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INFLUENCE OF CHAIN-LENGTH UPON SOME PHARMACOLOGICAL PROPERTIES OF S-ALKYL *ISO*THIOUREAS

BY

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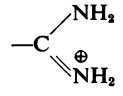
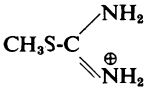
This study is one of a series in which an attempt has been made to explain, as far as possible in physicochemical terms, the distribution of certain pharmacological properties amongst *iso*thioureas and other organic bases of simple structure.

It has been shown in earlier papers (Fastier and Smirk, 1943, 1947; Fastier, 1944, 1948, 1949) that, whereas a variety of bases of formula X-C(: NH)NH_2 resemble one another closely in their pharmacological properties, substitution of a non-basic or of a feebly basic group for the ionized amidine group (I) of an active compound abolishes distinctive properties like pressor and adrenaline-potentiating activity.

(I) 

(II) 

Even when the amidine group is preserved, the introduction of substituents into it generally reduces activity and often removes it entirely. The chemical structure of the side-chain X does not seem to be of much importance so long as the compound ionizes freely to give a kation of small size. Yet merely lengthening a side-chain, as in higher homologues of S-methyl-*iso*thiourea (II), affects pharmacological activity profoundly.

Thus it would appear that physical as distinct from purely structural attributes play a large part in determining activity. Fastier and Reid (1948) therefore suggested that the quantitative differences in the effects of S-methyl-*iso*thiourea and its homologues might be due to differences in their distribution between the bulk aqueous phase, in which their concentration is measured, and the phase in which they combine with the cell receptors—the " biophase." Subsequently, Fastier and Hawkins (1951) compared the inhibitory effects of these *iso*thioureas on the amine oxidase activity of acetone-dried rabbit liver powder. Lengthening the S-alkyl chain up to ten carbon atoms caused a gradual but ultimately very considerable increase in inhibitory activity. The water-insolubility of their enzyme preparation enabled them to show that the inhibition produced by a long-chain *iso*thiourea is far more difficult to reverse by repeated washing and centrifugation of a treated specimen than that produced by an equi-inhibitory concentration of a short-chain *iso*thiourea. This

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finding supports the view that long-chain *iso*thioureas are distributed more favourably than their lower homologues between external phase and biophase; the hydrophobic properties of long-chain alkyl groups would ensure that a smaller proportion of the compound was removed from the biophase with each washing.

The experiments now to be described extend these observations. They were performed mainly in order to discover to what extent differences in the effects of S-alkyl-*iso*thioureas on some more complex biological systems can be explained by supposing that the derivatives which produce the most striking pharmacological effects are those which would be expected (on physicochemical grounds) to attain the highest concentration in the biophase for a given concentration in the aqueous phase.

METHODS

In the main set of experiments with rabbit intestine, short strips of ileum were suspended in constantly aerated Ringer-Locke solution at 37° C. in an organ bath of the type described by Burn and Dale (1922). The apparatus used for the " constant flow " experiments has been described previously (Fastier and Reid, 1949). It was so constructed that a constant stream of Ringer-Locke solution entered a small organ bath, from which it escaped by overflow. The Ringer solution was oxygenated, and drug solutions were added to it, before it entered the organ bath.

For organ bath experiments with guinea-pig ileum, the apparatus described by Gaddum and Lembeck (1949) was used. This permitted the contents of the bath to be changed automatically. Over a 125 sec. cycle there were washings at 30 sec. and 120 sec. The stimulant drug (e.g. histamine), when washed into the organ bath automatically in known concentration, remained for 30 sec. in contact with the strip, which then had 90 sec. for recovery

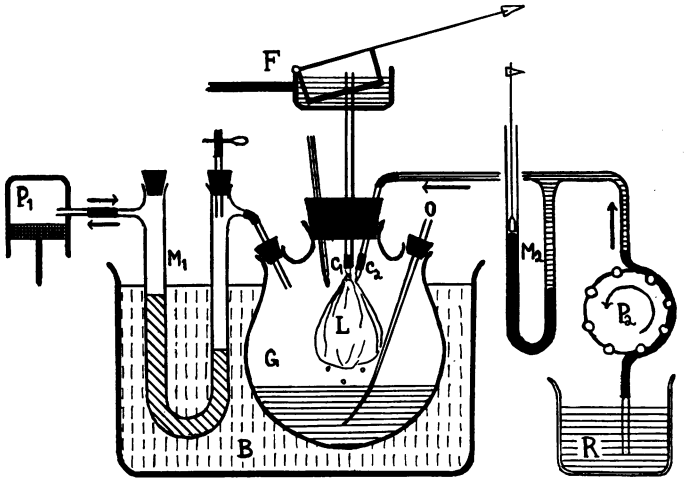


FIG. 1.—*Isolated rabbit lung preparation* (schematic). The lungs *L* are suspended in the glass " thorax " *G*, which is immersed in the constant temperature bath *B*. Pressure changes in *G*, effected by the artificial respiration pump P_1, are measured by the water manometer M_1. Consequent changes in intratracheal pressure are recorded by means of the float recorder *F* connected to the tracheal cannula c_1. The lungs are kept alive by pumping oxygenated Ringer solution from the reservoir *R* into the pulmonary artery cannula c_2 by means of the constant output pump P_2. M_2 measures the perfusion pressure. Accumulation of the perfusate in *G* is avoided by connecting the sharp-pointed tube *O* to a finely adjustable filter pump.

in fresh Ringer-Tyrode solution before the next cycle. In those experiments in which the stimulant drug was given in varied doses, it was injected into the bath immediately after the second washing of the cycle. Its time of contact with the strip was thus approximately 30 sec. The time for recovery (90 + *n*. 125 sec.) varied; large doses were never given in successive cycles. In order to ensure that the temperature of the Ringer solution entering the organ bath did not alter appreciably, each reservoir was connected to the organ bath by a long narrow glass coil placed in the outer bath, which was normally kept at a temperature of 34° C. The height of the reservoirs was such that the volume of fluid flowing past the strip in the 5 sec. taken for a washing was about five times the volume of the organ bath (2 ml.).

