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31. Issei Takayanagi : Chemicopharmacological Studies on Antispasmodic Action. XVIII. On the Antibarium Action of Papaverine-like Antispasmodics tested on Plexus-free Circular Muscle and Innervated Longitudinal Muscle.

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In the previous report,[1] it was suggested that the papaverine-like action is to be divided into two groups; (1) the non-specific inhibitory action, which is exerted through some physicochemical property of non-ionized molecule (I-Group), and (2) the specific non-competitive inhibitory action of strong basic compounds, which is effected by ionized molecule (II-Group).

Up to the previous report,[2] the non-competitive antiacetylcholine action was considered as the papaverine-like action and a high concentration of acetylcholine (ACh), instead of barium chloride, was used to contract isolated smooth muscles.

It has been reported that Ba^{2+}, though it also stimulates the smooth muscle directly, owed most of its action to stimulation of ganglion[3] and Parkes[4] raised an objection against the ordinary method of relaxing contraction of the ileums of guinea pigs produced by Ba^{2+} for the assay of the papaverine-like antispasmodics, for the reason that some antispasmodics showed an extraordinary high antibarium activity, which was supposed by him to depend mainly on their ganglionic inhibitory action and not on the papaverine-like musculotropic action.

In the present work, the mode of action of Ba^{2+} and its antagonists was examined with excised intact small intestines of mice, tracheal muscles of guinea pigs, ganglion-free circular muscles, and innervated muscles of cat small intestines.

Method

The Magnus method with 40-cc. organ bath and Tyrode solution were used as described in the previous reports[1] of this series. The muscle preparations used were as follows :

(1) Excised intact intestines of mice and guinea pigs.
(2) Isolated small intestine of cats.
(3) Longitudinal muscle strip with ganglion and circular muscle of cat small intestine.
(4) Longitudinal muscle strip of cat small intestine.
(5) Circular muscle strip with ganglion and longitudinal muscle of cat small intestine.
(6) Circular muscle strip (without ganglion cells) of cat small intestine.

This ganglion-free circular muscle strip was prepared by a method similar to that used by Magnus[5] and by Evans and Schild,[6] and the absence of ganglion cells was confirmed histologically. Some of longitudinal strips of cat small intestines were used, which were kept at 6° for 24~40 hr. Bath temperature used was 26°.

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1) K. Takagi, I. Takayanagi, K. Fujie : This Bulletin, **6**, 716 (1958).
2) K. Takagi, I. Takayanagi : *Ibid.*, **5**, 580 (1957).
3) W. Feldberg : J. Physiol., **113**, 483 (1951).
4) M. W. Parkes : Brit. J. Pharmacol., **10**, 95 (1955).
5) R. Magnus : Pflüg. Arch. ges. Physiol., **102**, 349 (1904).
6) D. H. L. Evans, H. O. Schild : J. Physiol., **119**, 376 (1953).

(7) The tracheal muscle of guinea pig was prepared according to the previous report.[1] Bath temperature was 38°. When $BaCl_2$ was used as spasmogene, the response of small intestine was not sensitive in the beginning of the experiment. A certain concentration of $BaCl_2$, 10^{-4} g./cc., was given repeatedly in the bath fluid for 1.5~3 hr., and the response became more sensitive and at last, constant. The small intestine treated in this way was used for the quantitative experiment described in this paper.

Results

(1) Antagonism between $BaCl_2$ and Some Antispasmodics on Intact Ileums of Mice

Dose-response curve of $BaCl_2$ on mouse small intestines was a logistic curve, whose slope was 2.3 and moved parallel by the drugs of II-group listed in Table I. In the

Table I. Competitive Antibarium Activity of the Antispasmodics of II-Group on the Small Intestine of Mice

Compound (II-Group)	Effective concn. (M)	Compound (II-Group)	Effective concn. (M)
Aspaminol	1.8×10^{-5}	Its methiodide	1.4×10^{-3}
Avacan	1.8×10^{-5}	2-Octyloxytriethylamine	2.8×10^{-4}
Benactyzine	3.2×10^{-6}	Its methiodide	4.8×10^{-3}
Thio-benactyzine	1.5×10^{-9}	Diphenhydramine	1.7×10^{-5}
2-(Diethylamino)ethyl benzoate	4.5×10^{-4}		

concentrations listed in Table I, they antagonized Ba^{2+} competitively and ACh non-competitively. An example of Aspaminol[7] (1,1-diphenyl-3-piperidinobutanol) is shown in Fig. 1. The drugs of I-group inhibited the response to Ba^{2+} non-competitively and also to ACh at the concentration given in Table II. In Fig. 2 is illustrated the inhibitory action of papaverine to $BaCl_2$, which mainly depresses the maximum response non-competitively. The three barbiturates tested here possessed mainly non-competitive, but also some competitive, inhibitory action to $BaCl_2$.

