Brit. J. Pharmacol. (1965), **24**, 789–797.

STUDIES ON ANTAGONISM OF MORPHINE MIOSIS BY NALORPHINE AS A DIAGNOSTIC TEST FOR NARCOTIC USAGE

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(*Received October* 6, 1964)

The effectiveness of nalorphine as a specific antidote against morphine overdosage is well established. So selective is this effect that, should nalorphine fail to antagonize deep respiratory depression that is known to be drug-induced, one can conclude that poisoning must have resulted from an agent unrelated to morphine or its surrogates. The usefulness of nalorphine as a diagnostic agent, however, finds greater application in chronic rather than acute morphine poisoning.

In 1953, Wikler, Fraser & Isbell suggested that nalorphine could be used as a reliable test for addiction to narcotics. They had noted that in patients tolerant to heroin, morphine or methadone, a single dose of nalorphine precipitated severe abstinence-like signs including mydriasis. However, the test as recommended is useful only for diagnosis of addicts with a relatively strong physical dependence on morphine or its related substitutes. In an attempt to apply the test on a known addict, it was noted that a small dose produced marked mydriasis and this occurred in the absence of other withdrawal signs (Terry & Braumoeller, 1956). On the other hand, when nalorphine was injected in non-addicts miosis resulted. These observations were studied further and as a result a standardized procedure was evolved which is used fairly extensively in the United States, Hong Kong and Singapore for detecting narcotic users. The popularity of the test may be attributed principally to its ease in application.

The pupil of a subject is measured under fixed lighting conditions, after which nalorphine (3 mg) is injected subcutaneously. After 30 min the pupil is again measured. Dilation of the pupil of 0.5 mm or more is considered to be an indication of narcotic use; pupillary constriction would indicate a negative result.

While the test is rapid and simple to perform, relatively little information is available with respect to its reliability and limitations. An assessment of the methods for measuring pupil size by the card pupillometer and other procedures has been made by Elliott & Way (1961). A comparison has been made of the results obtained in the field on narcotic addicts on parole with those obtained by chemical analysis of urine (Way, Elliott & Nomof, 1963). More recently the limitations of the test were further defined with respect to dose and frequency of narcotic administration (Elliott, Nomof, Parker, Dewey & Way, 1964).

These studies indicate that the nalorphine pupil-test is a useful screening procedure for the detection of narcotic usage. However, there is still much to be learned and it is not always possible to derive such information from addicts. Should the test prove to be applicable in laboratory animals, it would greatly facilitate defining its limitations. Hence we are reporting studies on the rabbit eye with respect to possible conditions which might alter the character of the test and which have not been reported for humans.

METHODS

Twenty-four adult albino rabbits between 1.7 and 3.2 kg in body weight were used in rotation to assess the effect of various drug treatments on changes in pupillary size. A rest period of at least 48 hr was allowed between the tests with different drugs. The experiments were conducted in a dark room, the sole source of light being a 60-W electric bulb at a distance of about 75 cm from the heads of the rabbits. The pupil size was measured with a pupillometer, which is a white card printed with a graduated vertical series of black dots with diameters ranging from 0.5 to 7.0 mm in 0.5-mm increments. The pupil was observed continuously for 1 min after retracting the eyelid and the minimal diameter size noted over this period was taken as the end-point.

Optimal conditions for determining effects of morphine and nalorphine on pupil size were determined empirically before assessing the influence of other drugs. The dose selected for morphine was 15 mg/kg of the sulphate injected intravenously into a marginal ear vein; the dose for nalorphine was 3 mg/kg of the hydrobromide injected intravenously. Antagonism of morphine miosis by nalorphine was always carried out 1 hr after the morphine injection and the influence of other drugs on this response was studied with six animals per drug. The dose employed for each of the other drugs was arbitrary and was selected on the basis of adequacy in producing known pharmacological effects.

