ANALGESICS AND THEIR ANTAGONISTS: SOME STERIC AND CHEMICAL CONSIDERATIONS

PART III. THE INFLUENCE OF THE BASIC GROUP ON THE BIOLOGICAL RESPONSE

BY A. H. BECKETT, A. F. CASY AND N. J. HARPER

From the Chelsea School of Pharmacy, Chelsea Polytechnic, London, S.W.3

Received June 20, 1956

ELSEWHERE[1,2] the thesis was advanced that the basic group of the molecule influenced analgesic activity and evidence was adduced in support. In morphine-type compounds, a gradual transition from analgesic to anti-analgesic activity occurred as the group was changed from *N*-methyl to *N*-ethyl, *N*-*n*-propyl and *N*-allyl.[3-5]

A number of authors have attributed this anti-analgesic effect to drug-antagonist competition for unspecified "cell sites", or "susceptible enzyme systems" thought to be involved in the analgesic metabolic process[6-8]. Beckett and Casy[9] outlined the physical and chemical characters of the hypothetical "analgesic receptor site" and speculated on the mode of its physical interaction with the drug. It seems reasonable to assume that the mechanism of action of an analgesic antagonist involves competition with an analgesic for the "analgesic receptor site", but "fit" at the receptor surface does not of necessity mediate an analgesic response[9].

If the analgesic is represented by A and the receptor by S, the reaction between the drug and the receptor may be represented by (1) where k_1 and k_2 are the rate constants for the association of the components and the dissociation of the complex respectively.

$$A + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AS \quad .. \quad .. \quad .. \quad (1)$$

The formation of this complex may be regarded as initiating a sequence of reactions which may be represented in the following way:

$$A + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} AS \xrightarrow{k_3} XS + Y \rightarrow \underset{\uparrow \text{FURTHER REACTION?}}{\underset{\uparrow \text{ANALGESIC RESPONSE}}{X}} + S + Y \quad .. \quad (2)$$

(I) ADSORPTION (II) REACTION (III) DESORPTION

X represents the compound which causes analgesic action, or an essential intermediate in a further sequence of reactions which produces the biological effect. The desorption of X from the receptor S regenerates the latter for further combination with the drug. Assuming all receptors must be filled to obtund "pain", if A is present in less than an effective concentration in the biophase in contact with the receptor site, there will be incomplete saturation of the receptors which will result in a decreased

concentration, or rate of formation, of X leading to a reduced analgesic response. Complete saturation of the receptor sites by a particular analgesic will result in the full analgesic effect for the drug, (within its own analgesic "potency", which is itself limited by other considerations) and further increase in concentration may prolong, but will not increase, the level of the action.

Mechanism of antagonism

An analgesic antagonist B may be considered to exert its effect by one of the following mechanisms.

(1) The antagonist B may react with the analgesic A to form a stable complex AB which thus removes A from possible combination with the receptor S. However, since such analgesic antagonists as nalorphine and (−)-3-hydroxy-*N*-allylmorphinan differ from their parent analgesics only in the replacement of the *N*-methyl group by an *N*-allyl group, and have the same configuration, this mechanism may be ignored.

(2) The antagonist B may combine with the analgesic receptor and the formation of this complex may be followed by, (*a*) failure to undergo reaction II (see equation 2), with the result that the essential intermediate X is not produced; or (*b*) reaction II may proceed only with great difficulty so that X is liberated so slowly that only very low levels of analgesia are produced; or (*c*) a reaction sequence differing from that caused by an analgesic is initiated, and one of the intermediates in this sequence fails to react with one of the enzyme systems or receptor surfaces implicated in the analgesic metabolic sequence.

The following observations indicate that analgesics and their antagonists are adsorbed upon the same receptor sites.

(i) Morphine and the antagonist, *N*-allylnormorphine have the same configuration.

(ii) (−)-Dromoran, an active analgesic, becomes an analgesic antagonist upon replacing the *N*-methyl by an *N*-allyl group, whereas the corresponding change in the analgesically inactive (+)-dromoran fails to yield an antagonist[10].

