smooth muscle cells. However, BNA at the concentration used in this study did not depolarize the resting membrane potential in guinea-pig papillary muscles[1].
In conclusion, BNA decreased ^{45}Ca influx and increased ^{45}Ca efflux. Both effects could explain the inhibition of the contractile responses induced by NE, 5-HT, KCl and $BaCl_2$ by BNA in isolated rat aortic strips.

1 Tamargo, J., Archs int. Pharmacodyn. *246* (1980a) 224.
2 Tamargo, J., Eur. J. Pharmac. *62* (1980b) 81.
3 Tamargo, J., Aleixandre, A., Rodriguez, S., and Garcia de Jalon, P., J. Pharm. Pharmac. *30* (1978) 455.
4 Furchgott, R., and Bhadrakon, S., J. Pharmac. exp. Ther. *108* (1953) 129.
5 Godfraind, T., J. Physiol. *260* (1976) 21.
6 Barrigon, S., Tamargo, J., and Garcia de Jalon, P., Experientia *34* (1978) 770.
7 Goodman, F., Weiss, G., and Adams, R., J. Pharmac. exp. Ther. *188* (1974) 472.
8 Bolton, T., Physiol. Rev. *59* (1979) 606.
9 Van Breemen, C., Mangel, A., Fahim, M., and Meisheri, K., Am. J. Cardiol. *49* (1982) 507.
10 Godfraind, T., and Kaba, A., Br. J. Pharmac. *36* (1969) 549.
11 Hudgins, P., and Weiss, G., J. Pharmac. exp. Ther. *159* (1968) 91.
12 Goodman, F., and Weiss, G., J. Pharmac. exp. Ther. *177* (1971) 415.
13 Bohr, D., Circulation Res. *32* (1973) 665.
14 Altura, B.A., and Altura, B.T., Anesthesiology *41* (1974) 197.
15 Hudgins, P., and Weiss, G.B., J. Pharmac. exp. Ther. *159* (1968) 91.
16 Hudgins, P., J. Pharmac. exp. Ther. *170* (1969) 303.
17 Kuperman, A., Altura, B.T., and Chezar, J., Nature *217* (1968) 673.
18 Northover, B., Br. J. Pharmac. *34* (1968) 417.

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Failure of naloxone to modify the depth of hypnotic trance

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Summary. Naloxone (10 mg/70 kg, i.v.) was used in normal volunteers to study a possible relationship between endorphins and the depth of hypnotic trance. No effect was found. The drug also failed to modify the subjects' level of alertness.

The endorphins have been implicated, though not always convincingly, in a number of psychic states, including various mental disorders[2,3], acupuncture[4], jogger's high[5-7] and placebo-induced analgesia[8]. One phenomenon of hypnosis, hypnotically induced analgesia, may also involve the endorphins though the conclusion is controversial[9,10]. We decided to investigate the possible participation of endorphins in another aspect of hypnosis, the depth of hypnotic trance. We used the opiate antagonist, naloxone, which blocks the endorphin receptor. If the depth of hypnosis were related to endorphins, then the drug might antagonize the depth of hypnotic trance.
Methods. The subjects were 16 healthy males ranging in age from 19 to 31. They were medical or pre-medical students. They became involved in the experiment because of their interest in hypnosis. All subjects were found to be hypnotizable in preliminary testing which utilized the criteria of Spiegel[11] and Spiegel and Fleiss[12]. Written consent forms and health questionnaires were administered. There were no indications of illness. The subjects were questioned about their use of psychoactive drugs. Most admitted an occasional use of alcohol and marijuana, but denied using other agents. They agreed to refrain from alcohol and marijuana for at least 72 h prior to each experimental session. The subjects were informed that after screening, they would be hypnotized by a psychiatrist skilled in hypnosis on 3 occasions following the i.v. administration of either naloxone or placebo during 2 of the trials. The remaining trial (no injection) would serve as a baseline.
The subjects were randomly divided into different groups, based on the possible sequences of naloxone, placebo and baseline. The placebo was the drug vehicle, as supplied by the manufacturer. During the baseline (no injection) sessions, as in sessions involving actual injections, a small bandage was placed on the subjects' forearm to prevent the hypnotist from recognizing whether or not an injection had been given. Only the person giving the injections was aware of the sequences of the treatments. The placebo was given in a dose of 1 ml/70 kg, i.v. The dose of naloxone was 10 mg/ml/70 kg, i.v. Although this dose is well above the standard clinical dose used to treat narcotic overdose, no side effects have been reported in the literature at this level[13]. The high dose was chosen because of evidence that some effects attributed to endorphins appear to be blocked only by large doses of naloxone and because of the efficacy of such doses in certain behavioral conditions in humans[2]. After treatment, a 15-min waiting period was allowed, to permit drug equilibration. After the waiting period, the subjects were hypnotized and the depth of trance was assessed according to the Hypnotic Induction Profile of Spiegel[11,12]. This test provides a score which measures the depth of hypnotic trance. The scores range from 0 (subjects who are refractory to hypnosis), to 10 (subjects who are hypnotizable and who experience a very deep trance). Spiegel and Fleiss[12] found that the mean score for a sample of 1674 normal subjects was 6.7 ± 2.9. Following the session with the hypnotist, the subjects were asked to rate their level of alertness according to the Stanford Sleepiness Scale[14]. This questionnaire provides a rating of sleepiness with scores ranging from 1 (fully alert) to 7 (almost asleep). The subjects were also asked to describe any subjective effects and whether they could identify when they received drug or placebo.

