

## THE EXCITABILITY OF THE MESENTERIC NERVE ENDINGS IN THE TOAD

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It is a well-known fact that quaternary ammonium bases can increase the excitability of motor nerve endings, and thereby set the innervating muscle into fibrillation<sup>2)</sup>, and especially, they have been reported to be highly sensitive to the so-called slow motor system<sup>18)</sup>; the effective concentration for frog's geniohyoideus muscle being  $10^3$ - $10^4$  times lower than for the fast motor system. As to the somatic sensory nerve<sup>16)</sup>, it has been observed at our laboratory that as the concentration of the drugs is raised impulse discharges are evoked in the order of C,  $\delta$  and  $\beta$ . Further the general tendency was found that the thinner the size of fiber, the lower the threshold to the ammonium bases. The author intended to test the adoptability of this law to the visceral sensory nerve.

### METHODS

*Animals.* Experiments were carried out on Japanese toads (*Bufo vulgaris formosus*) weighing 250-350 gr. In most cases, excised viscera were used, but in a few cases unexcised viscera in situ were tested for contrast. The cutaneous nerve-skin preparation of the frog was used for comparison with the splanchnic nerve. The room temperature was kept at 18°-25° C. The nerve-viscera preparation. The both side greater splanchnic nerves were picked out with the viscera from the belly, their branches being cut off at their junctions with the sympathetic chain. Sometimes the nerve preparation was provided with only the mesentery. For the purpose of comparing with the frog's skin-nerve preparation, a piece of the skin at the tibial region with its nerve was excised.

*Recording apparatus.* For recording the nerve impulses, a pair of silver-silver chloride electrodes and a four-stage C-R coupling amplifier of overall time constant 50 msec., and a cathode ray oscillograph were used.

*Stimulation.* Mechanical and chemical stimulations were applied. The former was made by touching with a glass thread and scraping, pricking with a glass rod, while distension of intestine was made by blowing air into a balloon inserted in the intestine, and the tension was measured with a mercury manometer. For measurement of chemical excitability of the receptors, the quaternary ammonium bases such as  $\text{NH}_4\text{Cl}$  and tetra-ethylammonium bromide (T.E.A.) were applied on the preparations step by step as their concentration was

lowered. Other chemical substances such as acid (HCl and acetic acid), acetylcholine, epirenamine, atropine and histamines were employed.

#### RESULTS

##### 1. *The effects of mechanical stimuli*

In most cases, there was found spontaneously no obvious impulse discharge at rest when the mesentery-splanchnic nerve preparation was fixed on a rubber base. But in a few preparations, monophasic triangular waves of  $30\ \mu\text{V}$  were observed in a frequency of 2-3 per minute. It was thought that these discharges were caused by irritation due to the preparation procedure. These spontaneous discharges were absent in the majority of the experiments and this fact made it very convenient to detect the discharges elicited by mechanical stimuli. Thus when the nerve endings or receptors were stimulated by glass rod stroking and scraping, two kinds of impulses,  $60\ \mu\text{V}$  and  $30\ \mu\text{V}$ , were observed during and after the stimulation.

##### a) *Two kinds of impulses*

The impulses could be differentiated into two types. One had the higher amplitude of  $60\ \mu\text{V}$  and rather of shorter duration of 2-3 msec., which was monophasic; the other had the lower amplitude of  $30\ \mu\text{V}$  with longer duration of 4-5 msec., which was also triangular monophasic, as shown in Fig. 1. Therefore, it may be said that the former was the fast conducted impulse and the latter the slow conducted.