(iii) When substitution of the hydroxyl groups of morphine-type compounds leads to a reduction in the analgesic activity, similar substitution in the corresponding *N*-allylnormorphine compounds leads to a reduction in anti-analgesic activity[3,4].

(iv) Antagonists based on the morphine or morphinan type structures, antagonise not only their parent molecules[10–15] but also many other active compounds[10,13–18].

This spectrum of antagonism is further demonstrated by the precipitation of the withdrawal phenomena by *N*-allylnormorphine in man[19] and monkey[20] addicted to any one of a range of analgesics. That antagonists seem to be adsorbed on the same receptors as analgesics and undergo comparable reactions less readily after adsorption, is indicated by the following:—

(*a*) *N*-allylnormorphine—an antagonist, possesses slight analgesic activity at high doses[13,21,22]; (*b*) alteration of the *N*-alkyl group of certain

analgesics can lead to compounds in which there is a gradual transition from analgesic to anti-analgesic activity as the groups are changed in the order, methyl, ethyl, *n*-propyl, allyl[3,4].

Scharenburg (unpublished, cited by Seevers and Woods[23]) claims to have shown that there is competition between nalorphine and synthetic analgesics of the pethidine, methadone and methorphinan type for cell sites in certain myelinated neurons. A similar phenomenon has been described for morphine and heroin[24]. Seevers and Woods[23] concluded from this and other evidence that "these compounds (morphine and synthetic analgesics), occupy receptors on certain myelinated neurons, and exert a pharmacologic or pathologic effect after occupation, and nalorphine competes successfully for the receptors ordinarily occupied by these agents, or displaces these agents after occupation".

Another possible way by which an antagonist may exert its effect is by directly blocking the reaction sequence initiated when a drug is adsorbed upon the receptor site subsequent to the formation of X (see later).

Nalorphine, in addition to antagonising the analgesic action of morphine, antagonises many of the other effects, e.g., respiratory and vasomotor depression. We are deliberately restricting our considerations in the present paper. The rapid reversal of morphine analgesia by nalorphine may be attributed to a direct central nervous stimulation by the latter rather than drug-receptor antagonism. The foregoing considerations and facts seem to favour the latter explanation, but the possibility of a dual mechanism of antagonism cannot be excluded (see also Miller and others[25]).

The reaction between an analgesic antagonist B and the analgesic receptor S may be shown thus:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \text{NEGLIGIBLE ANALGESIC RESPONSE} \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \text{(IV)} \uparrow \text{FURTHER REACTION?} \\ \mathrm{B} + \mathrm{S} \underset{k_5}{\overset{k_4}{\rightleftharpoons}} \mathrm{BS} \xrightarrow{k_6} \mathrm{XS} + \mathrm{Z} \rightarrow \mathrm{X} + \mathrm{S} + \mathrm{Z} \qquad .. \quad (3) \\ \qquad\qquad\quad \text{(I)} \qquad\qquad\quad \text{(II)} \qquad\qquad\quad \text{(III)} \\ \qquad\quad \text{ADSORPTION} \qquad \text{REACTION} \qquad\quad \text{DESORPTION} \end{array}$$

In these reactions, it is presumed that k_6 is very much smaller than the rate constant k_3 for the corresponding reaction of analgesics, resulting in X being formed only slowly so that there is insufficient concentration to give an analgesic response. The question of the possible constitution of X will be considered later.

The degree of inhibition of an analgesic A by an antagonist B will depend upon, (*a*) the relative concentration of A and B in the phase in contact with the receptor, and (*b*) the change in free energy upon formation of the complexes AS and BS.

It would appear that analgesics and their antagonists exert the particular effect under consideration, at the central nervous system[26], and consequently transport through membranes is involved before these drugs can reach the site of action. Although such factors as lipoid solubility,

chemical reactivity, and steric factors may affect the distribution of chemicals within the body, the similarity in structure and dissociation constants of analgesics and their antagonists, derived by alteration of the *N*-alkyl group only should ensure similar distributions in the body.