Measures of the depth of hypnotic trance and of sleepiness after the induction of hypnosis in subjects receiving no prior treatment (baseline), or pretreated with placebo (1 ml/70 kg, i.v.) or naloxone (10 mg/ml/70 kg, i.v.)

	Baseline	Placebo	Naloxone
Hypnotic induction Profile score	7.1 ± 0.4	6.2 ± 0.6	6.0 ± 0.8
Stanford sleepiness Scale score	2.5 ± 0.2	2.6 ± 0.6	2.6 ± 0.3

The values shown are means, $\pm$SE. N = 16.

Results. There were no statistically significant differences between the 3 treatment conditions relative either to the Hypnotic Induction Profile or to the Stanford Sleepiness Scale (table). There were no reports of unusual subjective states or sensations, and the subjects were unable to identify when they received naloxone.

Discussion. The data disclaim our hypothesis that endorphins might be released during hypnosis in such a way that they could influence the depth of hypnotic trance. The failure of the large dose of naloxone to change the scores of the Stanford Sleepiness Scale represents new data confirming in a quantitative way the lack of effect of this dose of naloxone in relation to levels of alertness. Our observation that subjects were unable to identify when they received naloxone corroborates further the lack of subjective effects of this drug in individuals who are not using narcotics. Given antagonism of the endorphin receptor, the pharmacological blandness of naloxone remains a major stumbling block to hypotheses which seek to relate psychological states to the endorphin system.

1 To whom correspondence and reprint requests should be sent (at the Department of Pharmacology).
2 Lehmann, H., Nair, N.P., and Kline, N.S., Am. J. Psychiat. *136* (1979) 762.
3 Davis, G.C., and Bunney, W.E., in: Neural peptides and neuronal communication, p.455. Eds E. Costa and M. Trabucchi. Raven Press, New York 1980.
4 Liao, S.J., Yale J. biol. Med. *51* (1978) 55.
5 Wagemaker, H., and Goldstein, L., Sports Med. Phys. Fitness *20* (1980) 227.
6 Colt, E.W., Wardlaw, S.L., and Frantz, A.G., Life Sci. *28* (1981) 1637.
7 Bortz, W.M., Angwin, P., Mefford, I.N., Boarder, M.R., Noyce, N., and Barchas, J.D., New Engl. J. Med. *305* (1981) 466.
8 Levine, J.D., Gordon, N.C., and Fields, H.L., Lancet *2* (1978) 654.
9 Frid, M., and Singer, G., Psychopharmacology *63* (1979) 211.
10 Nasrallah, S.M., Holley, T., and Janowsky, D.S., Lancet *1* (1979) 1355.
11 Spiegel, H., Ann. N.Y. Acad. Sci. *296* (1977) 129.
12 Spiegel, H., and Fleiss, J., Int. J. clin. exp. Hypnosis *24* (1976) 300.
13 Zaks, A., Jones, T., Fink, M., and Freedman, A., J. Am. med. Ass. *215* (1971) 2108.
14 Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., and Dement, W., Psychophysiology *10* (1973) 431.

