 mm. × 5 mm. It was measured accurately in 12 units by mounting the stimulating probe on a micromanipulator and mapping the field while monitoring the pressure applied, so that equal stimuli were applied in the area tested. The most

sensitive C units could be excited by stretching the skin as much as 10 cm. away from the receptive field. However, by using minimal stimuli, the field could be plotted. The results in Fig. 2 are typical. The field is small, 4 × 3 mm., and at its centre, i.e. at the most sensitive spot, the latency was least, the frequency of response highest, adaptation slowest and the after-discharge most persistent. Shifting the probe 1 mm. in any direction reduced the response by about one-half and further movement of 1 mm. made the stimulus ineffective unless the intensity was increased. With less sensitive mechanoreceptors and the thermal

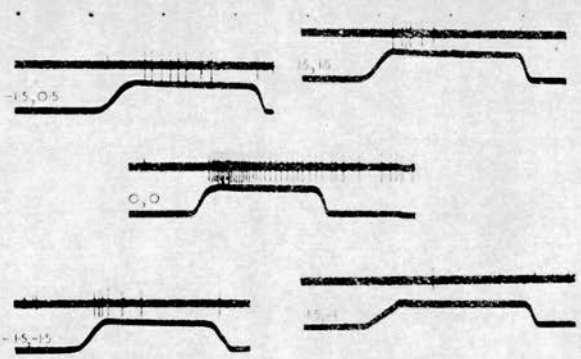


FIG. 2. Response of a C mechanoreceptor to pressure at the centre of the receptive field and at distances of 0·5–1·5 mm. away from the centre. Time marks at 1 sec. intervals in upper figures.

receptors the small size of the receptive field was more obvious because stretching the skin was less effective in exciting them.

Rate of adaptation. When a constant stimulus was applied there was an initial burst of impulses as the stimulus was being applied. The rate of discharge then fell slowly as the stimulus was maintained (Fig. 2). This phase of adaptation was slow, lasting at least one second and often longer; it was related to the extent to which the stimulus was above threshold. The most slowly adapting receptors were the cold receptors; these could maintain a steady discharge for minutes. If rapidly adapting C units exist, they were not found in the present work. An example of a very slowly-adapting mechanoreceptor is provided by the tension receptors in the viscera (Iggo, 1957*a*).

Fatigue. A property of the receptors which needs to be clearly defined is their reaction to repetition of the stimulus. When the stimulus is repeated at short intervals (seconds) the initial response is not sustained. This is true whether stroking, pressure or punctate stimulation is used, but the latter is more likely to be associated with a rapid onset of fatigue. This property is important when changes in sensitivity associated with the injection of chemicals are studied; e.g. in Fig. 6 the test stimuli were spaced at 1 min. intervals, so that transient changes in sensitivity may be missed. Receptors with myelinated afferent fibres did not display this property. The response of mechanoreceptors to mechanical stimulation of the skin could be abolished for several minutes by vigorously stroking the skin for 30 sec., an effect which may be relevant to the relief of itch by scratching or rubbing the skin.

After-discharge. A conspicuous property of the C afferent units is the way in which they continue to discharge impulses for several seconds after a brief stimulus is withdrawn. Only part of this after-discharge is an artifact due to the latency imposed by conduction time. The after-discharge is most conspicuous if the skin has been rested for 30 min. or longer before the stimulus. It is also related to the intensity of the stimulus, strong stimuli being followed by a more persistent discharge of impulses. The rate of discharge was always low during the after-discharge (5/sec. or less). Receptors with myelinated fibres did not have any after-discharge.

Rate of discharge. The maximal frequency at which the C fibres have been driven by natural stimuli was 100 impulses/sec. This frequency was not sustained for more than a few impulses. Firing at frequencies of 50 to 70 impulses/sec. was sustained for as long as 0·5 sec.; after this time adaptation reduced the rate of firing (see Figs. 2 and 4). The rate of firing has been measured in all fibres for which records are available, even though the maximal stimulus was not always used. For the mechanoreceptors the discharge during the first second of stimulation ranged from 15 to 40 impulses. Thereafter the frequency was less, falling to about 5 impulses/sec. The persistence of discharge depended on the intensity of stimulation. In the after-discharge the frequency fell to less than 1/sec.

These results for cutaneous C fibres agree with the figures for

visceral C fibres (Iggo, 1957*a*, *b*,) but do not confirm the conclusion reached by Douglas and Ritchie (1957*b*) that the maximal rate of firing in C fibres "is about 10 impulses/sec." This discrepancy, so far as the cutaneous fibres is concerned, is in part due to their use of rhythmical stroking of the skin. In single C fibres this causes an irregular discharge of impulses, the instantaneous frequency fluctuating between 50 and 5 impulses/sec. Their method of analysis appears to assume that the rate of firing in individual fibres is regular.

Evidence for specific receptors

Although there is convincing evidence for specificity of response among the cutaneous receptors with myelinated fibres this concept of specificity is still denied by some workers. Weddell (1957), largely on the basis of a failure to find histologically distinct structures in hairy skin and the cornea, has propounded the view that the temporo-spatial impulse pattern is an important factor in the signalling of different modalities of sensation. Apart from the failure to account for the ability of the skin to distinguish thermal and mechanical stimuli after they have been active for 30 seconds or longer, by which time any initial temporal differences will have disappeared, the hypothesis ignores a large body of electrophysiological data. Several categories of response have been found using the methods described earlier and the conclusion has been reached that these represent different classes of receptor.

Mechanoreceptors. These were excited by mechanical stimuli but were insensitive to thermal stimuli. They could however be excited by suddenly cooling or heating the skin, but the rate of discharge was low compared with their response to a mechanical stimulus, was short-lived, or when the skin was heated was followed by loss of response to mechanical stimulation. This last effect was attributed to damage to the nerve ending and was sometimes followed, after 10 minutes or longer, by some return of sensibility.

(a) *Hair receptors*: These could be excited by moving single hairs or groups of hairs, and single units fired at frequencies of 50/sec. when only the tips of hairs were moved (Fig. 3). Pressing on the skin was no more effective as a stimulus. The hair receptors included the most sensitive C mechanoreceptors; a steady

pressure of 50 to 100 mg. on the skin surface was sufficient to excite most of them. Several differences from hair receptors with myelinated fibres were found. (1) The A fibres gave a burst of impulses at high frequency, which lasted about 100 msec. as the hair was moved and again as it sprang back to its position of rest. The C hair fibres also gave a burst of impulses as the hair was moved, with a latency of 100–300 msec. due to conduction delay, but the burst was more prolonged, lasting perhaps 1 second. (2) If a hair was bent from its normal position in the skin and held in the new position the A fibres gave the customary burst as the hair was being moved and then fired at low frequency and irregularly. The C fibres on the other hand kept on firing

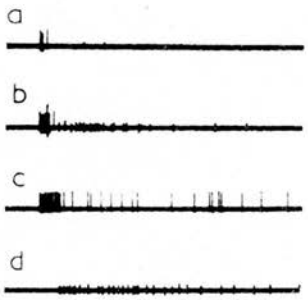


FIG. 3. Response of A and C fibres to movements of hairs. (a) very light movement of hairs; (b) stronger stimulus which excited both A and C hair receptors; (c) A receptor, bending hair to new position and holding it there throughout remainder of the record; (d) the same for C receptors. The receptors were in adjacent areas of skin. Time marks at 0·1 sec. intervals.

at about one-half the maximal rate, with a slow adaptation so that 4 seconds after the hair was moved they were still firing.

(b) *Touch or pressure receptors*: These were the type of receptor found most often. The reactions to mechanical stimulation were similar for all fibres in this group despite a wide range of sensitivity (Fig. 2). When tested by pressing nylon threads on the skin the most sensitive units had a threshold of 50 mg. and the least sensitive of 10 g., a ratio of 200:1. The most sensitive were not excited by moving hairs but were readily excited by lightly stroking the skin. The least sensitive receptors in this group were not excited by stroking the skin; pinching a fold of skin or firm pressure with a blunt probe was required to excite them. The less sensitive units also had a less conspicuous after-discharge and adapted more slowly to a sustained stimulus. Stretching the skin was an effective stimulus for all the C mechanoreceptors

and may have been an important component in many of the effective stimuli. Any stimulus sufficient to excite the least sensitive units will also excite all the other mechanoreceptors, so that a painful mechanical stimulus may excite all the C mechanoreceptors. Thus attempts to decide by the use of mechanical stimuli which of these fibres are "pain" fibres requires knowledge of the "pain" threshold.

Thermoreceptors. Two main categories of response were found (Iggo, 1958*b*). (1) Fibres which were excited when stimulus objects at 15° or more above the normal cutaneous temperature were put on the skin. (2) Fibres which were excited when stimulus objects about 20° or more below the normal cutaneous temperature were put on the skin.

Two other thermoreceptors were found which were silent at neutral skin temperatures and were sensitive to smaller changes in cutaneous temperature than the two main categories. One was excited by cooling the skin a few degrees and the other by raising the skin temperature a similar amount. Neither unit was examined in detail.

This diversity of response requires a terminology, preferably one that does not prejudge their rôle in sensation. The following terms will be used; C hot fibre (for 1), C warm fibre, C cool fibre and C cold fibre (for 2).

The thermoreceptors were relatively insensitive to mechanical stimulation of the skin; they were similar in this respect to the least sensitive mechanoreceptors. After repeated heating and cooling of the skin the mechanical threshold sometimes fell. When the skin was being explored before there had been extensive thermal stimulation it was often very difficult to find the receptor with a mechanical stimulus, e.g. firm pressure with a blunt metal probe or squeezing folds of the skin between fingers and thumb. The effect of skin temperature on mechanical threshold has been examined for only one hot fibre, a myelinated fibre (conduction velocity 17 m./sec., i.e. an A δ fibre). Its sensitivity was enhanced by heating the skin, e.g. at 33° there was a discharge of 15 to 17 impulses/sec. and at 53° to 60° a discharge of 25 to 37 impulses/sec. in response to the same mechanical stimulus.

Heating and cooling nerve trunks is known to excite some of their component fibres (see Granit, 1955) and a few experiments were done in which the saphenous nerve was heated or cooled

(Iggo, 1959). The series is too small to be more than a guide but thermal changes sufficient to excite endings in the skin failed to do so in most instances when the nerve trunk was exposed to them. Heating the saphenous nerve brought up a different response in fibres whose endings were not identified; this was an irregular recurring pattern of a gradually rising frequency of discharge, culminating in a burst of impulses followed by silence, the whole cycle taking 2 to 5 seconds.

C hot fibres. These were silent at resting skin temperatures of 26° to 33° and were not excited by lightly pressing smooth metal rods at temperatures below 48° on the skin. When rods

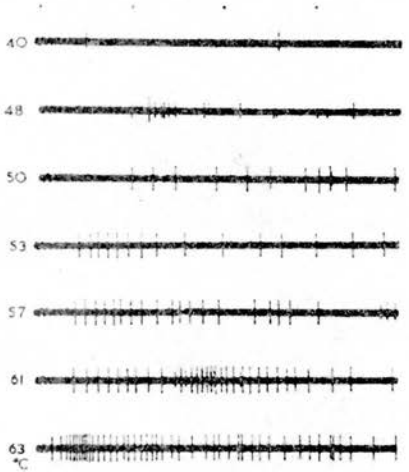


FIG. 4. Response of a C heat receptor to lightly touching the skin with 1 cm. diameter brass rod at the temperatures shown on the left. This strand also contained a C mechanoreceptor about 1 cm. away from the heat receptor. This mechanoreceptor was not excited by radiant heat, although the heat receptor was.

hotter than this touched the skin there was a burst of impulses which appeared with a shorter latency, at a higher frequency and for a longer time as the temperature of the rod increased (Fig. 4). Radiant heat has also been used, particularly during the preliminary stages of an experiment when heat receptors were being sought. With the stimulator most frequently used as a source of radiant heat (a coil of wire heated electrically to a bright red) the response was usually brief, lasting only a few seconds. The brevity of the response and its failure to persist even though the skin temperature still rose may have been due to heat block of the nerve fibre. The threshold for radiant heat was 44° on the surface and 35–40° subcutaneously for one heat receptor.

The temperature gradient across the skin was not steep when this receptor was firing.

The threshold temperature in man for pain caused by heating the skin is about 48° (Skouby, 1952) when thermodes are used; the C hot fibres just described may thus mediate part of the sensation, but not all of it since there are also heat receptors with A fibres (see above).

C fibres excited by cooling the skin. Three types of response were found. (1) *C cool fibres*: One unit similar to the myelinated cold fibres of Hensel and Zotterman (1951*a*) was

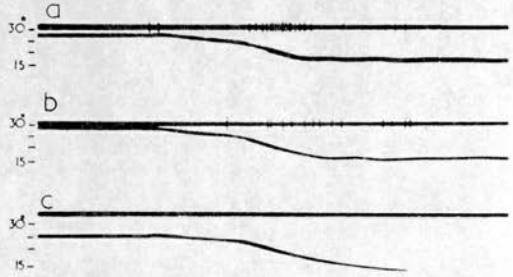


FIG. 5. The response of three C units to cooling the skin. (*a*) and (*b*) are sensitive mechanoreceptors and (*c*) a heat receptor. Sudden cooling caused a short burst of impulses at low frequency (1 to 5/sec.) in the mechanoreceptors. These units discharged at 50 impulses/second when the skin was lightly stroked. Note time scale, marks at 5 sec. intervals.

isolated. It was silent when the skin was at normal temperature and was excited by cooling the skin a few degrees. Further cooling led to a fall in the rate of discharge. (2) *Mechanoreceptors* which were excited when the temperature of a thermode on the skin was lowered by 4–10°. This effect was first noted by Douglas and Ritchie (1959). It appears to be a different phenomenon from the thermal responses of the fibres in (1) and (3). Thus the response of the mechanoreceptors to cooling the skin was a low-frequency (< 5/sec.) discharge of impulses and the main response was a burst of impulses which lasted 5–10 seconds (Fig. 5). Further cooling from the new low steady temperature sometimes aroused a second burst of impulses. The rate of

firing (< 5/sec.) was much less than could be aroused by mild mechanical stimulation of the skin (>50/sec.). This reaction to rapid cooling of the nerve endings or axon is shared by many receptors and nerve fibres including the A cold fibres (Dodt, 1953) and is not related to specific thermal responses.

(3) *C cold fibres*: These had thresholds, measured in three fibres by a thermistor on the skin surface, of 13°, 14° and 17°. The fibres discharged impulses steadily at frequencies of 2–10/second (Fig. 6). At the lower rates they continued for as long as 3 minutes. Although the steady discharge of impulses appeared at various frequencies in individual fibres the exact relation

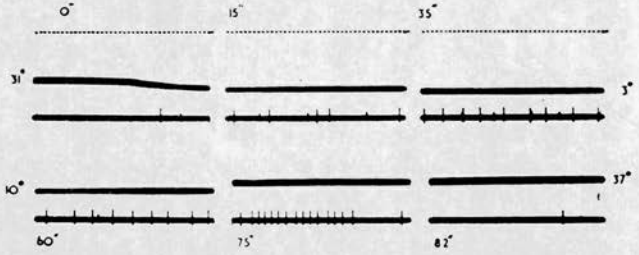


FIG. 6. The response of a C cold receptor to cooling the skin with ice. Cutaneous temperature registered by a thermistor resting on the skin. The ice was put on at the beginning of the first record and left on for 55 seconds. Just before 75 seconds a warm saline pad was put on the skin. Cooling the skin evoked a steady discharge of impulses, and during rewarming the frequency was higher, but the burst was brief. Time marks at 0·1 sec. intervals.

between temperature and rate of discharge has not yet been examined. Most units stopped firing when the skin was warmed either by removing the cold stimulus or by replacing it with a warm stimulus. One receptor (Fig. 6) was found, however, which fired faster as the skin was being rewarmed than it did at any stage during cooling. It was not excited by high temperatures unless the skin was being rewarmed from a very low temperature. The frequency of discharge during the rewarming phase was related to the temperature applied and the discharge was brief, lasting not longer than 10 seconds.

The relation of these thermal responses to the pain elicited on cooling human skin (Wolf and Hardy, 1941) was examined in ten subjects (Iggo, 1959, unpublished). Immersion of a

finger in water or an ice/salt mixture at $-10°$ to $+5°$ for 15 to 45 seconds caused persistent severe pain, even in the ischaemic finger when only second pain had survived. Plunging the cooled finger into water at 30° to 40° was in some of the subjects followed within 5 seconds by a brief flash of pain more intense than that felt during immersion in the cold fluid. A similar flash was felt in the ischaemic finger. In both Wolf and Hardy's experiments and in the experiments just mentioned the pain in ischaemic skin was felt in the absence of any sensation of cold. In the ischaemic finger there was an initial report of cold sensation when the finger was first put into water at 1° but this had gone before the pain appeared. These two sets of data, one on single C fibres and the other on sensation, lead to the conclusion that part of the pain experienced when the skin is drastically cooled is due to impulses in C cold fibres.

The results just described are clear proof that the C wave of the compound action potential is generated by axons which innervate a diversity of receptors. The hypothesis that all C fibres are pain fibres and innervate an undifferentiated non-discriminating network of nervous tissue has to be rejected. The C hair receptors and the most sensitive mechanoreceptors cannot be convincingly upheld as pain receptors. The least sensitive pressure receptors and the thermoreceptors on the other hand could all mediate pain.

One way to establish which are the C pain fibres would be to expose individual fibres of the above categories to pain-producing chemicals, on the assumption that all the pain fibres would be excited by such stimuli. Concentrated saline (30 per cent NaCl) is undoubtedly painful when put on to skin wounds, and Gessler (1957) reports that such a stimulus caused a high-frequency rhythmical discharge of impulses in cutaneous afferent fibres. However with single fibre preparations it was more effective in exciting A hair receptors than in exciting C receptors, and it excited a cut axon in the skin as effectively as the intact ending (Iggo, unpublished). Such a stimulus is quite non-specific and causes pain because it excites all the afferent fibres, including the pain fibres.

Close-arterial injection of pain-producing chemicals. Another more promising approach seemed to be the use of chemicals which in low concentrations (10^{-6} to 10^{-12} g./ml.) are

effective in provoking pain from human skin, either when pricked into intact skin (Emmelin and Feldberg, 1947) or when put on to blister bases (Armstrong *et al.*, 1953). Histamine (6–300 μg.), acetylcholine (20–60 μg.), mixtures of histamine and acetylcholine (in similar concentrations to those used for single injections of either), hydroxytryptamine (2–40 μg.) and impure bradykinin (3–4 $\times$ 10^{-4} g./ml.) were injected close-arterially into the femoral artery of cats, so that the chemicals were carried into the saphenous artery. At the same time electrical activity was recorded in fine strands dissected from the saphenous nerve (Fjällbrant and Iggo, 1959).

(a) *Effect on C fibres.* When strands containing many axons were used, injection of any of the chemicals except bradykinin excited some of the C fibres in the strands. The discharge reached a peak in 20 seconds and had fallen sharply by 1 minute and some irregular discharge persisted for several minutes. The rate of discharge in individual fibres, when this could be recognized, was less than 5 impulses/second. However, when single units of all the categories described above were tested and the effect of the injections on the response of the receptors to mechanical or thermal stimuli was tested there was no clear enhancement of activity. The brevity of the excitatory action of the chemicals and the inability of C receptors to respond uniformly to frequently repeated stimulation prevented any detailed analysis of the excitatory effects. A more prolonged action of the chemicals (histamine; histamine and acetylcholine; hydroxytryptamine) was to depress the response of receptors to adequate stimulation. The example shown in Fig. 7 was a C pressure receptor and its response to pressing on the skin was depressed for nearly one hour. It is clear that if the injections of the chemicals follow too closely on each other they may find the receptor still affected by previous injections.

(b) *Effect on A fibres*: The most striking effect of the chemicals was on the response of slowly-adapting pressure receptors, with afferent fibres in the Aγ range (20–40 m./sec.). These units sometimes had a very steady resting discharge which would continue, with minor fluctuations in frequency, at rates of 2–10 impulses/sec. for hours. Two minutes after the injection of histamine (33 μg.) the resting discharge was more than doubled and then began to fall until by 3 minutes it was absent. At this

time the receptor still responded to pressure, usually at a higher frequency than initially. This response then failed and the fibre was silent. (At this time mechanical or thermal stimuli could still excite the appropriate C fibres if there were any in the strand.) After 20 minutes the responses of the pressure receptor returned in reverse order to their disappearance. A similar experiment is illustrated in Fig. 8. Acetylcholine alone raised the resting discharge, histamine alone in the concentration used (20 μg.) did very little. An injection of both now gave the usual response to larger doses of histamine—enhancement at 2 minutes followed

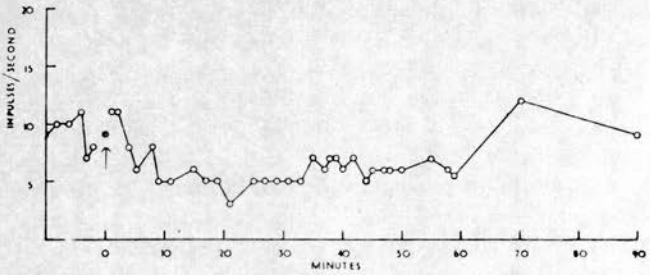


FIG. 7. The effect of a close-arterial injection of histamine (10 μg.) and acetylcholine (24 μg.) on the response of a C mechanoreceptor to repeated stimulation by pressing on the skin with a nylon thread. The injection was made at zero time. The stimulus was repeated at 1 minute intervals to avoid fatigue of the receptor.

at 3 minutes by depression and then recovery to the resting level of discharge. Bradykinin caused an uncomplicated enhancement of the resting discharge. Hair receptors with myelinated fibres were apparently unaffected by any of the chemicals used. The failure of acetylcholine to cause brief (10–20 sec.) excitation of all the cutaneous afferent fibres (Brown and Gray, 1948; Douglas and Ritchie, 1959) may be a concentration effect. But these groups of investigators made the injections closer to the skin and to a much smaller piece of skin than in the work described above.

These results fail to provide a satisfactory answer to the question, "Which are the C pain fibres?" Thus an irregular low frequency discharge in fibres which can be readily excited by lightly touching the skin cannot be regarded as likely to cause pain. Likewise an enhancement of the response of A

pressure receptors, although it may account for other cutaneous sensory phenomena (such as the hyperaesthesia in the region of a flare; Lewis, 1942), cannot be considered to be painful. There are other reasons for rejecting these results as likely to identify pain fibres. The close-arterial injection of hydroxytryptamine

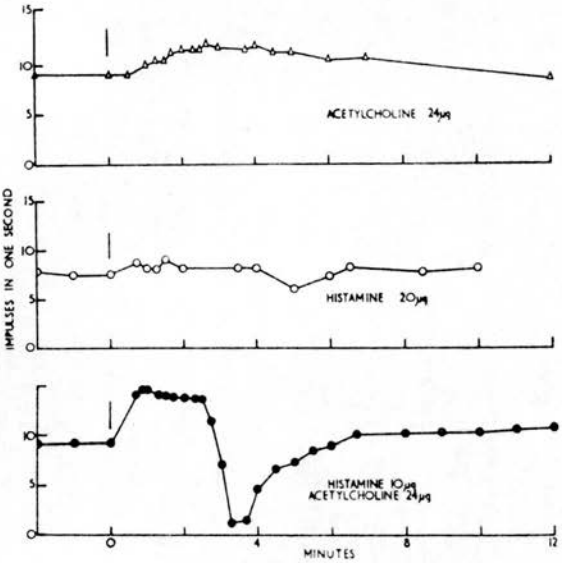


FIG. 8. The effect on the steady resting discharge of an A pressure receptor of the close-arterial injection of (*a*), acetylcholine, 24 μg., (*b*) histamine, 20 μg., and (*c*) histamine, 10 μg. and acetylcholine, 24 μg. The response in (*c*) is typical of the reaction of these pressure receptors to sufficient quantities of histamine and mixtures of histamine and acetylcholine, showing enhancement and then depression of the steady discharge of impulses.

into the brachial artery in man (Roddie, Shepherd and Whelan, 1955) aroused a persistent sensation from the skin but no pain. The close-arterial injection of hydroxytryptamine in dogs in an amount sufficient to evoke respiratory effects if given intravenously fails to provoke any of the usual reactions to painful stimulation (Douglas and Toh, 1953) and so does the injection of histamine or hydroxytryptamine into the arterial supply of the

rabbit's ear (Lembeck, 1957). The pain-producing chemicals thus may not be painful if they are put into the blood-stream instead of into the intact or damaged skin.

Conclusions

The results described make it clear that the unmyelinated cutaneous afferent fibres are not uniformly sensitive to all cutaneous stimuli. On the basis of the differences in sensitivity which were found an attempt was made to classify the receptors. There is a clear distinction possible between the mechanoreceptors and the thermoreceptors. Within each group further subdivisions could be made. The subdivisions were easier to make for the thermoreceptors because of the large differences in effective temperatures for the two main groups. The most obvious difference among the mechanoreceptors was that only some of them were excited by touching the hair. Other mechanoreceptors at least as sensitive as the hair receptors to punctate pressure were not excited. This difference may be due to some of the C fibres ending in the hair follicles (the accessory fibres) and others in the dermis. There was a wide range in sensitivity among the mechanoreceptors not excited by moving hairs, but no other difference in response to mechanical stimulation which would justify their further subdivision. These results are thus evidence that the numerous unmyelinated cutaneous afferent fibres, as well as the unmyelinated fibres, display a specific sensitivity to cutaneous stimuli. Evidence for specific differences among the unmyelinated visceral afferent fibres has also been found (Iggo, 1957*a*, *b*).

There is no reason to believe that all types of receptor with afferent C fibres have yet been examined by single fibre methods. So far none of the slowest C fibres described by Gasser (1955) have been isolated as single units. The results are, however, sufficient to refute any hypothesis which asserts that all nerve endings with afferent C fibres are identical in their responses to cutaneous stimulation. They also indicate that the C fibres may have a hitherto unrecognized importance both for reflex activity and for sensation.

DISCUSSION

Ritchie: The disagreement between your frequencies and ours may be only an apparent one, due to differences in our methods. Our estimate depends on a calculation, and I agree there is room for error. However, when we make the same sort of calculation for the δ fibres when the same sort of tactile stimuli are used, we do get reasonable frequencies of firing (about 100 impulses/sec.). If our calculation for the frequency of firing of C fibres were out by a factor of 5 or more, as might appear to be the case, we should be out by the same factor for the δ fibres—and this I think is unreasonable. We look at the whole population of fibres to get some sort of average value. Perhaps the stimuli we use are not so vigorous as yours.

Iggo: I cannot accept that. Your δ fibre frequencies are also low.

Ritchie: Our stimulus is applied over a diffuse area. The other possibility is that although the conduction velocities of the fibres that we both look at are of much the same order and thus of similar size, other factors as yet unknown might cause you to select fibres of one particular type.

Iggo: The response of individual fibres to repetitive stroking (4/sec.) is to discharge bursts of impulses. The instantaneous frequency varies between 50/sec and 5/sec. Your method assumes that there is a steady discharge, but clearly there is not. Another point is that there is a good deal of variation in the threshold—fibres which are mechanically sensitive are not all equally sensitive. The second assumption which you make is that you are working with a uniform population. Neither of these assumptions holds.

As for selection, I can only say that the fibres I have worked on seem to be the same as yours. You find that the whole of the first C wave goes out, and that it contains the only fibres excited by mild mechanical stimuli, and I find that my conduction velocities fit into that first C wave and in fact the whole C complex.

Ritchie: I am not trying to say that our method was better than yours, but to suggest how we may have got different results. From your argument one would also expect to find lower frequencies with the δ fibres than are usually found with other methods. But we did not.

Iggo: The impulses in myelinated fibres come in groups as in the C fibres, only more so.

Ritchie: Then we would have tended to be more in error with the myelinated than with the non-myelinated fibres. But in fact we seem to be getting reasonable values for the frequency of firing of δ fibres. I am aware that we make assumptions, but you also make assumptions. For example, you assume that you make a random selection, when there is evidence from studies on other nerve fibre populations that this does not usually occur. The fact that we have never found any significant amount of C fibres responding to heating whereas such fibres make up a large fraction of the C fibres you

have isolated would corroborate the view that some process of selection is occurring with the single fibre technique.

Iggo: In any one animal I may get a dozen single fibres, and there are thousands of C fibres in the saphenous nerve.

Ritchie: One point about selection is the enormous complexity of the structure of C fibres, for we know from electron microscopy that a single Schwann cell sheath may contain varying numbers of C fibres, some of which can be seen passing from one sheath to the next. These factors might make it easier to isolate some fibres than others; and as we do not know whether all modalities have the same morphological arrangement, the possibility must be emphasized that the sampling is biassed and not random in single-fibre studies.

Dodt: In the small medullated nerve fibres of the glossopharyngeal nerve of the frog a sudden jump in tongue temperature (8° to 24°) is followed by a strong phasic response, whereas cooling is quite ineffective. If the same stimulus is applied repetitively, the tongue must be kept at the low temperature for 10–30 seconds before the response to warming can be produced again. Thus "fatigue" of receptor processes can be seen in medullated as well as in unmedullated nerve fibres.

We have also done experiments on cats in which impulses of small medullated fibres from the lingual nerve were elicited by heating the tongue above 47°. Since these fibres did not respond to cooling or to lower constant temperatures we thought they should be called "pain" fibres. However, they did not respond to the very cold temperatures which produce cold pain in man. Therefore I am very pleased about Dr. Iggo's findings on the activity of unmedullated nerve fibres within the low temperature range.

Zotterman: Dr. Iggo, would cooling of these C fibres to very low temperatures produce a sensation of cold? I believe the result should be pain.

Iggo: These C fibres which fire at low temperatures may have something to do with cold pain. There does not seem to be any evidence of a cold sensation carried by C fibres at these low skin temperatures.

Ritchie: Could you speculate on the relative numbers of fibres responding only to touch, fibres responding only to cold and fibres responding to both these forms of stimulation?

Iggo: All the mechanoreceptors which I have taken out to test with cooling (one experiment; five fibres) were excited by mechanical stimuli and by cooling. But when cooled they gave a slow frequency (below 5/sec.) and a fairly brief discharge. These fibres would fire at 50/sec. when the skin was touched. On the other hand, there are the fibres which are excited at lower temperatures still and are insensitive to mechanical stimuli; I had only six of those altogether. Most (40 or 50) of the single fibres I looked at were probably mechanoreceptors; 14 others were heat-sensitive, and about six were cold-sensitive. It looks as if the mechanoreceptors are in the majority.

Dodt: How long does it take to produce signs of oxygen deficiency at your endings?

Iggo: I have not looked at these fibres in the cat during asphyxiation of the nerve or during any other form of block.

Douglas: Many C fibres discharge quite briskly in situations which one certainly cannot consider as painful, yet a single shock exciting the C group is painful. The C fibres subserving pain may therefore form only a small proportion of the C fibre population.

Adrian: These results agree with what has come out of your records, Prof. Zotterman. On the other hand you have shown that the cold receptors give a specific discharge.

Zotterman: Our main studies on thermal receptors were made on the lingual nerve which does not contain any afferent C fibres. Cutaneous δ and C fibres are more resistant to high and low temperatures than are the endings of the large myelinated fibres of the skin. When water at 50° was squirted over the tongue it abolished for some minutes the excitability of the large myelinated fibre endings responding to touch or pressure. Cooling the tongue to a low temperature also blocked the activity of the large fibres, leaving the signalling duty to small δ and C fibres.

Iggo: The hot C fibres are resistant to high temperatures, but the C mechano fibres are not. The C mechano fibres are damaged by temperatures which excite but do not damage the C hot fibres.

Discussion of paper by Sven Landgren continued from page 83.

Landgren: Not necessarily but it may very well be so. Signals of low intensity may not be able to force their way through the chain of synapses. If a group of afferent fibres are more sensitive to a particular modality it seems reasonable that this modality should be the most effective in penetrating the chain of synapses, and weak responses to other modalities may very well be sorted out.

Dawson: Stimulation of the cortex under a fairly deep anaesthesia produces a reduction in the firing of units in the cuneate nucleus with a latency of only about 5 msec. This effect seems to outlast quite deep anaesthesia and it looks as though there is a fairly direct pathway, of the type suggested by Wallenberg, from the cortex to the cuneate nucleus. This inhibition of the cuneate response by stimulating the cortex can still be obtained with a lighter anaesthesia but Dr. Schatz has shown that if at the same time the trigeminal area is stimulated, say by pinching the lip or tickling the whiskers, this will cause a very considerable increase in the cortical responses. This does not occur at the cuneate level at all because the units in the response there do not seem to be changed by the trigeminal stimulus. Have you tried cortical stimulation, Dr. Landgren?

Landgren: No. I would expect that stimulating the cortex would also activate the reticular formation.

Nicht im Handel

Sonderdruck aus Band XXIV, Heft 1—4, 1963
ACTA NEUROVEGETATIVA
Schriftleitung: E. Anderson, Bethesda, C. Coronini, Wien, A. Sturm, Wuppertal
Springer-Verlag in Wien

Department of Physiology, University of Edinburgh

New Specific Sensory Structures in Hairy Skin

By

A. Iggo

(Locke Research Fellow)

With 3 Figures

Frankenhaeuser (1949), *Maruhashi, Mizuguchi* and *Tasaki* (1952), and *Hunt* and *McIntyre* (1960) have described afferent fibres with a highly localised sensitivity to mechanical stimulation of the skin of the cat and rabbit, and used the expression "touch spot" to describe the receptive fields of the afferent nerve-fibres. The results to be described now deal with similar afferent nerve fibres dissected from the saphenous or sural nerves of 10 cats, either anaesthetized with chloralose or decerebrated. All 20 receptors examined electrophysiologically as single units were excited by mechanical stimuli applied to very small spots on the skin of the hind leg and foot. The weakest effective stimuli, 2 to 10 mg. *v. Frey* hairs, evoked a response from an area of skin about 100 to 300 μ^2 and to obtain a response from the surrounding skin not more than 1 mm. away, the stimulus had to be increased at least 50 times. Movements of surrounding hairs, by an amount more than sufficient to excite the rapidly-adapting hair receptors, was without any action on the slowly-adapting "touch spots". Pressing on the cat's hair was effective in exciting the axons only if the hair was pushed down on to the "touch spot". Carefully controlled movement of the hairs adjacent to the "touch spot" usually failed to excite the nerve-fibre, even when the hair was pulled, except that occasionally a discharge of impulses was elicited if the hair nearest the "touch spot" was moved in some appropriate way. These results suggest that the "touch spots" are organised in such a way as to be unaffected by displacement of the tips of the hairs and to respond to pressing on the skin. In agreement with *Frankenhaeuser* (1949) each "touch spot" appeared to be innervated by only one afferent nerve-fibre, although, as *Hunt* and *McIntyre* (1960) also report, a single axon sometimes innervated more than one "touch spot" (10 fibres to 1 spot each, 4 to 2 spots each, 1 to 3 spots). The diameters of the afferent fibres, assessed by measuring their conduction velocities (40 to 95 m./sec., 9 nerve-fibres) ranged 7 to 16 μ, so that they are in the same size group as the larger of the afferent nerve-fibres which innervate the hairs.

Mechanical sensitivity

The mechanical thresholds ranged from 2 to 10 mg. wt. A steadily maintained pressure on the sensitive spot was provided by a probe mounted on a micromanipulator. This evoked a very persistent, though irregular, discharge of impulses, which, after a quick phase of adaptation lasting less than 1 sec., continued at a slowly falling frequency for 5 minutes (Fig. 1) or longer. The highest frequency of discharge was obtained by the quick movement of a smooth glass rod or similar smooth object across the skin containing the sensory ending or by suddenly distorting the "touch spot" by a rapidly applied vertical movement of a smooth stimulating probe. In these conditions a burst of impulses lasting 5 to 100 msec., with the maximal rate of firing at the very high frequencies of 1100 impulses/sec., was elicited. The

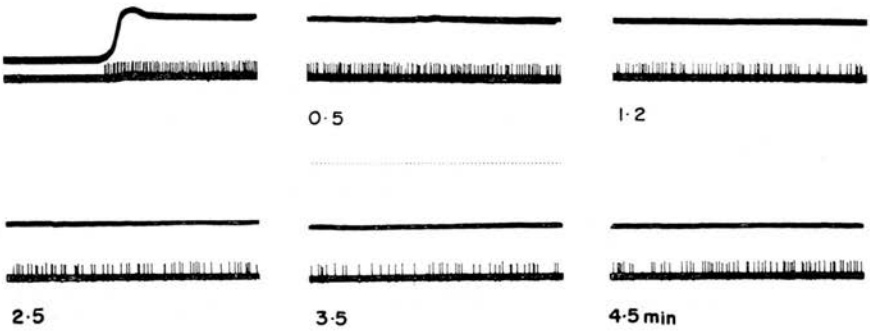


Fig. 1. The slowly-adapting discharge of impulses in the afferent nerve-fibre from a touch corpuscle, before and, at the indicated times, during the application of a mechanical stimulus. In each record the upper trace indicates the mechanical load and the lower trace shows the impulses in the afferent fibre. There was no resting discharge in the fibre in the absence of a mechanical stimulus and 4.5 minutes after the load was applied the fibre was still discharging. Time marks at 1/10th sec. intervals.

amplitudes of successive impulses in such a burst were progressively smaller particularly at the highest frequencies, presumably because the later impulses were travelling in the relative refractory periods of the preceding ones. On these results the receptors would be classed as *slowly-adapting mechanoreceptors.*

Thermal sensitivity

Cooling the skin elicited a discharge of impulses in the afferent nerve-fibres; e. g. suddenly lowering the temperature of the thermal stimulator from 40° to 20° C. caused a discharge of impulses in single fibres at frequencies not greater than 50/sec. if the afferent fibre was silent at the upper temperature. The discharge usually ceased before the skin had reached its new steady temperature which was measured with a thermistor mounted on the bottom of the thermal stimulator. It there was a steady discharge in the afferent fibre as a result of the pressure of the thermal stimulator on the skin then the steady discharge was influenced by the cutaneous temperature

(Fig. 2) as reported by *Witt* and *Hensel* (1959) and *Hunt* and *McIntyre* (1960). The peak rate of discharge in response to sudden severe cooling of the skin was, therefore, less than 5 per cent of the maximal response to a sudden mechanical stimulus. It is suggested elsewhere that this effect of cooling is related to the rate of adaptation of the receptor to mechanical stimulation (*Iggo*, 1962), and that it is unlikely to be concerned in thermal sensation.

Microscopical observations

The sensitive area of the skin was explored carefully using a fine hair as the mechanical stimulus, under binocular microscopic observation ($\times$ 40 to $\times$ 80) while recording from the afferent fibre. The "touch spots" were invariably, in more than 50 trials, found to be slightly raised roughly hemi-

Fig. 2. The effect on the discharge of impulses in an afferent fibre from a touch corpuscle (the same preparation that is illustrated in Fig. 1) of lowering and raising the cutaneous temperature. The four records are all taken from the same strip of recording paper but there is a time interval of about 20 seconds between each of them. The temperature of the water flowing through the thermal stimulator is indicated at the side of each tracing. At the left-hand-side of each record there is a steady discharge of impulses, due to the pressure of the thermal stimulator on the skin, and this discharge is modified when the temperature is changed. Time marks at 1/10th sec. intervals at the top.

spherical domes ranging in diameter from 150 to 250 μ and raised above the surrounding skin by about 100 μ. Touching the raised surface of the dome with the light *v. Frey* hair (2 to 10 mg.) aroused a discharge of impulses whereas the same stimulus was quite ineffective less than 1 mm. away on the surrounding skin. A very characteristic feature of the dome was the presence in it of a whorl or glomus of small blood vessels, probably capillaries, which were always filled with a rich flow of blood. This rich vascular supply was absent from the adjacent skin. The density of the domes or corpuscles in the hairy skin of the hind leg and foot ranged from 10 to 20 cm^2. Not all the domes were identified as "touch spots", but is was invariably the case that the "touch spot" afferent fibres, with the responses described above, ended in the domes. There are, however, other slowly-adapting mechanoreceptors in the cat's skin which differ in some respects from the present fibres and which do not end in the domes. Similar domes are present in the skin of the dog where they appear as hemispheres on the skin and are slightly larger; the diameters of the small sample so far examined ranged from 200 to 300 μ.

Histological observations

Two techniques, silver staining of sections by the Holmes method, and intravital staining with methylene blue, were used to examine the structure of the nerve endings in the touch corpuscles. All corpuscles examined (> 50) were innervated by a single axon which either reached the corpuscle unbranched or began to divide not more than 200 to 300 μ from the corpuscle, unless the afferent fibre supplied several corpuscles when it must have divided several millimetres away from the corpuscles. Further, more profuse, branching occurred as the axon entered the corpuscle and the branches took

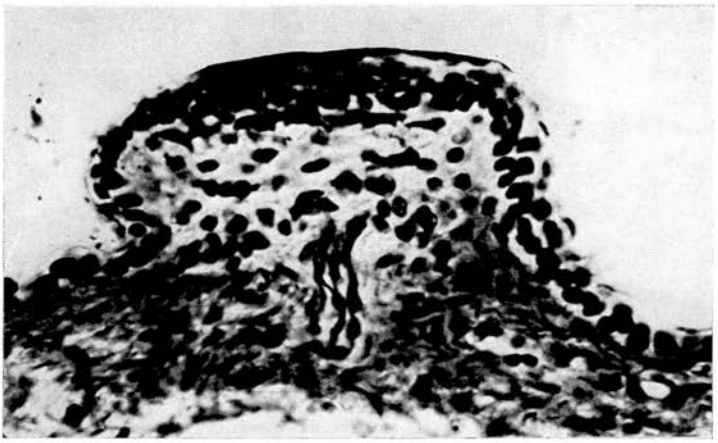


Fig. 3 a. A transverse section through a "touch corpuscle" on the surface of cat's skin. Holme's silver method. The afferent fibre, divided into three branches, can be seen entering at the middle of the corpuscle and the terminal discs of the fibre are oriented parallel to and just below the epithelium. The clear area just below the terminal discs is occupied by blood vessels.

a radial course to penetrate towards the epithelium (Fig. 3 a). An individual axon gave rise to as many as 15 branches which ended, as far as could be judged, just beneath the epithelium. The terminals of the axons had a very characteristic structure which was best revealed in methylene-blue-stained preparations, examined as whole mounts (Fig. 3 b). The terminals were roughly circular plates or discs up to 10 μ in diameter and 1 μ thick. Each disc was the terminal expansion of an axonal twig. These terminals probably correspond to the *Merkel's* discs which are commonly found in glabrous skin (e. g. *Miller*, *Ralston* and *Kasahara*, 1960). In the same methylene-blue-treated skin in which all the tactile corpuscles contain the discs, the axons ending about the hairs were also clearly visible and well-stained. No large discs similar to those found in the tactile corpuscles were evident although occasionally there were small expansions on the fibres about the hair follicles. This absence of large discs in the hair follicles agrees with all previous reports of hair follicle innervation and can be taken as an indication that the

method of methylene-blue staining adopted did not lead to the production of serious artifacts. Electron micrographical examination of these corpuscles has been started but no results are available yet.