The relative stability of the analgesic and anti-analgesic-receptor complex

The stability of these complexes will probably be influenced by the following factors.

(*a*) The shape of the molecule, which may affect the closeness of the fit of the complementary surfaces and consequently the strength of the ionic and van der Waals' forces bonding the drug to the receptor; (*b*) the dissociation constant of the basic group which will affect the ionic interactions of the drug and the anionic site; and (*c*) the presence or absence in the drug molecule of other groups in addition to the basic groups and the flat aromatic ring. These additional groups may increase the attractive or repulsive forces when in proximity to the receptor surface, and may cause steric effects which will alter the fit of the drug to the receptor.

The influence of the factor (*c*) may be thought to be reduced to a minimum by considering the competitive antagonism of an analgesic and its antagonist derived by alteration of the *N*-alkyl group only. Winter and others[13] reported that nalorphine antagonised many times its molecular equivalent of analgesic drugs, although the drug-antagonist ratio seemed to vary with different drugs. Huggins (unpublished, cited by Siebert and Huggins[7]), in what appears to be a study of respiratory depression, comments on a blocking ratio of one molecule of nalorphine to 67 molecules of morphine. It may be postulated that, using equal doses, nalorphine may penetrate to the central nervous system in slightly higher concentration than morphine since the former is less ionised at physiological pH. Nevertheless, the slight concentration difference would probably be nullified by the lower basic strength of nalorphine leading to a less strong ionic binding at the receptor than occurs with morphine.

The increase in drug-receptor attraction upon replacing the *N*-methyl by an *N*-allyl group may be attributed to the increase in non-bonded attractive forces between drug and receptor effected thereby.

If the free energy change upon the combination of the antagonist (B) with the receptor (S) is $\triangle F_1$, and the corresponding change for the combination of the analgesic (A) with the receptor is $\triangle F_2$, then

$$K_{BS} = \frac{[BS]}{[B]\,[S]} = \exp(-\triangle F_1/RT)$$

$$K_{AS} = \frac{[AS]}{[A]\,[S]} = \exp(-\triangle F_2/RT)$$

In concentrations in the vicinity of the receptor at which the analgesic and the antagonist compete on equal terms for the site,

$$[BS] = [AS].$$

$$\text{Then } \frac{[A]}{[B]} = \exp([\triangle F_2 - \triangle F_1]/RT)$$

If [A] = 40[B] and T = 310° A. (body temp. = 37° C.)

$$40 = \exp([\triangle F_2 - \triangle F_1]/RT) = \exp([\triangle F_2 - \triangle F_1]/[1{\cdot}986 \times 310])$$

$$\therefore \triangle F_2 - \triangle F_1 = 1{\cdot}986 \times 300 \times \frac{\log 40}{\log e} \text{ cal.}$$

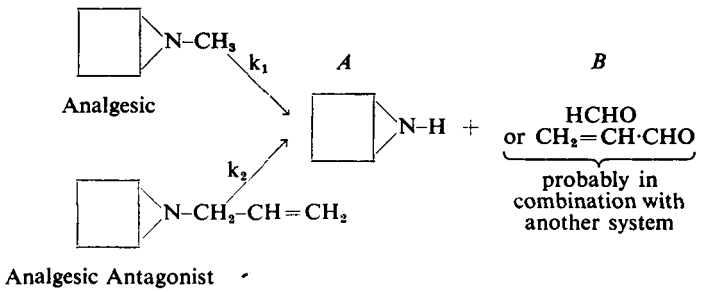
$$= 2270 \text{ cal.} = 2{\cdot}27 \text{ k. cal.}$$

Consequently, if the analgesic is present in the biophase in the vicinity of the receptor in 40 times the concentration of the antagonist, and the free energy change of BS formation is 2·27 k. cal. greater than that of AS formation, the two complexes would be formed in equal amounts. The difference of 2·27 k. cal. would be that expected for bonding involving an allyl as distinct from a methyl group since the former has a double bond in addition to 2 extra carbon atoms, and 5 carbon-carbon van der Waals' bonding forces would represent a free energy change of about 2·5 k. cal.[27].

