

## 1. Studies on Action Potential of Intestinal Smooth Muscle in Various Pathological State

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In recent year Bülbring et al first succeeded in recording the membrane potential of the taenia coli of the guinea-pig and subsequent studies by a number of other workers contributed to the elucidation of excitation and conduction mechanism of the smooth muscle cells. However, the majority of such studies were conducted in isolated intestinal segment and little attention appeared to be directed to the physiological or pathological effects of the other parts of the body. The data obtained in such works undoubtedly are quite important, but need critical evaluation, when considering the real mechanism.

Thus, it appears necessary to conduct experiment in whole living animal, not in the isolated intestinal segment.

In the present investigation, the membrane potential of the intestinal smooth muscle *in vivo* under the normal and various pathological conditions was studied.

The abdominal wall of the guinea-pig weighing approximately 400 gr. was opened along the median line under the anesthetized condition in the apparatus specially designed for the observation *in vivo*. And the edge of the incised wall was pulled by strings upwards.

The abdominal cavity of the animal was perfused with the Tyrode solution which was previously warmed at 38°C.

The membrane potential was measured by using a flexibly mounted microelectrode.

In the normal cases, the action potential and the resting potential were 60 to 40 mV and 10 to 40 mV respectively. The duration of each spike was 50 to 150 msec and the discharge frequency was 60 to 180 per minute.

The duration of the series of discharge lasted for 30 seconds to 4 minutes, and the resting period approximately 30 seconds to several minutes.

In the case of peritonitis, during the intermediate stage, the resting potential generally decreased and ununiformity of the spike discharges, prolongation of the duration of each spike and the period of the series of the spike discharge were recognized. In the later stage of peritonitis, the amplitude of each spike tended to become lower.

In strangulation obstruction the finding were almost similar to those observed in

the course of peritonitis.

The anoxia in the intestinal tract appeared to produce initial augmentation of the intestinal movement and subsequent decrease of the resting potential and the irregularity of the action potential. When the intestinal anoxia lasted more than 60 minutes, conductivity and excitability were lowered in all cases.

After cardiac arrest, however, under adequately aerated circumstance, the membrane potential of the intestinal smooth muscle could be restored. This might help to explain a part of mechanism of intestinal automaticity.

## 2. A Study of the Clinical Measurement of Uterine Contraction (The Second Report)

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As the measurement of uterine contraction, the Ballon Method for the inner measurement and the Guardring Labour Gauge with Strain Gauge for the outer measurement were used in our study. It is thought that this Ballon Method is the simplest and the most accurate method for the study of uterine contraction. In order to measure the internal pressure of the uterus, a direct method of inserting a needle in the organ is chiefly used in the United States. Since we reported on the process of uterine contraction last year, we are hereby to make a report on the situation where a woman in labour becomes aware of uterine contraction. We reported previously an average period of contraction lasted 124.3'' in the first period of labour and 108.6'' in the second period. However, in our present study an average period of 105'' was obtained and the women in labour feel the uterus for 55'' namely about a half of the actual contraction.

Between the period of contraction and the period which was recognized there is a correlation of +0.8. There is 13'' between the beginning of contraction and the time when it was recognized. It took 38'' from the end of recognition of contraction to the actual end of contraction.

An intensity of contraction when it was recognized was 11 mmHg and at the moment when the recognition of contraction was disappeared it was 15 mmHg. There are no correlativity between the period of pain and the intensity of contraction and between the time when the recognition was disappeared and the intensity of contraction.

Moreover, we reported a method to show an intensity of uterine contraction mathematically with Planimeter value of contraction-curve in order to show an activity of uterine contraction. We also reported a case of normal delivery last

year. (Average of 140 & fiducial limit of 300-60)

This time we report on the cases of prolonged pregnancy, early or premature ruptures of membranes and breech presentation. In case of prolonged pregnancy the Planimeter value by Ballon Method is same as a case of normal delivery except that contraction suddenly becomes intense 20 minutes before delivery. In case of early or premature rupture of membranes, though contraction is less intense than a case of normal delivery in the first period, it becomes same as a normal delivery in the second period. In case of breech presentation until an hour and twenty minutes before delivery the contraction is same as the normal delivery but later it becomes more intense. It is necessary to study more about the latter cases since there are poor in number. Planimeter value by Ballon Method is obtained by a calculation after delivery for the actual use. "Delivery Observation Machine" should show the changes of Planimeter value automatically. We have manufactured and are now testing such a machine in cooperation with Fukuda Electro for trial.

### 3. Action Potential of the Bowel in Rabbits Subject to cut the Rostral Portion of the Diencephalon

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Recently many observers have mentioned that the cortical area associates with the autonomic nervous system.

We paid attention to the hypothalamus, in which autonomic nervous system exists. We cut the impuls between the cortical area and the hypothalamus and observed the movement of the bowel, especially in duodenum and ileum. The rabbits were anesthetized with uretan and we cut the rostral portion of the diencephalon. Action potential were introduced directly from serous membrane of the duodenum and ileum. The date was obtained from the analysis of spike groups in every minute.

In all cases, amplitude of action potential and duration of group spikes prolonged. We could not find the remarkable exchanges in the number of spikes and spike groups. In these mentioned rabbits, the movement of duodenum and ileum were accelerating.

### 4. The Effect of Pin on the Stomach and Intestine

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The effect of anesthesia and emotion on the motility of the digestive system has been studied by many authors, but surprisingly little information has been obtained, remaining many unsolved problems. In order to cultivate this field, we tried to examine at first the effect of pain on the digestive system of the adult rabbit. The electrical and mechanical responses of the gastrointestinal smooth muscle to a pain stimulus were observed on the animal in situ with or without anesthesia, and a comparison was made between the responses in different anesthetic conditions.