The invariable association of a characteristic and constant physiological behaviour with a cutaneous organ which in turn had a characteristic structure is probably sufficient to establish the structure as a new cutaneous sense-organ. A second conclusion is that the particular physiological response, a slowly-adapting discharge to sustained mechanical stimulation, is dependent on the structure of the nerve endings. If this is so then the present results go some way to resolve the long-standing controversy concerning the relation between structure and function in afferent nerve-endings, by providing unequivocal evidence for such a relation. There are striking differences in behaviour in response to identical mechanical stimulation of the skin, between the rapidly-adapting axons supplying the hair follicles and the slowly-adapting axons supplying the touch corpuscles, and equally striking differences between the structure of the terminals of the axons. Whether this proposed association of a slowly-adapting response to mechanical stimulation with disc-like endings of the axons is generally true requires further experimental testing.

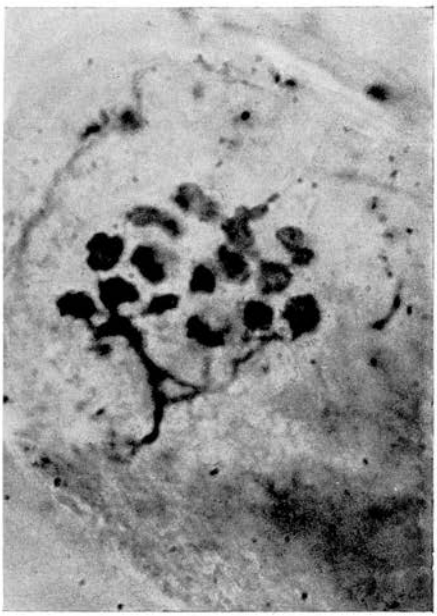


Fig. 3 b. A touch corpuscle in a methylene-blue stained preparation of cat's skin. The terminal discs, which are darkly stained, are arranged parallel to the surface of the corpuscle.

Summary

1. Cutaneous "touch spots" in hairy skin, with mechanical thresholds as low as 2 mg. weight, were identified by recording electrically from single myelinated cutaneous afferent nerve fibres in anaesthetised cats.

2. The discharge elicited by mechanical stimulation of a "touch spot" was slowly-adapting. Movements of the adjacent hairs were ineffective stimuli unless the hairs were pressed down on to the "touch spot". A brief low frequency afferent discharge could be elicited by lowering the temperature of the skin.

3. The "touch spots" could be seen on the surface of the skin in the living animal as hemispherical domes, 100 to 250 μ diameter. Histological examination of a "spot" showed a specialised epithelium, containing flat terminal nerve discs, 10 μ diameter, innervated by branches of a single thick myelinated axon.

4. It is suggested that the results provide strong support for the concept of a specialisation of function and structure in some cutaneous afferent nerves.

Zusammenfassung

1. Cutane „Berührungspunkte" mit einer mechanischen Reizschwelle von weniger als 2 mg wurden in der behaarten Haut durch elektrische Registrierung von einzelnen markhaltigen afferenten cutanen Nervenfasern bei anästhesierten Katzen aufgedeckt.

2. Die durch mechanische Reizung ausgelöste Entladung eines „Berührungspunktes" adaptierte sich langsam. Eine Bewegung der Haare an diesem Ort war als Reiz wirkungslos, ausgenommen dann, wenn die Haare auf den „Berührungspunkt" niedergedrückt wurden. Eine kurze niederfrequente afferente Entladung konnte durch Senkung der Hauttemperatur ausgelöst werden.

3. Die „Berührungspunkte" konnten an der Hautoberfläche bei lebenden Tieren als halbkugelige Kuppeln von 100 to 250 μ Durchmesser beobachtet werden. Histologische Untersuchungen einer solchen Stelle ergaben ein besonderes Epithel, das flache, terminale Nervenscheiben von 10 μ Durchmesser enthielt und das durch Zweige eines einzelnen dicken myelinisierten Axons innerviert wurde.

4. Die vorliegenden Untersuchungen stellen eine starke Unterstützung der Ansicht dar, daß bei einigen cutanen afferenten Nerven eine Spezialisierung der Funktion und Struktur vorhanden ist.

Résumé

1. Des «points de contact» cutanés dans la peau poilue, avec des seuils mécaniques aussi bas que 2 mg de poids, ont été identifiés par l'enregistrement électrique de résultats trouvés dans de différentes fibres nerveuses myéliniques cutanées afférentes chez des chats anesthésiés.

2. La décharge déclenchée par la stimulation mécanique d'un «point de contact» s'est adaptée lentement. Les mouvements des poils voisins étaient des stimuli inefficaces excepté que les poils étaient déprimés sur le «point de contact». Une brève décharge afférente de basse fréquence pouvait être déclenchée par la diminution de la température de la peau.

3. On pouvait voir les «points de contact» dans la surface de la peau chez l'animal vivant comme des domes hémisphériques d'un diamètre de 100 à 250 μ. L'examen histologique d'un «point» a montré un épithélium spécialisé contenant des disques nerveux terminaux plats d'un diamètre de 10 μ, innervés de branches d'un seul axon myélinisé épais.

4. On est d'avis que les résultats sont fortement en faveur du concept d'une spécialisation de la fonction et de la structure dans quelques fibres cutanées afférentes.

References

Frankenhaeuser, B., Acta physiol. Scand. *18* (1959), 68. — *Hunt, C. C.*, and *A. K. McIntyre*, J. Physiol. 153 (1960), 88. — *Iggo, A.*, This Symposium, 1962. — *Maruhashi, J., K. Mizuguchi* and *I. Tasaki*, J. Physiol. *117* (1952), 129. — *Miller, M. R., H. J. Ralston* and *M. Kasahara*, In: Cutaneous Innervation. p. 1, Pergamon Press, London. — *Witt, I.*, and *H. Hensel*, Pflügers Arch. Physiol. *268* (1959), 582.

Anschrift des Verfassers: Dr. *A. Iggo*, Department of Physiology, University of Edinburgh, Edinburgh (Scotland).

Printed in Austria

Reprinted from Excerpta Medica, International Congress Series No. 48,
containing abstracts of Free Communications read at the

XXII INTERNATIONAL CONGRESS OF PHYSIOLOGICAL SCIENCES

Leiden, September 10-17, 1962

612.794 + 612.88.079

A CUTANEOUS SENSE ORGAN IN HAIRY SKIN.

A. Iggo and A.R. Muir, Depts. of Physiology and Anatomy, Univ. Edinburgh, U.K.

'Touch spots' in cat hairy skin were examined by recording from single active afferent fibres dissected from the saphenous or sural nerves of anaesthetized cats. The mechanical thresholds were 2-10 mg wt. The maximal frequency of discharge, when the skin was stimulated mechanically, was 1100 imp./sec. Steady mechanical stimuli caused a slowly adapting discharge at 10-50 imp./sec. which lasted five min. or longer, with an irregular spacing of the impulses. Sudden lowering of the skin temperature by several degrees centigrade accelerated, for 5-30 sec, a steady discharge caused by constant pressure and vice versa. The touch spots were circular elevations of the skin surface. The epidermis of the elevation was modified and thicker than the surrounding epithelium. At the periphery of the elevation, cytoplasmic processes projected from the cells of the stratum basalis into a meshwork of fine collagenous fibres, which formed the core of the elevation. The sensory endings consisted of 'tactile cells' which hung along the lower border of the stratum basalis, being attached to this layer by many desmosomes and enclosed by the basement membrane of the epithelium. The nerve endings, containing many mitochondria, were invested by the tactile cell and lay just beneath its polylobulated nucleus. A clear space was seen beneath the nerve ending. Each touch spot was supplied by one thick myelinated axon, which branched profusely at the base of the elevation.

[*From the Proceedings of the Physiological Society*, 28–29 September 1962]
Journal of Physiology, **165**, 28–29*P*

The structure and function of cutaneous 'touch corpuscles' after nerve crush

By A. G. Brown and A. Iggo. *Department of Physiology, University of Edinburgh*

The saphenous nerve, in the thigh of anaesthetized cats, was crushed for 1 cm or resected for 3 cm, and structural changes in cutaneous 'touch corpuscles' were followed. Four days after crushing the nerve the 'tactile corpuscles' show degenerative changes; the normal thick epidermis is thinner, the 'tactile cells' along the lower border of the epidermis are smaller, their nuclei are centrally placed and their clear sacs are smaller and nervous tissue is absent. These changes are progressive and 20–30 days after nerve resection the epidermis is similar in appearance to the adjacent normal epithelium and 'tactile cells' are few or absent. The corpuscle is still recognizable by its tuft of capillaries and dermal papilla. When the nerve is crushed the degenerative changes are less complete than after resection; within 16–20 days axonal branches are reappearing in the touch corpuscle, and the epidermis is thickened with a conspicuous fimbriated basal membrane. 'Tactile cells' with large clear sacs are present after 25–30 days and numerous axonal twigs are present at the outer margins of the dermal papilla. By 100 days the tactile corpuscles appear normal.

Electrophysiological recordings were taken from fine strands dissected from the saphenous nerve, proximal to the lesion. From 5 to 20 days after crushing the nerve the axons were silent in the absence of an applied stimulus but mechanical stimuli evoked a brief burst of impulses, lasting 0·1–0·2 sec (Tinel's sign) with a threshold of 200–500 mg wt., in both myelinated and non-myelinated fibres. The sensitivity to mechanical stimuli was restricted and in the early stages was close to the regenerating nerve tip; it moved distally with time. In a minority of fibres a very regular discharge of impulses (about 50/sec) was present, and could be arrested by mechanical stimuli or could be evoked in other fibres by brief mechanical stimulation of the skin. After 16–20 days the sensitive spots were widely distributed in the skin innervated by the saphenous nerve. By 25 days there were two distinct types of receptive field: (*a*) a highly localized spot-like field (threshold > 200 mg), sometimes associated with 'touch corpuscles', and (*b*) a dispersed sensitivity about 1 cm^2 (threshold > 200 mg) similar in size to receptive fields of hair follicle afferent fibres. Twenty-seven to thirty days after the crush further differentiation in sensitivity was evident, with the emergence of a low-threshold (10 mg), slowly adapting response to pressure and a reaction to thermal changes by the touch corpuscles and a rapidly adapting, low-threshold response to hair movements by the hair follicle receptors.

From Olfaction and Taste. Proc. 1st Internat. Symposium, Stockholm, 1962
editor Y. ZOTTERMAN.

13.

THE SIGNIFICANCE OF THE TERMINAL STRUCTURE OF AFFERENT NERVE FIBRES

A. IGGO

Department of Veterinary Physiology, University of Edinburgh

SPECIALIZED structures, 100 to 300μ in diameter in the superficial layers of hairy skin in the cat, contain in their modified epithelium a layer of large cells, numbering 20 or more, each of which encloses a disc of nervous tissue, approximately 10μ wide and 1μ thick, densely packed with mitochondria, which is the terminal expansion of a branch of a myelinated afferent nerve fibre (Iggo and Muir, 1962). A discharge of afferent impulses in the myelinated fibre is most easily aroused by mechanical stimulation of the epidermis, threshold 3 to 5 mg, adapts slowly to steady pressure and can also be provoked by a fall in cutaneous temperature (Iggo, 1962). These properties, particularly the rate of adaptation, distinguish the axons from other similar myelinated axons innervating hair follicles in the same skin, and are always associated with the specialized region of dermis and epidermis, for which the name " touch corpuscle " is suggested.

If the skin was denervated by cutting the saphenous nerve the distinctive structure of the epidermis was disorganized, the layer of special cells disappeared and the epidermis became similar to the adjacent unspecialized parts, but the capillaries and Schwann cells remained in the dermis, at least for 30 days. When, after crushing the saphenous nerve, the afferent fibres were allowed to regenerate, the growing axonal tips did not reveal distinctive responses to mechanical and thermal stimuli, even when the growing axons had reached the dermis (Brown and Iggo, 1962). There was a brief discharge in all axons, with a relatively high threshold, in response to mechanical stimuli and no discharge in response to temperature changes. Two spatial patterns of mechanical sensitivity emerged among the large myelinated axons ; in one type mechanical stimuli were effective over areas of more than a square centimetre while in the other type the responsive areas were restricted to a few square millimetres, probably corresponding to hair follicle and " touch corpuscle " axons respectively.

There was a period after the nerves had reached " touch corpuscles " and were dividing in the dermal papilla when the response to mechanical stimuli was still undifferentiated. At this stage the epidermis, which earlier had shown signs of disorganization, had once more thickened but the

specialized basal cell layer was still absent. A few days after this the typical, low threshold, slowly-adapting response to mechanical stimulation reappeared, weakly at first, and with it a response to temperature changes. Coincident with this change was the re-appearance of the specialized epidermal cells, with their enclosed nerve discs and other features.

From these results it can be concluded that the afferent fibres do not possess any selective sensitivity when they are growing into the skin and that in some way the differentiation of the nerves depends on further development which occurs when the axons have reached their terminal sites. For the afferent fibre innervating a " touch corpuscle " this appears to be the formation of nerve discs in association with specialized cells at the base of the epidermis. Whether this type of differentiation or modulation of associated cells by the nerves, or vice versa, occurs with other afferent fibres is not known. The interesting results reported to the Symposium by Dr. Beidler, which establish a progressive change of epithelial cells within the taste bud, suggest that it also occurs there. In general, it can be asked whether the cells associated with the afferent nerve terminals are necessary only for the proper development of their selective sensitivity or whether the selective sensitivity depends more directly on the intervention of the associated cells as transducers.

REFERENCES

BROWN, A. G. B. and IGGO, A. 1962. *J. Physiol.*, in the press.
IGGO, A. 1962. *Acta Neurovegetativa*, in the press.
IGGO, A. and MUIR, A. R. 1962. *J. Anat.*, in the press.

Nicht im Handel

Sonderdruck aus Band XXIV, Heft 1—4, 1963
ACTA NEUROVEGETATIVA
Schriftleitung: E. Anderson, Bethesda, C. Coronini, Wien, A. Sturm, Wuppertal
Springer-Verlag in Wien

Department of Physiology, University of Edinburgh

An Electrophysiological Analysis of Afferent Fibres in Primate Skin

By

A. Iggo

(Locke Research Fellow)

With 13 Figures

If one of the principal motives in studying laboratory animals is to obtain a better understanding of human physiology then due care must be taken to verify, where possible, that the results apply to man. This is particularly true of the nervous system where there has been a marked development in its complexity in the more advanced animals. There may also be developments in the peripheral nervous system and for this reason the investigation reported here was started. The sensory mechanisms of the skin have been studied in general in three independent ways. A. Psycho-physical techniques in conscious man, together with the various techniques of interfering with the passage of impulses along the peripheral nerves in order to separate if possible the different modalities of sensation (see *Bishop*, 1960). B. Electrophysiological techniques applied to the lower animals, from frog to cat and dog, which have provided the basic evidence for the mechanisms of action of the primary afferent fibres. These techniques were pioneered by *Adrian* (1928) and *Adrian* and *Zotterman* (1926). Only recently has the attempt been made to apply the same techniques to man (*Hensel* and *Boman*, 1961). C. The histological examination of the skin in order to reveal the structures involved in sensation. This has a distinguished history and also has the advantage that the identical techniques can be used in studying all the biological material available. As a result a great deal has been published about the existence and non-existence of various nervous structures in the skin. In particular there has been general agreement among some histologists that the afferent innervation of hairy and non-hairy or glabrous skin is basically different (see *Montagna*, 1960). The evidence for comparable functional differences is slight. This problem of the existence of functional counterparts to the apparent histological differences is examined in the experiments to be described. A second major problem is the old and controversial question of the specificity of the primary afferent fibres to which the Symposium has already devoted some time. Common sensation has been divided, on the basis of psycho-physical studies, into at least four modalities;

touch/pressure, warm, cold, pain, and controversy has arisen about the independence of the nervous paths, and also on the subordinate question; are the primary afferent fibres differentiated by specific sensitivity of their ending to different forms of stimulation? The discovery of punctate sensitivity for cold, warmth and pressure by *Blix* (1884) and also of pain spots and the correlation of these spots with different nervous structures in the skin led to the rigidly formulated concept of structural specificity by *v. Frey* (1895). The apparent absence of organised afferent nerve-endings in hairy skin together with an apparently undiminished ability to recognise the various modalities of sensation (*Hagen, Knoche, Sinclair* and *Weddel*, 1953) led to an attempt to find some alternative mechanism to account for the ability of man to discriminate among different cutaneous stimuli. This has culminated in the frequently re-stated view that the nerve-endings are non-specific and that the various sensations arise from differences, ill-defined and experimentally unsubstantiated, in the pattern of discharge along afferent fibres from the skin. The pattern is assumed to depend in some way on both temporal and spatial variations in the discharge of impulses along afferent fibres, with endings of almost uniform sensitivity to cutaneous stimuli.

The extensive electrophysiological investigations so far made on the frog, toad, rat, cat and dog (*Adrian*, 1928; *Zotterman*, 1939; *Frankenhaeuser*, 1949; *Maruhashi, Mizuguchi* and *Tasaki*, 1952; *Douglas* and *Ritchie*, 1957; *Iggo*, 1959, 1960; *Hensel, Iggo* and *Witt*, 1960; *Iriuchijima* and *Zotterman*, 1960; *Hunt* and *McIntyre*, 1960; *Fjällbrant* and *Iggo*, 1961) have established, by a gradual refinement of technique and restatement of the problems, that the afferent fibres ending in the skin differ widely in response to identical mechanical, thermal and chemical stimulation. There is convincing evidence for differential sensitivity among the different fibres. There are also claims for "non-specificity" of afferent fibres (*Lele* and *Weddell*, 1959). Some recent experiments (*Iggo*, 1961 b) have also established that, at least for two types of afferent fibres in the cat and dog, there is a relation between structure of the nerve-ending and the physiological behaviour of the receptor, so the idea of structural specificity may need to be revised and restated. There are therefore two problems which might expect to be elucidated by an electrophysiological analysis of the afferent fibres in primate skin; a) do the afferent fibres which supply either hairy or glabrous skin differ in their response to identical cutaneous stimuli and b) are the cutaneous afferent fibres uniformly or differentially sensitive to mechanical and thermal stimuli? These two problems can be investigated in man as *Boman* and *Hensel* (1961) have so elegently demonstrated. In such experiments there are, however, limitations which prevent as detailed an examination as is necessary. They do, however, raise the very exciting prospect that it may in the future be possible to correlate the activity recorded electrically in an afferent fibre with the sensation aroused by the same activity in that particular fibre. Another approach is to investigate other primates which have a skin innervation which, according to *Hagen* et al. (1953), is indistinguishable from that of man.

The remainder of this paper is an account of some experiments on 7 monkeys and baboons. The animals were anaesthetized with chloralose and/or nembutal and since an experiment could be made to last 24 hours it was possible to make an exacting quantitative examination of individual afferent fibres. The technique (*Iggo*, 1958, 1960) was based on the dissection of fine filaments from the median or musculo-cutaneous nerve of the arm. These nerves were selected because they supplied both hairy and glabrous skin and made possible the examination of both types of skin in the same experiment. Quantitative mechanical and thermal stimuli were used in exploring the skin and in testing the sensitivity of the endings of individual fibres to various stimuli. The diameters of the afferent fibres were assessed by measuring the conduction velocities of the individual afferent fibres (*Iggo*, 1960) and using the factor of 6 for the relation between diameter in microns and the conduction velocity in metres/second (*Hursh*, 1939) to convert from one to the other. Some of the results have been published (*Iggo*, 1961 a) and the remainder are in the course of publication. To simplify the presentation of the results they are grouped into categories. The validity of the classification will, it is hoped, be established by the evidence. Three main categories are made; a) *mechanoreceptors*, these are the afferent fibres with endings very sensitive to mechanical stimulation of the skin and relatively less sensitive to thermal stimuli, b) *thermoreceptors*, afferent fibre endings more sensitive to thermal stimuli than the previous group and relatively insensitive to mechanical stimulation and c) *nociceptors*, those afferent fibres with a high mechanical and/or thermal threshold when compared with either a) or b).

a) *Mechanoreceptors*. Two principal types were found, those which adapted rapidly to mechanical stimulation and those which adapted much more slowly. The *rapidly-adapting* group comprised about 25 per cent of the sample; those ending in the hairy skin had diameters ranging from 2 to 8 micra and those in glabrous skin 6 to 11 micra (with one exception 3 μ). The 15 fibres ending in *hairy skin* could each be excited by moving the tips of the hairs and responded with a brief burst of impulses. Direct mechanical stimulation of the skin gave a discharge only as the load was being applied and again when the load was removed. During the steady mechanical stimulation there was no response. The mechanical thresholds of the receptors were very low, ranging from 5 to 20 mg. when tested by a *v. Frey* hair on the skin. The sizes of the receptive fields, measured at threshold intensity in only three of the fibres, ranged from 1.5 to 5 cm^2. Stronger stimuli gave a very wide field and were effective presumably because of spread of the stimulus through the skin or because of movement of the hairs. Five of the fifteen fibres were excited by lowering the cutaneous temperature (Fig. 1) but the discharge was weak, only 3 to 5 impulses as the temperature was falling rapidly on the surface of the skin from 40 to 20° C. This group of fibres was thus indistinguishable from similar fibres ending in hairy skin of the cat and dog. The 11 fibres ending in *glabrous skin* were very effectively excited by the rapid movement of a smooth probe across the skin. Steady mechanical stimulation directly on the most sensitive spot gave a discharge of impulses

only when the load was applied and as it was removed (Fig. 2). The mechanical thresholds ranged from 50 to 300 mg. (*v. Frey* hair). Without exception the fibres all failed to be excited when the skin temperature was raised or lowered. The properties of these rapidly-adapting tactile fibres ending in glabrous skin raises the interesting question — "Are they homologous with the fibres ending around the hair follicles?". If they are then the fibres might be expected to lack any conspicuous terminal expansions if the proposal (*Iggo*, 1961 b) that there is a correlation between structure and function in cutaneous nerve-endings is generally valid.

Fig. 1. Monkey. The discharge of impulses in a myelinated afferent fibre from a hair follicle, when the skin temperature was lowered suddenly from 42° C to 21° C. Impulses on upper tracing, temperature on lower tracing. Time at 1 sec. intervals, above (*Iggo*, 1961 c).

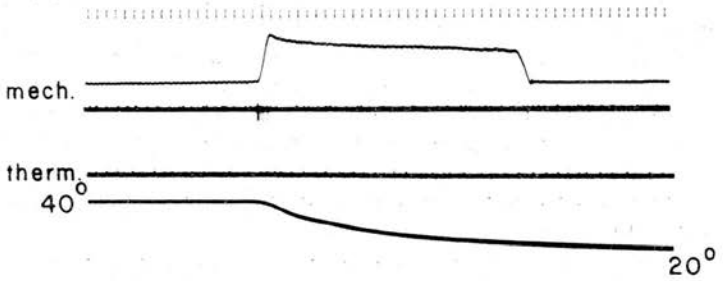


Fig. 2. Monkey. Rapidly-adapting mechanoreceptor in glabrous skin. The upper record shows the brief response to mechanical stimulus, a discharge at "on" and at "off". The lower record shows the failure of a sudden fall in skin temperature, from 40° C to 20° C, to excite the fibre. Time marks at 1/10 sec. intervals (*Iggo*, 1961 c).

Slowly-adapting mechanoreceptors. All the fibres in this group would continue to discharge for several minutes when the skin was pressed on steadily. In addition to a classification based on location of the nerve-endings in either hairy or hairless skin there was another property which made a further subdivision of this group desirable. Some (6) of the afferent fibres ended, so far as could be judged from threshold mechanical stimulation of the skin, in a very cirumscribed area or spot, not more than 250 microns^2, whereas the remainder (25 fibres) had larger receptive fields, not less than 1 mm^2. The former are referred to as "touch spots" the latter as "touch fields". One reason for making this distinction is that in the cat a similar difference in type of receptive field is associated with a structural difference in the endings (*Iggo*, 1961 b).

Touch spots. The 6 slowly-adapting fibres in this group had mechanical thresholds ranging from 1 to 300 mg. The specimens in hairy skin were not always more sensitive than the fibres ending in glabrous skin, e. g. some of the latter had thresholds as low as 20 mg. The afferent fibres were large,

6 to 9 microns diameter. The three fibres ending in hairy skin could all be excited by lowering the skin temperature and one of the two fibres ending in glabrous skin was not. One fibre ending in glabrous skin was particularly interesting because of the relation of its receptive field to the morphology of the skin. This unit was most sensitive to pressures on the centre of an epidermal ridge, and was excited by a 20 mg. *v. Frey* hair at this position. When the probe was moved only 200 microns in either direction along the ridge, or if it was moved into the valley at either side of the ridge, it became

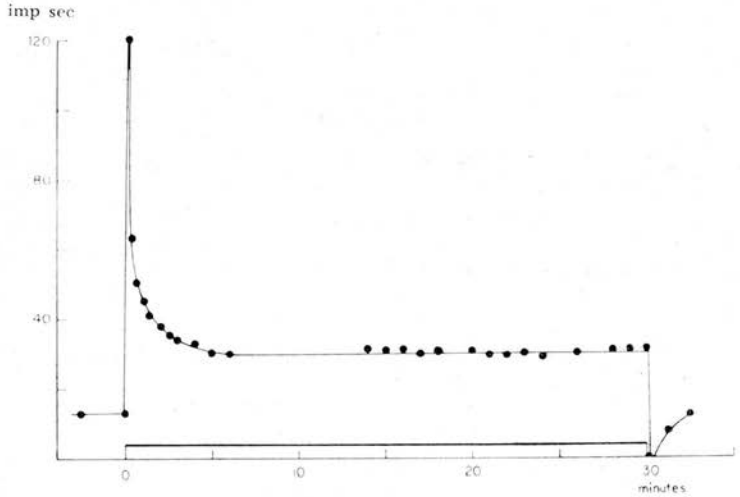


Fig. 3. Monkey. A slowly-adapting mechanoreceptor in hairy skin. The frequency of discharge in the afferent fibre is plotted. A steady mechanical stimulus was applied to the skin at 0 time and removed 30 minutes later. For at least 20 minutes there was a very steady discharge of impulses after a period of more rapid adaptation lasting about 5 minutes (*Iggo*, 1961 c).

ineffective unless the force was increased at least 10 times. This very highly localized sensitivity of the ending suggests that, as has been proposed by *Cauna* (1954), the *Meissner's* corpuscles which have a very characteristic position immediately under the epithelium in the epidermal ridge, are very sensitive to mechanical stimulation. If this hypothesis, that the *Meissner's* corpuscles are slowly-adapting mechanoreceptors, is validated by future experiments it will provide further support for the idea that there is some relation between structure and function.

Touch fields. The fifteen fibres in this group which ended in hairy skin had diameters ranging from 5 to 13 microns and their mechanical thresholds for stimulation of the skin ranged from 5 to 50 mg. All the fibres could be excited by moving hairs but were clearly distinguished from the rapidly-adapting hair receptors by their very slowly-adapting response to mechanical stimuli, and also because they gave a persistent discharge when the hairs were moved to, and held in, a new un-natural position. The regularity of discharge (Fig. 3) even after 30 minutes of maintained mechanical stimula-

tion of the skin was remarkable, and one unit kept firing at an almost constant frequency for more than 20 minutes. These units sometimes had a steady resting discharge in the absence of an applied mechanical stimulus and this activity could be modified easily by moving the skin, e. g. by stretching it. In response to a sudden mechanical stimulus the rate of firing

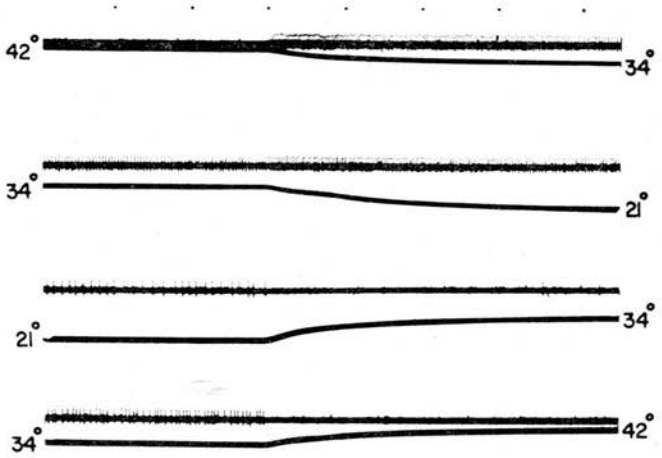


Fig. 4. The same myelinated fibre as in Fig. 3. The records show the effect of cooling and heating the skin. Between each record there was an interval of 20 seconds. Time marks at 1 sec. intervals (*Iggo*, 1961 c).

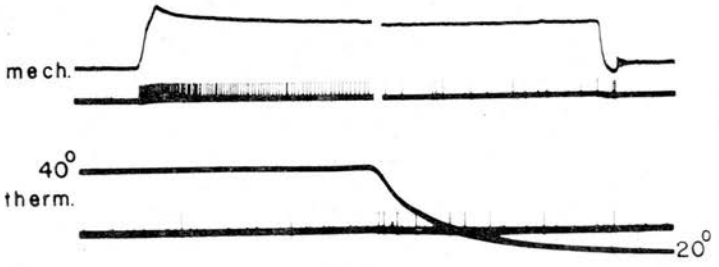


Fig. 5. Monkey. A slowly-adapting mechanoreceptor (touch field) in glabrous skin. The upper records show the response to mechanical stimulation; there is a gap of 60 sec. between the two parts of the record. The lower record shows the effect of suddenly lowering the skin temperature from 40° C to 20° C.

exceeded 900/sec. In addition to their high mechanical sensitivity these fibres were excited by lowering the temperature of the skin (Fig. 4) and the regular discharge of impulses caused by the pressure of the thermal stimulator on the skin was dependent on the temperature, in much the same way that *Hensel* and *Witt* (1959) and *Hunt* and *McIntyre* (1960) found for afferent fibres in the cat.

In the glabrous skin 10 fibres, classified as "*Tactile field*" units were found and had diameters ranging from 3 to 8 μ. All adapted slowly to

steady mechanical stimulation of the skin and, with one exception at the margin of the hairy skin, all the mechanical thresholds were higher (approx. 5 to 20 times) than for the fibres with similar properties in hairy skin. For six fibres they were about 180 mg. and for one it was more than 1 g. The exceptional fibre had a threshold of 20 mg. The receptive fields were measured carefully at mechanical threshold. They varied from 1 to 3 mm². and extended across 2 or more epithelial ridges. Lowering the skin temperature elicited a discharge of impulses from them (Fig. 5) but the sensitivity was much less than in the comparable fibres in hairy skin. These fibres are therefore very similar to the "touch fields" units in hairy skin except for their higher thresholds to cutaneous stimuli.

One general deduction that can be drawn from results so far described, is that the basically similar types of response can be obtained from both

Fig. 6. Cat. The identification of the active cold fibre in a multifibre strand of the saphenous nerve. A, Spontaneous discharge of a single C cold fibre and effect of electrical stimulation (underlined) of the saphenous nerve. B, Record with expanded sweep corresponding to that part of A underlined. The first impulse (marked X) is the spontaneous cold fibre discharge, the second is from another C fibre excited by electrical stimulation. C, The same discharge as in A, but with stronger electrical stimulation. There is now a gap in the series of impulses. D, Expanded sweep. Both impulses were caused by electrical stimulation, the first is identical with the second in the upper record, the second is in the cold fibre. The conduction velocity was 1.1 m./sec. (*Hensel, Iggo* and *Witt*, 1960).

hairy and glabrous skin. The major difference appears to be in threshold, a difference which is perhaps accounted for by the thickness of the epidermis. This is thicker in glabrous skin and therefore would be expected to insulate the underlying receptors from externally applied stimuli. An exception to this rule is provided by the receptors underlying the slowly-adapting "touch spots" in glabrous skin. If these are *Meissner's* corpuscles they may, as *Cauna* (1954) has suggested on physical grounds, be expected to have a greater sensitivity than other mechano-receptors in glabrous skin. He has suggested that the arrangement of the epidermal ridges and the properties of the stratum corneum enhance the transmission of a mechanical stimulus through the epidermis on to the *Meissner's* corpuscles. That the epidermis does act as an insulating barrier is indicated by the much greater sensitivity of a receptor to a mechanical stimulus from the dermal than from the epidermal surface of the skin.

b) *Thermoreceptors.* The afferent fibres to the cat's tongue which have endings with a high thermal sensitivity and a low mechanical sensitivity (*Zotterman* and coll.) have become the archetypes of thermal receptors. The results are usually transferred to the skin in discussion, even though the

lingual nerve is more akin to a visceral than a cutaneous nerve. Recently, however, the cutaneous thermally-sensitive afferent fibres supplying the skin of the cat, rat and dog (*Iggo*, 1959; *Hensel, Iggo* and *Witt*, 1960; *Iriuchijima* and *Zotterman*, 1960) have been isolated as single units. The quantitative studies of *Hensel* and coll. established the existence of afferent fibres with a very high sensitivity either to heating or to cooling of the skin. The threshold was as low as 0.2° C. The afferent fibres were proved by an exact technique to have conduction velocities less than 2.5 m./sec. (Figs. 6, 7), i. e. the fibres

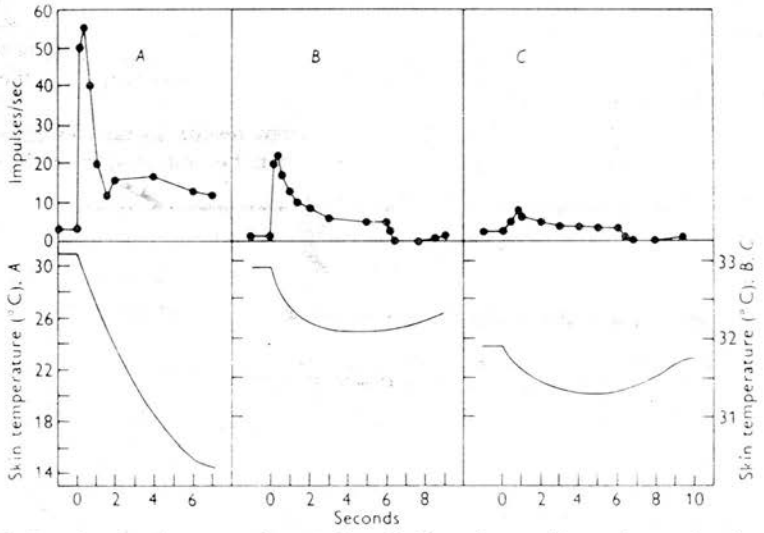


Fig. 7. Cat. Impulse frequency of a single cold fibre when cooling and warming the skin (fibre No. 3). The conduction velocity of the fibre was 1.5 m./sec. The left-hand temperature scale refers to A, and the right-hand scale to B and C (*Hensel, Iggo* and *Witt*, 1960).

were almost certainly non-myelinated. This result was most unexpected but was subsequently confirmed by *Iriuchijima* and *Zotterman* (1960) and extended by them to include the rat and dog. Psycho-physical studies combined with *nerve-block experiments* in man had indicated that the afferent fibres mediating thermal sensation in man had conduction velocities faster than 4 m./sec. (*Gasser*, 1953), although *Landau* and *Bishop* (1953) have more recently brought forward evidence that in man too, some thermal sensation may be mediated along non-myelinated fibres in peripheral nerves.

Nineteen single afferent fibres with a high thermal sensitivity have now been examined in detail in the monkey (*Iggo*, 1961). All the endings were excited by a fall in the cutaneous temperature and were either in the hairy skin on the radial side of the hairy forearm, or in the glabrous skin of the palm, thumb, first or second finger. The receptive fields, measured with a thermal stimulator which had a tip diameter less than 75 μ, were (Fig. 8) small, ranging from 0.25 to 0.6 mm.^{2}. Each fibre ended in only one temperature sensitive spot in the skin. These small areas of maximal thermal sensi-

tivity may correspond to the cold spots in human skin first reported by *Blix* (1884).

The diameters of the afferent fibres were, with two exceptions, in the range of the smallest myelinated fibres, 1 to 3 μ (conduction velocity 3.6 to 15 m./sec.) and thus are comparable to the lingual thermal fibres of the cat and dog (*Iriuchijima* and *Zotterman*, 1961). The exceptional fibres were comparable to the thermoreceptors in the skin of the cat, rat and dog and had conduction velocities below 1.5 m./sec. They were probably non-myelinated, with diameters below 1 micron. The monkeys thus differ markedly in this respect from the cat, rat and dog in which all the thermoreceptors

Fig. 8. Cold spot in monkey skin. Each record shows the discharge of impulses, in a non-myelinated cold fibre in the monkey, caused by lightly placing a cold needle tip on the hairy skin. The central record was taken at the most sensitive spot and the other 4 records at not more than 1 mm. away in each direction. Time marks at 1/10 sec. intervals (*Iggo*, 1961 a).

examined in detail had afferent fibres with conduction velocities below 2.5 m./sec., i. e. were non-myelinated fibres, less than 1 μ diameter.

All 19 thermoreceptors examined as single units in the present experiments were excited by lowering and inhibited by raising the cutaneous temperature. No receptors excited by small increases in skin temperature were found, although in multi-fibre preparations, not examined in detail, there were some fibres comparable to the heat receptors of the cat (*Iggo*, 1959). The peak frequency of discharge on suddenly cooling the skin, by lowering the temperature of the thermode resting on the surface from 40° to 20° C, was about 150 impulses/second. A striking and characteristic property of these cold-sensitive fibres was the grouping of impulses into bursts separated by intervals of silence (Fig. 9). This pattern of discharge returned when the temperature was once again steady. The frequency of the adapted discharge depended on the temperature of the skin and was maximal, not usually greater than 20/sec., for each fibre at some temperature in the range 20° to 35° C. Many of the fibres had a regular grouped discharge of impulses at cutaneous temperatures above 35° C but not above 40° C, so that they were active at temperatures above the neutral temperature of the skin. On two occasions there was a discharge above 46° C which

corresponds to the paradoxical discharge reported by *Dodt* and *Zotterman* (1952). Although a fibre might be silent at the higher temperatures it would nevertheless fire when the skin was cooled to a temperature above that at which there was an adapted discharge, and would become silent again after a few seconds.

Exact measurements of the thermal sensitivity were not made but all the fibres could be excited by temperature changes of less than 0.5° C and the grouped discharge could be altered by much smaller changes than this, less than 0.05° C/sec. A comparison of the thermal sensitivity with the threshold of a cold spot in human skin revealed a comparable sensitivity at threshold.

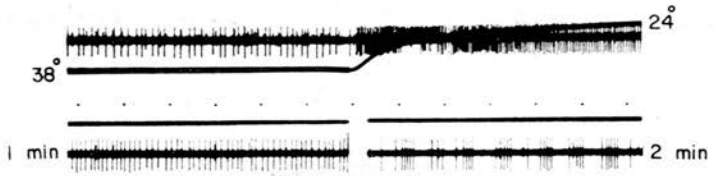


Fig. 9. Monkey. The discharge of impulses in a cold receptor in response to alteration in the temperature of the skin. In each record the upper trace shows the impulses in the fibre and the lower trace shows the temperature of the skin. Initially the skin had been held at 38° C and just before the start of the upper record was allowed to cool slightly. This produced the regular discharge of impulses. The water flowing through the thermode was then suddenly switched to 24° C. As the cutaneous temperature was falling there was an acceleration of the discharge which was maximal before the skin had reached the new steady temperature. One minute after the change the skin temperature was still falling very slowly and the discharge was still regular. Two minutes after the change the temperature was steady and the grouped discharge had made its appearance. Eight minutes later the grouped discharge was still present. The time marks are at 1 sec. intervals (*Iggo*, 1961 a).

Eight of the fibres with myelinated afferent fibres had endings in hairy skin and an equal number in glabrous skin. The general behaviour of both groups of fibres, as well as the non-myelinated fibres which ended in hairy skin, was similar. The thermal sensitivity of some of the fibres ending in glabrous skin was as high as other fibres ending in hairy skin, e. g. a 5° C fall in temperature gave a maximal discharge at 60/sec. compared with 70/sec., for fibres in glabrous and hairy skin respectively. Other units in glabrous skin were less sensitive. These results imply either that the glabrous thermoreceptors were more sensitive than the hairy skin thermoreceptors, or that for some reason, perhaps the position of the terminals in the skin, the insulating action of the epidermis was less than for some of the mechanoreceptors.

None of the receptors could be excited by innocuous mechanical stimulation such as moving hairs, stroking or pressing on the skin. Some units were excited by pressing a small probe firmly on the skin using a force (5 g. weight) about 2000 times the threshold of the most sensitive cutaneous mechanoreceptors. The thermal and mechanical receptive fields were coincident so it is likely that the mechanical stimulus was acting on the temperature sensitive elements.

