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INHIBITION OF SMOOTH MUSCLE CONTRACTILITY BY INDOLE AND SOME INDOLE COMPOUNDS

BY

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Among the indole compounds arising in bacterial decomposition of tryptophane, those having the strongest effect on muscle contractility seem to be indole, skatole, and β-indolylethylamine. Their pharmacology has been studied for some time, but only qualitative, often contradictory results have been reported. Thus, whereas indolylethylamine stimulates smooth muscle (Guggenheim and Löffler, 1916), indole and skatole inhibit the contractility of skeletal, heart or smooth muscle (Danilewsky, 1908a, b; Guggenheim and Löffler, 1916; Waddell, 1927; Yanai, 1935; Garcia Blanco, del Castillo, and Rodeles, 1941; and Ets and Feinberg, 1942), although in a few instances they can also stimulate it (Danilewsky, 1908b; Biebl, 1929; and Ets and Feinberg, 1942). The action of indole, skatole, indolylacetate, and L-tryptophane on striated muscle sensitivity to potassium ions and acetylcholine has been more recently studied by Torda and Wolff (1945). According to their observations, indole increases the response of the frog rectus abdominis to acetylcholine, whereas indole, skatole, indolylacetate, and L-tryptophane increase the response to potassium ions.

The main part of this study has been devoted to an analysis of the effect of the indole compounds on the response of guinea-pig ileum to acetylcholine, potassium ions, and histamine. Experiments have also been carried out with duodenum, colon, uterus, and striated muscle preparations. In the latter we have entirely confirmed Torda and Wolff's findings. With smooth muscle organs we find that indole compounds produce the opposite effect: they strongly antagonize the action of acetylcholine and other muscle stimulants. A preliminary account of this work has been given elsewhere (Izquierdo and Stoppani, 1950).

METHODS

Muscle Preparations.—Guinea-pig ileum or colon, rat or mouse colon, rabbit duodenum or rat uterus were washed and set up in the bath, at 37° C., filled with 20 ml. Tyrode fluid (0.8 g. NaCl; 0.02 g. KCl; 0.01 g. $CaCl_2$; 0.1 g. $NaHCO_3$; 0.01 g. $MgCl_2$; 0.005 g. NaH_2PO_4; 0.1 g. glucose and water up to 100 ml.) through which was bubbled a mixture of O_2 with 5% CO_2. The contractions were recorded on a kymograph with an isotonic lever 14 cm. long (magnification: ×4). Responses in the presence of indole compounds were intercalated between responses to the stimulant alone. After each contraction the bath fluid was renewed three times and the organ left at rest for 10 minutes until the next contraction was elicited. Unless stated otherwise, the indole compounds were added immediately after the last washing, remaining therefore 10 minutes in contact with the organ before the subsequent addition of stimulant. The experiments with striated muscle were carried out at room temperature (20°–28° C.) following Chang and Gaddum's (1933) technique. The rectus anterior abdominis of *Leptodactylus ocellatus* (L.) Gir. freshly removed from the animal, or kept for 12 hours in Ringer solution with eserine 10^{-}, was set up in a bath, as above. For these experiments Ringer solution was used (0.6 g. NaCl, 0.01 g. KCl, 0.01 g. $CaCl_2$, 0.01 g. $NaHCO_3$ and water up to 100 ml.).

Reagents.—Indole, skatole, indolylacetic, and β-indolylpropionic acids, β-indolylethylamine, L-tryptophane, and histamine hydrochloride were obtained from E. Kodak Co.; potassium indoxylsulphate (indican) from T. Schurchardt; and acetylcholine from Hoffman-La Roche Co. The indole compounds were added to the bath fluid dissolved in 0.2–2.0 ml. and neutralized when necessary.

Expression of Results.—The concentrations of the indole compounds are usually represented by their molarity. The inhibitions reported are the average of not less than three measurements. The standard error of the mean has been calculated when necessary.

