CLXX. STUDIES ON THE RELATIONSHIP BETWEEN CHEMICAL CONSTITUTION AND PHYSIOLOGICAL ACTION.

V. MOLECULAR DISSYMMETRY AND PHYSIOLOGICAL ACTIVITY.

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When a molecularly dissymmetric substance possesses pharmacological activity it is frequently, although not invariably, found that one optical isomeride is considerably more potent than the other. The work carried out on this subject has been reviewed by Cushny [1926], who himself made a detailed pharmacological comparison of the antimeric adrenalines and hyoscyamines. Largely as a result of his studies on these substances, Cushny regarded optical activity as a factor which is quite distinct from general structure in determining the magnitude of the specific pharmacological activity of a molecule, and this view is, we believe, the one which is currently held.

Cushny regarded the difference between the physiological activities of two optical isomerides as analogous to the different behaviours of the antimeric forms towards optically active acids and was of the opinion that an explanation of the influence of molecular dissymmetry on physiological activity would be found along the lines of this analogy, possibly, for example, by the more active isomeride forming a compound with an optically-active specific receptor in the tissues which possessed different physical properties from that formed with its enantiomorph.

It is the purpose of the present communication to elaborate an alternative view according to which there appears to be no reason for differentiating between molecular dissymmetry and structure in regard to the manner in which they influence physiological activity. On the contrary, it is considered that the different physiological activities of optical isomerides may frequently be ascribed to circumstances which are identical with those which cause different symmetrical molecules to exhibit different physiological activities; in other words, that molecular dissymmetry is, of itself, without influence on physiological activity, and that both it and the optical activity with which it is associated are accidental accompaniments, in optical isomerides, of different molecular arrangements which differ in their ability to cause the development of a particular physiological effect for precisely the same reasons that two structural isomerides so differ.

Before considering any definite examples, the theoretical basis of our suggestion must be explained. This starts from the postulate that a drug is attached to its specific receptor in the tissues in such a manner that a considerable proportion of the drug molecule is involved. If an asymmetric carbon atom is

present, three of the groups linked to this atom may be concerned in the process. The exact nature of the attachment is immaterial to the main argument, but it is our view that this may be effected either by normal valencies or by adsorptive or other forces; or it may be of a loose type, somewhat analogous to the attachment of a glove to the hand, involving the contour of a large part of the molecule rather than points on its surface; or, finally, a combination of these possibilities may be involved. Whatever the mechanism, the position of affairs may be represented by the diagrams in Fig. 1. Of these, III and IV represent, in the conventional manner, two enantiomorphs, while VI depicts diagrammatically the surface of the specific receptor in the tissues. For the drug molecule to produce a maximum physiological effect it must, according to the above postulate, become attached to the receptor in such a manner that the groups B, C and D in the drug coincide respectively with B', C' and D' in the receptor. Such coincidence can only occur with one of the enantiomorphs (III), and this consequently represents the more active form of the drug. Let it now be supposed that the dissymmetry of III is abolished by replacing the group A by a second group B. The resultant molecule, represented by V, retains unchanged that part of the structure of III, *i.e.* the base BCD of the tetrahedron, which is concerned with its attachment to the specific receptor and must therefore be considered capable, despite the absence of molecular dissymmetry, of exerting its physiological activity with an intensity numerically equal to that of III, except in so far as this activity is changed by modifications in such of its properties as are not directly concerned with its attachment to the receptor. It will be noted that V possesses a second face, shaded in the diagram, containing the groups BCD. This face, however, corresponds with the base of IV and, like this, cannot be brought into coincidence with the receptor.

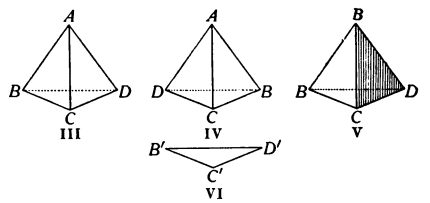
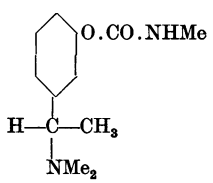


Fig. 1.