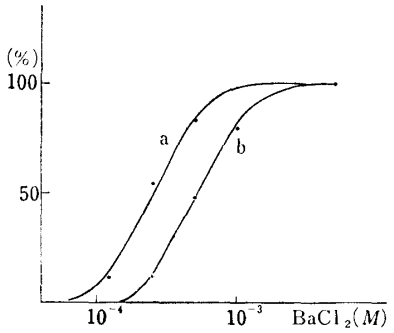

Fig. 1. Parallel Shift of Dose-Response Curve of $BaCl_2$ (a) by Aspaminol ($1.8\times10^{-5}M$) (b) (mouse ileum)

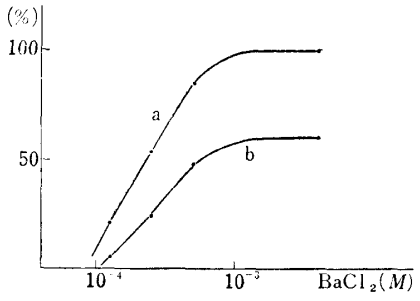

Fig. 2. Non-competitive Inhibition of Dose-Response Curve of $BaCl_2$ (a) by Papaverine ($1.8\times10^{-5}M$) (b) (mouse ileum)

Table II. Non-competitive Antibarium Activity of the Antispasmodics of I-Group on the Small Intestine of Mice

Compound (I-Group)	Effective concn. (M)	Compound (I-Group)	Effective concn. (M)
Papaverine	1.8×10^{-5}	Isopentyl benzoate	3.1×10^{-4}
Dihydroneuspasverine	5.1×10^{-5}	Isopentyl butyrate	8.2×10^{-4}
Isopentyl benzilate	3.8×10^{-5}	Pentobarbital	5.5×10^{-4}
Isopentyl phenylacetate	7.0×10^{-5}	Barbital	4.6×10^{-3}
Isopentyl mandelate	4.5×10^{-5}	Methylhexabital	2.2×10^{-3}

7) K. Takagi, *et al.*: Yakugaku Zasshi, **73**, 541 (1953).

Table III. Non-competitive Antibarium Activity of the Antispasmodics of I- and II-Groups on the Tracheal Muscles of Guinea Pigs

Compound	Effective concn. (M)
Papaverine	1.8×10^{-5}
Dihydroneuspasverine	7.0×10^{-5}
Isopentyl benzilate	3.8×10^{-5}
Isopentyl phenylacetate	5.9×10^{-4}
Isopentyl mandelate	4.5×10^{-4}
Isopentyl benzoate	4.0×10^{-4}
Isopentyl butyrate	8.2×10^{-4}
Pentobarbital	2.2×10^{-4}
Barbital	3.8×10^{-3}
Methylhexabital	2.2×10^{-3}
Aspaminol	2.2×10^{-4}
Avacan	3.1×10^{-4}
Benactyzine	4.2×10^{-4}
Thio-benactyzine	3.7×10^{-5}
2-(Diethylamino)ethyl benzoate	4.0×10^{-4}
Its methiodide	2.2×10^{-3} (no effect)
N,N-Dimethyl-2-octyloxyethylamine	1.8×10^{-3}
Its methiodide	2.3×10^{-3} (no effect)

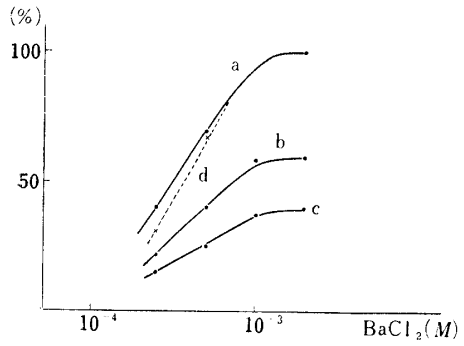

Fig. 3. Effect of Various Compounds to $BaCl_2$ on the Tracheal Muscles of Guinea Pigs

(a) $BaCl_2$ alone (b) Papaverine $1.8\times10^{-5}M$ (c) Aspaminol $2.2\times10^{-4}M$ (d) N,N-Dimethyl-2-octyloxyethylamine methiodide $2.3\times10^{-3}M$ (dotted line)