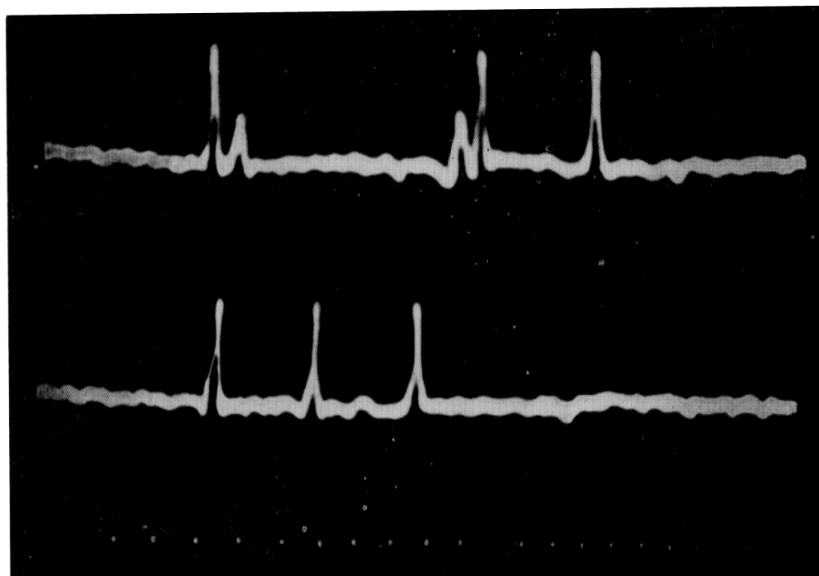


FIG. 1. Upper record shows two kinds of impulses;  $60\ \mu\text{V}$  and  $30\ \mu\text{V}$  which were evoked by glass rod stroking of the mesentery. Lower record shows only fast impulses,  $60\ \mu\text{V}$ , which were evoked by touching with glass thread. Time marks 200 c/s.

Though other mechanical stimuli were employed, no other potential than these two were detected from the mesentery. On the other hand, when the frog's skin was stroked with the glass rod, the impulse discharges led from the cutaneous nerve could be divided into three groups of potentials; of  $240\ \mu\text{V}$ , of  $90\ \mu\text{V}$ , and of  $30\ \mu\text{V}$  (Fig. 2). It may thus be considered that in the toad's splanchnic nerve there are no  $\beta$  impulse discharges amounting to  $240\ \mu\text{V}$ , the highest potential, which can be led from the mammalian mesentery and the frog's skin preparation.

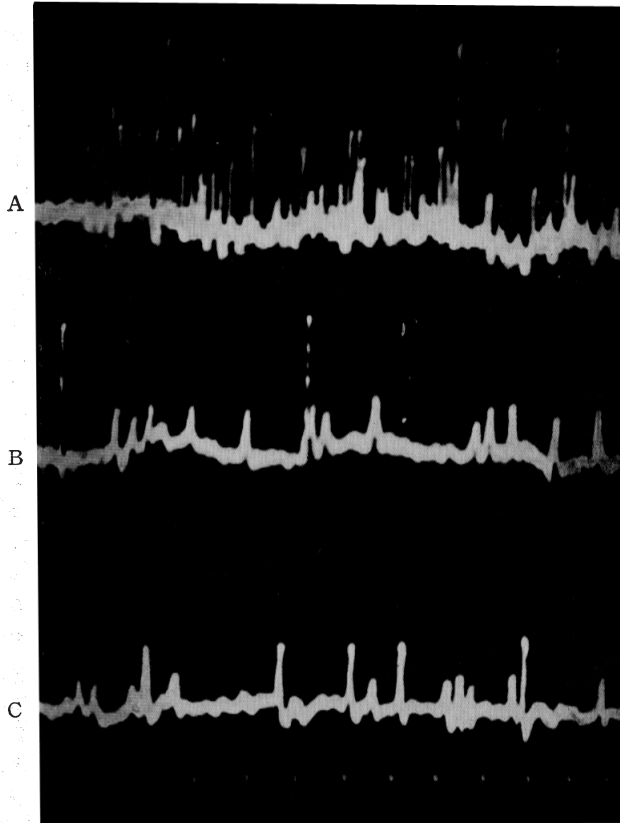


FIG. 2. The nerve impulses in response to stroke on the toad's mesentery with a small glass rod (C) are compared with those led from frog's cutaneous nerve (A and B). Time marks 200 c/s.