Manometric Experiments.—The ileum oxygen consumption was measured with Warburg's direct method (see Umbreit, Burris, and Stauffer, 1949) and cholinesterase activity with Ammon's (1933) technique. Rat brain homogenate and blood cells stroma were used for true cholinesterase, and diluted horse serum for pseudo cholinesterase (cf. Todrik, Fellowes, and Rutland, 1951; and Augustinsson, 1950).

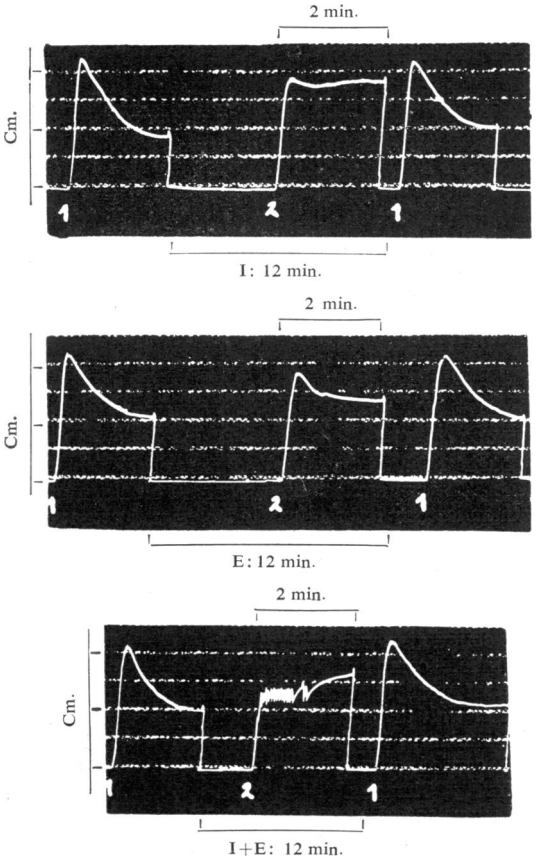

FIG. 1*a*.—Responses of the guinea-pig ileum to 5.0 μg. acetylcholine; I, in the presence of 0.4 mg. indole, and E, in the presence of 0.08 mg. skatole. 20 ml. bath.

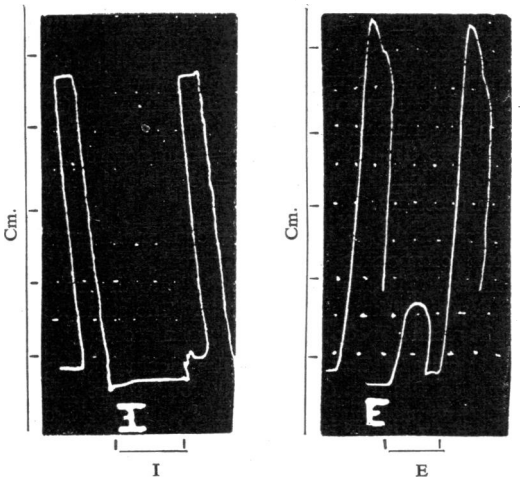

FIG. 1*b*.—Responses of the guinea-pig ileum to 0.1 (I) or 0.4 (E) μg. acetylcholine. I, in the presence of 2.1 mg. indole, and E, in the presence of 0.5 mg. skatole. 20 ml. bath.

RESULTS

Experiments on Guinea-pig Ileum

Stimulant: Acetylcholine.—Skatole and indole strongly affect the intestinal responses to acetylcholine. Both compounds diminish the height of contractions which, however, are better sustained (Fig. 1*a* and *b*). The base line remains unmodified. In the presence of indole, skatole, and acetylcholine, automatic movements of the ileum are frequently observed, which do not occur in the presence of acetylcholine alone. The effects of indole and skatole are additive as shown in Table I.

The inhibitions produced by 9×10^{-4}M indole (with 6.0 μg. acetylcholine as stimulant) after 10 sec., 30 sec., 5, or 10 min. incubation with the organ are respectively 51.5, 70.0, 82.0, and 87.0%. Skatole, 6×10^{-4}M, incubated with the organ for 10 sec., 5, or 10 min., produced inhibitions of 72.0, 94.0, and 93.0% respectively. Therefore, to obtain maximal effects, in all the experiments the organ has been left for 10 min. with the indole compound before adding the stimulant. Indole and skatole inhibitions are reversible, as, after the inhibitor has been removed by washing, the intestine again responds normally to acetylcholine (Fig. 1). However, in some instances recovery takes place at a slower rate and before the normal height is attained smaller contractions may be observed. This also happens with potassium ions as stimulant (Fig. 3).