If, as it seems possible, competition between the antagonist and the drug for the supposed analgesic receptor site is the major mechanism involved in reversal of analgesic action by an antagonist, the rapid character of the reversal requires consideration. It is possible that few analgesic receptor sites are available and that the reaction of AS to XS (2) and the utilisation of X is rapid; a relatively large excess of drug molecules possibly is necessary in the biophase to give continued receptor saturation. The antagonist molecules upon combination with the receptor could then be supposed to lead to an immediate antagonism of the analgesic response.

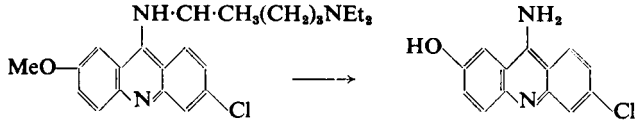
Consideration of the possible structure of X—the primary product of the reaction of an analgesic (and anti-analgesic) at the receptor site

If we assume that an analgesic and its antagonist analogue are adsorbed at the same receptor site, and our own observations support this concept, it may be useful to consider the possible nature of the first reactions involved. Since an anti-analgesic will antagonise a great variety of analgesics, it seems reasonable to assume that a common primary reaction step is involved for both. Because change in potency and change from an analgesic to an antagonist can occur by merely altering the *N*-alkyl group, it is now postulated that this group is involved in the primary reaction. This may be *N*-dealkylation by an oxidative mechanism resulting in small groups being removed more readily than larger ones. The reaction of an analgesic and its antagonist may be represented as follows, k_1 being much greater than k_2.



Either the dealkylated residue *A* or the alkyl fragment will be the essential intermediate involved in the sequence which produces analgesic activity. The latter possibility is precluded since some *nor*-compounds derived from active analgesics are not devoid of analgesic activity[28,29]. However, the low activity of the *nor*-compounds of morphine, pethidine and codeine, as compared with their parent molecule, does not invalidate the hypothesis that the *nor*-compounds are the essential intermediates. The assumption that the release of the *nor*-compounds at the receptor site in sufficient concentrations leads to an analgesic response does not imply that administration of these compounds by normal routes will give the same (or increased) effects. Differences will occur in the chemical reactivity, lipoid solubility and probably the membrane penetrating properties between the *nor*-compound and its parent molecule, e.g. *nor*-pethidine is extracted from a benzene solution of pethidine and *nor*-pethidine by a phosphate buffer solution[30].

Evidence is lacking concerning oxidative dealkylation by brain or central nervous system tissue but enzyme systems capable of effecting such reactions are known to be present in the body. Demethylation of a diverse range of compounds by animals and animal tissues has been demonstrated[31], e.g., demethylation of choline, monomethyl- and dimethyl aminoethanols (by dogs)[32]; monomethyl- and dimethylanilines (by rabbits)[33]; *N*-methyl and *NN*-dimethylsulphonamides (by man and mice)[34]; aminopyrine[35], ephedrine[36], methylamphetamine[37]. Evidence that dealkylations other than demethylations can take place is also available, e.g., the metabolism of phenacetin to *p*-aminophenol via phenetidine[38,39] and the de-ethylation of mepacrine[40]. The metabolism of mepacrine in man also demonstrates that large groups can be removed by dealkylation[41], the following change having been shown to occur:



Conversion of the removed alkyl groups to the corresponding aldehyde (methyl giving formaldehyde and ethyl, acetaldehyde) has been demonstrated in certain cases, e.g. ephedrine[42], amidopyrine[43] and monoethylaniline[44]. The dealkylation enzyme system, requiring both oxygen and reduced triphosphopyridine nucleotide, has been located in the microsomes of liver cells.

Bert and others[43] found that monomethyl-4-aminoantipyrine is more rapidly demethylated than the dimethyl analogue (amidopyrine) and showed that the size of the basic group has an influence on the ease of dealkylation (see Table I).