*b) Adaptation to mechanical stimuli*

With the mechanical stimuli, the fast impulse was elicited more selectively by touching with a fine glass thread than by other mechanical stimulations. As shown in Fig. 2, when the impulses were elicited by touching, one or a few appeared immediately after stimulation and lasted for  $1/25$ - $1/50$  sec. Next, when a pressure was applied by placing a weight on the ebonite plate of 10

mm in diameter, slow impulses were observed better than fast ones. In this case, the fast impulses were observed only at the beginning of stimulation, and later the slow impulses appeared continuously of which the adaptation was rather slower and took 7.8 sec. on an average, when the weight was 20 gr (Fig. 3). Though the pressure varied in strength, there was no remarkable change in the adaptation time. When the stimulation was interrupted and repeated as in the case of stroking and scraping, fast impulses as well as slow ones appeared very frequently and continued for a while after the stimulation was arrested.

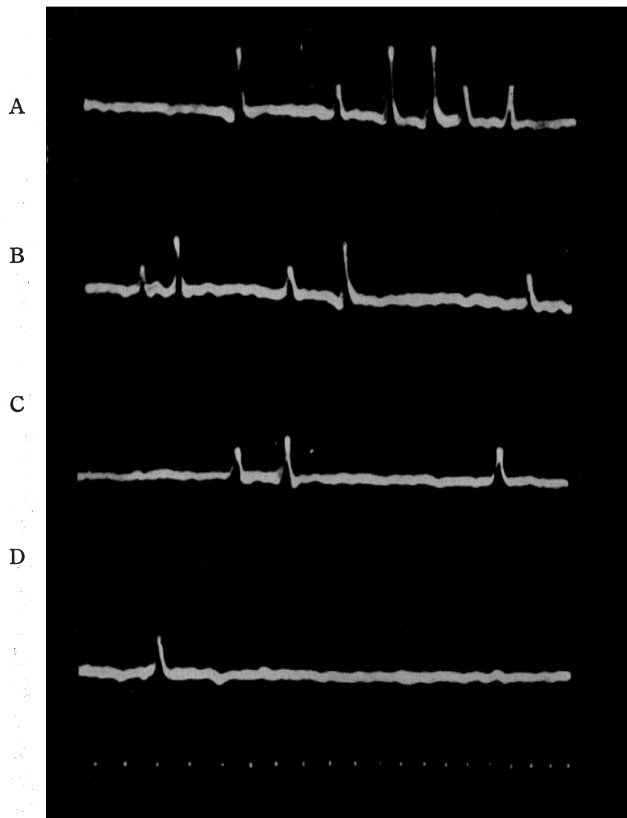


FIG. 3. Adaptation to pressure; A is a record immediately after application of a load of 20 gr having a basies area of 10 mm diameter, and B, C, and D were successively recorded at intervals of 2 sec. later each above respectively, showing that the fast impulse is more rapidly adapting than the slow. Time marks 200 c/s.

*c) Distribution of receptive spots*

The auther searched for receptive, sensitive spots to touch with a glass rod, 0.1 mm in diameter, and the results showed that they were distributed in spot-like manner on the mesentery, hence not diffusely as described by Tower<sup>17)</sup>.

Near the intestinal wall they were located somewhat more densely than at any other part. The schematical diagram of their distribution is shown in Fig. 4. There was found no routine method that could distinguish the receptive spots for fast impulses from those for slow.

Next, the influence of intestinal distension on the frequency of splanchnic nerve impulses was observed. The impulses began to appear when the intestinal pressure reached between 30–50 mm Hg, and their frequency inclined to increase in proportion to the pressure augmented, until it reached about 170–200 mm Hg, when the frequency became maximum. The increased excitability of the receptor was verified also when a very diluted solution of  $\text{NH}_4\text{Cl}$ ,  $10^{-9}$  g/ml. was applied to the mesentery stretched.

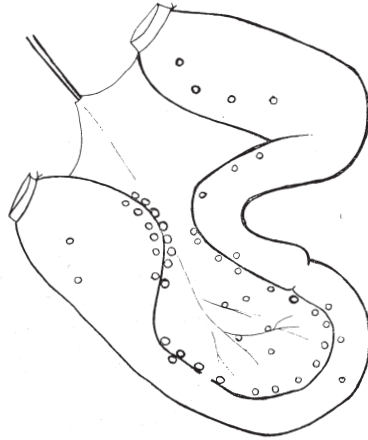


FIG. 4. A schematical diagram of the receptive spots, showing somewhat dense distribution near the intestinal wall, but sparse on the bowel.

