

第20回 日本平滑筋学会総会講演抄録

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特 別 講 演 I.

内視鏡による消化管運動の観察 —筋電図からみた平滑筋の運動と内圧の関与—

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は じ め に

内視鏡機器の改良, 進歩に伴ない, 内視鏡検査は安全かつ容易に施行出来るようになった。そして内視鏡検査の目的も単なる形態診断にとどまらず, 治療への応用にも向けられている。さらにこれらに加えて, 我々は内視鏡による消化管機能検査法の開発を目指し, 1973年に消化管筋電図の内視鏡的直視下誘導法を報告した。ここでは本法を中心に, 内視鏡検査を用いた消化管運動の臨床的検討について, 吾々の成績を述べる。

I. 内視鏡的直視下誘導法の開発の意義

消化管運動を観察しようとする臨床的な試みはこれまでに多数報告されている。ところで内視鏡機器が優れた観察及び運搬手段であることに着目した吾々は, 消化管運動を直接に観察すると同時に, 内視鏡鉗子孔を通した小さな装置により, 消化管運動を表現する各種のデータを記録することを考えた。そこでまづ消化管筋電図の管内誘導法をとりあげた。そして, 多くの改良をへて, 現在では内視鏡の挿入可能な消化管全域にわたり, 消化管運動を観察しながら, 同時に目的部位の消化管筋電図を記録することが可能となった。ただ内視鏡機器の挿入は極めて非生理的な条件を作り出すことが心配されたが, 内視鏡操作に関する検者の熟練度, 被検者の協力, 内視鏡機器の改良例えば細径 scope の使用, 前処置の工夫などの諸因子に十分な配慮を施すことによって, 従来の各種

の機能検査と変わらない条件下にて本法を行いうるものと考えている。

II. 内視鏡的直視下誘導法に関する装置

臨床応用を目的として試作された電極は, 吸引電極, バルーン電極, 針電極の3種類であった。このうち, 吸引電極は目的とする消化管粘膜に電極を長く固定しておくことが困難であった。バルーン電極も消化管内での運搬が難かしいうえに, バルーンの両面に装着した針電極が広い管腔をもつ臓器ではうまく管壁にあたらなかった。これに對して, 双極針電極では, 手許操作により管壁に刺入した針の深さが調節されるため, 目的部位に

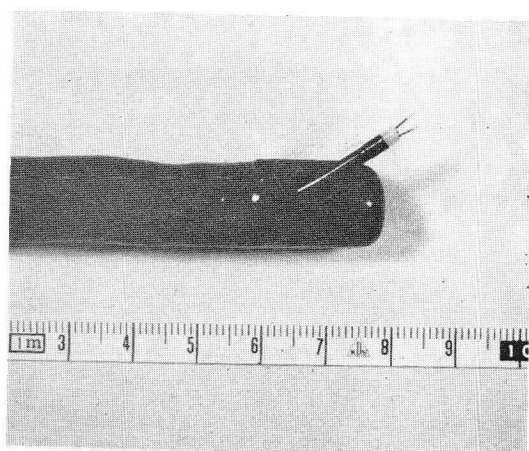


図 1. 内視鏡の鉗子孔に装着された双極針電極

確実に電極を留置することが出来た。

吾々の製作した銀製の双極電極は長さ 10 mm で、先端の 1~2 mm を除き、コーティングしてある。極間距離は 1 mm であり、各種の scope の鉗子孔を通るように設計してある (図 1)。

本法は開発当初、シールドルーム内で行い、内視鏡光源や amplifier, recorder はルーム外に置き、夫々の機器、検者、被検者の間に 1 点アース集中方式をとったが、現在では特別なシールドルームは使用していない。

III. 本法の操作上の実際

被検者の前処置は、上部消化管では xylocaine スプレーによる咽頭麻酔を、下部消化管では xylocaine ゼリーによる肛門部麻酔を行い、特殊な前投薬は使用していない。内視鏡操作では、送気送水は観察に必要な最小限の量を用いている。とくに細径 fiberscope では胃体部への scope の挿入に際して少量の送気を必要とするのみで、速かに前庭部に到達出来る。不必要なアングル操作により、scope 先端を消化管壁にあてることは避けるべきである。被検者の苦痛や緊張状態を招かないためにも、手馴れた内視鏡操作で短時間に、目的部位に到達することは、本法の重要なポイントであると考えている。

目的部位での針電極の壁への刺入は、粘膜面に充分近づき、可能なかぎり直角方向から刺入する必要がある。

IV. 良好な筋電図を得るには

胃体上部や大腸脾彎曲部では、筋電図への心電図の混入が生じる。この心電図波形を判別するためには筋電図の記録時に心電図を併記するのがよい。

刺入した針電極の消化管壁内での深さは、筋電図所見に大きく影響する。すなわち粘膜又は粘膜下への浅い刺入では、典型的な spike の出現が不明瞭である。この場合でもより深く刺入しなおすことによって、明瞭な筋電図波形が得られる。ただ電極刺入の実際においては斜方向且つ遠距離からの刺入になりやすいため、深部への刺入が不十分なままでも稀には存在する。また十二指腸より肛側の臓器では壁がうすいために、深部刺入時に稀には被検者が軽度の疼痛や圧迫感を訴え、筋電図上にも極めて小さな針状突起様パターンが連続して出現することがある。この場合、針電極を僅かに引き抜くことによって明瞭な筋電図が描出出来る。

検査中の呼吸運動は浅くゆっくりした状態を維持させる必要がある。

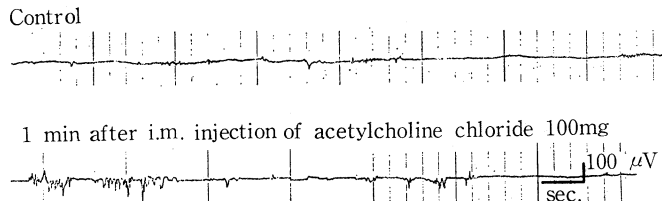


図 2. 胃運動に対する薬剤効果 acetylcholine chloride の投与により、直視下に観察される蠕動及びそれに同期した spike burst の出現は著明となる。

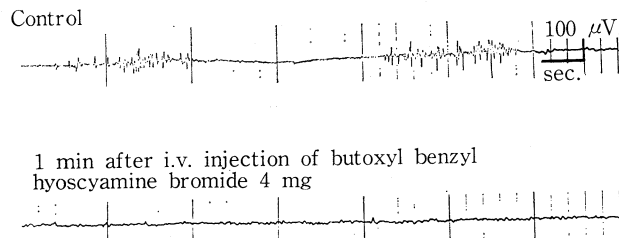


図 3. 胃運動に対する薬剤効果 butoxyl benzyl hyoscyamine bromide の投与にて蠕動及び spike burst の出現は抑制される。

時定数については、内視鏡的に観察される蠕動運動と同期して出現する spike burst のみを検出の対象としたため、小さな time constant (0.01 秒) で記録することにした。消化管運動を亢進又は抑制する薬剤の投与によって、これらの蠕動及び spike burst はその出現頻度が著明となったり (図2), 消失したりするので (図3) 我々は, spike burst が消化管運動を反映する指標の1つと考えている。

V. 消化管の各部位における筋電図

16 mm シネにて撮影した内視鏡像により、消化管各部の運動を供覧したが、その際に本法にて得られた消化管各部位の筋電図を示す。

a) 食道筋電図 (図4)



図4. 食道筋電図

ごく小さな基線の変動を混じた粗な spike 群から成っており、大動脈拍動や呼吸による影響を受けやすい。時定数をさらに小さくした条件で導出する必要がある。

b) 胃筋電図 (図5, 6)

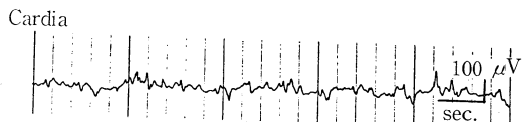


図5. 胃噴門部の筋電図

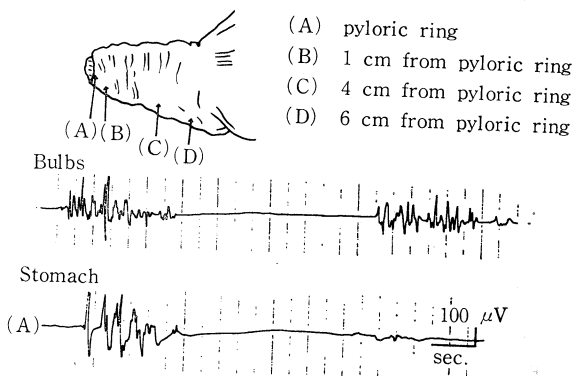


図6. 胃筋電図 幽門輪に近いほど, spike の振巾は大である。

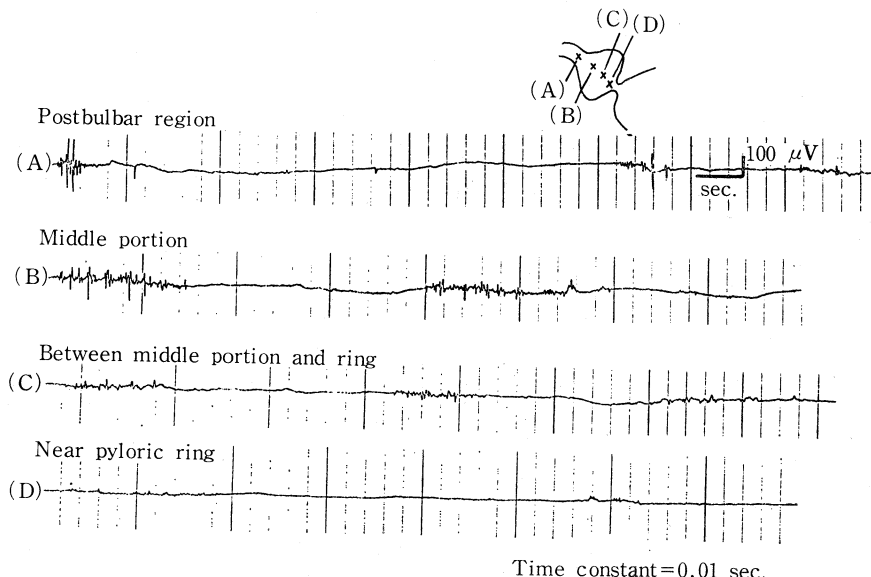


図7. 十二指腸球部筋電図 幽門輪から離れるにつれて spike の振巾は大となる。

噴門部では明瞭な spike 群はみられず, 心拍動や呼吸の影響も大きい. 我々は胃運動のペースメーカーの位置を噴門部後壁よりの“いわゆる集合点”に想定しているが, 現在までのところ, 確証を得るまでには至っていない. 胃体部では幽門側に向うに従いがい, 漸次 spike 群が明瞭となる. 特に胃角から幽門輪に至る部位では振巾の大きな spike 群が整然と出現する. しかも幽門輪に近づくほど, spike の振巾が大きくなるが, これは胃壁の固有筋層の状態を反映しているものと考えられる. 典型例では幽門輪の開閉運動に同期して spike 群の出現がみられる.

c) 十二指腸筋電図 (図7)

幽門輪の筋電図に類似した鋭い spike 群として

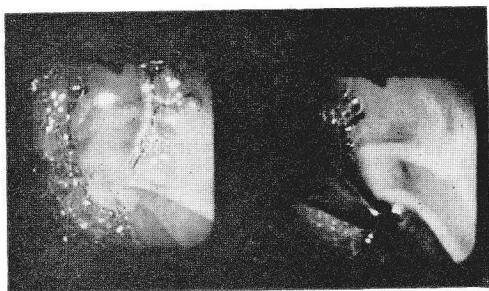


図 8. 内視鏡的直視下誘導法による十二指腸乳頭部からの筋電図の導出

左: 十二指腸乳頭部の内視鏡像

右: 同部に針電極を刺入し Oddi 氏括約筋の筋電図を記録している.

みられ, 他臓器の筋電図に比較して spike の振巾は大きく, 出現頻度も密である. 球部筋電図は内視鏡的に観察される球部運動と明らかに同期しており, artefact も少なく, 安定した筋電図が得られる. 球部筋電図は幽門輪に近接した部位では spike の振巾は小さく, 球後部に近づくにつれて振巾は大となる.

d) Oddi 氏括約筋筋電図 (図 8, 9)

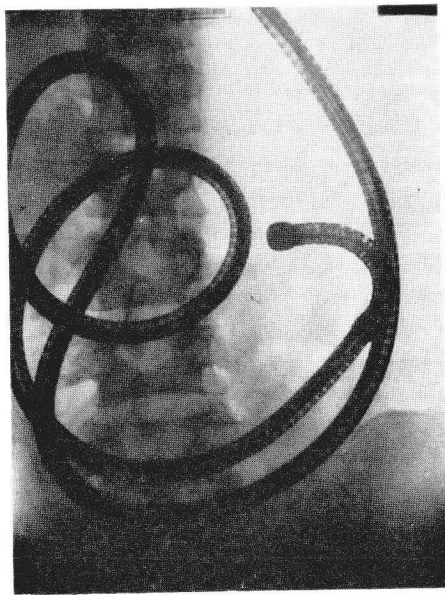


図 10. Push 式小腸 fiberscope による空腸の観察, 引き続き空腸の筋電図を記録することが出来る.