Pithed rat hind-quarters were perfused at a constant rate with Ringer-Locke solution that had been aerated with oxygen containing 5 per cent carbon dioxide, as described by Fastier and Smirk (1947). S-Alkyl-*iso*thiourea salts were dissolved in Ringer-Locke solution to give the dilutions specified. The compounds tested were some of those used for previous studies.

The apparatus employed for an isolated rabbit lung preparation is illustrated in Fig. 1. Ringer-Locke solution containing 0.1 per cent gelatin was used as the perfusing medium. It was pumped through the lungs at a constant rate of about 30 ml./min. by a rotary pump. The pressure within the glass " thorax " was varied within a range of 5–8 cm. of water. Similar techniques have been described by Daly (1927), von Euler (1932), and others.

RESULTS

Experiments on isolated rabbit intestine

According to the length of the S-alkyl chain, *iso*thioureas of formula $CH_3(CH_2)_n S.C(:NH)NH_2$ either raised or lowered the tone of ileal strips when added to the organ bath to give a concentration of M/10,000. Typical results are illustrated in Fig. 2*a*. Only the first four members of the series increased tone regularly. S-*n*-Amyl- and S-*n*-hexyl-*iso*thiourea produced irregular effects; sometimes they increased tone but more often they lowered it. Purely depressant effects were obtained with S-*n*-heptyl-, S-*n*-octyl-, S-*n*-nonyl-, and S-*n*-decyl-*iso*thiourea in this concentration. Still higher members of the series were not sufficiently soluble for their effects to be compared with those of the above.

Similar results were obtained when the test concentration was M/100,000. S-Methyl-*iso*thiourea and its nearer homologues increased tone, if they had any effect at all, whereas S-*n*-heptyl-*iso*thiourea and higher homologues decreased tone and reduced the sensitivity of the strip to such agents as histamine and acetylcholine. As the series was ascended, it became increasingly difficult to reverse the depressant effect of an *iso*thiourea by repeatedly washing the muscle with plain Ringer-Locke solution.

" *Constant flow* " *experiments*.—These were performed to see if there was any obvious relationship between the direct effects of *iso*thioureas on muscle tone and the changes which they produce in sensitivity to certain local hormones.

The effects of S-alkyl-*iso*thioureas alone were not dissimilar to those obtained with the ordinary organ bath technique. Apparent differences (cf. Figs. 2*a* and 2*b*) can be attributed to the fact that when a constant flow technique is used, the concentration of *iso*thiourea in the bath does not remain constant; it will rise rapidly within a second or two of the injection of the drug into the tube supplying Ringer solution to the bath, then fall away more gradually as fresh Ringer solution is pumped

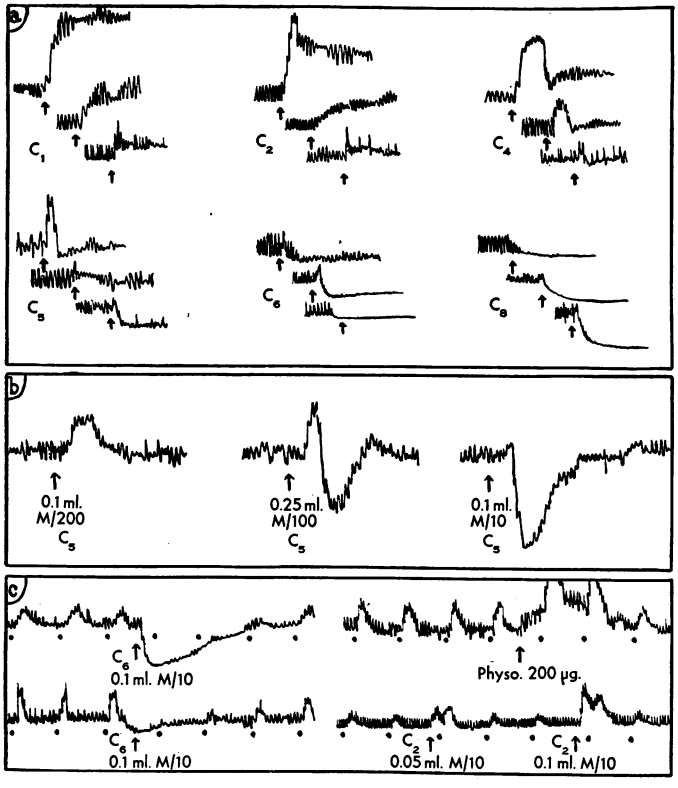


FIG. 2.—Isolated rabbit intestine. Facsimiles of kymograph records obtained by (*a*) ordinary, and (*b*, *c*) "constant flow" technique. (*a*) Effects of M/10,000 S-methyl-, S-ethyl-, S-*n*-butyl-, S-*n*-amyl-, S-*n*-hexyl-, and S-*n*-octyl-*iso*thiourea (C_1, C_2, C_4, C_5, C_6, and C_8 respectively). (*b*) Effects of graded doses of S-*n*-amyl-*iso*thiourea. (*c*) Effects of S-ethyl-and S-*n*-hexyl-*iso*thiourea (C_2, C_6) and of physostigmine upon the sensitivity of intestinal strips to acetylcholine, a small test dose of which was given at each dot.

forward. Fig. 2*b* shows that the injection of 0.1 ml. of 0.1 M- S-*n*-amyl-*iso*thiourea (C_5) caused a brief rise in tone followed by a more lasting fall. Such a response indicates that moderate concentrations of S-*n*-amyl-*iso*thiourea raise tone, whereas high concentrations lower it. In accordance with this view it was found (i) that the injection of a small dose (0.1 ml. of an 0.1 M solution) produced only a rise in tone, and (ii) that as dosage was increased the depressant stage became more and more pronounced. Near homologues of S-*n*-amyl-*iso*thiourea were also found to have a dual action on muscle tone, but there was no indication of stimulation with any of the doses of S-*n*-nonyl- or S-*n*-decyl-*iso*thiourea that were tried.