The solutions of drugs were: morphine sulphate (1%), nalorphine hydrobromide (0.1%), meprobamate (8%), homatropine hydrobromide (2%), physostigmine salicylate (0.5%), reserpine (0.02%; dissolved in a 1 : 1 : 2 mixture of ethyl alcohol, propylene glycol and water), chlorpromazine hydrochloride (0.25%) and amphetamine sulphate (1%). These were administered at fixed time intervals between the morphine and nalorphine injections. Measurements of pupil size were made before morphine administration, 1 hr after morphine (−60 min), at 10- to 20-min intervals up to the time of nalorphine administration (0 min), and at 1, 5, 10, 20 and 40 min after nalorphine.

The assessment of pupillary changes to nalorphine was also made in rabbits chronically treated with morphine. The animals were given morphine sulphate (15 mg/kg) twice daily by subcutaneous injection for 3 weeks. Subjectively, this treatment appeared to produce a considerable degree of tolerance to morphine in the animals with respect to sedative and respiratory actions but not to its miotic effects. On the day of the test the regular subcutaneous administration of morphine was withheld and an intravenous dose of the drug (15 mg/kg) was substituted. At 1, 4, 8, 16 and 32 hr after this injection, nalorphine (3 mg/kg) was injected intravenously and pupil size was measured in the usual manner 5, 10, 20 and 40 min after the nalorphine. Six animals were used at each time interval.

At the end of the above tests, the routine daily injections of morphine were continued over a period of 1 to 3 weeks and the animals were used to assess the effect of the same drugs used previously in normal animals. As described above, an intravenous dose of morphine was substituted for the subcutaneous one on the day of the test; the effect of morphine by this route on the pupil response to nalorphine was identical to that in the previous experiments on control rabbits.

RESULTS

Effect of morphine on the pupil size of normal rabbits

Miosis occurred in twenty-three out of twenty-four rabbits given morphine sulphate (15 mg/kg, intravenously). One rabbit showed no change in pupillary size. A plot of the mean change in pupillary diameter of the twenty-four rabbits against time is shown in

Fig. 1. While maximal miosis usually occurred at about 20 min, in several animals the change was apparent within 5 min. The maximal diminution of pupillary diameter in each animal varied from −0.5 to −3.5 mm and the mean decrease was −1.7 mm. In more than two-thirds of the rabbits, definite miosis was still present 10 to 11 hr after the injection. At the end of 21 hr, nearly one-half of the rabbits had a slight mydriasis, three still had a slight miosis, and in the rest the pupils had reverted to their original sizes.

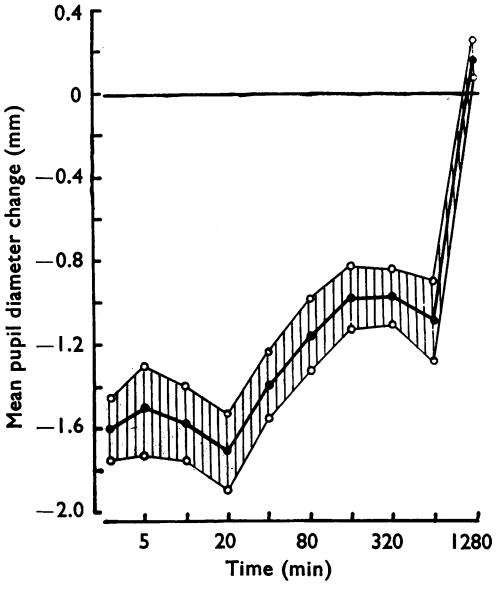

Fig. 1. Time/response curve of pupillary size of rabbits after intravenous injection of 15 mg/kg of morphine sulphate. The shaded area represents the standard error. The first change was recorded at 30 sec after injection. Abscissa on log scale. Ordinate gives mean changes in diameter.