The movement and electrical activity of the stomach and small intestine were usually inhibited by pentobarbital anesthesia and gradually recovered with time after awakening. Under a deep anesthesia almost no response to the stimulus was observed as expected, but after awakening a sustained and marked response was elicited not only in the mechanical and electrical activities of the smooth muscle but also in the intra-abdominal pressure. In majority of the cases there was observed a temporal inhibition of the movement of the intestine and a slight increase in spike height and frequency of the electrogram, suggesting a rise in the resting tension in their consequence. The pain stimulus also caused an increase in the intra-abdominal pressure accompanied by a decrease in the average intra-thoracic pressure. Accordingly, a cause of these pressure changes was presumed to be the lowering of the diaphragm, which was lately confirmed by recording electromyogram of the diaphragm.

## 5. Dysfunction of the Auerbach's Plexus

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In this study we intended to know whether the ganglion free bowel segment could be experimentally produced and a pathophysiologic mechanism of the intestine might be detected.

### 1. Method of experiments;

The injection of iodine derivatives or nerve blocking agents into the intestine through the mesenteric vessels was disappointing. Then a distal segment of the dog colon was perfused for 4 hours with Tyrode's solution as to produce a prolonged anoxic condition. However, from the anatomic point of view, we were con-

fronted with a great difficulty in reconstruction of the impaired drainage vein which resulted in postoperative circulatory disturbances. Hence, the same procedure was applied to the terminal portion of the ileum of the cat. After 4 hours perfusion the clamps are released, the blood flow is immediately reestablished. Six among 15 cats were alive more than one month and their result were evaluated. They did not show no tendency of constipation or obstruction during the first one to two months. In sacrificed animals, the irrigated segments looked quite normal, but showed irregular contractions, if extrinsic stimulations given, that strengthened by physostigmine.

2. Light microscopic observations;

1) Fibrous thickning of the serosa. 2) No particular finding in the smooth muscle tissue. 3) Fibrous thickning of the connective tissue in the submucosal layer. 4) Destruction and regeneration of the villi of the mucosal membrane and marked increasing of the lymphatic follicles. 5) The Auerbach's plexus presents a slight decrease of the ganglionic cell with atrophic cytoplasm and dark stained nucleus without any other definite degenerative change. 6) The conventional silver impregnation technique reveals decreased postganglionic neurites and increased connective fibers.

3. Electronmicrography;

Axons with Schwann cells were less scattered in the perfused area. No disappearance of ganglion cell observed. Electromicrographic density, however, generally increased and irregularity of structures were remarkable.

4. Electromyographic observations;

It was recorded by the suction electrode method. The normal pattern of the terminal ileum of the cat consists of regular slow waves with intervals of 4 to 5 sec and of spike waves, which overlaps on the slow waves and appears when peristalsis passes under the tip of the electrode. If an extrinsic mechanical stimulation is applied to the perfused area, the spike frequency increases far more than that in normal. We might call its condition "TETANUS" that many cells under the electrode become a state of hypermotility. When we compare the perfused portion with the preganglionic denervated segment, the excitability of the latter is transient and does not last long.

Our purpose to produce experimentally an aganglionic segment was not so successful as expected, however, a certain degree of degenerative changes to the intramural nerve system was observed.

## 6. Comparative Studies on the Motility of the Normal, Denervated and Aganglionic Thiry-Loops

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The comparative studies are carried out on the motility of the normal, denervated and aganglionic THIRY-loops of dogs.

The records obtained on the isometric contraction reveal that as for the level of the diastolic pressure or the tonicity in the resting state the aganglionic loop is in the highest rank among others, the normal one in the lowest and the denervated one in the intermediate, whereas as for the pressure developed or the tension developed the ranks are reversed. These facts suggest that the normal intestine contracts far more efficiently than the intestine lacking in some of its nervous elements.

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## 7. The Effect of Vestibular Stimulation on the Electromyograms of the Dog's Stomach

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The effects of vestibular stimulations on the EMG of the stomach were observed with dogs.

The vestibular stimulations we used were as follows:

1. Irrigation of an external auditory canal with 100 cc of 0°C water.
2. Pendular rotation with amplitude of 90 degrees and period of 2 seconds for 5 minutes.
3. Direction-fixed rotation with angular velocity 360 degrees per second for 5 minutes.
4. Electric stimulation (5 volts, 10 miliampere, mesopulse) on bilateral retro-auricular regions for 5 minutes.

We used the method of recording, which Takeda et al. (Prof. Takeda's Surgical Clinic, Osaka University) had designed.

The results obtained were as follows:

1. The irrigation of cold water made prolongation of discharge intervals by about 2-4 seconds or irregular fluctuation for about 10-30 minutes.
2. After the pendular rotation and the direction-fixed rotation, the discharge

intervals prolonged by about 2–4 seconds for about 10 minutes, or fluctuated irregularly for about 30–60 minutes.

3. Electric stimulation caused the irregular fluctuation of discharge intervals for 90 minutes or so.

## 8. Studies on Methods of Electromyogram of the Smooth Muscle

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Various kinds of myographic pattern obtained from the gastrointestinal smooth muscle have been noted by many authors, but many discrepancies are found among them. These differences presumably come from the following reasons;

1. EMG is fairly different due to kinds of animals and organs.
2. Even in the same organ, the electrical excitability is not constant from time to time.
3. The most important factor is the fact that obtained patterns largely depend on the type of electrodes.

These three factors are related to the histoanatomic structure characteristic of the smooth muscle. In comparison with the heart muscle, for instance, each smooth muscle cell is small, interstitial tissue among the muscle is more abundant, electrical conduction velocity is lower and limited to smaller area, and its activity is not as simple but rather complicated.

From the point of clinical application, the methods are demanded to allow steady recording for a long observation and facility of operation.

The purpose of this paper is to discuss following two items.

### ELECTRODES:

1. Ultramicroelectrode method of intracellular lead applied in situ which we have reported in previous papers, is the most valuable measure to record the cell activity, but it is accompanied by technical difficulties and skillfulness.
2. During impaling of the ultramicroelectrode, series of only a few mV of spike heights are recorded for a period of a few minutes. This seems to represent extra-cellular action potentials close to the firing cells.
3. Sometimes intracellular lead is not achieved despite of repeated impaling. In such a case, if the electrode is pressed against the muscle tissue, a small (about 5–10 mV) but steady recording occasionally obtained. The reason for this phenomenon is that, the mechanical pressure produces a decrease in membrane resistance

of the cell, so that the electrical change is effectively picked up extracellularly. The same phenomenon was recently reported by Bortoff.