The effect of several concentrations of indole and skatole on the responses to acetylcholine is shown in Fig. 2. In these experiments, in a preliminary trial a dose of stimulant drug producing a submaximal effect was given repeatedly until the

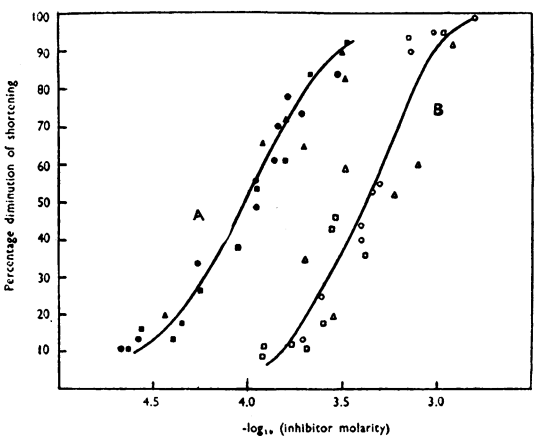

FIG. 2.—Effect of skatole (curve A) and indole (curve B) on the responses of guinea-pig ileum to acetylcholine (●,○), potassium ions (■,□) and histamine (▲,△). Acetylcholine: 5.0–0.2 μg. KCl: 3.0 mg. Histamine: 11.2–1.5 μg. 20 ml. bath.

preparation became stabilized. The doses required to produce these effects ranged from $10^{-6.25}$ to 10^{-8} for acetylcholine and from $10^{-6.15}$ to $10^{-7.73}$ for histamine. For potassium a single dose was used. The effect of inhibitors was then assayed as described under "Methods." The concentration-effect curves obtained are sigmoidal and indicate that relatively small changes in the concentration can strongly modify the extent of the inhibition. The similar shape of the curves for indole and skatole suggests that the mechanism of the inhibition may be the same for both, although the dissociation of the inhibitor-effector complexes are different. The activity of the inhibitor drug has been expressed in terms of pI_{50}, which is the $-\log_{10}$ of the inhibitor molarity reducing by 50% the response to the stimulant. The pI_{50} is 3.4 for indole and 4.0 for skatole, which are equivalent to dilutions of 4.7 and 1.3×10^{-5} respectively. The percentage reduction of the contractions does not seem to be dependent on the concentration or the nature of the stimulant used (Fig. 2).

TABLE I

ADDITION OF THE ACTIONS OF INDOLE AND SKATOLE ON THE GUINEA-PIG ILEUM

Inhibition calculated from experiments as illustrated in Fig. 1. Average response of the ileum to acetylcholine, 2.4 cm. Measurements in triplicate.

Stimulant	Inhibitor	Percentage Diminution of Shortening
Acetylcholine:		
5.0 μg.	1.8×10^{-4}M-indole	12.8±3.6
5.0 μg.	2.7×10^{-5}M-skatole	13.8±3.6
5.0 μg.	Indole and skatole	31.4±2.4
KCl:		
3.0 mg.	1.5×10^{-4}M-indole	10.4±2.2
3.0 mg.	4.2×10^{-5}M-skatole	15.0±0.4
3.0 mg.	Indole and skatole	30.0±1.7

With reference to the prolongation of contraction of the ileum by indole and skatole, it seemed possible that this might be connected with inhibition of cholinesterase, since Waelsch and Rackow (1942) have reported inhibition of serum cholinesterase by indole 10^{-2}M. However, when tested on true or pseudo cholinesterase preparations, indole (2×10^{-3}M) and skatole (6×10^{-4}M) scarcely affected (8 to 12% inhibition) the activity of the enzymes and no explanation for indole or skatole contractures can be provided thereby.