While these major changes in tone were taking place, the sensitivity of the preparation to such agents as acetylcholine and adrenaline was determined by injecting a small dose of one of them at 3 minute intervals, as indicated in Fig. 2*c*. When the concentration of an *iso*thiourea was sufficiently high for it to decrease tone, the strip was rendered less sensitive to acetylcholine and also to histamine, nicotine, and potassium salts. The response to a test dose of adrenaline was also reduced, but possibly only because the *iso*thiourea had already produced such a considerable diminution of tone that little more relaxation was possible.

In five experiments in which a small dose of physostigmine (200 µg. of the salicylate) was given in between test doses of acetylcholine, it was invariably found

that the response to acetylcholine was increased most markedly at the time when the effect on muscle tone was maximal. With an *iso*thiourea, on the other hand, the stage of increased tone was not accompanied regularly by an increased sensitivity to acetylcholine. Sometimes the effect of the latter was definitely reduced, while that of a test dose of adrenaline might be increased. No connexion between the tonus-increasing effect of an *iso*thiourea and its effect upon sensitivity to acetylcholine, adrenaline, or histamine could be detected.

Experiments with guinea-pig intestine

Ileal strips were taken from the guinea-pig, in preference to the rabbit, for quantitative experiments in order that spontaneous changes in the tone and motility of a strip might be avoided as far as possible. For the same reason Ringer-Tyrode solution was used in place of Ringer-Locke, and experiments were conducted at 34° C. Drug solutions were introduced into the bath automatically whenever this was possible.

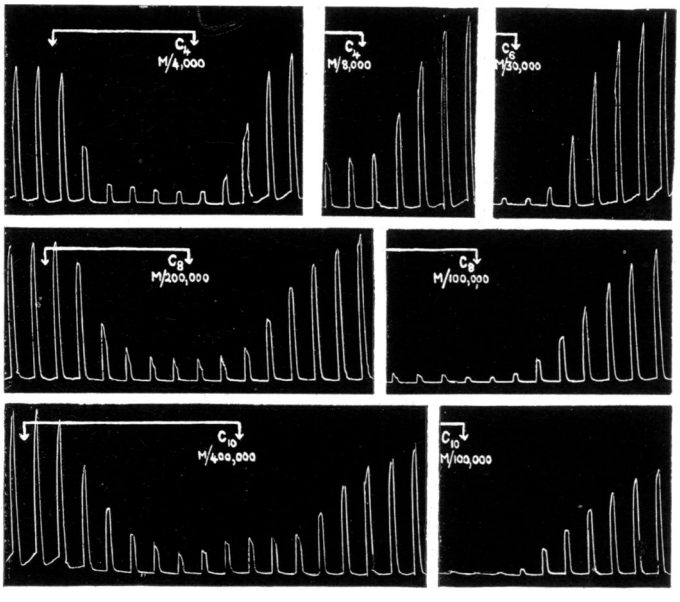


FIG. 3.—Guinea-pig ileum. Contractions elicited at 2 min. intervals by exposure to M/2,000,000 histamine for 30 sec. The anti-histamine effects were all produced on the one strip by S-*n*-butyl-, S-*n*-hexyl, S-*n*-octyl-, and S-*n*-decyl-*iso*thiourea respectively (C_4, C_6, C_8, and C_{10}).

Fig. 3 illustrates the effects of some S-alkyl-*iso*thioureas on the sensitivity of guinea-pig ileum to a fixed dose of histamine given every 125 seconds. It can be seen that when the two "plain" Ringer solutions which enter the bath alternately (viz., that containing the histamine and that used for the subsequent washing of the strip) are replaced by solutions containing in addition an inhibitory concentration of an *iso*thiourea, a state of equilibrium is eventually reached, as judged by the constancy of the response to histamine.

As sensitivity to histamine or to acetylcholine seldom altered much under these conditions, even in the course of hours, it was not difficult to discover equi-inhibitory centrations of various *iso*thioureas by testing each at several concentrations. Typical figures for S-*n*-butyl-, S-*n*-hexyl-, S-*n*-octyl-, and S-*n*-decyl-*iso*thiourea were

M/2,000, M/15,000, M/80,000, and M/200,000 respectively. For equi-inhibitory concentrations of these *iso*thioureas, the number of washings required for a state of equilibrium to be reached was not constant; the higher homologues exerted their full effect more slowly (Fig. 3). Likewise, when the administration of an *iso*thiourea was discontinued, the original sensitivity of the strip to histamine or acetylcholine was regained more slowly after treatment with a long-chain *iso*thiourea than after treatment with a lower homologue; two to three times as many washings with plain Ringer-Tyrode solution might be required in order to remove one of the higher homologues. The response to histamine was antagonized to a somewhat greater extent than that to acetylcholine. This was shown by giving alternately doses of histamine and acetylcholine which produced equal submaximal contractions and finding which of the two was antagonized more strongly by different *iso*thioureas.

In a further series of experiments the concentrations of both drugs were varied: before any *iso*thiourea was administered, the agonist (acetylcholine, carbachol, histamine) was tested over a wide range of doses, the largest of which was at least a hundred times that needed to produce a maximal contraction. The same doses were then given in the presence of an *iso*thiourea, in order to see in what way the concentration-action relationship for the agonist was modified by the *iso*thiourea.