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Chromatographic separation of coelomic fluid from *Holothuria polii* (Echinodermata) and partial characterization of the fractions reacting with erythrocytes

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Summary. Coelomic fluid preparations from *Holothuria polii* were passed through a Bio-gel A5m column. The 3 separated protein peaks possess hemagglutinating or hemolytic activity against rabbit erythrocytes. Electrophoretic and immunochemical methods showed that 2 identical protein subunits characterized hemagglutinins of different size. Hemolysin differs from hemagglutinin in molecular weight and organization of subunits.

Coelomic fluid from certain echinoderm species possesses naturally occurring hemolytic and also hemagglutinating activity against erythrocytes of several vertebrate species. Chemico-physical investigations of sea-urchin[2], star-fish[3] and holothurian[4] hemagglutinins showed that, except for those of the sea-urchin *Hemicentrotus pulcherrimus*, they are protein or protein-like substances. The same methods also suggest that hemolysins are thermolabile proteins active, in alkaline medium supplemented with Ca^{2+}, in lysing vertebrate erythrocytes[5,6]. In previous papers[4,6] we showed that hemagglutinins and hemolysins from *Holothuria polii* are proteins which differ in some of their chemico-physical properties. The present report concerns a partial molecular characterization of the active fractions obtained by chromatographic separation of the coelomic fluid.

Materials and methods. Holothuria polii Delle Chiaje specimens were collected in the Gulf of Palermo. Coelomic fluid was centrifuged at 400×g to remove the cells and then, after dialysis with phosphate buffered saline at pH 7.4 (PBS), stored at −20 °C.

Hemagglutination and hemolysis assays have been previously described[4,6]. The end titer was taken to be the reciprocal of the highest dilution of the coelomic fluid giving a clear agglutination after gentle shaking, or revealing hemolysis (at least 10%).

To determine protein content the Folin-Ciocalteau method as described by Lowry et al.[7] was used. Bovine serum

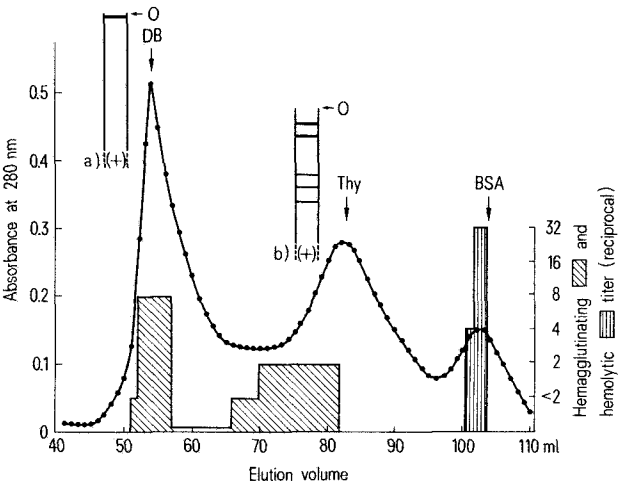

Figure 1. Bio-gel A5m elution pattern of 10-fold concentrated *Holothuria polii* coelomic fluid showing the distribution of hemagglutinating and hemolytic activity. The eluting buffer was phosphate buffered saline pH 7.4, the column size 1.5×90 cm. The elution volumes of dextran blue (DB), thyroglobulin (Thy) and bovine serum albumin (BSA) are indicated. Inset: diagrams which represent developed portions of 7.5% polyacrylamide gel electrophoresis; (a) 1st peak, (b) 2nd peak, (O) origin.