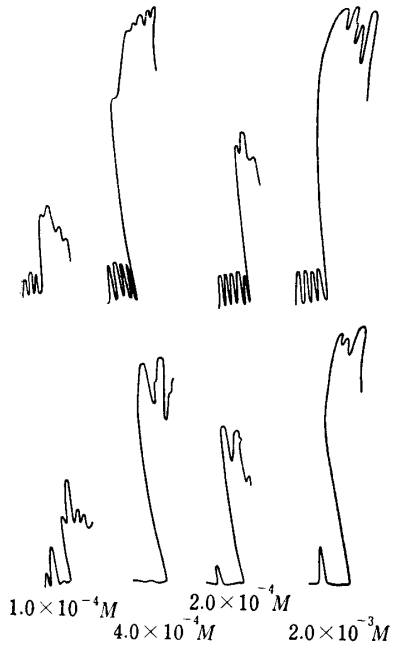

Fig. 4. Response of Longitudinal Muscle Strip of Cat Small Intestine to $BaCl_2$

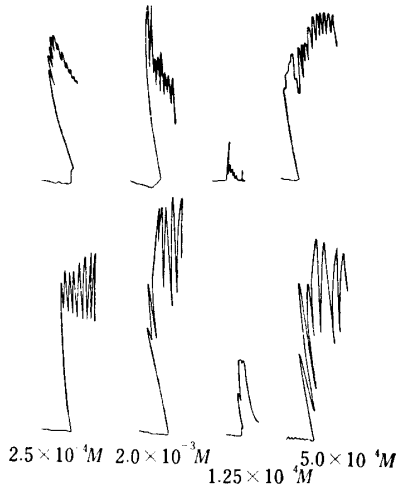

Fig. 5. Response of Circular Muscle Strip (without ganglion cells) of Cat Small Intestine to $BaCl_2$

On the other hand, the drugs listed in Table III, which include those of I- and II-groups, antagonized Ba^{2+} non-competitively on the tracheal muscle at the cited concentration (Fig. 3).

(2) Experiments on Various Muscle Strips prepared from Cat Small Intestines

a) Effect of $BaCl_2$: Contractions of longitudinal muscle (Fig. 4), plexus-free circular muscle (Fig. 5), and strips of longitudinal muscle with circular muscle had almost a similar magnitude to the same range of concentration of $BaCl_2$. These results did not agree with that of Evans and Schild[6] and of van Esveld,[8] who recognized that the response of ganglion-free circular muscle had different quality, that is, an increase of rhythmical peristalsis, from the innervated longitudinal muscle, which showed usual spastic contraction. This discrepancy might depend on the experimental method and on the bath temperature, because they used the Trendelenburg's method, where the pressure of the content of a tubular intestinal preparation is recorded at 38°.

The slope of dose-response curve of $BaCl_2$ on ileum of mice and cats, and tracheal muscle of guinea pigs was greater than that of ACh.

b) Antibarium action on antispasmodics: Aspaminol, which had been proved to exert papaverine-like action in the ionized form,[1] was found to have a competitive antibarium activity on the five kinds of smooth muscle preparations of cats, i.e. Aspaminol ($1.8\times10^{-5}M$) shifted the dose-response curve of $BaCl_2$ competitively. Avacan (isopentyl 2-(2-dimethylaminoethylamino)-2-phenylacetate) ($1.4\times10^{-5}M$) exhibited similar activity. These concentrations were not significantly different from those on isolated ileum of mice listed in Table I.

The compounds of I-group, such as papaverine ($5.1\times10^{-5}M$), isopentyl benzoate ($4.8\times10^{-4}M$), pentobarbital ($3.8\times10^{-4}M$), and isopentyl butyrate ($8.2\times10^{-4}M$), mainly exerted non-competitive antibarium action on all the preparations.

c) Activity of ACh and antiacetylcholine agents on various kinds of preparations: Dose-response curve of ACh tested on isolated small intestines, on longitudinal muscle, and on longitudinal muscle with circular muscle, lies between the concentration of 10^{-6} and $10^{-8}M$, and shifted parallel by Aspaminol of 1.4×10^{-5}. On the contrary, the concentration range of ACh was between 10^{-4} and $10^{-6}M$ on ganglion-free circular muscle and circular muscle with longitudinal muscle (Fig. 6), and these facts indicate that the circular muscle itself has 1/100 lower sensitivity to ACh than longitudinal muscle, irrespective of the presence of ganglion cells.