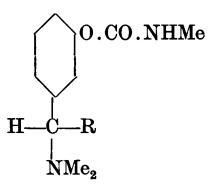
In order to apply this generalised argument to a particular drug it must be decided which of the four groups linked to the asymmetric carbon atom are concerned in the attachment of the drug molecule to the receptor. Such a decision can be reached with a fair degree of certainty for *l*-adrenaline from a consideration of the abundant evidence which is available relating structure to sympathomimetic action, and there seems little doubt that the groups in question are the basic, the aromatic, and the alcoholic hydroxyl groups. Our reasons for this conclusion, as well as our views regarding the mechanism of attachment, are briefly as follows. (1) The basic group is a feature common to all the sympathomimetic bases. From its nature it appears probable that this group is linked to the receptor by means of normal valencies. (2) The aromatic group is also necessary for the development of any appreciable activity. Whether or no this is attached by normal valencies is more problematical. We think, however, that it should be regarded as a possibility that the group, as a whole, "fits" the receptor, but that when it carries phenolic hydroxyl groups in the *m*- or *p*- positions it becomes more firmly attached by means of normal valencies. (3) The alcoholic hydroxyl group is necessarily concerned in the attachment, since it is the presence of this group which is responsible for the outstanding activity of *l*-adrenaline. It is nevertheless difficult to say whether it is combined chemically with the receptor or whether it merely causes a better "fit" of the drug to the receptor.

Of the four groups linked to the asymmetric carbon atom in *l*-adrenaline it is thus the hydrogen atom, represented by *A* in III, which plays no part in the attachment of the drug to the receptor. If this group were replaced by a hydroxyl group a symmetrical compound corresponding with V would result which, according to the above argument, would possess a physiological activity equal to that of *l*-adrenaline except in so far as this activity was modified by changes in the physical properties of the compound due to the presence of the second hydroxyl group. Unfortunately such a compound is not capable of existence and cannot, therefore, be utilised to test our hypothesis. A similar test, scarcely less satisfactory, can however be made. If IV be regarded as representing *d*-adrenaline, it is clear that the hydroxyl group (*B*) cannot be concerned with the attachment of the drug to its receptor, but that this must be brought about by the face *ACD* involving the hydrogen atom (*A*), and the smaller physiological activity of this isomeride is attributed to the less perfect combination which results. By substituting a hydrogen atom for the hydroxyl group in *d*-adrenaline a symmetrical molecule would be obtained which, on the hypothesis which we have advanced, should combine as readily as *d*-adrenaline with the receptor and should therefore exhibit, with the above reservation regarding physical properties, the same physiological activity as this compound. The symmetrical compound in question is 3 : 4-dihydroxy-β-phenylethylmethylamine. While the activity of this compound has not been compared directly with that of *d*-adrenaline, data are available from which the relative activities of the two bases can be calculated. Thus, according to Barger and Dale [1910], the pressor activity of *dl*-adrenaline is seven times as great as that of 3 : 4-dihydroxy-β-phenylethylmethylamine, while Cushny's results show that *l*-adrenaline is 12–15 times as active as *d*-adrenaline. It follows that *d*-adrenaline is 1·07 to 0·87 times as active as 3 : 4-dihydroxy-β-phenylethylmethylamine, a result which corresponds with that deduced from our hypothesis much more closely than might have been anticipated.

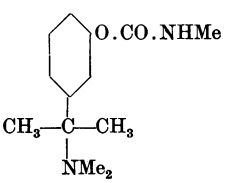
One such favourable result cannot, of course, be regarded as establishing the correctness of our suggestion, and we have therefore attempted to obtain further experimental support for it with another group of drugs, namely the synthetic miotics which have formed the subject of the preceding papers of this series (for references, see Stedman [1931]). Among these drugs, miotine (VII)



VII



VIII



IX

possesses exceptional miotic activity, and one which is considerably greater than that of the methylurethane of *m*-hydroxybenzyldimethylamine (VIII; R = H). Its large activity is thus associated with the introduction of the methyl group into the side-chain, which simultaneously creates an asymmetric carbon atom in the molecule. We were inclined originally to ascribe the high physiological activity of this substance to the latter circumstance, but the view outlined above now offers an alternative explanation. According to this view miotine, and the miotics of this class in general, must become attached to their receptor by means of the basic and $NH(CH_3).CO.O.C_6H_4$-groups, since these two groups

are together responsible for the physiological properties of these compounds. Since the presence of the methyl group in miotine is associated with high physiological activity it must further be assumed that, in the more active isomeride, this group causes a more perfect combination to take place between drug and receptor than would occur in its absence. In the less active isomeride, on the other hand, the methyl group must be directed away from the receptor and consequently cannot directly influence the fixation of the drug. It is thus clear that a compound of the formula IX, in which the molecular dissymmetry of miotine has been abolished by the introduction of a second methyl group, should possess a physiological activity equal to that of the more active form of miotine except in so far as this activity is modified by changes in the physical properties of the compound. Similarly, the less active form of miotine should possess an activity equal to that of VIII (R = H).