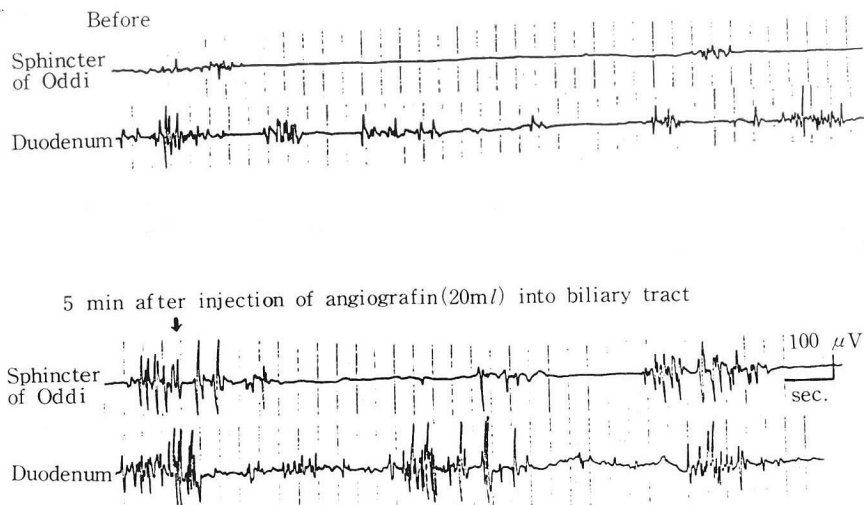


図 9. Oddi 氏括約筋の筋電図 angiografin の注入による胆導内圧の上昇により, 夫々異ったリズムを示していた Oddi 氏括約筋の筋電図と近傍十二指腸の筋電図はほぼ同期して来る.

Oddi 氏括約筋の十二指腸筋に対する独立性は未だ議論の対象となっているところである。吾々の検討では、2チャンネルの試作 fiberscope を用いて、Oddi 氏括約筋と近傍十二指腸筋から筋電図の同時記録を行うと、両者間に明かなリズムのずれを認める例と、ほぼ同期したリズムをもつ例とに分かれ、前者の例が多く認められた。さらに内視鏡直視下に胆管内に造影剤を注入し、胆道内圧を高めると、総胆管末端の運動は増強し、5分後には亢進した十二指腸運動とほぼ同期するリズムを示めた。このことから我々は Oddi 氏括約筋の独立性が示唆されたと考えている。

e) 小腸の筋電図 (図 10, 11, 12)

push 式小腸 fiberscope を Treitz 靱帯より肛側に挿入することによって空腸の筋電図を記録する

ことが出来る。回腸の筋電図は大腸 fiberscope を Bauchin 弁を介して回腸末端へ挿入すること

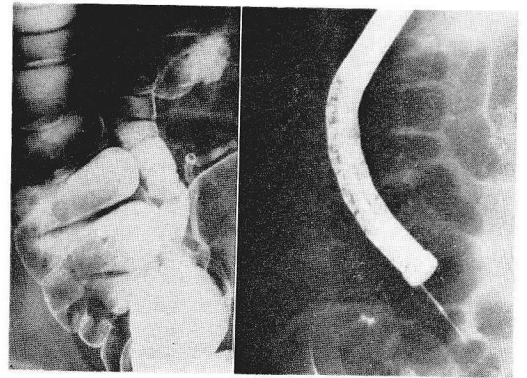


図 13. 内視鏡の直視下誘導法による Bauchin 弁からの筋電図の導出を示す。



図 11. 内視鏡の直視下誘導法により得られた空腸の筋電図。(Treitz 靱帯から 20 cm の位置)。上より下に経時的に続く。時定数 0.01 秒

Time constant

0.05 sec.

0.01 sec.



図 12. 内視鏡の直視下誘導法により得られた回腸末端の筋電図。時定数を 0.05 秒と 0.01 秒とによって同時記録した。

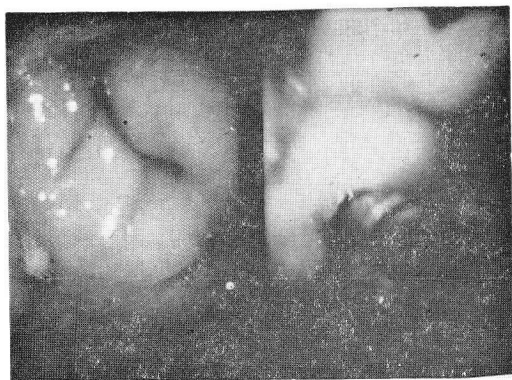


図 14. Bauchin 弁の内視鏡像
左: 正常の Bauchin 弁の運動を示す。
右: Bauchin 弁へ針電極を刺入し、筋電図を記録しているところ。

よって記録出来る。しかし深部小腸では内視鏡のアングル操作に難点があるため、目的部位からの導出は必ずしも容易ではない。空腸筋電図は Treiz 靱帯附近では十二指腸筋電図に類似するが、肛側に向かうに従い、粗な spike が出現するパターンへと変化する。

f) Bauchin 弁の筋電図 (図 13, 14, 15)

大腸 fiberscope の観察では回腸末端部は運動亢進時に Bauchin 弁口を介して、盲腸内に翻転する動きを示す。この場合、Bauchin 弁の筋電図として、運動に同期した spike 群が記録される。ただ深部大腸への scope の挿入は極めて難かし

く、手馴れた術者によらなければ、被検者の苦痛や緊張を伴ないやすく、安定した筋電図は記録されないことが多い。

g) 大腸筋電図 (図 15, 16)

上行結腸は呼吸などの artefact が混入しやすいうえに、spike 自身も不明瞭である。横行結腸から、下行結腸、S 状結腸へと進むにつれて、spike は明瞭で振幅は大となる。

大腸筋電図の記録にあたっては、前処置として用いる薬剤の影響を無視出来ないため、我々は原則として残渣の少い注腸食を 2 日間投与し、洗腸後 16 時間以上の絶食(補液による栄養補給も検査前 6 時間以上に行なう)を行ったのち、本法を施行した。

VI. 本法を用いた臨床的検討

(a) 内圧・筋電図の同時継続記録による直腸運動

腸内圧の測定には telemetering capsule を用いたが、本装置は Radio capsule (National TFH-101 型)、アンテナ・増巾・記録装置から成っている(図 17, 18)。Radio capsule は内圧を感知すると、それに相当した変調を呈する電波を発信する。この capsule を大腸 fiberscope を用いて直腸に置き、次いで内視鏡直視下に針電極をその近傍に刺入し、fiberscope のみを抜去したのち、背

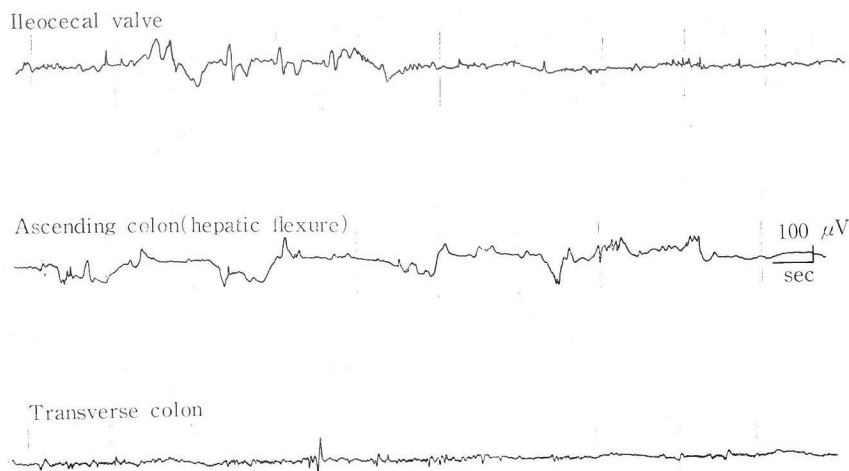


図 15. 内視鏡的直視下誘導法により得られた大腸の筋電図. Bauchin 弁及び上行結腸の筋電図には artefact が混入しやすい。横行結腸では明瞭な spike 群を記録出来る。

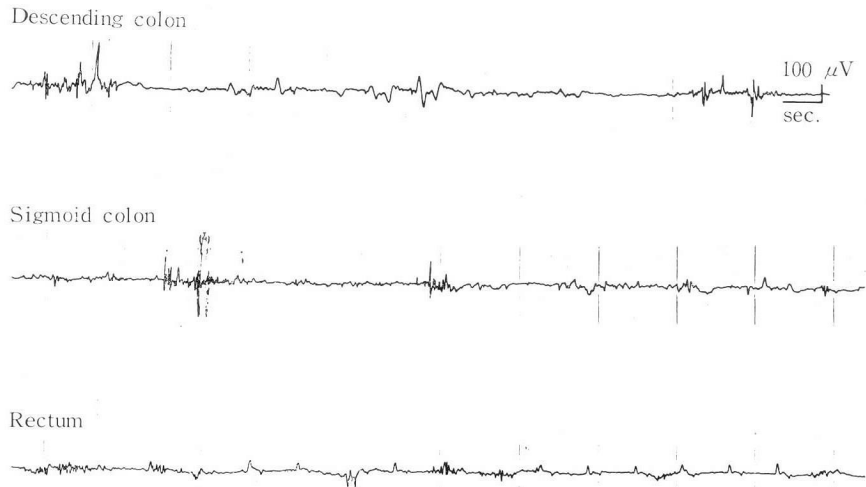


図 16. 内視鏡的直視下誘導法により得られた大腸筋電図
下行結腸, S 状結腸へと進むにつれて spike 群は明瞭となる.

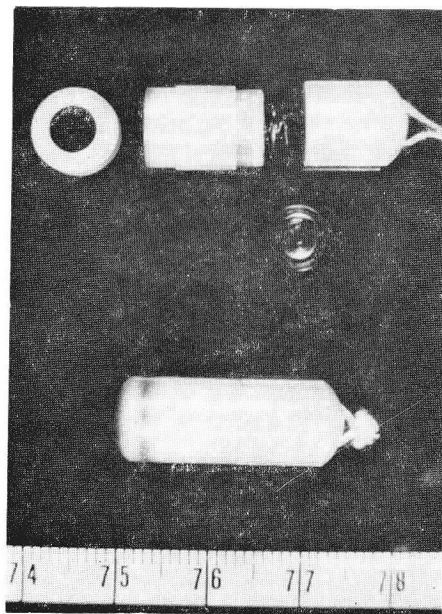


図 17. Radiocapsule の本体

臥位で直腸の内圧・筋電図を同時記録した.

8 時間におよぶ継続記録の間, 大腸運動は spike 群が連続して出現する活動期と, 散発的に spike を認めたり, 時には全く平坦となる静止期とをくり返えすことが内圧曲線及び筋電図上で捉えられた (図 19). 検査中に被検者は短時間眠ったが, この期間にも活動期, 静止期を区別出来たものの直腸運動は極めて不明瞭となった (図 20).



図 18. テレメーターリングカプセルの各種装置

直腸運動に対する薬剤効果を AOC-Tetragestrin 及び Scopolamine butylbromide について検討したが, 前者では運動の亢進が, 後者ではその抑制が内圧曲線及び筋電図上に認められた.

(b) 胃癌の筋電図

胃癌の浸潤が胃壁の深層に及ぶと局所の運動に異常が生じることは X 線検査上注目されて来たが, 吾々は本法を用いて胃癌の筋電図を検討した. すなわち, 前庭部に位置する胃癌例で, 幽門輪から口側へと漸次胃筋電図を記録すると, 進行癌では病巣内から記録された筋電図には spike 群は認められなかったが, 癌巣周辺からは, 幽門輪からの距離に対応した明瞭な spike 群が記録された (図 21). 一方, 早期癌特に粘膜内癌では癌巣内から記録された筋電図には周辺部のそれと同様

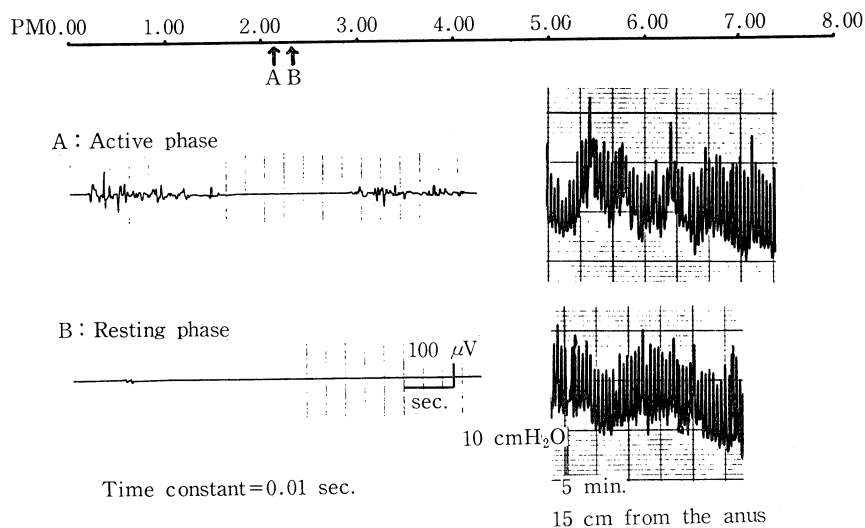


図 19. 内圧・筋電図の同時記録による直腸運動（覚醒時）
spike 群の出現する活動期と不明瞭な静止期が区別されるが、内圧曲線上でも両時期が区別される。時定数 0.01 秒