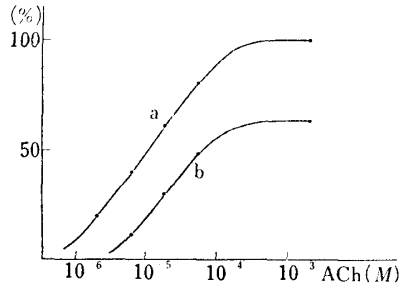

Fig. 6. Effect of Aspaminol ($1.4\times10^{-5}M$) to Dose-Response Curve of ACh on Circular Muscle Strips (without ganglion cells)

The same figure was obtained on circular muscle with ganglion cells and with longitudinal muscle

(a) ACh alone (b) with Aspaminol

In accordance with this fact, the atropine-like activity of Aspaminol ($1.4\times10^{-5}M$) was weaker, that is, the parallel shift of the response curve was more slight, on the circular muscle preparations than on the longitudinal muscle. It was reported also by van Esveld[8] that higher concentration of atropine was necessary to antagonize ACh on circular muscle of cats.

8) L. W. van Esveld: Arch. exptl. Pathol. Pharmakol., **134**, 347 (1928).

Table IV. Non-competitive Antiacetylcholine Potency Ratios tested on Small Intestines of Cat (Agonist : ACh, $1.4\times10^{-3}M$)

Antagonist \ Preparation	Isolated small intestine (2)	Longitudinal muscle strip (4)	Circular muscle strip without ganglion (6)	Longitudinal muscle strip with ganglion and circular muscle (3)	Circular muscle strip with ganglion and longitudinal muscle (5)
Aspaminol	343	289	201	230	210
Avacan	98	89	92	98	108
Benactyzine	230	213	230	192	231
Diethylaminoethyl thiobenzilate	19200	18100	18030		
2-(Diethylamino)ethyl benzoate	1.2	0.9	0.8		
N,N-Dimethyl-2-octyloxyethylamine	1.3	4.8	1.2		
Its methiodide	0.8	0.3	0.8		
Isopentyl phenylacetate	10	18	21		
Isopentyl mandelate	92	69	68		
Isopentyl benzoate	5.2	6.2	3.0	5.0	8.2
Isopentyl butyrate	2.1	2.3	1.2	1.4	1.9
Phenobarbital	0.9	0.6	1.2	0.6	0.9
Barbital	0.5	0.5	0.2		
Papaverine	100	100	100	100	100

The non-competitive antiacetylcholine activity of various compounds on each preparation of cat ileum is summarized in Table IV, where high concentration of ACh ($1.4\times10^{-3}M$) was used as spasmogenes. The difference of the activity of each compound was small among the five preparations of cat ileum.

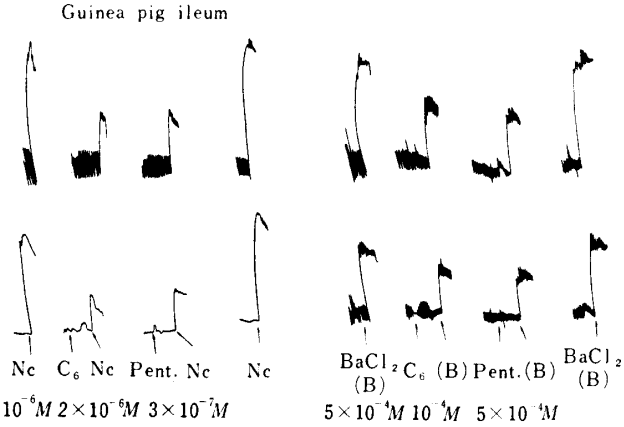

Fig. 7.
Response to Nicotine Tartrate (Nc) or $BaCl_2$ (B) inhibited by Hexamethonium (C_6) or Pentolinium (Pent.) on Small Intestine of Guinea Pig

d) Antagonism of ganglion-blocking agents and atropine to nicotine and $BaCl_2$: Contraction of ileum of mice and of guinea pigs induced by $10^{-8}M$ of nicotine (Fig. 7) was inhibited by lower concentration ($10^{-6}\sim10^{-7}M$) of Hexamethonium and of Pentolinium, of which $10^{-4}M$ or higher concentration was necessary to inhibit the contraction elicited by Ba^{2+} (Fig. 7). Although nicotine did not contract the ganglion-free circular muscle of cat up to the concentration of $5\times10^{-4}M$, $BaCl_2$ induced contraction at $6\times10^{-4}M$, which was inhibited by Hexamethonium and/or Pentolinium (Fig. 8). Hexamethonium antagonized competitively not only Ba^{2+} (Fig. 9) but also nicotine on the ileum of guinea pigs (Fig. 10).

If atropine acts on the ACh-receptor on plain muscle, the agent might antagonize nicotine non-competitively, which excites ganglion cells exclusively. A non-competitive depression of maximum response of nicotine was found by atropine with a small parallel shift (Fig. 10), which was thought to be a ganglionic blocking action of atropine. On the contrary, the dose-response curve of $BaCl_2$ was shifted in parallel by atropine $4.8\times10^{-4}M$.

