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THE SPASMOLYTIC PROPERTIES OF THIOBENZILIC ACID ESTERS AND RELATED COMPOUNDS

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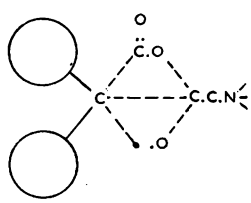
In the absence of any clear understanding of the causes underlying the clinical occurrence of spasm in smooth muscle, the search for agents by which it might be relieved has always been pursued empirically. There are conditions, such as asthma, in which drugs reducing spasm by parasympatholytic action may be undesirable, on account of side effects, and for which specific antihistamines are not satisfactorily effective. For such conditions a non-specific inhibitor of smooth muscle tonus and action might be the type of drug required. Papaverine exerts this kind of spasmolytic effect, but has certain disadvantages. To investigate this problem a series of compounds was screened for papaverine-like activity, using the commonly adopted criterion of reduction of barium-induced spasm in isolated intestine.

Spasmolytic activity appears to be particularly associated with certain molecular structures (Blicke, 1944; Bovet and Bovet-Nitti, 1948; Burtner, 1951; Bergel and Parkes, 1952). Atropine-like antagonism of acetylcholine on isolated strips of intestine is, in general, associated with a structure consisting of a short chain of atoms linking a tertiary or quaternary nitrogen atom to a carbon atom bearing aromatic or other ring systems, as may be schematically represented thus:



Compounds of this type may show, in addition, activity in reducing spasm produced by other agents such as histamine or barium chloride.

The substances tested were synthesized in the chemical laboratories of the Research Department, Roche Products Ltd., and consisted of a series of basic esters of thiobenzilic acid,

$$Ph_2C(OH)CO.S.(CH_2)_nNRR',$$

and derivatives, described by Morrison and Konigstein (1949), and a few derivatives of benzilic acid esters (Morrison, Konigstein, and Cohen, 1950), together with ethers and thioethers of the type

$$Ph_2C(OH)CH_2O(\text{or S})\,(CH_2)_nNRR'.$$

METHODS

Isolated Guinea-pig Ileum.—Lengths of guinea-pig ileum were suspended in a 30 ml. bath containing oxygenated Tyrode solution at 37° C. (composition: NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.01%, $MgCl_2$ 0.01%, NaH_2PO_4 0.005%, $NaHCO_3$ 0.1%, glucose 0.1%). Addition of test drugs was regulated automatically by a device similar to that recommended by Schild (1947); two baths were usually employed simultaneously.

Solutions of drugs were diluted with distilled water and added to the bath in volumes of not more than 1 ml. Test substances were allowed 2 min. contact with the gut before the addition of the spasmogenic drug, which remained in the bath for a further 15 sec., except for barium chloride and nicotine, which usually required 30 sec. for full effect. After each spasmogenic drug the bath was drained and refilled twice at an interval of 1 min., the cycle being completed by a resting period usually of 4 min., but up to 15 min. where necessary, as, for example, when using nicotine. Responses of the gut were recorded by a lever with a frontal writing point.

Each substance tested as an antagonist was compared directly with a standard drug for action against a repeated dose of spasmogenic agent chosen to give a suitable submaximal response. Usually a four-point assay routine of the type described by Schild (1947) was used. The number of assays varied with the apparent activity of the compound, and an activity of interesting magnitude would be accorded two or three assays on each of several lengths of gut, preferably from more than one animal.

Drugs used to stimulate the gut were acetylcholine chloride, histamine acid phosphate, barium and potassium chlorides, and nicotine hydrochloride. The

drugs chosen as standard antagonists were atropine sulphate, mepyramine hydrochloride, and, against both metallic salts and nicotine, papaverine hydrochloride.

Rabbit and rat ileum were also used to assay anti-Ba activity in the same way as described for guinea-pig ileum; rabbit ileum was further used to assay activity in reducing spontaneous motility in comparison with papaverine.

Isolated Rat Uterus.—This organ was suspended in oxygenated Locke's solution at 36° C. and stimulated by barium chloride. Substances were assayed for antagonistic activity in comparison with papaverine.