In analgesics, it has been shown using *N*-methyl-^{14}C labelled morphine, codeine, and pethidine, that *N*-demethylation occurs in rats and man[30,45–48]. The *nor*-compounds have not always been isolated although evidence confirming their presence has been reported. Burns and others[30] have isolated the demethylation products of pethidine, while

Plotnikoff and others[48], also using pethidine, established the presence of $^{14}CO_2$ in the expired air and identified *nor*-pethidine in the urine of rat and in man by counter current distribution. Brossi and others[49] recently reported that (+)-3-methoxyl-*N*-methylmorphinan was excreted as such, and in the form of three demethylated derivatives. On the other hand Shore and others[50] reported that neither of the isomers of 3-hydroxy-*N*-methylmorphinan are demethylated by intact animals or *in vitro* by a

TABLE I

DEALKYLATION OF VARIOUS AMINES

Substrate	4-Aminoantipyrine formed (μ moles)	Substrate dealkylated (per cent.)
Monomethyl-4-aminoantipyrine	1·93	36
Monoethyl-4-aminoantipyrine	0·60	11
Monobutyl-4-aminoantipyrine	0·56	9
Dimethyl-4-aminoantipyrine	0·62	11
Diethyl-4-aminoantipyrine	0·29	6
Dibutyl-4-aminoantipyrine	0·10	1

(5 μ moles of each alkylamine were incubated with liver homogenate. From Bert and others[48].)

demethylating enzyme present in liver. This last observation does not invalidate the present hypothesis because the very low concentration of the *nor*-compound, even after complete demethylation of the drug localised in the central nervous system, would pass undetected by the techniques adopted. The evidence provided by the work of Miller and Elliott[26] is apparently far more damaging to the present hypothesis. Using morphine-*N*-methyl-^{14}C, codeine-*N*-methyl-^{14}C and 2-^{14}C(±)-methadone, they determined the distribution of these analgesics in the central nervous system of the rat; peak levels correlated with pharmacological activity as measured by the pain reaction time method. Countercurrent distribution studies indicated that unaltered codeine and methadone (at least 90 per cent. unchanged) were present in the central nervous system 30 minutes after drug administration. The present hypothesis is therefore only tenable if relatively few of the analgesic molecules penetrating to the central nervous system are responsible for the biological response.

Recent investigations[51] have shown that the reduced, triphosphopyridine nucleotide dependent, microsomal enzyme system, which can demethylate morphine and other phenanthrene analogues—methadone and pethidine, is inhibited by certain *N*-substituted *nor*-morphines (the *N*-allyl and *N-iso*butyl derivatives exert the greatest action). The *N*-allyl compound has no effect on the enzymatic *N*-demethylation of cocaine, the side chain oxidation of hexobarbitone or the de-esterification of pethidine. It is of interest that *N*-allylnormorphine can itself be deallylated, but no evidence is available concerning the rate of deallylation in comparison with the rate of conversion of other *N*-alkyl*nor*morphines to *nor*-compounds.

The above hypothesis, that dealkylation is the primary step subsequent to adsorption of the drug upon the receptor site implies that, if the *nor*-compounds could be introduced directly into the biophase about the receptor, analgesic activity at least equal to that of the parent analgesics

would result. Investigations using morphine and *nor*-morphine were carried out in attempts to provide information on this point.

Pharmacological testing and results

Subcutaneous injections of morphine sulphate (2 mg./kg.) into rats gave much greater analgesic effects than those obtained using 50 mg./kg. doses of *nor*-morphine[52].

Solutions of the two compounds were injected intracisternally into mice and the degree of analgesia determined using a modification of the Singh Grewal method[53]. The results demonstrated that *nor*-morphine was rather more active than morphine at equal dose levels. Intravenous injection (mice) gave results indicating that *nor*-morphine had about 10 per cent. of the analgesic activity of morphine by this route; the onset of analgesic action using *nor*-morphine was preceded by short-lived convulsions in the animals.