(3) Synergism between Papaverine-like Drugs

The object of this experiment is to discriminate the site of action of two kinds of

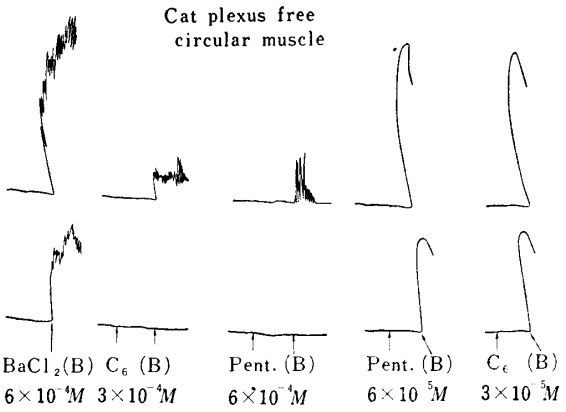

Fig. 8. Antagonism of Hexamethonium (C_6) and Pentolinium (Pent.) to $BaCl_2$ (B) on Circular Muscle strip (without ganglion cells) of Cat Small Intestine

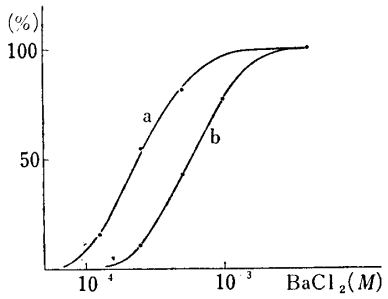

Fig. 9. Parallel Shift of Dose-Response Curve of $BaCl_2$ (a) by Hexamethonium (C_6) ($2.8\times10^{-4}M$) (b) (mice small intestines)

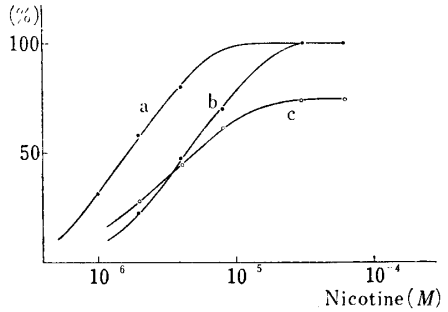

Fig. 10. Parallel Shift of Dose-Response Curve of Nicotine Tartrate (a) by Hexamethonium (C_6) ($1.7\times10^{-6}M$) (b) and Non-competitive Antagonism by Atropine ($6\times10^{-6}M$) (c) (on the small intestines of guinea pigs)

papaverine-like substances. If two drugs, which have the same site of action, are given at the same time, the combined effect would be additve, and in the case of two drugs having different sites of action, the combined effect would be potentiation. As spasmogenes, a high concentration of ACh of $5\times10^{-4}M$ was used exclusively in this experiment. The response to ACh was inhibited to 38.2% in the presence of Aspaminol ($5.2\times10^{-6}M$) and to 24.4% in the presence of Avacan ($1.0\times10^{-5}M$).

When the same concentration of the two drugs was given simultaneously, the response was suppressed to 16.3%. In the case of potentiation, the expected effect is 9.3%, derived through multiplying each response ($=0.382\times2.224\times100$) and, in additive effect, it must be 18.0%, according to equation (1):

$$\frac{y'-y}{y}=\left(\frac{[A]}{K_A}\right)^m+\left(\frac{[B]}{K_B}\right)^m \quad \cdots\cdots(1)$$

where y' is the response elicited by ACh of $5\times10^{-4}M$, y is the response, A and B are non-competitive antagonists, K_A and K_B are dissociation constants of A and B, each with the receptor, and m is the constant.

Aspaminol, an antispasmodic of II-group, and papaverine, that of I-group, are expected to exert a potentiative effect, because they have different site of action according to the previous experiments. The reponse to ACh was inhibited to 38.2% in the presence of Aspaminol ($5.2\times10^{-6}M$) and to 24.4% with papaverine ($1.0\times10^{-5}M$). When the same concentration of the two drugs was given simultaneously, the response was suppressed to 8.2%. In the case of potentiation, the expected effect is 11.4%, derived through multiplying each response ($=0.382\times0.299\times100$), and in addititive effect, it must be 20.7% according to equation (1). It was concluded from the synergistic effect of the two drugs, therefore,

that Aspaminol and Avacan would have the same site of action, and Aspaminol and papaverine would have a different site of action.