Isolated Air-perfused Lung.—The preparation used was similar to that described by Arunlakshana and Schild (1950). Guinea-pig lungs, removed from animals stunned and bled, were perfused via the pulmonary artery with the fluid described by McDowall and Thornton (1930) at 36° C. under a constant pressure of 15 cm. Air was led into the trachea at a constant rate and escaped through scarifications in the lung surface. The pressure set up in maintaining the rate of air flow was recorded. The bronchial vessels were constricted by perfusing with fluid containing barium chloride. Drugs were tested for antagonism either by adding them to the fluid to be perfused, in the required dilution, or by injecting doses into the stream immediately above the cannula.

Isolated Tracheal Muscle.—The preparation used was the tracheal chain, first described by Castillo and de Beer (1947). This was suspended in Thornton's fluid or in Krebs-Henseleit fluid at 37° C. Dr. D. F. Hawkins has found that if the bath is aerated with oxygen the *p*H rises to 8.4 and the guinea-pig tracheal chain takes on a high resting tonus; this has been used in the detection of relaxant activity. When the gas bubbling through the fluid contains 5% CO_2 the *p*H falls to 7.4 and the guinea-pig preparation is fully relaxed, as is that of the rabbit under either condition. These chains can be stimulated by spasmogenic agents and have been used to determine spasmolytic activity.

Coronary-perfused Heart.—The Langendorff preparation of the rabbit heart was perfused with Locke solution at 37° C., under a constant pressure of 120 cm. fluid. Beat and coronary flow were recorded. Solutions of test substances were diluted in Locke's solution and injected slowly in small volumes above the aortic cannula. Coronary dilator activity was assayed in comparison with papaverine.

Substances were supplied either as hydrochlorides and dissolved in water for testing, or as base and dissolved with the aid of equivalent hydrochloric acid.

Results

Isolated Guinea-pig Ileum.—The results of assays upon this organ are given in Table I, where activities are expressed relative to that of the standard antagonist used for comparison. Among the series of thioesters studied was the thio-analogue of "Trasentin" (β-diethylaminoethyl diphenylthioacetate, Ro 3-0235), the spasmolytic activity of which was reported by Dupré, Lévy and Tchoubar (1946) and also by Ramsay and Richardson (1947).

In this series considerable activities in antagonizing ACh, Ba and K were found among the thioesters of benzilic acid. Comparison of the diethylaminoethyl thioesters Ro 3-0235, Ro 3-0226 and Ro 3-0297 shows that substitution of the hydroxyl group by hydrogen reduced atropine-like activity, whereas introduction of a methyl group considerably reduced ability to anatagonize both ACh and Ba. Comparison of the pairs of compounds Ro 3-0290 and Ro 3-0298, Ro 3-0299 and Ro 3-0303, Ro 3-0305 and Ro 3-0306 further demonstrates the dependence of activity upon the hydroxyl group. Etherification of this group also reduced activity—compare, e.g., Ro 3-0292 with Ro 3-0290.

Activities among the aminoalkanethiol esters of benzilic acid appeared to be influenced both by the substituents on the nitrogen atom and by the length and character of the alkylene chain. The relationships may be seen from Table II. In the aminoethylthiol esters studied, anti-ACh activity was high with the diethylamino- member and even higher with the dimethylamino- (Ro 3-0378, Table I), but was progressively reduced in the piperidino- and morpholino- compounds. A similar tendency appeared to exist in the series derived from aminopropylthiol. The effect of chain-length upon anti-ACh activity showed differences between the diethylamino- and the piperidino- series; activity in the former decreased progressively with increase in chain-length, whereas in the latter it was low, rising suddenly to twice that of atropine with the piperidinohexyl member.

The more active compounds are very potent Ba antagonists, some being effective at dilutions of 1 in 50 million. Many of the mean activities, however, are associated with considerable variance; a number of the compounds—particularly the most active—gave widely different activities at different periods. For these and other reasons to be discussed later it is not possible to ascribe significance to any differences or trends which may appear to follow changes in structure, as is possible for anti-ACh activity.

Activities in antagonizing the effects of K ions also did not fall into a clearly defined pattern in this series. None of the compounds showed any appreciable antihistamine activity.