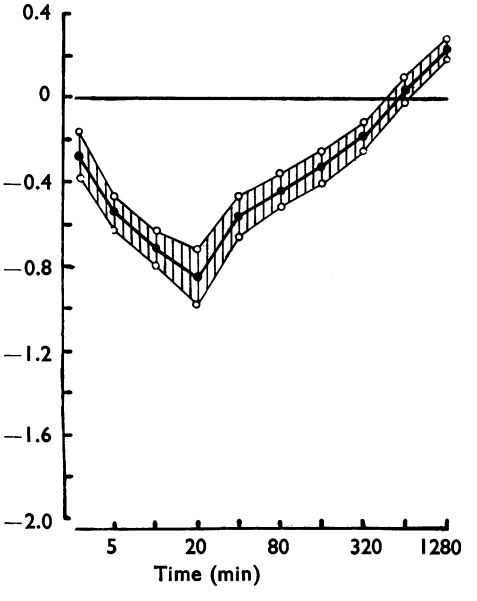

Fig. 2. Time/response curve of pupillary size of rabbits after intravenous injection of 3 mg/kg of nalorphine hydrobromide. The shaded area represents the standard error. Presentation as in Fig. 1.

Effect of nalorphine on the pupil size of normal rabbits

The change in pupil size after nalorphine was determined in the same twenty-four rabbits after a rest period of at least 2 days after the morphine experiments. Nalorphine hydrobromide (3 mg/kg, intravenously) produced pupillary constriction in twenty-three rabbits. Slight mydriasis occurred in the animal which failed to exhibit miosis after morphine. The miosis with nalorphine was less than that previously observed with morphine, the maximal mean decrease being −0.85 mm with a range of −0.5 to −2.0 mm. As with morphine, the mean peak effect occurred at 20 min but some animals exhibited maximal effects as early as 5 min and some not until 40 min (Fig. 2). Among the twenty-three rabbits showing pupillary constriction after nalorphine, three initially exhibited a prompt but transient mydriasis; 5 min after the injection, however, the pupils were constricted.

Reversal by nalorphine of morphine miosis—the nalorphine test

The same twenty-four rabbits were treated again with morphine sulphate as in the earlier experiment to produce miosis. When nalorphine (3 mg/kg) was injected 1 hr later, mydriasis occurred in twenty-three of the twenty-four rabbits. The previously " abnormal " rabbit now exhibited miosis. In most of the rabbits, the pupils dilated maximally almost immediately after the administration of nalorphine, the mean increase being 1.7 mm. The antagonistic effect of nalorphine persisted in more than two-thirds of the animals for at least 20 min and in more than one-half of the animals for at least 160 min (Fig. 3).

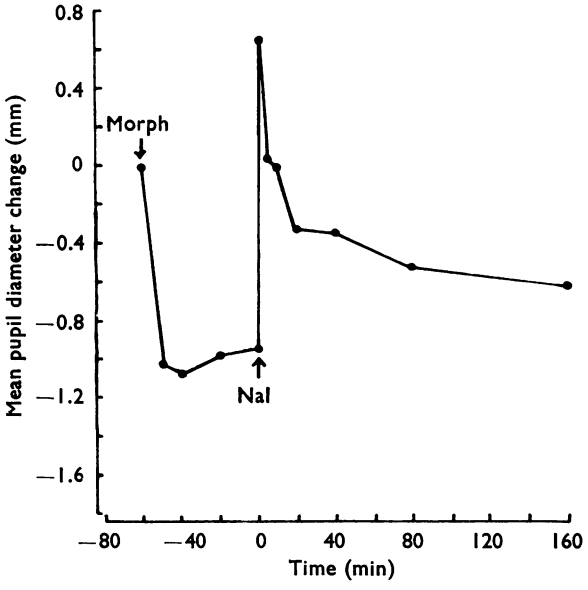

Fig. 3. Time/response curve showing reversal by nalorphine of morphine (Morph) miosis in normal rabbits. Values are means for twenty-four rabbits. Ordinate gives mean changes in diameter. Abscissa gives times before and after injection of nalorphine (Nal).