Discussion

Against the classical interpretation that Ba^{2+} has a musculotropic spasmogenic activity, some workers believe that it has partly musculotropic and partly ganglionic activity.

a) Evans and Schild[6] proposed that Ba^{2+} has some ganglionic activity, because the difference of activity arose through the presence or absence of ganglion cells. (b) Feldberg[3] revealed that the contraction due to Ba^{2+} can be depressed by Hexamethonium, which is believed to act on ganglion.

c) Some antispasmodics such as diethylaminoethyl thiobenzilate has especially higher antibarium activity on guinea pig ileum than on other plain muscle organs. Parkes[4] interpreted this fact to depend upon plentiful presence of ganglion cells in the former preparation.

(1) The constrictive activity of Ba^{2+} on various kinds of ileum preparation was compared with those of ACh and nicotine (tartrate) and shown in Table V. It is observed

Table V. Comparison of the Effective Molar Concentration of $BaCl_2$ with those of Acetylcholine and Nicotine

Preparation*	ACh (M)	$BaCl_2$ (M)	Nicotine (M)
Mouse isolated small intestine (1)	$10^{-8}\sim10^{-6}$	$10^{-4}\sim10^{-3}$	10^{-6}
Cat ileum			
Isolated intact intestine (2)	$10^{-8}\sim10^{-6}$	$10^{-4}\sim10^{-3}$	10^{-6}
Longitudinal muscle strip (4)	$10^{-8}\sim10^{-6}$	$10^{-4}\sim10^{-3}$	10^{-6}
Circular muscle strip with ganglion (5)	$10^{-6}\sim10^{-4}$	$10^{-4}\sim10^{-3}$	
Circular muscle strip without ganglion (6)	$10^{-6}\sim10^{-4}$	$10^{-4}\sim10^{-3}$	Inactive at 5×10^{-4}
Guinea pig tracheal strip (7)	$10^{-6}\sim10^{-4}$	$10^{-4}\sim10^{-3}$	

* Number in parentheses indicates the preparation described in the experimental method. (Tested on isolated small intestine of mice, different smooth muscle preparations of cat small intestine at 26°, and tracheal strip of guinea pigs at 38°)

here that Ba^{2+} has almost similar activity on different kinds of smooth muscle preparations, including tracheal muscle strips, in quality of contraction and in effective concentration. ACh has higher potency on intact ileum and on longitudinal muscle strip than on circular muscle strip with and without ganglion cells. The discrepancy of the activity of ACh is probably derived not from the presence or absence of ganglion, but from the difference between longitudinal and circular muscles. On the contrary, contraction by nicotine, which could be observed on excised intact ileum and also on longitudinal muscle with plexus, could not be observed on plexus-free circular muscle in concentrations up to $5\times10^{-4}M$. This agrees with the experiments of Gasser[9] and of Magnus,[10] but not with those of Evans and Schild[6] and of van Esveld,[8] and it must be cited again that bath temperature was maintained exclusively at 26° in the present experiment.

(2) The antagonistic activity of ganglion-blocking agents and antispasmodics to the above three agonists is summarized in Table VI, where type of antagonism and effective concentration are given. For example, Hexamethonium antagonized Ba^{2+} competitively in a concentration of $3\times10^{-4}M$, on the longitudinal muscle strip of cat ileum. Ganglion-blocking agents such as Hexamethonium and Pentolinium inhibited Ba^{2+} contraction of some ileum preparations and the antagonism was competitive, but they antagonized nicotine in 1/100 or lower concentration. If Ba^{2+} and nicotine have the same site of action, Hexamethonium or Pentolinium must each antagonize them in a similar concentra-

9) H. S. Gasser : J. Pharmacol. Exptl. Therap., **27**, 395 (1926).
10) R. Magnus : Pflüg. Arch. ges. Physiol., **108**, 1 (1905).

TABLE VI. Comparison of Antagonistic Activity of Ganglion-blocking Agents and Antispasmodics to $BaCl_2$, Acetylcholine, and Nicotine on Different Kinds of Smooth Muscle Preparations