In order to test these deductions, certain of the properties of the compounds VII, VIII (R = H) and IX have been compared. In addition some urethanes of the general formula VIII, in which R = Et, Pr$^{\alpha}$, and Ph, have been similarly examined in order to obtain data from which the influence of increased hydrocarbon content on the physiological activity of this type of substance might to some extent be judged. Measurements have also been made with the corresponding methiodides. The latter, however, clearly contribute little to the present discussion, since the methiodide from miotine does not show any markedly greater activity than that of its parent substance (VIII; R = H). A description of the various compounds examined will be published elsewhere.

Results.

Miotic activities. The above urethanes were first compared with respect to their action on the pupil. For this purpose, one drop of a solution of the hydrochloride or methiodide in physiological saline was instilled by one of us (E. S.) into the eye of a cat. To ensure that the drops were, as far as possible, of the same size, the same pipette was used throughout the experiments. Individual variations in different animals were avoided by carrying out all the tests on one animal. Alternate eyes were used, but on no occasion was an experiment carried out until both eyes were completely normal. This usually necessitated an interval of 24, and in some cases 48, hours between successive experiments. The results are tabulated below. Slight salivation usually occurred shortly after the instillation of the drug; with Nos. 6 and 12, however, salivation was considerable. Racemic miotine was necessarily used for these experiments, since,

	Methylurethane	Conc. %	Miosis
1.	*m*-NHMe.CO_2.C_6H_4.CH_2.NMe_2, HCl	0·1	Slight but distinct after 20–25 mins.
2.	*m*-NHMe.CO_2.C_6H_4.CHMe.NMe_2, HCl	0·1	Very marked; commenced in 15 mins. and reached maximum in 25 mins.
3.	*m*-NHMe.CO_2.C_6H_4.CMe_2.NMe_2, HCl	0·1	Similar to 2 but effect somewhat greater
4.	*m*-NHMe.CO_2.C_6H_4.CHEt.NMe_2, HCl	0·1	Similar to 1 but effect greater
5.	*m*-NHMe.CO_2.C_6H_4.CHPr$^{\alpha}$.NMe_2, HCl	0·1	Similar to 1 and 4
6.	*m*-NHMe.CO_2.C_6H_4.CHPh.NMe_2, HCl	0·1	Nil
		1·0	Slight after 30–40 mins.
7.	*o*-NHMe.CO_2.C_6H_4.CH_2.NMe_2, HCl	0·1	Commenced in 15 mins. Probably greater than 1
8.	*p*-NHMe.CO_2.C_6H_4.CH_2.NMe_2, HCl	0·1	Nil
9.	*m*-NHMe.CO_2.C_6H_4.CMe_2.NMe_3I	1·0	Distinct after 15–20 mins.; maximum in 25–30 mins.
10.	*m*-NHMe.CO_2.C_6H_4.CHEt.NMe_3I	1·0	Very slight after 30 mins. Less than 9
11.	*m*-NHMe.CO_2.C_6H_4.CHPr$^{\alpha}$.NMe_3I	1·0	Nil
12.	*m*-NHMe.CO_2.C_6H_4.CHPh.NMe_3I	1·0	Slight after 30–40 mins.

at the time they were carried out, the antimeric forms were not available. This, however, does not invalidate the results. According to our argument a compound of formula IX should possess an activity equal to that of the more active form of miotine and hence greater than that of *dl*-miotine. This involves, it is true, the assumption that the two forms of miotine differ in activity, but this assumption, as will be seen from a later section, has now been verified.

The above results are clearly in accordance with our hypothesis. The general effect of increasing the hydrocarbon content of the molecule is to diminish its miotic activity. This is exemplified by the activities of 4 and 5, which are much smaller than that of miotine (2). On the other hand, the introduction of the second methyl group into miotine with the production of 3, although this necessarily increases the hydrocarbon content of the molecule, is associated with an increase in miotic activity.