The sizes of the receptive fields, the thermal sensitivity and persistence of discharge at constant temperature taken together with the size of the afferent fibres suggest that if, as seems likely, man and the monkey have similar cutaneous receptors, then the receptors described here have a crucial role in thermal sensation. They are probably the cutaneous cold receptors,

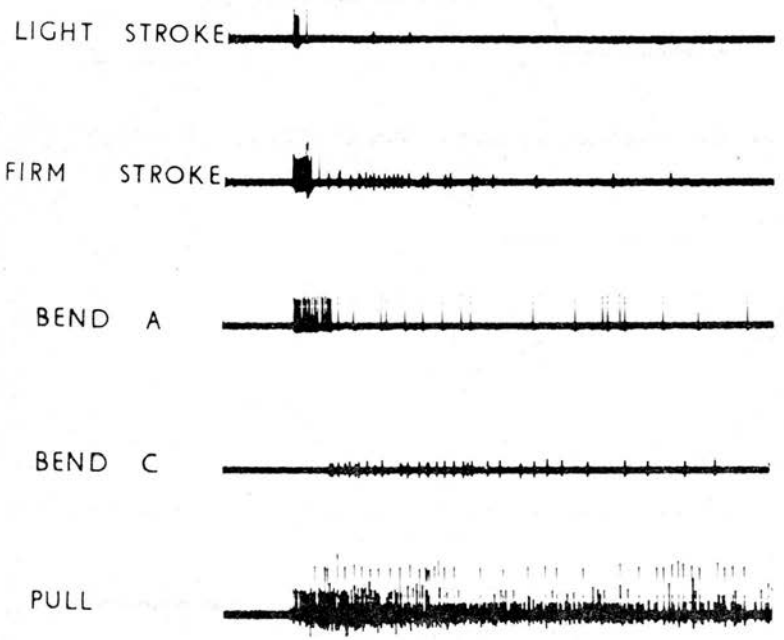


Fig. 10. Cat. Impulses in a multi-fibre strand of the saphenous nerve, to illustrate the differences in sensitivity of cutaneous myelinated (A) and non-myelinated (C) fibres. Light stroke-moving the tips of the hairs, Firm stroke-brushing the hairs, Bend A-moving hairs to a new position and holding them there, Bend C-moving another group of hairs in the same way excites 2 C fibres, Pull-pulling the hairs excites many fibres not stimulated by bending (*Iggo*, 1960).

specifically signalling thermal conditions in the skin and thus of the environment. Whether warm receptors also exist, i. e. afferent nerve-endings specifically excited by small rises in skin temperature, cannot be decided on the present results. They have been described in other animals and the failure to find any may reflect only the actual paucity of warm receptors, and indeed warm spots are distributed much less thickly in human skin than are the cold spots.

Non-myelinated afferent fibres. All the small sample (7) of single C fibres so far examined in the monkey ended in hairy skin. Of these, 5 were classed as mechanoreceptors. Three of these had mechanical thresholds less

than 500 mg. and the other two were above 5 g. The three more sensitive units Fig. 10 were excited by innocuous stimuli such as brushing the tips of the hairs or stroking or pressing on the skin. Such a high sensitivity confirms the conclusion already reached in the cat, that a proportion of the non-mye-

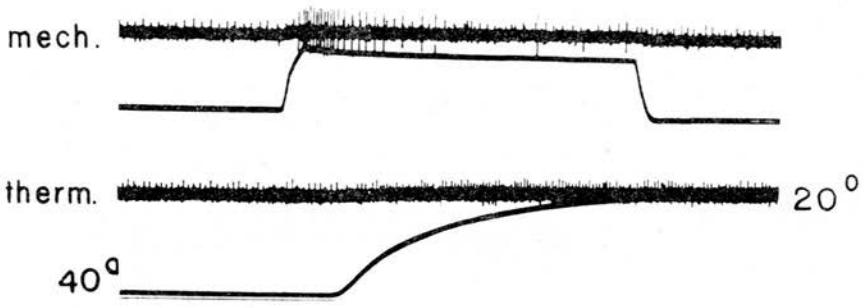


Fig. 11. Monkey. Impulses in a non-myelinated afferent fibre in response to (a) pressing on the skin with a sharp needle point and (b) suddenly lowering the skin temperature from 40° C to 20° C. Time marks at 1 sec. intervals (*Iggo*, 1961 c).

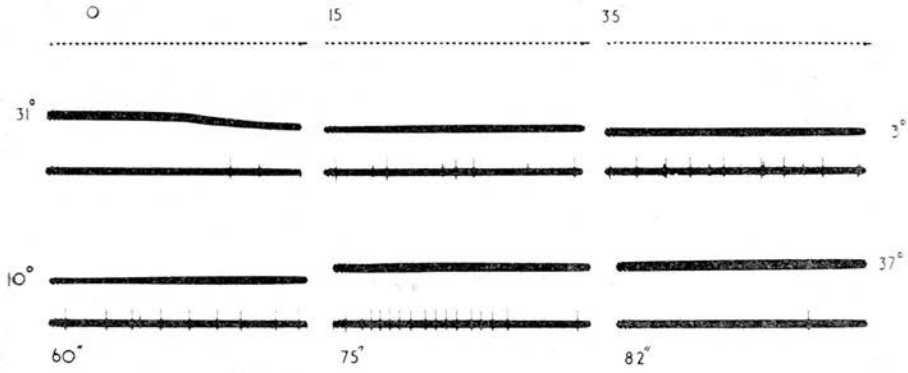


Fig. 12. Cat. The response of a cold receptor and another fibre to cooling the skin with ice and then re-warming it. In each record the upper tracing is the surface temperature recorded by a thermistor and the lower tracing shows the impulses in two afferent fibres. The records were taken at the following times: (A) When ice was put on the skin, (B) 15 sec. later, (C) 35 sec. later, (D) 60 sec. later, just after the ice had been removed, (E) 75 sec., just after a pad of cotton-wool soaked in water at 55° C was put on the skin, this record shows part of the re-warming burst of impulses and (F) 82 sec. The fibre giving the smaller impulse was not excited by rewarming the skin. Time at 1/10 sec. intervals (*Iggo*, 1959).

linated afferent fibres are too sensitive to be easily accepted as nociceptors. As in the cat some of these fibres were excited weakly by lowering the temperature of the skin. The remaining units were excited only by strong mechanical stimuli and most effectively by squeezing or pricking the skin. One of the fibres Fig. 11 was superficial and may have had an epidermal ending. This unit was unaffected by severe thermal stimuli whereas the other unit

could be excited by temperatures about ± 10° C from the neutral temperature. This (Figs. 12, 13) unit could be a prototype of the much sought "pain receptor". However the first unit, as well as the existence of other high temperature sensitive units, not examined as single fibres, raises the question of "modality-specificity" of the pain receptors (*Iggo*, 1959). The evidence pre-

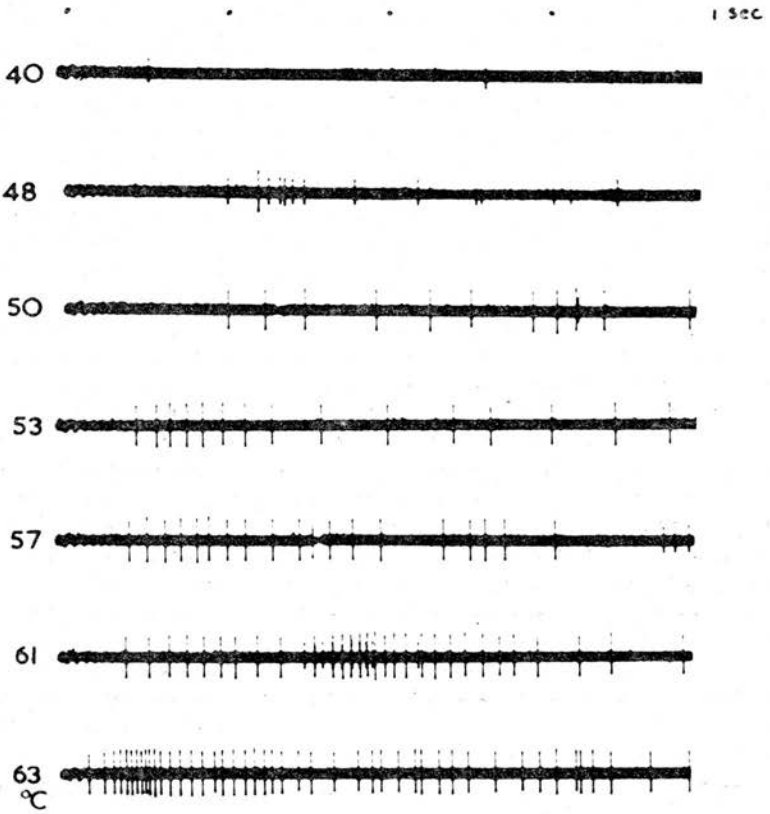


Fig. 13. Cat. The response of heat receptor (fibre No. 3) when a brass rod (10 mm. diameter) at the temperatures indicated was put on to the skin and left there for the duration of the record. The small impulses seen in the upper two tracings are from a mechanoreceptor, 10 mm. away from heat receptor, and were evoked by the mechanical effect of putting the hot rod on the skin. The heat receptor on the other hand, was not excited by such a mechanical stimulus. Time in seconds at the top of the figure (*Iggo*, 1959).

sented so far is very convincing in establishing a strongly marked differential sensitivity among the cutaneous afferent fibres of the monkey. The high degree of selective sensitivity afforded by the thermoreceptors with myelinated fibres raises a question concerning the significance of the well-documented examples of fibres with a mechanical sensitivity which are also excited by cooling the skin in the cat (*Witt* and *Hensel*, 1959; *Hunt* and *McIntyre*, 1960; *Douglas, Ritchie* and *Straub*, 1960; *Iggo*, 1960; *Boman* and

Hensel, 1961). Most investigators have been cautious in their interpretation of these results, but the fibres have been cited as examples of a dual-specificity of cutaneous receptors or even (*Lele* and *Weddell*, 1959) as an example of non-specificity. *Hunt* and *McIntyre* (1960) pointed out that in the hairy skin of the cat there were apparently two types of fibres, one supplying the hairs which was not excited by lowering the skin temperature and the other ending in slowly-adapting fibres which had a conspicuous sensitivity to temperature. This result has not been fully confirmed in more recent work on the cat in which it was found that some of the fibres supplying the hairs could be excited, if only weakly, by rapidly cooling the skin (*Iggo*, 1961 c). The present experiments on the monkey have shown that all of the slowly-adapting mechanoreceptors in hairy skin were excited by cooling, as were many of the rapidly-adapting fibres innervating hair follicles. This invariable association of greater thermal sensitivity with long-lasting response to mechanical stimulation was also found in the glabrous skin. Despite the lower sensitivity to cooling, which led to the rapidly-adapting units failing to respond, the slowly-adapting units which were affected by cooling. At the extreme of rapid adaptation lie the Pacinian corpuscles which *Loewenstein* (1961) has shown, in his elegant experiments, to be entirely without response to cooling, even of the almost naked nerve-ending. To argue from these results that the rapidly-adapting mechanoreceptors have a very high degree of specificity and the slowly-adapting receptors have a dual specificity, over-looks the possibility that there is some connection between rate of adaptation and the expression of a thermally induced change. This may occur in all afferent endings but, because of the time-constant of the electrical changes, is revealed most clearly in the slowly-adapting fibres. On this hypothesis the excitatory effect of suddenly lowering the cutaneous temperature becomes a phenomenon which, while it may modify touch/pressure sensation, is without significance for thermal sensation. Such an hypothesis, and indeed all hypotheses about the role of different types of peripheral afferent behaviour in sensation, requires an analysis of the central nervous paths in order to test the idea that fibres of unique peripheral specificity enter separate central paths.

Earlier in this paper two questions were raised to which, it was hoped, an investigation of the cutaneous innervation in the monkey might contribute an answer. The first was — "Are there functional differences between afferent fibres ending in hairy and in glabrous skin?" The results indicate that there are no striking differences and that basically similar types of receptors are to be found in each, some adapt rapidly and others adapt slowly. There are however quite large differences in sensitivity, particularly for some of the slowly adapting receptors, and it is suggested that the variation may be attributable to the insulating properties of the epithelium which is much thicker in glabrous skin. Certainly there are no contrasts as great as might be expected from the reported differences in the morphology of the nerve-endings. The second question was — "Are the differences in sensitivity to mechanical and thermal stimulation of the skin sufficient to support a differentiation of the afferent fibres into modality-specific groups?" This

question can be answered in the affirmative, at least as far as it concerns a separation into mechanoreceptor (of several types) and cold receptors. A subsidiary question was raised by the excitation of some highly sensitive mechanoreceptors by cooling the skin. It was suggested that the phenomenon was in some way related to the rate of adaption of the ending to mechanical stimulation and that it probably had no significance for thermal sensation.

Insufficient results were collected, in these preliminary studies, to make any contribution to the identification of the nociceptors. Recent experiments in the cat (*Iggo*, 1959; *Fjallbrant* and *Iggo*, 1961) indicate that this problem may not be amenable to an easy solution.

Summary

Knowledge of the mechanisms of cutaneous sensation at present depends on 3 lines of evidence a) psycho-physical b) electrophysiological data from animals and c) histological studies of man and animals. Two major problems have arisen; a) the significance of the apparent histological differences between hairy and non-hairy or glabrous skin, b) do the peripheral afferent fibres differentiate between mechanical, thermal and chemical stimuli to a degree sufficient to justify a classification as mechanoreceptors and themoreceptors? Experimental electrophysiological results using afferent fibres dissected from forearm nerves of monkeys are described. Both hairy and glabrous skin gave basically similar types of responses in myelinated afferent fibres to cutaneous stimulation, except that the thresholds were in general higher for nerves ending in glabrous skin. The data are convincingly in favour of the idea that there are substantial differences in specificity of the nerve endings.

Zusammenfassung

Die Kenntnis über die Funktion der Haut als Sinnesorgan ist das Ergebnis von 3 Untersuchungsrichtungen: a) Psycho-physikalische Untersuchung, b) elektrophysiologische Untersuchung bei Tieren, c) histologische Untersuchungen bei Mensch und Tier.

2 Hauptprobleme wurden aufgeworfen:

1. Die Auffälligkeiten der histologischen Unterschiede zwischen behaarter und nicht behaarter Haut,

2. die Frage, ob die peripheren afferenten Fasern zwischen mechanischen, thermischen und chemischen Reizen in genügendem Ausmaß unterscheiden können, so daß ihre Klassifikation in Mechano- und Thermoreceptoren gerechtfertigt ist.

Ergebnisse elektro-physiologischer Versuche an afferenten Fasern am Unterarm des Affen werden beschrieben. Sowohl die behaarte als auch die unbehaarte Haut ergaben grundsätzlich ähnliche Arten von Antworten der afferenten myelinisierten Nerven nach Hautreizen. Lediglich die Reizschwelle war in den Nervenendigungen der unbehaarten Haut im allgemeinen höher. Die Ergebnisse sprechen eindeutig für die Ansicht, daß es wirkliche Unterschiede hinsichtlich der Spezifität von Nervenendigungen gibt.

Résumé

La connaissance des mécanismes de la sensation cutanée dépend, à présent, de trois lignes d'évidence: a) de dates psycho-physiques, b) de dates électrophysiologiques d'animaux et c) d'études histologiques de l'homme et des animaux. Il y a deux problèmes: a) la signification des différences histologiques apparentes

afférentes périphériques différencient entre des stimuli mécaniques, thermiques entre la peau poilue et la peau non-poilue ou glabre, b) la question si les fibres et chimiques d'un degré qui suffit pour justifier leur classification comme des mécanorécepteurs et des thermorécepteurs. On a décrit des résultats électrophysiologiques expérimentaux en faisant usage de fibres afférentes prises de nerfs des avant-bras de singes. La peau poilue et la peau glabre ont montré des types au fond semblables de réponses dans des fibres afférentes myéliniques à une stimulation cutanée, à l'exception des cas où les seuils étaient, en général, plus hauts pour des nerfs terminant dans la peau glabre. Les dates sont, de manière convaincante, en faveur de l'idée qu'il y a des différences substantielles en ce qui concerne la spécificité des bouts nerveux.

References

Adrian, E. D., The basis of sensation. Christopher (Publishers) Ltd., London, 1928. — *Adrian, E. D.,* and *Y. Zotterman,* J. Physiol. *61* (1926), 151 and 465. — *Blix, M.,* Zschr. J. Biol. *20* (1884), 141. — *Cauna, N.,* Anat. Rec., Philadelphia, *119* (1954), 449. — *Dodt, E.,* and *Y. Zotterman,* Acta physiol. Scand. *26* (1952), 358. — *Douglas, W. W.,* and *J. M. Ritchie,* J. Physiol. *138* (1957), 19 and 31. — *Douglas, W. W., J. M. Ritchie* and *R. W. Straub,* J. Physiol. *150* (1960), 266. — *Fjälibrant, N.,* and *A. Iggo,* J. Physiol. *156* (1961), 578. — *Frankenhäuser, B.,* Acta physiol. Scand. *18* (1949), 68. — *Frey, M. v.,* Ber. Sächs. Ges. (Akad.) Wiss. *47* (1895), 166. — *Gasser, H. S.,* Res. Publ. Ass. Nerv. Ment. Dis., N. Y., *23* (1943), 44. — *Hagen, E., H. Knoche, D. C. Sinclair* and *G. Weddell,* Proc. Roy. Soc., London, Biol. Sc. *141* (1953), 279. — *Hursh, J. B.,* Amer. J. Physiol. *127* (1939), 131. — *Hensel, H.,* and *K. Boman,* This Symposium (1961). — *Hensel, H., A. Iggo* and *I. Witt,* J. Physiol. *153* (1960), 113. — *Hunt, C. C.,* and *A. K. McIntyre,* J. Physiol. *153* (1960), 88. — *Iggo, A.,* J. Physiol. *142* (1958), 110. — *Iggo, A.,* Quart. J. Exper. Physiol., London, *44* (1959), 362. — *Iggo, A.,* J. Physiol. *152* (1960), 337. — *Iggo, A.,* Proc. Roy. Physiol. Soc. Ed. *1961 a* (in the press). — *Iggo, A.,* This Symposium (1961 b). — *Iggo, A.,* In preparation for publication (1961 c). — *Iriuchijima, J.,* and *Y. Zotterman,* Acta physiol. Scand. *49* (1960), 267. — *Iriuchijima, J.,* and *Y. Zotterman,* Acta physiol. Scand. *51* (1961), 283. — *Landau, W.,* and *G. H. Bishop,* Arch. Neurol. Psychiatr., Chicago, *69* (1953), 490. — *Lele, P. P.,* and *G. Weddell,* Exper. Neurol. *1* (1960), 334. — *Loewenstein,* This Symposium (1961). — *Maruhashi, J., K. Mizuguchi* and *I. Tasaki,* J. Physiol. *117* (1952), 129. — *Montagna, W.,* Cutaneous Innervation. Pergamon Press, London, 1960. — *Witt, I.,* and *H. Hensel,* Pflügers Arch. Physiol. *268* (1959), 582. — *Zotterman, Y.,* J. Physiol. *95* (1939), 1.

Anschrift des Verfassers: Dr. *A. Iggo,* Department of Physiology, University of Edinburgh, Edinburgh (Scotland).

[*From the Proceedings of the Physiological Society*, 9–10 December 1960.]
Journal of Physiology, **155**, 52–53*P*.

Non-myelinated afferent fibres from mammalian skeletal muscle

By A. Iggo*. *Physiology Department, University of Edinburgh*

Electrical activity was recorded from fine strands dissected from the nerves to the gastrocnemius and soleus muscles in cats. The non-myelinated fibres were identified by measuring the conduction velocity in the nerve trunk or from the nerve-endings. In the fibres reported here the velocities ranged from 0·4 to 1·6 m/sec. In addition to these fibres there were others in which the conduction velocity in the muscle was about 1–2 m/sec, but in which it was 3–10 m/sec in the main nerve. These latter fibres probably correspond to the Group III fibres recently described by Bessou & Laporte (1960).

For all except one fibre there was only a small region of the muscle or tendon (less than 5 mm^2) from which a discharge of impulses could be evoked. A persistent discharge of impulses could be provoked by sustained pressure on the sensitive part of the muscle, and even after 30 sec of stimulation the rate of firing was almost as high as initially with some fibres. Mechanical thresholds ranged from 5 to 50 g wt. No fibre was excited by stretch or contraction of the muscle, even when loads of several kilograms were placed on the tendon. Many of the fibres responded with a persistent discharge of impulses when the mechanically sensitive part of the muscle was warmed or cooled. Most of the units were excited by both high and low temperature, above 41° C and below 25° C, but a few were most sensitive about 28–32° C.

All the fibres tested were excited by application of the classical deep-pain stimulant—a small quantity of 5 % NaCl solution. The rate of firing was as high as 30 impulses/sec and could persist for 30 sec. This stimulus was, however, non-specific since all the other muscle afferent fibres tested were also excited, including muscle-spindle afferent fibres in which the rate of firing was as high as 40 impulses/sec. Muscle ischaemia, another well-known cause of muscle pain, was produced by occluding the popliteal artery, the femoral artery and in some experiments the abdominal aorta. It never aroused a very high-frequency discharge of impulses in the C fibres, even when combined with intercurrent tetanus. There was often a low-frequency discharge, less than 1/sec, and in some fibres there were bursts of impulses lasting up to 3 sec at 10–20 impulses/sec. Restoration of the blood flow usually abolished the activity but on one occasion was associated with an enhancement of the irregular discharge of impulses.

REFERENCE

Bessou, P. & Laporte, Y. (1960). *J. Physiol.* **155**, 19*P*.

* Locke Research Fellow.

NON-MYELINATED VISCERAL, MUSCULAR AND CUTANEOUS AFFERENT FIBRES AND PAIN

A. IGGO

BEYOND the certainty that afferent nerve fibres mediate organic pain lies the intractable task of identifying which are the particular fibres; as witness the persistent attempts to establish experimentally their properties. One fruitful result of earlier investigation has been to exclude the largest afferent fibres and to limit the range of speculation (Adrian, 1931; Zotterman, 1939; Gasser, 1943). The extent to which pain is an exclusive function of all the smaller fibres is not settled but assertions that the non-myelinated fibres are uniformly sensitive to damaging or near-damaging stimuli are now made more cautiously than even a few years ago. The principal reason for this change is that quantitative information is now accumulating about the smallest, numerically preponderant, afferent nerve fibres principally as a result of the application of single fibre electrophysiological techniques combined with quantitative stimulation of the receptors (Douglas & Ritchie, 1957; Iggo, 1957 *et seq.*; Hensel, Iggo & Witt, 1960; Iriuchijima & Zotterman, 1960).

The principal and unforeseen results revealed (*a*) that in mammals the nerve-endings of the non-myelinated afferent fibres ending in skin comprise a range covering the full gamut of sensitivity found among cutaneous receptors, and (*b*) that individual fibres had small receptive fields. The individual fibres, particularly those most highly sensitive, usually displayed a very selective sensitivity as Professor Zotterman (1962) has just been describing. The fibres within any group distinguished by sensitivity to one form of energy, *e.g.* thermoreceptors, displayed a wide range of sensitivity, from those excited by quite innocuous stimuli, almost at the threshold of human sensation, to the other extreme of almost certainly noxious stimuli. Between the extremes there lay an almost continuous variation in threshold. No clear-cut boundaries within a group of fibres based on threshold differences were obvious, although the extremes of the range were clearly dissimilar. The problem now is to devise tests to distinguish what might be called the 'innocuous' mechanoreceptors and thermoreceptors from nociceptors with similar properties but much higher thresholds, on the assumption that such a distinction is valid.

The results obtained using single fibre electrophysiological tech-

In The assessment of pain in man & animals. UFAW Symposium 1961
ed C.A. KEELE & R. SMITH

niques to analyse the behaviour of non-myelinated afferent fibres (Table I) will be dealt with under three headings: (*a*) abdominal and pelvic viscera; (*b*) skeletal muscle, and (*c*) cutaneous afferent fibres.

Visceral afferent fibres. The abdominal and pelvic viscera have not been as fruitful a source of afferent fibre studies as the thoracic viscera, in part because the fibres are predominantly non-myelinated. The Pacinian corpuscles, with large myelinated fibres, are an exception and these have become a prototype of the rapidly-adapting mechanoreceptor (Gray, 1959; Loewenstein, 1959). It is only recently that the reflex experiments which established the existence of other visceral

Table I

Classified results for single non-myelinated afferent fibres from viscera, muscle and skin, referred to in the text

	No.	Cond. Vel. m./sec.	Sensitivity			5% NaCl
			mech.	therm.	chem.	
Mechanoreceptors						
Oesophagus ..	2	0·5–1·5	+++	—	○	○
Stomach	43		+++	—	○	○
Intestines (small)	11	(2–12 goat)	+++	—	○	○
Urinary bladder ..	12		+++	—	○	○
Urethra	1		+++	○	○	○
Skin: cat	41	0·5–2·5	+++	±	+[1]	+
monkey ..	3		+++	±	○	○
Chemoreceptors						
Stomach: acid ..	5	1–5	+	—	○	○
alkali ..	9		+	—	○	○
Thermoreceptors						
Skin: warm ..	5	0·6–1·5	+	+++	—	○
cold	5		+	+++	—	○
Muscle	1	0·6	+	++	○	+
Nociceptors						
Skin: mechano ..	24	0·7–1·2 (1 @ 17)	+	—	+[1]	+
thermo $> \pm 10°$ C.	25	0·5–1·2	+	++	+[1]	+
Muscle: belly ..	6	0·6–1·5	+	○	○	+
tendon ..	11		+	±	○	+
TOTAL	204					

+ = excitatory, — = depressant, ○ = not tested, [1] = principal action was depressant, chem. = histamine, 5-HT, bradykinin.

The specificity was not absolute, each fibre displayed a relatively greater sensitivity to one or another form of stimulation.

mechanoreceptors and chemoreceptors have been supported by afferent fibre studies of the receptors (Paintal, 1954, 1957; Iggo, 1955, 1957a, b).

The ruminant animal was used initially and has a particular convenience since the abdominal vagus in these animals contains a much higher proportion of myelinated fibres than it does in other laboratory animals (Iggo, 1956). All 19 single fibres so far collected in the sheep and goat were mechanically sensitive. They were excited by distending different parts of the stomach and, in addition, were all excited by gastric contractions under both isotonic and isometric recording conditions. These results suggest that they are 'in series' with the contractile elements in the gastric wall. Vagal afferent fibres with a similar response (Fig. 1) were found in the cat (Iggo, 1955, 1957a) and again, with one exception, all 69 fibres were excited by both distension and contraction of the oesophagus, stomach or intestine. The principal difference from the sheep and goat was that the conduction velocities in the fibres from these alimentary mechanoreceptors were less than 2·5 m./sec., *i.e.* the afferent fibres were non-myelinated except for a solitary oesophageal fibre (7 m./sec.) (Iggo, 1957a). Similar receptors were also found in the pelvic nerves supplying the urinary bladder (Iggo, 1955). The simple alimentary canal of the cat allowed the intramural position of the receptors to be established. None of the five fibres tested was affected by removal of the mucosa or submucosa, and the nerve fibres probably ended in the muscularis externa. This fits well with their behaviour during muscular contractions. The properties of these mechanoreceptors, in addition to revealing the afferent basis of alimentary reflexes, provide a simple explanation for an otherwise confusing clinical finding, namely that the sensation from an over-distension of the stomach (atonic dilatation) can be confused with that from a contracted stomach (hypertonic stomach) (Hurst, 1911). Likewise the pain of intestinal colic and of distension probably arise in the same afferent fibres. There is, however, no proof that any of the fibres have a particular function and information about thresholds is not sufficiently precise to give a definite answer.

In addition to the alimentary mechanoreceptors there is another quite distinct class of visceral receptor, for which there is reflex evidence (Thomas, Crider & Mogan, 1934; Titchen, 1953). These are the slowly-adapting *pH-sensitive afferent fibres*, which were found to end superficially in the gastric mucosa (Iggo, 1957b). Two categories were distinguished, based on sensitivity to the pH of the solution bathing the mucosa; acid- (Fig. 1, c–e) or alkali-sensitive fibres. The thresholds were below pH 3 or above pH 8 respectively and the sensitivities were differential. The receptors were indifferent to the tonicity

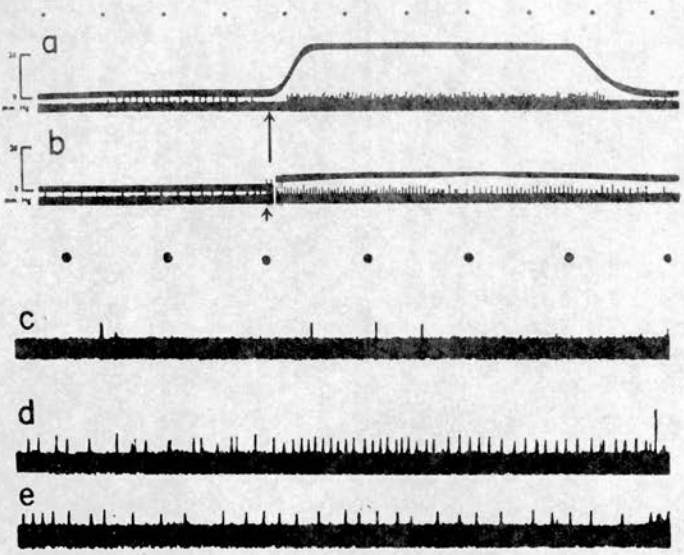


Fig. 1. The responses of a single duodenal mechanoreceptor to (*a*) distension and (*b*) contraction of a short length of the duodenum of a cat. The apparent delay in response to the distension, signalled by the upper tracing, was due to long conduction time in the non-myelinated afferent fibre. (*From:* Iggo, *Quart. J. exp. Physiol.* **42**, 130–43.)

The response of an acid-sensitive receptor in the intact stomach with a vagal afferent fibre when the stomach was irrigated with (*c*) 0·03 N HCl pH 1·5 and (*d*) 0·1 N HCl, pH 1. The low bottom record (*e*) was taken 30 sec. after (*d*) and illustrates the slowly-adapting nature of the receptor. (*From:* Iggo, *Quart. J. exp. Physiol.* **42**, 398–409.)

of salt solutions within the range 0–0·6 M, and to the chemical composition of the solutions used; pH appeared to be the determining factor. Although the fibres could be excited by rapidly stroking the mucosa, they were much less sensitive than the mechano-receptors described above and were not excited by mild distension or by contraction of the stomach. These receptors presumably contribute to gastro-intestinal vago-vagal reflexes influencing gastric motility and secretion (Harper, Kidd & Scratcherd, 1959). They may also mediate the pain of gastric hyper-acidity; for example, in a patient with hyper-acidity the pain was abolished after partial neutralization of the gastric contents had brought the pH above 2 (W. I. Card, personal communication). Whether some of these receptors function exclusively as visceral nociceptors is not known.

Muscle afferent fibres. More is known of the central connexions and reflex actions of the small muscle afferent fibres than of the pro-

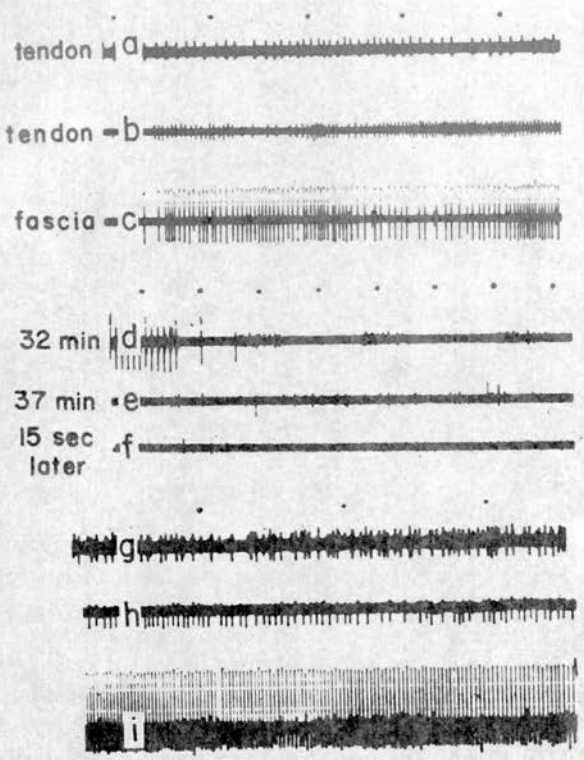


Fig. 2. The responses of non-myelinated afferent fibres in skeletal muscle. The upper six records (*a–f*) were all taken from the same strand of the nerve to lateral gastrocnemius/soleus muscle. The upper three records show the response in single fibres to squeezing different parts of the tendon or fascia of the muscle. Each record is from a different fibre. The next three records show the effect of prolonged ischaemia, combined with inter-current tetanus. (*d*) was taken after 32 minutes of ischaemia and shows the artefacts of the last twelve shocks to the muscle nerve and the afferent discharge which ensued. (*e*) shows the activity in the nerve after 37 minutes of ischaemia, and (*f*) shows the virtual disappearance of the discharge within 15 seconds of restoring the blood flow. The lower three records (*g–i*) are taken from other experiments and show the discharge aroused by the injection of small quantities of 5 per cent NaCl solution into the muscle: (*g*) non-myelinated fibres ending in the belly of the muscle; (*h*) a small myelinated fibre ending on the tendon, and (*i*) a muscle spindle afferent fibre.

a high-frequency discharge in muscle spindle afferent fibres, a result which was confirmed by Paintal (1960) who also reports that the Group III muscle fibres were not excited by asphyxia. There is no evidence that this discharge enters consciousness or that it excites

the idea of 'selective sensitivity' of the nerve-ending. This term 'selective sensitivity' is to be understood as meaning a heightened, not an exclusive, sensitivity to one form of energy—mechanical or thermal. The available evidence justifies the division of cutaneous fibres into at least two categories: (*a*) mechanoreceptors, and (*b*) thermoreceptors. A third category, nociceptors, may have to be added but the evidence from single fibre studies, while suggestive, is as yet inconclusive.

The cutaneous C-fibres, which comprise about 80 per cent of all the cutaneous afferent fibres, when examined as single units showed a preponderance of units sensitive to low-intensity mechanical stimulation (Iggo, 1960), in good agreement with the results of Douglas and Ritchie (1957) using their whole-nerve technique. The most sensitive fibres, although less excitable than the receptors of myelinated cutaneous fibres (threshold 2 mg.wt.) were excited by moving the tips of the hairs or by a force of 20 mg.wt. on the skin, and the least sensitive required a force of 7·5 g.wt. A conspicuous feature, particularly of the more sensitive fibres, was an after-discharge which might continue for 10 seconds or even longer and was especially prominent when the skin had been left undisturbed beforehand. The after-discharge was depressed by repeated stimulation of the skin. The initial response to a mechanical stimulus was also reduced tem-

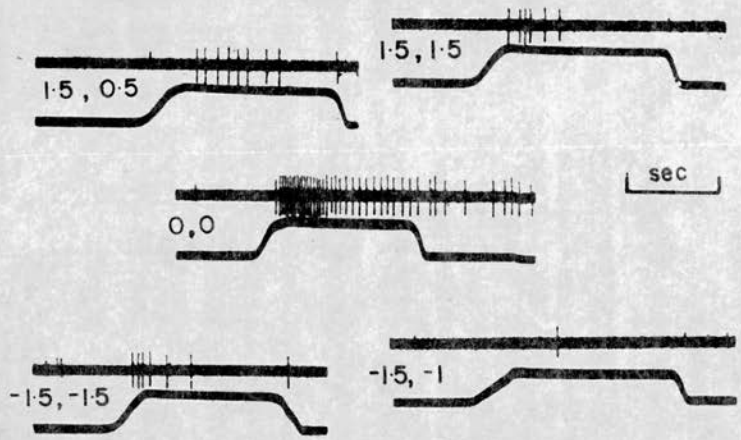


Fig. 3. The discharge of impulses in a non-myelinated cutaneous afferent fibre in response to mechanical stimulation of the skin. Each record was obtained when a spot on the skin was pressed with a probe. The final load was about the same (2 g.wt.) each time and the distances of the probe from the most sensitive central spot are indicated in millimetres. An after-discharge is seen only at the central position. (*From:* Iggo, *J. Physiol.* **152**, 337–53.)

porarily by repeated rubbing or stroking of the skin. Many of these mechanically-sensitive fibres could be excited weakly by suddenly lowering the temperature of the skin, 2° C. or more. This property is shared by most mechanoreceptors, particularly those which adapt fairly slowly to mechanical stimulation and it is probably to be regarded as an instance of the failure of 'receptor-specificity' to be absolute rather than, as suggested by Douglas, Ritchie and Straub (1960), indicating a 'dual-specificity'. All the mechanoreceptive fields of the C-fibres were small, less than 5 × 5 mm. (Fig. 3) and thus the fibres innervated a smaller area of skin than the large myelinated fibres innervating the hair follicles.

In addition to the numerous mechanoreceptors with afferent C-fibres there was another class—the slowly-adapting thermally-sensitive C-fibres. The majority were excited by either a rise or a fall in temperature in the range 3°–50° C. (Fig. 4). Only exceptional fibres were excited by both a rise and a fall and then only by large changes in temperature. The most sensitive fibres had thresholds so low,

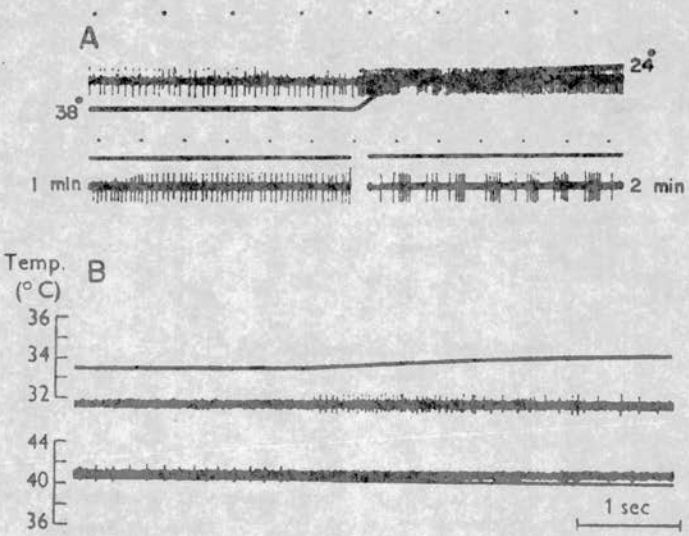


Fig. 4. Sensitive thermoreceptors in the skin. (*A*) Monkey. The upper records are in a small myelinated fibre from a sensitive cold receptor in the forearm and show the effect of lowering the temperature from 38° to 24° C. The steady resting discharge is accelerated. One minute later the temperature was still falling slowly and the discharge was continuous. After 2 minutes the temperature was steady and the impulses appeared in groups. (*From:* Iggo, *Proc. R. Phys. Soc. Edinb.* 1962.) (*B*) Cat. The lower records are from a non-myelinated fibre from a receptor excited by raising the skin temperature less than 0·3° C. The discharge from this warm receptor stopped when the temperature was lowered a similar amount. (Hensel *et al.*, *J. Physiol.* (1960) 153, 113–26.)

$\triangle T \pm 0{\cdot}1°$ C. (Hensel *et al.*, 1960), that they were comparable in sensitivity to human thermal discrimination. The least sensitive units required a change of 10° C. or more before they responded. The mechanical thresholds of the fibres were high (1·5–10 g.wt.) when compared with the most sensitive mechanoreceptors with non-myelinated afferent fibres (Iggo, 1959). All the cutaneous thermoreceptors so far examined in detail in the cat, rat and dog have had non-myelinated afferent fibres (Iggo, 1959; Hensel *et al.*, 1960; Iriuchijima & Zotterman, 1960). In the monkey, on the other hand, the majority of the thermoreceptors so far isolated in both hairy and glabrous skin have had small myelinated fibres (Iggo, 1961c), but no obvious difference could be seen between the responses to temperature changes of the myelinated and non-myelinated fibres. A conspicuous difference from the thermoreceptors in the cat, rat and dog was the very characteristic grouping of impulses into short bursts (Fig. 4). Both the origin and significance of this grouping are obscure.

Within the categories of mechanoreceptors and thermoreceptors there exist differences in sensitivity and in the time-course of the response to an appropriate, constantly maintained, stimulus. The variation in threshold sensitivity was wide, from 20 mg. to 5,000 mg. wt. for the mechanoreceptors and from $\triangle T \pm 0{\cdot}1°$ C. to $\triangle T \pm 10°$ C. for thermoreceptors. This wide range together with the failure of the endings to be absolutely specific adds to the difficulty of a precise classification of individual fibres. On the grounds of threshold alone the least sensitive mechano- and thermoreceptors could be classed as nociceptors. The evidence indicates that among the C-fibres there are fibres which could function as high-temperature nociceptors (Fig. 5), low-temperature nociceptors and strong-pressure nociceptors (Iggo, 1959, 1960). These results lead to the conclusion that instead of, or in addition to, the classical 'pain-receptor' equally sensitive to all damaging or near-damaging stimuli, there may also be nociceptors with some degree of 'modality-specificity'.