TABLE I

SPASMOLYTIC ACTIVITIES OF VARIOUS BENZILIC ACID DERIVATIVES ON ISOLATED GUINEA-PIG ILEUM

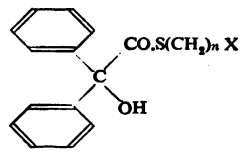
Anti-acetylcholine activities assayed against atropine, anti-barium and anti-potassium activities against papaverine, and anti-histamine activities against mepyramine

Ro Series No.	Formula			Activity against Spasm due to: ACh (Atropine = 100)	$BaCl_2$ (Papaverine = 1)	KCl	Histamine (Mepyramine = 100)
				Ph_2:C.CO.S.CH.CH_2.R^3			
Thioesters				R^1 R^2			
	R^1	R^2	R^3				
3–0235	H	H	NEt_2	20±10 (5)†	150±87 (5)*; 27±9 (5)	6·2±1·3 (5)	0·5 (3)
3–0226	OH	H	NEt_2	112 (2)	(5150±1150 (5)*; 73±30 (3)	17·5±12 (3)	0·15
3–0297	Me	H	NEt_2	2·65 (3)	13·3±1·3 (3)	3·8 (3)	0·45 (3)
3–0378	OH	H	NMe_2	163±9 (5)	2·32±0·6 (3)	1·96 (2)	—
3–0348	OH	H		2·7±2·4 (5)	90±20 (4)	10–200 (7)	<0·01
3–0368	OH	H		0·025 (3)	0·5 (2)	0·36 (3)	0·005 (3)
3–0296	OH	H	CH_2NEt_2	65±4·3 (3)	875±410 (5)*; 23·5±7 (3)	310 (2)	0·03
3–0290	OH	H		6·2±1·6 (3)	91±27 (8)*; 10·3±3·4 (5)	70±21 (5)	7·5
3–0292	OEt	H		1	3·2±1·2 (5)	5	0·15
3–0298	Me	H		0·2	3·3±0·8 (5)	5·4±0·6 (5)	0·1
3–0299	OH	H		1·12 (2)	28±3·4 (4)	5·8±0·64 (3)	<0·01
3–0303	Me	H		0·12 (2)	1·35 (2)	1·5	0·02
3–0305	OH	Me		2·7 (4)	10±4·7 (5)	12·5	0·01
3–0306	Me	Me		0·2	<1	1	<0·1
3–0341	OH	H	$(CH_2)_2NEt_2$	1·1 (8)	7·75±6 (6)	5 (3)	0·005 (5)
3–0342	OH	H	$(CH_2)_3NEt_2$	0·22 (5)	4±0·6 (4)	10·8±5·65 (8)	0·03 (4)
3–0320	OH	H		6±1·5 (3)	50·5±25 (4)*; 17·2±8 (4)	41±10 (4)	0·1 (2)
3–0367	OH	H		200±95 (12)	2·2 (2)	1·5 (2)	0·025 (2)
				Ph_2:C.CO.O.CH_2.CH_2.NEt_2			
Benzilic Acid Derivatives				R^1			
	R^1						
3–0256	OH			50 (2)	20	50	—
3–0131	$OCH_2CH_2NMe_2$			0·5 (4)	7·5 (2)	12 (2)	0·2
				Ph_2:C.CH_2.A.CH_2.CH_2.R^3			
Ethers				R^1			
	R^1	A	R^3				
3–0327	OH	O	NMe_2	0·5 (5)	0·5 (4)	4–10 (2)	0·02
3–0328	OH	O	NEt_2	0·5 (3)	3·3±0·72 (4)	9·15±1·9 (5)	0·01
3–0354	$OCH_2CH_2NMe_2$	O	NEt_2	0·18 (2)	1·8 (2)	4·2 (3)	0·15 (2)
3–0380	OH	S	NMe_2	1·2±0·17 (3)	9·3±7·8 (3)	0·75 (2)	0·05 (2)
3–0326	OH	S	NEt_2	1·85 (2)	79·5±12·5 (11)	19·5±7·5 (5)	0·1 (2)
3–0373	OH	S	CH_2NEt_2	4·3±2·2 (3)	0·46±0·02 (4)	14·2±8·3 (3)	0·025
3–0353	OH	SO_2	NEt_2	0·4 (7)	1·5±0·5 (6)	1·15 (2)	0·001 (3)

*Values obtained at different periods (see text). † Mean values ± S.D The figures in parentheses give the number of assays from which the mean is derived.