The influence of various drugs on the nalorphine test

Homatropine. A 2% solution was instilled into the conjunctival sac of morphine-treated animals 40 min before the administration of nalorphine. After the pupils had been greatly dilated by homatropine, they did not respond to nalorphine (Fig. 4) except possibly in one instance when a slight increase in pupillary diameter (+0.5 mm) was noted 10 min after the injection of nalorphine.

Physostigmine. A 0.5% solution was instilled into the conjunctival sac of morphine-treated rabbits 40 min before the administration of nalorphine. The pupils became markedly constricted and did not respond to nalorphine except for one rabbit which exhibited a slight constriction (−0.5 mm) 20 min after the injection of nalorphine (Fig. 4).

Amphetamine. An intravenous injection of 10 mg/kg was given 20 min before injecting the morphine-treated rabbits with nalorphine. In all six animals with pupils constricted by morphine, amphetamine elicited marked mydriasis within 2 min, the pupils increasing

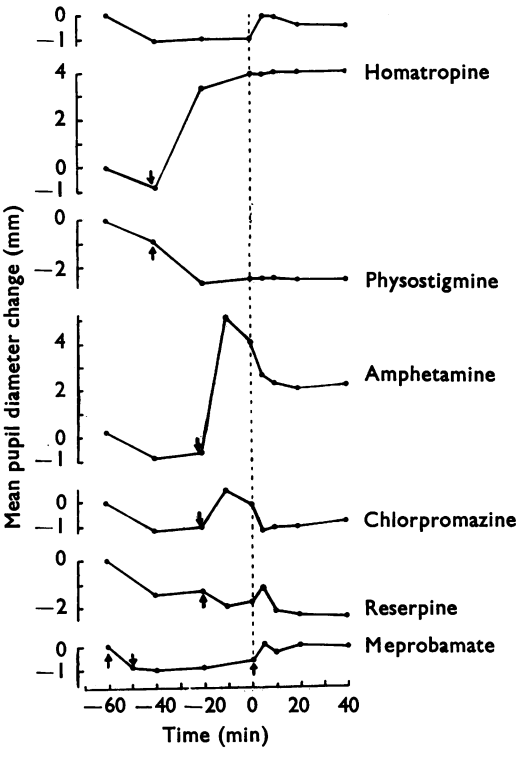

Fig. 4. Effect of various drugs on the antagonism by nalorphine of morphine miosis. Six rabbits were used for each drug. The top curve represents the response to nalorphine in morphine-treated rabbits without other drugs. Morphine was given at time —60 min; nalorphine was given at the interrupted line; other drugs were given at the arrows. Ordinate gives mean changes in pupil diameter. Abscissa gives times before and after nalorphine.

from —1.0 mm to +5.0 mm. On challenging with nalorphine the pupils constricted somewhat but the pupils remained very much enlarged over their premorphine state, the mean size 5 to 40 min after nalorphine being more than +2.0 mm (Fig. 4).

Chlorpromazine. A dose of 2.5 mg/kg was injected intravenously 20 min before the nalorphine test. In five of the six rabbits an almost instantaneous response was elicited. The pupils dilated considerably, reverting to or even exceeding the original sizes before the injection of morphine. After 20 min, just before the challenge with nalorphine, the mydriasis, although somewhat smaller, was still present (Fig. 4). After nalorphine, the pupils in all these five rabbits constricted, thus giving a negative result. One rabbit responded to chlorpromazine by further miosis, and after the injection of nalorphine there was no change in pupillary size.

In control experiments when six rabbits were given chlorpromazine without previous treatment with morphine, five rabbits exhibited definite mydriasis and one had no change in pupillary size. After nalorphine all the six rabbits exhibited pupillary constriction.