Test organ	Agonist	I-Group Papaverine (M)	II-Group Aspaminol (M)		Ganglion blockers (M)		Atropine (M)
					Hexamethonium	Pentolinium	
Mouse or guinea pig ileum (1)[b]	$BaCl_2$	NC[c] 1.8×10^{-5}[a]	C[c] 1.8×10^{-5}		C 4.3×10^{-4}	C 5×10^{-4}	C 4.8×10^{-4}
	ACh	NC 1.8×10^{-5}	NC 1.8×10^{-5}	C 4×10^{-8}	C 5.2×10^{-5}	C 3.8×10^{-5}	C 8.0×10^{-9}
	Nicotine				C 4.3×10^{-6}	C 5.0×10^{-7}	NC 6.0×10^{-6}
Cat ileum (2)	$BaCl_2$	NC 1.8×10^{-5}	C 1.8×10^{-5}		C 2.8×10^{-4}	C 5.0×10^{-4}	
	ACh	NC 1.8×10^{-5}	NC 1.4×10^{-5}				
	Nicotine				C 1.7×10^{-6}	C 3.0×10^{-7}	
Cat ileum, longitudinal muscle strip with ganglion and circular muscles (3)	$BaCl_2$	NC 1.8×10^{-5}	C 1.8×10^{-5}		C 3.0×10^{-4}	C 6.0×10^{-4}	
	ACh	NC 2.0×10^{-5}	NC 1.8×10^{-5}	C 1.4×10^{-8}			
	Nicotine				C 7.0×10^{-6}	C 6.0×10^{-7}	
Cat ileum, longitudinal muscle strip (4)	$BaCl_2$	NC 2.6×10^{-5}	C 1.8×10^{-5}		C 3.0×10^{-4}	C 6.0×10^{-4}	
	ACh	NC 2.0×10^{-5}	NC 1.4×10^{-5}	C 1.4×10^{-8}			
	Nicotine				C 7.0×10^{-6}	C 6.0×10^{-7}	
Cat ileum, circular muscle strip without ganglion (6)	$BaCl_2$	NC 2.6×10^{-5}	C 1.8×10^{-5}				
	ACh	NC 2.0×10^{-5}	NC 1.4×10^{-5}	C 1.4×10^{-5}			
Guinea pig tracheal muscle strip (7)	$BaCl_2$	NC 1.8×10^{-5}	NC 1.4×10^{-4}				
	ACh	NC 1.8×10^{-5}	NC 1.2×10^{-4}				

a) The effective concentrations are in molecular concentration.
b) Number in parentheses indicates the preparation described in the experimental method.
c) NC : Non-competitive antagonism. C : Competitive antagonism.

tion. Even in plexus-free circular muscle they antagonized Ba^{2+} in the same way as in intact ileum. It may be concluded that Ba^{2+} has different site of action from nicotine, and the two ganglion-blocking agents combine with the barium-receptor and nicotine-receptor each with different affinity. It is very probable that the barium-receptor is present on smooth muscle of small intestine and that the ganglioplegics can combine with it, but the ganglionic action of Ba^{2+} cannot be accepted from the above results.

Dose-response curve of nicotine was obtained on guinea pig small intestine and Hexamethonium shifted the curve in parallel, which was again inhibited non-competitively by atropine. The competition between atropine and nicotine can probably be elucidated by the action of atropine to nicotine-receptor on ganglion cells, but the non-competitive inhibition of nicotine contraction by atropine may be produced only through a mechanism in which atropine blocks the stimulation produced by nicotine on ganglion cells at ACh-receptor on plain muscle. The fact that atropine inhibited the contraction due to Ba^{2+} competitively does not prove that Ba^{2+} acted on ganglion.

(3) Antagonism between Ba^{2+} and antispasmodics of II-group was competitive and that between Ba^{2+} and papaverine of I-group was non-competitive in all the ileum preparations. This is in line with the division of antispasmodics into the two groups by

applying Ferguson's rule, which was reported in the preceding paper.[1)]

The mode of antagonism of the antispasmodics to Ba^{2+} was quite similar not only between innervated and plexus-free smooth muscles of small intestines, but also between longitudinal and circular muscles, and the effective concentration was in parallel with that necessary to produce non-competitive antiacetylcholine action in all the smooth muscle preparations tested. In this way, it is considered that II-group antispasmodics combine with Ba^{2+}-receptors as a cation and drug-receptor complex blocks ACh non-competitively and Ba^{2+} competitively.

It is to be added here that atropine inhibited the contraction due to nicotine of guinea pig ileum in 1000 folds or higher concentration than that at which atropine competitively antogonizes the contraction by ACh. This fact was elucidated from several causes by Ambache[11)] but it is supposed that the correspondingly higher concentration of ACh would be freed locally in the post-ganglionic junction, when ganglion cells are excited by nicotine.

Table VII. Inhibition Ratio (%) of Papaverine and Aspaminol to the Contraction produced by Various Concentrations of $BaCl_2$ tested on Small Intestines of Mice

Inhibitor \ Concn. (g./cc.) of $BaCl_2$	2×10^{-4}	10^{-4}	5×10^{-5}
Papaverine ($1.8\times10^{-5}M$)	45	41	55
Aspaminol ($1.8\times10^{-5}M$)	20	43	77

(4) Since it has been revealed now that the antibarium action is qualitatively different between I-group and II-group antispasmodics, the comparison of the potency ratio to papaverine as standard must be practiced under careful consideration. The inhibition ratio (%) of papaverine and Aspaminol to the contraction produced by various concentrations of $BaCl_2$ in the absence of inhibitors is calculated in Table VII from Figs. 1 and 2. In the lower concentration of barium chloride, inhibition by Aspaminol becomes greater, that is to say, the potency ratio of Aspaminol to papaverine becomes greater.