The miotic activities of the methylurethanes of the isomeric hydroxybenzyldimethylamines (1, 7 and 8) have been re-examined since, according to Stedman [1929], they are in the order $o > p > m$ while White and Stedman [1931] state that the *m*-compound is more active than the *p*. In agreement with the latter result the order is now found to be $o \geqslant m > p$.

Inhibitory activities towards liver esterase. It has been shown by Stedman and Stedman [1931; 1932] that, in addition to possessing characteristic pharmacological properties, urethanes of the type under consideration also inhibit the activity of liver esterase. Since the suggestion which we have made regarding the influence of molecular dissymmetry on physiological activity applies equally well to the influence of the same factor, either in substrate or inhibitor, on enzymic activity, we have compared the inhibitory activities of the above urethanes on the liver esterases from the cat and the pig. As in the miosis experiments, *dl*-miotine was employed.

The enzyme preparations were made by extracting the desiccated liver powder with dilute ammonia, precipitating some impurities from this extract with acetic acid, and then dialysing the centrifugate, according to the detailed procedure previously described. A method, based upon that of Knaffl-Lenz [1923], has however now been employed for following the hydrolysis of the substrate. To a solution of 0·25 cc. of methyl butyrate in 100 cc. of water warmed to 30° were added 10 drops of a solution of bromothymol blue and sufficient NaOH to bring the solution to about p_H 7·6 as shown by the indicator. A suitable volume of the enzyme or enzyme-inhibitor solution was then added and the p_H of the solution maintained as constant as possible at the above value by the addition, drop by drop as required, of 0·02 *N* NaOH. Burette readings were taken at regular intervals. The inhibitor was left overnight in contact with the enzyme.

The results obtained with liver esterase from the cat are given in Table I. Since all the inhibitors examined were of the general formula

$$NHMe.CO_2.C_6H_4.CRR'.NMe_2$$

they are described in this and the following tables by the groups which are represented by RR′ in this formula. It should be mentioned that considerable difficulty was experienced in obtaining consistent results with the enzyme from the cat and many measurements had to be discarded as worthless. The cause of this inconsistency was ultimately traced to the fact that the acidity of an extract of liver esterase from this animal slowly increases on standing. Now, the inhibitory activities of the urethanes in question vary with the p_H of the solution in which they are in contact with the enzyme. Since the measurements with

Table I.

Enzyme: liver esterase from cat. Substrate: methyl butyrate. p_H 7·6. t = 30°. Final conc. of inhibitors: 4×10^{-7} molar.

Inhibitor	Alkali in 5 min. periods	Total	Percentage inhibition
Hydrochlorides			
Control	4·2, 4·2, 4·1, 4·2	16·6	—
H, H	1·2, 1·2, 1·1, 1·2	4·7	72
H, Me	1·2, 1·3, 1·2, 1·3	5·0	70
Me, Me	1·0, 0·9, 1·0, 1·0	3·9	77
H, Et	1·3, 1·3, 1·3, 1·3	5·3	68
H, Pr[a]	1·2, 1·2, 1·4, 1·3	5·1	69
H, Ph	1·7, 1·5, 1·8, 1·6	6·6	60
Hydrochlorides			
Control	4·6, 5·0, 4·8, 4·7	19·1	—
H, H	1·1, 1·3, 1·3, 1·3	5·0	74
H, Me	1·0, 1·2, 1·1, 1·1	4·4	77
Me, Me	0·9, 0·9, 1·1, 1·0	3·9	80
H, Et	1·2, 1·2, 1·3, 1·3	5·0	74

the individual drugs were usually made seriatim, they were carried out with a solution which was becoming progressively more acid and hence were not comparable. In the experiments recorded in Table I, this difficulty was overcome by carrying out each group of experiments simultaneously, the enzyme solution having been brought to p_H about 7·6 by the addition of alkali immediately prior to mixing it with the inhibitors. A buffer could hardly be employed, since the amount necessary to prevent the change in acidity would have interfered with the accuracy of the subsequent titration. We are unable definitely to explain the cause of this liberation of acid. It did not appear to be due to the action of micro-organisms since the enzyme extract was saturated with chloroform in order to keep it sterile. The most probable explanation is that it was caused by the presence of another enzyme, possibly an oxidase, in the extract.