Reserpine. A dose of 0.2 mg/kg was injected intravenously 20 min before the nalorphine test. In all six rabbits reserpine increased the miosis induced by morphine given 40 min

previously. The challenge with nalorphine caused mydriases in all rabbits, but it was much briefer than in animals not previously treated with reserpine (Fig. 4). In five of the six rabbits the mydriasis lasted for only 5 min, whereas, in the majority of the control twenty-four morphine-treated rabbits receiving no reserpine, the mydriasis elicited by nalorphine lasted for more than 160 min. In an additional experiment, the same dose of reserpine in six rabbits which had not been treated with morphine also produced miosis; however, the pupil reacted to nalorphine by further constriction.

Meprobamate. An intramuscular injection of 100 mg/kg was given 10 min after morphine and 50 min before nalorphine. There was no definite pattern in the pupillary changes after meprobamate; three rabbits had slight dilation, two had slight constriction, and one had no change. After the challenge with nalorphine, the test was not affected qualitatively, except for one rabbit which had a slight constriction (−0.5 mm) 10 min after the administration of nalorphine. All the other five rabbits responded to nalorphine by mydriasis (Fig. 4).

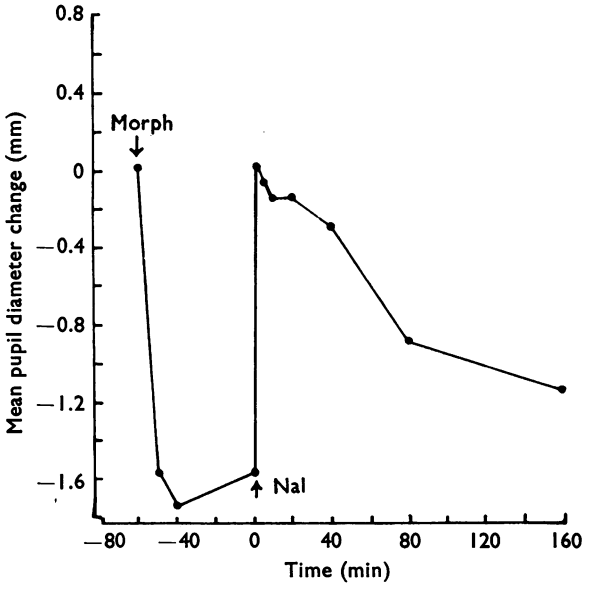

Fig. 5. Effect of nalorphine on the pupil size of chronically morphine-treated rabbits. Six rabbits were used for each drug. Presentation as in Fig. 3.

Effect and its timing of nalorphine on the pupillary response of chronically morphine-treated rabbits. Rabbits maintained on daily subcutaneous injections of morphine were tested with nalorphine. An intravenous dose of morphine was substituted for the subcutaneous injection on the test day and the challenge with nalorphine was carried out at 1, 4, 8, 16 and 32 hr later, using six animals at each time. At 1 hr after the morphine, nalorphine caused a rapid and marked mydriasis similar in onset, degree and duration to that produced in animals treated with a single dose of morphine (Fig. 5). The nalorphine test was still positive in all the chronically morphine-treated animals at 4 and 8 hr, but the mean maximal change in pupillary response was less than that at 1 hr (Table 1). At 16 hr, the

TABLE 1
PUPILLARY RESPONSE OF CHRONICALLY MORPHINE-TREATED RABBITS CHALLENGED WITH NALORPHINE AFTER VARIOUS TIME INTERVALS

Time after last morphine (hr)	Pupillary response	
	Positive/tested	Mean maximal change (mm)
1	6/6	+1·50
4	6/6	+1·25
8	6/6	+0·33
16	3/6	−0·25
32	0/6	−0·58

test was negative in three rabbits and equivocably positive (no change of pupillary size) in the other three. At 32 hr, all six animals showed miosis after nalorphine.

The influence of various drugs on the nalorphine test in chronically morphine-treated rabbits. The same six drugs (homatropine, physostigmine, amphetamine, chlorpromazine, reserpine and meprobamate), when given in the same dosage and by the same route as those used earlier in normal rabbits, modified the nalorphine test in the chronically morphine-treated rabbits with respect to onset, degree of change and duration in a manner very similar to that in the normal rabbits.