The author is indebted to Prof. K. Takagi for suggesting this investigation as well as for constant guidance during the course of this work.

Summary

It was concluded from the foregoing experiments through Magnus method (at 26° and 38°) with (1) excised intact intestines of mice and guinea pigs, (2) longitudinal strip, (3) longitudinal muscle strip with ganglion and circular muscle, (4) longitudinal muscle strip without circular muscle, (5) circular muscle strip with ganglion and longitudinal muscle, and (6) circular muscle strip (without ganglion cells) of cat small intestine, and (7) tracheal strip preparation of guinea pig that :

(1) Ba^{2+} had almost similar activity on different kinds of smooth muscle preparations, in quality of contraction and in effective concentration. Ganglion-blocking agents inhibited Ba^{2+}-contraction of the ileum preparation, and the antagonism was competitive, but they antagonized nicotine in 1/100 or lower concentration on all the ileum preparations. Therefore, Ba^{2+} must have a site of action other than that of nicotine and the ganglion blocking agents combine with barium-receptor and nicotine-receptor, each with different affinity. It is very probable that the barium-receptor is present on smooth muscle of small intestine and the ganglioplegics can combine with it and that the ganglionic action of Ba^{2+} cannot be revealed.

11) N. Ambache : Pharmacol. Rev., **7**, 467 (1955).

(2) Antagonism between Ba^{2+} and strongly basic antispasmodics (pKa>8.5) of II-group was competitive and that between Ba^{+2} and papaverine of I-group, which would exert through some physicochemical property of non-ionized molecules, was non-competitive on all the smooth muscle preparations used.

(3) Since the antibarium action is qualitatively different between I-group and II-group antispasmodics, the comparison of the potency ratio to papaverine as standard must be made under careful consideration.

(4) ACh and atropine-like antispasmodics were proved to have higher potency on intact ileum and longitudinal muscle strip than on circular muscle strip with and without ganglion cells.

(5) Atropine inhibited the nicotine-contraction of guinea pig ileum non-competitively in 1000 times or higher concentration than that at which atropine competitively antagonizes the contraction by ACh.

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32. Makoto Shirasaka and Masako Tsuruta : Microbiological Transformation of Steroid. V.[1] Hydroxylation of Steroid by *Sclerotium hydrophilum*.

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Among the hydroxylation of steroids by fungi, 11α-hydroxylation is comparatively common but this is often accompanied by 6β-hydroxylation in majority of cases. *Rhizopus arrhizus* used by Peterson and others[2] is the representative of such fungi.

During examination of numerous fungi, *Sclerotium hydrophilum* was found to carry out 11α- and 6β-hydroxylation of Reichstein's compound S at the same time. Application of this fungus to various other steroids showed the formation of chiefly 6β,11α-dihydroxy compound from progesterone and deoxycorticosterone, about equal amounts of 6β- and 11α-hydroxy compounds from 17α-hydroxyprogesterone, as in the case of the compound S, and 6β- and 15β-hydroxy-11-oxo compounds from corticosterone. Consequently, this fungus was found to have a kind of substrate specificity.

Fermentation of *Sclerotium hydrophilum* using potato decoction as a medium and by shake culture, as will be described later, with progesterone as the substrate and paper chromatographic examination of the concentrated ethyl acetate extract showed the presence of unreacted progesterone and a spot with much greater polarity than that. The concentrate was dissolved in benzene with warming and the crude crystals that separated on cooling were recrystallized from methanol to granular crystals (I) of m.p. 236~241°. Its analytical values indicated it to be dihydroxyprogesterone and the constants of (I) and its diacetate, obtained by the usual acetylation with acetic anhydride and pyridine, and their infrared spectra, were in good agreement with those of 6β,11α-dihydroxyprogesterone[1] and its 6,11-diacetate.[1]

The same fermentation of this fungus with 17α-hydroxyprogesterone as the substrate and paper chromatographic examination of the concentrated extract showed the presence of some unreacted 17α-hydroxyprogesterone and two spots with greater polarity than that.

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1) Part IV : This Bulletin, **9**, 159 (1961).

2) D. H. Peterson, *et al.* : J. Am. Chem. Soc., **74**, 5933 (1952); **75**, 408, 412, 416 (1953).