## 2. The effect of chemical substances

### 1) The threshold of the receptors to

#### *quarternary ammonium bases and the effect of acetylcholine*

From the receptors the two kinds of impulses were found to be evoked by chemical stimulation. The concentrations of T.E.A. and  $\text{NH}_4\text{Cl}$  sufficient to stimulate these nerve endings were measured. The mesentery-splanchnic nerve preparation was soaked in a constant volume of Ringer's solution in which  $\text{NH}_4\text{Cl}$  was added and the threshold concentration was measured. In this experiment the preparation was made to receive the same concentration throughout.

The results were as follows; slow impulse of  $30 \mu\text{V}$  in spike height appeared at the concentration of  $7.0 \times 10^{-4}$  g/ml on an average and fast impulse of  $60 \mu\text{V}$  at a concentration of  $11 \times 10^{-4}$  g/ml. As the concentration increased, the slow and fast impulses increased in frequency. By such a method, the impulses appearing early tended to fade after a while. This was not due to adaptation but believed to be due to concentration of the medicament attacked to the receptors at the beginning. In consequence the following filter paper method was applied; namely, filter papers containing the medicaments of various concentrations were attached to the mesentery. As a result slow impulses of  $30 \mu\text{V}$  occurred at the concentration of  $1 \times 10^{-4}$  g/ml of  $\text{NH}_4\text{Cl}$ , and fast impulses of  $60 \mu\text{V}$  began to appear together with the slow when the concentration was raised to over  $1 \times 10^{-3}$  g/ml of  $\text{NH}_4\text{Cl}$ . The concentration of T.E.A. was also measured the same as with  $\text{NH}_4\text{Cl}$ . The slow impulse elicited by T.E.A. Ringer began to appear at the concentration of  $2.0 \times 10^{-3}$  g/ml on an average, and its frequency increased in proportion to the concentration. Next, at the concentration of  $6 \times 10^{-3}$  g/ml, fast impulses of  $60 \mu\text{V}$  began to appear together with the slow, but only temporarily. When the filter paper containing T.E.A. of vari-

ous concentrations was placed on the mesentery, the slow impulse began to appear in the concentration range of  $1.0 \times 10^{-4}$ – $2.0 \times 10^{-4}$  g/ml and the fast ones in  $5.0 \times 10^{-4}$ – $6.0 \times 10^{-4}$  g/ml. On increasing the concentration, first the slow impulse was abolished by paralysis in the range of  $10^{-2}$ – $10^{-1}$  g/ml, and next the fast impulse as in the case with  $\text{NH}_4\text{Cl}$ . In some cases with T.E.A., rhythmical discharges were observed, namely at  $10^{-3}$  g/ml; after both impulses continued for some time they developed into bursts for about 10–30 sec, and the pauses between the bursts were 20–30 sec or 1 minute at the longest. The repetition of bursts continued for 2–5 minutes.

The measurement shows that the difference in the concentrations for the slow and fast impulses was about 10 times that with  $\text{NH}_4\text{Cl}$  and about 5 times that with T.E.A. To both stimulants the slow impulse showed lower thresholds than the fast. Furthermore, it is interesting to note that the liminal concentrations of these drugs for evoking both the slow and the fast impulses in the toad's mesenteric nerve were nearly equal respectively to those in the frog's cutaneous nerve. It has been reported by some investigators that acetylcholine enhances the excitability of the motor nerve or the sensory nerve endings. In this experiment, the slow and the fast impulses were observed in  $10^{-4}$ – $10^{-3}$  g/ml of acetylcholine. Below  $10^{-4}$  g/ml both impulses were not evoked constantly, but if  $\text{NH}_4\text{Cl}$  Ringer was added up to  $10^{-5}$  g/ml, these two impulses began to appear in high frequency. It was confirmed that  $\text{NH}_4\text{Cl}$  and acetylcholine act potentiating each other.