DISCUSSION

The pupillary response observed in albino rabbits after the injection of morphine, after nalorphine, and nalorphine following morphine, appears to be very similar to that which can be elicited in humans. It seems reasonable, therefore, to conclude that at least some of the results obtained in the present experiments can be extrapolated for making interpretations of the limitations of the pupil test in humans, especially with respect to dose/response and time effects as well as possible drug influences on the morphine and nalorphine pupil response.

In establishing the optimal conditions for obtaining consistent pupillary changes in the rabbit it was necessary to standardize the morphine and nalorphine dosages as well as the times for the administration of both drugs. This means that in field tests on addicts, where the amount and time of administration of a narcotic are unknown, the nalorphine test is liable under certain conditions to give a negative test. Our studies reveal that a variety of factors can modify the nalorphine pupil test for narcotic use. The amount of narcotic present in the body, the mode of administration of nalorphine and the timing of the test, as well as the presence of other drugs, are all important considerations.

The effectiveness of the nalorphine test appears to be directly dependent on the amount of morphine present in the body, since the incidence of positive tests after administration of morphine more or less parallels the known patterns of brain levels of and duration of response to morphine (Way & Adler, 1960, 1961). Strong positive nalorphine responses were recorded at 1 and 4 hr after morphine, after which the response became progressively weaker and after 32 hr no positive tests were noted. Since morphine is excreted in man largely within 24 hr (Way & Adler, 1960, 1961), the likelihood of obtaining a positive nalorphine test in the narcotic user after 24 hr is rather small, despite statements of law-

enforcement officials to the contrary (Brown, 1961). The findings have additional support with respect to our unpublished experiences with addicts and in a study with treated addicts given single and repeated injections of morphine (Elliott *et al.*, 1964).

Dosage and timing of the nalorphine administration also appear to be important considerations. A single dose of nalorphine may be insufficient to elicit pupillary changes in heavily addicted persons, as shown by the fact that, during the preliminary design of our experiments, the pupils were less responsive to nalorphine in animals given 20 instead of 15 mg/kg of morphine. Moreover, with the 15 mg/kg dose, maximum antagonism of morphine effects with nalorphine did not coincide consistently with peak morphine effects at 20 min. In accord with these findings in rabbits we have found that, after large doses of heroin (50 to 100 mg, intravenously) in addicts a positive pupil test to nalorphine does not always occur if the test is performed too early after heroin administration.

The generally recommended test for nalorphine calls for only a single reading at 30 min after its subcutaneous injection. In establishing optimal conditions for the test in rabbits, most consistent pupillary changes were noted with nalorphine given intravenously instead of subcutaneously. However, even with intravenous nalorphine there is considerable variation in the peak response, so that with a single reading at one fixed time interval a positive test may sometimes be overlooked. This conclusion is also substantiated by our experiences with heroin addicts who occasionally gave a negative test at 30 min but reacted positively either at 20 or 40 min.

Our findings also indicate that various drugs can modify the morphine and nalorphine test in a variety of ways. Powerful drugs acting on the pupils, such as homatropine or physostigmine, can render the pupil unresponsive to nalorphine. Reserpine greatly diminished the period to which the pupils were responsive to nalorphine. Chlorpromazine and amphetamine converted a positive test to a negative one. While differences in species sensitivity to drugs may render it difficult to duplicate exactly these findings in addicts, and, indeed, this may be too much to expect, the important point is that drugs can greatly modify the morphine and nalorphine pupillary response. If the particular drugs we studied do not show parallel responses, in humans, almost certainly there should be other drugs that can modify the nalorphine test. These considerations are of practical importance since undoubtedly addicts have tried numerous drugs to beat the test. An investigation of this aspect is in progress.