It was found that the response of a strip to the agonist might be modified qualitatively as well as quantitatively. Thus, in the presence of M/20,000 S-*n*-hexyl-*iso*thiourea, the response to a dose of acetylcholine which was just sufficient to produce a maximal contraction of the untreated strip would be reduced by some 30–60 per cent; the response to smaller doses would be reduced to a greater extent (Fig. 4); while the response to larger doses would be modified radically. When one of these very large doses was given, the muscle would begin to contract rapidly, but a period of inhibition would quickly follow (Fig. 4), then sometimes a slowly developing contraction. Similar results were obtained with M/100,000 S-*n*-octyl-*iso*thiourea and with S-*n*-decyl-*iso*thiourea. In their presence it was impossible to reproduce

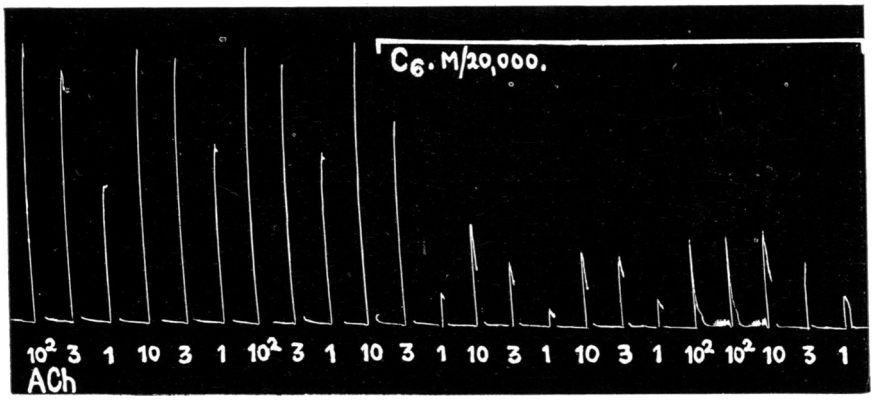


FIG. 4.—Guinea-pig ileum. Contractions elicited by acetylcholine given at 2 min. intervals in concentrations of from 1–100 μg. per 200 ml. S-*n*-hexyl-*iso*thiourea (C_6) antagonizes the effect of the various test doses of acetylcholine. It so modifies the response to the largest dose of acetylcholine that the rapid contraction which this produces initially is not sustained until the wash-out 30 sec. later.

the maximal contraction of the untreated strip by giving even very large doses of histamine or of acetylcholine. However, a large contraction could still be obtained by injecting 0.1–0.3 ml. of an isotonic solution of barium chloride into the organ bath.

It was then noticed that, even when no *iso*thiourea was present, the response to a large dose of the agonist was seldom a slowly developing contracture. As dosage was increased, the initial rate of contraction increased (Fig. 5*a*); but, sooner or later, a stage was reached at which the strip would begin to relax before the agonist was washed out of the bath. This relaxation is unlikely to be due to a local reflex, as the phenomenon was still obtained in the presence of high concentrations (60–100 mg./ml.) of hexamethonium bromide (*vide* Feldberg, 1951). It was accentuated when the muscle lever was loaded more heavily (Fig. 5*c*), and also when the temperature of the bath was raised from 29° C. to 39° C.

As indicated in Fig. 4 the main effects of an *iso*thiourea upon the response to a large dose of the agonist was to increase the tendency for a rapid contraction to be followed by relaxation. The phenomenon was more pronounced with acetylcholine as agonist than with carbachol or histamine. It was observed not only with *iso*-thioureas, but also with salts of such simple bases as *n*-decylamine (Fig. 5*b*); it is evidently not specific for amidine derivatives.

Experiments on perfused rat hind-quarters

When the test dose was 0.1 ml. of an 0.1 M solution of the *iso*thiourea, the normal effect of S-methyl-, S-ethyl-, and S-*n*-propyl-*iso*thiourea was a 30–100 mm. increase in perfusion pressure due to vasoconstriction (Fig. 6*a*). Higher homologues also increased perfusion pressure when given in this dose, but their action became emphatically biphasic as the series was ascended. The pressure would rise steeply; fall

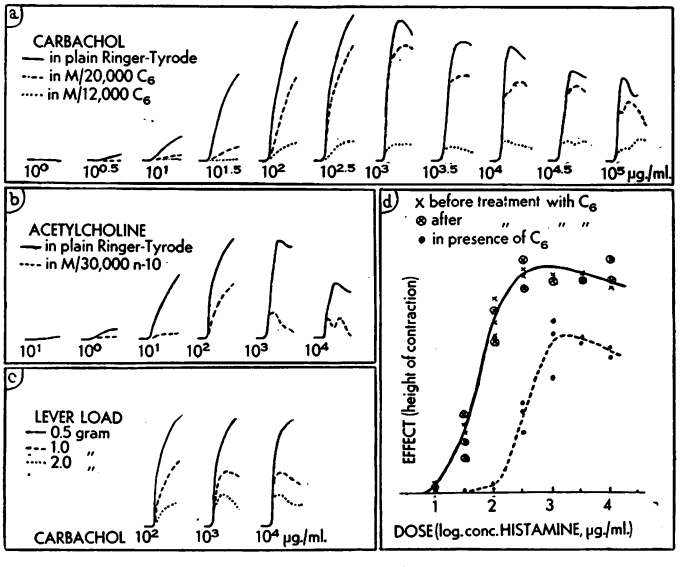


FIG. 5.—(*a*) Facsimiles of kymograph records showing the contractions of guinea-pig ileum produced by various concentrations of carbachol acting for 30 sec. S-*n*-hexyl-*iso*thiourea (C_6) antagonizes the action of carbachol. (*b*) A similar experiment with acetylcholine and *n*-decylamine (*n*-10). (*c*) The response of an ileal strip to various doses of carbachol modified by loading the muscle lever more heavily. (*d*) Log. dose-effect curves for histamine in plain Ringer-Tyrode (upper) and in the presence of M/15,000 S-*n*-hexyl-*iso*thiourea (lower).