Some additional, more discriminating, tests are clearly necessary to supplement the usual tests for nociceptors. The use of chemicals is attractive, particularly since the 'blister-base' technique of Professor Keele and his colleagues has allowed a wide variety of chemicals to be tested on human skin. Some chemicals used, *e.g.* KCl solutions and hypertonic NaCl solutions, are effective not because they excite nociceptors to the exclusion of other afferent fibres, but because they excite all afferent fibres including the nociceptors. Gessler (1957) using the severe osmotic stimulus of a 30 per cent NaCl solution could excite many afferent fibres in the skin of frogs and rats. In some unpublished experiments on cat skin it was found that when hypertonic saline solutions (5 per cent NaCl w/v) were

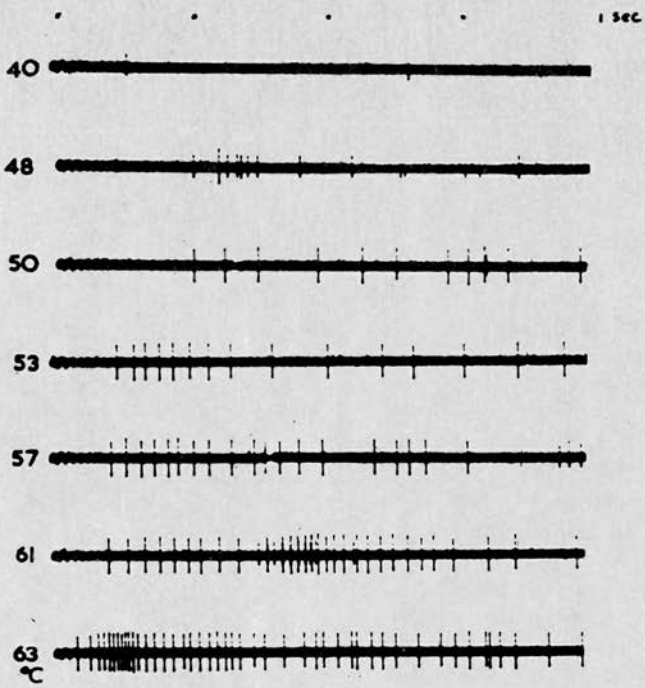
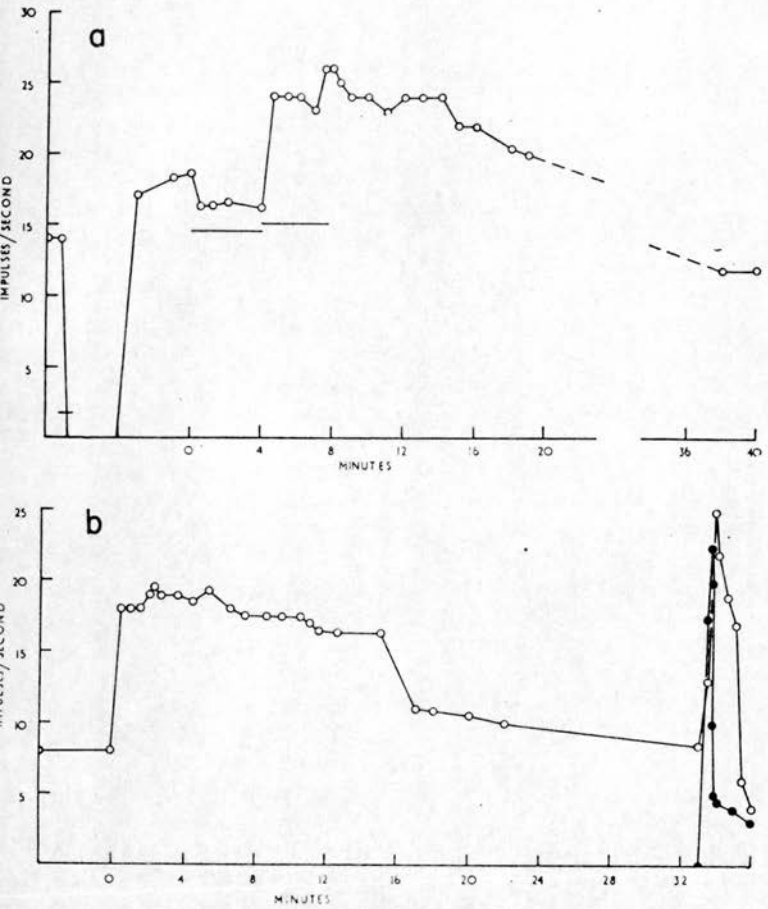


Fig. 5. The response from a non-myelinated fibre (conduction velocity 1·0 m/sec.) with a cutaneous ending, excited by heating the skin. The stimulator was a brass rod (10 mm. diameter) at the temperature indicated at the left-hand side and remained on the skin for the duration of each record. The smaller impulses seen in some records are from a mechanoreceptor about 10 mm. away from the heat-sensitive unit. They were evoked by the mechanical stimulus, and the unit was insensitive to temperature in range tested. The thermal unit was not excited by the mechanical stimulus so that these two units provide a clear illustration of the phenomenon of the 'peripheral specificity' of cutaneous afferent fibres. (*From:* Iggo (1959). *Quart. J. exp. Physiol.* **44**, 362–70.)

placed on superficial cuts all myelinated and non-myelinated afferent fibres were excited, including those cut off from their endings in the skin. This type of response presumably accounts for the pain caused by salt solutions put on open wounds. The cuts themselves caused only a very brief discharge of impulses. This type of stimulus causes pain, not because it excites only nociceptors but because it excites all fibres, and the proper description is 'pain aroused by an inclusive stimulus', to distinguish it from the pain aroused, *e.g.*, by heating the skin to 40°–50° C., which is probably due to the excitation of specifically-sensitive fibres. This latter could be termed 'pain aroused by an exclusive stimulus'. Even with heating, however, there may be an



ig. 6. The effect of (*a*) histamine pricked into the skin and (*b*) bradykinin and ıen a mixture of histamine and acetylcholine injected close-arterially, on the ischarge of impulses in cutaneous afferent fibres from the saphenous nerve of the at. The open circles in each graph show the rate of firing in a slowly-adapting ~~ec~~hanoreceptor with a myelinated afferent fibre. In the upper graph there was a eady discharge which disappeared temporarily when superficial cuts were made the skin. At zero time histamine (10^{-3} g/ml.)was put on the skin without any ffect. At 4 minutes it was pricked into the skin and caused an increase in the ıte of steady firing in the fibre. The lower graph shows that the injection of $00\mu g$. of impure bradykinin also caused the acceleration of the steady discharge a similar fibre. After 33 minutes a mixture of histamine (10 μg.) and acetyl-ıoline (24 μg.) was injected. In addition to a strong excitatory action on the ıyelinated fibre there was also a discharge of impulses in several non-myelinated bres (filled circles) which had not been excited by the bradykinin. The injected istamine caused a depression of the steady discharge after the initial enhance-ment. (Fjällbrant & Iggo, 1961.)

altered discharge from other cutaneous receptors. Thus, any steady discharge in slowly-adapting mechanoreceptors will be temporarily reduced (Witt & Hensel, 1959) so that even in this instance the alteration in cutaneous afferent inflow is complex.

Some other chemicals which excite pain (Armstrong, Dry, Keele & Markham, 1953) might fall into the category of 'exclusive pain-stimulants'. These are histamine, 5-hydroxytryptamine and bradykinin. The last is particularly potent when injected close-arterially into the skin, muscles or viscera in dogs (Braun, Guzman, Horton, Lim & Potter, 1961). These chemicals have recently been pricked into the skin or injected close-arterially into the saphenous artery of cats, while recording from cutaneous afferent fibres dissected from the saphenous nerve (Fjällbrant & Iggo, 1961). With few exceptions the non-myelinated fibres tested were depressed by these chemicals. Nevertheless some small fibres were excited. The only example identified was a high-threshold heat receptor and this unit responded with bursts of impulses each lasting a few seconds, separated by longer intervals of silence. The most dramatic changes were seen in slowly-adapting cutaneous mechanoreceptors with myelinated afferent fibres (Fig. 6). These were excited by histamine, 5-hydroxytryptamine and impure bradykinin. The period of exaltation of responsiveness, which lasted 2–4 minutes with histamine and 5-HT, was followed by a depression of sensitivity whereas bradykinin caused only an enhanced response. Other more sensitive mechanoreceptors have since been found to possess similar properties (Iggo, unpublished results) so that none of the chemicals can be regarded as acting as 'exclusive pain stimulants'. This approach so far has not been encouraging and it might reflect the illusory nature of the idea that the nociceptors can actually be identified, with certainty, in the peripheral nerves.

Although it is necessary to construct a detailed account of the activity in all peripheral nerve fibres in response to natural stimulation of the body, it is equally necessary to follow the impulses centrally. This central search may turn out to be required for the complete analysis of 'pain'. It is clear, however, that no longer can all activity in the non-myelinated fibres of peripheral nerve be regarded as exclusively nociceptive. On the other hand, neither can all afferent fibres be regarded as identical, in terms of the sensitivity of their endings to natural stimuli.

REFERENCES

ADRIAN, E. D. (1931). *Proc. Roy. Soc. B.* **109**, 1.
ARMSTRONG, D., DRY, R. M. L., KEELE, C. A. & MARKHAM, J. W. (1953). *J. Physiol.* **120**, 326.
BESSOU, P. & LAPORTE, Y. (1958). *C.R. Soc. Biol., Paris* **152**, 1587.
BESSOU, P. & LAPORTE, Y. (1961). *Arch. ital. Biol.* **99**, 293.

J. Physiol. (1961), **159**, *pp.* 461–478
With 7 text-figures
Printed in Great Britain

ELECTROPHYSIOLOGICAL INVESTIGATIONS ON RENSHAW CELLS

BY J. C. ECCLES, ROSAMOND M. ECCLES, A. IGGO* AND A. LUNDBERG†

From the Department of Physiology, Australian National University, Canberra, Australia

(*Received* 12 *June* 1961)

When Renshaw cells are synaptically activated by antidromic volleys entering the spinal cord through ventral roots, the initial frequency of discharge is often over 1500/sec; and, after a few impulses at above 1000/sec, the frequency progressively declines until the terminal responses are 20 or more milliseconds apart (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Eccles, Eccles & Fatt, 1956; Frank & Fuortes, 1956; Curtis & Eccles, 1958*a*, *b*). The initial high frequency certainly indicates that a very intense synaptic stimulation is evoking discharges immediately the cell has recovered from the absolute refractoriness following the previous discharge. It has been generally assumed that the later progressive decline in frequency of discharge results from a smooth continuous decline in the intensity of synaptic stimulation; nevertheless, there are experimental observations that suggest a discontinuity between an intense initial phase of synaptic stimulation and a subsequent low and prolonged activity. For example, the blocking drug, dihydro-β-erythroidine hydrobromide is very effective in suppressing all but the first two or three discharges, which persist with almost unchanged latency and frequency even after very large doses (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956; Curtis & Eccles, 1958*b*; Brooks & Wilson, 1959). A further indication of an intense initial synaptic excitatory action is provided by the lengthened interval that often disturbs the rhythm between the second and third discharges (Eccles, Fatt & Koketsu, 1954, Fig. 6G, H; Frank & Fuortes, 1956, Fig. 12B).

In the present investigation synaptic stimulation of Renshaw cells has been examined both by studying the effect of variation in intensity of the stimulus on the time course of the repetitive response, and by employing intracellular recording to give the time course of the synaptically induced

* Present address: Physiology Department, University of Edinburgh.
† Present address: Department of Physiology, University of Göteborg, Sweden.

depolarization. A further variant of the intensity of synaptic stimulation has been obtained by making use of the depression that follows a conditioning volley in the same presynaptic pathway to the Renshaw cell. A systematic examination has also been made of the synaptic activation of Renshaw cells by antidromic volleys from a large variety of muscle nerves.

METHODS

The general experimental procedures with micro-electrode recording from the lumbosacral cord of anaesthetized spinal cats were similar to those already described in publications from this laboratory (Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Eccles & Fatt, 1955). Renshaw cells were recorded from extracellularly, and also, with varying degrees of success, intracellularly. They were synaptically excited by antidromic volleys in motor axons, which were set up by stimulation either of ventral roots or of various muscle nerves. In the latter case the appropriate dorsal roots had been severed so as to eliminate synaptic activation either through interneuronal pathways or via axon collaterals of reflexly discharging motoneurones. Many muscle nerves were mounted on stimulating electrodes, and could be rapidly tested in turn by a rotary switch device (see Eccles, Eccles & Lundberg, 1957). The nomenclature of the various muscle nerves is given in the description of Table 1.

RESULTS

Time course of synaptic activation of Renshaw cells

Variations in the intensity of the synaptic stimulus. When the antidromic volley in the alpha fibres of the ventral root was progressively decreased in size, the intensity of excitation of Renshaw cells decreased stepwise, as revealed both by the frequency and by the duration of their discharge (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954; Frank & Fuortes, 1956). Renshaw cells also displayed several intensities of response when excited by maximal antidromic volleys in the alpha fibres of different muscle nerves (Renshaw, 1946; Eccles, Fatt & Koketsu, 1954). By graded submaximal stimulation of the muscle nerve evoking the most intense response of a Renshaw cell that cell could be made to respond in a manner closely resembling the responses evoked by maximal volleys in the less effective nerves. For example, Fig. 1A and G are virtually identical; as also Fig. 1B and H; and Fig. 1C and J, K, L. Thus it may be concluded that the responses evoked by antidromic volleys from different muscle nerves vary solely on account of the effectiveness of the respective synaptic excitatory actions, i.e. the variations have a purely quantitative basis. In parenthesis it may be noted that a similar conclusion has been reached for the monosynaptic activation of motoneurones for different afferent pathways, both homonymous and heteronymous (Eccles *et al.* 1957). It is therefore justifiable to employ both submaximal and maximal volleys from different muscle nerves (cf. Fig. 2A–E) when investigating the effects of variation in the amount of synaptic excitatory action on Renshaw cells.

When there was a smooth decline in the frequency of the repetitive response evoked by a maximal antidromic volley, as in Fig. 2E, progressive weakening of the synaptic excitation (E–A) caused, first, a shortening of the response and a more rapid decline of the frequency; but the first two or three discharges were much less affected until the stimulus was so weak that all later discharges had been eliminated (Fig. 2A, B). When the reciprocal of the response interval, i.e. the frequency, was

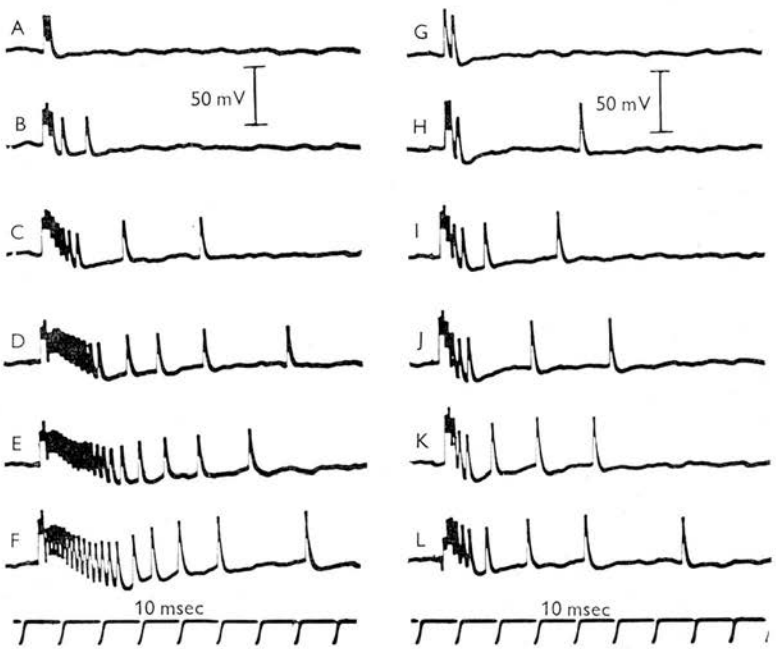


Fig. 1. Intracellular responses of a Renshaw cell evoked by submaximal alpha volleys from the medial gastrocnemius nerve, of increasing size from A–D and by maximal volleys in E, F. G–L are responses of same cell evoked by maximal alpha volleys from a series of other muscle nerves: plantaris, soleus, anterior biceps, inferior gluteal, lateral gastrocnemius and posterior biceps respectively. The series of A–E was taken a little later than F–L, the resting potential and spike potential having meanwhile declined. Same voltage and time scales throughout.

plotted for the successive discharges (Fig. 2G), it might be inferred for the range of responses of Fig. 2A–F that the synaptic stimulation diminished first in duration and only later in intensity. However, this inference is inadmissible because the initial very high frequencies of discharge (Fig. 2C–F) would be largely determined by the duration of Renshaw-cell refractoriness.

When a Renshaw cell was activated by various sizes of antidromic volley in the ventral root (Fig. 2H–L), the respective repetitive responses

may be plotted (Fig. 2M), as in Fig. 2G, to show the time course of the decline of frequency. Comparison of Fig. 2G and M shows that the two modes of grading synaptic excitation of Renshaw cells yielded closely parallel results, as has already been illustrated by the respective series of records, A–E and G–L in Fig. 1.

When attempting to derive the time course of the synaptic excitatory action from curves such as those of Fig. 2G and M, it may first be assumed

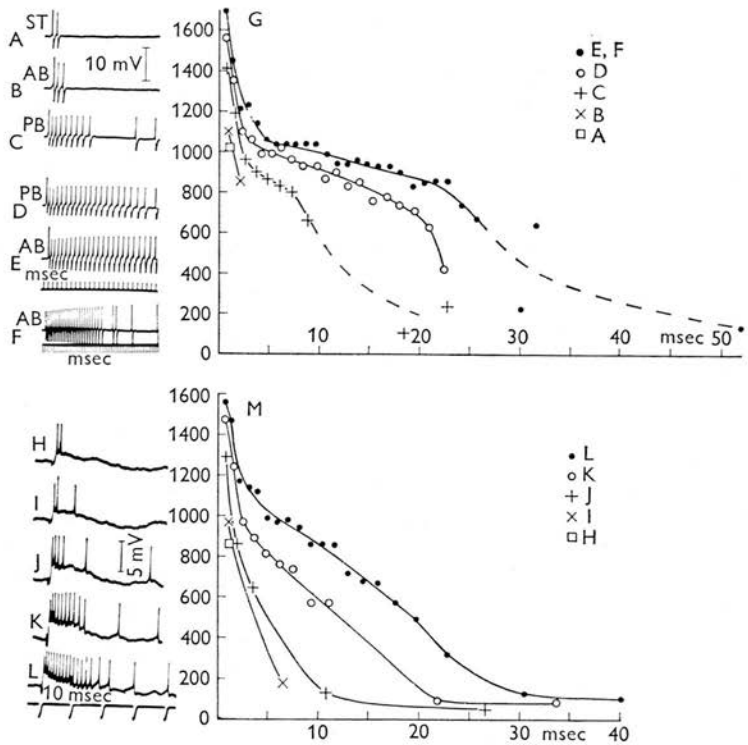
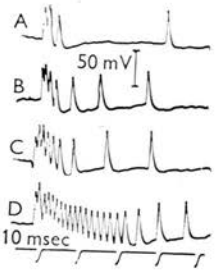


Fig. 2. A–F are responses of a Renshaw cell recorded extracellularly and evoked respectively by antidromic volleys from the three muscle nerves that were effective: maximal semitendinosus, submaximal anterior biceps, submaximal posterior biceps, maximal posterior biceps, and maximal anterior biceps at fast (E) and slower speed (F). The intervals between the successive responses are plotted as frequencies (ordinates) in G, much as has been done for interneuronal responses by Hunt & Kuno (1959), the abscissae being the time of each discharge after the initial discharge. Thus the curves of G (see symbol lettering) plot the time courses of the frequencies of discharge for each of the responses in A–F. The terminal portions of two curves are shown as interrupted lines because of the scatter of the points. H–L are another series for the repetitive responses of another Renshaw cell (with poor intracellular recording) evoked by an antidromic volley in L7 ventral root, which was maximal in L and with four grades of submaximal stimulation in H–K. The intervals between the successive spike discharges are plotted in M in the same way as in G, as again indicated by the symbol lettering.

that the various motor-axon collaterals converging on a Renshaw cell have similar time courses for their synaptic excitatory action. At least there has been no significant difference between the motor-axon collaterals for the motoneurones supplying different muscles, and no difference would be expected between axon collaterals from the lower-threshold and the higher-threshold alpha motor fibres. When there was a sufficiency of convergent impulses on a Renshaw cell, the initial intensity of activation was so high that the frequency (over 1500/sec) would be close to the limit set by the refractoriness of the cell; and even with considerably lower frequencies (around 1000/sec) refractoriness must be a significant factor in setting the frequency. The curves for the more powerful synaptic stimulations thus give a misleading impression of the time course for the intensity of the synaptic stimulus, because the refractoriness of the discharging cell prevents the initial intense phase from evoking a commensurately high frequency of discharge. The curves for the less powerful synaptic stimulations also give an erroneous time course, because a synaptic stimulus below threshold is given a zero value, as occurs after the first two or three responses with the weakest stimuli in Fig. 2G, M. However, some indication of the time course of the synaptic stimulus may be derived by noting the times at which the same response frequencies were produced by stimuli of different strengths. For example, the peak frequency in Fig. 2A was comparable with the frequency at a point 10 msec later in Fig. 2E, so it is probable that the respective intensities of synaptic stimulation were then similar. A complicating factor would be introduced by the possible action of accommodation in slowing the frequency in the latter part of Fig. 2E. Likewise, in the series of Fig. 2H–M, the peak frequency of the weakest response (Fig. 2H) was comparable with the frequency of the strongest response (L) at a point 10 msec later. An intermediate stimulus strength (J) showed a decline from an initial frequency of 1290/sec to 135/sec at 10 msec later; yet, with the response evoked by the maximum stimulus (L), the initial frequency of 1560/sec had declined only to 860/sec at 10 msec later. Evidently the synaptic stimulus was much greater in this latter case, but it could cause only a limited increase in the initial frequency. In general, therefore, the curves of Fig. 2G, M may be interpreted as indicating that the synaptic excitatory action produced by a single impulse had a very intense phase lasting 2 or 3 msec followed by a slowly declining tail of much lower intensity.

As already mentioned, an abnormally long interval may occur between the second and third responses to a very powerful synaptic stimulation. For example, in Fig. 3 this was regularly present in responses evoked by a maximum antidromic volley in the medial gastrocnemius nerve (D), but did not occur with the responses to the weaker stimulations provided by

other muscle nerves (A–C). The break in rhythm is further illustrated by the filled circles in the plotted curves (E). A regular observation has been that the lengthened interval is a little too brief (by about 0·2 msec in Fig. 3E) to be explicable merely as due to the dropping out of one response.



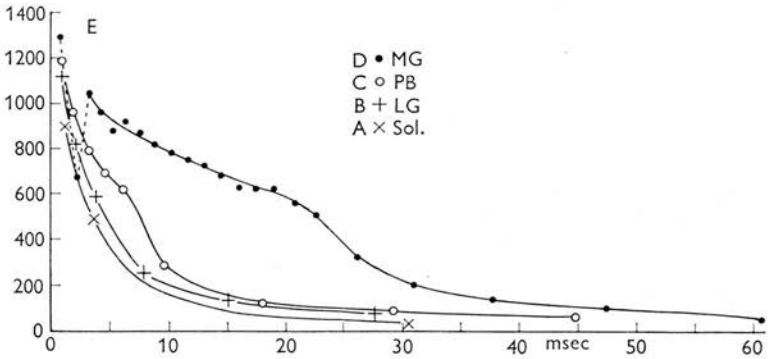


Fig. 3. A–D, repetitive Renshaw cell responses evoked by maximal antidromic volleys in the various muscle nerves as indicated. Same cell as in Fig. 1, with a faster sweep speed, in order to show the break in rhythm between the second and third responses in the largest response (D). In E the measurements from the series partly shown in A–D are plotted as in Fig. 2G, M. The plotted points for the medial and lateral gastrocnemius volleys are the means of two closely similar records. The curve joining the first three points for medial gastrocnemius (●) is drawn as a dotted line.

Similar findings occur with antidromic volleys set up by graded stimulation of the ventral root (Fig. 4A–D). The break in the rhythm was not present with the two weakest stimuli (A, B), but was very evident with the strongest stimulus (C), particularly when observed at high speed (D). Again, the lengthened interval was about 0·2 msec too brief to be attributable to the dropping of one discharge. Presumably, when extremely large the synaptic depolarization caused some disorganization of the spike-generating mechanism, just as occurs with cathodal depression of nerve

fibres or motoneurones (Coombs *et al.* 1955). Sometimes a small abortive spike was detectable at the time of the missing discharge (cf. Eccles, Fatt & Koketsu, 1954, Fig. 6G, H).

Another common type of disorganization of the spike mechanism is illustrated in Fig. 4E–H. Here the spike had a double composition resembling the compound IS–SD spike of motoneurones, and in the initial

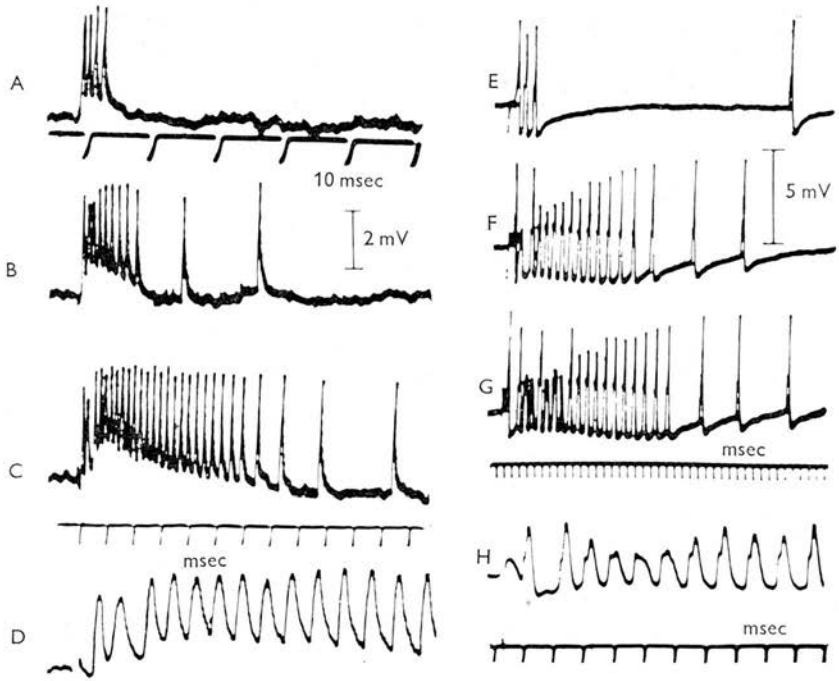


Fig. 4. A–D, series of Renshaw cell responses recorded intracellularly as in Fig. 3A–D, but for a different cell and evoked by antidromic volleys set up by stimulation of L7 ventral root, submaximal in A, B and maximal in C. D is same response as C, but at a faster sweep speed as shown above it. Same potential scale throughout. (D, H are at lower amplification.) E–H, responses evoked in another Renshaw cell as in A–D, G and H being evoked by maximal antidromic volleys in L7 ventral root. H at the sweep speed indicated below it.

high-frequency phase most of the spike potentials were largely composed of the initial small spike. In the fast record (H) it is seen that this initial small spike was fairly uniform throughout the response, while there was great variability in the later spike. Presumably this fragmentation of the spike is attributable to the high frequency of spike generation, rather than to the intensity of the background synaptic depolarization. It did not occur with the lowest frequency of discharge evoked by the weakest stimulation (Fig. 4E). On analogy with motoneurones it is probable that

the initial small spike was associated with the discharge of an impulse along the axon (Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957*a*, *b*).

Excitatory post-synaptic potentials of Renshaw cells. On rare occasions the intracellularly recorded Renshaw cell responded by a depolarization

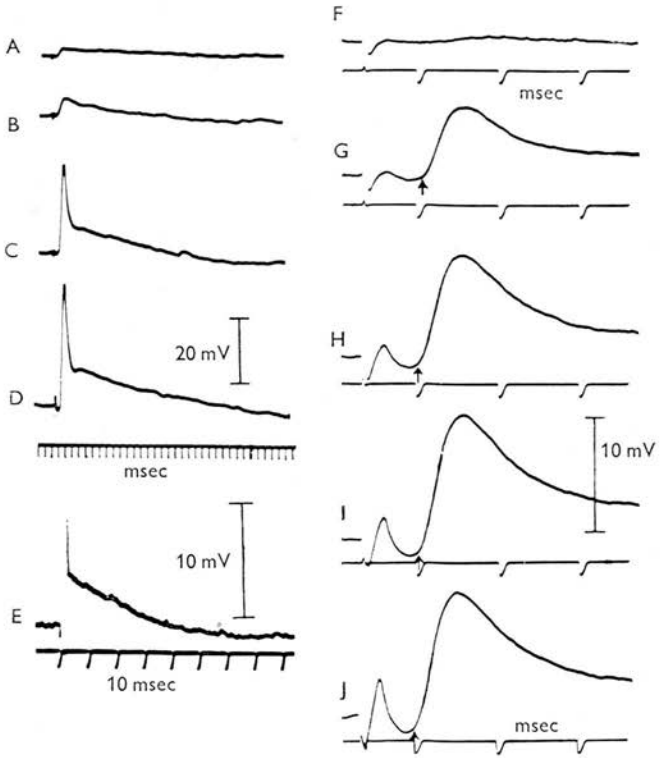


Fig. 5. Excitatory post-synaptic potentials (EPSPs) recorded intracellularly from a Renshaw cell with a recorded membrane potential initially of −60 mV. A–D show graded sizes of EPSPs evoked by submaximal (A–C) and maximal (D) antidromic volleys in L7 ventral root. E shows maximal response recorded at slower sweep speed and with a long time constant (1 sec) of the amplifier. F–J are fast records of EPSP evoked by progressively increasing antidromic volleys, as shown by the initial diphasic wave with first deflexion upwards. The arrows indicate times of onset of the EPSPs. Series E–J at higher amplification than A–D, as indicated. Upward deflexions signal positivity relative to the indifferent earth lead i.e. membrane depolarization.

(the excitatory post-synaptic potential, EPSP) uncomplicated by spike potentials. A response of this type has already been illustrated (Eccles, Fatt & Koketsu, 1954, Fig. 6I), but it was at that time suggested that the initial brief peak of depolarization was a spike potential. A much larger

potential of the same type is illustrated in Fig. 5. The responses of this Renshaw cell were so unusual that its identification could be regarded as established only after the full investigation described below.

The membrane potential was about −60 mV for the first records of Fig. 5; it gradually declined thereafter. A maximum alpha volley in the L7 ventral root evoked the large (36 mV) brief depolarization (D) that after about 2 msec merged into a much slower decaying depolarization. The full duration of this depolarization is shown in E to be about 60 msec. Progressive diminution of the alpha volley from maximum size (D) was associated with a diminution of both the fast and slow components of the depolarization (C). With the responses to smaller volleys the fast component was relatively lower, being but little above the slow component in B, and at about the same level in A. This is also well shown in the very fast records of Fig. 5, F–J. The fast component was barely identifiable in the weakest response (F), but showed progressive increment in G, H and I, there being no further increase when the alpha volley finally reached maximum (J).

The onset of the fast response was obscured in F–J, as it was superimposed on the downward (negative) deflexion of the extracellularly recorded soma spike potential of the alpha motoneurones. However, it can be detected as a sharp bend in the curve at the points marked by the arrows, which give it a latent period of about 0·4 msec from the phase of maximum positivity in the field potential produced by the approaching antidromic impulse. If a small allowance be made for the time of propagation of the antidromic impulse up the motor-axon collaterals, the actual synaptic delay for the onset of the depolarization of the Renshaw cell would correspond well with the value of about 0·3 msec calculated for other synaptic delays in the central nervous system (Eccles, 1957).

The smooth contour and relatively long duration (2 msec) of the brief depolarization establishes that it was not due to the superposition of a brief spike-like process on a more prolonged EPSP. This conclusion receives further support from the various gradations in its size (Fig. 5A–D, F–I). It may, therefore, be concluded that the potentials illustrated in Fig. 5 are EPSPs set up in a Renshaw cell by the summed action of several excitatory impulses converging on this cell. At least several of these impulses generated EPSPs having fast and slow components.

In Fig. 6A–H, from the same cell, a second maximum alpha volley was set up in the L7 ventral root at various intervals after the first. Even at relatively long intervals there was a considerable diminution both of the fast and slow components of the second EPSP. As shown by the plotted points (Fig. 6J) derived from the series partly illustrated in A–H, full recovery took about 150 msec, and at the shortest intervals (0·7–5 msec)

the depression was to about 40% of the control. As measured by the addition to the slow EPSP produced by the testing volley, the slow component of the EPSP was even more depressed at the shortest intervals. The time course of recovery was approximately the same for both the fast

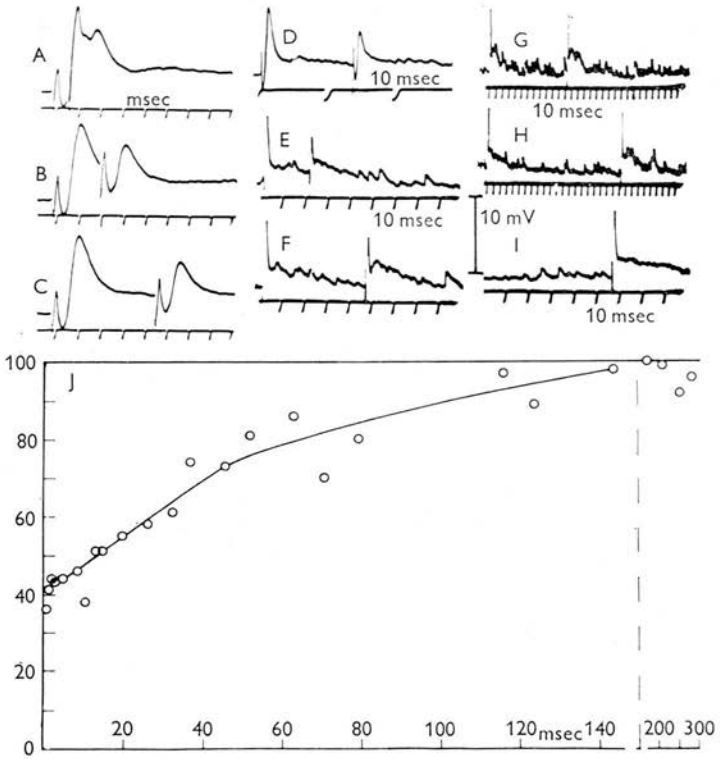


Fig. 6. A–I, same Renshaw cell as in Fig. 5, but responses evoked by two maximal antidromic volleys at various intervals as indicated by the stimulus artifacts. Note changes in sweep speed as indicated by the time bases for each record. Same potential scale throughout. J. Sizes of initial brief EPSP evoked by the second volley (calculated as percentages of the mean control size) are plotted against stimulus intervals for the series partly shown in A–I. Note the great compression of the time scale beyond the interrupted line at 150 msec.

and slow components. Renshaw (1946, Fig. 5) illustrated the depression of discharge that occurred with conditioning by a preceding antidromic volley in the same nerve fibres. There was considerable depression with a volley interval in excess of 50 msec, and more recently (Eccles, Fatt & Koketsu, 1954) it has been reported that the depression persisted for as long as 100 msec. Presumably this depression of Renshaw cell discharges is sufficiently explained by the depression of the second EPSPs illustrated in Fig. 6.

Patterns of activation of Renshaw cells

The input pattern from individual muscle nerves on to Renshaw cells has now been examined in conditions where nearly all the muscle nerves arising from the lower lumbar and upper sacral cord could be stimulated. The only nerves to hind-limb muscles which were not stimulated were the obturator nerve and sartorius nerve. The convergence on to Renshaw cells could thus be examined in almost full detail. Nine of the cells were dominated by one muscle nerve, which was at least twice as effective as any other muscle nerve in causing a discharge of impulses from the cell (Figs. 1F–L, 3A–D, 7A–D). All the remaining cells were excited really effectively by only 2 or 3 muscle nerves.

TABLE 1

The convergence of antidromic volleys in muscle nerves on to 18 Renshaw cells. Each muscle nerve was tested separately and in the columns are the numbers of impulses discharged from the respective Renshaw cell in response to a maximal antidromic volley in the alpha motor fibres. Numbers in bold type show the contribution from the dominant muscle nerves. The symbols labelling the columns correspond to the following muscle nerves, and the same convention is employed in the text: Q, quadriceps; SM, semimembranosus; SG, superior gluteal; FDL, flexor digitorum longus+flexor hallucis longus; Per., peroneal; Pl., plantaris; Pop., popliteus; LG, lateral gastrocnemius; AB, anterior biceps; ST, semitendinosus; IG, inferior gluteal; PB, posterior biceps; MG, medial gastrocnemius; Sol., soleus; T, posterior tibial nerve (flexor digitorum brevis plus medial and lateral plantar). Cells 10, 11 and 18 are illustrated in Figures 7, 2 and 1, respectively

Renshaw cell number	L6 and upper L7					Mid L7					Lower L7 and S1			
	Q	SM	SG	FDL	Per.	Pl.	Pop.	LG	AB	ST	IG	PB	MG	Sol.
1	6	5	**13**	.	.	.	.	.	.	.	.	.	.	.
2	.	.	**13**	.	2	.	.	.	.	.	.	.	.	.
3	.	.	**10**	.	5	.	.	.	.	.	.	.	.	.
4	.	.	**7**	2	.	.	.	.	.	.	7	.	.	.
5	.	3	**7**	3	**8**	.	.	.	.	.	.	.	.	.
6	.	.	**10**	3	**3**	.	.	3	**8**	**3**	3	.	.	.
7	.	**17**	4	.	8	.	5	.	.	.	.	.	.	.
8	.	**16**	.	.	8	.	.	.	.	.	3	.	.	.
9	.	.	.	.	.	**14**	9	9	.	.	.	1	.	.
10	.	.	.	.	.	6	.	**18**	.	.	.	.	8	6
11	.	.	.	.	.	.	.	.	**32**	2	.	**28**	.	.
12	.	.	.	.	.	.	.	.	**20**	9	.	**13**	.	.
13	.	.	.	.	.	.	.	.	.	**10**	.	**12**	1	.
14	.	.	.	.	.	.	.	.	.	.	5	**22**	.	.
15	.	.	.	.	.	3	.	6	.	5	7	**12**	9	.
16	.	.	.	.	.	.	.	**26**	.	.	.	.	**26**	**17**
17	.	.	.	.	.	.	.	5	2	.	5	4	**13**	.
18	.	.	.	.	.	2	.	8	7	.	7	10	**21**	4

Table 1 has been prepared so that the muscle nerves are arranged in the approximate sequence of their motoneuronal nuclei along the long axis of the ventral horn. When they are arranged in this way, an important feature of the input pattern to these 18 Renshaw cells becomes con-

spicuous; namely, that Renshaw cells tend to be excited by collaterals from the axons of adjacent motoneuronal nuclei, as for example in Fig. 7. When 2 or 3 muscle nerves were dominant, they were invariably from adjacent nuclei in the cord. An apparent exception to this rule (cell 4),

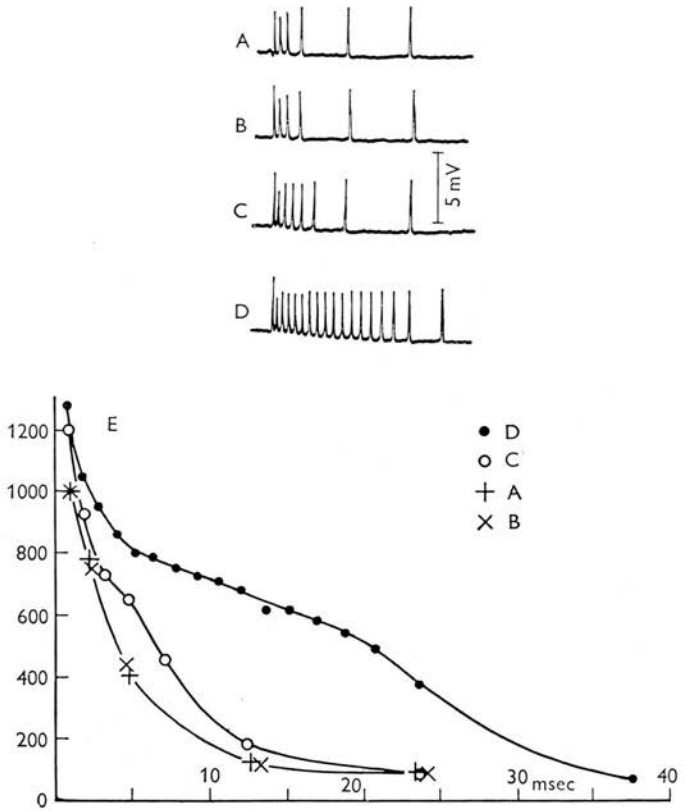


Fig. 7. A–D, Renshaw cell responses recorded intracellularly as in Fig. 3 and evoked by maximal antidromic volleys in the muscle nerves. A, plantaris; B, soleus; C, medial gastrocnemius; D, lateral gastrocnemius. All other antidromic volleys were ineffective. E, Responses illustrated in A–D are plotted as in Fig. 2G, H, the lettering of the symbol giving the reference to the records A–D. Note that the curves for A and B are virtually identical.

with equally effective inputs from superior and inferior gluteal nerves, can be accounted for by the imperfection of the tabular presentation. These two nuclei overlap in the ventral horn, and furthermore they are in the same cell column (Romanes, 1951). The influence of location was also apparent with the less effective muscle nerves. Thus cell 18 (Fig. 1) was excited most effectively by MG, but was also excited by PB, IG, AB,

Sol., Pop., LG and Pl. These nuclei are adjacent to MG, whereas Q, SM, SG and Per. were without effect and are more cephalad in the spinal cord. Motoneuronal nuclei in the same cell column in the ventral horn but separated longitudinally are less effective in exciting a Renshaw cell than are nuclei at the same level of the cord, but in different cell columns. Thus cells 7 and 8, which were fired optimally by SM, were not excited by AB, PB or ST, even though the latter are in the same cell column; whereas SG, Per. and Pop. were effective, yet lie in different cell columns in the ventral horn.

DISCUSSION

Synaptic excitation of Renshaw cells

The intracellular records of the EPSPs in Fig. 5 show a time course that corresponds closely with the time course that was postulated for the synaptic excitatory action generating the repetitive discharges of Figs. 1–4, 7. There is even direct evidence that submerged beneath the spike potentials of Figs. 1, 2H–L, 2A–D, 4A–D, there was an EPSP having a brief intense phase and a prolonged tail. For example, in the weakest response of Fig. 4A the four spikes were superposed on a depolarization that had a total duration of about 6 msec. With the next stronger response (B) the depolarization was about 10 msec in duration, but the second and third spikes were reduced in size, possibly owing to cathodal depression exerted by the large depolarization on which they were superposed. Finally, with the largest response (C) the spikes were superposed on a long tail of depolarization over 30 msec in duration, and between the second and third spikes appeared the break in rhythm that probably is further evidence of the initial intense phase of depolarization. The depolarizations of Fig. 4 were much less than those of Fig. 5, but the recording conditions were much inferior in Fig. 4, where the membrane potential and the spike potentials were only about -10 mV and 4 mV respectively.