## 2) *The effect of histamine*

It was observed that the peristaltic movement of intestines was slightly accelerated by  $\text{NH}_4\text{Cl}$  or T.E.A. in the range of  $10^{-3}$ – $10^{-2}$  g/ml. On the other hand, it is a well known fact that the peristaltic movement is accelerated by histamine, so that histamine was selected for comparison with  $\text{NH}_4\text{Cl}$  and T.E.A. Histamine-2HCl was applied in the range of  $10^{-4}$ – $10^{-2}$  g/ml by dilution with Ringer which is suitable for acceleration of intestinal movement. When the preparation was dipped, the intestinal movement was apparently accelerated and the blood vessels were dilated. But no impulse was detected. As was in case of  $\text{NH}_4\text{Cl}$  or T.E.A., in order to obtain the nerve impulse, the histamine solutions should be further diluted. Next, more diluted solutions of histamine-2HCl and histamine-2 $\text{H}_3\text{PO}_4$  were prepared up to  $10^{-12}$  g/ml. Several small pieces of filter paper were soaked respectively in each one of the diluted solutions, and each piece was put on the preparation.

In the case of histamine-2HCl, the slow impulse was observed with the lower limit of  $10^{-9}$  g/ml. The frequency of the impulses increased in proportion to the concentration, maximum being in the concentration of  $10^{-8}$ – $10^{-7}$  g/ml. Above this concentration, the spike height became gradually smaller, the frequency also decreased, and finally no discharge could be detected at  $10^{-4}$  g/ml. With all these diluted solutions of histamine-HCl the fast impulse could not be observed. In the case of histamine-2 $\text{H}_3\text{PO}_4$ , the slow impulse began to appear at the concentration of  $10^{-10}$  g/ml the same as with histamine-2HCl. Its frequency increased with the concentration and became maximum at the concentration of  $10^{-8}$ – $10^{-7}$  g/ml. Above this concentration, the frequency decreased

gradually, and the discharge disappeared at  $10^{-5}$  g/ml. It was found that there was adiscernible difference in the concentrations, between histamine-2HCl and histamine-2H<sub>3</sub>PO<sub>4</sub>, but the fast impulse was not observed in the range of these concentrations for the slow, if the concentration was increased, the nervous activity became completely paralyzed, because no response was detected by stimulation even with mechanical stimuli.

### 3) *The effect of atropine sulphate and epinephrine hydrochloride*

In the above experiment, the slow impulse was found to be not connected with the contraction of bowel. In this experiment, it was examined whether the impulse was elicited by the relaxation of the bowel or not. When the mesentery-splanchnic nerve preparation with the bowel was treated with 0.5-0.05% atropine sulphate, relaxation of the bowel appeared, but no impulse was observed. With this condition the intactness of the nerve tissue was confirmed in the following way; whether on stimulation with the glass rod, the slow and fast impulses could still apparently be evoked.

When epinephrine hydrochloride was used, apparent contraction was observed in the capillaries, the mesentery becoming pale. Under this condition, it was observed that the impulses were normally evoked by stroking with a glass rod, but no impulse at rest. Thus it was clarified that atropine or epinephrine could not provoke the nervous impulse inspite of producing contraction and relaxation in the bowel, showing that mere contraction or relaxation caused by drugs did not excite the nervous tissue.

### 4) *Effect of acids*

With hydrochloric acid, the slow impulse was evoked at  $3 \times 10^{-5}$  g/ml, liminal concentration. When the concentration was increased step by step to  $10^{-2}$  g/ml, the frequency of the slow impulse increased with concentration, but the fast impulse did not appear.

With acetic acid, the slow impulse was initiated at  $10^{-3}$  g/ml, liminal concentration, and the frequency increased in proportion to the concentration. But the fast impulse appeared to mix with the slow impulse at the concentration of  $10^{-2}$  g/ml. It was confirmed that the receptors for the slow impulse respond easily to these acids as noxious stimuli.