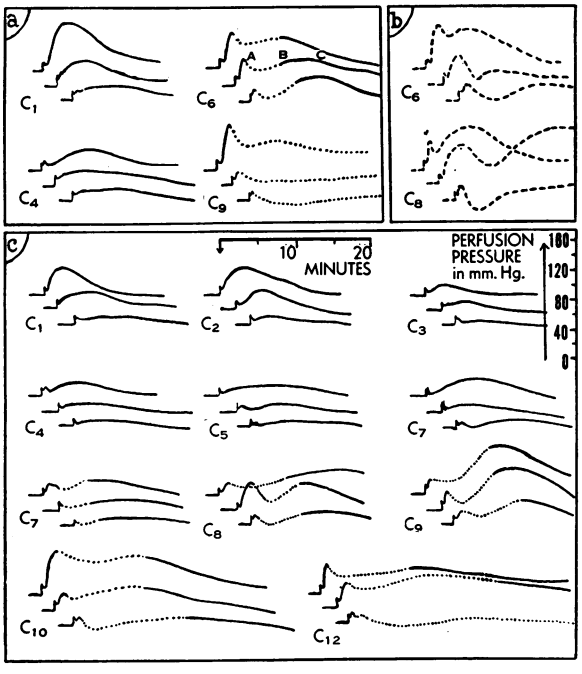


FIG. 6.—Pithed rat hind-quarters preparations. Each curve shows the vasoconstriction produced by injecting 0.1 ml. of (*a*) and (*b*) an M/10 and (*c*) an M/100 solution of one or other of the *iso*thioureas of formula $CH(CH_3)_2S.C(:NH)NH_2$. The three curves shown for each *iso*thiourea are representative of those obtained in 5–10 experiments. In (*a*) and (*c*) the broken portion of a curve represents a phase during which test doses of adrenaline (0.2–2.0 μg.) produced smaller vasoconstrictor responses than usual. The effects of S-*n*-hexyl- and S-*n*-nonyl-*iso*thiourea shown in (*b*) were obtained with preparations which were being perfused with Ringer-Locke solution containing ergotoxine (1 : 200,000); this raised the initial perfusion pressure from 30–50 mm. of Hg to 100–150 mm. and reversed the response to adrenaline.

back again, sometimes to the initial level or even below it; then rise and fall again less abruptly (Fig. 6*a*).

If the vessels of the hind-quarters, which normally have but slight tone, were constricted considerably by perfusing them with Ringer-Locke solution containing adrenaline, large falls in perfusion pressure could then be obtained with S-*n*-hexyl-*iso*thiourea and higher homologues, and with S-aralkyl-*iso*thioureas (Fig. 7*b*). Long-chain alkyl *iso*thioureas still produced vasoconstriction, however, if the tone of the vessels was raised by perfusing ergotoxine (1 : 200,000) in place of adrenaline

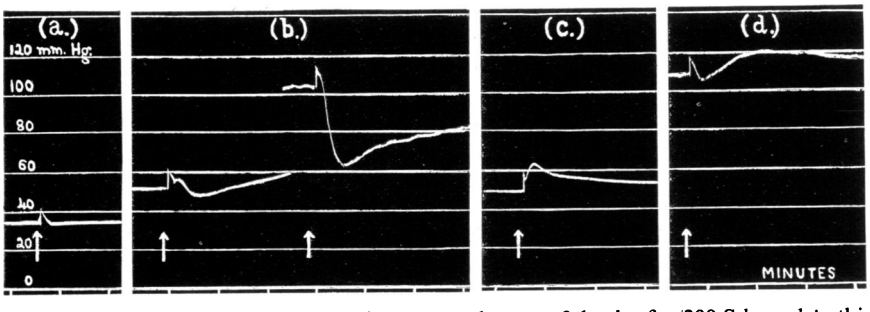


FIG. 7.—Pithed rat hind-quarters preparation. At each arrow 0.1 ml. of M/200 S-benzyl-*iso*thiourea was injected. (*a*) Normal perfusion pressure. (*b*) Tone of perfused vessels gradually increased by perfusing 1 : 100,000 adrenaline. (*c*) Five minutes after perfusion with adrenaline had been discontinued. (*d*) Tone of vessels increased by perfusing them with Ringer-Locke containing 1 : 10,000 barium chloride.

(Fig. 6*b*). A similar experiment in which barium was used in place of adrenaline to restore the tone of the vessels to something like the normal level is illustrated in Fig. 7*d*. In view of these findings it was thought of interest to go through the records of an earlier series of experiments (Fastier and Reid, 1948), in which effects of *iso*-thioureas on the sensitivity of perfused rat blood vessels to adrenaline were studied, to see if the direct effects of *iso*thioureas on vessel tone bore any close relationship to anti-adrenaline effects.

Typical results have been illustrated as follows. In Fig. 6 only some of the perfusion pressure tracings have been represented by continuous curves. The broken portions of the other curves show the periods during which test doses of adrenaline produced smaller vasoconstrictor responses than usual. It can be seen that anti-adrenaline effects were obtained almost invariably when there was a pronounced " dip " in the perfusion pressure tracing. These changes in sensitivity to adrenaline are not the *result* of the perfusion pressure changes elicited by the *iso*thiourea. Thus, when adrenaline was given at the points marked A, B, and C in Fig. 6*a*, the vaso-constrictor response to the second dose was almost twice as large as that to the third dose of adrenaline and some ten times larger than that to the first, yet the perfusion pressure level was almost the same for each injection.

With S-methyl-*iso*thiourea and its nearer homologues, the effect of a 0.1 ml. dose of an 0.1 M solution did not persist for more than 10–20 minutes. A second equal dose could be given 30 minutes later without the occurrence of " tachyphylaxis." With S-*n*-hexyl-*iso*thiourea and higher homologues, on the other hand, an interval of 30 minutes between the injection of 0.1 ml. doses of 0.1 M solutions was insufficient to prevent tachyphylaxis. The more pronounced dip in the perfusion pressure tracings suggested that enough of the *iso*thiourea had remained from the first dose, despite the continuous passage of fresh Ringer-Locke solution through the vessels, for the second dose to have produced a cumulative effect. The effects of S-*n*-nonyl- and S-*n*-decyl-*iso*thiourea were apparent for upwards of an hour after their injection.

Tachyphylaxis was less evident with smaller doses. Provided that the interval between injections was kept at 20 or 30 minutes, the response to successive injections of one or other of the first seven members of the series in a dose of 0.1 ml. of the 0.01 M solution did not alter appreciably. Typical responses are illustrated in Fig. 6*c*. The effects of the *n*-octyl, *n*-nonyl, and *n*-decyl derivatives were more persistent than those of lower homologues; even 30–40 minutes after their administration the response to adrenaline and to other *iso*thioureas was affected.

