kg, respectively; group 4 – mannitol, 2 g/kg; group 5 – saline, 1 ml/kg. The same treatment schedule was used for serum sodium determinations, and at 0′, 15′, 30′ and 60′ after morphine injection, eight rats per dose were killed by decapitation, blood was collected, and sodium concentration in serum determined by flame spectrophotometry (Zeiss PMQ II; emission at 589 nm).

Significances were determined using Student's t-test. Variability is expressed as the SE of the mean.

Results and discussion. Hydrochlorothiazide, acutely injected at the doses of 5 and 10 mg/kg i.p., had a poor but significant analgesic effect per se; moreover, at all the doses tested, it greatly potentiated and prolonged the antinociceptive activity of morphine (table), this effect being longer-lasting with the highest dose. Mannitol, which at our dose level induces essentially a water diuresis, neither modified per se the pain threshold, nor affected the effect of morphine. Sodium concentration in serum was significantly reduced only by the highest dose of hydrochlorothiazide (table).

Several biochemical studies have examined the mechanism of the effect of sodium on opiate receptors[8]. The enhancement of antagonist binding by sodium appears to be elicited by an accelerated dissociation of the endogenous opiate molecules from the opiate receptor[9,10]. Opiate receptor binding involves distinct high-and low-affinity sites[11]. The reduction in agonist binding produced by sodium is mediated by an abolition of high-affinity agonist binding sites[8].

However, the present results do not seem to indicate a clear-cut relationship between the morphine-potentiating effect of hydrochlorothiazide and the reduction in serum sodium concentration.

In fact, under our conditions, sodium concentration was significantly reduced only by 10 mg/kg of hydrochlorothiazide, whereas morphine analgesia was also significantly potentiated and prolonged by 5 and 1 mg/kg of hydrochlorothiazide.

It is possible that serum sodium concentrations do not exactly reflect the contemporaneous situation in tissues, and that the morphine-potentiating effect of hydrochlorothiazide nevertheless depends on a reduced Na^+ availability at the opiate receptor level. However, our present data can neither definitely prove such a possibility, nor exclude that this effect of hydrochlorothiazide is independent of its natriuretic activity.

1 This work was supported in part by grants from Consiglio Nazionale delle Ricerche, and by Ministero della Pubblica Istruzione, Roma.

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0014-4754/85/020265-02$1.50 + 0.20/0

Ocular instillation of naloxone increases intraocular pressure in morphine-addicted patients: A possible test for detecting misuse of morphine

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Summary. The effect of conjunctival instillation of naloxone on intraocular pressure has been examined in morphine-addicted patients as compared to non-addicted healthy volunteers. Morphine-addicted subjects showed a lower basal value of intraocular pressure as compared to the control volunteers. The instillation of naloxone caused a normalization of intraocular pressure to a level similar to that of control volunteers. This test seems to be a useful screening method for detecting morphine addiction.
Key words. Morphine addiction; naloxone; conjuctival instillation; intraocular pressure.

Introduction. Pupillary constriction is induced by local application of morphine in the rabbit[1] and human eye[2,3]. While the topical administration of the opiate antagonist naloxone is unable to antagonize miosis induced by an acute parenteral dose of morphine conjuctival instillation of naloxone reverses miosis induced by morphine[3]. Moreover, naloxone induces mydriasis in morphine-addicted patients after conjunctival instillation[4], and this test has been suggested as a possible screening method in detecting morphine dependence. Patients addicted to morphine or heroin exhibit an increased aqueous outflow associated with a decrease in intraocular pressure[5]. Furthermore, the existence of opioid peptides has recently been demonstrated in the ox eye[6].

The present experiments were aimed at investigating the effect of local administration of naloxone on intraocular pressure in patients addicted to morphine. The results of the present work suggest the use of this naloxone test in detecting morphine addiction.

Methods. The study was performed on 10 patients (eight males and two females) at the Provincial Hospital 'Garibaldi' in Catania, 18–29 years old, who had been abusing morphine for at least one year, 100–200 mg/day. Four nonaddicted healthy volunteers (two males and two females, 20–24 years old) were studied as controls. 4 h after the last i.v. morphine administration to addicted patients, basal values of intraocular pressure were measured on both eyes of addicted and control subjects with a Schiötz tonometer (Sbisà, Florence, Italy). 1 h later the naloxone test was performed by instilling two drops (0.1 ml) of 20% naloxone saline solution in the right eye of addicted and control subjects. Only saline solution was instilled in the left eye. Measurements of intraocular pressure, started 30 min after the instillation of the drug, were performed four

times on a period of 15 min (a measurement every 5 min). All measurements of intraocular pressure were performed between 13.00 and 15.00. Statistical differences were calculated with the paired t-test.