Indican (2×10^{-4}M), indolylacetate (4.3×10^{-4}M), indolylpropionate (4.2×10^{-4}M), and L-tryptophane (2.4×10^{-4}M) do not depress the acetylcholine responses.

Indican and indolylethylamine directly stimulate the ileum (Table II), which confirms earlier observations by Guggenheim and Löffler (1916) with indolylethylamine, and furthermore indolylethylamine slightly potentiates acetylcholine.

TABLE II

ACTION OF INDICAN AND INDOLYLETHYLAMINE ON ACETYLCHOLINE CONTRACTIONS OF THE GUINEA-PIG ILEUM

Acetylcholine (μg.)	Indole Compound	Height of Contraction Measured from Tracing (cm.)	Percentage Variation
0·3	None	2·8±0·1	—
0·3	2×10^{-4}M-indican	2·9±0·2	+0·3±0·1
2·0	None	3·8±0·1	—
2·0	4×10^{-4}M-indolylethylamine	4·5±0·1	+18·4±0·2

Responses (cm.) to 2.0 10^{-4}M-indican: 1.0±0.1; to 4.0 10^{-4}M-indolylethylamine: 1.1±0.2. The duration of these contractions was short and acetylcholine was added when the organ had returned to the previous relaxed state. All observations were made in triplicate.

Stimulant: Potassium Ions.—Skatole and indole diminish the contractions due to potassium ions and acetylcholine to the same extent, but they cause no prolongation of the contraction such as happens with acetylcholine (Fig. 3). The effect of the two

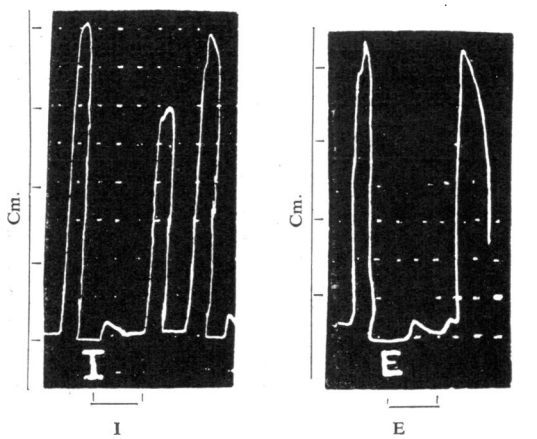

FIG. 3.—Responses of the guinea-pig ileum to 3.0 mg. KCl; I, in the presence of 1.5 mg. indole, and E, in the presence of 0.8 mg. skatole. 20 ml. bath.

compounds on the potassium contractions are additive as shown in Table I. Indican (9×10^{-4}M), indolylacetate (7×10^{-4}M), indolylethylamine (2.2×10^{-4}M), and L-tryptophane (2.4×10^{-4}M) do not affect significantly the potassium contraction.

Stimulant: Histamine.—The ileum responses to histamine (11.2–1.6 μg.) are depressed by indole and skatole in the same way as the potassium contraction. The concentration-effect curve of each compound for histamine is the same as that for potassium and acetylcholine (Fig. 2).

Effect of Indole and Skatole on the Oxygen Consumption of the Intestine

Intestinal contractility depends on the oxygen supply to the organ (Furchgott and Shorr, 1950), and as indole inhibits cell respiration (Quastel and Wheatley, 1933) it seemed of interest to find out whether there was some relationship between inhibition of contraction and depression of respiration of the intestine. Small pieces of guinea-pig ileum (26.0–41.0 mg., dry weight) were therefore suspended in Krebs-Ringer-phosphate solution and their oxygen uptake measured in Warburg manometers. The oxygen uptake (Qo_2: −4.1) did not change significantly after 15 min. incubation with concentrations of indole (1.7×10^{-3}M) or skatole (2×10^{-4}M) which strongly diminish the contractility.