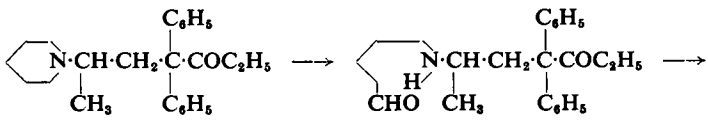
N-Allylnormorphine antagonised the analgesic action of intracisternally injected *nor*-morphine in doses comparable to those required to antagonise the action of morphine.

(The authors are grateful to Dr. M. F. Lockett and Mr. M. J. Davis for carrying out the above pharmacological testing which will be published elsewhere.)

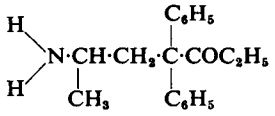
Mr. A. F. Green tells us that preliminary tests using the peristaltic reflex of isolated guinea-pig ileum show that *nor*-morphine has an inhibitory activity of about 10 per cent. that of morphine.

The greater activity of *nor*-morphine than morphine upon presentation of the drugs close to the analgesic receptors (intracisternally), in contrast to the negligible activity of *nor*-morphine upon subcutaneous injection, is consistent with the above hypothesis. The antagonism by *N*-allylnormorphine of the *nor*-morphine, as well as the morphine response, may be attributed to the former blocking the analgesic receptor so that although the essential metabolite X (*nor*-morphine) is available, it fails to be incorporated into the reaction sequence resulting in analgesia unless present upon the analgesic receptors.

The above discussion of oxidative dealkylation of analgesics has involved the consideration of compounds possessing one relatively small alkyl group attached to the *N*-atom (e.g., morphine and pethidine type compounds). The application of this hypothesis to methadone and thiambutene-type compounds possessing dialkylamino groups is self evident. (Methadone is demethylated by a reduced triphosphopyridine nucleotide dependent enzyme system[54].) Analogous compounds possessing piperidino, morpholino and pyrrolidino groups have high analgesic activity. It is presumed that ring opening and dealkylation occurs by an oxidative mechanism as shown below (I to III).



(I) (II)



(III)

Although it is suggested that increasing the size of the alkyl group attached to the nitrogen atom in morphine and pethidine-type compounds leads to anti-analgesic activity due to the greater difficulty of dealkylation, it is necessary to stress that the presence of electrical dipoles in the alkyl chain may affect the rate of dealkylation, e.g., the high activity of *N*-β-phenylethyl*nor*pethidine[55], despite its large alkyl group, may be attributed to this factor.

Summary

1. The mode of action of analgesic antagonists is considered in terms of competition with analgesics for the analgesic receptor surface.

2. The hypothesis is advanced that analgesics and their antagonists undergo a similar chemical reaction subsequent to adsorption, the rate constant for the former being very much greater than that for the latter.

3. Oxidative dealkylation to produce *nor*-compounds is presumed to be the first step in the reaction sequence leading to analgesia.

4. *Nor*-morphine has been shown to have a greater analgesic activity than morphine upon intracisternal injection into mice.

The authors wish to express their thanks to Mr. A. F. Green for supplying the *nor*-morphine and to Dr. M. F. Lockett and Mr. M. J. Davis for carrying out the pharmacological testing.

