Brit. J. Pharmacol. (1961), 17, 433–441.

SIMILARITY BETWEEN RECEPTORS RESPONSIBLE FOR THE PRODUCTION OF ANALGESIA AND LENTICULAR OPACITY

BY

MARTA WEINSTOCK

From the Department of Pharmacology, St. Mary's Hospital Medical School, London

(*Received August* 14, 1961)

Evidence is presented that the receptors responsible for the mediation of analgesia by morphine-like drugs are similar to those which are involved in the production of a reversible lenticular opacity. The activity of a number of compounds in mice on the lens was closely correlated with analgesic potency in this species. Stereospecificity for isomers with D configuration was demonstrated for both effects. Nalorphine only antagonized the lenticular opacity activity of those drugs the analgesic action of which it abolished.

The appearance of reversible lenticular opacity in rodents by the acute administration of analgesic drugs has been reported by Weinstock, Stewart & Butterworth (1958) and by Weinstock & Stewart (1961). It is generally accepted that these drugs combine with specific receptors in the central nervous system in order to elicit an analgesic response. The evidence for the existence of such receptors is based on the structural requirements for potency (Braendon, Eddy & Halbach, 1955), the importance of stereochemical configuration (Beckett & Casy, 1954; Beckett, Casy & Harper, 1956), and the specific antagonism by nalorphine. As preliminary experiments had indicated that the formation of an opacity was confined to analgesic drugs of the morphine type, a study was undertaken to determine whether the receptors involved in these two actions of morphine-like drugs were similar.

METHODS

Male and female mice of a Pirbright (Albino) strain, weighing 18 to 22 g, were used.

Analgesic activity

The modification of the hot-plate method of Eddy & Leimbach (1953), described by Janssen & Jageneau (1957), was used for measuring analgesic activity.

The reaction times of mice were determined twice before, and 15, 30, 45, 60, 90, 120, and 180 min after subcutaneous injection of the drug. All animals whose mean initial reaction-time exceeded 10 sec, or in which the two readings differed by more than 100%, were discarded. An analysis of the average of the two reaction times (measured to 0.2 sec) of some 2,300 mice, estimated before injection, showed that there was no significant difference between the readings of male and female mice, the mean reaction time of males being 6.0 ± 0.2 sec and that of females 6.0 ± 0.2 sec.

A response was considered positive if the reading after injection was greater than 30 sec, or if it exceeded the initial mean reading by a factor of three or more. The number of such positive responses was noted at each of the various times. The largest number of positive

responses at each dose of each analgesic drug was converted to a percentage and used in the plotting of a dose-response curve from which the ED50 was calculated.

Opacity-producing activity

Mice were placed in containers in groups of 10 and examined at 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after subcutaneous injection of the drug. The number of mice showing any degree of opacity at each of the above times was determined.

All drugs were administered as aqueous solutions of their salts, in a constant volume of 1.0 ml./100 g. The compounds listed in Table 2 were injected subcutaneously in doses ranging from 1 to 20 mg for the opacity tests, and from 0.1 to 10 mg for the measurement of analgesic activity, in increments of 0.2 or of 2 mg until an effect was obtained. If no opacity was produced in doses below 20 mg, and this quantity was not lethal, larger doses were given until 50% mortality occurred.

The ED50 values for opacity and analgesia for all active drugs were determined at the time of maximum activity. At least three doses of each compound were administered. A minimum of 30 mice was used for each dose. The ED50 values and their fiducial limits of error were computed by the method of probit analysis.

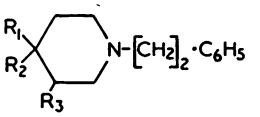
All doses are expressed in mg/100 g body weight.

Structure–activity relationships

A new series of compounds, well suited to the study of structure–activity relationships, was made available for our use by Dr A. H. Beckett, the structures of which are shown in Table 1.