The series of Fig. 2H–L similarly shows the spikes superposed on a prolonged depolarization up to 30 msec in duration, which is better seen when there were few spikes in H–J, than in L, for the depolarization was there largely submerged by the after-hyperpolarization that followed each spike. This after-hyperpolarization was very prominent in Figs. 1 and 4E–H. Evidently the generation of impulses had occurred at a locus remote from the site of recording.

It is surprising that the very large EPSPs of Figs. 5 and 6 failed to generate spikes. Comparable observations have recently been made with the cells of origin of the dorsal and ventral spino-cerebellar tracts (Eccles, Oscarsson & Willis, 1961; Eccles, Hubbard & Oscarsson, 1961), and with some cells of the dorsal horn (Eccles & Krnjević, 1959). It was suggested

that the spike-generating mechanism was suppressed by the depolarization resulting from injury by the micro-electrode. With motoneurones also the depolarization resulting from severe injury eliminates the production of spikes by the EPSP, there being a transitional phase of local responses. In all these examples the elimination of the spikes does not greatly affect the EPSPs. With motoneurones the time course is shortened as a consequence of the diminution of the membrane time constant (Eccles, 1961), the time course of the EPSP approaching nearer to that of the currents generating the EPSP. It may be concluded that the EPSPs of Figs. 5 and 6 give reliable information on the time course of the synaptic excitatory action that evoked the high-frequency discharges in other Renshaw cells. The high intensity of the brief initial synaptic excitation furthermore provides a sufficient explanation of the failure of large doses of dihydro-β-erythroidine to suppress the first one or two discharges (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956; Curtis & Eccles, 1958*b*; Brooks & Wilson, 1959).

Nevertheless, an important question still remains to be answered. It can be assumed that the EPSPs of Fig. 5 were generated by a virtually synchronous bombardment of the Renshaw cell by one impulse in each of several motor-axon collaterals. There is no evidence of any delayed bombardment via interneurones, which would be revealed by irregularities on the declining phase of the EPSP. Thus an explanation is required for the fast and slow components of the EPSPs produced by single impulses.

One suggestion would be that the initial rapid decline in the EPSPs of Fig. 5 is due to rapid destruction of acetylcholine by cholinesterase that is localized close to the site of its liberation. The subsequent low intensity of EPSP could then be attributed to the action of acetylcholine that had diffused beyond the zone of high cholinesterase activity. Unfortunately there are no observations of the effects produced by anticholinesterases on EPSPs such as those of Fig. 5. However, the action of anticholinesterases on the repetitive discharges of Renshaw cells indicates that the initial intense phase of action is not changed (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1956), the only significant action being the intensification and great prolongation of the later low frequency component of the response.

Possibly, therefore, diffusion accounts for the initial rapid decline in intensity of action of the synaptic transmitter. However, on account of the electric time constant of the cell membrane, the rate of decline of the EPSPs of Fig. 5G–J will be much slower than that of the transmitter (cf. Curtis & Eccles, 1959). It may be assumed that diffusion out of the synaptic cleft would cause the transmitter concentration in the synaptic cleft to decline much faster than the EPSPs of Fig. 5G–J (cf. Eccles &

Jaeger, 1958, Fig. 3B). The prolonged residuum of action could then be due to the more diffuse action of acetylcholine on the membrane receptors adjacent to synapses. It has recently been shown (Miledi, 1960) that such receptors normally exist in an extensive zone around the region of the neuromuscular junction.

In the slower records of Fig. 6 there is clear evidence of brief depolarizing potentials occurring in random fashion. Since they have a time course rather like EPSPs, they may be assumed to be examples of the synaptic noise that has been reported on motoneurones and attributed to background internuncial bombardment (Brock, Coombs, & Eccles, 1952). In the anaesthetized preparation in which the activity of Fig. 6 was recorded, repetitive discharges of impulses from motoneurones and so up to motor-axon collaterals are unlikely to be the cause of all the activity seen in Fig. 6E–H. At least seven of these potentials occurred in Fig. 6I before the antidromic stimulus, hence the random potentials of Figs. 6E–H were not generated by the antidromic stimulation. However, there are synaptic connexions from interneurones to Renshaw cells (Curtis, Phillis & Watkins, 1961) and discharges from such interneurones could cause the spontaneous activity in Fig. 6. Alternatively, the small potentials could be comparable to the miniature end-plate potentials first described by Fatt & Katz (1952), and shown by them to be due to the liberation of quanta of transmitter. It should be remembered that the transmitter is the same as at the neuromuscular junction, and that the axon collaterals making synaptic connexions with Renshaw cells are indeed branches of the same motor nerve fibres that produce the miniature end-plate potentials of muscle. Possibly such miniature synaptic potentials are responsible for the late irregular discharges seen in such records as Fig. 2J, K, L and for the spontaneous discharges that are given by many Renshaw cells (Eccles *et al.* 1956; Curtis & Eccles, 1958*a*).

Convergence on to Renshaw cells from different muscle nerves

The convergence of muscle nerves on to a Renshaw cell was investigated by testing in turn the responses of the cell to single maximum alpha volleys in the motor fibres of the various muscle nerves. Conceivably this technique would demonstrate an arbitrarily restricted field of motoneurones from which a Renshaw cell could be excited during normal activity, for in such activity the repetitive firing of motoneurones might lead to the recruitment of Renshaw cells that were not excited by single volleys. This possibility was tested in one experiment by investigating the effect of repetitive synaptic activation of Renshaw cells. If a single volley failed to excite a Renshaw cell, a succession of volleys was also ineffective. The convergence revealed by the technique used in the present

work will thus also be present during the repetitive firing of motoneurones during normal reflex activity.

Individual Renshaw cells in Table 1 tend to be dominated by one or two muscle nerves. When there was only one dominant line, the Renshaw cell might be labelled as belonging to that particular muscle. When there was more than one dominant line, a convergence of synergists was sometimes evident, as in cells 13 and 16 in Table 1. Other Renshaw cells, however, were dominated by muscles of unrelated reflex functions, e.g. cells 11 and 12 were excited maximally by the hip extensor, AB, and the knee flexor, PB. The possibility of interaction between antagonists at the same joint is, however, limited by the topographical arrangement of the motoneuronal nuclei in the spinal cord. The knee extensors, Q, are more cephalad than the knee flexors, PB and ST, the centres of the respective nuclei being separated by more than one segment. This separation is sufficient to prevent any significant interaction between those antagonists. The ankle extensors, MG, LG and Sol., are more caudal than the ankle flexors (supplied by the deep peroneal nerve), but there is more overlap of the respective nuclei than with the knee antagonists. The location of motoneurones supplying the ankle antagonists thus again limits the possibility of convergence on to the same Renshaw cell, but there may also be other factors which limit the convergence.

SUMMARY

1. A detailed investigation has been made of the repetitive responses evoked in Renshaw cells by various sizes of antidromic volleys in alpha motor fibres. Comparable responses of a Renshaw cell were observed whether the size was varied by graded submaximal stimulation of a ventral root or by antidromic volleys in the different muscle nerves that converged on the same cell.

2. An analysis of the responses when there was a smooth decline in frequency from an initial maximum indicated that the synaptic stimulation had an initial very intense phase, about 2–3 msec in duration, followed by a much longer phase of low intensity. The very intense initial phase was also indicated by the break in rhythm sometimes observed between the second and third discharges, and by the high resistance of the initial 2 or 3 discharges to the depressant action of drugs such as dihydro-β-erythroidine hydrobromide.

3. An intense initial phase of synaptic depolarization was also revealed by intracellular recording from Renshaw cells. Usually the time course was obscured by superimposed spikes; but these spikes were occasionally absent and the full time course of the synaptically induced EPSP was revealed. There was an initial depolarization about 2 msec in duration

and up to 36 mV in height and a later slowly declining tail up to 60 msec in duration.

4. The EPSP evoked by a second antidromic volley was depressed for as long as 150 msec after a preceding volley, which correlates with previous reports of a prolonged depression of the discharges that a second antidromic volley evokes from Renshaw cells.

5. An investigation has been made of the pattern of activation of Renshaw cells from a large variety of muscle nerves, and its significance has been discussed.

REFERENCES

Brock, L. G., Coombs, J. S. & Eccles, J. C. (1952). The recording of potentials from motoneurones with an intracellular electrode. *J. Physiol.* **117**, 431–460.

Brooks, V. B. & Wilson, V. J. (1959). Recurrent inhibition in the cat's spinal cord. *J. Physiol.* **146**, 380–391.

Coombs, J. S., Curtis, D. R. & Eccles, J. C. (1957*a*). The interpretation of spike potentials of motoneurones. *J. Physiol.* **139**, 198–231.

Coombs, J. S., Curtis, D. R. & Eccles, J. C. (1957*b*). The generation of impulses in motoneurones. *J. Physiol.* **139**, 232–249.

Coombs, J. S., Eccles, J. C. & Fatt, P. (1955). The electrical properties of the motoneurone membrane. *J. Physiol.* **130**, 291–325.

Curtis, D. R. & Eccles, J. C. (1959). The time courses of excitatory and inhibitory synaptic actions. *J. Physiol.* **145**, 529–546.

Curtis, D. R. & Eccles, R. M. (1958*a*). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. *J. Physiol.* **141**, 435–445.

Curtis, D. R. & Eccles, R. M. (1958*b*). The effect of diffusional barriers upon the pharmacology of cells within the central nervous system. *J. Physiol.* **141**, 446–463.

Curtis, D. R. Phillis, J. W. & Watkins, J. C. (1961). Cholinergic and non-cholinergic transmission in the spinal cord. *J. Physiol.* **158**, 296–323.

Eccles, J. C. (1957). *The Physiology of Nerve Cells.* Baltimore: Johns Hopkins Press.

Eccles, J. C. (1961). Membrane time constants of cat motoneurons and time courses of synaptic action. *Exp. Neurol.* **4**, 1–22.

Eccles, J. C., Eccles, R. M. & Fatt, P. (1956). Pharmacological investigations on a central synapse operated by acetylcholine. *J. Physiol.* **131**, 154–169.

Eccles, J. C., Eccles, R. M. & Lundberg, A. (1957). The convergence of monosynaptic excitatory afferents onto many different species of alpha motoneurones. *J. Physiol.* **137**, 22–50.

Eccles, J. C., Fatt, P. & Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

Eccles, J. C., Fatt, P., Landgren, S. & Winsbury, G. J. (1954). Spinal cord potentials generated by volleys in the large muscle afferent fibres. *J. Physiol.* **125**, 590–606.

Eccles, J. C., Hubbard, J. I. & Oscarsson, O. (1961). Intracellular recording from cells of the ventral spino-cerebellar tract. *J. Physiol.* **158**, 486–516.

Eccles, J. C. & Jaeger, J. C. (1958). The relationship between the mode of operation and the dimensions of the junctional regions at synapses and motor end-organs. *Proc. Roy. Soc.* B, **148**, 38–56.

Eccles, J. C. & Krnjević, K. (1959). Potential changes recorded inside primary afferent fibres within the spinal cord. *J. Physiol.* **149**, 250–273.

Eccles, J. C., Oscarsson, O. & Willis, W. D. (1961). Synaptic action of Group I and II afferent fibres of muscle on the cells of the dorsal spino-cerebellar tract. *J. Physiol.* **158**, 517–543.

Fatt, P. & Katz, B. (1952). Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* **117**, 109–128.

Frank, K. & Fuortes, M. G. F. (1956). Unitary activity of spinal interneurones of cats. *J. Physiol.* **131**, 425–435.

FUORTES, M. G. F., FRANK, K. & BECKER, M. C. (1957). Steps in the production of motoneuron spikes. *J. gen. Physiol.* **40**, 735–752.

HUNT, C. C. & KUNO, M. (1959). Properties of spinal interneurones. *J. Physiol.* **147**, 346–363.

MILEDI, R. (1960). Junctional and extra-junctional acetylcholine receptors in skeletal muscle fibres. *J. Physiol.* **151**, 24–30.

RENSHAW, B. (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *J. Neurophysiol.* **9**, 191–204.

ROMANES, G. J. (1951). The motor cell columns of the lumbo-sacral spinal cord of the cat. *J. comp. Neurol.* **94**, 313–363.

DISTRIBUTION OF RECURRENT INHIBITION AMONG MOTONEURONES

By J. C. ECCLES, ROSAMOND M. ECCLES, A. IGGO and M. ITO

J. Physiol. (1961), **159**, *pp.* 479–499
With 10 *text-figures*
Printed in Great Britain

DISTRIBUTION OF RECURRENT INHIBITION AMONG MOTONEURONES

BY J. C. ECCLES, ROSAMOND M. ECCLES, A. IGGO* AND M. ITO†

From the Department of Physiology, Australian National University, Canberra, Australia

(*Received* 12 *June* 1961)

In recurrent inhibition the discharge of impulses from motoneurones exerts an inhibitory influence not only on motoneurones of the same species (homonymous motoneurones), but also on other motoneurones of diverse function (Renshaw, 1941; Lloyd, 1946, 1951; Eccles, Fatt & Koketsu, 1954; Holmgren & Merton, 1954; Granit, Pascoe & Steg, 1957; Henatsch & Schulte, 1958; Kuno, 1959; Wilson, 1959; Brooks & Wilson, 1959; Wilson, Talbot & Diecke, 1960). It is now generally agreed that the recurrent inhibitory pathway runs via motor axon collaterals to special interneurones in the ventro-medial part of the ventral horn (Renshaw, 1946), which in turn discharge impulses that directly inhibit motoneurones (Eccles, Fatt & Koketsu, 1954; Frank & Fuortes, 1956; Eccles, Eccles, Iggo & Lundberg, 1961). Undoubtedly recurrent inhibition provides a negative feed-back whereby motoneuronal discharge causes inhibition that is extensively distributed to neighbouring motoneurones of diverse function. There is no evidence that recurrent inhibition extends more than one segment along the cord, and it is strictly ipsilateral.

In the earlier investigations the distribution of recurrent inhibition appeared to have no functional significance in reflex co-ordination and a general suppressor function on motoneuronal excitability was postulated (Renshaw, 1941; Eccles, Fatt & Koketsu, 1954; Holmgren & Merton, 1954; Hammond, Merton & Sutton, 1956). Subsequent investigators have attempted to discern additional functional meanings. Granit *et al.* (1957) found that recurrent inhibition was particularly exerted on tonic alpha motoneurones, which was confirmed by Kuno (1959), and proposed that recurrent inhibition served to stabilize the discharge of tonic motoneurones at low frequencies even during strong gamma excitation of muscle

* Present address: Physiology Department, University New Buildings, Edinburgh, Scotland.

† On leave from: Department of Physiology, Faculty of Medicine, University of Tokyo, Tokyo.

spindles (Granit & Rutledge, 1960). Brooks & Wilson (1959) also proposed a function related to the gamma-loop activation of motoneurones, but with a more general distribution to motoneurones of all types. When gamma motoneurones activate muscle spindles of a muscle, they evoke the discharge of Group Ia afferent impulses, which monosynaptically excite not only the motoneurones of that muscle, an homonymous action, but also the motoneurones of synergic muscles, and even of apparently unrelated muscles (Eccles, Eccles & Lundberg, 1957*a*; Eccles & Lundberg, 1958). These latter types of activation are usually weaker, and hence are more readily suppressed by the recurrent inhibition driven by the discharging motoneurones. Thus recurrent inhibition would tend to confine the operation of the gamma-loop mechanism to the alpha motoneurones belonging to the muscle containing the discharging annulo-spiral endings (cf. Granit & Rutledge, 1960), and hence subserve finesse of movement. Finally, Wilson, Talbot & Diecke (1960) investigated the distribution not only of recurrent inhibition, but also of the recurrent facilitation that was originally reported by Renshaw (1941) and later overlooked because it was depressed in anaesthetized preparations (Wilson, 1959; Wilson & Talbot, 1960). On the basis of this additional information they proposed that, besides the inhibitory stabilization of level of excitation of motoneurones and the sharpening of effectiveness of monosynaptic excitation, the recurrent facilitation serves to enhance the level of excitation of flexor motoneurones, which otherwise would be dominated by the more powerfully activated extensors.

The present investigation has been undertaken in relation to these various postulated functions of recurrent inhibition, which are tested by a more extensive survey of the distribution of recurrent inhibition than has hitherto been attempted. Altogether over 400 motoneurones belonging to fourteen different muscles of the cat hind limb have been studied intracellularly in order to discover the incidence of recurrent inhibition in response to antidromic volleys in fourteen different muscle nerves. In the original survey there were only thirty-six motoneurones and eight types of antidromic volley (Eccles, Fatt & Koketsu, 1954). The only other intracellular survey was restricted to twenty-eight gastrocnemius-soleus motoneurones and three types of antidromic volley (Kuno, 1959). A preliminary report has been published (Eccles, Iggo & Ito, 1960).

METHODS

The ten cats used were lightly anaesthetized with pentobarbital sodium. The spinal cord was cut at the upper lumbar region (lumbar 1 or 2) and all the ipsilateral dorsal roots from sacral 3 to lumbar 5 were cut. Up to fifteen nerves supplying the muscles of the hind limb were dissected free in the leg and set up for electrical stimulation. All the motor nuclei innervating knee and ankle muscles lie in the lumbar 6 and 7 and sacral 1 segments and

almost all the limb-muscle nerves, including the inferior and superior gluteal nerves, arising from this part of the cord were tested. A full investigation of the Renshaw inhibition for lumbar 5 and 6 segments has not been attempted because of the technical difficulty of working with many of the muscle nerves originating from these segments.

The techniques for intracellular recording from motoneurones have already been published in detail (Eccles, Fatt, Landgren & Winsbury, 1954; Coombs, Eccles & Fatt, 1955*a*). Single micro-electrodes filled with either 0·6 M-K_2SO_4-agar, or occasionally 3 M-KCl, were used. With KCl-filled electrodes the inhibitory potentials were tested at different membrane potential levels to see whether the inhibitory potential was masked by a change in the inhibitory equilibrium potential due to the leakage of Cl^- ions from the electrode (Coombs, Eccles & Fatt, 1955*b*; Eccles, Eccles & Lundberg, 1957*b*). The time constant of the amplifier (500 msec) was sufficiently long for recording the recurrent or Renshaw inhibitory post-synaptic potentials (RIPSPs) without appreciable distortion. Invasion of a motoneurone by an antidromic impulse in its own axon was prevented by passing between the micro-electrode and earth a brief pulse (0·1–1·2 msec) of hyperpolarizing current (see Fuortes, Frank & Becker, 1957; Coombs, Curtis & Eccles, 1957). In this way it was possible to employ a maximum antidromic volley in the homonymous nerve without the complication of an after-hyperpolarization in the motoneurone under observation. While thus avoiding depression of the Renshaw IPSP on account of superposition on the after-hyperpolarization, there was, nevertheless, still some depression because the IPSP was superposed on, and hence occluded by, the hyperpolarization that decayed for many milliseconds after the blocking pulse. By comparing the IPSPs of Fig. 1 *R* and *T*, the occlusion is seen to diminish the Renshaw IPSP to about 60 %. Throughout this paper there has been no correction for this effect, hence all homonymous IPSPs will be underestimated.

Nomenclature. The following abbreviations have been used, especially in figures: AB = anterior biceps; SM = semimembranosus; ST = semitendinosus; PB = posterior biceps; MG = medial gastrocnemius; LG = lateral gastrocnemius; Sol = soleus; Pop = popliteus; Pl = plantaris; FDL = flexor digitorum longus + flexor hallucis longus; Per = peroneal, supplying all three peroneal muscles as well as tibialis anticus, extensor digitorum longus and brevis; SG = superior gluteal; IG = inferior gluteal; T = tibial, supplying flexor digitorum brevis and the small foot muscles; Q = quadriceps; Grac = gracilis.

RESULTS

Post-synaptic potentials generated by recurrent inhibition (RIPSP)

As illustrated in Figs. 1, 2 and 3, maximum antidromic volleys in the motor fibres of many muscle nerves produce RIPSPs in a motoneurone. These diverse RIPSPs vary greatly in size, but have similar time courses: a central latency of rather more than 1 msec; a maximum at about 5 msec later; and a total duration of about 40 msec (cf. Eccles, Fatt & Koketsu, 1954). Since the equilibrium potential for the RIPSP (about -80 mV) is only about -10 mV from the resting potential (Coombs *et al.* 1955*b*), a linear increase in the inhibitory synaptic action will give a progressively diminishing increase in the size of the RIPSP, i.e. there is an interaction, or occlusion, between superimposed RIPSPs, which increases with the sizes of the RIPSPs (Fig. 3). Usually, the RIPSPs generated by single muscle volleys have not been larger than -2 or -3 mV, so there is no serious error in employing the amplitudes of the RIPSPs in Figs. 1 and 2

as measures of the respective inhibitory synaptic actions without making any correction for occlusion.

A total of 474 motoneurones were collected in the present experiments. As judged by the magnitude and instability of the resting and action potentials, many of the motoneurones were damaged by the penetration of the micro-electrode, and consequently were discarded as unreliable when assembling the statistical analyses in Tables 1 and 2. Because the sulphate-filled electrodes tended to be noisy and to block frequently, the resting potential measurements were not considered to be sufficiently reliable to provide an index of the condition of the cell. Instead, the size of the antidromic spike potential has been used and all cells with a spike potential of less than 50 mV were rejected.

In order to compare the total amount of recurrent inhibition which

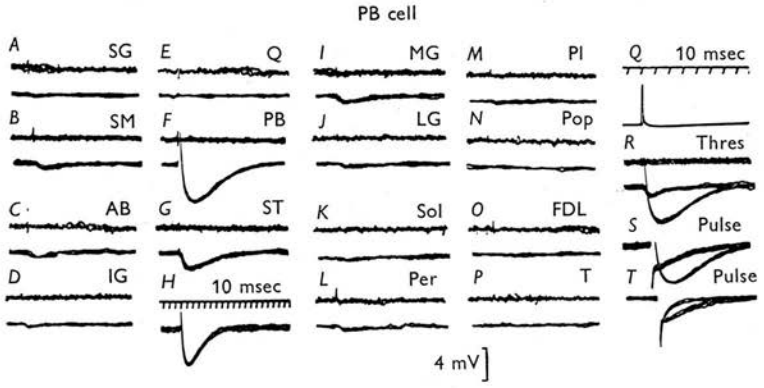


Fig. 1. Intracellular records from a posterior biceps motoneurone to illustrate the recurrent or Renshaw post-synaptic inhibitory potentials (RIPSPs) generated by antidromic volleys from different muscle nerves. Lower traces of *A–P* give the responses evoked by maximum antidromic volleys from the various muscle nerves as indicated by the symbols, which may be identified by reference to the paragraph, under Methods, labelled 'nomenclature'. Downward deflexion signals increasing negativity of the micro-electrode tip, i.e. hyperpolarization, *H* shows PB response at a slower sweep speed, all other records being at speed given in time scale of *Q*. Upper traces were taken from the dorsal surface of the cord. In *R* the RIPSP from PB was sometimes recorded since the stimulus was at the threshold of the axon itself. When the axon was activated the RIPSP was obscured by the larger after-hyperpolarization. In *S* a rectangular pulse was applied in about 50 % of the records, so preventing the invasion of the cell. In *T* the effect of such a pulse alone (upper record) is compared to the pulse plus the posterior biceps volley. The difference between them indicates the size of the autogenous RIPSP. Spike evoked by the PB volley was 89 mV (*Q*); conduction velocity of the axon was 70 m/sec; duration of after-hyperpolarization (AHP) was 100 msec (*H*); resting potential (RP) was −67 to −62 mV. Micro-electrode was filled with 0·6M-K_2SO_4 plus 1 % agar. Same potential scale for records except *Q*. Records in this and subsequent figures consist of superposed sweeps.

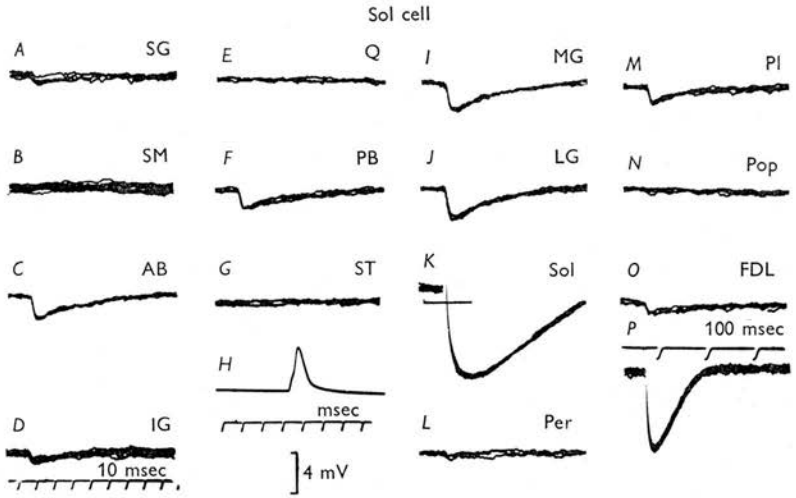


Fig. 2. Intracellular records as in Fig. 1, but from a soleus motoneurone in order to show the wide field of neurones from which it receives Renshaw inhibition. The size of the autogenous RIPSP is indicated by a line on the soleus record (*K*), where the large after-hyperpolarization follows the antidromic spike potential. This cell had a resting potential of −60 to −63 mV; the spike potential was 80 mV (*H*); the duration of the after-hyperpolarization was 140 msec (*P*); and the conduction velocity of its axon was 67 m/sec. Same potential scale for records except *H*, and time scale of *D* for all except *H* and *P*.

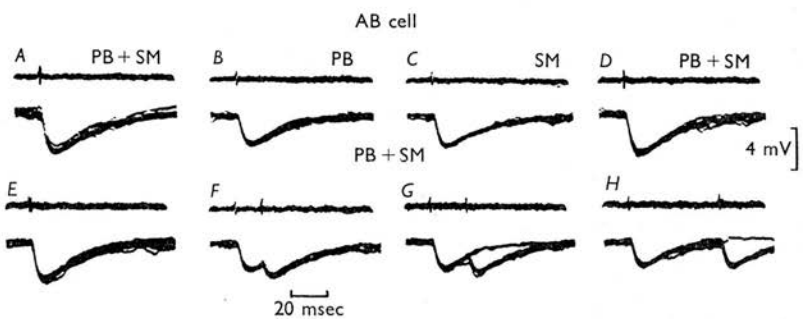


Fig. 3. These records from an anterior biceps motoneurone illustrate the RIPSPs produced by interaction of antidromic volleys from two different muscle nerves. In each record the upper trace is from a lead on the surface of the cord and the lower is the intracellular record. The RIPSPs were generated by maximal volleys in the alpha motor axons in posterior biceps (PB) and semimembranosus (SM) alone and together as indicated by the symbols; with synchronized volleys in *A*, *D* and *E* and with SM following PB at various intervals in *F*–*H*. At 50 msec there was almost no sign of the occlusion which was so evident in *E*–*G*. Antidromic spike (not shown) was 90 mV; autogenous RIPSP by pulse method was 3·2 mV; resting potential, −62 mV.

could be generated in different motoneurones, the peak amplitudes of the RIPSPs produced in an individual motoneurone by maximum antidromic volleys from each muscle nerve alone have been added together to give a cumulative figure (the sum of the autogenous and heterogeneous in Table 1), which was as large as 22 mV in one Sol motoneurone. These aggregate values give a useful basis for comparison between different species of motoneurones, but it is evident that synchronous volleys in all the muscle nerves could not generate such a large RIPSP which would be

TABLE 1. The mean values for the action potentials, conduction velocities, duration of after-hyperpolarizations and Renshaw IPSPs for the motoneurones of the hind limb. The nuclei are arranged in descending order of duration of after-hyperpolarization. The RIPSP is entered under autogenous RIPSP, i.e. that generated by volleys in the motoneurone's own muscle nerves and heterogeneous RIPSP, i.e. the sum of the individual RIPSPs generated by volleys in all the other muscle nerves. The standard error of each mean is given, with the number of cells tested in parentheses

Motoneuronal nucleus	Action potential (mV)	Conduction velocity (m/sec)	After-hyperpolarization: Duration (msec)	After-hyperpolarization: % of cells longer than 110 msec	Renshaw IPSP (autogenous) (mV)	Sum of all Renshaw IPSPs (heterogeneous) (mV)
Soleus	67 ± 3·2 (11)	64 ± 1·8 (12)	186 ± 3·5 (9)	100	1·46 ± 0·22 (7)	12·8 ± 1·6 (11)
FLD + FHL	66 ± 3 (15)	75 ± 2·1 (14)	118 ± 5·6 (13)	54	0·5 ± 0·25 (13)	3·4 ± 0·95 (16)
Plantaris	67 ± 2·3 (23)	75 ± 2·2 (15)	110 ± 4·9 (22)	45	1·16 + 0·36 (9)	3·5 ± 0·65 (23)
Semimembranosus	72 ± 2·1 (10)	75 ± 5 (6)	110 ± 4 (10)	40	2·47 ± 0·12 (6)	6·6 ± 1·55 (6)
Anterior biceps	64 ± 1·8 (29)	76 ± 1·7 (31)	109 ± 3·9 (27)	37	2·06 ± 0·28 (17)	5·3 ± 0·36 (29)
Lateral gastrocnemius	66 ± 2·3 (24)	79 ± 2·1 (16)	105 ± 4·3 (19)	31	1·32 ± 0·11 (16)	4·1 ± 0·56 (24)
Medial gastrocnemius	62 ± 2·3 (32)	73 ± 1·1 (21)	103 ± 4·1 (25)	40	0·93 ± 0·2 (19)	2·9 ± 0·5 (30)
Gluteal	68 ± 2·3 (19)	—	103 ± 2·9 (18)	21	1·52 ± 0·2 (12)	2·9 ± 0·45 (19)
Posterior biceps	64 ± 1·6 (43)	79 ± 0·4 (46)	99 ± 2·5 (39)	10	1·52 ± 0·34 (29)	3·1 ± 0·49 (44)
Popliteus	69 ± 3·4 (7)	80 ± 2·9 (9)	98 ± 5 (6)	16	1·37 ± 0·25 (4)	3·8 ± 0·32 (7)
Peroneal	65 ± 2·1 (18)	85 ± 7·3 (4)	95 ± 2·5 (11)	18	0·45 ± 0·23 (8)	1·4 ± 0·42 (18)
Semitendinosus	66 ± 2·9 (13)	78 ± 0·5 (15)	89 ± 4·0 (9)	0	1·5 ± 0·25 (12)	1·9 ± 0·49 (13)

far beyond the potential required to reach the equilibrium potential for the IPSP. Not only would there be occlusion between the RIPSPs generated by the different muscle volleys, but a further occlusive influence would occur at the level of the Renshaw cells themselves, because there is a very effective convergence of the axon collaterals from the motor fibres to many different muscles on to the same Renshaw cell (Eccles, Fatt & Koketsu, 1954; Eccles *et al.* 1961).

With a few very sigificant exceptions the largest RIPSP was generated by an antidromic volley in the muscle nerve to which the impaled motoneurone belonged (Table 2, Fig. 9). The measurement of this autogenous RIPSP required that antidromic invasion of the impaled cell should be blocked by means of a short hyperpolarizing pulse through the recording

electrode as described in Methods. This autogenous RIPSP was largest with semimembranosus and AB motoneurones (Table 1). Exceptions to the rule were soleus and flexor longus digitorum motoneurones (Figs. 2, 9, 10; Table 2), where the autogenous RIPSP was smaller. The small number of alpha motor fibres in the soleus nerve presumably accounts for the poverty of its autogenous RIPSP.

Factors governing the intensity and pattern of the RIPSP received by a motoneurone

Type of motoneurone. Measurements of axonal conduction velocity and duration of after-hyperpolarization have been made on 146 alpha motoneurones. After pooling these motoneurones into eight categories according to conduction velocity, it is found that the mean durations of the after-hyperpolarization for each velocity category lie along the curve shown in Fig. 4. Evidently, those axons with conduction velocities of less than 70 m/sec come from motoneurones with relatively long after-hyperpolarizations. This confirmation of earlier investigations (Eccles, Eccles & Lundberg, 1958; Kuno, 1959) provides further support for the assumption that cells with long after-hyperpolarizations are tonic alpha motoneurones. If an arbitrary figure of 110 msec is taken to be the lower limit for the after-hyperpolarizations that are classed as belonging to tonic motoneurones, then the Sol sample in Table 1 exclusively comprised tonic motoneurones, whereas at the lower end of the scale ST was exclusively phasic. However, such a rigid criterion for tonic and phasic classification must be regarded as of doubtful validity.

Motoneurones with long after-hyperpolarizations always received a larger total RIPSP than motoneurones with brief after-hyperpolarizations, though the receptive field may be just as extensive. For example, a comparison of Figs. 1 and 2 shows typically that a soleus motoneurone received a larger aggregate RIPSP from much the same receptive field as for the PB motoneurone. Both the aggregate RIPSP and the duration of the after-hyperpolarization have been determined for 210 motoneurones. After pooling these motoneurones into eleven categories according to the duration of the after-hyperpolarization, the mean aggregate RIPSP was determined for each category and plotted as in Fig. 5. It is seen that there is an approximately linear relationship, the motoneurones with the longest after-hyperpolarizations having the largest aggregate RIPSPs. If an arbitrary figure of -9 mV is taken to indicate a very large aggregate RIPSP, then 75 % of the Sol motoneurones are in the group having very large RIPSPs. The SM comes next with 50 % of its cells in that same group followed by AB with 17 %.

If the assumption that all alpha motoneurones with long after-hyperpolarizations are tonic is correct, the present results indicate that the tonic alpha motoneurones are more strongly inhibited by the recurrent collaterals than are the phasic motoneurones. This conclusion is in agreement with the results of the reflex experiments of Granit *et al.* (1957). There do not, however, appear to be two distinct populations of motoneurones;

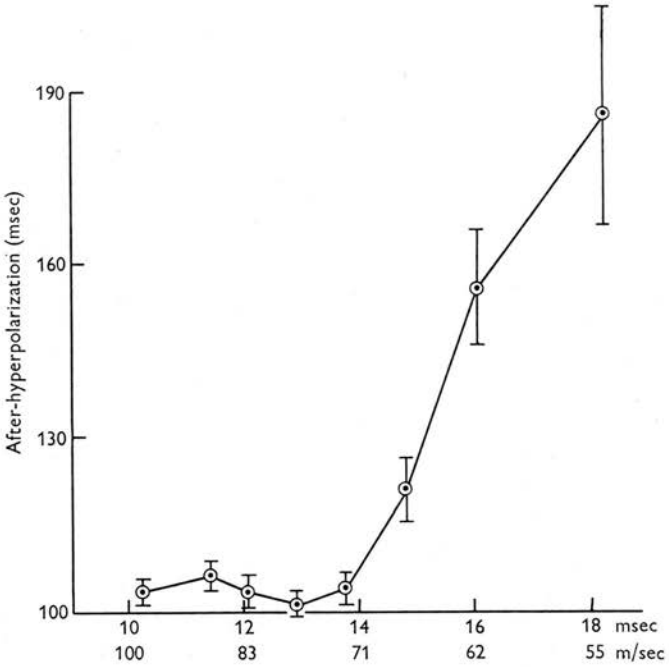


Fig. 4. Relationship between the conduction velocities of the axons and the durations of the after-hyperpolarization from 227 motoneurones. The times to travel 1 m are plotted as abcissae in linear scaling and the equivalent conduction velocities are also shown. On correction (assuming a $Q_{10} = 1{\cdot}7$) for the relatively low temperatures of the preparations (usually 34–35° C) the inflexion on the curve occurs at approximately 80 m/sec. The vertical lines indicate the sizes of the standard errors of the means at each point. Further description in text. The population was grouped into classes at intervals of 10 msec on the conduction velocity scale, except at either end where, because of the small sample available, the classes were larger.

all transitions exist both with the conduction velocities (Fig. 4) and with the after-hyperpolarizations (Fig. 5). The gamma motoneurones, on the other hand, do not develop a detectable RIPSP even though their axons are much more slowly conducting than those of the tonic alpha motoneurones (Granit *et al.* 1957; Eccles, Eccles, Iggo & Lundberg, 1960).

Location of the motoneurone. It was reported by Renshaw (1941) and by Eccles, Fatt & Koketsu (1954) that the effectiveness of recurrent inhibi-

tory action from muscle nerves on to a motoneurone was related to the proximity of this motoneurone to the motor nuclei of the various nerves. On the other hand, while recognizing the possible influence of proximity, Wilson *et al.* (1960) stressed particularly that a meaningful functional pattern could be discerned. Before attempting to relate the present results to the concepts developed by Wilson *et al.* three different procedures will be adopted in order to see how far the new evidence conforms to the

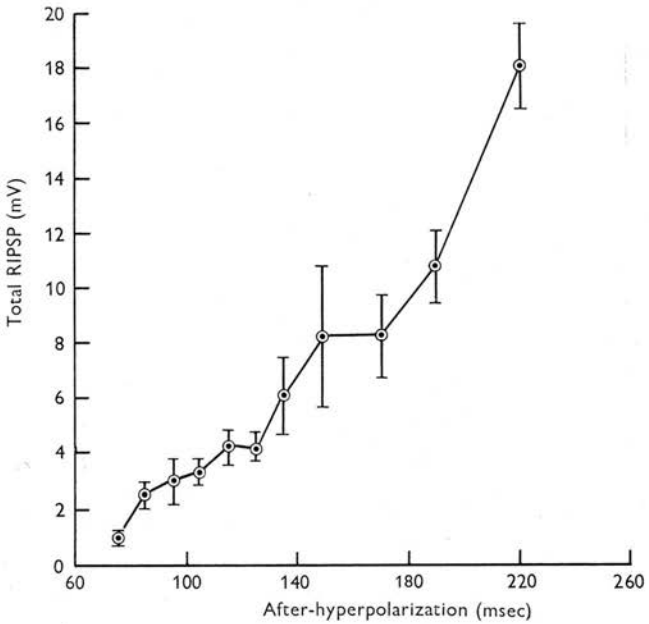


Fig. 5. Illustration of the relationship between after-hyperpolarizations of 210 motoneurones and the sums of all RIPSPs received by them from all the muscle nerves tested. Vertical lines indicate the sizes of the standard errors of the means. Further description in text. The population was arbitrarily grouped into classes at 10 msec intervals along the abscissa, except for the last four classes which were made larger to increase the size of the sample for statistical analysis.

proximity principle. In (i) and (ii) individual cells were tested whilst in (iii) populations of cells were examined.

(i) Figure 6 shows that when several cells in the same nucleus of one animal (AB in this example) were examined at the same segmental level, there was a fairly consistent pattern in the effectiveness of different muscle nerves in generating the RIPSP. The relative potency of these different nerves can be related in part to the proximity of the respective motor nuclei to the AB nucleus. For example, PB, SM and ST motoneurones lie in the same column of cells in the ventral horn and at overlapping segmental levels (Romanes, 1951), while the other potent nerves in Fig. 6

were MG and LG, which have their motoneurones just dorsal to the AB motoneurones, and IG with a just ventral location. These results for a single nucleus sampled at one level of the cord are illustrative of many series in the present investigation and are in general agreement with the proximity hypothesis.

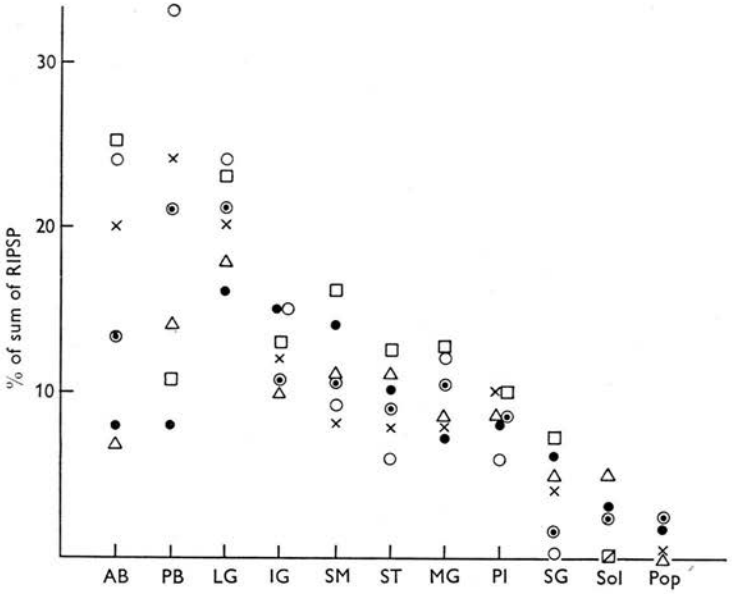


Fig. 6. In six anterior biceps motoneurones indicated by the six different symbols the RIPSPs received from all eleven nerves are plotted as percentages of the sums of all the RIPSPs. These neurones were all in the same region of the anterior biceps nucleus of one cat. The nerves are arranged approximately in descending order of effectiveness.

(ii) In an alternative procedure the RIPSP production has been determined for motoneurones in the same nucleus but at several different segmental levels. For this investigation the PB nucleus is particularly appropriate since it extends through nearly two segments. For example, the RIPSPs produced in PB cells by the most effective three muscle nerves were recorded at three segmental levels several millimetres apart, the mean values being plotted in Fig. 7*A*. The much greater effectiveness of the AB volley at the most rostral level (level I at lower L7) may be correlated with the more rostral location of its motor nucleus. This segmental gradient of effectiveness is even better illustrated in the larger sample of motoneurones plotted in Fig. 7*B*, where the mean RIPSPs recorded from all species of motoneurones were plotted as in Fig. 7*A*. The motor nuclei of PB, MG and IG correspond closely in their caudal extension, which is

several millimetres beyond the AB nucleus, as is indicated in the locations marked in Fig. 9. The segmental gradients of effectiveness of various muscle nerves in generating RIPSPs are thus in good agreement with the segmental locations of their respective motor nuclei.