4. The small tip of the hook electrode is one of the useful methods for EMG recording, however, if it is used with a D.C. amplifier, the recording becomes unstable because of the amplifier itself. Therefore, an A.C. amplifier is usually used with the time constant being limited to the range of 0.01 second. The latter method, however is far from ideal, because of elimination of the significant slow components and recording of only spike discharge which occasionally confused with artifacts. Therefore, a special care is necessary to differentiate a real spike discharge from a resembling noise, this seems to be almost impossible.

5. Suction electrode method which we have been studying for years, has a less artifact, and picks up slow waves corresponding well with ultramicroelectrode method.

Myographic patterns recorded by ultramicroelectrode, suction electrode, pressure electrode and hook electrode with various time constant (0.01–4.0 sec.) as well as with D.C. amplifier were compared. The results were shown by slides (omitted in this paper).

#### SLOW WAVES:

Another problem in smooth muscle electromyogram is how to deal with the slow waves. We are impressed by the fact that slow components are characteristic of and peculiar to different organs as well as different animals. We could say that each gastrointestinal organ has its own pattern of myogram composed of characteristic slow waves and accompanying spike groups.

Therefore, elimination of the slow waves by use of an amplifier with short time constant is not the method of choice.

We would recommend the time constant of at least 3–4 sec. for this purpose.

## 9. Electrical Activities of Smooth Muscle in Digestive Tract with Planted Electrode Method

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Recently, more attempts have been made to observe the electrical activities of smooth muscle in digestive tract and amount of informations had been reported. But most of them were performed experimentally in anaesthetized animals. Our study was undertaken to examine the electrical activities of digestive tract in unan-



aesthetized animals for a long time.

#### Method;

Dogs weighing approximately 10–12 kg, were used for our study and they were first operated under anaesthesia.

The electrodes, insulated with “Resin or Cashew” except for their tips, were planted in the wall of digestive tract (intramuscular) of dogs and fixed to their serosa with thread. The electrodes were consist of two 180 $\mu$  silver wires with a distance of 1 mm. between the tips. Moreafter, these wires were led to back of dogs through their abdominal cavities. The electrical activities were recorded with an two-channel ink-writting instrument. Then the dogs were in state of free exercise and electrographical observations were simultaneously made. By this method, the electrical activities of digestive tract had been recorded during 48 days (maximum 64 days).

#### Results;

In comparing with data from the anaesthetized dogs, the unanaesthetized dogs having the planted electrodes had showed the higher activities in their digestive tract than the former and this tendency was so commonly remarkable in intestine rather than in stomach.

When amount of feed were given, all of the intestinal peristalsis had earlierly accentuated before the stomach.

As changes with position of bodies, generally, the electrical activities had showed a depressive tendency in sitting or standing state. In the relationship between stomach and duodenum, it was most appearant in either electrical activities or peristalsis that the spindle-burst in duodenum was found rhythmically after in stomach.

These facts were all recognized in the unanaesthetized animals with electrodes chronically planted in their digestive tract.

We will contineously study on changes of their electrical activities with other conditions.

## 10. On the EMG of the Canine Intestine, Induced from the Intra- and Extra-Canalicular Method

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At the extracanalicular induction, unipolar or bipolar electrode of silver needle, was inserted into the intestinal wall from its serous surface and fixed to the serous membrane of the intestine. At the intracanalicular induction, the bowel was cut

and the same electrode was inserted from the mucous membrane and fixed. At the balloon method, the same electrode, settled to the soft balloon surface, was introduced into the intestine. The balloon was distended by the air was insufflated, and so the electrode was inserted into the intestinal wall from its mucous surface. Results were as follows:

1) The E.M.G., which was induced from serous surface, revealed a typical pattern of the intestinal myogram. Discharge groups, which consisted of one or more, spike-like discharges, appeared with the same rhythm.

2) The E.M.G., which was induced from the mucous surface, after cutting the intestine, revealed the similar pattern as the former except a little movement of the base line, sometimes was accompanied some artefacts.

3) At the E.M.G., induced by the balloon method, it was difficult to fix the electrode, and the movement of the base line and the artefacts mixed in the E.M.G., so that the action potential could not distinguished from the artefacts. But, when the anesthesia was profound and apnea occurred, the spike pattern was acquired by the balloon method, was similar as the extra- and intracanalicular induction.

As the results, it is evident that the E.M.G. induced by the balloon method was not different from that of the intra- and extra-canalicular induction.

Further investigation should be needed for the clinical application of the balloon method.

## 11. Electromyographic Study on Transverse Cutting and Gastro-Gastrostomies of the Canine Stomach

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Adult 20 dogs were subjected to the experiments. Transverse cutting of the stomach and end-to-end anastomosis was performed, and bipolar electrode of silver needle was inserted into the gastric wall from its serous surface, and the E.M.G. of the canine stomach was induced. The results were as follows:

1) In the normal untreated dogs, normoperistaltic discharge, arising from the body and reaching to the antrum was 98 %, and anti-peristaltic discharge, arising from the pyloric ring and reaching to the body was 2 %.

2) Transverse cutting at pyloric ring and end-to-end anastomosis was performed and the only normoperistaltic discharges arising from the antrum or the body were observed in all five cases.

3) Transverse cutting between the antrum and the body, and end-to-end anasto-

mosis was performed. On the E.M.G., induced from the antrum anti-peristaltic discharges was 89%, normoperistaltic discharge 11%.

4) Transverse cutting between the body and the antrum, and end-to-end anastomosis was performed. The E.M.G., induced from the oral portion of the anastomosis revealed only the normoperistaltic discharge. Otherwise the E.M.G., induced from the anal portion, revealed dominant anti-peristaltic discharges, though both normo and anti-peristaltic discharges appeared.