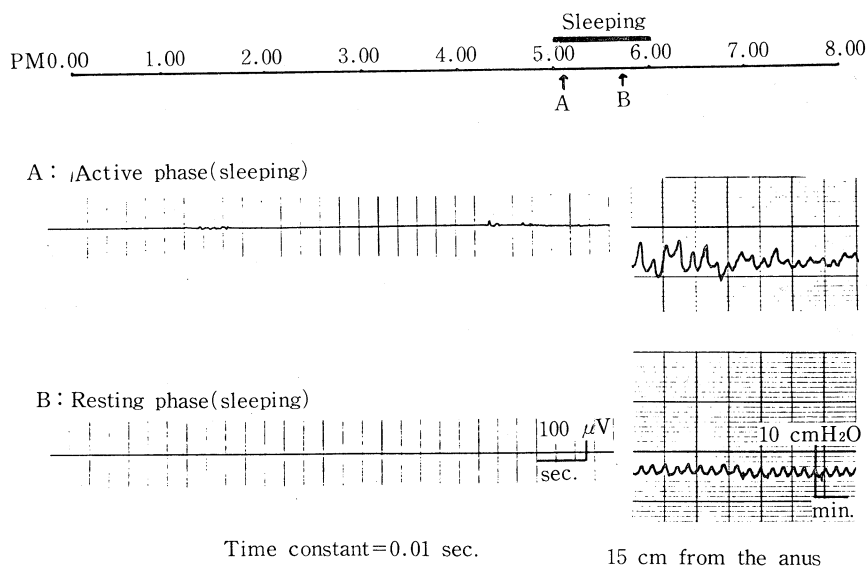


図 20. 内圧・筋電図の同時記録による直腸運動（就眠時）
活動期及び静止期を区別することが出来る。時定数 0.01 秒

の spike 群が認められた。結局、胃癌の壁内深達度が進むにつれて、癌巣内からの筋電図は不規則から平低化へと変化し、癌による固有筋層の破壊は消化管局所の運動を阻害することが確認された（表 1）。

(c) 消化管ホルモンの十二指腸球部運動に対

する効果（表 2）

十二指腸球部は各種の消化管ホルモンにとってその局在の場として、また自律神経の関与による作用の場として注目されている。また球部運動を本法を用いて検討する際、最も安定した筋電図波形が得られることを確認している。そこで十二指

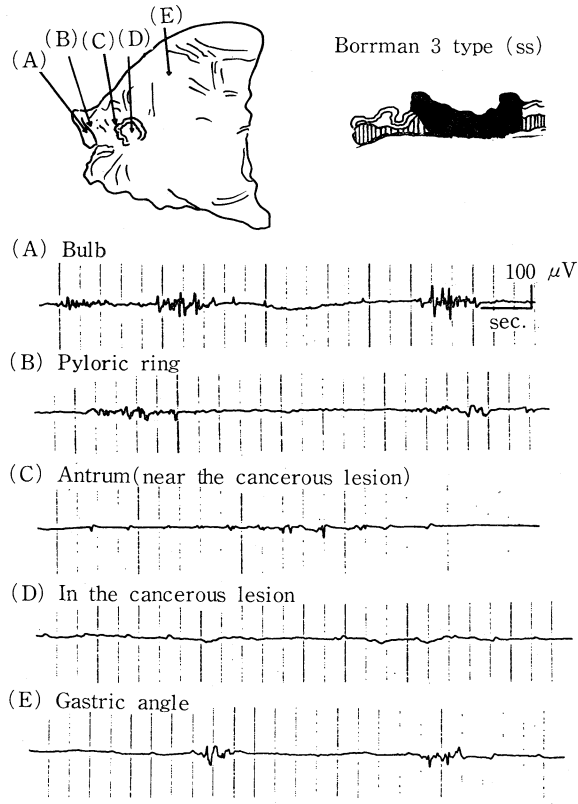


図 21. 胃癌 (進行癌) の筋電図
癌巣からは明瞭な spike 群は記録されない。

表 1. 胃癌の深達度別にみた胃筋電図所見
壁の壁内深達度が進むにつれて, 癌巣からの筋電図は平低化する。

EMG findings			
Depth of cancer invasion	Normal	Irregular or low amplitude	Flat
Mucosal	4	—	—
Submucosal	2	2	1
Muscular	—	3	2
Serosal	—	1	6

腸球部を目的部位として消化管ホルモンの運動に対する効果を検討した。AOC-Tetragastrin, Secretin, CCK-PZ についてみると, いずれの消化管ホルモンでも 1 回大量静注法ではその効果にばらつきが強く認められ効果判定が困難であった。持続静注法では, Tetragastrin で運動亢進を認め

表 2. 消化管ホルモンの球部運動に対する効果
AOC-Tetragastrin は運動亢進を, Secretin は運動抑制を示めたが CCK-PZ は一定傾向を示さなかった

<i>HORMONES</i>						
Change of movement on EMG	Gastrin		Secretin		CCK-PZ	
	Inj.	Inf.	Inj.	Inf.	Inj.	Inf.
Increase	10	9	0	1	2	4
Unchanged	5	2	2	2	4	3
Decrease	4	1	7	10	3	5
Total	19	12	9	13	9	12

Inj.: Bolus i.v. injection, Inf.: I.V. infusion

た。しかし, 大量静注や反復静注では運動抑制がみられたことより, 大量の Tetragastrin 投与はその薬理効果及び 2 次的な生理効果が重なって, 生体に複雑な反応を示すことが考えられた (図

Duodenum

Control

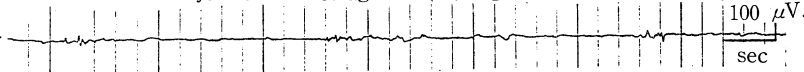
1 min after bolus i.v. injection of Tetragastrin(3 γ /kg)Second bolus i.v. injection of Tetragastrin(3 γ /kg) 6 min after first injection

図 22. 球部運動に対する消化管ホルモンの効果
AOC-Tetragastrin の反復大量投与は運動抑制を示した. 定時数 0.01 秒

Control



1 min after i.v. infusion of secretin(2 Units/kg/hr)



図 23. 球部運動に対する消化管ホルモンの効果
Secretin の持続静注法による投与で運動抑制が認められた. 定時数 0.01 秒

22).

また Secretin は運動抑制を示めした (図 23).
CCK-PZ は持続静注法でも一定の傾向を示さなかったが, このことは本製剤の投与量や純度に関係しているものと考えられた.

VII. 本法の将来

今後, 複数の channel をもつ生理機能検査用 fiberscope の開発により, 内圧, 血流, 筋電図などのデータを同時に且つ長時間に亘り記録することや, 同時に多数点からのデータを記録することが可能になると思われる. 図 24 は多チャンネル

胃 fiberscope により, 2 本の双極針電極を前庭部の離れた部位に置き, 同時記録しているところである (図省略).

おわりに

内視鏡的直視下誘導法による筋電図の導出については, その臨床的応用の有用性は十分強調出来るようであり, また約 300 例での経験からは未だ偶発症を認めていないことより安全性も強調出来るものと考えている.

本講演の機会を与えて頂いた本学会の会長横山正松教授並びに司会の労をとって頂いた田北周平名誉教授に深甚の謝意を表します.

特 別 講 演 II

Functions of Auerbach's plexus

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The propulsive motility in the small intestine and the unidirectional progress of intestinal contents have long been the subject of researches in physiology of movements of the digestive tract. Bayliss and Starling (1899) have concluded from their experiments on dog's small intestine that the peristaltic movements are true coordinated reflexes started by mechanical stimulation of the gut, and carried out by the local nervous mechanism in Auerbach's plexus. Both investigators have shown that the local stimulation of the gut produces excitation above and inhibition below the stimulated spot. Since this phenomenon is the fundamental mechanism of food transport in the gut, they named this phenomenon the law of the intestine. Although the law of the intestine has been questioned by numerous observers, several investigators (Thomas, 1951, Hukuhara and coworkers, 1958) have proved excitation above and inhibition below excited spot. Hukuhara and his associates (1958) observed that the chemical and mechanical stimulation of intestinal mucosa in anesthetized dog caused excitation above and inhibition below, whereas they proved that the mechanical stimulation of muscular coat caused relaxation of the muscle both above and below the point of stimulation. They distinguished two intrinsic intestinal reflexes, a mucosal reflex conforming to Bayliss and Starling's law and a muscular reflex. They suggested that the failure of some investigators to elicit responses in accordance with the law of the intestine might be due to failure to distinguish between the two reflexes. Nakayama and Nanba (1961) reported that the electrical activities of the intestinal muscle showed the analogous behaviour to that which Hukuhara and his coworkers have proved in mucous reflex and muscular reflex.

Auerbach's plexus lying between the longitudinal and circular muscle layers was discovered by Auerbach (1864) a long time ago. However, the direct physiological study of this plexus has not been done by any investigator. Since I have noticed that the longitudinal muscle of the rabbit small intestine could be easily separated from the circular muscle coat and Auerbach's plexus adheres to the peeled longitudinal muscle layer, that was already observed by Mangus (1904) on the cat small intestine and by Ambache (1964) on the guinea-pig small intestine, I intended to make researches on the electrophysiological properties of Auerbach's plexus (Yokoyama, 1966).

Auerbach's plexus consists of a mesh of nerve fibers with ganglion cells at nodes. From the studies of Bayliss and Starling, Hukuhara and coworkers, it can be thought that Auerbach's plexus acts as a reflex arc when the peristaltic movement occurs. For the analysis of the intrinsic nervous control in the cases of peristaltic movement, it seems to be necessary to make clear the properties of excitation conduction in Auerbach's plexus and to

clarify the properties of afferent as well as efferent pathways of the reflex arc in Auerbach's plexus.

METHODS AND RESULTS

I. Excitation conduction in Auerbach's plexus (Yokoyama *et al.* 1977)

Nerve impulse conduction in Auerbach's plexus of the rabbit small intestine was investigated by analysing its evoked potentials as the response to a single electrical stimulus given to this plexus. Figure 1 shows the experimental arrangement (A) and evoked potentials of Auerbach's plexus (B). The peeled longitudinal muscle strip of the rabbit small intestine was suspended horizontally in Krebs-Henseleit solution which was kept at 38°C and constantly gassed with 95% O₂-5% CO₂. A pair of tungsten wire tapered electrolytically to 10 µm, insulated except at tips and aligned parallel 0.5 mm apart, were used as the

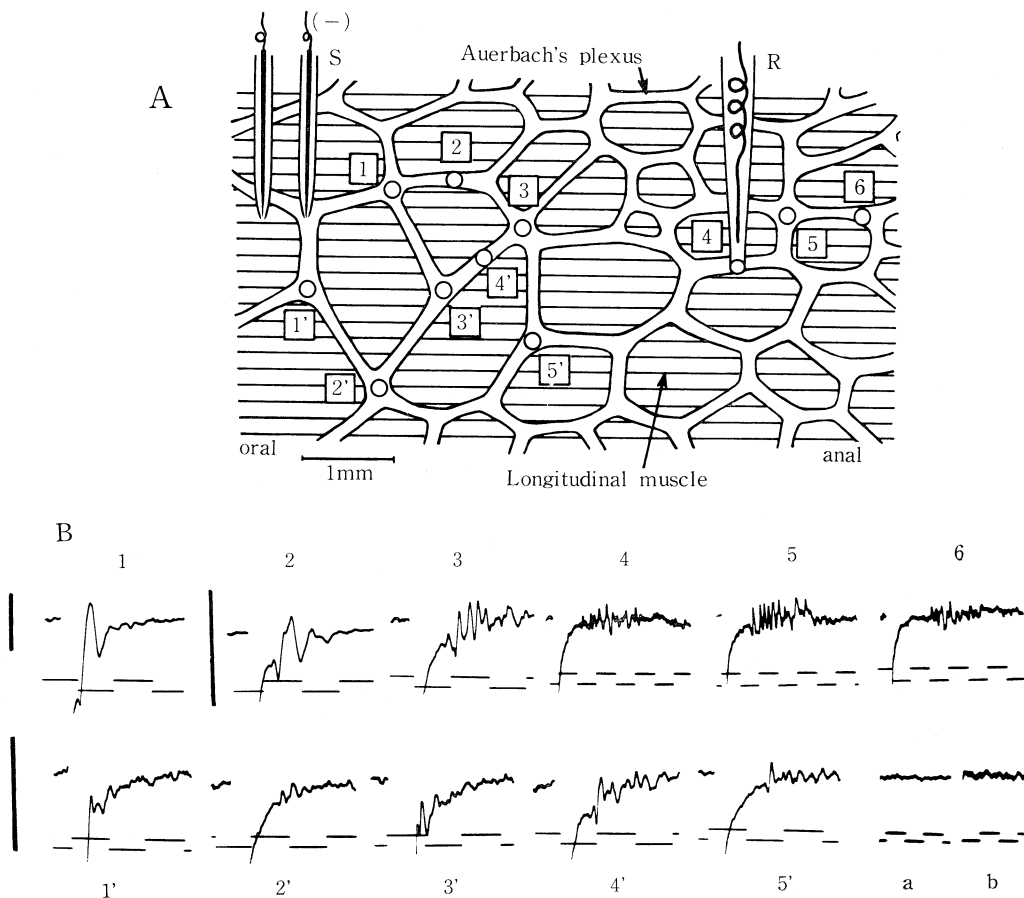


Fig. 1. Scheme of Auerbach's plexus of rabbit ileum (A) and evoked potentials obtained from various spots (B). S, stimulating electrode (cathode). Circles on the plexus arranged with number show spots where recording electrode (R) was placed. Action potentials were elicited by a single supramaximal stimulus (0.1 ms, 10 V). Inset number 1, 2, 3 and 1', 2', 3' etc. indicate records obtained from spots (1, 2, 3 and 1', 2', 3' etc). Two vertical bars show 0.1 mV; short bar is applicable to record 1 (noise level is shown in a); long bar to all other records (noise level, in b). Time calibration: 100/s.

stimulating electrodes. The cathode electrode was set on the ganglion (Fig. 1A, S), the anode one was in contact with the longitudinal muscle. A glass electrode (R) of which tip diameter was 20–30 μm was used for recording evoked potentials.

When the distance between the stimulating cathode electrode and recording electrode (interelectrode distance) was 1 mm, two spike waves were recorded. When this distance was further increased, evoked potential waves became multiple and small (Fig. 1B), until beyond 15 mm in the longitudinal direction and beyond 3 mm in the circular direction they could not be recorded. Evoked potentials recorded from spots situated along the longitudinal direction were larger in their amplitude than those recorded from spots situated along the oblique and circular direction. This fact means that in Auerbach's plexus nerve impulses spread through multiple pathways, conducting mainly along the longitudinal direction.