TABLE II
ACTIVITIES OF THIOESTERS ON ISOLATED GUINEA-PIG ILEUM

Values as in Table I

General formula 

	X= —NEt_2				X= —N⬡				X= —N⬡O			
n	Ro Series No.	Anti-ACh	Anti-Ba	Anti-K	Ro Series No.	Anti-ACh	Anti-Ba	Anti-K	Ro Series No.	Anti-ACh	Anti-Ba	Anti-K
2	3–0226	112	5150±1150* 73±30	17·5±12	3–0348	2·7±2·4	90±20	10–200	3–0368	0·025	0·5	0·36
3	3–0296	65±4·3	875±410* 23·5±7	310	3–0290	6·2±1·6	91±27* 10·3±3·4	70±21	3–0299	1·12	28±3·4	5·8±0·64
4	3–0341	1·1	7·75±6	5								
5	3–0342	0·22	4±0·6	10·8±5·65	3–0320	6±1·5	50·5±25* 17·2±8	41±10				
6					3–0367	200±95	2·2	1·5				

*Values obtained at different periods.

Ro 3-0256 (β-diethylaminoethyl benzilate) was first tested by Halpern (1938) and later by Lehmann and Knoefel (1942), by Ing, Dawes, and Wajda (1945), by Buchel, Lévy, and Pernot (1948) and by Lands (1951). The value reported here for anti-ACh activity is similar to those found in tests on rabbit intestine by Halpern, by Lehmann and Knoefel, and by Lands, though the values reported by previous workers for anti-Ba activity are far less than those reported here. Ro 3-0256 was included in this series for comparison with the corresponding thioester Ro 3-0226; Table I shows that the thio-linkage may be associated with somewhat higher activity against ACh than the normal ester linkage.

The compound Ro 3-0131 (β-diethylaminoethyl diphenyl (β-dimethylaminoethoxy)-acetate) was reported upon by Forbes and Marshall (1951). The anti-Ba activity of Ro 3-0131 reported here, relative to that of papaverine, was of the same order as that calculated from the pA_2 values given by these authors. Table I shows that etherification of the benzilic hydroxyl group reduced all types of spasmolytic activity.

The substitution of the ester linkage by an ether linkage reduced all activities (Table I : compare Ro 3-0256 with Ro 3-0328, and Ro 3-0131 with Ro 3-0354). The thioether Ro 3-0326 and the corresponding thioester Ro 3-0226, however, appeared to possess similar activity against both Ba and K although the ether lacked anti-ACh activity. Similar reduction in atropine-like activity distinguished the thioether Ro 3-0380 from the thioester Ro 3-0378. Oxidation of the sulphur linkage in Ro 3-0326 to form the compound Ro 3-0353 decreased all activities markedly.

Isolated Ileum of Rabbit and Rat.—A number of substances shown in Table I were also tested on isolated rabbit ileum, principally for activity in reducing spontaneous motility and antagonizing spasm due to $BaCl_2$. A few were also tested against Ba spasm on isolated rat ileum. The results are given in Table III. No direct correlation could be detected between the activities of most of the compounds and those obtained with guinea-pig ileum. However, some of the figures relating to anti-Ba effects for the rabbit correspond with those for the rat.