TABLE 1

STRUCTURES OF SOME DERIVATIVES OF 1-PHENETHYLPIPERIDINE



Series and number	R_1	R_2	R_3
A1	C_6H_5–	–OH	–H
A2	*o*–$C_2H_5.C_6H_4$–	–OH	–H
A3	*o*–$CH_3.C_6H_4$–	–OH	–CH_3
A4	*p*–$CH_3.C_6H_4$–	–OH	–CH_3
A5	CH:C–	–OH	–H
A6	N:C–	–OH	–H
E1	C_6H_5–	–O.CO.CH_3	–H
E2	*o*–$CH_3.C_6H_4$–	–O.CO.CH_3	–CH_3
E3	*o*–$CH_3O.C_6H_4$–	–O.CO.CH_3	–CH_3
E4	*m*–$CH_3.C_6H_4$–	–O.CO.CH_3	–CH_3
E5	*p*–$CH_3.C_6H_4$–	–O.CO.CH_3	–CH_3
E6	*o*–$CH_3.C_6H_4$–	–O.CO.C_2H_5	–CH_3
E7	*m*–$CH_3.C_6H_4$–	–O.CO.C_2H_5	–CH_3

RESULTS

The ED50 values for analgesic and opacity-producing activity of a number of established drugs, as well as those of the new A and E series, are shown in Table 2, with the fiducial limits of error and slopes of regression lines expressed in mg/100 g of the free base. The LD50 of most is given for comparison.

The results show that all compounds which produced an opacity had analgesic activity. However, apomorphine and A4 had no effect on the lens although they had apparent analgesic activity. As the ED50 for opacity was usually about five

TABLE 2

A COMPARISON OF THE ED50 VALUES FOR ANALGESIA AND OPACITY OF A NUMBER OF COMPOUNDS

All doses are in mg/100 g free base. The numerals in parentheses indicate the % effect obtained by the given dose when it was not possible to obtain an ED50. With some substances no analgesia and no lens opacity activity was found even with the quantities designated (−)25 and (−)50 as with nalorphine

Compound	ED50 analgesia	Fiducial limits %	Slope "b"	ED50 opacity	Fiducial limits %	Slope "b"	LD50
Morphine	0·43	80·4–121	1·58	3·06	87·5–114	1·72	39·6
Codeine	3·73	70·8–131	1·52	14 (30%)	—	—	14·2
Dihydrocodeine	2·84	79·6–121·0	1·87	12 (40%)	—	—	19·4
Diamorphine	0·174	75–126	1·58	0·37	82–118·8	3·13	22·8
Normorphine	5·79	67–121·7	4·69	11 (30%)	—	—	10·4
Nalorphine	(−)25	—	—	(−)50	—	—	51·2
Levorphanol	0·170	80·3–121·2	1·84	0·34	82·6–117·8	3·4	10·7
Levomethorphan	0·58	77·5–129·6	1·87	2·50	79·4–121·0	2·94	7·83
Levallorphan	(−)20	—	—	(−)25	—	—	—
Apomorphine	3·01	75·8–132·2	3·46	(−)10	—	—	14·9
Papaverine	7·71	92–108·4	10·87	15·3	87–114·6	5·76	20·3
Pethidine	1·84	77·5–129·6	2·07	3·77	81·6–118·4	3·22	20·8
(±)-Methadone	0·40	86·8–114·9	2·61	0·80	89·5–111·7	3·54	3·56
Dextromoramide	0·078	75·2–126·8	2·1	0·189	78–129	3·01	14·0
E1	0·213	79·6–121·2	1·8	0·910	83·8–114	2·28	—
E2	0·080	89·6–111·7	1·95	0·443	90–109·2	3·24	20·9
E3	0·272	69·2–132·8	2·0	0·564	69·6–132	3·01	—
E4	0·577	74·6–128	1·7	2·78	87·2–114·2	3·99	—
E5	0·702	77·5–129	1·91	3·34	80·3–121	3·07	—
E6	0·400	80·2–121	1·27	1·184	85·5–117·2	2·82	—
E7	2·65	75·2–126·8	2·1	6·29	80·6–121·2	3·10	—
A1	(−)10	—		(−)10	—	—	15·0
A2	(−)5·0	—		(−)7·5	—	—	8·6
A3	1·45	89–113	4·43	8·2	88·3–113·5	6·98	9·46
A4	1·175	64–137	4·9	(−)5·0	—	—	6·0
A5	(−)15·0	—		(−)20·0	—	—	37·5
A6	2·5	68·5–131·8		3·0 (25%)	—	—	6·3

times that for analgesia, it was possible that an opacity was not seen with these two compounds because the dose necessary was above the LD50.