5) After transverse cutting between the body and cardia, and anastomosis was performed, the pyloroplasty was performed. The E.M.G. induced from the anal portion of the anastomosis revealed only normoperistaltic discharges and no anti-peristaltic discharge.

As a result, the effect of the pyloroplasty to the peristaltic movement of the stomach was shown on the electromyogram of the stomach.

## 12. Electrophysiologic and Radiocinematographic Study of Pyloric Sphincter Action of the Rabbit Stomach

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Pyloric sphincter action of the stomach in gastric evacuation was investigated on 60 rabbits by means of electromyography of the gastric and duodenal wall muscles with the radiocinematography and the concomitant electromanometric measurement of the pressure of the stomach and duodenum.

The results obtained are as follows:

1. The pyloric sphincter prevents rapid out-flow of the gastric contents into the duodenum and carries on a slow gastric evacuation little by little co-operating with the antral peristalsis. The duodenal contents are carried on by the different, and specific, peristalsis of the duodenum from that of the stomach.

2. Pyloric sphincter action in the gastric emptying is little influenced by a simple pyloromyotomy and is not disturbed until a partial resection of the sphincter muscle layer is performed. From these results it is conceivable that the partial resection of the pyloric sphincter in normal stomach disturbs the gastric evacuation co-operating with the antral peristalsis.

3. However, in the gastrectomized (proximal) animals the gastric emptying is rather hastened by the partial resection of the pyloric sphincter. This fact suggests that disturbed gastric emptying after proximal gastrectomy is due to in-cooperation between the pyloric sphincter action and the antral peristalsis caused by interception

of the vagal supply on the antrum rather than pylorospasm, thus pylorotomy in the gastrectomized animals makes it easy to empty the gastric contents by the pressure gradient.

### 13. The Difference in Smooth Muscle in Accordance with Various Portions of Stomach

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The gastric motility was investigated on the visible peristalsis, motility pattern, electrical activity, anatomical muscular construction, electrical muscular sensibility and tonus against intraluminal pressure at each portions of canine stomach. And on the human stomach the correlation between the anatomical muscular construction and location of the gastric ulcer was studied.

The results were:

- 1) The difference in motilities in accordance with each portions of stomach particularly difference between antrum and body is confirmed.
- 2) Both of peristalsis and tonus are considered to be governed chiefly by the circular muscle and its difference in anatomical construction and development among each portions should produce the motility difference.
- 3) The difference in motilities and muscular construction between active antrum and less active body may constitute one of important contributing factor to the development of gastric ulcer.

### 14. Electro-Physiological Studies on the Motility of the Esophagus and the Cardia

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Despite a large number of literatures concerning to the motility of the esophagus, there are a few reports regarding its electromyogram. The studies on the motility of lower part of the esophagus and the cardia, by means of electromyographic measurement and their internal pressure simultaneously recorded by transducer, have been carrying out experimentally.

Electromyogram were recorded by Mutiplex Monitor-Recorder ME-91D, using a coaxial depolar needle electrode or a dipolar fishing hook electrode at 1 mm. intervals, 0.005 second as time constant and 0.5 cm per second as recording speed.

Internal pressure curve were obtained by transducer connected to Electromanometer MPF-4, using a rubber tube inserted in each parts. Physiologic cardial and respiratory action potential, which had influence on electromyogram of esophagus and cardia, were obtiterated on the basis of the data obtained in preliminary experiment.

As the first report of this study, here, the result of experiment in normal dogs are presented.

In the lower part of the esophagus, following three types of spike were noted in the electromyogram during its movement which was determined by internal pressure: (1) average frequency of 8.7 times of single spike per one minute and average amplitude of  $132.2 \mu\text{V}$ , (2) average frequency of 13.5 times per one minute, average amplitude of  $94.7 \mu\text{V}$  and average duration of 0.9 second, (3) average amplitude of  $100 \mu\text{V}$  spike lasting for several minutes.

In the cardia, following three types of spike were also noted: (1) average frequency of 9.7 times of single spike per one minute average amplitude of  $147.0 \mu\text{V}$ , (2) average frequency of 16.2 times per one minute, average amplitude of  $102.5 \mu\text{V}$  and average duration of 1.2 seconds, (3) average amplitude of  $100 \mu\text{V}$  spike lasting for several minutes.

According to the results of electromyogram taken in the present experiment, it is impossible to elucidate each origin of these above mentioned types of spike obtained both in the lower part of the esophagus and the cardia, and additional experiment will be necessary.

## 15. Colonic Movements in Transport of Intraluminal Contents

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Dogs and cats anesthetized with urethan and morphine are used. In order to record easily action potentials of the colon, all the small intestine is extirpated except the duodenum and the terminal part of the ileum. To eliminate the reflex

effects, the splanchnic, hypogastric and colonic nerves are severed. D-C or R-C coupled amplifiers (time constant : 1.5 sec.) are used for recording action potentials.

## RESULTS

1. The antiperistalses usually are observed in the proximal colon, which originate in the colonic sphincter and propagate to the cecum.

When Ringer solution of 5 ml. is injected into the proximal colon through the ileo-cecal sphincter, the antiperistalses cease transitorily and then become gradually more powerful and the spike potentials increase in number and amplitude. Despite that the direction of the contraction waves is antidromic, the fluid is transported to the anal side. The colon relaxes for a while, after the fluid has been evacuated.

2. When the bolus made of cotton wool or plastic materials covered with vaseline is inserted into the lumen of the proximal colon through the ileo-cecal sphincter, the intestine contracts powerfully at the region oral to the bolus and this contraction propagates gradually anad, by which the bolus is transported to the anal side. The author designates this contraction wave as the greater peristalsis. The spike discharge in the greater peristalsis is 3 to 15 seconds in duration, several times of that in the antiperistalsis. Even if the greater peristalsis is observed like a single tonic contraction, the spike discharge sometimes consists of two groups of spike potentials.