TABLE III
ACTIVITIES ON ISOLATED ILEUM OF RABBIT AND RAT
(Papaverine = 1)

Ro Series No.	Rabbit Ileum		Rat Ileum
	Against Spontaneous Motility	Against Ba Spasm	Against Ba Spasm
3–0226	74·0±41 (5)*	3·0± 1·0 (3)	7·5± 4 (6)
3–0235	9·3± 7·8 (7)	2·2± 0·8 (5)	21·0± 7 (5)
3–0290	6·0± 3·0 (6)	38·5±12·0 (4)	38·0±11 (5)
3–0296	6·0 (2)	3·5	—
3–0299	6·5	0·75 (2)	—
3–0305	0·7 (2)	2·5± 1·3 (3)	—
3–0320	17·0± 6·5 (4)	16·0± 0·5 (4)	20·0± 6 (7)
3–0326	2·5± 1·07 (7)	5·0± 2·2 (6)	—
3–0373	1·6 (2)	—	—

* Mean values ± S.D. The figures in parentheses give the number of assays from which the mean is derived.

Other Isolated Organ Preparations.—A number of compounds were tested on other preparations: for relaxant activity on the tracheal chain of the guinea-pig; for anti-Ba activity on the rat uterus, the tracheal chain of the rabbit and the isolated air-perfused lung of the guinea-pig; and for coronary dilator activity on the Langendorff preparation of rabbit and guinea-pig heart. The results are collected in Table IV.

TABLE IV
ACTIVITIES ON VARIOUS ISOLATED ORGAN PREPARATIONS
(Papaverine = 1)

Tracheal chain, guinea-pig, pH 8·4, relaxant activity	
Ro3–0226 0·01	Ro3–0326 0·015
Ro3–0299 0·02	Ro3–0348 0·01
Tracheal chain, rabbit, pH 7·4, reduction of Ba spasm	
Ro3–0299 0·5	Ro3–0348 1·0
Ro3–0320 0·1	Ro3–0368 0·5
Ro3–0326 0·12	
Air-perfused guinea-pig lung, reduction of effects of Ba perfusion	
Ro3–0290 1·0	Ro3–0326 1·0
Ro3–0320 ca. 1	
Langendorff preparation, coronary dilator activity	
Ro3–0226 0·15 (guinea-pig)	Ro3–0296 1·0(guinea-pig)
Ro3–0235 1·0 ,, 1·0 (rabbit)	Ro3–0299 0·25 ,,
Ro3–0290 1·0 ,, 0·7 ,,	Ro3–0320 1·5 ,,
Ro3–0292 0·4 (rabbit)	Ro3–0326 0·25 ,,
Rat uterus, reduction of Ba spasm	
Ro3–0226 1·0 (3 assays)	Ro3–0326 0·2
Ro3–0320 1·0 (2 ,,)	

The activities of the compounds in these tests were but a fraction of those on intestine, except that, in reducing ACh spasm in the guinea-pig tracheal chain, Ro 3-0226 was as active as atropine, a result similar to that with guinea-pig ileum.

Further Tests on Guinea-pig Ileum.—Because the action of barium upon intestine is at least partly mediated by stimulation of ganglion cells (Ambache, 1949; Feldberg, 1951), it seemed possible that the inhibition of barium spasm in guinea-pig ileum might be exerted upon ganglia. Some of the compounds were therefore compared with papaverine against the stimulant action of nicotine hydrochloride on isolated guinea-pig ileum. Re-determinations of anti-Ba activity were also made for two of the compounds, Ro 3-0235 and Ro 3-0226—which, as Table I shows, gave different results at different periods—and the values obtained were only a fraction of any found earlier. Inquiry revealed that the animals used for these tests, and those with which the earlier tests were performed, were from different sources. Anti-barium and anti-nicotine activities of five compounds, four of which were highly active in earlier tests and one of which appeared to be weak, were therefore re-determined on lengths of ileum from guinea-pigs of two different strains. In addition, a few animals, obtained from the same source as those in the earlier tests, were used to determine the anti-Ba activity of Ro 3-0226; similar determinations were also made for this compound on gut from animals of yet a fourth strain. The results are given in Table V. The activities, relative to papaverine, were much lower against both barium and nicotine with animals of strain II than were those originally obtained with strain I. The values with strain III, however, were of the order of the original results. Ro 3-0226 was a potent Ba antagonist with animals of strain IV and of the strain corresponding to strain I, secured later than the original group. Similar differences were found for activities against nicotine in strains II and III, in which some of the compounds were more potent against nicotine than against Ba. Indeed, the most active of the compounds, Ro 3-0348, was effective in reducing responses to nicotine in dilutions of the order of 1 in 10^9. It should be emphasized that the differences in relative activity were due entirely to differences in activity of the test compounds, papaverine being consistently effective in dilutions of