Experiments on Other Tissues

Large Intestine.—Guinea-pig, mouse, or rat colon was used. The colon wall is thicker than the ileum, especially in the rat and the guinea-pig, which might affect the diffusion of stimulants or inhibitors to the muscle fibre. Nevertheless, indole and skatole depress the contractility, their action being more marked on the descending colon. Furthermore, they also diminish the gut tonus (Fig. 4),

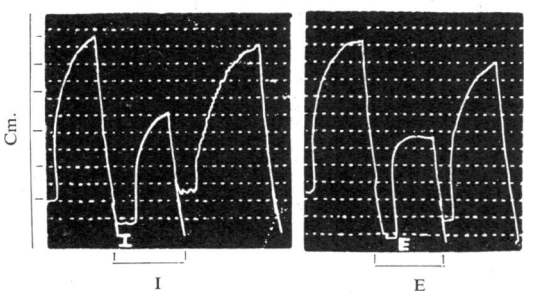

FIG. 4.—Responses of the guinea-pig colon to 3.5 μg. acetylcholine; I, in the presence of 1.8 mg. indole, and E, in the presence of 2.5 mg. skatole. 20 ml. bath.

and when acting together there is summation of effects (Table III). Skatole is somewhat less effective on the colon than on the ileum, whereas indole depresses the responses of both organs to the same extent.

Rabbit Duodenum.—Indole and skatole diminish the height of the rabbit duodenum contractions, but do not affect their frequency (Fig. 5).

Rat Uterus.—Indole and skatole diminish both the height and frequency of contractions of the rat uterus (Fig. 6), and its responses to the pituitary oxytocic hormone. Indole (6×10^{-4}M) and skatole (4×10^{-4}M) depress by 54 and 34%, respectively, the contractions elicited by 0.1 i.u. oxytocic hormone (a " Pitocin " preparation).

TABLE III

ACTION OF INDOLE AND SKATOLE ON ACETYLCHOLINE CONTRACTIONS OF THE COLON

Average response to the stimulant (measured from the tracing) of the rat colon, 5.0 cm ; the mouse colon (ascending), 3.6 cm ; and mouse colon (descending), 5.0 cm. Observations were made in triplicate.

Organ	Inhibitor	Depression of Tonus Measured from the Tracing (cm.)	Acetylcholine (μg.)	Percentage Diminution of Shortening*
Rat colon (ascending)	8×10^{-4}M-indole	0·5±0·1	0·7	69·3±0·3
,, ,,	4×10^{-4}M-indole	0·0	0·7	36·7±1·7
,, ,,	2×10^{-4}M-skatole	0·2±0·0	0·7	21·2±3·0
Mouse colon (ascending)	8×10^{-4}M-indole	0·0	15·0	30·6±3·8
,, ,,	2×10^{-4}M-skatole	0·0	15·0	17·7±0·8
,, ,,	Indole and skatole	0·0	15·0	69·5±1·5
Mouse colon (descending)	8×10^{-4}M-indole (*a*)	0·7±0·0	3·0	79·0±3·6
,, ,,	4×10^{-4}M-indole (*b*)	0·0	3·0	33·5±1·5
,, ,,	2×10^{-4}M-skatole	0·0	3·0	38·2±2·2
,, ,,	Indole (*b*) and skatole	1·1±0·0	3·0	61·0±3·2

* Change of tonus accounted for.

Striated Muscle.—When tested on the *Leptodactylus ocellatus* (L.) Gir. anterior rectus abdominis, indole potentiates acetylcholine, while indole, skatole, indican, and indolylpropionate potentiate the effect of potassium ions (Table IV). Indolylacetate (5.9×10^{-4}M), indolylethylamine (2.2×10^{-4}M), and L-tryptophane (2.4×10^{-4}M) do not affect the responses to acetylcholine or potassium.

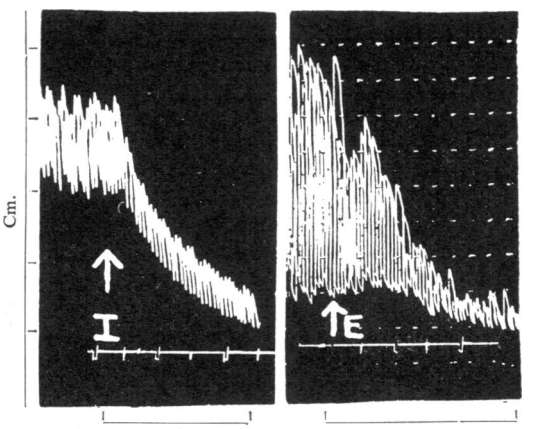

FIG. 5.—Effect of indole on contractions of the rabbit duodenum. I, addition of 1.1 mg. indole. E, addition of 2.5 mg. skatole. Time marks, 30 sec. 20 ml. bath.