### 3. *Conduction velocity and diameter spectrum of the greater splanchnic nerve fibers*

It was questioned and examined to what groups in Erlanger and Gasser's classification the fibers conducting these two impulses belong. For this purpose, the conduction velocities of two kinds of impulses, the slow and the fast, were measured. As a measuring method, Ito's method<sup>6)</sup> was adopted, and used to measure the conduction velocity of the cutaneous nerve of the rabbit. This method was convenient to measure slower conducted impulses. A brief explanation on this method is as follows; three electrodes were prepared, one earthed, the other two connected to the grid of the first stage of the amplifier. Thus, each impulse could be picked up twice, as it passed two grid electrodes. In the records taken in this way the slow impulse presented two peaks and the fast a notch on the rising phase of the spike (as shown in Fig. 5). Caution

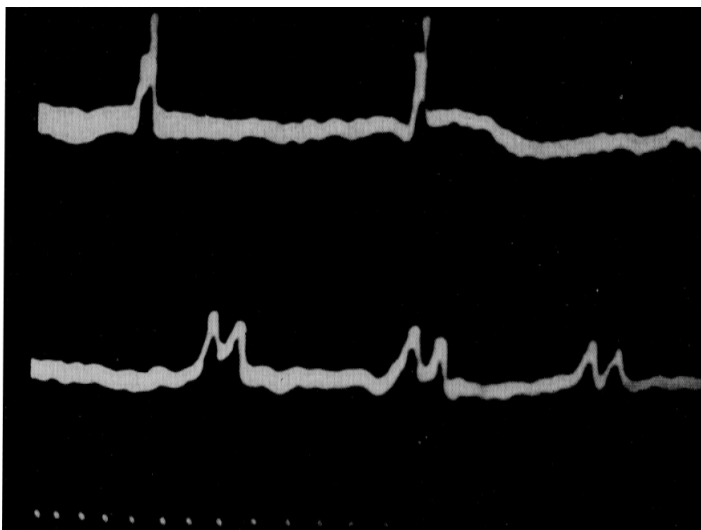


FIG. 5. The action potentials recorded by Ito's method; superimposed potentials showing a notch in the upper tracing of every two fast impulses led through two glid electrodes. Lower tracing shows the slow impulses which have two peaks. Time marks 500 c/s.

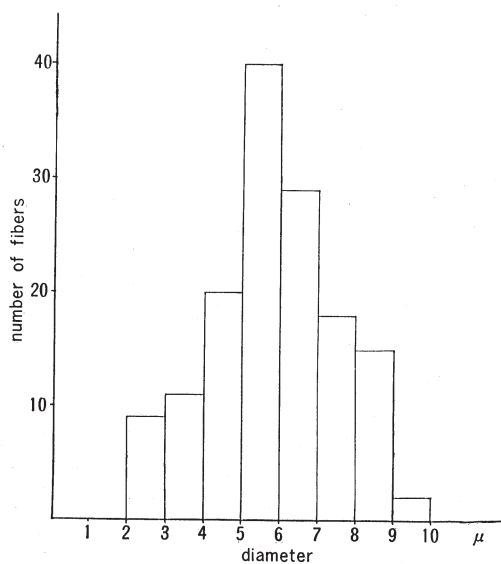


FIG. 6. A fiber diameter spectrum obtained from two medium size filaments in the greater splanchnic nerve, showing a peak at 5-6  $\mu$  and distributing from 2  $\mu$  to 10  $\mu$ .

was paid not to produce impulses too frequently, otherwise their peaks and notches could hardly be distinguished. So, in this case, the frequency of im-



pulse was kept as low as possible. The results at a room temperature of 18° C, showed that the velocity of the slow impulse was 1.3 m/sec and that of the fast 15.6 m/sec. From the above data it was assumed that these slow and fast impulses had respectively velocities corresponding to C and  $\delta$  fiber.

Next, the fiber diameter of the splanchnic nerve was measured. The splanchnic nerve was divided into 4-5 filaments which were put of an average of 46.2-88.2  $\mu$  in diameter. Further these filaments consisted of 40-110 myelinated fibers. A histogram of a medium size filament is shown in Fig. 6. The diameter of myelinated fibers ranged from 2  $\mu$  to 10  $\mu$ , showing a peak at 5-6  $\mu$ , but C fibers could not be counted by means of supravital methylene blue staining.