Experiments on perfused rabbit lungs

The action of acetylcholine on the preparation illustrated in Fig. 1 is manifested by an increase in perfusion pressure and a decrease in tidal airflow (Fig. 8). The former effect may be attributed to constriction of pulmonary blood vessels, the latter to constriction of the airways. Fig. 8 shows how the bronchoconstrictor action of acetylcholine on the preparation is augmented by physostigmine and antagonized by atropine, as would be expected for a muscarinic effect.

With this preparation, potentiation of muscle-contracting actions of acetylcholine by *iso*thioureas could be readily demonstrated. In six of seven experiments treatment with S-methyl-*iso*thiourea brought about a definitely enhanced response to acetyl-choline (Fig. 8). Long-chain *iso*thioureas greatly decreased the sensitivity of the

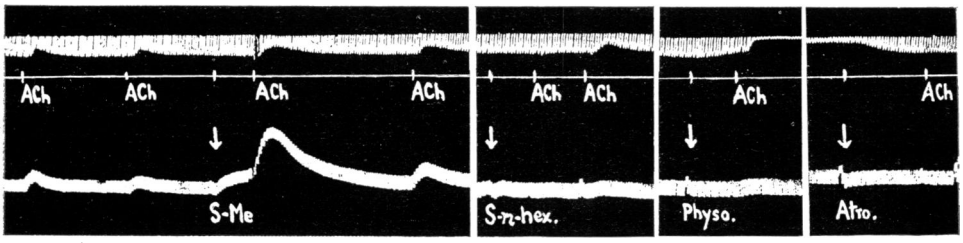


FIG. 8.—Isolated rabbit lung preparation. Doses of acetylcholine (50 μg. at ACh) given at 5 min. intervals produce temporarily a decrease in tidal airflow (upper record) and an increase in perfusion pressure. The response to acetylcholine is potentiated when it is injected shortly after a dose of S-methyl-*iso*thiourea (0.5 ml. of a M/10 solution at S-Me), but antagonized by an equivalent dose of S-*n*-hexyl-*iso*thiourea (S-*n*-hex.). Effects of physostigmine (200 μg. at Physo) and of atropine (250 μg. at Atro) are shown for comparison.

preparation to acetylcholine when they were given in the same molar doses (0.2–0.5 ml. of 0.1 M solutions). Smaller and less persistent anti-acetylcholine effects were obtained when S-*n*-hexyl-*iso*thiourea was given in equivalent amount, and a subsequent period of enhanced sensitivity to acetylcholine could usually be detected.

DISCUSSION

It has been found for several effects of the S-alkyl-*iso*thioureas of formula $CH_3(CH_2)_nS.C(:NH)NH_2$ on smooth muscle preparations that the molar concentration required to produce a given intensity of effect decreases steadily as the series is ascended up to the *n*-decyl derivative. Experiments of the type depicted in Fig. 3 indicate that potency increases by a factor of 2–3 with the addition of each methylene group. Approximately the same increase in potency has been noted previously for the inhibitory effects of S-alkyl-*iso*thioureas on amine oxidase (Fastier and Hawkins, 1951). Now chain-length affects to the same extent such physical properties of homologues as depend upon a distribution between two phases (Ferguson, 1939). We therefore attribute the logarithmic, 2–3-fold increase in pharmacological activity with the addition of each methylene group to changes in those physical properties of *iso*thioureas which influence the distribution of a homologue between aqueous phase and biophase.

Two explanations, not mutually exclusive, can be given for the finding that more washings with plain Ringer solution were needed to reverse the spasmolytic effect on gut of a long-chain *iso*thiourea than that of a lower homologue producing equal inhibition (Fig. 3). If a state of equilibrium is approached during each washing, then the amount of *iso*thiourea removed will be determined largely by the partition coefficient. Fastier and Hawkins (1951) thought this to apply to their experiments with amine oxidase. In our organ bath experiments the washings occurred at much shorter intervals, probably far too quickly to permit equilibration during a washing. Under these conditions the most important factor is likely to be the mean time required for an *iso*thiourea to diffuse through the biophase from the receptors to the aqueous phase. Naturally the higher *iso*thioureas will diffuse more slowly. It is significant that approximately as many washings with an *iso*thiourea solution are needed for the full inhibitory effect to be obtained as are needed with plain Ringer solution for the subsequent reversal of the inhibition (Fig. 3).

Homologous series of amidine derivatives have been used for several other studies of the influence of chemical structure upon pharmacological activity. The structure-activity relationships found with corresponding series of amidines, guanidines, and *iso*thioureas for such properties as trypanocidal and antibacterial actions (King, Lourie, and Yorke, 1937; Fuller, 1942), production of hypoglycaemia (Shikinami, Yonechi, Kawai, and Hosono, 1930; Broom, 1936), and inhibition of inactivation of adrenaline by the liver (Dawes, 1946) show a broad resemblance. Normally, as a series is ascended, the intensity of a given action increases steadily to a maximum and then falls off again. The " cut-off " may occur at the same point in a series for several properties. It is probably determined by solubility in the aqueous phase, for when chain-length is increased, homologues must be eventually obtained which are not sufficiently soluble in aqueous solution for them to be transported in adequate amount to the site of action.

One exception to this rule is provided by certain pharmacological properties of di-amidine derivatives which are not shared by mono-amidine derivatives, e.g. the inhibition of histaminase (Blaschko, Fastier, and Wajda, 1951) and the liberation of histamine *in vivo* (MacIntosh and Paton, 1949). It is not surprising that the influence of chain-length on such properties is harder to discern because, when two functional groups are involved in the production of a particular action, the distance between these groups is likely to be an important factor in determining activity (Paton and Zaimis, 1949).