References

1. Beckett, *J. Pharm. Pharmacol.*, 1956, **8**, 848.
2. Beckett, Casy, Harper and Phillips, *ibid.*, 1956, **8**, 860.
3. Clarke, Pessolano, Weijlard and Pfister, 3rd *J. Amer. chem. Soc.*, 1953, **75**, 4963.
4. Green, Ruffell and Walton, *J. Pharm. Pharmacol.*, 1954, **6**, 390.
5. Braenden, Eddy and Halbach, *Bull. World Hlth. Org.*, 1955, **13**, 937.
6. Lasagna, *Arch, intern. Med.*, 1954, **94**, 532.
7. Seibert and Huggins, *Proc. Soc. exp. Biol., N.Y.*, 1953, **82**, 518.
8. Landmesser, Cobb and Converse, *Anesthesiology*, 1953, **14**, 535.
9. Beckett and Casy, *J. Pharm. Pharmacol.*, 1954, **6**, 986.
10. Fromherz and Pellmont, *Experientia*, 1952, **8**, 394.
11. Unna, *J. Pharmacol.*, 1943, **79**, 27.
12. Ovahovats, Winter and Lehman, *ibid.*, 1953, **109**, 413.
13. Winter, Ovahovats, Flataker, Lehman and Lehman, *ibid.*, 1954, **111**, 152.
14. Eckenhoff, Elder and King, *Amer. J. med. Sci.*, 1952, **223**, 191.
15. Benson, O'Gara and van Winkle, *J. Pharmacol.*, 1952, **106**, 373.
16. Bornstein, Yorburg and Johnson, *J. Amer. med. Ass.*, 1953, **151**, 908.
17. Huggins, Glass and Bryan, *Proc. Soc. exp. Biol., N.Y.*, 1950, **75**, 540.
18. Smith, Lehman and Gilfillan, *Fed. Proc.*, 1951, **10**, 335.
19. Wilker, Carter, Fraser and Isbell, *ibid.*, 1952, **11**, 402.
20. Irwin and Seevers, *J. Pharmacol.*, 1952, **106**, 397.
21. Eddy (cited by Lasagna, ref. 6).
22. Beecher and Lasagna, *J. Pharmacol.*, 1954, **113**, 4.
23. Seevers and Woods, *Amer. J. Med.*, 1953, **14**, 546.
24. Kolb and Du Mez, *Publ. Hlth. Rep., Wash.*, 1931, **46**, 698.
25. Miller, Gilfoil and Shideman, *J. Pharmacol.*, 1955, **115**, 350.
26. Miller and Elliott, *ibid.*, 1955, **113**, 283.

27. Albert, *Selective Toxicity*, Methuen and Co. Ltd., London, 1951, pp. 25–26.
28. Miller and Anderson, *J. Pharmacol.*, 1954, **112**, 191.
29. Macdonald, Woolfe, Bergel, Morrison and Rinderknecht, *Brit. J. Pharmacol.*, 1946, **1**, 4.
30. Burns, Berger, Lief, Wollack, Papper and Brodie, *J. Pharmacol.*, 1955, **114**, 289.
31. Challenger, *Chem. Rev.*, 1945, **36**, 315.
32. Fuchs, *Z. Biol.*, 1938, **98**, 473.
33. Horn, *Z. Physiol. Chem.*, 1936, **242**, 23.
34. Lewis and Tager, *Yale J. Biol. Med.*, 1940, **13**, 111.
35. Brodie and Axelrod, *J. Pharmacol.*, 1950, **99**, 171.
36. Axelrod, *ibid.*, 1953, **109**, 62.
37. Axelrod, *ibid.*, 1954, **110**, 315.
38. Muller, *Therm. Mh.*, 1882, **2**, 355.
39. Mahnert, *Dtsch. med. Wschr.*, 1888, **14**, 1027.
40. Taggart (unpublished, see ref. 43).
41. Sexton, *Chemical Constitution and Biological Activity*, Spon Ltd., London, 1953, p. 86.
42. Axelrod, *J. Pharmacol.*, 1955, **114**, 430.
43. Bert, La Due, Gaudette, Trousof and Brodie, *J. biol. Chem.*, 1955, **214**, 741.
44. Brodie, *J. Pharm. Pharmacol.*, 1956, **8**, 1.
45. Elliott, Tolbert, Adler and Anderson, *Proc. Soc. exp. Biol., N.Y.*, 1954, **85**, 77.
46. Adler, *J. Pharmacol.*, 1952, **106**, 371.
47. March and Elliott, *Fed. Proc.*, 1952, **11**, 373.
48. Plotnikoff, Elliott and Way, *J. Pharmacol.*, 1952, **104**, 377.
49. Brossi, Halfinger and Schnider, *Arzneimit.-Forsch.*, 1955, **5**, 62.
50. Shore, Axelrod, Adrian, Hagben and Brodie, *J. Pharmacol.*, 1955, **113**, 192.
51. Axelrod and Cochin, *Fed. Proc.*, 1956, **15**, 395.
52. Green (private communication).
53. Singh Grewal, *Brit. J. Pharmacol.*, 1952, **7**, 433.
54. Axelrod (unpublished, see ref. 42).
55. Perrine and Eddy, *J. org. Chem.*, 1956, **21**, 125.