Conduction velocities of nerve impulses in Auerbach's plexus were 0.3–0.5 m/s, and chronaxie of nerve was 0.06–0.11 ms.

Multiple spike waves, which were recorded at a interelectrode distance larger than 2 mm, were reduced in their number and amplitude, when hexamethonium (1×10^{-4} g/ml) was given to the bath solution or oxygen supply was stopped. However, hexamethonium application or oxygen lack did not abolish evoked potentials, even when they were recorded at relatively long interelectrode distance of 5–10 mm. These facts indicate that some nerve fibers pass in Auerbach's plexus with synaptic transmission and the other distinct nerve fibers pass in a relatively long distance without the synaptic transmission.

Multiple spike waves evoked in aboral direction at a relatively long interelectrode distance of 5 mm (Fig. 2a) were in general larger in their amplitude and number than those recorded in oral direction (Fig. 2b). It can be thought that nerve fibers conducting impulses with synaptic transmission are running in Auerbach's plexus more numerous in the aboral direction than in the oral direction.

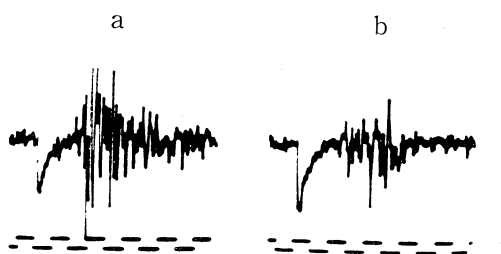


Fig. 2. Difference of evoked potentials depending on direction of excitation conduction. Jejunum preparation. Interelectrode distance was 5 mm. Stimulus: 0.2 ms, 10 V. a: action potentials evoked in aboral direction. b: action potentials evoked in oral direction. Both action potentials were led on same length and between same two ganglia. Note that amplitude of evoked potentials led aborally was larger than that of evoked potentials led orally. Calibration: 0.1 mV and 100/s.

Tetrodotoxin (1×10^{-7} g/ml) abolished evoked potentials 1.5 min after its application. At this stage rhythmic contractions of the longitudinal muscle continued without a change in their rhythm. The recovery occurred about 30 min after washing the preparation many times with Krebs solution.

II. *Afferent pathways (Yokoyama and Ozaki, 1974)*

It is thought that the afferent pathways of the reflex arch in relation to peristalsis is activated by the gut distension or the chemical stimulation of mucosa in the gut.

Spontaneous neuron discharges could be recorded occasionally, when a glass pipette electrode of which tip diameter was $20\ \mu\text{m}$ was placed on a local spot of a node of Auerbach's plexus. Figure 3 shows one example. Spontaneous neural discharges appeared with frequencies of 10–15 Hz and with amplitude of $50\text{--}70\ \mu\text{V}$, the duration of each spike was 1.5–2.0 ms. When these spontaneous discharges appeared as regular rhythmic spike potentials with the same amplitude, they might be thought to originate from a single neural unit in a node of Auerbach's plexus. That these spontaneous discharges are of origin of the nervous element is based on the following 2 points; 1) they could be obtained when the recording electrode was placed on a node of Auerbach's plexus and not obtained when it was placed on the longitudinal muscle layer. 2) the application of tetrodotoxin abolished these spontaneous discharges but it did not affect the muscle electrical and mechanical activities. Since the recording of electrical activity was extracellular, muscle action potentials could be recorded always as the periodic burst of large spike potentials (M in Fig. 3) with intervals of 3–4 s corresponding to rhythmic contraction. Using a peeled longitudinal muscle flap with adherent Auerbach's plexus which was connected to a intestinal segment, the effects of distension and chemical simulation of gut upon both neural and muscular discharges were investigated.

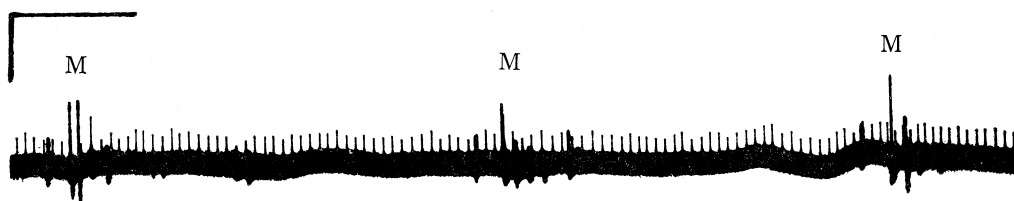


Fig. 3. Spontaneous muscular and neural discharges led from a local spot of node of Auerbach's plexus in jejunal longitudinal muscle strip. M, muscle action potentials appearing as burst of spike potentials. Spontaneous neural discharges appeared with frequency of 10–15 Hz and with amplitude of $50\text{--}70\ \mu\text{V}$. Calibrations; 0.1 mV and 1s.

When the intestinal segment was distended by elevation of inner pressure 5 cm high, neural discharges increased in their frequency from 10–15 Hz up to 30–60 Hz. In the period of distension (about 15 s) the intervals of bursts of muscle action potentials decreased and several peristaltic movements were observed. The higher was the inner pressure, the larger was the frequency of neural discharges. It is noteworthy that some spontaneous neural discharges were not affected by gut distension.

For analysing the nervous participation in the effects of gut distension, the effects of several drugs were investigated. Tetrodotoxin ($1 \times 10^{-7}\ \text{g/ml}$) stopped spontaneous neural discharges, while it did not affect muscle action potentials as well as muscle contraction. In the presence of tetrodotoxin, the gut distension did neither provoke neural discharges nor cause any change of muscle contraction. Atropine ($1 \times 10^{-6}\ \text{g/ml}$) did neither affect the spontaneous neural discharges nor the excitatory effect of gut distension upon neural discharges but in the presence of atropine the gut distension caused in general

the inhibition of intestinal movements. Mn Cl_2 (1×10^{-4} g/ml) suppressed the electrical and mechanical activities of the longitudinal muscle, whereas it did not stop spontaneous neural discharges. In the presence of MnCl_2 , the gut distension caused the increase of frequency of neural discharges, while it did not affect the muscle contraction.

From the results above described it can be concluded that the distension of the small intestine caused the excitation of the stretchreceptors in the gut wall and increased the frequency of discharges of distinct neurons which may participate in the occurrence of the peristaltic movement.

In a few instances, however, the unit discharges of a node did not respond to the gut distension but responded to the perfusion of HCl solution through the intestinal segment. In one example the unit discharges showed the frequency of 10–20 Hz when the inner pressure was zero. By elevation of the inner pressure up to 5 cm high, spontaneous neuron discharges did not change. When HCl solution (0.1 N) was introduced, the frequency of unit discharges increased up to 60–70 Hz.

It was concluded that there exist several sorts of neuron in the node of Auerbach's plexus and the one responds to the distension of the intestinal canal and the other responds to the chemical stimulation of the mucosa.

III. *Efferent pathways*

To clarify the properties of efferent pathways in Auerbach's plexus, repetitive stimuli were applied to a local spot of a node of Auerbach's plexus for 10–20 s and their effects on the electrical and mechanical activities of the longitudinal muscle and on circular muscle contractions were investigated. A stimulus frequency of 50 Hz was generally used for the nodal stimulation because of our findings that some neurons within the plexus which discharge at frequencies of 10–20 Hz increases their frequency rate up to 30–60 Hz during the distension of the intestinal canal or during the perfusion of mucosa with HCl solution. In order to avoid direct excitation of muscle, rectangular pulses of 0.1 ms duration and 10 V intensity were used. Such a pulse was supramaximal to produce evoked potentials of Auerbach's plexus (Yokoyama et al, 1977) but repetitive stimuli with such pulses applied to the longitudinal muscle did not cause any change of muscle activity. For stimulation of a node, a pair of tungsten wires (S_a , S_o in Fig. 4A, S in Fig. 7A) was used. The wires were tapered electrolytically to $5 \mu\text{m}$, insulated except at tips. The cathode electrode was placed always on a node and the anode electrode was placed carefully on the longitudinal muscle layer. Care was taken not to place the anode electrode on a node or nerve bundle of Auerbach's plexus.

Part A. Effects of nodal stimulation on spontaneous electrical activity of the longitudinal muscle (Yokoyama and Ozaki, 1978)

Repetitive electrical stimuli applied to a node of Auerbach's plexus situated anal (S_a in Fig. 4A) to the recording site caused in general a shortening of interburst intervals (Fig. 4B, b-c and 4C) of muscle action potentials, indicating the excitation of the longitudinal muscle. Repetitive stimuli applied to a node situated oral (S_o in Fig. 4A) to the recording site caused generally an increase in interburst intervals (Fig. 5A, c-d and 5B) indicating the inhibition of the longitudinal muscle. In a few examples, repetitive stimuli applied to a node caused the mixed form of excitation and inhibition, and on other several examples the

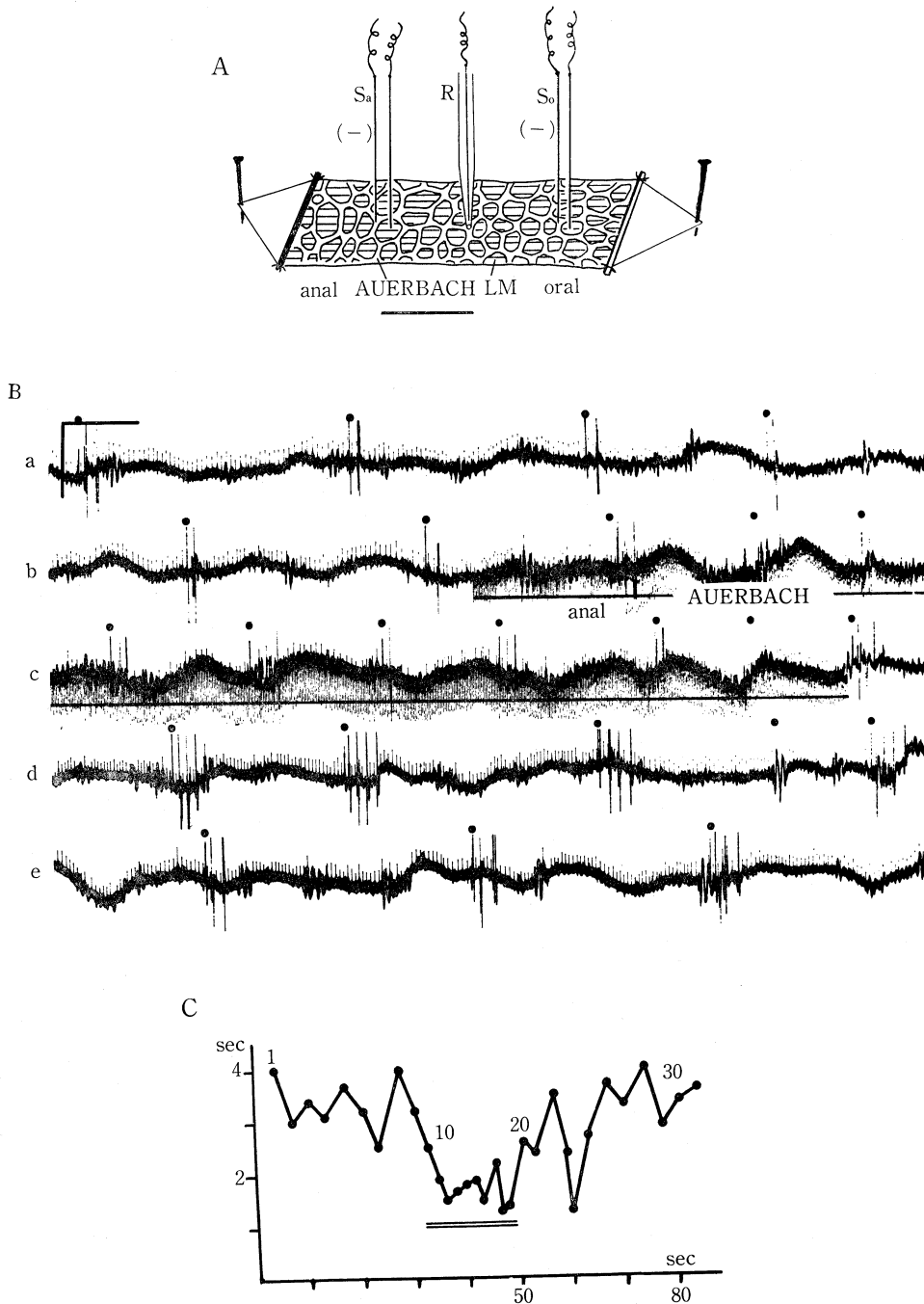


Fig. 4. A: Schematic representation of the experimental arrangement used for recording muscle action potentials and for stimulating a node of Auerbach's plexus. R, recording electrode. S_o , S_a ; stimulating electrodes on oral and anal sides. LM, longitudinal muscle. Horizontal bar shows length of 5 mm and direction of longitudinal axis of the small intestine. B: Effect of stimulating a node on the electrical activity of orally located muscle. Recording electrode was placed on a node. Interelectrode distance was 6 mm. a, b, c etc. continuous recording of the electrical activity of the longitudinal muscle (jejunum). Muscle action potentials appeared as periodic bursts (marked with •) of large spikes intermingled with spontaneous neural discharges of