Another exception is provided by " excitatory " properties of amidine derivatives, such as pressor activity and adrenaline-potentiating activity. Here the difficulty of gauging the influence of chemical structure upon activity appears due to the fact that amidine derivatives may have dual effects upon muscle preparations; the excitatory effect is obtained only if the concentration of the drug falls between lower and upper limits. This has already been demonstrated for effects upon sensitivity to adrenaline. All the homologues between S-methyl- and S-*n*-decyl-*iso*thiourea, and various other amidine derivatives, have been shown capable of either increasing or decreasing the response of perfused rat blood vessels to adrenaline, according to the dose given (Fastier and Reid, 1948). Similar findings have been described in this paper. Thus S-*n*-amyl-*iso*thiourea and its nearer homologues clearly have dual effects upon the tonus of isolated rabbit intestine (Fig. 2*b*). Dual actions on gut could not, however, be obtained with long-chain *iso*thioureas. In their effects on intestinal tone long-chain *iso*thioureas seem to differ qualitatively from lower homologues; not just quantitatively, as in their effects upon sensitivity to adrenaline. They resemble in this respect the long-chain alkyl-trimethyl-ammonium bases and the long-chain choline esters. The latter have been shown to antagonize the excitatory actions of their lower homologues by combining with the same receptors though they have no excitatory actions themselves (Clark and Raventos, 1937; Swan and White, 1944). In such cations it must be presumed that the hydrocarbon radical influences not only the distribution of the cation between external phase and biophase but also its capacity to react with receptors in the biophase.

Analogous effects of aliphatic amines

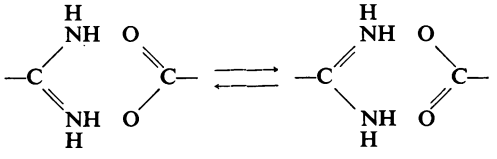
The function of the " head " of an *iso*thiourea cation must now be considered. Are the various excitatory and inhibitory actions described in this and in earlier

papers peculiar to amidine derivatives ? The results of sieving experiments (Fastier, 1944) indicate that strong basicity is necessary for activity, but it remains uncertain whether the basicity must be conferred by an amidine group.

The primary amines of formula $CH_3(CH_2)_nNH_2$ have approximately the same basicity ($pK_a \approx 10$) as the *iso*thioureas under investigation. Since the first three or four members of the amine series show little resemblance in their pharmacological properties—described by Alles (1941), Barger and Dale (1910), Dunker and Hartung (1941), and others—to the short-chain *iso*thioureas, the amidine group apparently does more than provide the cation with a charged head.

Walker (1949) has put forward the view that the combination between a drug containing an unsubstituted amidine group and protein anionic groups is not just a simple electrostatic attraction between oppositely charged bodies; resonance should permit the formation of doublet ion-pairs (III) with a relatively rigid configuration of minimum potential energy, the implicit hydrogen bond being short and comparatively strong.

(III)



If an amidine derivative is bound more strongly to receptors than the corresponding amine, we should look for pharmacological analogues of short-chain *iso*thioureas amongst amines of comparable absorptability, viz. those whose side-chains are long enough for the van der Waals attraction between them and the receptor to compensate for the smaller affinities of the end groups (Albert, personal communication).

Aliphatic primary amines with 5–7 carbon atoms in the side-chain have been examined by a number of pharmacologists, including Barger and Dale (1910), Nakamura (1925), Alles (1941, 1946), Proetz (1943), Jackson (1944), Lewis (1946), and Swanson and Chen (1946). Their effects are distinctly more " sympathomimetic " than those of short-chain amidine derivatives like S-methyl-*iso*thiourea. Nevertheless these amines, too, may cause the contraction of ileal strips and of several other smooth muscle preparations which are relaxed by adrenaline. For such reasons it has been argued that these aliphatic amines resemble acetylcholine and potassium more closely than adrenaline in their pharmacological properties, their effects being comparable to the nicotine-like and muscarine-like effects of the alkyl-trimethyl-ammoniums and other bases whose chemical relationship to acetylcholine is more obvious (Alles, 1941). Somewhat similar views have been expressed concerning the mode of action of short-chain amidine derivatives (Fastier, 1949). Judging from the results obtained when aliphatic amines have been tested under experimental conditions similar to our own, we think that those with 5–7 carbon atoms in the side-chain resemble short-chain amidine derivatives sufficiently closely in their pharmacological properties to suggest that their main modes of action are essentially similar, however they may be labelled.

It is of interest that the higher homologues of pressor aliphatic amines behave like those of pressor amidine derivatives. Alles (1941) found that for the homologues

between *n*-butylamine and *n*-nonylamine the addition of each methylene group increased relaxant activity on isolated rabbit ileum by a factor of 2–3. He observed, moreover, that there was a close relationship between the concentrations required to produce a minimal degree of relaxation and those required to antagonize the effects of small doses of acetylcholine or adrenaline. The lethal toxicity (for mice) increased quite regularly with the addition of each methylene group. Alles therefore suggested that the lethal toxicity of aliphatic amines may be related to their ability to render end-organs insensitive to normal excitatory agents.

Localization of depressant effects

In the experiments on perfused rat blood vessels, substantial vasoconstrictor effects have been obtained even with long-chain *iso*thioureas (Fig. 6). These results differ from those of Fastier and Vane (unpublished), who tested *iso*thioureas in comparable doses on cat hind-limbs perfused *in vivo*; they found that S-*n*-amyl-*iso*thiourea and higher homologues produced strong vasodilator effects when injected into the femoral artery of the perfused limb, as do numerous S-aralkyl-*iso*thioureas (Dawes and Fastier, 1950). Now the vessels of the cat hind-limb preparation, unlike those of the rat hind-quarters preparation, have their innervation intact; they have normal tone. It has been found that if the tone of the rat blood vessels is raised to a comparable level by adding adrenaline to the perfusing Ringer-Locke solution, then vasodilator effects which resemble closely those obtained in the cat hind-limb with long-chain alkyl and with aralkyl *iso*thioureas can be obtained in the rat hind-quarters also (Fig. 7). Yet if ergotoxine is used in place of adrenaline for increasing tone, even such bases as S-*n*-nonyl-*iso*thiourea produce effects which are still predominantly vasoconstrictor (Fig. 6*b*). We think it significant that the " dip " in perfusion pressure tracings like those illustrated in Fig. 6 should coincide with a period of reduced sensitivity to adrenaline. These results suggest that the dilator effects of long-chain *iso*thioureas on blood vessels which are under nervous control depend in part at least upon a reduced response to adrenergic stimuli.