(iii) There is also a dorso-ventral gradient in the patterns of RIPSP distribution. For example, Fig. 8 is formed by assembling the various motoneurones and their associated motor fibres into four groups that correspond to their dorso-ventral location, the dorso-ventral sequence being: FDL+Pl; MG, LG, Sol; PB, AB. ST; SG. For each group of motoneurones the RIPSPs are plotted as percentages of the aggregate RIPSP.

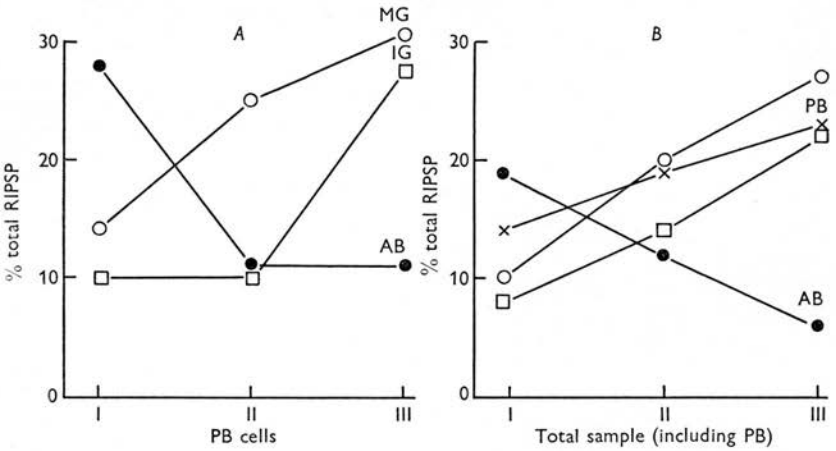


Fig. 7. *A*. In one cat posterior biceps motoneurones were investigated at different segmental levels: (I) in the lower L7 region; (II) 1 mm more caudally; and (III) in the upper S1 region. At each site the means of the RIPSPs from each source (MG, IG and AB) are expressed as percentages of the sum of all RIPSPs received. In *B*, mean RIPSPs in all the motoneurones in that same cat (including the posterior biceps that are alone plotted in *A*) are plotted for the three regions in the same way as in *A*, but RIPSPs produced by PB volleys are added. Crosses refer to PB volleys, otherwise symbols as in Fig. 7*A*.

There is seen to be a general tendency for the more ventrally located motoneurones to develop large RIPSPs in response to antidromic volleys to the corresponding motor nuclei, and in particular FDL and Pl volleys were quite ineffective on the two most ventral groups. Likewise the two most dorsal groups of motoneurones generate very little RIPSP in response to SG volleys. Neither FDL nor Pl, the most dorsal nuclei, give or receive RIPSPs of the same order of magnitude as more ventrally situated nuclei.

In order to compensate as far as possible for the incomplete sampling imposed by the experimental conditions, the results for all the experiments were pooled and the input pattern for each muscle nucleus was calculated.

The average value for the RIPSP from each muscle nerve to the average cell of the particular motoneuronal nucleus was then plotted on a histogram in Fig. 9. The importance of proximity is still evident. The peroneal nucleus at the rostral end of the series is not affected by the volleys in the muscle nerves of the more caudally located nuclei; reciprocally the motoneurones of the three most caudal nuclei (PB, MG and Sol) develop little if any RIPSP in response to volleys to the more rostrally located nuclei.

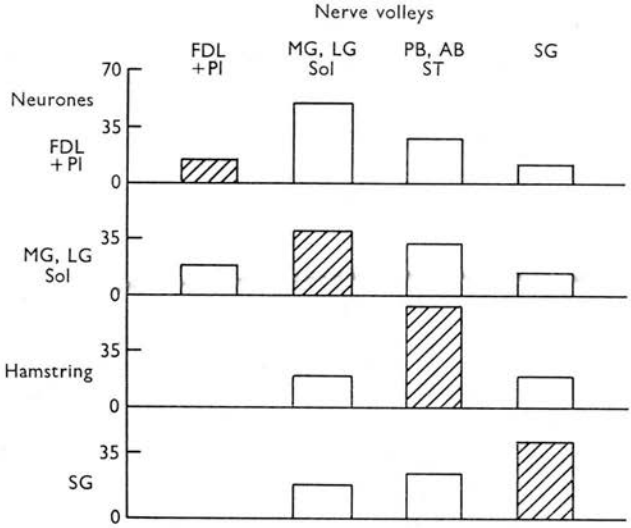


Fig. 8. The amounts of the RIPSPs from different sources are plotted as percentages of the sums of all the RIPSPs. The motor nuclei are arranged in horizontal rows (in relation to their dorso-ventral position): the most dorsal nuclei on the uppermost line; the most ventral on the lowermost. The corresponding nerve volleys are arranged in the vertical columns as indicated by the symbols. The height of each block is a measure of the sum of the RIPSPs which are produced by the antidromic volleys of its column, this sum being expressed as a percentage of the total RIPSPs of the motoneurones belonging to that row. With the hatched areas the RIPSP was produced in motoneurones by volleys of the same group.

The RIPSPs produced in Sol motoneurones provide an illustration of the patterns displayed in Fig. 9. The largest RIPSPs are produced by Pl, LG, AB, PB, MG volleys. The respective nuclei are either intermingled with Sol (LG and MG) or lie immediately dorsal (Pl) or ventral (AB, PB). The least effective volleys belong to nuclei more rostrally located (SM, SG, FDL, Per). An exceptional position is, however, occupied by ST which produces a very small RIPSP despite the proximity of the ST and Sol motor nuclei.

In order to display the pattern of distribution, the mean RIPSPs have been calculated for each type of antidromic volley acting on each type of

motoneurone. In Table 2 the volleys are arranged in columns and the motoneurones in rows. The antidromic volleys which were least effective were Per and FDL, which belong to the nuclei situated most dorso-laterally, and hence furthest from the location of most of the Renshaw cells (Eccles, Fatt & Koketsu, 1954). These motoneurones were also amongst the lowest recipients of RIPSP. Thus it seems that promixity

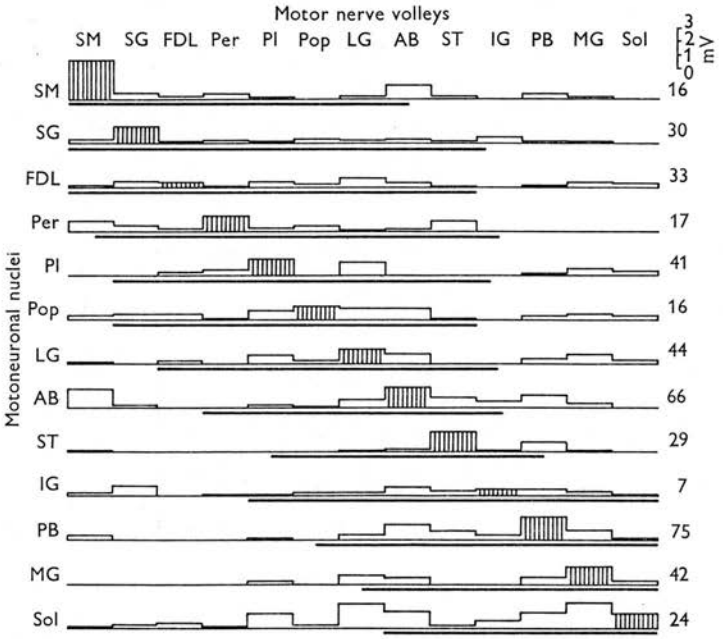


Fig. 9. The mean amounts of the RIPSPs received by all types of motoneurones are plotted for each type of antidromic volley in horizontal rows as indicated by the symbols. The motoneurones are arranged in vertical sequence on the left, the descending order representing relative rostro-caudal position. The RIPSPs produced by the various nerve volleys are arranged in vertical columns as indicated by the symbols above. Numbers to the right indicate the number of motoneurones in each category; the voltage scale applies to all potentials. The approximate longitudinal extent of each nucleus is indicated by the thick lines under each entry.

of the motoneuronal nuclei to the location of the Renshaw cells is an important factor in determining the size of the RIPSP. The poverty of action of Per volleys is presumably related to the extreme lateral course of their axons in the spinal cord (Balthasar, 1952), the motor axon collaterals consequently having a particularly long developmental path in order to reach the Renshaw cells.

Functional relationship of the motoneurones. In Table 2 the mean RIPSPs evoked by antidromic volleys in 387 motoneurones producing

extension and flexion at the various limb joints are tabulated with respect to functional relationship. Unfortunately the Table is incomplete: there were no hip flexors and the number of knee extensor (Q) motoneurones was so small that their RIPSPs were not included in the Table. The Q antidromic volleys were tested on all motoneurones, but uniformly had no action.

TABLE 2. The relation between functional groups of motoneuronal nuclei and the size of the RIPSPs generated in them by antidromic volleys in muscle nerves. The motoneurones producing extension and those producing flexion at hind-limb joints have been grouped and arranged vertically, while the muscle nerves stimulated have been arranged horizontally in the same order. Synergic groups are in bold figures and antagonists are in italic figures

		Nerve volleys										
		Extensors							Flexors			
		Hip		Ankle			Digits		Knee		Ankle	
Motoneurones	No.	AB (mV)	SM (mV)	MG (mV)	LG (mV)	Sol (mV)	Pl (mV)	FDL (mV)	PB (mV)	ST (mV)	Per (mV)	Total (mV)
AB	66	**1·9**	**1·4**	0·42	0·66	0	0·22	0	0·96	0·82	0	6·38
SM	16	**1·07**	**3·0**	0·2	0·2	0	0·1	0·2	0·5	0·2	**0·4**	5·87
MG	42	0·55	0	**1·3**	**0·68**	**0·23**	0·23	0	0·52	0	*0*	3·51
LG	44	0·84	0·12	**0·74**	**1·2**	**9·3**	0·71	0·2	0·4	0	*0*	4·51
Sol	24	1·3	0·1	**2·0**	**1·9**	**1·1**	1·1	0·37	1·2	0·22	*0·1*	9·39
Pl	41	0	0	0·52	1·0	0·3	**1·2**	**0·24**	0·23	0	*0·41*	3·9
FDL	33	0·37	0·1	0·44	0·7	0·32	**0·5**	**0·4**	0·16	0·12	*0·1*	3·21
PB	75	1·15	0·4	0·78	0·48	0·1	0·15	0	**1·7**	**0·6**	0	5·36
ST	29	0·20	0·12	0·1	0·1	0	0	0	**0·75**	**1·5**	0	2·77
Per	17	0·2	0·7	*0*	*0·1*	*0*	*0·2*	*0·2*	0	0·7	1·1	3·20
Totals		7·58	5·94	6·50	7·02	2·35	4·41	1·61	6·42	4·16	2·11	—

RIPSPs are seen to be particularly large within synergic groups, which are in bold figures in the Table. For example, with the synergic group of hip extensors the AB and SM volleys are particularly powerful on AB and SM motoneurones. Other examples are the ankle extensors (Sol, MG and LG), and the knee flexors (PB and ST). Evidently the large autogenous RIPSPs are just special examples of the large RIPSPs regularly occurring in the interaction between members of synergic groups. In all these examples the large RIPSPs could be attributable to proximity of the respective motoneuronal nuclei; there may be no significance in the functional relationship.

The RIPSPs produced in motoneurones by antidromic volleys from antagonist muscles at the same joint are shown in italic figures. These RIPSPs are always very small and are sometimes absent. It can be stated that RIPSPs between antagonist motoneurones are negligible. Again, however, this may not depend on their opposed functional relationship. In many cases there is a considerable distance between the antagonist motoneuronal nuclei. An exception would be the FDL, Pl and Per motor nuclei; the latter normally lies just lateral to the former two throughout almost the whole L7 segment.

Apart from the synergists at a particular joint, there is a tendency for the largest RIPSPs to be evoked by antidromic volleys from extensors on to extensor motoneurones. However, this relates to the most effective antidromic volleys, AB, MG and LG, rather than to all extensor antidromic volleys, and AB, MG and LG volleys are also effective on flexor motoneurones. Another relatively powerful antidromic volley is from the knee flexor, PB. On the other hand the knee extensor, Q, has little or no action on the extensors at other joints (Figs. 2, 10), an exception that is presumably attributable to the extensive segmental separation between Q motor nucleus in L5 and upper L6 segments, and the nuclei of all the other extensor muscles (in Table 2). Another general statement is that, apart from synergists, flexor antidromic volleys produce little or no RIPSP in flexor motoneurones.

Though there is much evidence that proximity of motoneuronal nuclei has an important influence on the size of the RIPSP, there is also evidence that other factors must be envisaged. For example, in Table 2 ST volleys are very poor at producing RIPSPs in Sol and G motoneurones. Reciprocally Sol and G antidromic volleys are also very poor in producing RIPSPs in ST motoneurones, yet the respective nuclei are in close apposition in the lower L7 and upper S1 segments, the ST being just ventral to the G and Sol motoneurones.

Excitatory post-synaptic potentials generated by antidromic volleys

In both anaesthetized and decerebrate cats, Renshaw (1941) regularly found a facilitation of monosynaptic reflex discharge when the conditioning antidromic volley entered the cord at a different segmental level from the tested muscle nucleus. Since Wilson (1959) has shown that the time course, as measured by changes in the size of the monosynaptic reflex, is longer for recurrent facilitation than for the inhibition, recurrent facilitation might be expected to be evident as a later depolarization. The latency of the facilitation was about 1 msec longer than for the inhibition, which Wilson (1959) suggested was due to at least one more interneurone in the facilitatory path.

Depolarization was never large in the present experiments though occasionally a small late depolarization was recorded (Fig. 10*K*). In order to see if a depolarization was being concealed by a larger hyperpolarization, the membrane was shifted to the equilibrium potential for the RIPSP (approximately -80 mV) by passing a steady current through the intracellular electrode and across the cell membrane, but no additional recurrent excitatory post-synaptic potentials (REPSPs) were revealed by this procedure. Except for one unusually large REPSP of 0·4 mV, all REPSPs were 0·1 mV or less. The total number of times that such a trace

as that of Fig. 10*K* was detected was 32 in a total of about 6000 trials. On 14 of these occasions a flexor motoneurone was depolarized, a peroneal motoneurone being involved ten times; which corresponds to the findings of Wilson, Talbot & Diecke (1960). The next most frequent examples were the extensor motoneurones, AB (8) and FDL (6).

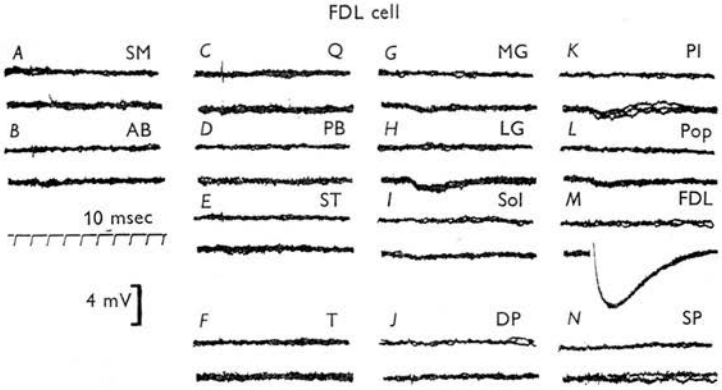


Fig. 10. The RIPSPs from all sources are shown for an FDL motoneurone as in Fig. 1. Note the small REPSP from plantaris (*K*). The antidromic spike was 58 mV; duration of the after-hyperpolarization was 110 msec and the resting potential was −54 mV.

DISCUSSION

In attempting to correlate the present results on the distribution of the RIPSP with the distributions reported by Wilson, Talbot & Diecke (1960), account must be taken of several differences between the two investigations. Recurrent facilitations were far more prominent in the investigation of Wilson *et al.* than were REPSPs in the present investigation, a difference readily explicable by the selective depression exerted by barbiturates on recurrent facilitation (Wilson & Talbot, 1960), although Renshaw (1946) observed large facilitations in cats anaesthetized with Nembutal. However, the present investigation has the advantage that detailed quantitative information is provided for many hundreds of motoneurones. Furthermore, the autogenous RIPSP was regularly measured, whereas autogenous recurrent inhibition was not investigated by Wilson, Talbot & Diecke (1960). Thus, the present investigation is much more comprehensive in respect of recurrent inhibition, but makes virtually no contribution on recurrent facilitation. It may be noted that there is on the whole very good agreement between Table 1 and the preliminary results of Eccles, Fatt & Koktesu (1954, Table 1) with relatively few motoneurones. The low values there reported for most autogenous RIPSPs arise because

in many cases they were submaximal, the stimuli being just below the threshold of the motoneurone's axon.

There is a very satisfactory degree of agreement between the present results on the distribution of RIPSP and those of Wilson, Talbot & Diecke (1960) on recurrent inhibition, particularly if allowance is made for the occasions when recurrent inhibition was masked by recurrent facilitation. For example, in agreement with Wilson, Talbot & Diecke (1960), there is in Table 2 virtually no RIPSP between the ankle extensors and flexors in either direction (G and Sol on the one hand and Per on the other); but there is a considerable RIPSP within each synergic group. There is similar agreement between the two series of investigations on the motoneurones responsible for extension and flexion of the digits, where likewise there is a negligible RIPSP between antagonistic motoneurones, which contrasts with the fairly large RIPSPs within each synergic group. Finally, there is agreement on additional interactions that occur between flexor and extensor motoneurones independently of functional grouping; G + Sol volleys produce an RIPSP in PB and ST motoneurones, which in Table 2 is seen also to be reciprocal; PB and ST volleys produce an RIPSP in AB motoneurones, which also is reciprocal in Table 2, at least for PB; finally Wilson, Talbot & Diecke (1960) reported that SM inhibits and AB facilitates Per motoneurones, though in Table 2 both actions were weakly inhibitory.

These last examples were reported by Wilson, Talbot & Diecke (1960) as being exceptions to their postulated general pattern of distribution of recurrent inhibition and facilitation. According to this pattern antidromic volleys from extensor muscles inhibit extensor motoneurones and facilitate flexors, while flexor antidromic volleys inhibit flexors and have little or no action on extensors. But in Table 2 there are several instances in which extensor volleys produce quite large RIPSPs in flexor motoneurones (AB → PG; G → BP; SM → Per); and similarly with flexors to extensors (PB → AB; ST → AB; PB → Sol). In fact, if the synergic groups be deleted from Table 2, there is on the average little more RIPSP from extensors to extensors (mean −0·35 mV) than from extensors to flexors (mean −0·28 mV) or flexors to extensors (mean −0·30 mV). Possibly, Wilson, Talbot & Diecke (1960) underestimated such types of recurrent inhibition because they were masked by recurrent facilitation. It can be concluded that the results documented in Table 2 provide many examples of distribution of RIPSP that do not conform with the postulates of Wilson, Talbot & Diecke (1960). In Table 2 there is no support for their proposal that the RIPSP has a distribution corresponding to that of Ib inhibition, which is very largely restricted to extensor motoneurones (Eccles *et al.* 1957*b*).

On the other hand, in Table 2 there is much support for the postulate that the distribution of RIPSP is related to the proximity of motoneuronal nuclei regardless of function. For example, AB and PB motoneurones are in the same neuronal column and have a considerable segmental overlap, but they have quite different functions, hip extension and knee flexion respectively. Yet in Table 2 there is a remarkable parallelism between the two motoneuronal types in respect both of action of the antidromic volleys, and of generation of RIPSPs by the motoneurones. However, there are several exceptions to the simple proximity hypothesis that was proposed by Eccles, Fatt & Koketsu (1954): ST motoneurones lie in a column just ventral to G and Sol motoneurones, and are at the same segmental level, yet there is virtually no interaction in either direction; the very poor interaction between the contiguous Per and FDL cell columns is another example. This latter case may arise on account of the lateral trajectory of the Per motor axons, as mentioned above, but the former case seems to require some functional discriminatory factor between flexor and extensor motoneurones in respect of RIPSP connexions.

The patterns of Renshaw cell connexions give an opportunity for investigating problems relating to the manner in which inhibitory connexions are established in development and to the possibility of changing the connexions in response to altered motoneurone function consequent on cross-union. Certainly the patterns are much less discriminative than with the Ia inhibitory action, which very largely operates between antagonists at a joint (Laporte & Lloyd, 1952; Hunt & Perl, 1960). Yet mere random growth and connexion seems inadequate to account for some of the observed specification of connexion or lack of connexion. Undoubtedly, a major factor governing the pattern of distribution is that the linkage by recurrent inhibition can be established only over short distances, regardless of the functional significance of the connexion if it could be established. The absence of inhibitory interconnexion between the motoneurones of knee flexors and extensors has been mentioned above. Instead there is strong recurrent facilitation in both directions (Wilson, Talbot & Diecke, 1960). This recurrent facilitation can operate over longer distances, which is presumably attributable to the one or more additional interneurones in the pathway (see Wilson, Talbot & Diecke, 1960; Wilson, Diecke & Talbot, 1960).

The present results agree with those of Kuno (1959) in fully confirming the important postulate of Granit *et al.* (1957) and Granit & Rutledge (1960) that tonic alpha motoneurones receive much more recurrent inhibition than phasic alpha motoneurones (cf. Holmgren & Merton, 1954). The tonic motoneurones were identified by the long duration of their after-hyperpolarization (Eccles *et al.* 1958; Kuno, 1959) and it was shown (Fig. 5)

that there was almost a linear relationship between the duration of after-hyperpolarization and the amount of RIPSP that a motoneurone received. On the basis of these two criteria it must be concluded that there are not two discrete categories of fast and slow motoneurones, but that all transitions exist (cf. Kuno, 1959). The specially large RIPSPs of motoneurones with long after-hyperpolarizations are further evidence that some specific factors control the development of recurrent inhibitory pathways in addition to mere proximity.

The general functional significance of recurrent inhibition is still an open question. Perhaps, as suggested by Wilson, Talbot & Diecke (1960), there are several functions. (i) There can be no doubt that the very wide distribution cutting across all functional classification must give recurrent inhibition a general suppressor action on motoneurones of diverse type, such as was originally suggested by Eccles, Fatt & Koketsu (1954). (ii) The high level of RIPSP distribution to tonic motoneurones would also act to stabilize the frequency of discharge during the maintenance of postures as proposed by Granit *et al.* (1957). (iii) Recurrent inhibition certainly would sharpen the operation of the gamma-loop activation of muscle as implied by Brooks & Wilson (1959); but it has yet to be demonstrated that such an action is functionally important in enhancing the precision of movement. (iv) Wilson, Talbot & Diecke (1960) incorporate recurrent facilitation in developing their concept that recurrent actions mediated through Renshaw cells tend to heighten the excitation of flexor motoneurones and depress the extensors, so helping to maintain a balance which otherwise would be weighted in favour of the more powerfully excited extensors. (v) Since tonic alpha motoneurones are special targets for recurrent inhibition, the intensive motoneuronal discharge subserving rapid movements would inhibit specifically the tonic motoneurones. This action would be functionally desirable, else the slowly contracting and relaxing muscles would impede the rapid movements. Thus recurrent inhibition would have the important function of suppressing all discharges from tonic motoneurones during the rapid movements of running or jumping. The desirability of this suppression was pointed out by Denny-Brown (1928) in his pioneer investigations on fast and slow muscles, and he observed suppression of discharges to soleus under such conditions.

There is experimental evidence for all these proposed actions of the recurrent pathways through Renshaw cells. It remains for further investigation to determine their relative importance in the control of posture and movement.

SUMMARY

1. The technique of intracellular recording has been employed in investigating the recurrent or Renshaw inhibitory post-synaptic potentials (RIPSPs) that are produced by a wide variety of antidromic motor volleys in the motoneurones of the seventh lumbar and first sacral regions of the cat spinal cord.

2. The total amount of the recurrent inhibition was measured as the aggregate of all the RIPSPs received by motoneurones of each type. It was much larger for motoneurones with long after-hyperpolarization, particularly for soleus motoneurones. For the whole population of motoneurones there was a significant relationship between the duration of the after-hyperpolarization and the size of the aggregate RIPSP.

3. The position of any motoneurone in the cord, whether in the rostral-caudal or in the dorsal-ventral dimensions, was related to the size and origin of the RIPSPs that it received. In general the closer the proximity of the motoneuronal nuclei the larger the size of the RIPSP that an antidromic volley in the axons of one produced in the motoneurones of the other.

4. Antidromic volleys in the nerves to flexor and extensor muscles exhibited approximately the same effectiveness in generating RIPSPs. Extensor motoneurones, however, received a larger aggregate RIPSP than did flexor motoneurones.

5. Occasionally an antidromic volley produced a small excitatory post-synaptic potential (the recurrent or Renshaw EPSP).

6. There is a general discussion of the various suggestions that have been made regarding the functional significance of the Renshaw or recurrent inhibition.

REFERENCES

BALTHASAR, K. (1952). Morphologie der spinalen Tibialis—und Peronaeus-Kerne bei der Katze: Topographie, Architektonik, Axon- und Dendritenverlauf der Motoneurone und Zwischenneurone in den Segmenten L_6–S_2. *Arch. Psychiat. Nervenkr.* **188**, 345–378.

BROOKS, V. B. & WILSON, V. J. (1959). Recurrent inhibition in the cat's spinal cord. *J. Physiol.* **146**, 380–391.

COOMBS, J. S., CURTIS, D. R. & ECCLES, J. C. (1957). The interpretation of spike potentials of motoneurones. *J. Physiol.* **139**, 198–231.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955*a*). The electrical properties of the motoneurone membrane. *J. Physiol.* **130**, 291–325.

COOMBS, J. S., ECCLES, J. C. & FATT, P. (1955*b*). The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *J. Physiol.* **130**, 326–373.

DENNY-BROWN, D. (1928). *On the Essential Mechanism of Mammalian Posture.* D.Phil. Thesis, University of Oxford.

ECCLES, J. C., ECCLES, R. M., IGGO, A. & LUNDBERG, A. (1960). Electrophysiological studies on gamma motoneurones. *Acta physiol. scand.* **50**, 32–40.

ECCLES, J. C., ECCLES, R. M., IGGO, A. & LUNDBERG, A. (1961). Electrophysiological investigations on Renshaw cells. *J. Physiol.* **159**, 461–478.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1957*a*). The convergence of monosynaptic excitatory afferents on to many different species of alpha motoneurones. *J. Physiol.* **137**, 22–50.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1957*b*). Synaptic actions on motoneurones caused by impulses in Golgi tendon organ afferents. *J. Physiol.* **138**, 227–252.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1958). The action potentials of the alpha motoneurones supplying fast and slow muscles. *J. Physiol.* **142**, 275–291.

ECCLES, J. C., FATT, P. & KOKETSU, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor-axon collaterals to motoneurones. *J. Physiol.* **126**, 524–562.

ECCLES, J. C., FATT, P., LANDGREN, S. & WINSBURY, G. J. (1954). Spinal cord potentials generated by volleys in the large muscle afferents. *J. Physiol.* **125**, 590–606.

ECCLES, R. M., IGGO, A. & ITO, M. (1960). The distribution of recurrent inhibition among motoneurones. *J. Physiol.* **153**, 49–50*P*.

ECCLES, R. M. & LUNDBERG, A. (1958). Integrative patterns of Ia synaptic actions on motoneurones of hip and knee muscles. *J. Physiol.* **144**, 271–298.

FRANK, K. & FUORTES, M. G. F. (1956). Unitary activity of spinal interneurones of cats. *J. Physiol.* **131**, 425–435.

FUORTES, M. G. F., FRANK, K. & BECKER, M. C. (1957). Steps in the production of motoneurone spikes. *J. gen. Physiol.* **40**, 735–752.

GRANIT, R., PASCOE, J. E. & STEG, G. (1957). The behaviour of tonic α and γ motoneurones during stimulation of recurrent collaterals. *J. Physiol.* **138**, 381–400.

GRANIT, R. & RUTLEDGE, L. T. (1960). Surplus excitation in reflex action of motoneurones as measured by recurrent inhibition. *J. Physiol.* **154**, 288–307.

HAMMOND, P. H., MERTON, P. A. & SUTTON, G. C. (1956). Nervous gradation of muscular contraction. *Brit. med. Bull.* **12**, 214–218.

HENATSCH, H. D. & SCHULTE, F. J. (1958). Reflexerregung und Eigenhemmung tonischer und phasischer Alpha-Motoneurone während chemischer Dauererregung der Muskelspindeln. *Pflüg. Arch. ges. Physiol.* **268**, 134–147.

HOLMGREN, B. & MERTON, P. A. (1954). Local feedback control of motoneurones. *J. Physiol.* **123**, 47–48*P*.

HUNT, C. C. & PERL, E. R. (1960). Spinal reflex mechanisms concerned with skeletal muscle. *Physiol. Rev.* **40**, 538–579.

KUNO, M. (1959). Excitability following antidromic activation in spinal motoneurones supplying red muscles. *J. Physiol.* **149**, 374–393.

LAPORTE, Y. & LLOYD, D. P. C. (1952). Nature and significance of the reflex connections established by large afferent fibers of muscular origin. *Amer. J. Physiol.* **169**, 609–621.

LLOYD, D. P. C. (1946). Facilitation and inhibition of spinal motoneurones. *J. Neurophysiol.* **9**, 421–438.

LLOYD, D. P. C. (1951). After-currents, after-potentials, excitability, and ventral root electrotonus in spinal motoneurones. *J. gen. Physiol.* **35**, 289–321.

RENSHAW, B. (1941). Influence of discharge of motoneurons upon excitation of neighbouring motoneurones. *J. Neurophysiol.* **4**, 167–183.

RENSHAW, B. (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *J. Neurophysiol.* **9**, 191–204.

ROMANES, G. J. (1951). The motor cell columns of the lumbosacral spinal cord of the cat. *J. comp. Neurol.* **94**, 313–363.

WILSON, V. J. (1959). Recurrent facilitation of spinal reflexes. *J. gen. Physiol.* **42**, 703–713.

WILSON, V. J., DIECKE, F. P. J. & TALBOT, W. H. (1960). Action of tetanus toxin on conditioning of spinal motoneurons. *J. Neurophysiol.* **23**, 659–666.

WILSON, V. J. & TALBOT, W. H. (1960). Recurrent conditioning in the cat spinal cord. Differential effect of meprobamate on recurrent facilitation and inhibition. *J. gen. Physiol.* **43**, 495–502.

WILSON, V. J., TALBOT, W. H. & DIECKE, F. P. J. (1960). Distribution of recurrent facilitation and inhibition in cat spinal cord. *J. Neurophysiol.* **23**, 144–153.

J. Physiol. (1961), **159**, *pp.* 500–506
With 4 *text-figures*
Printed in Great Britain

THE DOUBLE TWITCH OF THE GRACILIS MUSCLE

BY ROSAMOND M. ECCLES AND A. IGGO*

From the Department of Physiology, Australian National University, Canberra, Australia

(*Received* 8 *June* 1961)

An interesting double contraction of the gracilis muscle of the cat, in which the first component had the unusually short contraction time of about 5 msec, was reported by Buller, Eccles & Eccles (1960). This very fast contraction contrasts with the contraction time of the remainder of the muscle, which is about 25 msec and thus equivalent to other fast muscles in the cat hind limb. This very fast initial contraction has no known parallel in other cat limb muscles and is comparable with the contraction time of the extra-ocular muscles. If it was produced by motor units which were independently innervated by alpha motor axons, it would provide a mechanism for making extremely quick movements.

In the present investigation we have prepared single motor units of gracilis muscle by stimulating ventral root filaments to see if there was a separation of gracilis muscle into fast and slowly contracting motor units. No such segregation was found, and the rest of the paper describes an attempt to analyse the mechanism of the double twitch.

METHODS

The animals (four cats) were anaesthetized with pentobarbital sodium. The gracilis muscle was carefully freed from the surrounding connective tissue without interruption of its blood or nerve supply. The remainder of the leg muscles were denervated. Either one thread was tied around its tendon of insertion (Fig. 1 G) or the tendon was divided into four parts (Figs. 2 and 3), each being independently secured to the strain gauge (Statham G 1-8-350 or G 1-80-35). The methods of recording twitches from muscles kept at a controlled temperature have already been described in detail (Buller *et al.* 1960).

Stimuli were applied, via an isolation transformer, to the whole or part of the L 6 ventral root for the gracilis muscle; or to L 7 ventral root for the lateral gastrocnemius muscle.

Single motor units of the gracilis muscle were obtained by teasing apart filaments of L 6 ventral root. It was often possible to dissect the filaments into a small portion containing only one active motor axon, which could then be stimulated.

* Nuffield Royal Society Commonwealth Bursar, on leave from the Physiology Department, University of Edinburgh.

RESULTS

The isometric twitch of the gracilis muscle caused by stimulating electrically the L 6 ventral root is shown in Fig. 1. Two components of the twitch are clearly seen, with the very fast component demarcated from the rest of the contraction by a step on the rising phase. The contraction times were 5–10 msec and 16–20 msec for the two components, and depended on the resting tension (Fig. 1). These twitches of the gracilis are very similar to those reported by Buller *et al.* (1960, Fig. 8). The relative amplitudes of the two components could be changed by altering the resting tension of the muscle. The first component was relatively larger at low tensions (*A* in Fig. 1) and increased more slowly in amplitude than the second component as the resting tension of the muscle was increased.

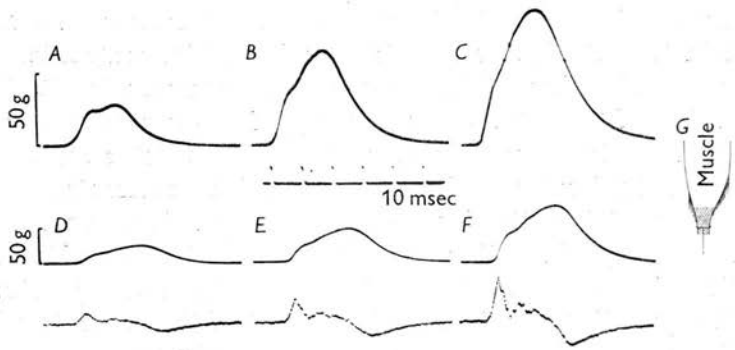


Fig. 1. Isometric twitches of the gracilis muscle are recorded in response to stimulation of L 6 ventral root. *A–C* show the effect of increases in resting tension, the stimuli being maximal for all motor axons. *D–F* show the effect of progressively increased stimulus intensity, the resting tension remaining constant. The lower traces in *D–F* are differentiated records of the twitches which were recorded by one thread tied around the main aponeurosis of the muscle (see diagram). Time scale constant *A–F*. Tension scales for *A–C* and *D–F* respectively are given on the left. *G* illustrates the method of attaching the thread to the tendon.

When the stimulus intensity used to excite the ventral root was varied from threshold response to maximal response of the muscle, the two components of the twitch were present at all intensities (Fig. 1 *D–F*) and the first component was not proportionately larger at the low stimulus intensities. This result shows that the very fast component of the twitch was not due to muscle fibres innervated by alpha motor axons having specially low thresholds, which might be expected by extrapolation from the general relationships for alpha motor axons: the lower the threshold and the faster the conduction of the motor axons, the faster the contraction time of their motor units (Eccles, Eccles & Lundberg, 1958).

Twitches in single motor units of the muscle illustrated in Fig. 1 had contraction times for the peaks of the twitches similar to those for whole-muscle twitches. Each motor unit twitch also had some indication of the very fast component, usually as a convexity on the rising phase of the contraction. Twitch tensions ranged from 1·5 to 7 g (mean 5·7 ± S.E. 0·5 g, $n = 33$). The tetanus:twitch ratio ranged from 2·5 to 16, the lower the twitch tension the greater the ratio. For 8 motor units with twitch tensions less than 4 g the ratio was 9–16, whereas for the 4 motor units with twitch tensions of 10–14 g the ratio was only 2·5–5. The units with low ratios also had twitches with an earlier and more rapid rise of tension.

The above results suggested that the presumed very-rapidly-contracting muscle fibres in the gracilis muscle were not organized separately from the rest of the extrafusal muscle fibres, since, in two cats, none of the 42 single motor units had made only a very fast component in the twitch.

The records in Fig. 1 were obtained by the method of recording adopted by Buller *et al.* (1960). The aponeurosis of the muscle, which is inserted into the medial border of the tibia, was separated from the underlying aponeurosis of the sartorius muscle and the thread tied around it was connected to a strain gauge (Fig. 1). Close examination of the gracilis muscle during dissection showed that the curved ventral margin of the muscle gave rise to a thin sheet of fascia which was inserted into the superficial fascia overlying the gastrocnemius muscle. The two insertions of the gracilis were most obvious when the knee was flexed. In subsequent experiments the main aponeurosis was split longitudinally into three parts and a thread was tied to each. A fourth attachment to the muscle was prepared by tying a thread around the fascia inserted into the gastrocnemius (Figs. 2 and 3). With these four attachments to the muscle it was hoped to distribute more evenly the tension applied to the muscle during recording. All threads could be attached simultaneously or in various combinations, at approximately equal tensions, to the strain gauge.

With the new method of recording, the form of the twitch of the whole muscle depended on the part of the muscle from which the twitch was recorded. The smoothest rising phases, with no indication of a step, were recorded from the middle part of the muscle, *B* and *C* in Fig. 2. The very fast component was most conspicuous at the curved ventral border (Fig. 2*D*), i.e. when recording from the fascia which inserts into the gastrocnemius. There was also a smaller very fast component at the dorsal curved border of the muscle, Fig. 2*A*. The presence of the two parts of the twitch are most clearly revealed by the differentiated twitch records in Fig. 2.

The twitch was almost free from any initial step if the contracting fibres of single motor units were in the dorsal (rostral) half of the muscle; the

contraction was maximal at about 20–25 msec (Fig. 3*A*, *B*). When the active muscle fibres of a single motor unit were in the ventral (caudal) part of the muscle, a pronounced step at 8–10 msec was often present in the twitches (Fig. 3*G*). The contraction of a motor unit in the dorsal part of the muscle usually developed only a very small tension when it was recorded from the opposite edge of the muscle and vice versa; compare *A* and *B* with *C* and *D*, and *G* and *H* with *E* and *F* in Fig. 3. The step in the twitch,

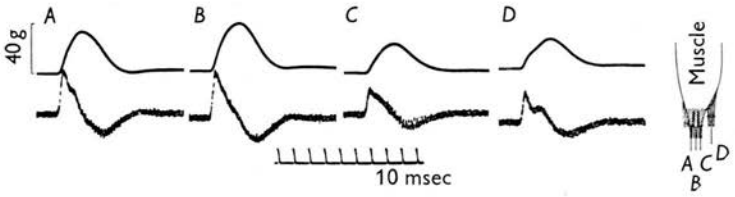


Fig. 2. Isometric twitches of the gracilis muscle recorded on stimulation of part of the L 6 ventral root. The twitches were recorded from each of the four attachments in turn, *A* being the rostral edge of the muscle and *D* the caudal. The diagram illustrates the method of recording from the muscle. Differentiation of the twitches, in the lower records, illustrates the existence of two components at the outer leads.

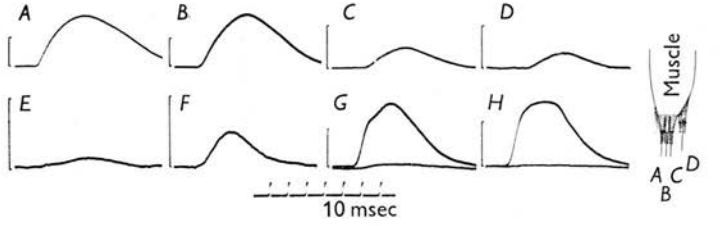


Fig. 3. Isometric twitches in 2 motor units from the gracilis muscle, recorded at each of the 4 leads from the muscle in turn (see diagram). The tension scales all indicate 10 g. The motor unit in *A–D* was at the rostral side of the muscle, and the unit in *E–H* was on the caudal edge. The tetanus:twitch ratios from *A–H* were respectively 5·4, 4·3, 7, 9, 7·4, 6·5, 4·2 and 4·3.

when present, was always most obvious in the part of the muscle which contained the active fibres as determined both by visual observation during the twitch and by the tendinous slip which gave the maximal tension. The maximal tensions for the two motor units in Fig. 3 were 18·5 and 16·1 g and were developed at leads 2 and 3 respectively. Visual inspection confirmed that the active fibres were in the dorsal part of the muscle for the upper unit and in the ventral part of the muscle for the lower unit. Although none of the 9 units was exclusively very-rapidly-contracting, some units had a large first component which was accentuated at low resting tensions.

The very fast component of the gracilis twitch was not, therefore, due to muscle fibres separately innervated by very-rapidly-conducting α motor axons. It was strongly influenced by the resting tension of the muscle and was most conspicuous when the contraction was recorded at the margins of the muscle where there is the greatest difficulty in obtaining uniform tension in the muscle fibres. These results suggested that the very fast component of the gracilis twitch was due not to the existence of special types of very fast muscle fibres, but rather to the non-uniform conditions in recording the contraction. In order to see if the very fast component could be produced in other fast skeletal muscles, an experiment was done with the plantaris and lateral gastrocnemius muscles. These were chosen

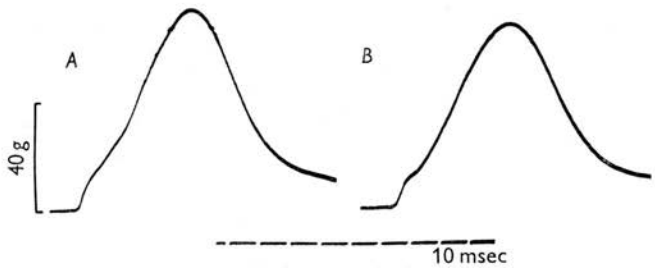


Fig. 4. Isometric twitches in lateral gastrocnemius muscle, recorded under low resting tension, to stimulation of the L 7 ventral root. In *A* the plantaris muscle, although not attached to the strain gauge, also contracted. In *B* the only contraction was in lateral gastrocnemius after section of the nerve to plantaris muscle. A very fast component is present in both *A* and *B*.

since they are closely united proximally and thus mimicked to some extent the conditions found in the gracilis muscle. The tensions on the two muscles could be changed independently. thus allowing the effect of unequal tension in the muscle mass to be analysed. A distinct very fast component, about 20 % of the peak amplitude of the twitch, was present when either the lateral gastrocnemius (Fig. 4*A*) or the plantaris muscle was under low resting tension. The step which marked off the very fast component at low tensions was still distinguishable as a small difference in slope at the start of the twitch at intermediate tensions. It was not detectable at higher resting tensions. This agrees with the results from the gracilis muscle in showing that the step arises in that part of the muscle under greatest tension but that it is conspicuous only when the muscle is at low resting tension. When the nerve to the plantaris was cut, so that only the lateral gastrocnemius contracted on stimulation of the L 7 ventral root, the initial step was at least as prominent as before (Fig. 4*B*).