When the greater peristalsis propagates to the anal side, the motility within the extent of about 10 cm anal to the greater peristalsis is remarkably inhibited and it continues until the greater peristalsis arrives at the recording portion.

3. It may be considered that the excitatory state at the region oral to the bolus is produced by the mucosal intrinsic reflex elicited by insertion of the bolus and the inhibitory state at the region anal to the greater peristalsis or the bolus is produced by both the mucosal and muscular intrinsic reflexes.

## 16. Electron Microscopic Study of the Uterine Smooth Muscle (Report II)

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In the previous report I mentioned that in the electrone microscopic observation of a mouse and the human uterine smooth muscle, the myofilament taken as the minimum unit of contraction of muscles distinguished itself during the period of pregnancy, especially in the outer layer of human uterus.

In the experiment the myofilaments of the mouse were not clearly seen, but in

using a rabbit as a test material, the myofilaments could be quite clearly observed. As the result I could confirm that there exists a dense area arranged in periodic order in all the myofilaments of animals and that the changes of the myofilaments during the period of pregnancy have the same tendency of distinguishing themselves as those of human bodies.

The observations above mentioned were made quite easily by improving embedding when sampling—prescribing equal quantity of stylen-methacrylate and n-butyl-methacrylate, and later, epon-epoxy.

Besides this I have changed the conditions of hormone in order to find out what caused the changes of pregnancy.

1. At the end of two weeks and four days after castration the mouse could not show any difference from non-pregnant one so far as the myofilaments are concerned.

2. From the end of three weeks after castration I injected estradiolbenzoate 0.04 milligramme (2000 unit) for seven days and progesterone 1 milligramme for three days subcutaneously. On the contrary some of the myofilaments became obscure, especially the dense area remarkably decreased.

3. In prescribing chorionic gonadotropin 0.15 milligramme (300 unit) for four days, no clear cells of the muscles could be caught by the electron microscope: this might be due to the decrease of the muscle layer.

As mentioned above, I have made the experiments, as the first trial, to see how hormone influence on the uterus though I am not sure whether the various conditions in hormone by prescribing may to some degree differ from those in the last period of human pregnancy both in quality and in quantity.

## 17. Fine Structures and Physiological Properties of Snail Retractor Pharynx Muscle

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When fine structures of snail retractor pharynx muscles were observed by an electron microscope, smooth muscle fibres of about 10  $\mu$  in diameter ran parallel with each other from the pharynx to the columella and each muscle fibre was combined by connective tissues. A number of unmyelinated nerve fibres, 0.2  $\mu$  in diameter, which were surrounded by Schwann cells were observed between muscle fibres but, near the end of these nerve fibres, they lost Schwann cells and multiply innervate the muscle fibres. No end-plate type junctions as found in the skeletal

muscle fibres were observed. The fibre membrane was separated with a space of about  $0.1 \mu$  from the membrane of adjoining ones. Sometimes paralleled membranes showed defect of the membrane of about  $0.5 \mu$  and protoplasmic continuity of the muscle fibres was observed. The end of one muscle fibre was connected to another with a thick membrane which was different from the membrane of other parts, i.e. these is also a possibility of longitudinal continuity between fibres. These structures may support the possibility of the impulse transmission from one muscle fibre to another.

When the nerve supplying the retractor pharynx muscle was stimulated by electric shocks, neuromuscular junctional potentials were produced with weak stimuli and action potentials with strong ones. The action potentials conducted with a velocity of about 1 m/sec which was the same as that of nerve fibre. These could be observed not only with indirect stimulation but also with direct one. This indicates that the muscle fibres are multiply innervated by nerve fibres and agrees with histological findings.

The effect of various drugs on the neuromuscular transmission of this muscle was examined. When the muscle was immersed in  $5 \times 10^{-6}$ g/ml ACh, no appreciable effect of ACh was observed.  $5 \times 10^{-5}$ g/ml curare produced spontaneous discharges in the muscle. The muscle contracted soon after Ringer solution was substituted for curare. On the other hand, the neuromuscular junction of this muscle showed very high sensitivity to noradrenaline and adrenaline.  $10^{-5}$ g/ml noradrenaline induced spontaneous discharges and contractions of muscle, but the response of the muscle in this solution to electric stimulation was hardly changed.  $5 \times 10^{-6}$ g/ml adrenaline produced similar effect on the muscle to noradrenaline. The muscle showed relatively higher sensitivity to noradrenaline than to adrenaline.  $5 \times 10^{-4}$ g/ml Priscol, which inhibits adrenergic substances, inhibited the transmission from the nerve to the muscle until the muscle could not produce action potentials but junction potentials only. Guanethidine of  $5 \times 10^{-3}$ g/ml had the same effect on the neuromuscular transmission. If reserpine was injected into the body cavity of the snail for 4 days with a dose of 10 mg/kg/day, the spontaneous potential discharges which can usually be recorded from the muscle became grouped and showed a lower frequency than the control, but the muscle responded to electric stimulation. When reserpine was injected for more than 5 days, the spontaneous discharges became very few, but the muscle responded to indirect stimulation.

From the above results, it may be concluded that the muscle is multiply innervated and that nervous excitation liberates adrenergic substance at the nerve ending, which generate junctional potentials in the muscle leading to the action potential and contraction of the muscle.



## 18. Formation of a Valve-like Mechanism with Denuded Ileal Ring

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It is a notable fact that the urinary bladder can regenerate and the smooth muscle, too, may be able to be seen in the new regenerated bladder wall. When the urinary bladder has been completed, one naturally expect that the new bladder will be given the urinary continence. The authors have been trying a series of experiment on formation of a valve-like mechanism with a denuded ileal ring.