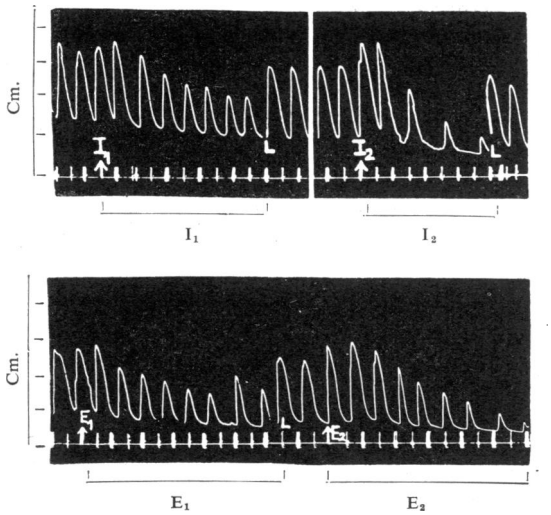

FIG. 6.—Effect of indole and skatole on rat uterus contractions. I_1, addition of 1.3 mg. indole; I_2, 2.6 mg. indole; E_1, 0.5 mg. skatole; E_2, 1.0 mg. skatole. Time marks, 30 sec. 20 ml. bath.

TABLE IV

ACTION OF INDOLE AND SOME INDOLE COMPOUNDS ON THE CONTRACTIONS OF THE FROG RECTUS ABDOMINIS

Stimulant	Indole Compound	Height of Contractions Measured from Tracing (cm.)	Percentage Variation
Acetylcholine:			
2·0 μg. ..	None	2·8±0·0	—
2·0 ,, ..	$1·1\times10^{-4}$M-indole	3·8±0·2	+35·6±0·1
20·0 ,, ..	None	2·3±0·0	—
20·0 ,, ..	4×10^{-5}M-skatole	2·3±0·0	0·0±0·0
KCl:			
3·0 mg. ..	None	2·6±0·1	—
3·0 ,, ..	$8·5\times10^{-4}$M-indole	2·9±0·1	+10·3±0·06
3·0 ,, ..	None	3·4±0·1	—
3·0 ,, ..	6×10^{-5}M-skatole	4·4±0·1	+29·4±0·05
3·0 ,, ..	None	1·5±0·1	—
3·0 ,, ..	9×10^{-4}M-indican	2·0±0·1	+33·1±0·11
3·0 ,, ..	None	1·7±0·1	—
3·0 ,, ..	7×10^{-4}M-indolyl-propionate	2·0±0·1	+17·6±0·10

Observations were made in triplicate.

DISCUSSION

It seems to be the rule that the contractility of smooth muscle organs (as observed with duodenum, ileum, colon, and uterus) is depressed by indole and skatole. It has been shown by Feldberg (1951) that histamine acts on the muscle fibre whereas potassium and acetylcholine act both on the muscle fibre and the myenteric plexuses. Since the action of these three drugs is equally depressed by indole and skatole, it seems likely that the latter act on the final common path of the three drugs, namely the smooth muscle fibre itself. This is confirmed by the finding that indole and skatole also inhibit the action of the pituitary oxytocic hormone on the uterus (see Stehle, 1950).