DISCUSSION

The conclusion to be drawn from the present results is that the very fast component in the twitch is not peculiar to the gracilis muscle, although it may be particularly large in it under some conditions. The phenomenon is unlikely to have any physiological significance, since it has its origin in the method of recording and is most prominent in the flaccid muscle. It probably arises because the gracilis muscle is not uniform and different series compliances may exist. This may be expressed as a small part in the middle having a small series compliance compared to that of the mass of the muscle which lies on either side. Hill (1951) has demonstrated that an increase in the series compliance increased the contraction time (time to peak) in frog muscle. If it is assumed that this is applicable to mammalian muscle, it seems possible that the compound gracilis contraction is derived initially from those fibres with extremely small series compliance and the later part of the contraction originates from muscle fibres with a large series compliance in the recording system. This mechanism could account for the presence of an apparently very fast contracting component in the twitch when, in fact, all the muscle fibres had similar contraction times.

SUMMARY

1. The apparent double isometric twitch of the gracilis muscle was analysed by recording (*a*) from the whole muscle while stimulating the ventral roots and (*b*) from single motor units prepared by stimulating filaments dissected from the ventral roots.

2. The shape of the twitch depended on the method of recording and could also be varied by altering the resting tension on the muscle. The very fast component was most conspicuous and relatively largest at low tensions.

3. No motor units were found with a pure very fast contraction and a very quick component in the twitch was most evident in motor units at either edge of the muscle, particularly the caudal edge.

4. The very fast component, when present in motor units, was largest in those parts of the muscle where the tetanus:twitch ratio was least.

5. A double twitch could also be produced with both the plantaris and gastrocnemius muscles.

6. It was concluded that there were no separately organized very-rapidly-contracting muscle fibres in gracilis muscle.

REFERENCES

BULLER, A. J., ECCLES, J. C. & ECCLES, R. M. (1960). Differentiation of fast and slow muscles in the cat hind limb. *J. Physiol.* **150**, 399–416.

ECCLES, J. C., ECCLES, R. M. & LUNDBERG, A. (1958). The action potentials of the alpha motoneurones supplying fast and slow muscles. *J. Physiol.* **142**, 275–291.

HILL, A. V. (1951). The effect of series compliance on the tension developed in a muscle twitch. *Proc. Roy. Soc.* B, **138**, 325–329.

PREGANGLIONIC SYMPATHETIC ACTIVITY IN NORMAL AND IN RESERPINE-TREATED CATS

By A. IGGO and MARTHE VOGT

J. Physiol. (1960), **150**, *pp.* 114–133
With 8 text-figures
Printed in Great Britain

PREGANGLIONIC SYMPATHETIC ACTIVITY IN NORMAL AND IN RESERPINE-TREATED CATS

BY A. IGGO AND MARTHE VOGT
From the Departments of Physiology and Pharmacology, University of Edinburgh

(*Received* 7 *July* 1959)

Reserpine causes a loss of noradrenaline and of 5-hydroxytryptamine from the brain, and many attempts have been made to correlate the clinical effects of reserpine with this loss. One of the difficulties in attempting such a correlation lies in the lack of knowledge about the part played by noradrenaline and 5-hydroxytryptamine in normal brain function. Regions containing the central representation of the sympathetic system (hypothalamus, reticular formation of mid-brain and of medulla) are rich in both these amines. If the stores of the amines have an essential role in central sympathetic activity, one might expect such activity to be impaired after the administration of reserpine. The following observations would appear to support such a possibility: peripheral adrenergic neurones fail to excite the tissues they innervate when reserpine has reduced their stores of amines to 10 % or less (Muscholl & Vogt, 1958); continued sympathetic activity, elicited by drugs which stimulate the sympathetic centres, is accompanied by a reduction in the noradrenaline content of these centres, a fact which demonstrates some connexion between nervous activity and noradrenaline turnover (Vogt, 1954).

The object of this work, on which a preliminary note has been published (Iggo & Vogt, 1959), was to test central sympathetic activity in cats in which reserpine administration had reduced the cerebral noradrenaline and 5-hydroxytryptamine to very low figures. Efferent discharge of the preganglionic fibres of the cervical sympathetic was used as a measure of central sympathetic activity. In order to assess possible changes produced by reserpine, it was necessary first to observe the preganglionic sympathetic activity of normal cats in the same experimental conditions, and to devise some simple means of eliciting reproducible changes in activity.

In most of the earlier work (Adrian, Bronk & Phillips, 1932; Bronk, Ferguson, Margaria & Solandt, 1936; Bronk, Pitts & Larrabee, 1940), which has provided the fundamental information on sympathetic discharge, records were derived from whole nerves, whereas in the

present work recordings were obtained from small nerve strands. It was therefore necessary to establish a normal base line for this type of recording.

METHODS

Cats were used for all the experiments. They were anaesthetized with chloralose (80 mg/kg I.V. in 0·9 % NaCl solution) after induction with ether. The reserpine-treated animals had to be induced very slowly with a minimal amount of ether so as to avoid the danger of cardiac failure, and the chloralose was also given very slowly. As a result, some of the cats were only lightly anaesthetized after receiving the full dose of chloralose. If the anaesthesia did not deepen sufficiently within 30 min, a supplementary dose of chloralose was given. During the experiments, which lasted 4–11 hr, further injections of chloralose were given as required in order to prevent the appearance of jerks in response to sensory stimuli.

Intravenous injections. The drugs were injected into a cannula introduced into a femoral vein and connected to a burette.

Recording methods. Electrical activity in the sympathetic fibres was recorded in the mid-cervical region. The right cervical sympathetic nerve was exposed by making a mid-line incision through the skin and separating the neck muscles. The nerve was dissected to free it from the carotid artery but was usually left attached to vagus. A black Perspex plate was placed underneath the nerve, and the dissection of fine strands from the nerve was done on the plate. A trough formed by tying the edges of the skin wound to a metal ring fixed above the neck was filled with paraffin, so that the sympathetic nerve was completely immersed in the paraffin.

The fine strands were dissected from the sympathetic nerve with the techniques and precautions which have been described (Iggo, 1957). No special attempt was made to isolate single units. Electrical activity was recorded by placing the central ends of the fine strands which had been cut distally across a pair of Ag–AgCl wire electrodes. The potentials picked up by the electrodes were amplified with a condenser-coupled amplifier, displayed on a cathode-ray tube and also fed into an audio amplifier. The amplified action potentials, blood pressure signal, respiratory signal and acoustic signal were simultaneously recorded photographically on the same piece of moving bromide paper. Most of the figures in this article are from such photographs. The electrocardiogram was recorded in the initial experiments through leads inserted under the skin of the right foreleg and the left hind leg.

Blood pressure. The cats were given heparin after all the necessary dissection was finished. The left femoral artery was cannulated and connected to a mercury manometer so as to give a continuous visual check on the arterial pressure. A lumbar puncture needle was introduced into the right femoral artery, and the needle was connected to an electromanometer, based on a design published by Green (1954), and used for photographic records of the pressure. This manometer had a frequency response of 35/sec with the long cannula used, and so reproduced faithfully the pressure changes in the artery.

Respiration. Changes in the intratracheal pressure were recorded by inserting a hollow needle, connected to a condenser manometer, into the rubber connexion of the tracheal cannula. The manometer was not sufficiently sensitive to record changes of pressure when the tracheal cannula was open and the cat was breathing naturally, but gave satisfactory records of respiratory efforts during asphyxia with the tracheal cannula closed, or when the cat was artificially ventilated by a pump. Visual observations were made of the relation between respiration and blood pressure when the cat was breathing naturally.

Acoustic stimuli. Clicks were produced by using a large switch, which was opened and shut when required. A signal of the clicks was obtained by putting the switch in a circuit which fed a current to the Y-plates of the cathode-ray tube. Thus when the switch was opened or closed a condenser discharge was recorded.

Reserpine treatment. Reserpine (Serpasil; Ciba Ltd.), 1 mg/kg, was injected intraperitone-

ally into cats on four consecutive days, and the experiment was carried out on the fifth day. There was always loss of weight, and occasionally one of the doses was reduced to half when the cat appeared to be in a poor condition. All the cats drank a great deal of milk, but had to be roused to take their food. The degree of ataxia and sedation tended to be less on the last day of treatment. Miosis, relaxation of the nictitating membrane and blepharospasm were very evident.

RESULTS

Normal cats

Effects of anaesthetic on the level of activity. Chloralose was used as an anaesthetic in order to avoid the depressing effect of barbiturates. As a result, however, the cats sometimes exhibited jerks in response to sensory stimuli. To prevent any change in the recording conditions which might have resulted from movement of the nerve strand on the recording electrodes, additional anaesthetic was given whenever jerks were present. The level of activity in the efferent fibres was, however, diminished as anaesthesia was deepened. This made the comparison of activity between animals less quantitative and also obscured the long-term changes in activity in any animal in which slowly developing drug actions were followed.

Efferent activity in anaesthetized normal cats. The pattern of efferent activity depended to some extent on the number of fibres in a strand. In order to sample as many preganglionic fibres as possible the strands were not dissected down to single fibres. The dissection was carried to the point of allowing activity in individual fibres to be followed with some certainty. This was done because when long term changes were followed it was necessary to be satisfied that the recording conditions had not changed, and the only satisfactory way to do this was to be able to recognize the impulses in individual fibres. Examples of the general features of the discharge of preganglionic fibres are given in Figs. 1*A* and 8*A*. Impulses appeared in bursts, but there was rarely a silent interval longer than 1 sec unless the animal was deeply anaesthetized. The behaviour of individual fibres was easier to follow when there were only a few active fibres in the strand (see Fig. 2*A*). In the undisturbed cat, the individual cells discharged at low rates, often less than 1/sec, so that frequently one cell discharged only one impulse within each of the bursts seen in the multifibre records. If a cell fired several times during a burst of activity the frequency of discharge was rarely higher than 30/sec and there were seldom more than a few impulses at such frequencies. The waves of potential recorded from intact sympathetic nerves by previous workers were probably generated by summed impulses in many, but not all, of the fibres in the nerve, each firing once or at most a few times during one wave.

Several rhythms of activity could be recognized. The most clear-cut was

a *respiratory rhythm* (Fig. 1*A*). Normal respiration does not show on these tracings; since. however, there was always a rise in blood pressure at the end of inspiration, the respiratory rhythm can be identified on the tracings by reference to these pressure rises. Thus Fig. 1*A* shows two such rises in blood pressure during two breaths. The electrical activity during these blood-pressure maxima was always at a minimum. A similar form of electrical discharge was observed by Dontas & Nickerson (1957) in splanchnic nerves. In the cervical sympathetic, the units which became inactive

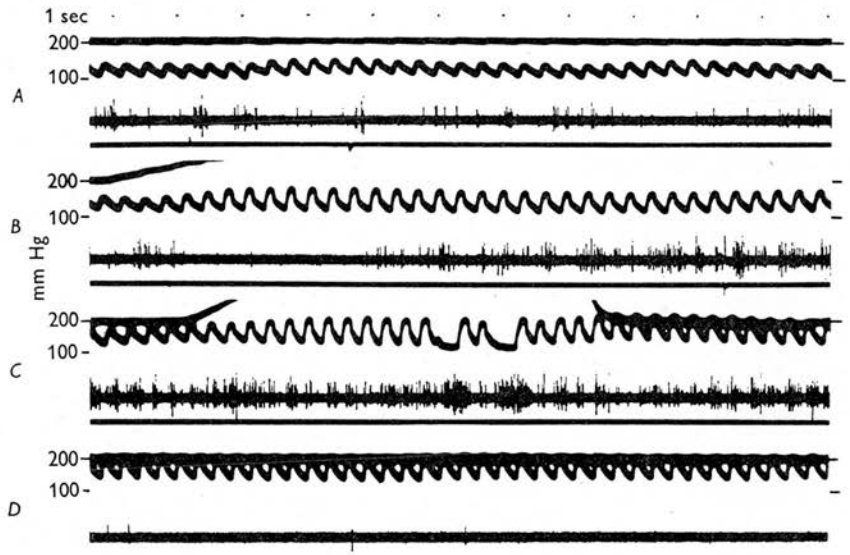


Fig. 1. Normal cat (no. 8). Electrical activity recorded in preganglionic fibres dissected from the cervical sympathetic nerve. Each record shows, from above down, respiration signal (inspiration upwards), arterial pressure (calibration in mm Hg on the left), impulses in the nerve strand, and signal marker for clicks (absent from *D*). *A*, normal activity, with two interposed clicks. The blood pressure rises at the end of each inspiration but, as described in Methods, the recorder for respiration was not sensitive enough to give a deflexion for normal breathing. Records *B* and *C* show the effect of asphyxia; the tracheal cannula was closed at the beginning of *B* and opened again at the end of *C*; two extra-systoles occur in the middle of *C*. *D* immediately after *C*. Time marker, seconds (top of *A*).

during the respiratory rises in blood pressure could also be silenced by pressor doses of adrenaline. Further evidence that the rhythm was secondary to the vascular changes was obtained in records taken during the early phase of, and during recovery from, asphyxia. Thus, for example, in the left of Fig. 1*B*, the abolition of activity coincided with the rise in blood pressure and not with the much more prolonged inspiratory effort. After the trachea was reopened the blood pressure remained elevated and

the efferent activity was suppressed, even though the respiratory activity was enhanced (Fig. 1*D*).

Adrian *et al.* (1932) ascribed the respiratory rhythm they observed in the rabbit to a central interaction between the respiratory and vasomotor centres, whilst Bronk *et al.* (1936, 1940) argued that, in the artificially ventilated cat, it was partly due to an inhibitory effect of the pulmonary stretch receptors. In cats breathing naturally the present work showed a consistent relation between the peaks of the respiratory blood-pressure fluctuations and the inhibition of the efferent discharge. This relation was still evident when the trachea was closed (Fig. 1*B*). In these conditions, however, the pulmonary stretch receptors were not excited during inspiration, and therefore cannot have been responsible for the inhibition of efferent activity. There are obviously a number of mechanisms responsible for respiratory rhythms.

One single unit was isolated in which the discharge of impulses appeared to be directly linked to respiratory activity (Fig. 2). There was a burst of impulses during every inspiration. The discharge of impulses in this unit was not abolished by pressor doses of adrenaline. During asphyxia (Fig. 2*B*, *C*) the discharge was closely linked to the respiratory efforts. This relation can be seen in the figure, in which the end of inspiration is marked by a downward movement of the base line. Thus the first breath after closing the trachea (during the expiratory pause) was longer than normal and the discharge of impulses was also carried on for longer (Fig. 2*B*). As the subsequent breaths became deeper, the efferent discharge was further enhanced. After air was allowed once more into the lungs, the efferent activity reverted to a lower frequency (Fig. 2*D*), and was not abolished, as happened in most other fibres, after a period of asphyxia (see p. 122). The discharge of this exceptional unit thus corresponds closely to the activity described by Adrian *et al.* (1932) in the rabbit, and is quite different from the behaviour to be expected if the efferent discharge had been inhibited by pulmonary stretch receptors, as in the experiments of Bronk *et al.* (1936).

Cardiac rhythm. Although the electrical activity in the undisturbed cat appeared in bursts, there was never any conspicuous relation of these bursts to the heart rate. A definite cardiac rhythm, however, appeared when a high level of efferent discharge was associated with a rise in blood pressure. This combination was found during the later phases of asphyxia, as in Fig. 1*C*. This record also shows details of the relation between the bursts of impulses and the cardiac activity. Two arterial pressure waves are virtually absent in this record, presumably because of extra-systoles. There was a prolonged burst of impulses when the normal pulse was absent. Each burst started 150–200 msec after the preceding peak and

ended shortly after the peak of the next wave. By this fortunate chance it became possible to relate the start of the burst of impulses to the fall of the preceding pulse wave and its end to the pressure wave with which it coincided. Thus the conclusion reached by Adrian *et al.* (1932) and Bronk *et al.* (1936, 1940), that the cardiac rhythm had its origin in a stimulation of the baroreceptors which in turn inhibited the vasomotor centre, is shown to be valid for the individual pulse. One might, therefore, infer that there is a latent period of about 200 msec between stimulation of the baroreceptors and inhibition of the preganglionic sympathetic outflow. That these

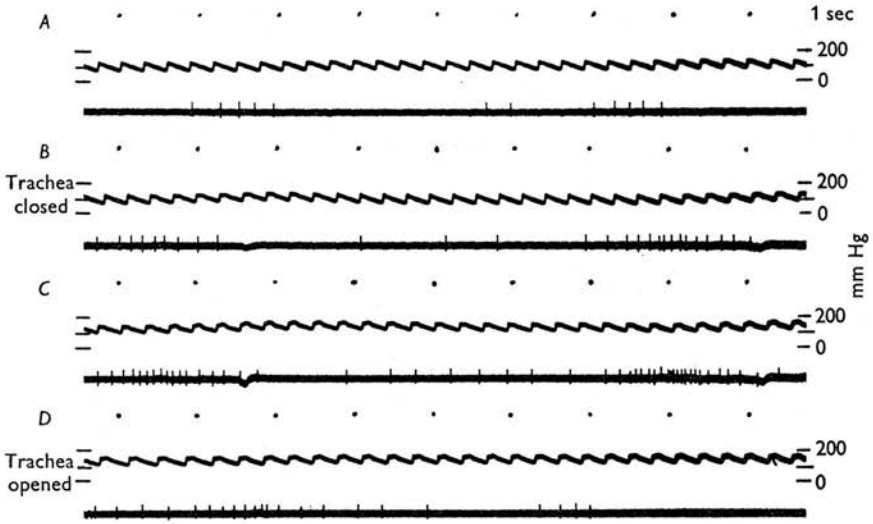


Fig. 2. Normal cat (no. 5). Impulses in a single preganglionic sympathetic fibre: respiratory rhythm. On each record: time (sec), blood pressure (calibration in mm Hg on the right), action potentials. *A*: impulses during two breaths; the peaks of activity coincide with inspiration (not indicated on the record). *B* and *C*: activity during asphyxia. Here the respiratory cycle is recognizable by a short downward movement of the base line which occurred at the end of each inspiration. Trachea closed during the expiratory pause just before *B*; the activity is enhanced during the next breath. 15 sec interval between *B* and *C*; trachea opened just before *D*; the activity quickly returned to the pre-asphyxial pattern; there was no inhibition like that shown in Fig. 1*D*.

bursts of impulses represent vasomotor activity is not certain but is suggested by the fact that they are abolished by a pressor dose of adrenaline.

Bronk *et al.* (1936), in their Fig. 7, also show an example of the presence of cardiac rhythm when the blood pressure was raised, and its disappearance when the pressure was allowed to fall.

A cardiac grouping of the impulses does not necessarily occur when there is an enhanced efferent outflow. For example, there was a massive efferent discharge which showed no cardiac rhythm following an intravenous

injection of a large dose of morphine. This was at a period when the blood pressure was still unchanged, so that one of the conditions in which cardiac rhythm was found, namely high blood pressure, was not satisfied.

Sympathetic activity during Traube waves. The regular waves, known by various names, which appear in most blood pressure records, have been attributed by Guyton & Harris (1951) to 'presso-receptor-autonomic oscillation'. There was one particularly clear example of these Traube waves in the present work. It occurred during a period of asphyxiation in

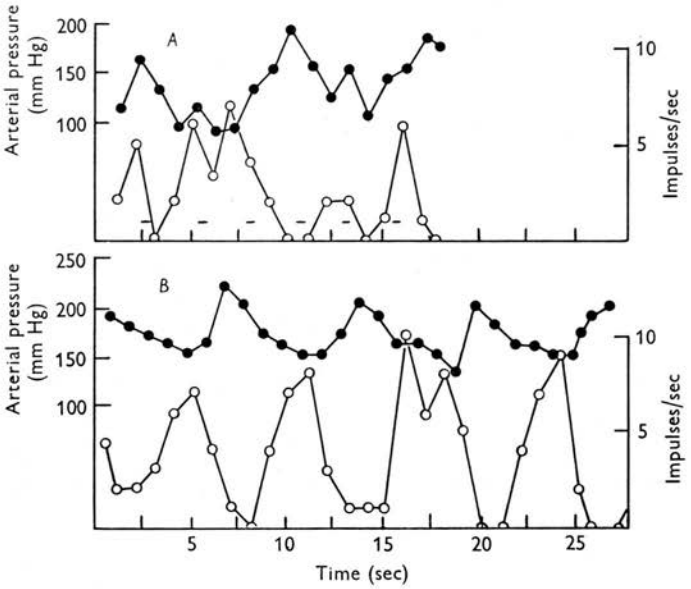


Fig. 3. Normal cat (no. 7). Traube waves in the blood pressure and efferent activity in the cervical sympathetic nerve. The filled circles represent the diastolic blood pressure and the open circles the total efferent activity per second in a multi-fibre strand. In *A* the cat was ventilated by a respiration pump, and the peak of inspiration is indicated by the short horizontal strokes at the bottom of the picture. *B* shows the activity after the respiration pump was turned off; the cat did not attempt to breathe and the Traube waves are very clear.

a cat which had been artificially ventilated. After the pump had been turned off the cat did not attempt to breathe for 1 min, and during this time the Traube waves were present, uncomplicated by respiratory variations in blood pressure. A rhythmical discharge of impulses was also present in the sympathetic fibres (Fig. 3*B*, lower record). It appeared at a gradually increasing frequency as the blood pressure was falling, and then gradually fell off again as the blood pressure rose once more. The period of the Traube rhythm was 6 sec and the efferent outflow lasted 3–4 sec. The blood pressure did not begin to rise until about 2·5 sec after the first

impulses appeared in the strand, and began to fall again about 1 sec after the last impulse in the group. The oscillations can be ascribed to delays in two components of the stabilizing mechanism, (*a*) the central delay along the path, pressoreceptor–central nervous system–preganglionic cell and (*b*) the sluggishness of the blood pressure in responding to changes in the efferent outflow. On the basis of the present results the delay along the central pathway is 200–300 msec and the delay in effector response about 2·5 sec. On this interpretation the Traube waves arise largely because of effector delay.

The Traube wave can be disrupted but not abolished by breathing (Dornhorst, Howard & Leathart, 1952). This is shown in Fig. 3*A*, in which the cat was on artificial respiration (pump strokes marked in the figure). Here complex fluctuations of blood pressure are superimposed on the Traube waves, the electrical record showing equal complexity of rhythm.

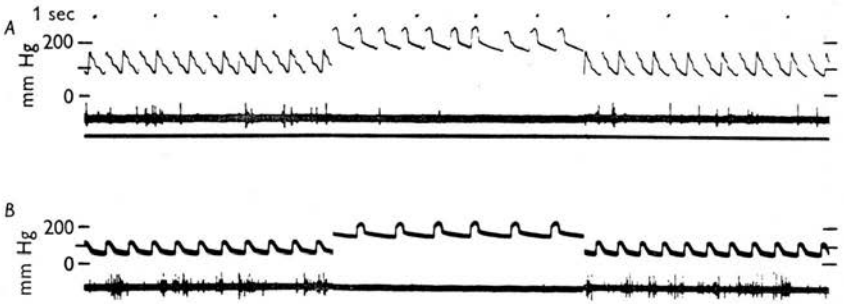


Fig. 4. The effect of intravenous injections of adrenaline on the blood pressure and the efferent discharge in a normal cat (*A*, no. 11), and in a reserpine-treated cat (*B*, no. 6). On the left, the control periods; in the centre, the records 20 sec after an injection of 7 and 5 μg respectively of adrenaline; on the right, records taken 4 min later. Blood pressure calibration (mm Hg) on the left. Time marker, 1 sec (top tracing).

Effect of drug-induced blood pressure changes on efferent activity. As has been previously reported by Adrian *et al.* (1932), most of the activity in the cervical sympathetic nerve can be modified by drugs which alter the blood pressure. When the blood pressure of the chloralosed cat was raised by the intravenous injection of 5–10 μg adrenaline (Fig. 4*A*), the efferent activity became less, and was absent in most of the fibres when the systolic pressure was above 200 mm Hg. At these pressures there was obvious cardiac slowing, the rate falling by as much as one half. Conversely, the efferent activity could be enhanced by lowering the blood pressure with an intravenous injection of 6 μg histamine.

The sensitivity of different fibres to rise in blood pressure varied. Some units were readily silenced, whereas others, e.g. the respiratory unit of

Fig. 2, were resistant. The different units in a strand dropped out at different times as the blood pressure rose, and the activity reappeared in the reverse order.

Effect of asphyxia on the efferent discharge. Temporary closure of the tracheal cannula was used as a convenient way to excite the sympathetic nervous system. The effect in normal cats was influenced by two factors, the depth of anaesthesia and the type of respiration. If the cat was lightly anaesthetized and breathing naturally, the effect was quick in onset. Deeper anaesthesia delayed the rise in efferent outflow. If the lungs were inflated by a respiration pump, there was usually some degree of over-ventilation, since the stroke was adjusted to prevent any respiratory efforts on the part of the animal. As a consequence the onset of the response was later still and initial vigour of changes in efferent activity less.

Asphyxiation of normal cats led after 5–10 sec to a progressive rise in arterial pressure and, as Adrian *et al.* (1932) have reported, to an enhancement of efferent outflow. There was a more persistent and higher frequency of discharge in the fibres already active, and new fibres started firing. At the end of a period varying between 10 and 45 sec the outflow was nearly continuous (Fig. 1*C*). The discharge, however, was not steady; the two patterns of activity described in preceding sections, the cardiac rhythm and the rhythm due to respiratory fluctuations of blood pressure, persisted throughout the enhanced activity. After the air supply was restored by opening the trachea, the efferent activity quickly subsided, often within 5 sec. During this period, the blood pressure remained at the same, or rose to an even higher, level than that reached at the end of the asphyxia. The sudden disappearance of the efferent activity can be accounted for by the fact that the inhibitory effect of the high blood pressure was no longer opposed by the central stimulatory effects of asphyxia.

When normal cats were ventilated with a respiration pump, animals which previously had given a brisk response to asphyxia sometimes did not breathe for a whole minute after the pump had been turned off; the efferent discharge was then also unchanged. Presumably there had been some degree of over-ventilation, although the pump stroke had been so adjusted as to keep the blood pressure at the initial level. This observation illustrates a difficulty in comparing the reactions of the normal and the reserpine-treated cat. All the treated cats had to be ventilated by pump during the experiments to prevent anoxia, so that the possibility of over-ventilation was always present to bedevil the comparison of the two types of preparation.

The one unit which had a primary respiratory rhythm and which was relatively resistant to inhibition by adrenaline also continued its individual pattern during asphyxia (Fig. 2 and p. 118). When the trachea was re-

opened the activity reverted to its original pattern and was not suppressed by the continued high arterial pressure.

The experiments were often terminated by clamping off the trachea. Such 'terminal' asphyxia caused a continuous discharge of impulses in the sympathetic fibres. The discharge became regular about 2–3 min after closure of the trachea, and at different times in different fibres. The steady discharge ranged from 2/sec in some fibres to 25/sec in others. The discharge lasted for up to 9 min and continued after the heart had stopped pumping blood. In all experiments fibres were found which had been silent in the undisturbed animal and which became active in the later stages of asphyxia.

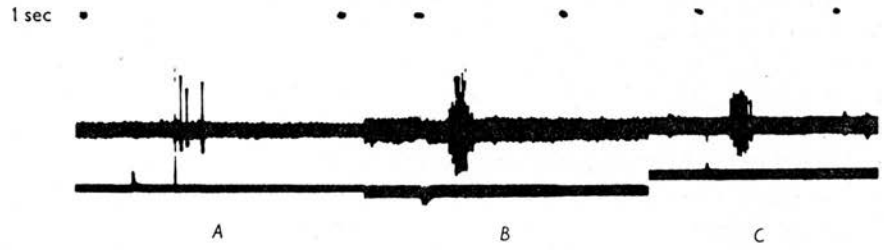


Fig. 5. Normal cats, nos. 7(*A*), 8(*B*), 9(*C*). Response of the sympathetic system to clicks. Each record was selected from a silent part of the base line in order to make the effect clearer. In each instance the click has excited several fibres, and in *B* and *C* there is repetitive firing of individual units. Time marker, 1 sec.

Sympathetic response to acoustic stimuli. Though all cats were anaesthetized sufficiently deeply not to respond to a sudden noise with a jerk, a click usually elicited a burst of impulses. Often new units were excited whilst some of the fibres already firing in the normal bursts appeared unaffected. The time course of the efferent discharge was always the same. Within 100–200 msec of the click there was a burst of impulses lasting 100–300 msec. The individual fibres discharged 1–5 impulses, depending on the excitability of the animal and the intensity of the stimulus. When a fibre fired several times the frequency of discharge could be as high as 100 impulses/sec. Examples are shown in Figs. 1*A*, 8*A* and 5. The response is seen best when there is little other activity (Fig. 5); in Fig. 1*A* the response can be identified by the fact that a new unit which had previously been silent discharged at each of the clicks. Individual units did not respond consistently to repetition of the same stimulus. There appeared to be a relation between the level of activity of a neurone and the likelihood that it would be excited by a click, but the relation was difficult to put on a quantitative basis. Thus a unit with a marked respiratory rhythm did not respond to a click during expiration but did respond during inspiration; later in the experiment, when the anaesthesia had become lighter, the

unit could be excited at any stage of the respiratory cycle. Another unit in the same strand, otherwise silent, fired in response to the click at all phases of the respiratory cycle.

Frequent repetition of the acoustic stimulus led to a failure of the response. After 3 or 4 clicks applied within 10 sec the response was absent, but a short rest of, say, 30 sec was sufficient to restore the response. Electrical stimulation of the saphenous nerve produced a similar response, but was not often used owing to the danger of eliciting reflex movements.

Effect of morphine on the sympathetic system. Morphine was tried as a stimulant for the sympathetic system because it is known to excite the sympathetic centres of the cat (Elliott, 1912). Small doses given intravenously had an uncertain effect on the efferent outflow; some enhancement was apparent but the origin of the effect was in doubt, since morphine is a respiratory depressant and the increased outflow might in part have been secondary to under-ventilation arising from this depression. This was controlled in the later experiments by the use of artificial respiration. When large doses of morphine (3–7 mg/kg) were given intravenously, there was an immediate slight rise in the blood pressure followed by a slowly developing deep fall. The efferent activity was much enhanced within 25 sec, at which time the blood pressure was normal. The activity remained at a high level while the blood pressure was falling and later gradually fell off; within half an hour it was coming in bursts which were associated with the convulsions appearing at that time. In one animal the discharge became very regular, about 10/sec, during the period of enhanced activity, thus giving a most unusual picture. There were various peripheral signs of sympathetic stimulation, such as a dilated pupil and a contracted nictitating membrane.

Reserpine-treated cats

Efferent activity in the undisturbed reserpine-treated cat. Sufficient reserpine was given to cause the disappearance of a large proportion of the catecholamines from the hypothalamus, mid-brain, medulla oblongata and the post-ganglionic sympathetic adrenergic neurones. Actual mean losses of noradrenaline, for two cats in which estimations were carried out after the same treatment, were 90·2 % from the hypothalamus and 94·7 % from the superior cervical ganglia.

Strands of the cervical sympathetic containing active efferent fibres were very readily obtained in these animals. This might indicate that the over-all discharge was greater than normal, but it is impossible to be perfectly certain about it. The activity in individual fibres was more continuous than in normal cats (Fig. 7*A*), even when the blood pressure was above 100 mm Hg; all the animals were ventilated by a respiratory

pump in order to remove any asphyxial drive to the sympathetic nervous system. Some units, in particular, discharged more regularly than had ever been observed in normal cats. Bursts of impulses were present but no respiratory or cardiac rhythm was apparent (Fig. 6).

The blood pressure in the reserpine-treated animals was very steady and exhibited no respiratory rhythm, and we think that for this reason the efferent discharge did not show the fluctuations to be seen in normal

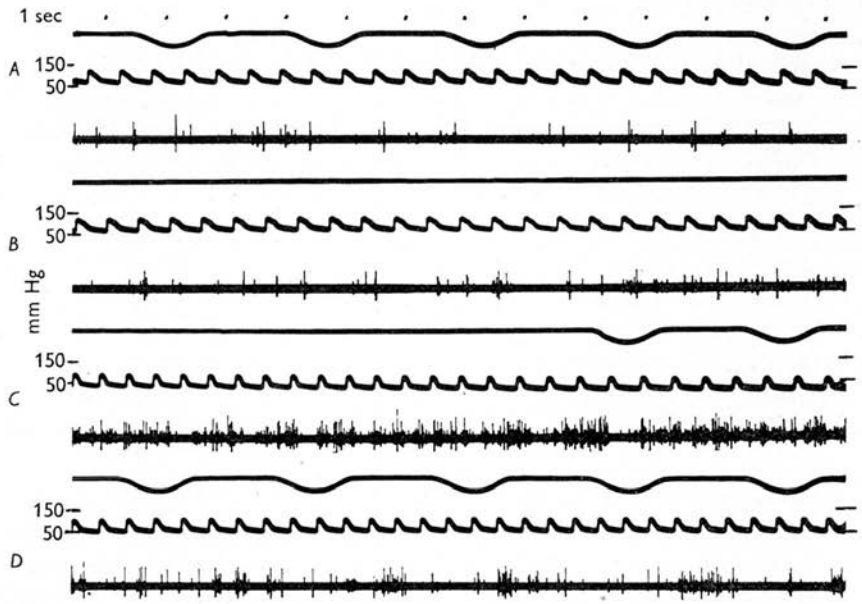


Fig. 6. Reserpine-treated cat (no. 6); effect of asphyxia on efferent sympathetic activity. The cat was artificially ventilated. Top tracing, respiration pump; second tracing, blood pressure (calibration in mm Hg); bottom tracing, efferent discharge. *A*, base line activity. *B* and *C*, activity during asphyxia, interval of 15 sec between *B* and *C*; the pump was turned off at the beginning of *B* and was turned on towards the end of *C*; during asphyxia increase in the efferent discharge but fall in blood pressure. *D*, 2 min after the end of asphyxia; blood pressure not quite back to normal and efferent activity still enhanced.

animals. A cardiac rhythm was seen on some occasions, when the blood pressure was rising after an intravenous injection of adrenaline. There was no cardiac rhythm during asphyxia as there was no rise in blood pressure (see below).

Acoustic stimuli were effective in eliciting bursts of impulses as in the normal animal. The discharge was sometimes more difficult to arouse and was often lost in the high background activity, but was otherwise similar to the response in normal animals; as in the normal preparation not all fibres could be made to respond.

Asphyxia. In reserpine-treated cats, as in the normal animal, closing of the trachea led to an increase in the sympathetic efferent outflow. There were, however, several important differences in the response. The efferent activity was slower in building up. This may have been due to the use of artificial ventilation in the reserpine-treated cats and not to a direct effect of the drug. The ventilation was adjusted to give a stable arterial blood pressure, about 100 mm Hg, but nevertheless the cats might have been over-ventilated.

A second difference from the normal cats was that the enhanced activity persisted after the respiration pump was started again (see Fig. 6). The main reason for this must have been the reversal of the normal blood pressure response to asphyxia. Soon after occlusion of the trachea the blood pressure and heart rate of the reserpine-treated cat started to fall instead of to rise, and on release of the occlusion recovery of the blood pressure was very slow, so that sympathetic activity continued to be stimulated. The absence of the normal rise in pressure and of tachycardia was, of course, caused by the functional failure of the vasoconstrictor nerves, the nervi accelerantes and the adrenal medulla, all of which had lost their stores of catecholamines. That the blood pressure was not even maintained but fell was probably a result of cardiac failure from lack of oxygen in the absence of the tonic action of the sympathetic system on the heart.

A subsidiary reason for the slow return of sympathetic outflow to the resting level might have been that the normal cat increased its respiratory volume by taking larger and faster breaths after a period of asphyxia, so that the normal blood gas levels were quickly restored. In the reserpine-treated animals, the rate and stroke of the pump were not altered so that a longer time would elapse before recovery was complete.

The enhanced sympathetic outflow during the post-asphyxial period failed to elicit any peripheral circulatory response, as is seen by the slowness of the recovery and by the absence of any overswing of blood pressure or pulse rate at any phase of the recovery period (Figs. 6 and 7).

Prolonged asphyxia led, as in normal cats, to a very steady discharge of impulses in individual fibres, at frequencies as high as 25/sec. Terminal asphyxia in the reserpine-treated cat revealed that, at rest, not all the sympathetic fibres had been active so that in this respect also the two preparations were similar. The absence of any peripheral effect of central sympathetic activity did not lead to extreme over-activity of the sympathetic centres.

Adrenaline. The pressor action of adrenaline appeared to be more prolonged than in normal cats. During the pressor effect all the preganglionic sympathetic activity disappeared (Fig. 4*B*). Thus the central mechanisms

for stabilizing the blood pressure were still active, irrespective of the inactivation of some of the effector pathways. Furthermore, an injection of adrenaline, after having produced an initial rise in blood pressure and heart rate, led to a bradycardia as the pressure rose higher still. Thus, wherever the efferent pathways were intact, central control of blood pressure proceeded in the normal way.

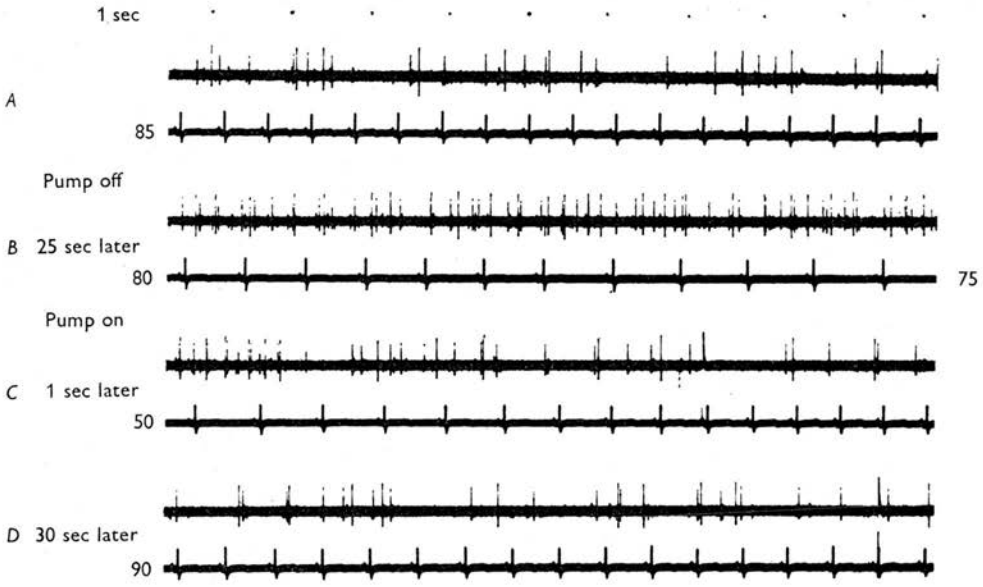


Fig. 7. Reserpine-treated cat (no. 3); effect of asphyxia on efferent sympathetic activity. This preparation shows the regularity of the efferent discharge better than cat 6 (Fig. 6). Top tracing, efferent activity; bottom tracing, electrocardiogram. *A*, base-line activity during artificial respiration; the pump was then turned off for 45 sec. *B*, after 25 sec of asphyxia; increase in the frequency of firing. *C*, immediately after the resumption of artificial respiration. *D*, 30 sec later; heart rate, blood pressure and discharge back to normal. Efferent activity not abolished at any phase of the recovery from asphyxia (contrast normal cat, Fig. 1). Time marker, 1 sec (top tracing); blood pressure (mm Hg) marked at sides.

Morphine. Intravenous injections of morphine in doses of 4–7 mg/kg were given to two cats which had had reserpine previously. In each instance there was a slight increase in efferent activity shortly after giving the morphine, but the effects were less dramatic than in normal animals.

Acute effects of reserpine. Bein (1955) has reported immediate effects of an intravenous injection of reserpine on the electrical activity of a cardiac nerve. He used a cat anaesthetized with chloralose and a dose of 0·1 mg/kg. The electrical activity in the nerve started to decrease 10 min after the injection and had disappeared in 50 min; at this time bradycardia was

apparent. We repeated Bein's experiment in order to see whether a similar decline in activity would occur in the cervical sympathetic nerves.

One difficulty in following changes in efferent activity for an extended period is that the recording conditions may change or that the preparation may deteriorate. When, however, as was done in the present work, the activity is recorded from small strands which permit the identification of impulses in individual fibres, this possibility is much reduced.