TABLE V
STRAIN DIFFERENCES IN ACTIVITIES AGAINST BARIUM AND NICOTINE ON ISOLATED GUINEA-PIG ILEUM
(Papaverine = 1)

Ro Series No.	Against Barium					Against Nicotine	
	Strain I* (Original)	Strain II	Strain III	Strain IV	Strain I (Later)	Strain II	Strain III
3–0226	5150±1150 (5) 73±30 (3)	11·8±4·2 (15)	88·5±52 (9)	55±32 (4)	161±75·5 (12)	218±67 (8)	850±650 (12)
3–0235	150±87 (5) 27±9 (5)	ca. 5	86±39 (10)			ca. 5	57±37 (13)
3–0326	79·5±12·5 (11)	ca. 5	118±64 (11)			16±5 (10)	85±60 (6)
3–0348	90±20 (4)	ca. 1	148±96·5 (10)			870±166 (9)	2750±1300 (10)
3–0367	2·2 (2)	ca. 2	31±8·8 (7)			ca. 20	70±25 (6)

* Values from Table I.

the order of 1 in a million. Gut from all strains contracted in response to the same order of concentration of stimulant agents.

Feldberg (1951) showed that hexamethonium, in a dilution of 1 in 150,000, antagonized the stimulant actions of Ba and nicotine on the isolated guinea-pig ileum. However, with gut from guinea-pigs of Strain II, hexamethonium was ineffective against Ba or nicotine contractions. With strain III it was possible to reduce nicotine contractions with hexamethonium in dilutions of the order of 1 in a million, the activity relative to papaverine being 2.44 $\pm$ 2.15 (9 assays). Effects against Ba were, however, so variable that it was not possible to obtain an assay.

Discussion

There are few records of substances many times more powerful than papaverine in reducing intestinal contractions due to barium. Jackman, Bolen, Nachod, Tullar, and Archer (1949) described 1-*cyclo*-pentylidene - 3 - dimethylamino - 1 - phenylpropane as 66 times more active than papaverine against barium spasm, and Becker, Ananenko, Glenwood, and Miller (1946) reported 1-*cyclo*-hexyl-1-phenyl-3-piperidinobutane to be 9 times as active as the reference drug. Few other substances have been described as more than 2 or 3 times as active as papaverine (see Bergel and Parkes, 1952). The order of activity found among the thioesters reported here is therefore outstanding and, indeed, suggests a specificity against barium corresponding to that of atropine against acetylcholine, or of such antihistamines as mepyramine against histamine. Only one or two of these compounds also possess strong activity against acetylcholine, of the order of that of atropine, and none is appreciably effective against histamine. High activity against barium in this series tends to be associated with high activity against potassium.

That the high anti-barium—and, to a lesser extent, anti-potassium—activity shown, on the guinea-pig ileum, by the compounds reported here may be due to a specific inhibitory action upon ganglion cells is supported by their even higher order of activity against nicotine, in the few instances in which this has been demonstrated. Further evidence is provided by the considerably lower order of activity against barium spasm in smooth muscle organs other than gut, in the responses of which ganglion-cell activity should play a smaller part. It has already been noted that ileum and tracheal chain preparations did not differ in sensitivity towards the inhibitory activity of Ro 3-0226 on acetylcholine, which Feldberg (1951) showed to be little affected by ganglion-blocking action in its effects upon intestine. The results also show that the thioesters are considerably weaker than papaverine in reducing the tone of tracheal muscle and coronary vessels, suggesting that papaverine-like activity in reducing smooth muscle tone plays little part in the strong spasmolytic activity that these substances exhibit on preparations of intestine.

Again, the highest order of activity against barium spasm was observed on the gut of the guinea-pig ; the compounds were less effective on rabbit and rat gut. Feldberg (1951) showed that hexamethonium is less effective as a nicotine antagonist on rabbit than on guinea-pig intestine, and considered this difference to reflect the extent to which ganglion-cell activity is involved in the responses to nicotine in the two species. Similar conclusions could apply to the differences in responsiveness towards the compounds described here.