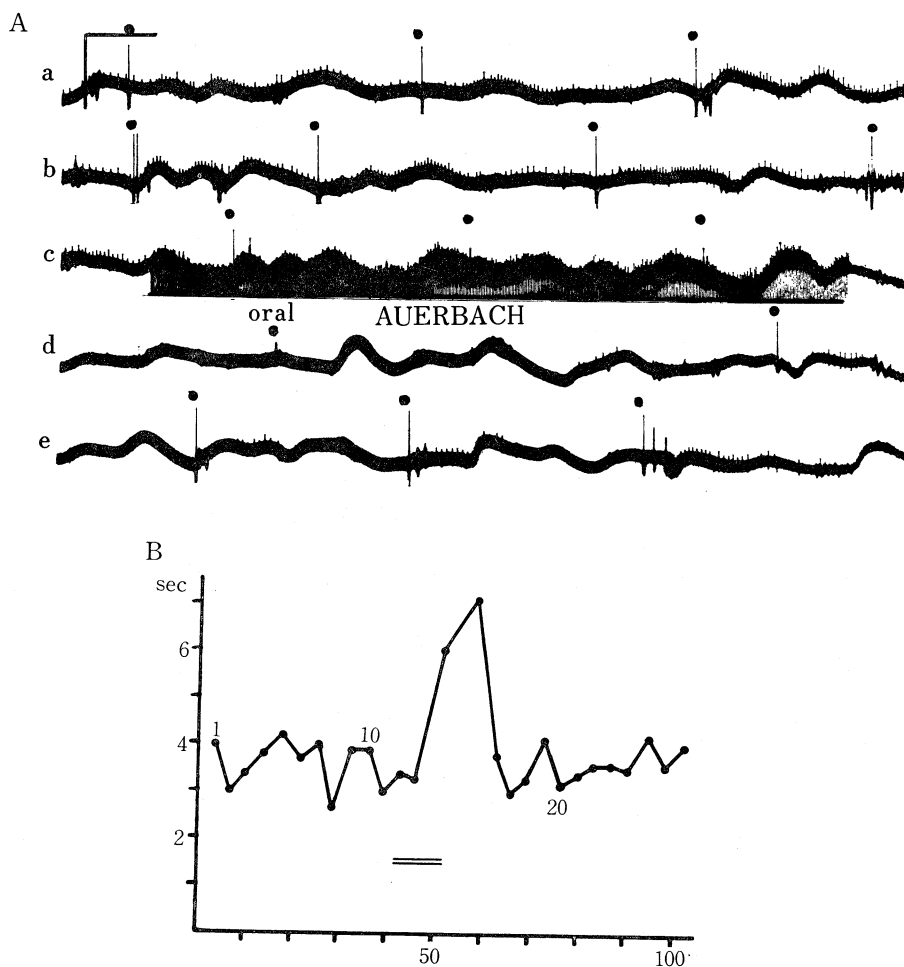


Fig. 5. A: Effect of stimulating a node on oral side on muscle action potentials (ileum). Further explanation is same as in Fig. 4B. Interelectrode distance was 5 mm. B: Change in interburst intervals of muscle action potentials evoked by nodal stimulation. Curve was obtained from experiment shown in A. Further explanation is the same as in Fig. 4C. Lengthening of interval occurred on 14-15 th intervals.

nodal stimulation with repetitive stimuli did not cause any change of electrical activity of the longitudinal muscle.

Occurrence number of excitation and inhibition caused by the nodal stimulation is summarized in Table 1. From the results shown in Table 1 it can be concluded that high frequency electrical stimulation of a node situated anally to the recording point generally produced excitation of the longitudinal muscle, whereas stimulation of a node situated orally

Fig. 4. (continual)

small spikes. Stimuli (50 Hz, 0.1 ms, 10 V) were applied to a node during the period marked with the base line. Calibration; 0.1 mV and 1 s. C: Change of interburst intervals of muscle action potentials evoked by node stimulation. Filled circles show interburst intervals measured from experiment shown in B. Period of stimulation was marked with double lines. Number on curve shows interval number of successive bursts of muscle action potentials. Shortening of time course in s. Ordinate: interburst interval in s. Abscissa:

Table 1. Occurrence number of excitation and inhibition of muscle electrical activity

Location of stimulated node	Number of effects				
	E	I	$\frac{E+I}{I+E}$	No effect	Total
Anal	46	5	8	7	66
Oral	6	50	6	4	66

E, excitation; I, inhibition. Muscle action potentials were recorded at the middle of preparation.

to the recording point generally produced inhibition of the longitudinal muscle. It seems that a polarity for influence of nodal stimulation on muscle activity exists in the longitudinal muscle of the rabbit small intestine.

In order to examine the polarity of the nodal stimulation more directly, electrical stimuli (50 Hz, 0.1 ms, 10 V) were applied for about 20 s to a node at the middle of a preparation and the effects on electrical activity of the longitudinal muscle on both oral and anal sides were investigated. Excitation of the longitudinal muscle orally situated and inhibition of the longitudinal muscle situated anally (oral E: anal I) occurred in 46 cases of 86 trials (53%), (oral E: anal E) in 8, (oral E: anal E+I) in 8, (oral I: anal I) in 4, (oral I+E: anal I+E) in 1, (oral E: anal no effect) in 5, (oral no effect: anal I) in 7, and (oral no effect: anal no effect) in 7. Figure 6 shows one example of oral excitation and an al inhibition produced by nodal stimulation at the middle of the preparation. Thus, a polarity of longitudinal muscle activity, excitation above and inhibition below a stimulated point, was also suggested in this series of experiments.



Fig. 6. Effect of nodal stimulation on electrical activity of both oral and anal longitudinal muscle (jejunum). Both interelectrode distances were 5 mm. a, b, c: continuous recordings of muscle action potentials of both oral (upper curves) and anal (lower curves) sides. Electrical stimuli (50 Hz, 0.1 ms, 10 V) applied to a node at middle of preparation caused excitation of orally located muscle and inhibition of anally located muscle (b). Stimulation period was marked with a straight line (b). Calibration, 0.1 mV and 5 s.

Part B. Effects of nodal stimulation upon mechanical activity of the longitudinal muscle (Yokoyama and Ozaki, 1978)

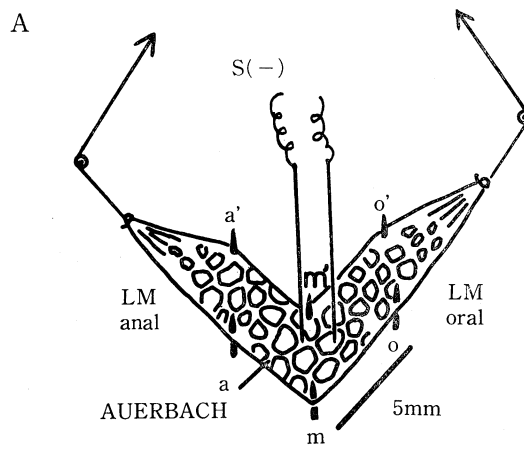
Excitation or inhibition of the muscle electrical activity reflects only the change of muscle activity at a local recording site. The question arises whether the repetitive electrical stimulation of a local spot in a node affects the muscle contractions of both oral and anal sides, and whether any polarity is associated with muscle responses to nodal stimulation. Experimental arrangement is shown in Figure 7A. Longitudinal muscle strips, about 0.3×2.0 cm, were fixed at 6 points and preparations were bent at the middle to an angle of 90° so as to avoid the influences of muscle contractions of both sides on each other. Contractions of both orally and anally situated longitudinal muscle strips (LM oral, LM anal) were recorded isotonicly on a smoked drum. Repetitive electrical stimuli were applied to a localized spot of a node at the middle of preparation using a metalelectrode of which tip diameter was $5\mu\text{m}$.

Stimulation applied to a node caused an increased frequency of muscle contractions and tone elevation on the orally situated longitudinal muscle strip in 57 cases of 64 trials (89%). An inhibitory effect occurred only in 1 case, a mixed form of excitation and inhibition in 2 cases, and no effect was observed in 4 cases. Upper curves in Fig. 7B show one example of excitatory effect.

The lower curves of Fig. 7B show an example of the effects of nodal stimulation on the anally situated longitudinal muscle strip. An increased frequency of muscle contraction appeared at first and then the muscle relaxation followed. Detectable relaxation was produced at a stimulus frequency of 5 Hz and it reached a peak at frequencies of 10–20 Hz, strong relaxation occurred at a frequency of 50 Hz and the degree of relaxation declined with higher stimulus frequency. Quantitatively, nodal stimulation (50 Hz, 0.1 ms, 10 V) caused excitation of anally situated muscle in 38 cases of 64 trials (59%), inhibition in 25 (23%), a mixed form of excitation and inhibition in 9 (14%), and no effect in 2 (3%). The results are summarized in Table 2. It is remarkable that in this series of experiments the excitatory effect on anally located muscle occurred in 59%, while the inhibitory effect occurred in 23%. These findings do not agree with those obtained in Part A. The reason of this discrepancy will be explained in the part of discussion.

Part C. Effects of nodal stimulation on both longitudinal and circular muscles (Ozaki and Yokoyama, 1973)

Using the L-formed longitudinal and circular muscle strips, effects of repetitive electrical stimulation of a centrally situated node upon the mechanical activities of both muscle strips were examined. Nodal stimulation produced predominantly excitation of the orally directed longitudinal muscle, excitation or inhibition or mixed form of excitation and inhibition of the anally directed longitudinal muscle and mainly excitation of the circular muscle. It is remarkable that the produced excitation of the circular muscle always appeared with higher threshold values of frequency and strength of stimuli and with a longer latency than those of the longitudinal muscle. Table 3 shows values of latencies of evoked excitation of both longitudinal and circular muscles.



B

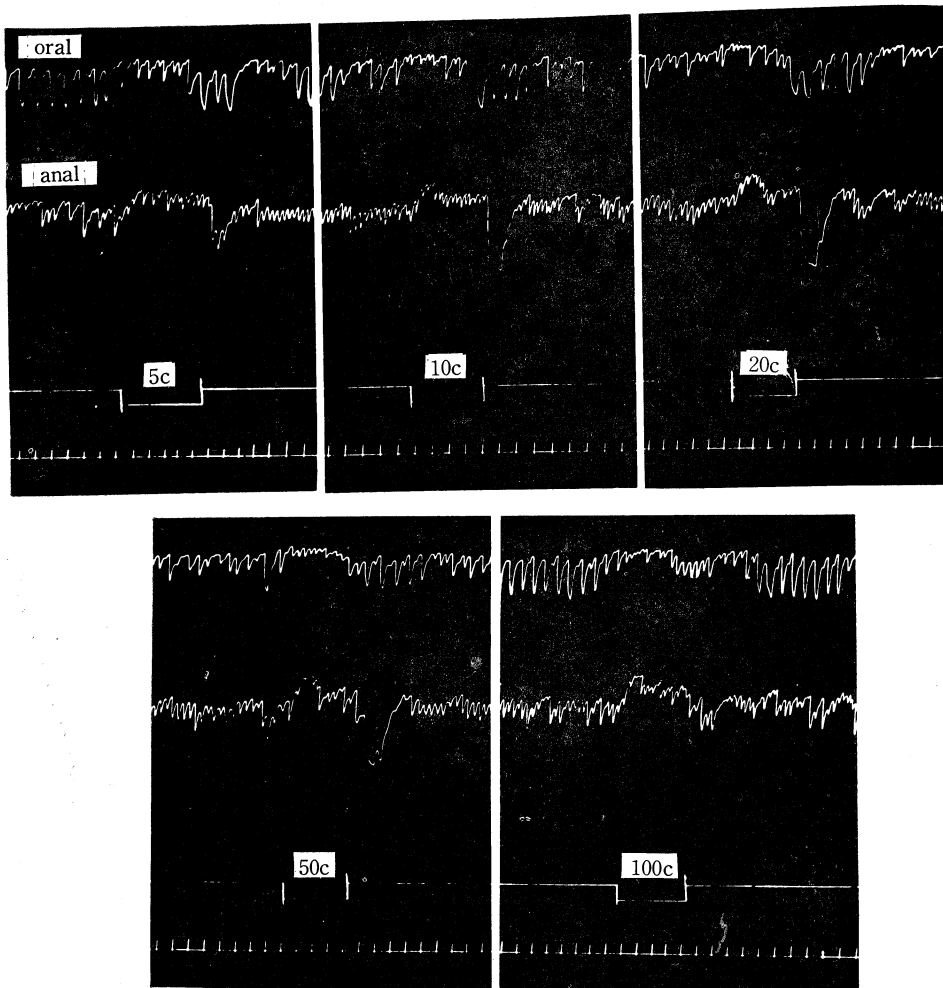


Table 2. Number of occurrences of excitation and inhibition of longitudinal muscle contractions

Location of muscle strip	Number of Effects				
	E	I	$\frac{E+I}{I+E}$	No effect	Total
Oral	57	1	2	4	64
Anal	38	15	9	2	64

E, excitation; I, inhibition. On both oral and anal sides, excitation or inhibition of contractions were provoked by stimulation of the node in the middle of preparation.

Table 3. Changes of latencies of evoked excitation of circular and longitudinal muscles by increasing stimulus frequency. Latencies were measured from record of Ozaki's experiment (unpublished).

Frequency of stimuli (Hz)	2	5	10	20	30	40	50
Latency in circular muscle (s)		25.4	21.6	10.8	7.2	6.4	5.0
Latency in longitudinal muscle (s)	14.4	13.2	10.8	7.2	3.6	3.2	3.3
Delay		12.2	10.8	3.6	3.6	3.2	1.7

Effects of drugs on muscle excitation and inhibition produced by nodal stimulation (Yokoyama and Ozaki, 1978)

Atropine (1×10^{-6} g/ml) abolished or suppressed the excitation of the longitudinal muscle but it did not abolish the excitation of the circular muscle. It can be concluded that the excitation of the longitudinal muscle produced by nodal stimulation was mainly cholinergic. However, the existence of the non-cholinergic neuron can not be excluded, since in several cases, especially in cases of excitation of the circular muscle, atropine did not abolish the excitation and the excitatory effect always remained. When the excitation or a mixed form of excitation and inhibition was produced by nodal stimulation on anally situated longitudinal muscle, atropine abolished the excitation and the inhibition was revealed or potentiated.