A short ileal segment of a dog, 2 to 3 cm in length, was isolated from the end of the ileum. The segment had an intact mesenterial attachment. The continuity of the remnant ileum was reconstructed by the end-to-end anastomosis. The ileal segment was of a ring form, which was cut longitudinally and was made flat. Then, the exposed mucosa and underlying submucosal layer were removed completely. Thus, the muscle layer was exposed on the cut surface of the ileal segment. The denuded ileal ring wrapped up a stoma of an artificial anus at the sigma. The serosa of the sigma was directly brought into contact with the denuded surface of the ring. Through this procedure, the ileal ring made a constriction at the tract of the sigma by compressing it. However, as the ileal ring possessed elasticity, the stool could be passed through this constriction by the abdominal stress, while, without abdominal stress, on the contrary, no stool could be seen in the artificial anus. When a finger was inserted into the artificial anus, the elastic movement could be felt, widening and narrowing, on the finger, though it was not controlled by the animal's will. The authors could show the constriction of the sigma roentgenologically. The ileal muscle layer adhered, histologically, with the serosa of the sigma and was made thicker than the original thickness.

In the urinary tract, the smooth muscle could regenerate as the authors had reported at the last meeting. And, the smooth muscle in the digestive tract, too, may be able to regenerate and show a similar histological findings as the natural sphincter has. The authors will continue the study further for making a valve-like mechanism in the clinical meaning.

## 19. Enzymic Inactivation of Oxytocin

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### I. Introduction

Fekete (1930) discovered the inactivation of oxytocin by pregnancy serum. The inactivation is enzymic in character. The enzyme is estimated by bioassay and called "Oxytocinase" or "Pitocinase".

Tuppy (1957) reported that oxytocinase might be an aminopeptidase which acts on half-cystine residues in amino-terminal position of oxytocin, and that the enzymic release of  $\beta$ -naphthylamine from 1-cystine-di- $\beta$ -naphthylamine could be made use of for an assay of oxytocinase.

### II. Biochemical Characteristics

The activity is stable in room temperature.  $Q_{10}$  of reaction is 2.2~1.2 ( $10^{\circ}\text{C}\sim 37^{\circ}\text{C}$ ). Optimum pH and temperature are 7.4 and  $37^{\circ}\text{C}$ . The activity does not be decreased by dialysis and contained between 48 and 52% saturation of ammonium sulphate. The maximal velocity of reaction is  $6.66 \times 10^{-7}$  Moles/Lt./Min. Michaelis constant is  $9.09 \times 10^{-4}$  Moles/Lt. Most of metal ions inhibit the activity. EDTA inhibits the activity remarkably. The inhibition is reversed by the addition of  $\text{Ca}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{Mn}^{++}$  or  $\text{Ni}^{++}$ . SH-reagents and -compounds does not show SH-activity of CAP. Therapeutic concentration of chinine ( $10^{-3}$  Moles/Lt.) inhibits the activity. Oxytocin in higher concentration than  $10^{-1}$  Moles/Lt. inhibits the activity as a competitive substrate. The activities of CAP and Oxytocinase (assayed by blood depressor method of fowl) of pregnancy serum are demonstrated only in the fraction of  $\alpha_1$ -globulin of continuous paper electrophoresis. Both activities involved in the same fraction, analysed by gradient elution of liquid chromatograph with DEAE-Cellulose adsorbents.

These properties of CAP shows the same as those of Oxytocinase.

### III. Clinical Observation

The ratio of CAP activities in pregnancy to non-pregnancy sera is much higher than any other aminopeptidases. There is a rectilinear relationship between the activities of CAP and Oxytocinase in the same pregnancy sera.

CAP is contained in body fluids and tissue homogenates. Pregnancy serum shows the highest activity among body fluids. The activity of serum is low in non-pregnant women, men and fetus, unchanged by menstrual cycle and diseases, does not

increase even during pregnancy in animals.

During pregnancy, the activity rises progressively until labor. The larger rate of the increase, the earlier the labor begins. The activity is higher in hydramnios, threatened premature labor and twins, lower in fetal death in utero, hydatidiform mole and choriocarcinoma.

The activity increases usually as labor progresses and reaches to a maximum peak at the expression of placenta, successively begins to disappear until the activity of non-pregnant women at fourth week postpartum.

The activity rises remarkably during the induced labor by oxytocin drip. Oxytocin injected in postpartum and non-pregnant women, occurs no change of the activity. In vitro oxytocin (final concentration  $10^{-2} \sim 10^{-7}$  iu/ml) does not affect the activity. In the higher concentration, as described, oxytocin inhibits the activity on the contrary.

These clinical findings may suggest the proportional levels of the activity of CAP and endogenous oxytocin in blood, and that CAP may be released by the stimulation of oxytocin into the blood from some organs, for instance the pregnant uterus including placenta.

## 20. The Effect of Vitamin B<sub>1</sub> on the Uterine Motility

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The effect of vitamin B<sub>1</sub> on the uterine motility of non-pregnant rabbit was studied by means of chronic fistulization of the uterus. The uterine motility of rabbit is distinctly accelerated after administration of vitamin B<sub>1</sub>. But this effect does not appear immediately after the injection. The uterine motility increases gradually and reaches the maximum 24 hours after the injection. The results of measurement of the blood level and uterine content of vitamin B<sub>1</sub> reveals that the acceleration of the uterine motility may be due to the increase of vitamin B<sub>1</sub> content in the myometrium rather than that of the streaming blood.

To be more precise, the results are as follows: the vitamin B<sub>1</sub> level in the blood increases direct after the intravenous injection of B<sub>1</sub> and then decreases gradually to return to almost former level after 24 hours, while the uterine content of B<sub>1</sub> increases slowly to reach the maximum 24 hours after the injection, and it takes about 7 days to return to the normal value. Therefore it may be able to consider that vitamin B<sub>1</sub> has a chemical affinity to the myometrium and, consequently, the uterine motility will be accelerated according to the amount of vitamin B<sub>1</sub> content

in the myometrium. And also the sensitivity of the uterus to estrogen or oxytocin is enhanced by vitamin B<sub>1</sub> administration.