However, the differences between the activity of these compounds against barium and nicotine on the intestine of different strains of guinea-pig are more unexpected than those found between species. They may imply strain differences in the extent to which nervous elements are concerned in the response of the gut to barium and nicotine, or in the sensitivity of these elements towards inhibitors.

It has been remarked that the values given for relative activity against barium and nicotine are associated with a very high variance, frequently of the order of two-thirds of the mean value and rarely less than half. No amount of repetition has successfully reduced these differences, which occur whether many pieces of gut, from more than one animal, are used for only a single assay each or whether a number of assays are performed on a single piece of gut. The use of barium as a stimulant agent in tests for spasmolytic action has often been described as being associated with difficulties of this nature (see, for example, Lehmann and Knoefel, 1942) and here also the source of variability may lie in responsiveness of the nervous elements.

All these observations have implications for the question of papaverine-like spasmolytic activity. Papaverine is characterized by its power to reduce the activity of all smooth muscle preparations in similar effective concentrations, whatever the cause of activity, spontaneous or drug-induced—

a circumstance which justifies the description of its activity as "musculotropic." Early investigations employed responses to either histamine or barium in tests for such musculotropic inhibition, since these agents were considered to contract smooth muscle in a manner not as susceptible to specific antagonism as that of acetylcholine by atropine (see, for example, the review by Burtner, 1951). When specific inhibitors of histamine were found it was recognized that antagonism to this agent was not a suitable criterion of papaverine-like activity.

The evidence now presented suggests that antagonism to barium spasm may be as inappropriate as antagonism to histamine as a criterion of papaverine-like activity, since many of the compounds reported show an anti-barium activity many times that of papaverine on isolated guinea-pig ileum but little advantage over papaverine in reducing the activity of other forms of smooth muscle. The possibility of antagonism of the effect of barium upon gut by a mode of action different from that exerted by papaverine emphasizes that antagonism to barium should not necessarily be considered as evidence for papaverine-like properties. The unsuitability of this antagonism as the basis for a screening test is further demonstrated by the possibility of the dependence of relative activity upon the species, and even upon the strain, of animal used.

In consequence, it may be impossible to rely upon correspondence between results for anti-barium activity obtained by workers in different laboratories, or even in the same laboratory at different periods, unless it can be established that the same species, and also the same strain, of animal is used. Mention has already been made of the higher order of anti-barium activity found for diethylaminoethyl benzilate, Ro 3-0256, than that reported for this compound by Lehmann and Knoefel (1942) and Buchel *et al.* (1948). Results for some of the more active compounds shown in Table I demonstrate the variation in results at different periods, as work was not being restricted at this time to animals of one strain. It also seems likely that a relatively insensitive strain came into use at a certain point in the work, for high activities were noticeably absent among the compounds of higher serial numbers. The values for Ro 3-0367 (Table V) show that, although the original order of activity was not comparable with that of its homologues, that found later shows more correspondence. It is thus impossible to attempt to deduce any relationship between chemical structure and the values determined for anti-barium activity of the type found in this group.

Summary

1. A number of compounds, principally amino-alkanethio- esters of benzilic acid, have been screened, on guinea-pig ileum, for spasmolytic activity against contractions produced by acetylcholine, histamine, barium and potassium chlorides, and nicotine. High activities against barium, potassium, and nicotine, of an order not previously reported, were found among this group.

2. The compounds were less active on the isolated intestine of the rabbit and the rat than on that of the guinea-pig. No compound was more active than papaverine on other forms of smooth muscle, such as uterus, bronchial muscle and coronary vessels. It is suggested that the mode of action of these inhibitors involves the nervous elements of the intestinal plexuses.

3. Great differences in activity against barium and nicotine on the intestine of several strains of guinea-pig are discussed.

4. The specificity of these compounds for antagonism of barium on the isolated intestine, particularly on the guinea-pig, probably indicates a different mode of action from that of papaverine.

5. It is concluded that anti-barium activity does not constitute a valid basis for testing synthetic compounds for papaverine-like spasmolytic activity.

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