Hexamethonium (1×10^{-4} g/ml). When oral excitation and anal inhibition of the longitudinal muscle occurred by nodal stimulation, hexamethonium generally abolished both effects. This fact suggests the existence of the interneuron of which stimulation produces the oral excitation and the anal inhibition with cholinergic and nicotinic transmission of synapses. When the oral excitation and the anal excitation of the longitudinal muscle contractions were produced by nodal stimulation, hexamethonium abolished both

Fig. 7. A: Schematic representation of experimental arrangement used for recording contractions of muscle strips located on both oral and anal sides of a node and for stimulation of that node. LM oral and LM anal, longitudinal muscle strips on oral and anal sides. S(—), stimulating cathode electrode placed on a node at middle of preparation. o, o', m, m', a, a' are fixed points of preparation. Longitudinal muscle strip was bent at m and m' at an angle of 90°. B: Effects of nodal stimulation on contractions of longitudinal muscle strips (ileum). Electrical stimuli (5–100 Hz, 0.1 ms, 10 V) applied to a node caused excitation of orally located longitudinal muscle (upper curves) and excitation and inhibition of anally located longitudinal muscle (lower curves). Period and frequency of stimulation are indicated by signal and numbers of bottom trace. Stimulation frequency is indicated by 5c, 10c etc. (5 Hz, 10 Hz, etc.). Time marker, 6 s.

excitations and the anal inhibition was revealed.

Neither guanethidine (1×10^{-6} g/ml), bretylium (5×10^{-6} g/ml), dibenamine (1×10^{-6} g/ml) nor propranolol (1×10^{-6} g/ml) affected the inhibitory effect of nodal stimulation on both longitudinal and circular muscle. It is thought that the inhibitory effect of nodal stimulation on both longitudinal and circular muscles was non-adrenergic.

Tetrodotoxin (1×10^{-7} g/ml) abolished all excitatory and inhibitory effects of nodal stimulation on both longitudinal and circular muscles.

DISCUSSION AND SUMMARY

Auerbach's plexus which is thought to be a regulatory system functioning as a local reflex in relation to peristalsis may be schematically illustrated as Figure 8. Afferent pathways of the reflex arch are activated by mechanoreceptors in mucous membrane and in muscle layers, and by chemoreceptors in mucous membrane. The gut distension or the chemical stimulation of mucosa stimulates afferent nerve cells (AN in Auerbach's plexus and Meissner's plexus) which excite in a part the interneuron (Int. N) and in other part the excitatory neuron (EN') anallysituated. The evoked excitation of interneuron may produce excitation of orally situated excitatory neurons (EN) and that of anally situated inhibitory neurons (IN). The former produces the excitation of both the longitudinal and circular muscles orally situated

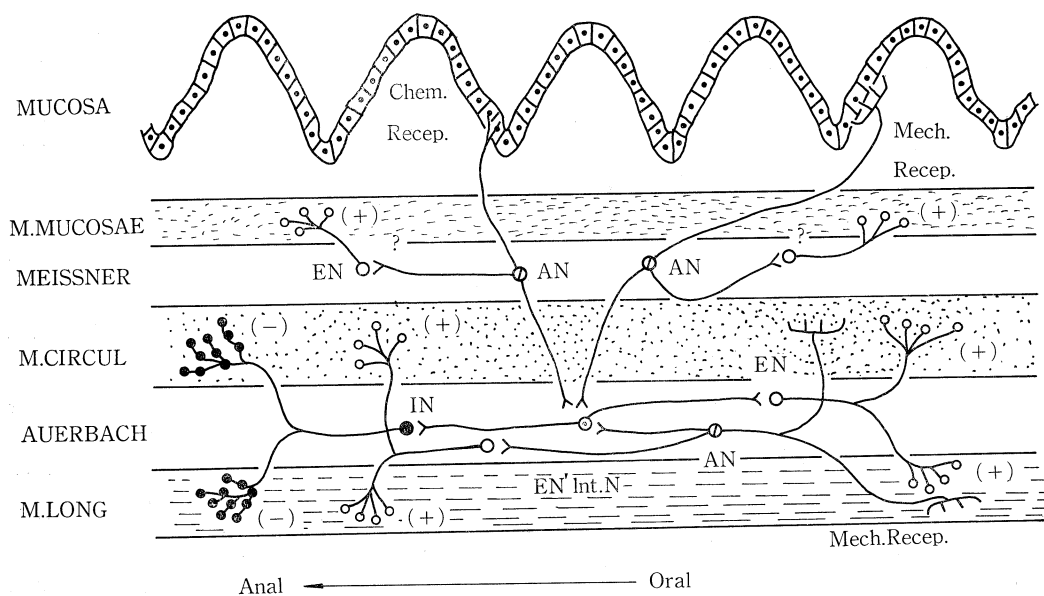


Fig. 8. Schematic representation of pathways in enteric system functioning as reflex archs in the case of peristalsis. Afferent processes (mechanoreceptor and chemoreceptor) of sensory neuron (AN) are activated by distension or chemical stimulation of gut. Efferent processes of sensory neuron impinge in one way on cell body of an interneuron (Int. N) and in another way, through side pathways of Auerbach's plexus, on cell body of descending excitatory neuron (EN') which causes the excitation of intestinal muscle anally situated. Efferent processes of interneuron run in one way in an oral direction and impinge on cell body of ascending excitatory neuron (EN) which causes the excitation of contractions of both longitudinal and circular muscles orally situated. Efferent processes of interneuron run in another way to an anal side and impinge on cell body of descending inhibitory neuron (IN) which causes the inhibition of contractions of both longitudinal and circular muscles anally situated.

and the latter produces the inhibition of the anally situated muscle layers. Existence of such a interneuron can be suggested by the fact that the nodal stimulation produced oral excitation and anal inhibition which were abolished by hexamethonium. Thus, the peristaltic movement caused by mechanical and chemical stimulation of the gut occurs as a local reflex. The polarity of effects of stimulation of Auerbach's plexus, namely excitation above and inhibition below stimulated spot, was proved in the longitudinal muscle. This fact can support the concept of the law of the intestine. However, there is a remarkable discrepancy between the results which were examined with electrical activity of the longitudinal muscle and those which were examined with mechanical activity of the longitudinal muscle. The electrical nodal stimulation produces mainly the excitation of electrical activity of the orally situated longitudinal muscle and the inhibition of the anally situated longitudinal muscle, whereas the nodal stimulation produces mainly excitation of contractions of the orally situated longitudinal muscle but it produces in many cases the excitation of contractions of the anally situated longitudinal muscle and the inhibition in rather a small number of cases. This discrepancy may be explained as follows.

The change in muscle electrical activity reflects only the action of the plexus on the small area of the muscle under the recording electrode, whereas the change in overall mechanical activity of the anal portion of the preparation reflects the total influence of the plexus on all the muscle cells located in this portion of the muscle strip. Thus, the former occurs as effects of rather a small number of nerve elements and the latter occurs as the sum of effects of a larger number of nerve elements. From the study of excitation conduction in Auerbach's plexus, it is known that nerve impulses propagate orally as well as anally through multiple synaptically interrupted pathways of the plexus network, mainly along the longitudinal axis of the small intestine. Although all the properties of neural pathways within Auerbach's plexus are not yet known in detail, there are the ascending excitatory pathways and the descending inhibitory as well as excitatory pathways that can be postulated from intracellular study of Hirst, Holman and McKirdy (1974) and Hirst and McKirdy (1974) and the results of III, Part B in this report. It may be presumed that the descending inhibitory action is mediated by the main longitudinal pathways of Auerbach's plexus, whereas the descending excitatory action is mediated by several side-pathways which are synaptically interrupted. Thus, the electrical activity of the anally situated muscle recorded at the spot along the main longitudinal axis was mainly inhibited, while the overall contractions of the anally situated muscle strip were augmented as a summed results of excitatory and inhibitory effects. Because the descending excitation covers the descending inhibition, as is indicated by the experiments with atropine and hexamethonium, the excitation of the mechanical activity of the anally situated muscle was observed in many cases. The above mentioned presumption is supported by the observation that the excitation of muscle electrical activity occurred by the nodal stimulation in place of inhibition, when the recording electrode situated 5 mm apart and anally along the main longitudinal axis was moved in the circular direction 3 mm off the main longitudinal axis. It is thought that the nodal stimulation could inhibit contractions of the anally situated longitudinal muscle, if the mechanical activity of very localized part of muscle situated along the main longitudinal axis was recorded.

It may be thought that ascending efferent pathways in Auerbach's plexus contain primarily excitatory neurons, whereas the descending efferent pathways contain both inhibitory and excitatory neurons. These facts are in agreement with the results in our study (Yokoyama *et al.* 1977) that synaptically interrupted nerve fibers in Auerbach's plexus run more numerous in an aboral direction than in an oral direction.

Hirst and McKirdy (1974) suggested the existence of an interneuron having efferent processes that impinge directly on the cell body of an inhibitory neuron running only in anal direction. In our study (Yokoyama and Ozaki, 1978) excitation of orally situated muscle and inhibition of anally situated muscle (oral E: anal I) evoked by nodal stimulation occurred in 46 cases of 86 trials dealing with muscle electrical activity (III, Part A) and this polarity was observed in 13 cases of 64 trials dealing with muscle contraction (III, Part B). In addition, it was demonstrated that this polarity was abolished in many cases by hexamethonium. These results suggest the existence of an interneuron (Int. N) having efferent processes which impinge on both cell bodies of ascending excitatory (EN) and descending inhibitory (IN) neurons and transmits impulses through nicotinic cholinergic synapses.

Whether the spontaneous neural discharges originate from motor neuron or afferent neuron or interneuron, can not be now decided definitely. Since spontaneous neural discharges were abolished in many cases by hexamethonium application, they were probably of origin of interneuron or motor neuron. However, in several cases, spontaneous discharges of neuron was not affected by hexamethonium. Such a neuron may be an afferent neuron in Auerbach's plexus.

From the results described in this report it may be concluded as the summary that a basic polarity exists within neural pathways of Auerbach's plexus that may be responsible, in part, for the pattern of muscle excitation and inhibition commonly referred to as the law of the intestine. The excitation above caused the constriction of the intestinal canal orally situated, the inhibition below causes the relaxation of the intestinal canal anally situated. The resulting local gradient of intraluminal pressure may move the intestinal contents from the higher to the lower pressure area. Thus, the anally moved contents may cause the similar mechanism successively. The peristaltic movements occur which transport the contents in the intestinal canal from oral part to anal part.

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特 別 講 演 III

Functions of Enteric Nerve Cells in Relation to Peristalsis

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The aim of this lecture is to review some of the results that have followed from experiments in which the membrane potentials of enteric neurones of the guinea-pig small intestine have been recorded with intracellular electrodes. Since our first experiments on Auerbach's plexus, in 1972, we have been ably assisted by Drs. Hugh McKirdy, Gene Silinsky, Ian Spence and Tim Neild. We wish to thank our colleagues for allowing us to report this work to your Society.

The main technical advance that enabled us to undertake these experiments was the inverted compound microscope. One of us (M.E.H.) was introduced to this apparatus by Professor A.K. McIntyre who is well known to many colleagues in Japan. Using this device, we looked at a number of preparations of small intestine in which the myenteric plexus adhered to the longitudinal layer. Since the longitudinal layer of the small intestine of guinea-pigs is so thin (some 5 cells thick) it was possible to visualize individual myenteric neurones in this preparation. However this smooth muscle is inclined to be spontaneously active *in vitro*. In our early experiments we used a variety of agents, including β agonists and non-specific smooth muscle relaxants to tranquilize the smooth muscle.

Eventually we found that if we were careful not to stretch the muscle and only pinned it out around the edges many preparations were not spontaneously active. However stimulation of the plexus always caused brisk contractions of the longitudinal muscle. Therefore most of our experiments on the myenteric plexus were done in the presence of atropine (usually 10^{-7} g/ml).

In a parallel series of experiments, Drs. S. Nishi and R.A. North were able to stabilize a single node of the myenteric plexus (guinea-pig small intestine) by the extensive use of small pins. This prevented contractions of the longitudinal muscle from disturbing the impalement. They used a NaCl-filled micropipette to stimulate the connectives running into the node and were able to record responses in the absence of atropine. It is of interest that their results were essentially the same as ours (see Nishi & North, 1973 a and Hirst, Holman & Spence, 1974).

In our initial experiments, preparations of about 1 cm^2 were pinned to a thin layer of silicone rubber, on the surface of a coverslip which formed the base of a tissue culture chamber. The myenteric plexus was stimulated transmurally by two Pt electrodes, one above and one below the preparation. This arrangement enabled us to record from myenteric neurones which were situated up to 1 cm from the stimulating electrodes.

Both Nishi and North and our group at Monash identified two types of neurones in the myenteric plexus. We have called the first type "synaptic" or S cells since they received extensive excitatory synaptic input. It was clear that these cells were very similar to autonomic ganglion cells ($\tau_m \simeq 20$ msec; R_{in} , 100–200 M Ω). Their ESPs were blocked by curare and C6 and could be mimicked by the iontophoretic application of acetylcholine. Excitatory synaptic potentials were recorded from cells both oral (above) or anal (below) the stimulating electrodes at distances of up to 1 cm.

During these experiments there was no evidence for postsynaptic inhibition. This is in contrast with the results of later studies on the submucous plexus (see below). The absence of postsynaptic inhibition in the myenteric plexus was rather surprising in view of the morphological observations of Gabella (1972). Many myenteric neurones or their processes received synaptic contacts from axons containing small dense core vesicles typical of noradrenergic nerves and it is well known that such axons cause inhibition of intestinal motility. Perhaps these synapses failed to evoke a postsynaptic response because the appropriate receptors were absent from the postsynaptic membrane. Evidence that noradrenaline and sympathetic nerve stimulation act presynaptically to depress the release of excitatory transmitter was found by Nishi and North (1973b) and Hirst and McKirdy (1974 a) respectively.