DISCUSSION

The papers were presented by DR. A. H. BECKETT.

DR. A. MCCOUBREY (London) said that pKa is of undoubted importance in all basic drugs, but he felt that attempts to relate pKa to analgesic activity would be unlikely to succeed, especially should the activity be mediated by a degradation product. Correlation could be expected only of the active species in either the ionised or unionised molecule provided factors such as excretion and detoxication were controllable or negligible. Attempts to extrapolate findings with nonspecific enzymes in liver to the more specific functions of nervous tissue may be misleading. He was glad that another worker was considering metabolic activation in the analgesic group. He could not, however, see much fundamental difference between morphine and normorphine, though one remembered the curious dissociation of properties in the sympathins. Incidentally adrenaline has been stated to be more effective as an analgesic than noradrenaline by the intracisternal route. He felt doubtful of the validity of analgesic assay figures derived from animals that had recently suffered convulsive seizures. He realised, of course, that Dr. Beckett had not been actively concerned here. The drug SKF–525A has been stated to prevent the demethylation process or any other detoxication process in liver, at the same time increasing the action of various drugs, though he was not sure whether figures have been quoted for pethidine or morphine.

DR. J. B. STENLAKE (Glasgow) said he was critical of the use of infra-red measurements to supplement the conclusions drawn from dissociation constant measurements. The authors were concerned finally with the conformation of the structure in aqueous solution. While infra-red measurements would be made in non-aqueous media, he was therefore critical of their value as supporting evidence, although he agreed with the postulated transannular effects. He suggested that the author should take some pK measurements in a mixed solution, because it had been shown that there was a shift of pK values as a concentration of non-aqueous solvent was brought down to water, and the direction of shift of pK was a measure or an indication of whether ionisation of the proton was away from nitrogen or oxygen. Measurements of that type would, he suggested, provide much more satisfactory confirmatory evidence than infra-red for annular structure in methadone and related compounds. He had looked in the papers for the dissociation constant of normorphine but had been unable to find it. His own guess was that normorphine being a secondary base would in fact be a stronger base than morphine, and that meant that in all probability there would be much lower penetration to the surface receptor site. That would explain the difference in results which had been obtained by intracisternal and subcutaneous methods of testing normorphine.

DR. A. H. BECKETT, in reply, said while there was a danger in extrapolating results using liver when considering central nervous tissue, nevertheless, the enzyme system which was involved in demethylation of *N*-methyl compounds was also present in central nervous tissue. The important fact had to be considered that when the morphine was converted to form *N*-ethyl, *N*-propyl and *N*-allyl-normorphine, there was a change from active analgesic into an analgesic antagonist, yet the analgesic antagonist had some analgesic activity in itself. Therefore, it seemed reasonable to postulate that some reaction involving the alkyl group was implicated in the action. It was significant that since the paper was presented, work had appeared by Brodie and his colleagues in which dealkylation of analgesics had been carried out and this had been antagonised by *N*-allylnormorphine. He agreed that dissociation constants in various mixed solvents were required and that for the conformation of the molecules infra-red measurements cannot be used as a complete argument because these cannot be made in aqueous conditions. The authors were submitting that it was reasonable to believe that that dissociation constant measurements indicated the conformations existing in aqueous conditions and that the infra-red measurements showed that such conformations existed. He had no figures for the dissociation constant of normorphine. He agreed that penetration would be an important factor, but he also suggested that conjugation involving the free hydrogen on the nitrogen atom would also be important.