Results. Addicted patients showed a lower basal value of intraocular pressure in both eyes as compared to control subjects (table). The instillation of naloxone in the right eye of addicted patients resulted in a significant increase ($p < 0.01$, as compared to the control left eye) in intraocular pressure, to a level similar to that of saline-treated eyes of non addicted healthy volunteers ($p > 0.05$). No difference in intraocular pressure of right and left eye was apparent after naloxone instillation in control subjects.

Discussion. The present results confirm our previous observation that patients addicted to morphine show an increased aqueous outflow associated with a decrease in intraocular pressure[5]. Moreover, since instillation of naloxone caused a remarkable increase in intraocular pressure in addicted patients, it is possible to speculate that local opiate receptors are involved in the regulation of intraocular pressure in man. Intraocular injection of morphine reduces intraocular pressure in rabbit[5]. Opiate receptors have been described in the iris of rabbit[1] and man[3]. Furthermore, the existence of opioid peptides has recently been demonstrated in the ox eye[6]. Thus, it is possible that the same population of opiate receptors in the eye affect pupillary diameter and intraocular pressure. Against this possibility is an old observation that morphine may lower intraocular pressure without increasing miosis[7].

Instillation of naloxone induces mydriasis in addicted patients[4]. This effect may be mediated by the liberation of noradrenaline accumulated during morphine abuse. In fact, morphine inhibits noradrenaline release in the cat nictitating membrane[8] and in the mouse vas deferens[9]. The same mechanism may be postulated in the effect of naloxone on intraocular pressure. Thus, naloxone applied topically to the eye provokes a local miniature withdrawl reaction that is limited to the functions of pupil and intraocular pressure, without general effect. In conclusion, beside the pupillary test described by Fanciullacci et al.[4], the naloxone test on intraocular pressure seems to be a useful screening method for detecting morphine addiction.

Effect of ocular instillation of naloxone on the intraocular pressure of morphine-addicted (n = 10) and control (n = 4) subjects. The drug was applied to the right eye 1 h after the basal measurements, while only saline was instilled in the left eye. Values are mean ± SE (in mmHg)

	Times of observation (after naloxone instillation)				
	Basal	30 min	35 min	40 min	45 min
Addicted					
Right eye	10.1 ± 0.8**	14.4 ± 0.8ff	14.7 ± 0.5ff	14.8 ± 0.3ff	14.0 ± 0.6ff
Left eye	10.0 ± 0.4**	10.1 ± 0.2	10.2 ± 0.2	9.8 ± 0.3	9.7 ± 0.4
Controls					
Right eye	14.0 ± 0.4	14.6 ± 0.4	13.8 ± 0.6	13.8 ± 0.6	13.7 ± 0.7
Left eye	14.6 ± 0.5	14.5 ± 0.7	13.5 ± 0.6	13.6 ± 0.5	13.4 ± 0.4

** Significantly different as compared to correspondent eye in control subjects ($p < 0.01$, paired t-test); ff Significantly different as compared to saline-treated left eye ($p < 0.01$, paired t-test).

Acknowledgments. The authors wish to thank the staff of the Neurological Division, Provincial Hospital 'Garibaldi', Catania.

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0014-4754/85/020266-02$1.50 + 0.20/0

Photoperiodically induced delayed insect metamorphosis: a larval oligopause in *Diatraea saccharalis*

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Summary. Sugarcane borers enter a state of delayed metamorphosis when exposed to a 12-h photophase at 21 °C. Larval feeding, growth, and molting continues but pupation is suppressed under these conditions.
Key words. Diapause; oligopause; sugarcane borer; *Diatraea saccharalis;* Lepidoptera; Pyralidae.

Diapause has long been recognized as a state of suppressed growth and development[3-6]. Delayed metamorphosis in the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae)[7,8], was thought to be similar to the closely related and well studied diapause of the southwestern cornborner, *D. grandiosella* Dyar, where there is a state of delayed pupation and no feeding or growth[9,10]. Sugarcane borer larvae, however, in response to a 12-h photophase at 21 °C enter a state of delayed metamorphosis, where pupation is suppressed but larval feeding, growth, and moulting continues. Mansingh referred to this condition as oligopause[5] but had no definitive studies as proof. Delayed metamorphosis in the sugarcane borer was photoperiodically induced for both male and female borers within the first 2 stadia. Delayed metamorphosis in borers reared[11] from egg hatch at 21 °C on photophases of fixed duration from 10–13 h was greater than 90% (fig. 1a) with shorter and longer photophases producing less than 60% induction (a type 1 response curve[3]). The percent delayed pupation was greatly reduced when borers were transferred from LD 14:10 to LD 12:12 at 10 days or later, but approximately 100% if the trans-