pable of sensing a minimum target of 5.08 cm in diameter at 9 m.
8. S. Lustick and D. D. Lustick, *Comp. Biochem. Physiol. A* **43**, 643 (1972).
9. J. Steen and I. B. Steen, *Acta Physiol. Scand.* **63**, 285 (1965).
10. S. Lustick, *Science* **163**, 387 (1969).
11. W. J. Hamilton III and F. Heppner, *ibid.* **155**, 196 (1967).
12. W. P. Porter and D. M. Gates, *Ecol. Monogr.* **39**, 227 (1969).
13. A bird's total surface area was calculated from the allometric equation $A_s = 10W^{.67}$, where W is the body weight. To calculate net radiation exchange the total surface area was subdivided from photographs illustrating relative illumination, orientation, and postural adjustments, into a dorsal surface (511 cm²) and a ventral surface (511 cm²). When the bird faced the sun its posture was such that only one-half the ventral surface (255 cm²) received solar radiation.
14. W. A. Calder and J. R. King, in *Avian Biology*, D. S. Farner and J. R. King, Ed. (Academic Press, 1974), vol. 4, p. 259.
15. We thank the U.S. Steel Corp. in Rogers City for allowing us to use their facilities. Supported in part by NSF grant DEB 7509290 (S.I.L.).

13 October 1977; revised 30 December 1977

Attenuation of Amnesia in Rats by Systemically Administered Enkephalins

Abstract. *The pentapeptides methionine-enkephalin and leucine-enkephalin are both able to reduce experimentally induced amnesia in rats. In contrast to the possible analgesic activity of these peptides, the anti-amnesic effect is seen after systemic administration of dosages of 30 micrograms or lower. The nature of the anti-amnesic effect is different for the two peptides.*

The pituitary hormone β-lipotropin (β-LPH) has behavioral activity, as exemplified by its ability to attenuate experimentally induced amnesia for a passive avoidance response in rats when administered prior to the memory retrieval test (*1*). Similar anti-amnesic activity has been reported for the adrenocorticotrophic hormone (ACTH) peptide ACTH 4-10 (*1*, *2*), a peptide whose amino acid sequence is identical to β-LPH 47-53. It has been suggested that β-LPH functions as a prohormone for behaviorally active peptides (*3*). Hughes *et al.* (*4*) identified two naturally occurring pentapeptides, which they termed methionine-enkephalin (Met-enkephalin) and leucine-enkephalin (Leu-enkephalin). The fact that the amino acid sequence of Met-enkephalin corresponds to β-LPH 61-65 prompted me to study the effects of these pentapeptides in an amnesia test. The results show that both peptides were active in reducing amnesia.

A common design for studies of amnesia is to train an animal on a one-trial passive avoidance task and to administer the amnesic agent shortly after the conclusion of training. Amnesia is then defined and measured as a loss of performance at a later retrieval test. I used CO_2 to induce amnesia. A variety of control studies have demonstrated that CO_2-induced loss of performance in amnesia tests is specific, that is, results from interference with some memory process (*5*). In the present experiments male Wistar rats, weighing approximately 200 g, were trained in a passive avoidance step-through apparatus (*6*). Rats were placed on an elevated illuminated run-