The second type of myenteric neurone did not appear to receive synaptic input. In our experiments the soma could only be excited in response to transmural stimulation if the electrodes were very close to the cell. Since these responses were not blocked by curare it may be assumed that they were due to the excitation of a cell process. Nishi and North (1973a) obtained similar results. Indeed, their method of stimulation, with a small focal electrode, provided evidence that cells without synaptic input were bipolar neurones with their processes at opposite poles of the soma. The injection of procion yellow by North has confirmed this observation (North & Nishni, 1976).

When the soma of these cells was stimulated with a long depolarizing current pulse it was apparent that, *unlike* most autonomic ganglion cells, only a limited burst of action potentials (APs) could be elicited. The reason for this behaviour is illustrated in Fig. 1. Soma action potentials led to the initiation of a long lasting (up to 10 sec) increase in membrane conductance which usually caused a large after-hyperpolarization, such as that illustrated in Fig. 1. The increase in conductance and associated hyperpolarization was clearly limited to the soma of these cells. Action potentials could still be initiated without a change in threshold, in the cell processes but these failed to evoke a soma AP during the most intense phase of the hyperpolarization.

It seems most likely that this hyperpolarization was caused by an increase in G_k which was due to the influx of Ca ions during the AP. It is generally accepted that tetrodotoxin (TTX) blocks the action potentials of most axons. This drug was also found to block action potentials in response to direct, intracellular stimulation of the soma of myenteric neurones which received excitatory synaptic input. However it was possible to evoke action potentials in the soma of cells *without synaptic input* in the presence of TTX. Evidence that such action potentials were due to a voltage-dependent increase in G_{Ca} was reported by Hirst and Spence (1973) and North (1973). It was noted that the threshold of TTX-resistant action potentials was higher than that of APs recorded in normal solution and

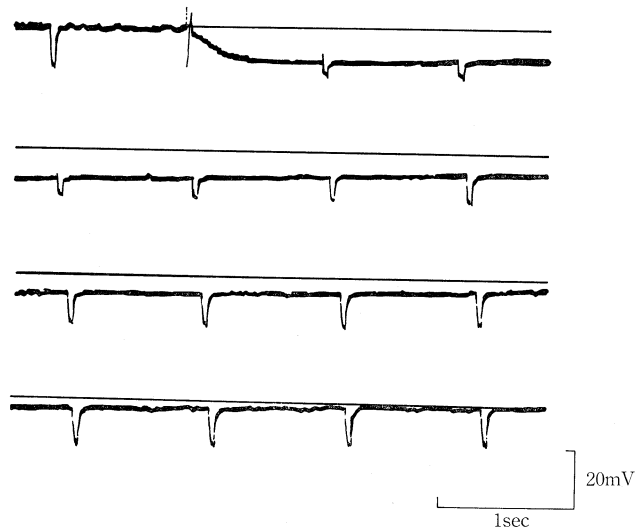


Fig. 1. Continuous intracellular recording from a myenteric neurone without synaptic input. A current pulse of constant intensity was passed through the recording electrode at intervals of about 1 sec; inward currents were used except for the second pulse in the upper trace in which an outward current pulse evoked an action potential (only the undershoot of this was recorded). Note the decrease in the amplitude of the change in membrane potential caused by inward current pulses throughout the period of after-hyperpolarization following the AP. The time course of the increase in conductance was comparable with the degree of hyperpolarization. (From Hirst, Holman, Prosser & Spence, 1973).

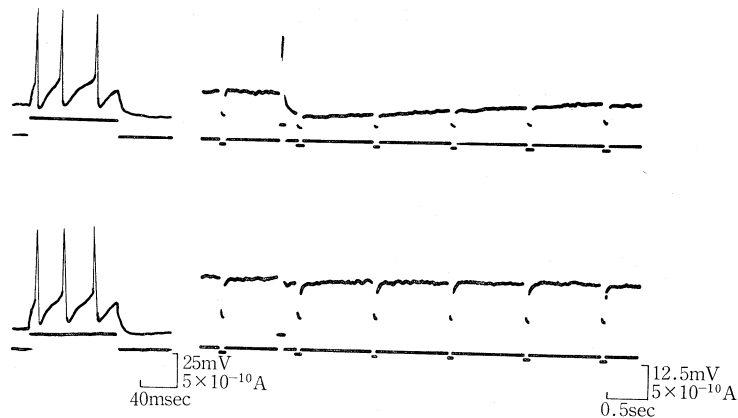


Fig. 2. Upper panels show response of a cell without synaptic input to direct stimulation (note different time scales). Lower panels show the effect of adding 0.5 mM Mn ions. The discharge of APs during the application of depolarizing current was unaffected but the after-hyperpolarization and increase in conductance was abolished. (From Hirst, Holman & Spence, 1974).

the rate of rise of these APs was reduced. It therefore seems likely that the membrane of the soma of these cells also contains voltage-dependent Na channels. As shown in Fig. 2, in the *absence* of TTX, Mn ions completely blocked the after-hyperpolarization without any effect on the AP. Since it is generally accepted that Mn blocks the influx of Ca ions it may be concluded that an increase in intracellular Ca is the cause of the after-hyperpolarization.

There has been a great deal of speculation about the possible function of these cells.

Not only do they appear to lack synaptic input but North and Nishi (1976) have shown recently that they also lack nicotinic receptors. They may well be sensory neurones but it must be emphasized that, so far, there is no direct evidence to support this suggestion. If one of their processes was sensitive to, for example, distension of the gut wall, and the other process made synaptic contact with another neurone it is interesting to speculate that the behaviour of the soma may influence the pattern of efferent discharge of these cells. It must be emphasized that nothing is known about the site of termination of their afferent process except that these must terminate within the myenteric plexus quite close to the soma or invade either the longitudinal or circular muscle layer.

Cells *with* synaptic input may function as interneurons, motor neurones to the smooth muscle, or inhibitory neurones to the smooth muscle. Unfortunately we have failed, so far, to detect any properties of myenteric S cells which might enable us to provide a sub-classification into these groupings. All S cells appear to be very similar.

During a recent series of experiments Hirst & Neild (unpublished observations) have made records from pairs of S cells within the same node. Fig. 3 shows records which are typical of those they obtained in some 30 such experiments. It is clear that when an AP was initiated in the soma of one cell this did not cause an excitatory synaptic potential in the second cell (and vice versa). Therefore it seems unlikely that interneurons and their post-synaptic target neurones are present in the same node.

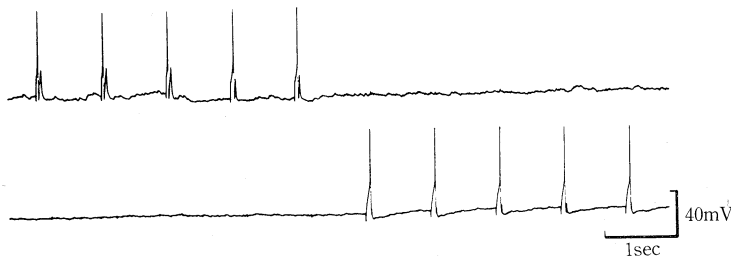


Fig. 3. Simultaneous records from two S cells within the same node of myenteric plexus. Action potentials were evoked by direct stimulation in one cell and afterwards, in the other. Note that APs failed to evoke EJPs or any other response in the unstimulated cell. (From G.D.S. Hirst & T.O. Neild, unpublished work).

There have been several suggestions that myenteric neurones, in isolated preparations of plexus such as those used in these experiments, were spontaneously active. This conclusion was based on experiments in which records were made with extracellular electrodes (see for example, Wood, 1975). We have failed to find any evidence for pacemaker activity in myenteric neurones with the use of intracellular electrodes (see Hirst et al. 1974). However it was clear that these neurones were extremely sensitive to mechanical stimulation. For example, it was only necessary to place a focal (NaCl) stimulating electrode against a node in order to obtain the continuous firing of an S cell for several hours (North & Williams, 1976). It is interesting to recall that motoneurons in the central nervous system are also mechanically sensitive (Alanis & Matthews, 1952). This behaviour may perhaps be a consequence of the lack of connective tissue elements in the immediate environment of both types of neurones. However the sensitivity of myenteric neurones to a localized

increase in pressure (touch) presents problems in the interpretation of records made with extracellular electrodes.

What do these results tell us about the role of these neurones in the peristaltic reflex? In order to try to answer this question Hirst and McKirdy (1974 b) developed the preparation which is shown in Fig. 4. This consisted of an intact segment of intestine, about 5 cm long, with a flap of longitudinal muscle and myenteric plexus attached to one end. The intact segment could be stimulated with transmural electrodes or distended by a small intraluminal balloon. When the membrane potential was recorded from S cells below the intact segment, many of these showed "spontaneous" ESPs. There did not seem to be any obvious pattern in this discharge and it did not appear to be correlated with any spontaneous contractions of the segment of intestine. Although a spontaneous discharge of ESPs was quite common in S cells *below* the segment of intestine this was only rarely observed in S cells *above* the segment.

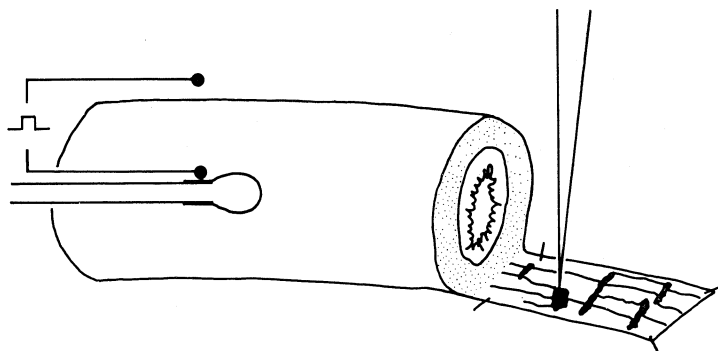


Fig. 4. Arrangement used to record from myenteric neurones in continuity with an intact segment of small intestine which could be stimulated by transmural electrodes or by an intraluminal balloon.

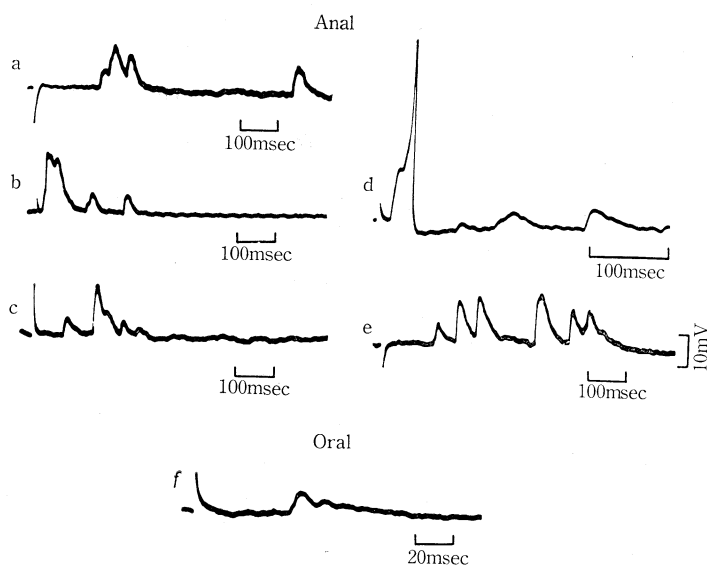


Fig. 5. Examples of the variety of responses recorded from myenteric neurones situated 2.5 to 3.0 cm from the stimulating electrodes. Records a to e were from neurones below (aboral) and record f from a neurone above (oral) to the site of stimulation. (From Hirst & McKirdy, 1974b).

Figure 5 shows the way in which myenteric neurones responded to transmural stimulation of the segment. In these experiments the neurones from which records were made were some 2.5 to 3 cm away from the site of stimulation. If they were situated *below* the segment they responded with a complex and often prolonged burst of ESPs. Very few neurones *above* the segment showed any response at all. The lowest trace is an example of the maximum response seen so far for a neurone situated above the segment.

Hirst and McKirdy (1974b) recorded from myenteric neurones below the segment, before, during and after the intraluminal balloon was distended. A surprisingly large proportion of neurones responded to distension with a discharge of ESPs indicating the great divergence of the action the sensory receptors involved in this reflex. Some neurones showed an initial burst of ESPs after a short latency (about 1 sec) but this was not maintained. The remaining S cells which responded to distension behaved quite differently, as is shown in Fig. 6. After a longer latency (2 sec or more) distension initiated a train of ESPs. This discharge was maintained throughout the period of distension and frequently continued after the distension had ceased.

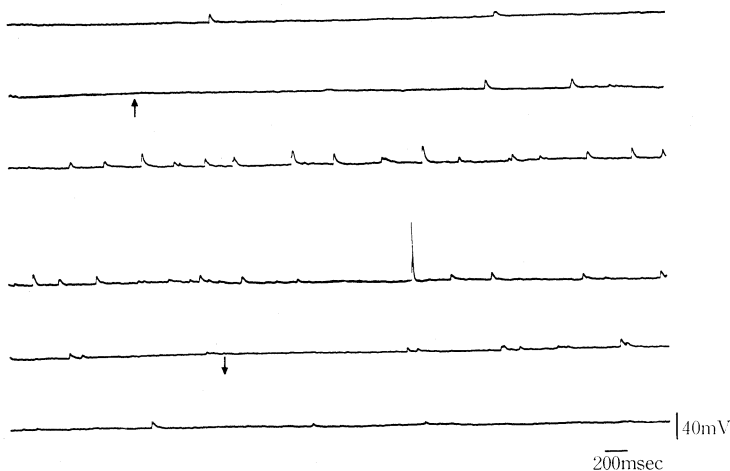


Fig. 6. Example of a myenteric neurone which responded to distension after a long delay and continued to show ESPs after the distension (indicated by arrows) was removed. (From G.D.S. Hirst & McKirdy, H.C., 1974 b).

There was no evidence for any overlap between these two populations of neurones. Fig. 7 is a histogram of the latencies observed in these experiments. All neurones that responded to distension with latencies of less than 2 sec or more, continued to show ESPs throughout the period of distension.