With the derivatives of morphine, including the morphinan series, activities in two tests for each effect were similarly related. Changes in potency which accompanied alterations in chemical structure in most of the 1-phenethyl-piperidine derivatives (A and E series) were reflected similarly in the two activities. As in the morphine series, and in many other synthetic analgesic drugs, esterification of the alcoholic hydroxyl group markedly increased activity. The position of the methyl substituent in the 4-phenyl ring appears to be of importance, producing the most active member when it is in the *ortho* position (E2). The acetoxy derivatives were more potent than the corresponding propionyloxy derivatives.

The relative activities of the various groups of compounds in the two tests are shown in Table 3, expressed as a ratio of the two ED50 values. The drugs are also listed in order of their efficiency in producing opacity and analgesia.

With the exception of that of morphine, the ratios of activity in the two tests fall into two distinct groups, with means of 2.13 ± 0.13 and 4.84 ± 0.45, respectively.

The relatively high ratio of the two ED50 values of morphine appears to be due to the fact that the slope of the regression line for its activity on the eye is

TABLE 3
RATIOS OF ACTIVITY OF COMPOUNDS ON THE EYE TO THAT OF ANALGESIC ACTIVITY

Compound	ED50 opacity/ ED50 analgesia	Order of activity	
		Analgesia	Opacity
Levorphanol	2·0	3	2
Papaverine	2·0	20	>17
(±)-Methadone	2·0	7	6
Pethidine	2·05	14	13
E3	2·08	6	5
Diamorphine	2·12	4	3
E7	2·38	16	15
Dextromoramide	2·40	1	1
A6	>1·5	15	14
Normorphine	>1·9	19	>17
Levomethorphan	4·3	11	9
E1	4·27	5	7
E6	4·62	8	8
E5	4·75	12	12
E4	4·8	10	10
E2	5·5	2	4
A3	5·65	13	16
Codeine	>3·75	18	>17
Dihydrocodeine	>4·2	17	>17
Morphine	7·1	9	11

much less than those of other drugs. The dose-response line for analgesic activity, however, does not so differ in slope. The typical regression lines obtained in the opacity test and those for analgesia are shown for a number of compounds in Figs. 1 and 2.

It had been noted that the time of onset of the opacity and that of maximum activity occur much later with morphine than for all other drugs. With most compounds the peak activity is reached 30 to 40 min after subcutaneous injection but is not seen until 75 to 90 min with morphine, particularly when larger doses are given. This may explain the shallow regression line seen with this drug.

At present there is no obvious explanation of why the remaining drugs should fall into either one of the two distinct groups. The route of administration does not appear to make any difference to the ratios of the ED50 values for the two actions. The analgesic activity and opacity-producing potencies of methadone, pethidine and morphine were determined when these drugs were given intraperitoneally and by mouth in addition to the subcutaneous route. The ratios of the ED50 values in the two tests are shown in Table 4.

It appears that the time of peak activity may influence the ratio to some extent, since the ratios for morphine were slightly reduced when maximum activity occurred earlier.

Stereochemical considerations

In all instances in which a compound had been resolved into optical enantiomorphs, on testing one was much more active than the other of each pair, and often the whole of the analgesic activity of the racemic mixture resided in one of the isomers (Beckett *et al.*, 1954). If it could be demonstrated that in a pair of optical