An inspection of Table I shows that the results run parallel with those obtained in the miosis experiments. Increase in the size of the alkyl group diminished the inhibitory activity of the urethane, while the introduction of the second methyl group into miotine increased it.

Tables II and III give the results obtained with liver esterase from the pig. The enzyme preparation used in the first series was made from a liver powder which was over a year old; that employed in the second was from a freshly prepared powder. This apparent duplication of experimental material was made because the activity towards methyl butyrate of an extract from an old powder is always considerably less than that from a new one, whereas the reverse holds when the substrate is tributyrin, and it was desired to ascertain if this diminution in activity was accompanied by any change in the inhibition phenomena. It can be seen from the tables that this is not the case. In these experiments the enzyme was left overnight in contact with the inhibitor in the presence of a small amount of buffer (p_H 8·9).

The results obtained with the enzyme from the pig do not at first sight appear to be so favourable to our hypothesis as do those described above. While they are satisfactory to the extent that the symmetrical compound (Me, Me) possesses a greater inhibitory activity than its isomeride (H, Et), the former is nevertheless considerably less active than miotine. When, however, it is recalled

Table II.

Enzyme: liver esterase from pig. Substrate: methyl butyrate. p_H 7·6. t = 30°.
Final conc. of inhibitors: hydrochlorides, $2·5 \times 10^{-7}$; methiodides, $2·5 \times 10^{-6}$ molar.

Inhibitor	Alkali in 5 min. periods	Total	Percentage inhibition
Hydrochlorides			
Control	1·8, 1·9, 2·0, 1·9	7·6	—
H, H	1·4, 1·4, 1·35, 1·45	5·6	26
H, Me	1·25, 1·15, 1·25, 1·3	4·95	35
Me, Me	1·3, 1·4, 1·4, 1·35	5·45	28
H, Et	1·4, 1·5, 1·45, 1·4	5·75	24
H, Pr$^{\alpha}$	1·4, 1·45, 1·55, 1·6	6·0	21
H, Ph	1·1, 1·1, 1·2, 1·1	4·5	41
Methiodides			
Control	2·0, 2·05, 1·95, 2·0	8·0	—
H, H	0·9, 1·0, 1·0, 0·95	3·85	52
H, Me	1·05, 0·95, 0·9, 1·0	3·9	51
Me, Me	0·9, 0·95, 0·9, 0·95	3·7	54
H, Et	1·05, 1·0, 1·05, 0·95	4·05	49
H, Pr$^{\alpha}$	1·05, 1·05, 1·05, 1·0	4·15	48
H, Ph	1·25, 1·2, 1·1, 1·15	4·7	41

Table III.

Enzyme: liver esterase from pig. Substrate: methyl butyrate. p_H 7·6. t = 30°.
Final conc. of inhibitors: hydrochlorides, $2·5 \times 10^{-7}$; methiodides, 5×10^{-7} molar.

Inhibitor	Alkali in 5 min. periods	Total	Percentage inhibition
Hydrochlorides			
Control	5·4, 5·5, 5·6, 5·5	22·0	—
H, H	3·3, 3·4, 3·4, 3·4	13·5	39
H, Me	2·7, 2·6, 2·8, 2·7	10·8	51
Me, Me	3·1, 3·3, 3·4, 3·3	13·1	40
H, Et	3·5, 3·4, 3·5, 3·5	13·9	37
H, Pr$^{\alpha}$	3·8, 3·9, 3·9, 3·9	15·5	30
H, Ph	3·2, 3·0, 3·1, 3·0	12·3	44
Methiodides			
Control	5·2, 5·4, 5·4, 5·4	21·4	—
H, H	3·6, 3·5, 3·6, 3·5	14·2	34
H, Me	3·6, 3·6, 3·5, 3·6	14·3	33
Me, Me	3·7, 3·7, 3·8, 3·7	14·9	30
H, Et	3·7, 3·7, 3·6, 3·6	14·6	32
H, Pr$^{\alpha}$	3·7, 3·6, 3·7, 3·7	14·7	31
H, Ph	3·5, 3·5, 3·5, 3·6	14·1	34

that the general effect of increasing the hydrocarbon content is to diminish the activity of the compound, it is clear that if the difference between the inhibitory activities of *d*- and *l*-miotine is, in this case, small the hydrocarbon effect might outweigh that due to configuration. Since the above experiments were carried out the preparation of the antimeric miotines by Macdonald and Stedman [1932] has enabled us to test this point, and it has, in fact, been found that there is little difference between the inhibitory activities of the two forms towards liver esterase from the pig, although the *l*-compound is slightly the more active. The results, therefore, can be regarded as consistent with our theory.