The next question was whether or not these two types of responses to distension, which were evidently mediated by quite different pathways, were associated with different responses in the smooth muscle. It was possible to make intracellular records from a flap of smooth muscle attached to an intact segment when this was distended by an intraluminal balloon. In the absence of atropine, distension caused a marked contraction, both around and above the site of distension. This contraction made it very difficult to study the changes in membrane potential of the smooth muscle. However, in the presence of atropine this contractile response was abolished. Fig. 8 shows records made by Hirst and

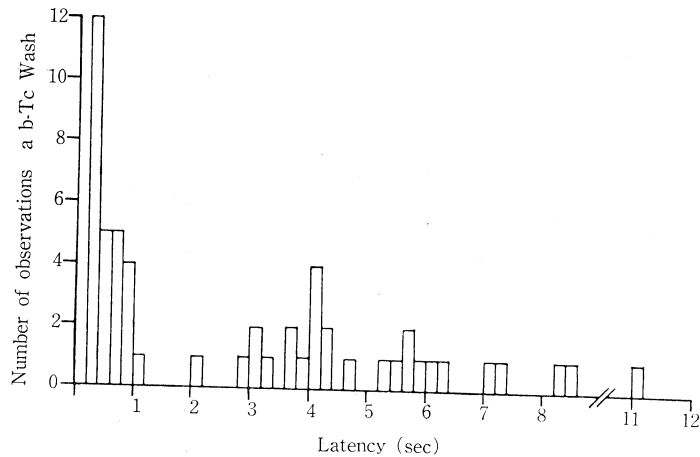


Fig. 7. Histogram showing the distribution of latencies between the onset of distension and the onset of a synaptic discharge in myenteric neurones below the site of distension. Each value for latency was the mean of 3 to 6 determinations for an individual neurone. (From Hirst et al., 1975).

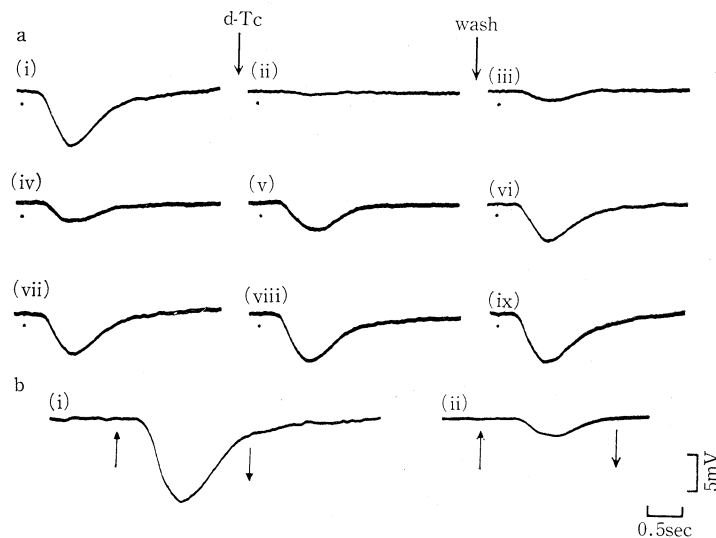


Fig. 8. Inhibitory junction potentials recorded from a flap of circular muscle attached below an intact segment of intestine. Records a i to a ix were responses to transmurial stimulation. As shown in a ii the IJP was blocked by d-tubocurarine (5×10^{-5} gm/ml), after 10 min. Records a iii to a ix were made at 2, 4, 6, 8, 10, 12 and 14 min respectively after washing out with control solution.

Fig. 8 b shows an IJP in response to distension. This was also depressed after 10 min exposure to curare, as shown in b ii. (From Hirst & Silinsky, 1974b).

McKirdy, (1974b) from the circular muscle of a flap of intestine some 2.5 cm below the region of electrical stimulation and distension. A short latency inhibitory junction potential (IJP) was recorded which was blocked by curare. In this and subsequent experiments the IJP was always of longer duration than that of an IJP evoked by close transmurial stimulation. However the IJP was never maintained even when the distension

lasted for several sec. Moreover, the latency of the IJP was short (about 1 sec). There was no evidence for a response having a long latency which was maintained throughout the period of distension.

It was much more difficult to do these experiments in the absence of atropine in view of the contractions of these preparations which occurred in response to distension. Some examples of the changes in membrane potential recorded from circular smooth muscle cells 2 to 3 cm below the site of distension are shown in Fig. 9. The distending stimulus evoked a short latency IJP. This was followed by a slow depolarization which initiated one or more action potentials.

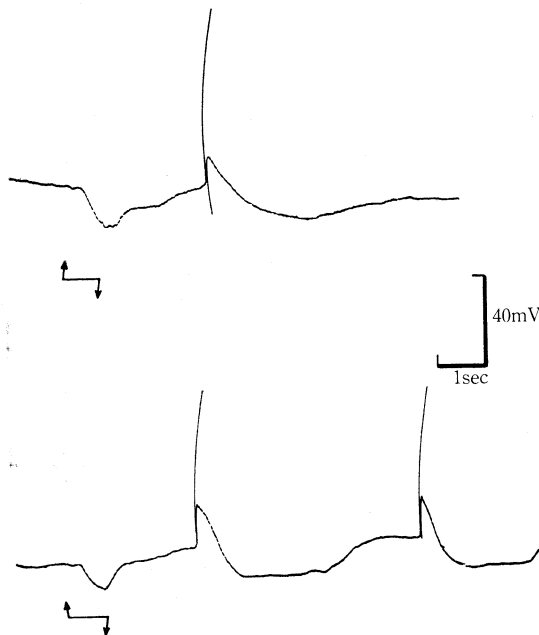


Fig. 9. Changes in membrane potential recorded from circular muscle below the site of distension, in the absence of atropine. The uneven baseline was due to contractions in response to this stimulus. Note distension (indicated by arrows) caused a short latency IJP followed by a slower depolarization which initiated APs. (This data was recorded on magnetic tape and subsequently displayed on a Grass Polygraph). (From G.D.S. Hirst & H.C. McKirdy, unpublished work).

We have therefore drawn the tentative conclusion that the short latency pathway in the intestine is associated with the descending inhibition of the circular coat which was first recorded graphically by Bayliss and Starling (1899). Thus the immediate response to distension is inhibition below the site of stimulation, which is caused by non-cholinergic non-adrenergic IJPs.

The second pathway appears to be associated with excitation of the smooth muscle—the long latency descending excitation which “passes down the intestine, driving the bolus ahead of it” (Bayliss and Starling, 1899).

During these experiments it became evident that descending excitation was a much more labile phenomenon than descending inhibition. It was abolished if the mucosa was injured or deliberately removed. In contrast, descending inhibition was clearly independent of an

intact mucosa and sub-mucous plexus. The next step then, was to learn about the physiology of the submucous plexus.

It has been possible to study the properties of the neurones of isolated preparations of submucous plexus which could be separated from the mucosa and the circular smooth muscle layer (Hirst & McKirdy, 1975). Segments of plexus about 1 cm² were used in these experiments and these were stimulated in the same way as described for the myenteric plexus. The first very obvious difference between the two plexuses was the absence of long nervous pathways in the submucous plexus. No responses have ever been recorded when the electrode was in a cell situated more than 0.6 mm from the stimulating electrodes.

Many submucous neurones showed ESPs which were indistinguishable from those of the myenteric plexus. Again, there was marked convergence of synaptic input on any one neurone and ESPs were blocked by curare.

In marked contrast with the myenteric plexus, some neurones which responded to stimulation with ESPs also showed ISPs. These ISPs were of relatively long latency (about 80 msec) and they lasted for 1-5 sec. Recently it has been shown that they are caused by an increase in potassium conductance (Hirst and Lang; 1976).

In contrast with ESPs, ISPs were not blocked by curare. In view of the numerous suggestions that 5-hydroxytryptamine (5-HT) might be a neurotransmitter in the peristaltic reflex, Hirst and McKirdy (1975) tested the effect bromo-lysergic acid (BOL) and methysergide. They found that these drugs blocked the ISP without diminishing the ESP. At this time we were joined by Dr. Silinsky who reminded us of the work of his colleagues, Drs. House and Ginsborg (in Edinburgh). It seemed that the ISPs recorded from submucous neurones were very similar to the secretory potentials recorded from the cockroach salivary gland in response to stimulation of nerves in which dopamine (DA) appeared to be the transmitter (see Ginsborg & House, 1976; Ginsborg, House & Silinsky, 1974). Both the cockroach secretory potential and the ISP recorded from submucous neurones were blocked by methysergide.

Hirst and Silinsky (1975) studied the effect of DA on submucous neurones using the method of micro-iontophoresis. They found that a brief pulse of DA could mimic the ISP provided it was applied to cells which received an inhibitory innervation. A secondary action of DA was observed in these cells and those without ISPs. This was a presynaptic inhibition of ESPs similar to that described for NA in myenteric plexus.

On the basis of this work it might be concluded that the inhibitory transmitter in the submucous plexus was DA. However Hirst and Silinsky (1975) found that NA had the same action as DA and appeared to be of the same order of potency. Experiments on extrinsically denervated segments of intestine have shown that the ISP must be caused by an intrinsic neurone. This procedure caused the loss of noradrenaline from the submucous plexus but did not cause a change in assayable levels of dopamine (Hirst and Jarrott unpublished observations). It would appear therefore that if either of these catecholamines were the transmitter at these synapses dopamine is the better candidate. The ultimate criterion for the identification of a neurotransmitter, that it is released in the appropriate amount when the inhibitory axon is stimulated, has yet to be satisfied.

It is perhaps of interest that 5HT caused depolarization of all the submucous neurones studied so far. This action was blocked by curare. T.O. Neild is currently attempting to

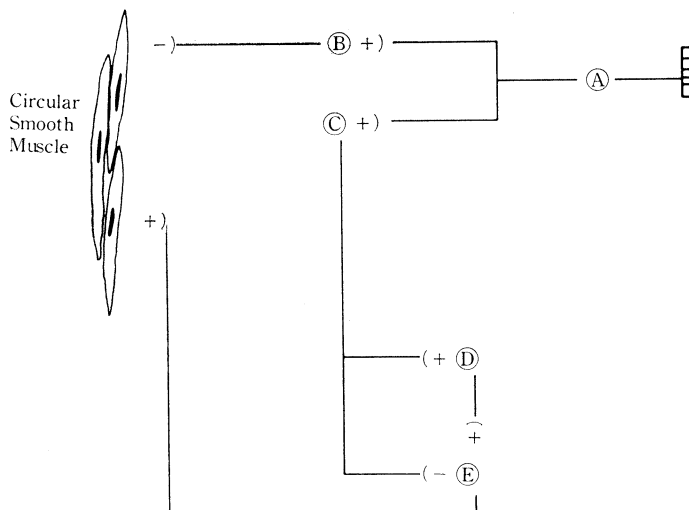


Fig. 10. A hypothetical scheme describing the possible connections of enteric neurones. Note that descending chains of cholinergic interneurons present in the myenteric plexus have been omitted from this diagram. The neurones labeled A to E might have the following functions:

- A: Primary afferent neurone whose process is sensitive to radial distension.
- B: Neurone whose axons cause direct inhibition of circular smooth muscle.
- C: A dopaminergic interneurone (site unknown)
- D: A cholinergic interneurone in the submucous plexus which shows "Bursting" activity after excitation by a dopaminergic axon and excites neurone E.
- E: A cholinergic neurone which excites the smooth muscle. Note that this is inhibited by a dopaminergic axon. See text for more detailed discussion.

sort out this rather unorthodox pharmacology, at least in the context of mammalian synapses.

Fig. 10 is a diagram of a scheme proposed by one of us (G.D.S.H.) which attempts to explain present observations, as well as those of many others from Bayliss & Starling up to the present time, utilizing the minimum number of neurones. In this diagram the cholinergic interneurons which link primary afferent neurones to their effector neurones (excitatory or inhibitory) have been omitted. It is suggested that distension causes excitation of a chain of cholinergic neurones which are arranged in a descending direction. One (or more) of these neurones excites an enteric inhibitory neurone (B) bringing about descending inhibition. It is tempting to invoke those cells of the myenteric plexus, which have a long lasting after hyperpolarization, but no synaptic input (A) to explain why this response is a transient one. Since this reflex can occur in the absence of the submucous plexus, the soma of the afferent neurones must be present in the myenteric plexus. However it should be emphasized that the site of the actual receptors has yet to be determined.

We would like to suggest that the ISPs of the submucous plexus are responsible for the delay of descending excitation. If excitation depends on the same sensory receptor as that for descending inhibition, then some mechanism must exist in the submucous plexus in order to maintain this response throughout the period of distension and beyond. Hirst and McKirdy (1975) reported the presence of a small number of cells in the submucous plexus which responded to stimulation with a long lasting ESP ($T^{1/2} \approx 100$ msec), which was followed by a long lasting (5 to 20 sec) series of oscillations in MP. This diagram suggests that cells such as (D) might be excited by the same dopaminergic neurone (C) which inhibits the

pathway for descending excitation. It must be emphasized that this proposal is pure speculation since virtually nothing is known about the physiology of these strange "bursting" neurones. The neurone labeled (E) leads to excitation of smooth muscle.

In conclusion, intracellular recordings from enteric neurones have begun to help to clarify our understanding of the peristaltic reflex. There are however, any gaps that need to be filled in. We still do not know what is the role of 5HT in the reflex, if any. We have much to learn about the effect of distension on *local* excitatory pathways. We do not even know what is the cause of spontaneous synaptic activity when a flap of myenteric plexus is attached to an intact segment of intestine.

At present it seems likely that at least three different neurotransmitters are involved - acetylcholine, possibly DA and the inhibitory transmitter which causes IJPs in the circular layer. There may well be others.

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