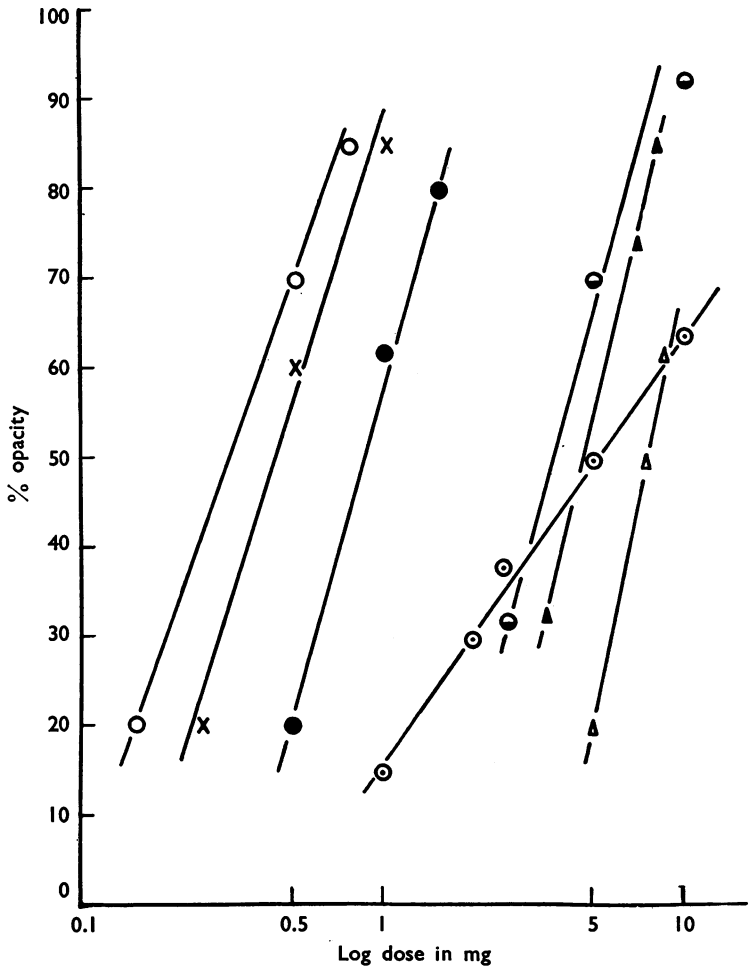


Fig. 1. Relation of opacity-producing activity to dose. Abscissa: log dose in mg/100 g body weight. Ordinate: number of mice showing opacity at a given time expressed as a percentage. ○ — ○ E2; X—X diamorphine; ● — ● methadone; ⊙—⊙ morphine; ◒ E7; △ — △ A2; ▲ — ▲ pethidine.

enantiomorphs the isomer with greater analgesic potency was also the more active in producing an opacity, then the receptor for lens opacity is probably similar to that involved in the mediation of analgesia.

Owing to the great difficulty in achieving a pure chemical separation of racemates into their optical constituents, it is almost impossible to obtain isomers in sufficient quantity to carry out adequate pharmacological tests. However, the ED50 values for analgesic and opacity-producing activity were determined for a number of isomeric pairs (Table 5).

The L(+)-isomers, dextrorphan and dextromethorphan, were completely devoid of either activity in the amounts used. With all other pairs, the more potent analgesic

drug was found to be the more effective one in producing an opacity. Furthermore, the ratios for the activity of one isomer to that of the other were constant for the two actions.

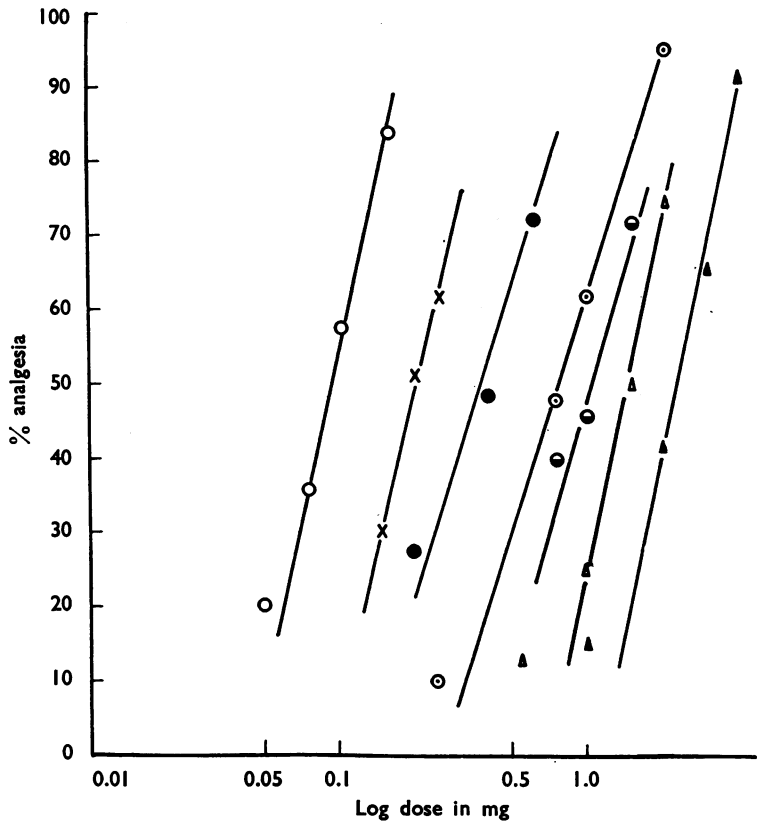


Fig. 2. Relation of analgesic activity to dose. Abscissa: log dose in mg/100 g body weight. Ordinate: Analgesic activity expressed as a percentage. O—O E2; X—X diamorphine; ●—● methadone; ⊙—⊙ morphine; ◒—◒ E7; △—△ A2; ▲—▲ pethidine.