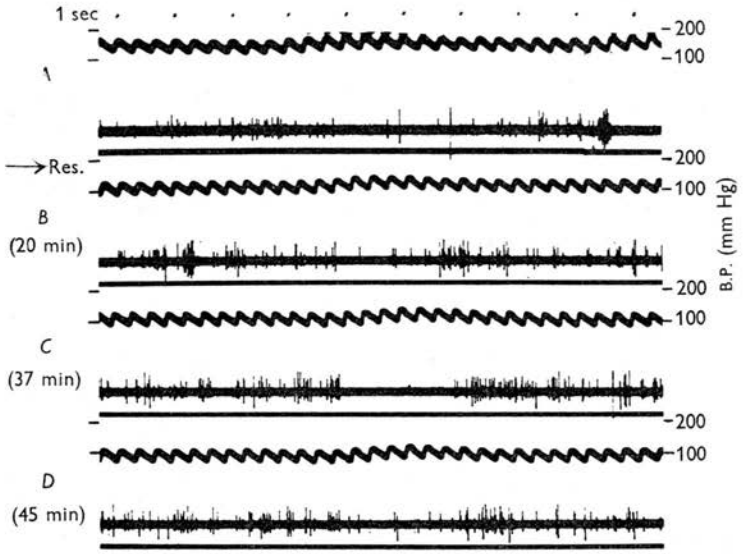


Fig. 8. Normal cat (no. 8); natural respiration. Changes in efferent sympathetic activity following the intravenous injection of reserpine (0·1 mg/kg). Each record shows the blood pressure (calibration in mm Hg on the right), impulses in the nerve strand, and the signal marker for clicks. The records have been aligned so that the rises of blood pressure at the end of each inspiration are in the centre of the picture. *A*, control period, followed immediately by the injection of reserpine (→Res.). *B*, 20 min; *C*, 37 min; *D*, 45 min later. The blood pressure had fallen within 20 min and was lower still at 45 min despite some increase in efferent discharge (*B*, *C* and *D*). No change in heart rate. In all tracings, the efferent activity is lowest during the respiratory rise in blood pressure, but the effect is most pronounced 37 min after reserpine (*C*). Time marker, 1 sec (top tracing).

When reserpine (0·1–0·2 mg/kg) was injected intravenously, a fall in blood pressure developed in the course of about 15 min in those cats in which the initial pressure was high; this dependence of the effect on the initial level has been reported by many authors (see Bein, 1956). Bradycardia was not observed. The effect on the electrical activity in the cervical sympathetic was small and not consistent. Twice a reduced activity was observed 20 min after the injection, but by 50 min the activity was greater than before the drug. In two other cats the only effect was that repre-

sented in Fig. 8, an increased over-all activity with an occasional longer pause between the bursts. These pauses coincided with the rises in pressure which formed part of the normal respiratory fluctuations. It is not possible to exclude some degree of recovery from the anaesthesia as a factor contributing to the enhanced activity.

One experiment was carried out in order to see whether a large intravenous dose of reserpine (1 mg/kg) would produce any effect on the sympathetic activity of a cat previously treated with reserpine (1 mg/kg for 4 days). No such effect was seen.

DISCUSSION

The results in the normal cats in most respects confirm the earlier descriptions of sympathetic activity given by Adrian *et al.* (1932) and Bronk *et al.* (1936, 1940). New information about the response of the sympathetic nervous system to abrupt sensory stimulation is given, in particular the brief and quick discharge of impulses following an acoustic stimulus. The use of fine strands in which the behaviour of individual units could be followed also revealed that, although a cell may not fire continuously at frequencies higher than 25/sec even during the intense stimulation of terminal asphyxia, some fibres at least can respond at much higher frequencies, if only for a short time.

The results on the cats which had been treated with high doses of reserpine were very clear-cut. Spontaneous preganglionic sympathetic activity was unabated, and perhaps enhanced, whilst the treatment had been thorough enough completely to abolish any peripheral sympathetic activity; the latter fact is best illustrated by the effects of asphyxia, which, after reserpine, were hypotension and bradycardia instead of hypertension and tachycardia. Though the experiments gave the impression that efferent preganglionic activity was greater than normal, the technique of recording from small strands of the nerve does not permit any definite statement to be made on the total outflow of sympathetic impulses; it was, however, clear that the discharge was more continuous than, and lacked the fluctuations of, the activity of the normal animal. The obvious explanation is that the lack of any peripheral effects of the central discharge caused a lack of inhibitory feedback which creates various rhythms in the normal resting cat. In both normal and reserpine-treated cats the sympathetic outflow was suppressed when the blood pressure rose, and most effectively by the injection of a pressor drug. That rises in blood pressure could not be produced in the reserpine-treated animal by the usual stimuli to which the normal animal responds with increased vasomotor tone and output of adreno-medullary hormones is, of course, a result of the inactivation of the

post-ganglionic adrenergic neurones and the adrenal medulla, and not of a failure of the centres; neither is it related to impaired transmission in autonomic ganglia, since transmission has been shown to be normal after treatment with reserpine (Trendelenburg & Gravenstein, 1958).

Acoustic stimuli and asphyxia increased the preganglionic electrical activity in the normal and in the reserpinized cat. When, however, air was again admitted to the lungs, the normal cat exhibited electrical silence whereas the reserpine-treated animal showed prolonged enhanced activity. The reason for this change is that, after reserpine treatment, the normal post-asphyxial rise in blood pressure is replaced by a prolonged period of hypotension which acts as a stimulus to the sympathetic centres. The observation that terminal asphyxia causes greatly enhanced discharge also in the reserpine-treated cat shows that, even in the complete absence of peripheral effects, the sympathetic centres do not discharge maximally under resting conditions.

The intravenous injection of morphine appeared to cause a smaller and more evanescent increase of sympathetic activity in the reserpine-treated cats. This may be an example of the central antagonism of reserpine and morphine found on injection of these drugs into the conscious cat (Gaddum & Vogt, 1956), but since dosage and experimental conditions were very different in the present work there might be other interpretations.

The experiments throw no light on the possible role of catecholamines and 5-hydroxytryptamine in central sympathetic activity. To conclude from the results that sympathetic activity of the brain is independent of these amines is not permissible, since these substances continue to be synthesized and never completely disappear from the tissue of the reserpine-treated animal. It is safe, however, to assert that the stores of these amines are not essential for normal sympathetic discharge and for normal responses of the autonomic centres to external or internal stimuli. In this respect the central sympathetic neurones behave quite differently from the post-synaptic adrenergic neurones, which cease to transmit impulses when their noradrenaline stores have been depleted by reserpine. This difference might be due to the fact that the function of noradrenaline in the brain is not one of transmitter in sympathetic pathways; but it could also be that central adrenergic neurones, if such neurones exist in these pathways, differ so much in their turnover of noradrenaline from the long peripheral nerves that they remain functioning at a low content of transmitter.

In view of the results obtained in this work it seems surprising that, as Bein (1955) observed, a single injection of a small dose of reserpine should arrest all efferent activity in the cardiac nerves. In the cervical sympathetic we saw no disappearance, and only occasionally some temporary diminution, of preganglionic discharge following an injection of reserpine.

5. Short acoustic stimuli elicited bursts of impulses with a latency of about 200 msec, the frequency of firing in individual fibres reaching 100/sec.

6. The injection of large doses of morphine was followed by a period of increased efferent activity.

7. After several days of treatment with reserpine (1 mg/kg), the sympathetic activity differed from normal only in the following points: (1) The discharge was more continuous, perhaps more frequent, and lacked a respiratory rhythm as also did the blood pressure. (2) Restoration of the air supply after asphyxia did not arrest the activity; discharge, greatly enhanced over the resting level, continued for as long as the fall in blood pressure which accompanied and followed asphyxia in these animals. (3) Intravenous injections of morphine stimulated the activity less than in the normal cat.

8. Injection of small doses of reserpine (0·1 mg/g) into normal cats in the course of an experiment did not greatly change, and never abolished, sympathetic discharge, irrespective of whether a fall in blood pressure had followed the injection.

9. The conclusion arrived at was that preganglionic sympathetic discharge, both spontaneous and in response to stimuli, was not fundamentally altered by doses of reserpine which abolished all peripheral sympathetic activity and caused severe depletion of the stores of noradrenaline and 5-hydroxytryptamine in the brain.

We are grateful to Ciba Laboratories Ltd., Horsham, for a generous supply of Serpasil.

REFERENCES

ADRIAN, E. D., BRONK, D. W. & PHILLIPS, G. (1932). Discharges in mammalian sympathetic nerves. *J. Physiol.* **74**, 115–133.

BEIN, H. J. (1953). Zur Pharmakologie des Reserpins, eines neuen Alkaloids aus Rauwolfia serpentina Benth. *Experientia*, **9**, 107–110.

BEIN, H. J. (1955). Significance of selected central mechanisms for the analysis of the action of reserpine. *Ann. N.Y. Acad. Sci.* **61**, 4–16.

BEIN, H. J. (1956). The pharmacology of Rauwolfia. *Pharmacol. Rev.* **8**, 435–483.

BRONK, D. W., FERGUSON, L. K., MARGARIA, R. & SOLANDT, D. Y. (1936). The activity of the cardiac sympathetic centres. *Amer. J. Physiol.* **117**, 237–249.

BRONK, D. W., PITTS, R. F. & LARRABEE, M. G. (1940). Rôle of hypothalamus in cardiovascular regulation. *Res. Publ. Ass. nerv. ment. Dis.* **20**, 323–341.

DONTAS, A. S. (1957). Effects of reserpine and hydralazine on carotid and splanchnic nerve activity and blood pressure. *J. Pharmacol.* **121**, 1–7.

DONTAS, A. S. & NICKERSON, M. (1957). Central and peripheral components of the action of 'ganglionic' blocking agents. *J. Pharmacol.* **120**, 147–159.

DORNHORST, A. C., HOWARD, P. & LEATHART, G. L. (1952). Respiratory variations in blood pressure. *Circulation*, **6**, 553–558.

ELLIOTT, T. R. (1912). The control of the suprarenal glands by the splanchnic nerves. *J. Physiol.* **44**, 374–409.

GADDUM, J. H. & VOGT, M. (1956). Some central actions of 5-hydroxytryptamine and various antagonists. *Brit. J. Pharmacol.* **11**, 175–179.

In the splanchnic nerves of the cat, Dontas (1957) found no diminution of preganglionic activity unless the dose was above 1 mg/kg. As the technical details of Bein's study have not been reported, it is impossible to decide whether the cardiac nerves respond differently from the cervical sympathetic or splanchnic nerves, or whether the experimental conditions were not comparable.

The fall in blood pressure which occurs soon after a single small intravenous dose of reserpine in animals with a high resting blood pressure has never been satisfactorily explained. It is tempting to suggest that this effect might be caused by the local action of catecholamines released from the region of the carotid sinus and carotid body on receptors in the carotid sinus. Palme (1944) has shown that local application of adrenaline to the tissue around the carotid sinus causes a precipitous fall in blood pressure, which is particularly pronounced in the rabbit but is also seen in the cat. He explains the effect as a stimulation of the carotid nerve by adrenaline, and Landgren, Neil & Zotterman (1952) have indeed demonstrated an increase in the discharge from the carotid nerve when adrenaline or other pressor agents were applied to the exposed carotid sinus of the cat. The effect was quite prolonged, and during that period carotid occlusion produced little or no rise in blood pressure. Impairment of the carotid sinus reflex also occurs after reserpine (Bein, 1953), and it may be that both this phenomenon and the fall in blood pressure have their origin in the action of locally released catecholamines on the carotid sinus.

SUMMARY

1. The action potentials in small strands of the preganglionic cervical sympathetic nerves were recorded in normal and in reserpine-treated cats anaesthetized with chloralose.

2. The activity in the undisturbed normal cat consisted of bursts of impulses, individual fibres contributing one or a few impulses to each burst. The frequency of discharge was often below 1/sec, but occasionally as high as 30/sec.

3. The activity was subject to fluctuations: (1) a rhythm related to the respiratory blood-pressure waves, the discharge being least at each pressure maximum; this activity disappeared when the blood pressure was raised with adrenaline; (2) a rare primary respiratory rhythm, rather insensitive to rise in blood pressure; (3) a cardiac rhythm, in which waxing and waning of activity occurred with each arterial pulse.

4. Asphyxia increased the discharge. Readmission of air into the lungs caused an abrupt standstill of activity, which was resumed as the asphyxial rise in blood pressure subsided.

GREEN, J. H. (1954). A manometer. *J. Physiol.* **125**, 4–6*P*.

GUYTON, A. C. & HARRIS, J. W. (1951). Pressoreceptor-autonomic oscillation: probable cause of vasomotor waves. *Amer. J. Physiol.* **165**, 158–166.

IGGO, A. (1957). Gastro-intestinal tension receptors with unmyelinated afferent fibres in the vagus of the cat. *Quart. J. exp. Physiol.* **42**, 130–143.

IGGO, A. & VOGT, M. (1959). The effect of reserpine on the electrical activity in preganglionic sympathetic fibres. *J. Physiol.* **147**, 14–15*P*.

LANDGREN, S., NEIL, E. & ZOTTERMAN, Y. (1952). The response of the carotid baroreceptors to the local administration of drugs. *Acta physiol. scand.* **25**, 24–37.

MUSCHOLL, E. & VOGT, M. (1958). The action of reserpine on the peripheral sympathetic system. *J. Physiol.* **141**, 132–155.

PALME, F. (1944). Zur Funktion der branchiogenen Reflexzonen für Chemo- und Pressoreception. *Z. ges. exp. Med.* **113**, 415–461.

TRENDELENBURG, U. & GRAVENSTEIN, J. S. (1958). Effect of reserpine pretreatment on stimulation of the accelerans nerve of the dog. *Science*, **128**, 901–903.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol.* **123**, 451–481.

J. Physiol. (1962), **161**, *pp.* 62–72
With 5 text-figures
Printed in Great Britain

THE MECHANISM OF ADRENALINE-INDUCED INHIBITION OF SYMPATHETIC PREGANGLIONIC ACTIVITY

BY A. IGGO AND MARTHE VOGT

From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge and the Department of Physiology, University of Edinburgh

(*Received* 28 *August* 1961)

When the blood pressure is raised the efferent discharge in many sympathetic nerve fibres is reduced and may disappear. This has been inferred from experiments on vascular reflexes (Heymans & Ladon, 1924) and established by direct electrical recording of impulses in both pre- and post-ganglionic sympathetic fibres, originally by Adrian, Bronk & Phillips (1932). Bronk, Ferguson & Solandt (1934) demonstrated that the inhibition could arise reflexly from the carotid sinus; the greater part of the effect can be abolished by cutting the carotid sinus nerves and the depressor nerves (Pitts, Larrabee & Bronk, 1941; Alexander, 1945), or the vagi and the sinus nerves (Gernandt, Liljestrand & Zotterman, 1946). There is a variable susceptibility to inhibition by a rise in blood pressure among different preganglionic fibres (Iggo & Vogt, 1960) which may be related to differences in function. Two principal methods have been used to raise the blood pressure in previous investigations, either the injection of pressor drugs or the mechanical obstruction of the aorta. Recently Marguth, Raule & Schaefer (1951) reported that intravenously-injected adrenaline was more effective than aortic obstruction in depressing sympathetic discharge, even when adrenaline caused a smaller rise in the blood pressure. This result suggested the possibility that adrenaline may inhibit sympathetic discharge not only on account of its pressor activity but also by some additional action either on the brain and spinal cord or directly on the baroreceptors. This latter possibility is suggested by the work of Palme (1944) and of Landgren, Neil & Zotterman (1952), which has shown that adrenaline, and also noradrenaline and Pitressin, can enhance the responsiveness of the baroreceptors when they are painted on the carotid sinus. However, the high concentrations of these substances required to alter the response of the baroreceptors raise some doubt as to whether intravenous injections have such a direct action. There is also some doubt about an action of adrenaline on the central nervous system, since intracarotid injections were less effective and had a longer latency of action than had

intravenous injections of the same amounts (Fischer, Raule & Seraphin, 1955). However, an action of adrenaline divorced from its pressor effects was postulated by the latter authors to account for the reduction in sympathetic discharge following the injection of adrenaline into adrenalectomized animals, in which adrenaline lacked its usual pressor activity. The theory was put forward that adrenaline acts directly on the spinal cord.

The present experiments were designed to compare quantitatively the relative effectiveness of injections of adrenaline and of other methods of raising the blood pressure in reducing sympathetic efferent activity. Observations were made before and after denervation of the baroreceptors and chemoreceptors. The results do not indicate that adrenaline has any potent inhibitory activity unrelated to its pressor effects.

METHODS

Experimental procedure. The six cats used were anaesthetized with ether, after a subcutaneous injection of atropine (0·25 mg/kg); anaesthesia was continued with an intravenous injection of chloralose (80 mg/kg) and supplemented with smaller doses of chloralose at intervals of several hours, as required. The arterial pressures were recorded from the femoral or brachial arteries by a mercury manometer recording on a kymograph paper, and simultaneously by an electrical transducer manometer recorded photographically with a double-beam oscilloscope. The second beam of the oscilloscope was used to display the amplified preganglionic action potentials picked up from small strands dissected from the cervical sympathetic nerve. The methods were the same as those used previously (Iggo & Vogt, 1960). All the injections were made into the right femoral vein and were washed into the circulation with 0·9 % NaCl solution. The substances used for injection were L-adrenaline (Burroughs Wellcome) 5–100 μg, L-noradrenaline (levo-Arterenol dextro-bitartrate, Bayer Products Ltd, dose expressed in weight of the base) 1–5 μg and vasopressin (Pitressin, Parke Davis and Co.) 2–6 i.u. To expand the circulatory volume blood was infused which had been collected from a donor cat by bleeding under ether into a flask containing heparin.

In two experiments the abdominal aorta was dissected free in order to occlude it when required with a polythene 'choker' (Hume & Nelson, 1954). In the first experiment it was approached through the thorax. In the second experiment, which was more successful because it avoided the need for artificial respiration, the aorta was exposed in the abdomen and 'chokers' were placed around it, one above the coeliac artery and one just below the superior mesenteric artery.

Denervation of the arterial baroreceptors and chemoreceptors. The carotid sinus nerves were dissected and identified near their entry into the sinus, and the depressor nerves were dissected out where they leave the superior laryngeal nerves. This approach may have left intact the baroreceptor innervation of the common carotid artery (Green, 1953). The cervical vagi were exposed high in the neck several millimetres below the origin of the superior laryngeal nerve. The nerves were cut in the following order: sinus nerve and depressor nerve on one side, then in the same order the corresponding nerves on the other side, and then the cervical vagi. Since atropine was injected subcutaneously at the beginning of each experiment there was no change in heart rate on vagotomy and one possible complicating factor in comparing the pre- and post-denervation activity of the sympathetic system was avoided.

Figures. The values for sympathetic efferent discharge in the graphs were obtained by measuring the total outflow during intervals of at least 5 and usually 10 sec or longer. This

was necessary because of the irregular nature of the discharge. When possible, the activity was measured for time intervals which corresponded to multiples of the respiratory cycle. This was done because the discharge frequently showed a respiratory rhythm. In the graphs the sympathetic activity is expressed as a percentage of the resting activity.

RESULTS

The activity of the sympathetic system was sampled by recording electrically from fine strands dissected from the cervical sympathetic nerve in the mid-cervical region and choosing a strand which contained a number of active sympathetic fibres (Fig. 1). The type of activity has been reported previously (Iggo & Vogt, 1960). The basal blood pressure in most experiments was 110–130 mm Hg when recorded with the mercury manometer.

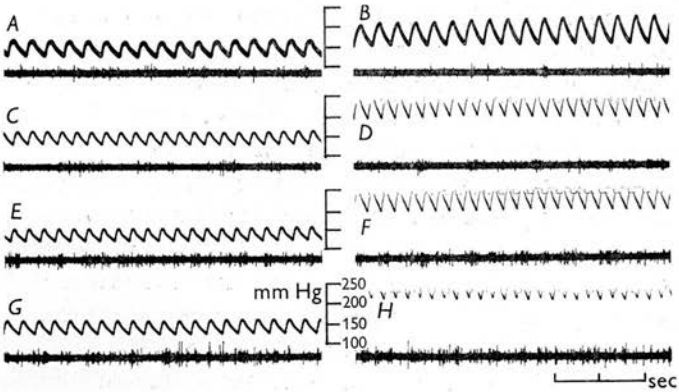


Fig. 1 (Cat 3). The effect of progressive denervation of the major baroreceptor and chemoreceptor regions on electrical activity in preganglionic sympathetic fibres of the cervical sympathetic nerve. The upper tracing in each record shows the femoral arterial blood pressure and the lower tracing the impulses in a strand of the cervical sympathetic nerve. *A*, intact cat; *B*, after the injection of 1·5 μg noradrenaline; *C*, basal activity after cutting the carotid sinus and depressor nerves bilaterally; *D*, injection of 5 μg noradrenaline; *E–H*, after cutting both vagi; *F*, injection of 5 μg noradrenaline and *H*, injection of 100 μg adrenaline. In *H* the blood pressure has risen to such a high level that the systolic pressure is off the top of the paper.

Comparison of different pressor agents

Intact cats. The following methods of raising the blood pressure were compared: intravenous injection of adrenaline, noradrenaline and Pitressin, transfusion of blood, and temporary obstruction of the abdominal aorta. The three drugs have in common that they increase vascular tone and that their local application sensitizes the sinus region to rises of blood pressure; no such effects are produced by the mechanical means used for raising the

blood pressure; these procedures distend rather than constrict the vessel walls while the pressure is rising.

If a comparison among different pressor agents is to have any value, it is necessary first to establish whether repetition of the same procedure gives reproducible results. Adrenaline (10 μg) was injected four times in one animal with the following increases in arterial pressure from the same base line: 63, 65, 66 and 68 mm Hg. The efferent activity was reduced by 65, 86, 80 and 40 % respectively. This variability in reduction of sympathetic discharge rules out any precise comparisons of the potency of different pressor agents.

In Fig. 2 the reduction in efferent activity, expressed as a percentage, is plotted (open signs) against the peak amplitude of the blood pressure change following the injection of adrenaline, noradrenaline and Pitressin in cat No. 3. On the whole, the greater the rise in blood pressure the greater was the inhibition of activity. There is no indication that adrenaline was more potent than either noradrenaline or Pitressin. The results of a similar, more detailed, comparison of intravenous adrenaline and aortic occlusion in cat No. 6 are shown in Fig. 3. Over a range of pressure rises of 20–105 mm Hg there was no indication that adrenaline was more potent than occlusion of the aorta. The time course of the changes in arterial pressure and in sympathetic activity following the injection of adrenaline and aortic obstruction are shown in Fig. 4. The blood pressure change was more abrupt with occlusion, and in some trials the arterial pressure was falling again at a time when the pressor response to an infusion of adrenaline had not yet reached its peak. This difference in the rate of change of pressure may account for the partial recovery of efferent discharge sometimes seen early during the occlusion and occurring only later after an injection of adrenaline. However, after prolonged (2–5 min) elevation of the blood pressure, by continued infusion of adrenaline or by persistent obstruction of the abdominal aorta, the inhibitory strengths of the two procedures were similar (Fig. 4).

When the attempt was made to raise the blood pressure by transfusing blood taken from a donor cat the results were not really comparable with the other procedures. Even after the infusion of 120 ml. of blood the arterial pressure did not rise higher than 145 mm Hg from a basal level of 115 mm Hg. Furthermore, the rate of rise of arterial pressure was slow compared to the response to intravenous adrenaline. The first infusion of 20 ml. of blood raised the arterial pressure from 115 to a peak of 140 mm Hg over 3 min and there was a further brief rise of 4–5 mm Hg with each additional 20 ml. of blood to give a final value of 145 mm Hg. The sympathetic discharge was reduced to 45 % with a 30 mm rise in blood pressure after the infusion of approximately 50 ml. of blood, whereas a similar

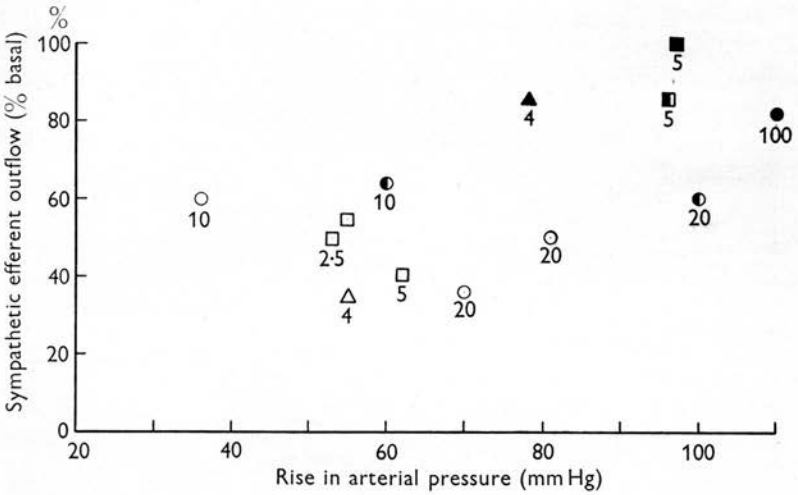


Fig. 2 (Cat 3). The reduction in sympathetic efferent activity by different pressor agents. The peak in arterial pressure above the basal level is plotted along the abscissa and the efferent activity expressed as a percentage of the resting level is plotted on the ordinate. The open symbols refer to the intact animal, the half-filled symbols to trials with the sinus and depressor nerves cut bilaterally, and the filled symbols to trials with both vagi also cut. The figures under the symbols indicate the dose of catecholamines (μg) and of Pitressin (i.u.). ○ adrenaline; □ noradrenaline; △ Pitressin.

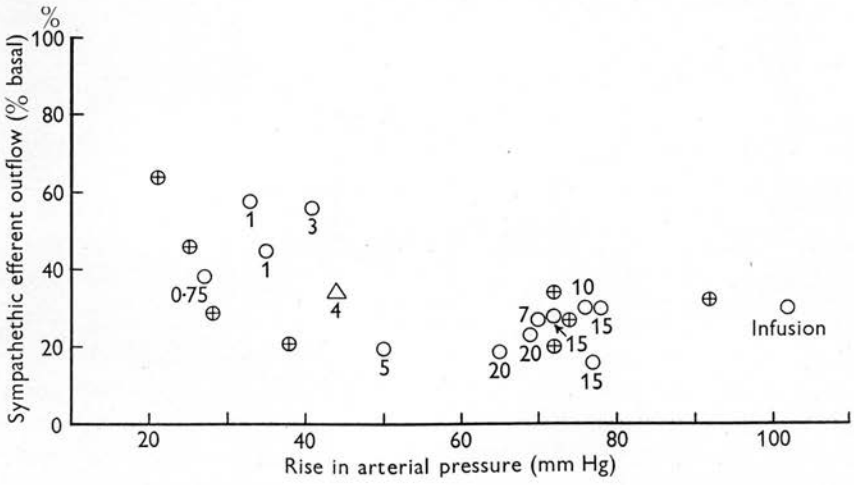


Fig. 3 (Cat 6). Comparison of the effects of adrenaline, Pitressin and aortic occlusion in a cat with the buffer nerves intact. Results plotted in the same way as Fig. 2. The figures under the symbols indicate the dose of adrenaline (μg) and of Pitressin (i.u.). ○ intravenous adrenaline; △ intravenous Pitressin: ⊕ occlusion of abdominal aorta.

reduction in activity after 10 μg adrenaline required about twice the rise in arterial pressure in the same cat. During 20 min of elevated blood pressure, caused by transfusion, the sympathetic efferent discharge returned slowly to about 60 % of the original value. After the blood transfusion the contour of the blood pressure pulse was flattened, the systolic peak being prolonged to a plateau (Fig. 5*d*). This would lead to a more

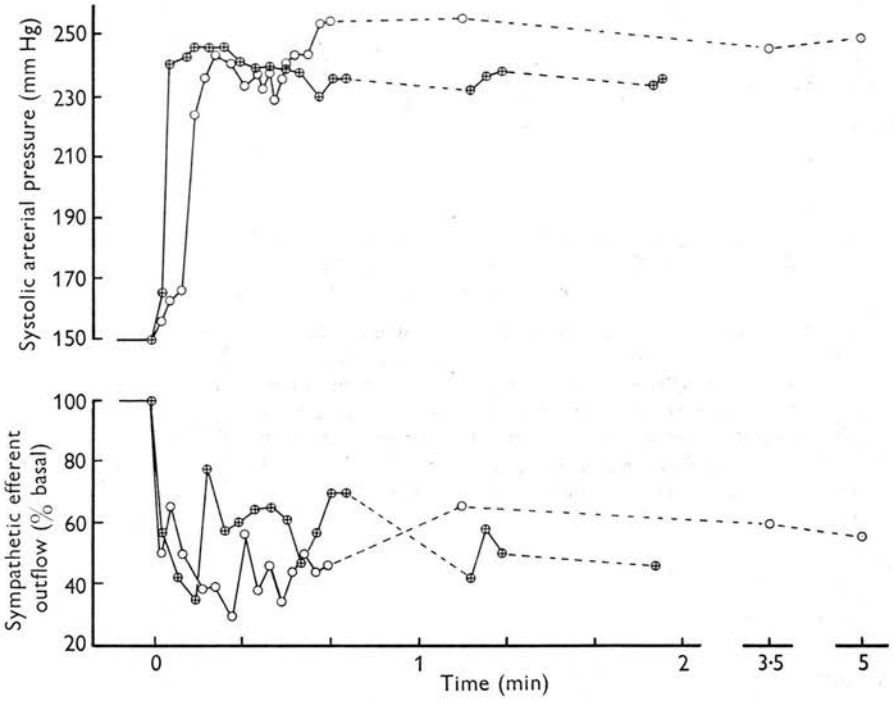


Fig. 4 (Cat 6). The effect of (*a*) an intravenous infusion of adrenaline, ○—○, and (*b*) prolonged aortic occlusion, ⊕—⊕, on the brachial arterial pressure (upper curves) and the cervical sympathetic activity, expressed as a percentage of resting activity (lower curves). A total of 70 μg adrenaline was infused over a period of 5 min.

persistent high-frequency inflow of impulses from the baroreceptors and may account for the greater inhibitory action of the transfusion at a lower mean level of blood pressure. Transfusion after cutting the carotid sinus and depressor nerves and the cervical vagi did not inhibit the sympathetic discharge, although a similar flattening of the arterial pressure pulse occurred.

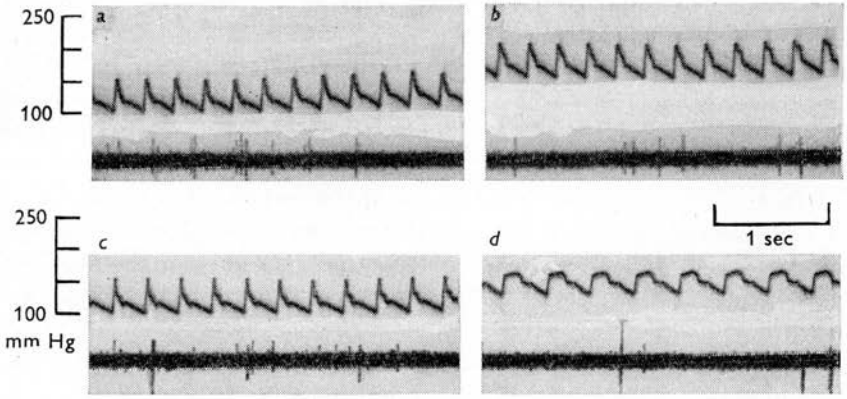


Fig. 5 (Cat 4). A comparison of the changes in contour of the arterial pressure wave following the I.V. injection of adrenaline and the transfusion of blood. Records show (*a*) before and (*b*) after the injection of 10 μg adrenaline; (*c*) before and (*d*) after the transfusion of 50 ml. blood. Note the prolonged systolic plateau in (*d*). The transfusion was as effective in reducing the sympathetic outflow (lower tracing in all records) as the I.V. adrenaline, even though the rise in arterial pressure was less. The B.P. calibrations are at the left-hand side. Time (1 sec) applies to all records.

Effect of denervation of baroreceptors and chemoreceptors

These experiments were done primarily in order to see whether adrenaline exerted an effect on sympathetic outflow by means other than by an action through the baroreceptors and chemoreceptors. They also gave some additional information on the afferent pathways involved in the depression of sympathetic activity.

To test the completeness of the carotid sinus denervation after section of the sinus nerve the effect on the arterial blood pressure of occluding the common carotid artery was examined. Only in one of the four cats was there, on one side, a small residual rise in blood pressure when the common carotid artery was clamped after cutting the nerve.

After each of the nerves (carotid sinus nerve, depressor nerve, cervical vagus) was cut there was a rise in systemic arterial pressure which persisted for different times in the various animals. In the experiment illustrated in Fig. 1 the blood pressure fell almost to the initial level a few minutes after each nerve was cut. The largest and best sustained rises of arterial pressure after denervation were obtained in the animal in which the blood volume had been expanded by a transfusion of 120 ml. of blood. In this cat the pressure was 140 mm Hg after transfusion and just before denervation, and it became steady at 210 mm Hg after cutting the sinus nerves, depressor nerves and the cervical vagi.

The effects of total baroreceptor and chemoreceptor denervation on sympathetic activity were striking. As is shown in Fig. 1 there was a dramatic increase in the sympathetic outflow (compare Fig. 1*A* and *E*). In some animals there was a large change after cutting only the sinus and depressor nerves, but in the experiment illustrated in Fig. 1 the largest increase appeared only when the vagi were cut.

There was also a variable disturbance of the response to experimentally induced hypertension. In the experiment shown in Fig. 1 the basal efferent activity did not increase substantially on cutting the sinus and depressor nerves (compare Fig. 1*A* and *C*) yet the inhibition by a rise in blood pressure clearly seen in section *B* (before denervation of carotid sinus and aortic arch) was negligible in section *D* (after denervation). This was so in spite of the fact that 5 μg noradrenaline instead of 1·5 μg was injected and the rise in blood pressure therefore was 50 mm Hg higher than before. In other experiments the basal efferent discharge was enhanced by cutting the sinus and depressor nerves and the sympathetic activity was strongly inhibited when the blood pressure was raised. In all animals, however, the suppressing action of hypertension on sympathetic discharge was almost completely absent after cutting all the nerves (sinus nerves, depressor nerves and cervical vagi). Very high levels of blood pressure failed to depress the sympathetic discharge in cats with completely denervated baroreceptors and chemoreceptors, as seen in Fig. 1*H* and Fig. 2 (black signs). Of particular importance is the failure of adrenaline, even at a dose of 100 μg, to reduce sympathetic outflow.

In its effect on basal sympathetic discharge, baroreceptor and chemoreceptor denervation resembled somewhat the effect of 'chemical sympathectomy' produced by prolonged treatment with reserpine (Iggo & Vogt, 1960). The discharge was more continuous and lacking in respiratory rhythm, and some individual cells discharged impulses very regularly. Whereas in both conditions there was unimpaired sensitivity to stimuli such as sudden noise, the responses to experimentally induced changes in blood pressure were quite different. After section of the carotid sinus nerves, depressor nerves and vagi there was no response in contrast to the very brisk response in the reserpine-treated cat. In the latter the reflex arc was, of course, intact except for the lack of transmitter at the postganglionic nerve-endings.

DISCUSSION

The results described in this paper confirm earlier reports that a temporary rise in blood pressure will suppress most of the efferent sympathetic activity in intact cats. This suppression depends principally on the extent and the rate of the rise in pressure, and secondarily on factors such as the

depth of anaesthesia. It is absent after denervating the major arterial baroreceptor and chemoreceptor pathways. Four of the five methods used to raise the arterial pressure in the present work were, as far as could be judged, equally potent in reducing sympathetic discharge. The size of the blood-pressure change, rather than the nature of the pressor agent, seemed to determine the response. The exception was provided by blood transfusion, with which, however, the changes in pressure were gradual and the blood-pressure pulse had a prolonged and flattened systolic peak. It proved more potent than the other methods in relation to the pressure level achieved, possibly because of the change in shape of the arterial-pressure wave.

The present results do not support the view that adrenaline has a direct central inhibitory action on sympathetic discharge. The possibility that a central action was masked by the more powerful reflex response to hypertension is ruled out by the finding that, after denervating the major baroreceptor and chemoreceptor pathways, adrenaline, even in large doses, was just as ineffective as the other pressor agents. However, this argument would be invalid if the supposed central action of adrenaline were exerted on the intracerebral or spinal part of these very pathways and would therefore disappear when the peripheral part of the reflex arc is interrupted.

The possibility remains that high local concentrations of adrenaline somewhere else in the central nervous system may inhibit sympathetic activity; this idea we did not think worth testing, seeing that intravenously administered adrenaline penetrates only very slightly into the brain.

An important role of an increase in the sensitivity of the baroreceptors was unlikely because, as already mentioned, only high local concentrations of pressor substances produce an inhibitory effect. Furthermore, such a role was practically ruled out by the finding that, for the same increment in blood pressure, adrenaline was no more potent an inhibitor of sympathetic outflow than aortic occlusion, which does not alter baroreceptor sensitivity.

Another factor which may participate in the pressor-induced inhibition of sympathetic outflow could be a reduction in chemoreceptor discharge. Diamond & Howe (1956) have shown that the chemoreceptor discharge may be reduced by a rise in blood pressure when the arterial pressures are below 100 mm Hg and the animal is therefore suffering some degree of anoxia. The reduction is presumably due to the increased blood flow through the carotid and aortic bodies. Landgren (1958), in addition, has shown that even when a cat is breathing 100 % oxygen, and the blood is presumably saturated with oxygen, there may be a chemoreceptor discharge at very low arterial pressures, which disappears when the blood

pressure is raised. Such a fall in chemoreceptor discharge would reflexly diminish sympathetic outflow. It is unlikely to have occurred in the present experiments, in which the basal blood pressures were between 110 and 130 mm Hg, and the cats showed no signs of anoxia. However, in the absence of detailed information, this question must be left open.

The difference between these findings and those of Marguth *et al.* (1951), who consider adrenaline a more potent inhibitor of sympathetic activity than aortic occlusion, may possibly be caused by the different time course of the two responses and the fact that some degree of adaptation occurs with time. If only the degree of inhibition at 14 sec, for example, had been examined in the experiment of Fig. 4, adrenaline would have scored over aortic occlusion, but the reverse holds for the state at 115 sec, and when the experiment is viewed as a whole the effects are very similar. We therefore conclude that adrenaline, like the other pressor agents, inhibits the sympathetic outflow principally by a reflex action through the arterial baroreceptors.

The contribution to sympathetic stabilization provided by vagal afferent fibres other than the arterial baroreceptors cannot be gauged accurately by the present experiments. It is clear that although pressor doses of adrenaline and noradrenaline might be ineffective in inhibiting the activity of the sympathetic centres after bilateral denervation of the carotid sinuses and the aortic arch as in Fig. 1*D*, yet there were, in the cervical vagi, additional afferent fibres with a powerful inhibitory action. This action can be inferred from the effect of cutting both vagi (Fig. 1*E–H*), which released still further the basal efferent sympathetic discharge.

SUMMARY

1. Preganglionic sympathetic activity was recorded from strands of the cervical sympathetic trunk in cats anaesthetized with chloralose.

2. The effect of raising the blood pressure by intravenous injections of adrenaline, noradrenaline or Pitressin, and by transfusion of blood or temporary occlusion of the aorta was examined before and after section of the sinus nerves, depressor nerves, and cervical vagi.

3. For the same rise in blood pressure, aortic occlusion and injection of the three hormones were indistinguishable in their inhibitory effects on sympathetic preganglionic discharge. All effects were abolished by cutting the carotid sinus and depressor nerves and the cervical vagi. The finding that adrenaline is no more potent than aortic occlusion in inhibiting sympathetic discharge is at variance with observations reported in the literature, perhaps because the different time course of the effects has not always been taken into consideration.

4. Expansion of the circulatory volume caused greater inhibition per increment in blood pressure than injection of adrenaline, but the pressor effects were so slow in developing that a valid comparison cannot be made.

REFERENCES

ADRIAN, E. D., BRONK, D. W. & PHILLIPS, G. (1932). Discharge in mammalian sympathetic nerves. *J. Physiol.* **74**, 115–133.

ALEXANDER, R. S. (1945). The effects of blood flow and anoxia on spinal cardiovascular centers. *Amer. J. Physiol.* **143**, 698–708.

BRONK, D. W., FERGUSON, K. L. & SOLANDT, D. Y. (1934). Inhibition of cardiac accelerator impulses by the carotid sinus. *Proc. Soc. exp. Biol., N.Y.*, **31**, 579–580.

DIAMOND, J. & HOWE, A. (1956). Chemoreceptor activity in the aortic bodies of the cat. *J. Physiol.* **134**, 319–326.

FISCHER, T., RAULE, W. & SERAPHIN, R. (1955). Über die Spontantätigkeit sympathischer Herznerven in Abhängigkeit von Narkose, Blutdruckänderungen, Atmung und Adrenalin. *Pflüg. Arch. ges. Physiol.* **262**, 72–91.

GERNANDT, B. E., LILJESTRAND, G. & ZOTTERMAN, Y. (1946). Efferent impulses in the splanchnic nerve. *Acta physiol. scand.* **11**, 230–247.

GREEN, J. H. (1953). A new baroceptor area in the cat. *J. Physiol.* **122**, 70*P*.

HEYMANS, C. & LADON, A. (1924). Sur le mécanisme de la bradycardie hypertensive et adrenalinique. *C.R. Soc. Biol., Paris*, **90**, 966–969.

HUME, D. M. & NELSON, D. H. (1954). Adrenal cortical function in surgical shock. *Surg. Forum*, 568–575.

IGGO, A. & VOGT, M. (1960). Preganglionic sympathetic activity in normal and in reserpine-treated cats. *J. Physiol.* **150**, 114–133.

LANDGREN, S. (1958), cited by HEYMANS, C. & NEIL, E. in *Reflexogenic Areas of the Cardiovascular System*, p. 186. London: Churchill.

LANDGREN, S., NEIL, E. & ZOTTERMAN, Y. (1952). The response of the carotid baroceptors to the local administration of drugs. *Acta physiol. scand.* **25**, 24–37.

MARGUTH, H., RAULE, W. & SCHAEFER, H. (1951). Aktionsströme in zentrifugalen Herznerven. *Pflüg. Arch. ges. Physiol.* **254**, 224–245.

PALME, F. (1944). Zur Funktion der brachiogenim Reflexzonen für Chemo- und Pressoreception. *Z. ges. exp. Med.* **113**, 415–461.

PITTS, R. F., LARRABEE, M. G. & BRONK, D. W. (1941). An analysis of hypothalamic cardiovascular control. *Amer. J. Physiol.* **134**, 359–383.