These studies furnish a pharmacological basis to explain some of the discrepancies previously observed between the nalorphine pupil tests on addicts and the chemical findings on urine. They confirm that the nalorphine pupil test for morphine usage has a fairly high degree of reliability but indicate that there are many factors which can alter the accuracy of the test. The test at best can furnish strong suggestive evidence with respect to narcotic usage but it cannot be regarded as an absolute criterion for forensic purposes.

SUMMARY

1. The applicability of the nalorphine test for narcotic usage was evaluated in rabbits given single or repeated injections of morphine. Under fixed lighting conditions changes in pupillary size were measured after morphine and morphine plus nalorphine, after which the influence of various drugs on the morphine and nalorphine pupil response was assessed.

2. The experiments yielded considerable results analogous to those obtained in addicts. A single injection of morphine (15 mg/kg, intravenously) elicited miosis with a mean maximal decrease in pupillary size of −1.6 mm and a duration of effect greater than 10 hr. Nalorphine (3 mg/kg, intravenously) also produced miosis; the mean maximal decrease was −1.0 mm and the duration of effect was between 5 and 10 hr. Nalorphine, given 60 min after morphine, produced a prompt dilation of the pupils of approximately 1.5 mm and the response was apparent even after 2 hr although its magnitude gradually diminished with time.

3. Nalorphine reversal of morphine miosis (positive test) was also obtained in animals chronically treated with morphine for at least 3 weeks. A positive response was consistently obtained up to 8 hr after the last morphine administration after which the incidence of positive reactions gradually decreased.

4. Various drugs modified the pupillary response to nalorphine similarly in animals given single or repeated injections of morphine. Homatropine and physostigmine rendered the pupils of morphine-treated rabbits unresponsive to nalorphine. Meprobamate did not alter the character of the test. Reserpine decreased the period during which morphine-treated animals yielded a positive test to nalorphine. Amphetamine and chlorpromazine converted a positive test to a negative one.

5. The applicability of the results for making interpretations related to human situations is discussed.

This study was supported in part by donations from the China Medical Board of New York, by Merck, Sharpe & Dohme and the United States Public Health Service (GM 1839). Nalorphine was generously supplied by Burroughs Wellcome. The authors are grateful for encouragement by Professor K. K. Cheng.

REFERENCES

BROWN, L. T. (1961). *The Enigma of Drug Addiction*, pp. 325 and 328. Springfield: C. C. Thomas.

ELLIOTT, H. W., NOMOF, N., PARKER, K., DEWEY, M. L. & WAY, E. L. (1964). Comparison of the nalorphine test and urinary analysis in the detection of narcotic use. *Clin. Pharmacol. Ther.*, **5**, 405–413.

ELLIOTT, H. W. & WAY, E. L. (1961). Effect of narcotic antagonists on the pupil diameter of non-addicts. *Clin. Pharmacol. Ther.*, **2**, 713–721.

TERRY, J. G. & BRAUMOELLER, F. L. (1956). Nalline: an aid in detecting narcotic users. *Calif. Med.*, **85**, 299–301.

WAY, E. L. & ADLER, T. K. (1960). The pharmacologic implications of the fate of morphine and its surrogates. *Pharmacol. Rev.*, **12**, 383–446.

WAY, E. L. & ADLER, T. K. (1961). The biological disposition of morphine and its surrogates, 1. *Bull. Wld Hlth Org.*, **25**, 227–262.

WAY, E. L., ELLIOTT, H. W. & NOMOF, N. (1963). Comparison of chemical tests with the pupillary method for the diagnosis of narcotic use. *Bull. Narcot.*, **15**, 29–33.

WIKLER, A., FRASER, H. F. & ISBELL, H. (1953). N-allylnormorphine: effects of single doses and precipitation of acute " abstinence syndromes " during addiction to morphine, methadone, or heroin in man (post addicts). *J. Pharmacol. exp. Ther.*, **109**, 8–20.