TABLE 4

EFFECT OF ROUTE OF ADMINISTRATION ON ACTIVITY RATIOS AND TIME OF ONSET OF OPACITY

Drug	Route	ED50 opacity/ ED50 analgesia	Opacity. Time of max. activity
Morphine	s/c	7·2	75–90 min
	i/p	6·1	60
	o/r	5·8	45–75
Methadone	s/c	2·0	30–40
	i/p	3·2	30–40
	o/r	1·8	20–45
Pethidine	s/c	2·05	30
	i/p	2·3	20–30
	o/r	2·0	30

TABLE 5

COMPARISON OF THE ACTIVITY OF PAIRS OF OPTICAL ISOMERS

Drug	Optical rotation	Configuration	Analgesia (mg/100 g)	Opacity (mg/100 g)	Activity ratios: D:L analgesia	Activity ratios: D:L opacity
(−)-Methadone	−	D	0·09	0·2	1 : 22	1 : 25
(+)-Methadone	+	L	2·0	5·0		
Levomethorphan	−	D	0·76	3·3	—	—
Dextromethorphan	+	L	>10·0	>10·0		
Levorphanol	−	D	0·30	0·6	—	—
Dextrorphan	+	L	>10·0	>10·0		
Dextromoramide	+	D	0·087	0·29	1 : 98	1 : 90
Levomoramide	−	L	8·5	26·0		

Nalorphine antagonism

Nalorphine is generally believed to reduce or prevent the analgesic action of morphine-like drugs by competing successfully for the receptor involved (Lasagna, 1954 ; Seibert & Huggins, 1953 ; Landmesser, Cobb & Converse, 1953).

Doses of the active drug were chosen that gave positive effects in both the opacity and hot-plate test, in approximately 70% of the animals. Even though nalorphine can antagonize many times its own weight of analgesic drug (Orahovats, Winter & Lehman, 1954), similar weights of nalorphine and the analgesic agent were given to ensure that any antagonism would be detected. The active drugs were injected subcutaneously immediately after nalorphine. The results in Table 6 show that nalorphine only prevented the production of an opacity by those compounds whose analgesic action it abolished.

TABLE 6

EFFECT OF NALORPHINE ON OPACITY AND ANALGESIA

(+) Complete antagonism. (−) No reduction of effect. (0) Action absent, therefore not tested

Drug	Antagonism by nalorphine: Analgesia	Antagonism by nalorphine: Opacity
Morphine	+	+
Codeine	+	+
Dimorphine	+	+
Dihydrocodeine	+	+
Normorphine	+	+
Levorphanol	+	+
Levomethorphan	+	+
Pethidine	+	+
E1	+	+
E2	+	+
E3	+	+
E4	+	+
E5	+	+
E6	+	+
E7	+	+
Papaverine	−	−
Apomorphine	−	0
(−)-Methadone	+	+
(+)-Methadone	−	−
Dextromoramide	+	+
Levomoramide	−	−
A3	−	−
A6	−	−
A4	−	0

DISCUSSION

These results show that the production of a reversible lenticular opacity is a characteristic action of analgesic drugs of the morphine type. Of the compounds tested, only those which produced an opacity had analgesic activity. It would be of great interest to know whether this relation will continue to be true when more new analgesic substances of widely differing chemical constitution are synthesized.

The hot-plate test, as a method of assessment of analgesia, does not distinguish specific analgesic drugs, such as morphine and pethidine, from several other central nervous depressants. For example, chlorpromazine was more active than methadone in the hot-plate test, but it is devoid of action on the lens (unpublished observation). Thus, although apomorphine and A4 showed some analgesic potency in the hot-plate test and had no opacity-producing activity, the failure of nalorphine to antagonize the effect of these drugs indicates that such analgesia as was found probably resulted from a depressant action at some other site (or sites) in the central nervous system and not through a combination with the specific analgesic receptor. However, papaverine, A3 and A6 did have some effect on the lens, but the doses required were in the lethal range, and neither the opacity nor the analgesia were antagonized by nalorphine. It is thus also clear that these compounds could hardly be producing either action by combining with the specific analgesic receptor.