Action on intestine. A detailed pharmacological comparison of *d*- and *l*-miotine, which have now become available, is being made by Dr A. C. White, who has informed us that, in those experiments which he has so far carried out,

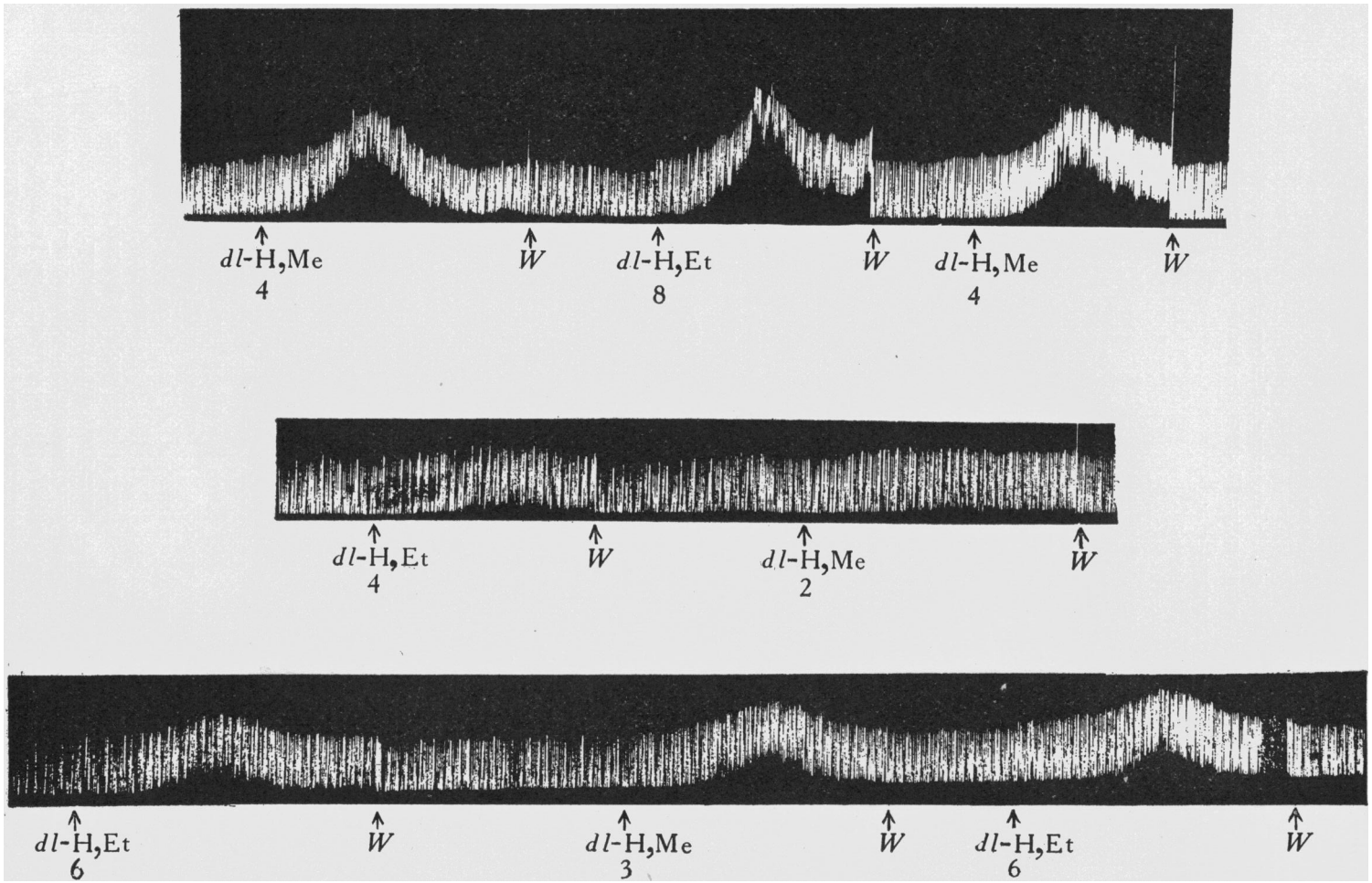


Fig. 2. Continuous tracing showing comparison of the activities of the hydrochlorides of *dl*-miotine (*dl*-H, Me) and of the methylurethane of *dl*-α-*m*-hydroxyphenyl-*n*-propyldimethylamine (*dl*-H, Et) on the isolated small intestine of the rabbit. Numbers represent parts of drug per 100,000,000 parts of Tyrode's solution. *W* = washed.