With isolated rabbit intestine, it has been found that concentrations of S-alkyl-*iso*thioureas which lower tone reduce the response of the strip to such agents as histamine, nicotine, and acetylcholine. Possibly these depressant effects are all related. Thus the fall in tone may be the result of a decreased response to excitatory hormones. Although *iso*thioureas show little chemical resemblance to acetylcholine or histamine, the possibility of their being able to compete with either is not altogether remote. It must be remembered that, whereas a compound may need to have several chemical groups in common with a neurohormone like acetylcholine for it to stimulate the same receptors (Ing, 1949), possession of but one of these groups may suffice for it to be able to combine with part of the receptor patch and so deny access to the natural effector, as Myers (1951) has shown recently for certain inhibitors of cholin-esterase. We have therefore made a quantitative study of the anti-acetylcholine and anti-histamine effects of S-alkyl-*iso*thioureas.

The results obtained cannot be explained by any simple theory of the interaction of antagonistic drugs. One well-known theory assumes that the pharmacological effect is proportional to the amount of the agonist present on the receptors and that the antagonist acts competitively according to the mass laws (Clark, 1937; Gaddum,

1943). The slope of the log. dose-effect curve has often been found to be equal to the slope predicted by this theory in its simplest forms, but not always. Our curves are steeper than the theoretical curves. Schild (1947) also has obtained steep curves for histamine with guinea-pig intestine. These steep curves can be explained by assuming that more than one molecule of the drug must combine with each receptor in order to make it respond. We would add, however, that the effects of small doses of the agonist may have been depressed for hours as the result of the administration of very large doses, although we tried to avoid this hazard (Eastman and Cantoni, 1946) by giving doses only at long intervals.

The theory based on the mass laws also predicts that the ratio of the doses of agonist producing any given effect in the presence and in the absence of the agonist is constant. The horizontal distance between the log. dose-effect curves should therefore be constant for competitive inhibition. This too has been found occasionally (Clark, 1937; Schild, 1949), but not always (Guarino and Bovet, 1949; Schild, 1949). Our results do not satisfy this condition in so far as the maximum effect obtained in the presence of the antagonist was generally smaller than the maximum effect obtained in its absence (Fig. 5*d*). This finding may mean that some fraction of the muscle contractile units is rendered completely inexcitable by the antagonist. Nevertheless our results cannot be explained by supposing that this fraction is constant; it has been found repeatedly that the response to small doses may be almost completely abolished, while the response to larger doses is reduced only by some 20–30 per cent.

We would emphasize that the mass-law theory assumes a state of equilibrium between the drugs and the receptors. It is unlikely to be applicable to cases like those considered here, where the response reaches a maximum and then decreases, despite the fact that the concentration of agonist in the bath remains constant (Fig. 5). As this kind of response is not obvious in records made with a slow-moving drum (cf. Figs. 4 and 5), false conclusions may be drawn from these. We have found that inhibitory concentrations of *iso*thiourea alter qualitatively the response of an intestinal strip to a large dose of histamine or of a choline ester. The muscle begins to contract rapidly as if a large increase in tone would result, but within a few seconds contraction is followed by relaxation. The same phenomenon has been observed occasionally, even in the absence of an *iso*thiourea with large doses of the agonist (Fig. 5*a*). It is of interest that several other preparations have been found to respond similarly to acetylcholine. Recently, McDowall and Watson (1951) have shown, in confirmation of the results of earlier workers, that the response of striped muscle to a large dose of acetylcholine is self-limiting. Contraction is followed within a few minutes by relaxation and thereafter the preparation will not respond to electrical stimulation or to another dose of acetylcholine so long as the previous dose remains in the bath. McDowall and Watson found that repeated minute doses of acetylcholine produced similar inexcitability. They suggest that this is because repolarization is prevented in the presence of acetylcholine.

It seems to us that the actions of amidine derivatives on muscle and nerve can be explained more simply by supposing that they effect some fundamental property like polarizability than by supposing that they affect tone and sensitivity to various neurohormones by unrelated, competitive actions.

SUMMARY

1. With the *iso*thioureas of formula $CH_3(CH)S.C(:NH)NH_2$ the intensities of anti-acetylcholine, anti-histamine, and tonus-decreasing actions on rabbit and guinea-pig ileum increase steadily as the series is ascended to about the *n*-decyl derivative. The "cut-off" probably occurs at this point because of the water-insolubility of higher homologues. The 2–3-fold increase in activity with the addition of each methylene group is attributed to an increasingly favourable distribution of the *iso*thiourea between aqueous phase and biophase, enabling each higher homologue to achieve a greater concentration at the site of action for a given molar concentration in the aqueous phase.

2. A quantitative study has been made of the anti-acetylcholine and anti-histamine effects of S-alkyl-*iso*thioureas on isolated guinea-pig ileum. The results cannot be explained by any simple theory of the interaction of antagonistic drugs. Inhibitory concentrations of an *iso*thiourea alter qualitatively the response of a muscle strip to a large dose of the stimulant; the maximal contraction of the untreated strip cannot be reproduced.

3. When given in somewhat smaller doses than those needed to decrease intestinal tone, some *iso*thioureas increase tone. Homologues containing 4–6 carbon atoms in the side-chain clearly have a dual action on gut. Lower homologues increase tone even when present in fairly high concentration, while higher homologues seem incapable of producing other than depressant effects on gut.

4. Short-chain *iso*thioureas can potentiate the bronchoconstrictor and vasoconstrictor actions of acetylcholine on perfused rabbit lungs, but potentiation of its tonus-increasing action of intestine could not be demonstrated. No connexion between the tonus-increasing effects of *iso*thioureas and their effects upon sensitivity to local hormones could be detected. However, the concentrations of different *iso*thioureas required to decrease intestinal tone and to dilate perfused blood vessels were found to approximate to the concentrations required to reduce the sensitivity of the same preparations to acetylcholine and to adrenaline respectively.

5. The influence of chemical structure upon the pharmacological activity of aliphatic amines is discussed with reference to the results obtained with amidine derivatives.

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