It is therefore suggested that this action on the lens can be used as the basis of a new method of screening potential analgesic drugs. This test has several advantages over existing screening procedures. No special apparatus is required and there is no dependence upon the subjective response of a small animal to a supposedly painful stimulus, the response to which can be very variable and often difficult to assess. Unlike methods which involve the measurement of reaction time, this test can distinguish between the morphine-like analgesics and other central depressant drugs. Fewer animals are needed for an accurate assessment of potency, and the slopes of a quantal assay procedure are significantly greater in the opacity test than in the hot-plate method (see Table 2).

The results of the opacity test in mice permit selection of compounds for study by other, more specific tests. If an opacity is produced, antagonism by nalorphine should next be ascertained. This will give further evidence that the compound is active similarly to morphine and its congeners.

Many workers have directed their efforts to finding a compound the analgesic activity of which is at least equal to that of pethidine, but which is non-addictive. So far, all of the known potent analgesic drugs whose actions are qualitatively similar to those of morphine have been found to be addictive. Also, in each case, characteristic antagonism was exhibited by nalorphine. It is therefore very likely that, once nalorphine antagonism of the opacity has been demonstrated, further investigation would fail to yield a non-addictive analgesic drug.

The similarity of the receptors responsible for the formation of the opacity to those involved in the mediation of an analgesic response is strikingly confirmed by the difference in activity found with optical isomers. This is further emphasized by the finding that the ratios of potency of one isomer to its optical enantiomorph

were the same for each action. In addition, these findings show that the active substance which combines with the receptor must be either the unchanged drug or a metabolite whose configuration is identical to that of the compound from which it is derived.

Although many theories concerning the mode of action of analgesic drugs stress the part played by specific receptors, their exact location remains obscure. On the other hand, the effect on the lens provides a readily accessible site for the study of the interaction between morphine-like drugs and their receptors.

The author wishes to thank Drs A. H. Beckett, P. A. J. Janssen and Mr A. F. Green for supplying the compounds for this investigation, and Dr H. C. Stewart for his helpful advice. This work was carried out during the tenure of an Aspro-Nicholas Research Fellowship.

REFERENCES

BECKETT, A. H. & CASY, A. F. (1954). Synthetic analgesics: stereochemical considerations. *J. Pharm. (Lond.)*, **6**, 986–999.

BECKETT, A. H., CASY, A. F. & HARPER, N. J. (1956). Analgesics and their antagonists: some steric and chemical considerations. Part III. *J. Pharm. (Lond.)*, **8**, 874–883.

BRAENDON, O. J., EDDY, N. B. & HALBACH, H. (1955). Synthetic substances with morphine-like effect. Relationship between chemical structure and analgesic action. *Bull. Wld Hlth Org.*, **13**, 937–998.

EDDY, N. B. & LEIMBACH, D. (1953). Synthetic analgesics. II, Dithienylbutenyl- and dithienylbutylamines. *J. Pharmacol. exp. Ther.*, **107**, 385–393.

JANSSEN, P. A. J. & JAGENEAU (1957). A new series of potent analgesics: dextro 2 : 2 diphenyl-3-methyl-4-morpholinobutyrylpyrrolidone and related amides. *J. Pharm. (Lond.)*, **9**, 381.

LANDMESSER, C. M., COBB, S. & CONVERSE, J. G. (1953). Effects of N-allylnormorphine upon the respiratory depression due to morphine in anaesthetised man with studies on the respiratory response to carbon dioxide. *Anaesthesiology*, **14**, 535–549.

LASAGNA, L. (1954). Nalorphine. Practical and theoretical considerations. *A.M.A. Arch. int. Med.*, **94**, 532–558.

ORAHOVATS, P. D., WINTER, C. A. & LEHMAN, E. G. (1954). Pharmacological studies of mixtures of narcotics and N-allylnormorphine. *J. Pharmacol. exp. Ther.*, **112**, 246–251.

SEIBERT, R. A. & HUGGINS, R. A. (1953). Conjugation of N-allylnormorphine by liver slices. *Proc. Soc. exp. Biol., N.Y.*, **82**, 518–519.

WEINSTOCK, M. & STEWART, H. C. (1961). Occurrence in rodents of reversible drug-induced opacities of the lens. *Brit. J. Ophthal.*, **45**, 408–414.

WEINSTOCK, M., STEWART, H. C. & BUTTERWORTH, K. R. (1958). Lenticular effect in mice of some morphine-like drugs. *Nature (Lond.)*, **182**, 1519–1520.