#### 4. Leading the afferent impulses from the dorsal roots

The question arises if the impulses observed in the above experiment are afferent or not; *i.e.* they might be antidromic impulses in efferent fibers caused by action of the drugs and mechanical stimulations near the end of the fibers. If these impulses are afferent, they will pass through the spinal ganglion, and be conducted along the dorsal roots. But if they are antidromic and elicited by stimuli at the postsynaptic site of sympathetic fibers, they might be blocked at the ganglion coeliacum. Based on the above hypothesis the impulses led from the splanchnic nerve had to be reexamined in respect to afferent nature. The impulses were made to be led from the dorsal roots of the 4th to 7th segments. The method employed was as follows; the toad was fixed in the ventral position, and after laminectomy the dorsal roots were exposed. The roots were then, cut off just at the connection with the spinal cord, the spinal ganglion being provided with the nerve. One of these isolated nerves was put on the electrode, wherefrom the action potentials were recorded. Two methods of stimulation were employed, one with the glass rod stroking through the abdominal window, and the other the injection of 0.1%  $\text{NH}_4\text{Cl}$  Ringer. As results, both the slow and fast impulses could be recorded, just as was seen before.

#### DISCUSSION

It has been generally accepted that visceral sensation is protopathic and lacks the fast conducting fibers for getting information.<sup>17)</sup> In this respect, leaving aside the discussion whether it is protopathic in the toad's mesentery, the physiological aspects of its afferent impulse must be called in question. In this experiment, it was confirmed that the mesenteric nerve endings react to such stimuli as pressure or stretch and chemical agents, and the impulses evoked by these stimuli were divided into two kinds, the slow impulse and the fast impulse. Both are considered to be afferent. However, as the splanchnic nerve contains not only afferent fibers but autonomic efferent ones, one can not exclude antidromic impulses elicited by these drugs. Farthermore, Kure *et al.*<sup>9)</sup> reported on the so called *Spinalparasympathicus*, and recently Kotsuka *et al.*<sup>8)</sup> have said that besides the parasympathic fibers, the sympathetic fibers are contained in the dorsal roots at every level of the spinal cord. But the antidromic impulses can not be transmitted through the spinal ganglion, therefore, if the leading electrode was placed above the spinal ganglion, recorded impulses

should not be contaminated by antidromic ones. Moreover, nicotine was applied to the spinal ganglion, but the results remained unchanged.

The conduction velocities of these slow and fast impulses were found to be 1.3 m/sec and 15.6 m/sec at 18°C, corresponding respectively to C and  $\delta$  fibers in Zotterman's classification of the mammalian sensory nerve fibers, according to the conversion formula;

$$K^{20/10} = \frac{V_{38^{\circ}\text{C}}}{V_{18^{\circ}\text{C}}} \quad (\text{K: temperature coefficient})$$

This assumption may be supported by the fiber diameter spectrum ranging from 2  $\mu$  to 10  $\mu$ .

Nijjima<sup>11)</sup> described that there are two types of receptors in the toad's mesenteric nerve; the rapidly and slowly adapting ones in the mode of adaptation to pressure with a tambour on the mesentery. His findings may be supported with the above author's experiment.

Gernandt and Zotterman<sup>3)</sup> suggested that there are  $\beta$ ,  $\delta$ , and C fibers in the cat's mesenteric nerve. However, Gernandt and Zotterman, and McLeod pointed out that Paccinian corpuscles exist in the cat's mesentery, and therefrom  $\beta$  discharges may be considered to have been led. According to the author's observation, the toad's mesenteric nerve was distributed along the mesenteric arteries and on its way it separated from the arteries and branched off spirally windingly, but the tracing could not be made to any particular endorgan. Explored by mechanical stimulation, the mode of distribution of the receptors was spot-like. Furthermore, from the fact that these receptors were distributed relatively more densely near the intestinal wall than at other parts, it may be easily considered that the function of these receptors is related to the movement of intestine or the stretch of mesentery. The frequencies of both impulses increased as the pressure was raised from 30 mm Hg up to 170 mm Hg by blowing air into the balloon inserted in the intestine. Iggo<sup>4)</sup> and Paintal<sup>13)</sup> reported the existence of receptors on the stomach and the small intestine by recording the action potentials from the vagal nerve, that were named distension sensitive receptors or stretch receptors, and elucidated that these receptors play the role of information of mechanical distension or contraction of the intestine. Besides, Paintal<sup>14)</sup> confirmed the existence of distension insensitive receptor or chemoreceptor in the muscularis mucosa of the stomach and small intestine, and assumed that the role of these receptors may be act to inform the chemical nature of the intestinal contents. Iggo<sup>5)</sup> reported also that there were some receptors that reacted to change in pH by leading from the vagal nerve.