Accordingly, it may be well considered that the possibility of shortening the progress of labor by accelerating the uterine contraction with a large dose administration of vitamin B<sub>1</sub>. So we have attempted to give 150 mg Biotamin (B<sub>1</sub> preparation) orally to the pregnant women every day about one week before delivery and confirmed that the time of delivery was significantly shortened in the primiparae as compared with the control group, but it was not in the multiparae.

## 21. The Effects of Morphine upon the Motility of the Small Intestine

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1. The effects of morphine upon the motility of the aganglionic, denervated and normal THIRY-jejunal loops of unanesthetized dogs were studied.

a) The intravenous administration of the amount of morphine ranging from 0.001 to 1.2 mg/kg always produced an abrupt rise of tone accompanied with the decrease in superimposed rhythmic contractions in their amplitude. The tone was then gradually decreased accompanied with the increase in rhythmic contractions in their amplitude. At this stage the undulation of both tone and rhythmic contractions almost entirely disappeared.

b) It may be concluded that morphine acts as an excitant to the intestinal muscle itself, since the drug can raise the motility of the aganglionic loop.

2. Morphine could raise the intestinal motility which was previously depressed by atropine as well as by hexamethonium.

3. The intravenous administration of morphine produced a transitory retardation followed by acceleration in the flow of fluid through the intestinal segment.

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## 22. Contraction and Excess Relaxation of the Taenia Coli of the Guinea-pig

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When the dissected taenia coli was stimulated by 50/sec ac stimulus (5V/cm, for

3sec in longitudinal electric field), an excess relaxation was observed that the tension during relaxation temporarily fell below the resting level. Two possible explanations for this relaxation are considered; (1) the resting muscle maintains a certain tetanic tension by spontaneous discharge and (2) another relaxation process could take place actively beyond the resting state without accompanying the change in discharge.

Therefore, the correlation between the preceding contraction and excess relaxation was examined under various conditions, such as the change in field strength or stimulus duration, anaesthesia, anoxia and glucose removal. The contraction tension became larger and larger with the field strength 1 to 4V/cm for 3sec, but no change was seen in the depth of the excess relaxation. The same alteration was found by varying the stimulus duration in the range of 1 to 10sec at 5V/cm. The contraction was clearly decreased by adding procaine ( $10^{-8}$  to  $10^{-4}$  g/ml), while no change in the relaxation was observed. The relaxation elicited by adrenaline  $1 \times 10^{-7}$  g/ml was deeper than the excess relaxation. When the muscle was stimulated during the relaxation by adrenaline, the contraction tension was always augmented. The excess relaxation disappeared more quickly than the decrease of contraction on the cessation of oxygen supply to the bathing solution and the removal of external glucose.

These facts mentioned above may support the active relaxation mechanism. It was postulated that this active relaxation is closely related to the metabolic process in the muscle.

## 23. Transmembrane Potential of Iris and Ciliary Muscle in Human

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Intrinsic ocular muscle (a smooth muscle) exists in the iris and ciliary body; iris muscle provokes iris movement which controls the amount of rays coming into the eye, and ciliary muscle is considered to cause deformation of the lens and therefore controls the state of accommodation of the eye.

The ciliary body of mammals is covered with two epithelial layers which secrete aqueous humors.

The ciliary muscles in domestic animals (rabbit, mouse and cat etc.) are poorly developed than in humans and these membrane potential has never been reported.

Electrophysiological analyses of iris and ciliary muscles have been performed by Bürbring, Schubert and Jacobson etc..

The aim of present study is to measure the transmembrane potential of human iris

and ciliary muscles by means of intra-cellular microelectrode.

Five human eyeballs enucleated from retinal glioma (3) and cancer of maxillar sinus (2) were immediately placed in Ringer's solution and were sectioned at the equator along the frontal plane.

The electrode used were made by pulling capillary glass electrode tube made of telex (outer diameter 1 mm and inner 0.8 mm) pulled with an electrode puller of Katsuki type.

The anterior half was steeped in Hanks's solution kept at  $37^{\circ} \pm 0.1^{\circ}\text{C}$ . and saturated with oxygen.

The diameter of the electrode tip was less than  $1\mu$  and filled with three mol. solution of sodium chloride from electrode tip to root.

The electrical resistance of the electrode was 20 to 50 megohms.

(1) Transmembrane potential of iris

Transmembrane potential of the human iris averaged  $-60\text{mV}$  and no remarkable potential changes were detected at the pupillary margin, middle and collarette.

In 1954, Bürbring reported a potentials of  $-60\text{mV}$  in the sphincter muscle of the iris of albino rabbits and its value beeing changed according to the regions recorded.

(2) Transmembrane potential of ciliary epithelium

Transmembrane potential of the human ciliary epithelium of the these potentials averaged  $-20\text{mV}$  for pigmented and  $-40\text{mV}$  for non-pigmented respectively.

Bergman and Miller measured resting potential of rabbit's ciliary epithelium and obtained higher values than present results, i.e.  $-30\text{mV}$  for pigmented and  $-60\text{mV}$  for non-pigmented.

Such difference may be due to an anatomical differences between two species.

(3) Transmembrane potential of ciliary muscle

The membrane potentials of the human ciliary muscle ranged from  $-20\text{mV}$  to  $-75\text{mV}$  and the histogram shows two peaks.

This histogram shows a higher peak at  $-65\text{mV}$  and a small peak at  $-30\text{mV}$ .

The peak at  $-65\text{mV}$  represents membrane potential of ciliary muscles and the peak at  $-30\text{mV}$  may be induced from connective tissues during insertion of the electrode.

The magnitude of the potentials and its frequency distribution shows no difference all material used; the age change may therefore be negligible.

The spontaneous discharges and slow waves, usually observed in other smooth muscle cells, were not obtained from enucleated ciliary and iris muscle preparations.

Such disappearance of spontaneous discharges and slow waves, may possibly be due to unfavourable conditions since eye was enucleated and kept in an artificial solution; this, however, further elucidation is necessary.

## 24. Some Observations on the Membrane Potential of the Uterine Smooth Muscle Fiber of Pregnant Mouse

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The membrane resting and action potentials of the uterine smooth muscle fiber of late pregnant mouse were recorded by means of intracellular microelectrodes.