The guinea-pig ileum preparation is normally quiescent *in vitro*, probably owing to the fact that little acetylcholine is released under these conditions. When the intestine is *in situ* a continuous release of acetylcholine occurs, as shown by Feldberg and Rosenfeld (1933), and on this basis it is reasonable to compare the reactions of the tissues *in situ* to those of the isolated organs in the presence of acetylcholine. Our experiments may thus furnish some explanation of the contradictory results by other workers with indole and skatole in the whole animal. Thus, Ets and Feinberg (1942) obtained paralysis of the dog jejuno-ileum by injecting intravenously 25 or 60 mg. indole per kg., which if diluted evenly in the body water (cf. Fulton, 1946) would give a concentration of 3 to 8×10^{-4}M. These concentrations have been shown in our experiments to depress the smooth muscle response to acetylcholine, potassium ions, and histamine. On the other hand, the more sustained contraction of the ileum by acetylcholine, in the presence of indole and skatole, may explain the apparent stimulation of intestinal motility observed with these substances by Biebl (1929) and Ets and Feinberg (1942). *In vitro*, the inhibitions of spontaneous intestine motility observed by Danilewsky (1908a and b), Guggenheim and Löffler (1916), Waddell (1927), and Garcia Blanco *et al.* (1941) were obtained with 8×10^{-4} to 8.6×10^{-2}M-indole or 3.8×10^{-4}M-skatole (concentrations recalculated from the experimental data reported in the papers quoted), in good agreement with our own results.

With striated muscle preparations, our results, like Torda and Wolff's, contrast with those of Danilewsky (1908a and b), Waddell (1927), and Yanai (1935), who obtained depression of the contractility of the frog heart or gastrocnemius by skatole or indole, although it must be pointed out that below a concentration of 0.01% indole may stimulate the frog heart (Danilewsky, 1908b). The different behaviour of the amphibian rectus abdominis fits in with other structural and functional peculiarities of the muscle, described already by Kuffler (1949).

Among the several compounds studied, the least soluble in water are the most effective on muscle. It is evident that the presence of polar groups in the molecule increases the solubility and diminishes the pharmacologic action; in some instances, as with indican, the effect is even reversed. The detoxication of indole in the body by changing it into indican nullifies its ability to inhibit smooth muscle contraction. On the other hand, the

presence of an amino group in the side chain confers on the molecule the property of stimulating the intestine, and in this connection it is of interest that 5-hydroxytryptamine (enteramine) has been claimed to be the hormone of the intestinal enterochromaffine tissue (Erspamer and Asero, 1952).

Indole and skatole seem to be normal constituents of the intestinal fluids, and, as reported by Herter (1907), there may be up to 60 mg. indole and 10 mg. skatole in 100 g. faeces. The concentrations calculated from these figures tally with those found in our experiments to affect intestinal motility. It may therefore be considered probable that these substances can play a role in intestinal pathology, especially when the accessibility to the muscle layer of the gut is increased by lesions of the mucosae (Nicolai, 1941a and b, and 1942).

Summary

1. The effects of indole, skatole, indoxylsulphate, β-indolylethylamine, indolylacetate, β-indolylpropionate, and L-tryptophane on the responses of striated and unstriated muscles to their chemical physiological stimulants have been studied.

2. Indole and skatole diluted about 1 in 10^5 diminish the shortening of guinea-pig ileum by acetylcholine, potassium ions, and histamine, and also prolong the contraction of the ileum due to acetylcholine. The effect of these inhibitors are additive and cannot be attributed to block of the oxygen consumption of the intestine or to inhibition of cholinesterase activity.

3. Indolylethylamine slightly potentiates the effect of acetylcholine, leaving unaffected the response to potassium ions. Indoxylsulphate, indolylacetate, indolylpropionate, and L-tryptophane do not modify the response of the ileum to acetylcholine and potassium ions.

4. Indolylethylamine and indoxylsulphate cause a contraction of the guinea-pig ileum.

5. Indole and skatole diminish the tonus of the colon of the guinea-pig, rat, and mouse, and also diminish the responses to acetylcholine and potassium ions; their effect is stronger on the terminal part of the colon.

6. Indole and skatole diminish the automatic contractions of the duodenum of the rabbit and of the uterus of the rat, and also the responses of the latter to the pituitary oxytocic hormone.

7. Indole potentiates acetylcholine effects, and indole, skatole, indoxylsulphate, and indolylpropionate potentiate potassium effects, on the frog anterior rectus abdominis.

8. It is considered that the depressant effects of indole compounds are due to a direct action on the smooth muscle fibres.

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