However, in this work, these nerve endings in the toad's mesentery seemed to react not only to mechanical stimuli but also to chemical stimuli. The quarternary amines such as Ach,  $\text{NH}_4\text{Cl}$ , T.E.A. depolarized the sensory nerve endings or receptors as well as the motor nerve endings, as was described by Landgren *et al.*<sup>10)</sup> The threshold concentration of these drugs to evoke impulses in the toad's mesenteric nerve, were  $10^{-3}$ – $10^{-4}$  g/ml with Ach for both impulses, with  $\text{NH}_4\text{Cl}$   $7 \times 10^{-4}$  g/ml for the slow impulse and  $11 \times 10^{-4}$  g/ml for the fast impulse, and with T.E.A.  $2 \times 10^{-3}$  g/ml for the slow impulse and  $6 \times 10^{-3}$  g/ml

for the fast impulse. Jarret<sup>7)</sup> has reported the threshold concentration of Ach to the frog's cutaneous nerve endings to be  $10^{-6}$  g/ml. But the discrepancy between their data and the author's may be considered to lie in the different methods of application of Ach. Furthermore, as reported by Suzuki,<sup>16)</sup> the threshold values for C and  $\delta$  fibers in the frog's cutaneous nerve to the quarternary ammonium bases were in good accord with the above data on the toad's mesenteric nerve. It is interesting that the threshold concentrations demanded for the excitation of C and  $\delta$  fibers, of Ach,  $\text{NH}_4\text{Cl}$ , and allied substances have nearly constant values, respectively. It is a generally well-known fact that histamines and allied substances are irritant and concerned with pain and itching (Rothman<sup>15)</sup> 1943). With histamines and HCl, only the slow impulses were characteristically evoked and no fast impulse was detected. Particularly with histamines, it was found that they excite especially the smallest fibers, C fibers, and that at a very diluted concentration ( $10^{-6}$ - $10^{-10}$  g/ml). This fact may indicate the peculiar excitability of C fiber to histamine. Further the action potential caused by histamine was lowered in spike height as histamine was increased but enlarged in the positive after-potential. But  $\text{NH}_4\text{Cl}$  and histamines in such higher concentrations as would evoke the contraction of the intestine paralysed the nerve endings or the receptors, so afferent impulse can not be detected.

#### SUMMARY

1. The action potentials from filaments of the splanchnic nerve were recorded, when the toad's mesentery was stimulated mechanically or chemically. In the records, there were found two types,  $30 \mu\text{V}$  the slow impulse and  $60 \mu\text{V}$  the fast impulse. The conduction velocities of these impulses were 1.3 m/sec and 15.6 m/sec respectively, so they may be taken to be impulses from C and  $\delta$  fibers.

2. The mode of distribution of the receptive fields was spot-like and tended to be localized more densely towards the intestinal wall. It was demonstrated that the excitability of these nerve endings increased by stretching the mesentery, and both impulses were initiated so these nerve endings may be considered to discharge the impulses set up not only by pain-producing drugs but also by intestinal movement or stretch of the mesentery.

3. By chemical stimulation with quarternary ammonium bases, it has been confirmed that the threshold concentration for slow impulses was lower than for fast ones as was verified in the observation on the frog's skin, but the difference was not so great as in the skeletal muscle.

4. Histamines evoked characteristically only the slow impulses at very low concentration ( $10^{-6}$ - $10^{-10}$  g/ml), of course they could not apparently contract the bowel. At higher concentrations of histamines that could contract the bowel, the nerve endings become paralysed.

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