The current-voltage relation of the resting membrane was obtained by aid of double barrelled intracellular microelectrodes. Results obtained were as follows.

1) The mean resting potential of 41 mV was obtained from 60 trials and the maximum value was 68 mV.

2) Various parameters of one of typical action potentials elicited by electrical stimuli were measured. Magnitude of the action potential, 70 mV; overshoot, 14.3 mV; duration at 50% of the action potential, 24 msec; critical depolarization, 18 mV; mean rate of rise and fall at 40–80% of the action potential, 5.9 and 2.9 V/sec. These action potentials were the 'all-or-none type' responses.

3) In some of action potentials humps were found on the phase of depolarization and/or repolarization. Usually these action potentials did not appear in all-or-none manner. Some of the action potentials of different type which were smaller in magnitude and longer in duration were not also all-or-nothing responses. These kinds of action potentials might be electrotonic potentials of the neighbouring activities.

4) Values of the effective membrane resistance obtained were considerably lower than those of the intestinal smooth muscle fiber of cat reported by Barr<sup>1)</sup>.

### REFERENCE

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## 25. Pace-maker Sites in the Pregnant Rat Uterus

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It has been studied for long time in which part of the uterus the pace maker area exists. Recently it was detected by intracellular recording technique that the pace maker area of the uterus altered its localization according to hormonal conditions. In this experiments the localization of pace maker was also investigated intracellularly on pregnant rat uterus immediately before and after delivery.

Pregnant rat uterus was removed with its content and fixed in a warmed bath with supporting clips which made the intended area of the uterus not to move freely. In some experiments pregnant uterus was opened and extended on a paraffin block in situ length.

It was found that there were no significant differences between the results obtained immediately before and after delivery.

In a ravine of the fetal sacks several kinds of characteristic potential changes were recorded. There found most frequently potential changes which seemed to be slow potential oscillations. This potential oscillation was low in amplitude and had slow rates of rise and decay and as the recording electrode was moved from the region close to the parametrium away, this small potential oscillation increased its amplitude and developed to spike like form.

The small potential oscillation above described is sometimes recorded from deteriorated preparations or injured cells.

Therefore care is taken for avoiding this unnecessary confusion. The other characteristic wave form obtained from the ravine of fetal sacks is so called pace maker potentials which are rarely found compared with the slow potential oscillations. In addition to these characteristic potential changes, conducted spikes usually seen in the other part of the uterus can also be recorded in this region.

From the other part of the fetal sack except the ravine only conducted spikes are recorded but when the muscle is excised as a small strip from this part of the fetal sack, pace maker potential sometimes appears.

Consequently on pregnant rat uterus at term the characteristic potential changes such as slow potential oscillations or pace maker potentials are usually recorded in the ravine of the fetal sacks, especially in the area close to the parametrium.

There might be some discussion whether the slow potential oscillation is generative potential or not. Bozler has shown that in the upper limit of the ureter slow oscillatory changes could be recorded and it seemed to be generative potential. Analogizing the results obtained by Bozler the slow potential oscillation recorded in the ravine of the fetal sacks might be regarded as a generative potential.

Therefore in pregnant rat uterus at term the excitation might be initiated in the ravine of the fetal sacks, especially in the area close to the parametrium. The fact agrees closely with our previous results obtained by extracellular record.



26. The Electrical and Mechanical Activities of the Isolated Guinea-pig Ureter in the Various Ionic Environments

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The effects of changing the ionic environment on the spontaneous activities of the isolated guinea-pig ureters were studied. Sucrose-gap method was used. NaCl in Ringer-Locke's solution was totally replaced by isosmolar LiCl, LiNO<sub>3</sub> or sucrose.

Results obtained were: ...

1. After replacement by LiCl, spontaneous spike discharges were inhibited for several minutes or more, accompanied by a slight hyperpolarization. Then the frequency of discharges and phasic contractions which were associated with spike discharges were augmented, but the amplitude of contractions was still diminished. Weakened contractions continued for one hour or more. Tonic changes could not be seen.

2. Replacement by LiNO<sub>3</sub> evoked nearly the same changes in spontaneous activity as by LiCl.

3. Replacement by NaNO<sub>3</sub> evoked changes in tone, in addition to the change in phasic contractions mentioned above.

4. Immediately after sucrose replacement initiation or increased frequency phasic contractions and the increased tone occurred. Tone change continued for one hour or more, but was reversible. Phasic contractions continued for a shorter period.

5. Excess K initiated or augmented spike discharges accompanied by more or less remarkable depolarization according to the K-contraction.

6. Deprivation of Ca from Ringer's solution abolished rapidly the mechanical activities.

27. Studies on the Action Potential of Cat Ureter by Suction Electrode Technique

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1. The action potentials followed by the automatic peristalsis were observed in cat ureter, in situ.

2. The suction electrode was the injection needle plated with silver or prepared by embedding 0.2 mm silver wire into the 3-way glass tube (inner diameter of the points were 1.5~0.5 mm), and was sucked with appropriate syringe the sliding down of mercury-bulb. Indifferent electrode was put on fat tissue distance of about 1 cm from the ureter.

3. The action potentials by suction electrode did not show remarkable difference from that obtained by extracellular monopolar lead with needle-electrode or AgCl electrode, until obtaining 10 cm Hg negative pressure of suction. Over 10 cm Hg, the wave form sometimes showed similar one with that of intracellular recording (Rf. H. IRISAWA: Discussion at Kyushu University on March 16, 1962, Dr. Mollie E. Holman).

4. The action potentials obtained by suction electrode technique sometimes showed the similar wave form with that of intracellular potentials. It might be due to the inactivation of smooth muscle cells which were injured by the treatment of suction. Therefore, it seemed that the observed action potentials depended on the undamaged cells near the electrode. It was discussed that the glass capillary electrode having the large point like 20~100  $\mu$ , which is unsuitable for intracellular potential-recording, is more useful than the suction electrode.