the *l*-isomeride has proved to be the more active. In order, however, to obtain numerical data bearing on the subject of the present investigation we have compared the activities of a number of the urethanes mentioned above, including *d*- and *l*-miotine, on the isolated rabbit intestine. The following ratios, which refer to the concentrations required to produce equal action, were obtained for the pairs of urethanes indicated: *dl*-H, Me: *dl*-H, Et = 2:1; Me, Me: *dl*-H, Et = 2·7:1; *l*-H, Me: *d*-H, Me = 5:1; Me, Me: *l*-H, Me = 2:3; *dl*-H, Me: H, H = 12:1. From these figures the relative activities of the various compounds are deduced to be as follows:

H, H	*d*-H, Me	*dl*-H, Et	*dl*-H, Me	Me, Me	*l*-H, Me
1	5	6	12	16	24

According to the activities thus assigned to *d*- and *l*-miotine, the racemic compound should have an activity of (24 + 5)/2 = 14·5. Actually, it was found by direct comparison with the methylurethane of *m*-hydroxybenzyldimethylamine to be 12, which is in satisfactory agreement with that calculated.

An inspection of the above figures at once shows that the results obtained are in general accord with that anticipated from our hypothesis. The symmetrical compound Me, Me has an activity which is 3 times that of *d*-miotine and only slightly less (two-thirds) than that of *l*-miotine. Moreover, the diminution in activity due to what we have termed the hydrocarbon effect on passing from miotine to its next higher homologue is, when judged from the compounds *dl*-H, Me and *dl*-H, Et, 6 units. Assuming that the additional methyl group in Me, Me has produced the same effect, the activity for this compound, when corrected by the addition of 6 units, becomes 22, a value which is in satisfactory agreement with that obtained (24) for *l*-miotine. The only discrepancy between these results and our anticipations is the fact that *d*-miotine is 5 times as active as its lower homologue, whereas according to our deductions these substances should be equally active. We do not, however, regard this discrepancy as serious. It is possible that the hydrocarbon effect on passing from H, H to H, Me may be in the opposite direction from that associated with the change from H, Me to Me, Me or H, Et.

In order to illustrate the method adopted, and the degree of accuracy attained, in the comparison of the activities of the different urethanes, the tracings for *dl*-miotine and the methylurethane of α-*m*-hydroxyphenyl-*n*-propyldimethylamine (H, Et) are reproduced in Fig. 2. These tracings are typical of those obtained in the experiments recorded above. In each case a strip of the small intestine from the rabbit was suspended in Tyrode's solution and was treated alternately with the two drugs under comparison. It was invariably found that the response of the intestine was at first inconstant but became constant after several hours. At this stage the concentrations of the two drugs under comparison were sought which produced the same effect on the strip of intestine. Only the latter and significant portions of the tracing are reproduced in Fig. 2.

Summary.

1. It has been shown theoretically, on the assumption that three of the groups linked to the asymmetric carbon atom in an optically active drug are concerned in its attachment to its specific receptor in the tissues, that molecular dissymmetry and its associated optical activity have no direct influence on the magnitude of the physiological activity of a drug.

2. The same argument holds with respect to the relationship between molecular dissymmetry in a substrate molecule or in a specific inhibitor and their power of combining with an enzyme.

3. This theory has been shown to be in agreement with the fact that the pressor activities of *d*-adrenaline and 3 : 4-dihydroxy-β-phenylethylmethylamine are, within the limits of experimental error, equal.

4. A comparison of the miotic activities of a number of urethanes has given results which are also consistent with this theory. Similar results have been obtained in a comparison of the inhibitory activities towards liver esterase and of the activities on the isolated intestine from the rabbit of the same urethanes.

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