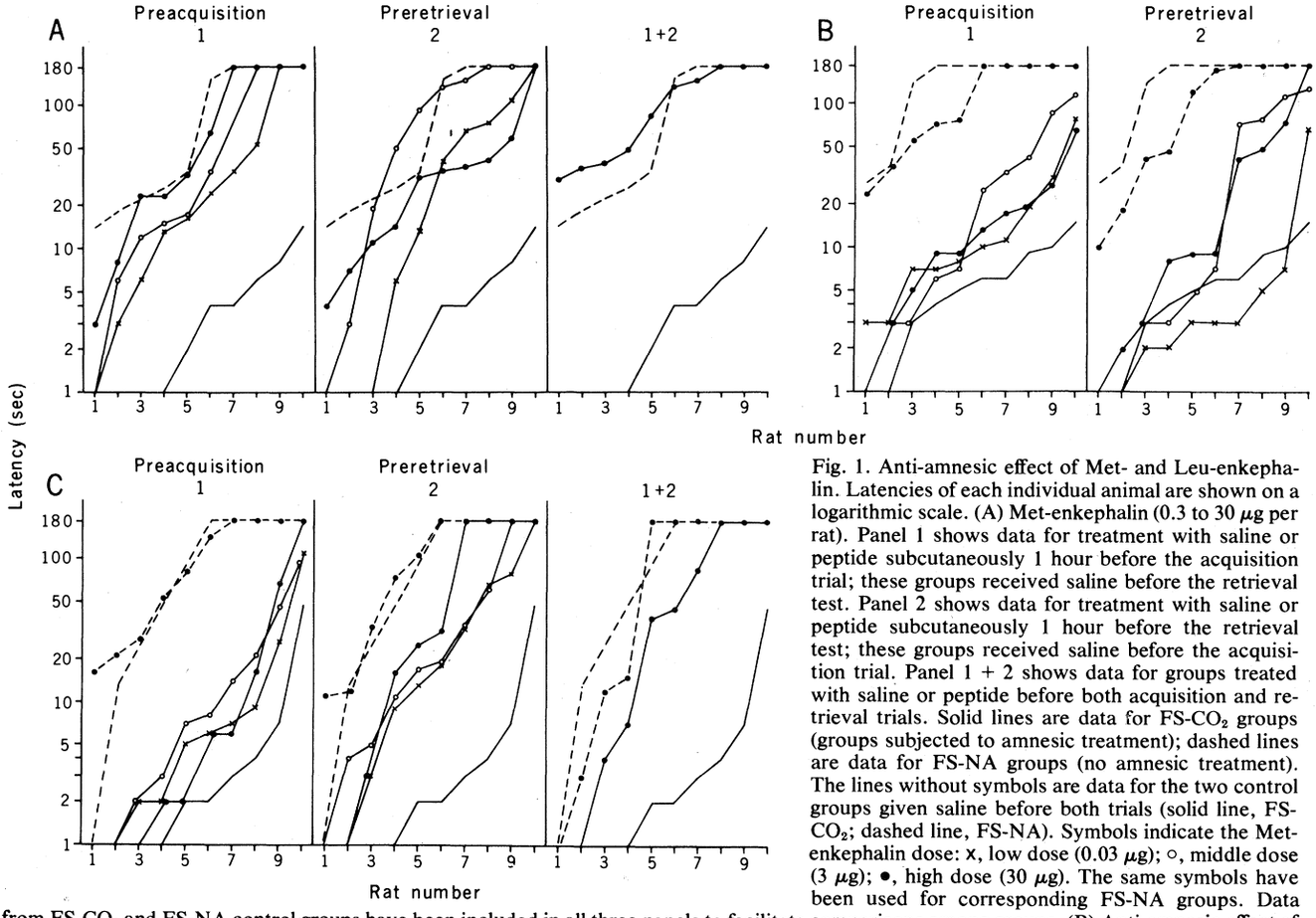

Fig. 1. Anti-amnesic effect of Met- and Leu-enkephalin. Latencies of each individual animal are shown on a logarithmic scale. (A) Met-enkephalin (0.3 to 30 μg per rat). Panel 1 shows data for treatment with saline or peptide subcutaneously 1 hour before the acquisition trial; these groups received saline before the retrieval test. Panel 2 shows data for treatment with saline or peptide subcutaneously 1 hour before the retrieval test; these groups received saline before the acquisition trial. Panel 1 + 2 shows data for groups treated with saline or peptide before both acquisition and retrieval trials. Solid lines are data for FS-CO_2 groups (groups subjected to amnesic treatment); dashed lines are data for FS-NA groups (no amnesic treatment). The lines without symbols are data for the two control groups given saline before both trials (solid line, FS-CO_2; dashed line, FS-NA). Symbols indicate the Met-enkephalin dose: x, low dose (0.03 μg); ○, middle dose (3 μg); •, high dose (30 μg). The same symbols have been used for corresponding FS-NA groups. Data from FS-CO_2 and FS-NA control groups have been included in all three panels to facilitate comparisons among groups. (B) Anti-amnesic effect of Met-enkephalin at lower doses; low dose, 0.0003 μg; middle dose, 0.003 μg; high dose, 0.03 μg. (C) Anti-amnesic effect of Leu-enkephalin; low dose, 0.03 μg; middle dose, 3 μg; high dose, 30 μg. The dose symbols are the same as those for Met-enkephalin.

0036-8075/78/0407-0083$00.50/0

way protruding from a dark chamber and allowed to enter the chamber through an opening in its front wall. On both days 1 and 2 of an experiment, two pretraining trials were given. On day 3 a single acquisition trial was conducted. The time taken by the animals to enter the chamber was recorded. Ten seconds after entering the chamber the rats received a foot shock (FS) (0.5 mA for 3 seconds) through the grid floor of the chamber. Shock was followed either by amnesic treatment with CO_2 (FS-CO_2 groups) or "sham" amnesic treatment (FS-NA groups). Amnesic treatment consisted of placing the animals in a box with 100 percent CO_2 until respiratory arrest occurred. The rats were then revived by artificial respiration and returned to their home cage. Since CO_2 itself does not affect later performance (*2*), control groups receiving CO_2 but no FS were not included in the present experiments. On day 4 retrieval was measured in a single trial by recording the time (latency) taken by the rats to enter the chamber. When a rat did not enter the chamber within 180 seconds, it was removed from the runway and a score of 180 seconds was assigned. The results were analyzed by means of the two-tailed randomization test for independent samples (*7*, *8*).

Met- and Leu-enkephalin (*9*) were dissolved in saline and a volume of 1 ml of saline or peptide solution was injected subcutaneously 1 hour before the acquisition trial (day 3) and 1 hour before the retrieval trial (day 4) according to the schedule given in the legend to Fig. 1. Each treatment group contained ten rats. Three experiments were conducted. In the first study Met-enkephalin was given in doses of 0, 0.3, 3, and 30 μg per rat; in the second, doses of Met-enkephalin were 0, 0.0003, 0.003, and 0.3 μg per rat; in the third, Leu-enkephalin was administered in doses of 0, 0.3, 3, and 30 μg per rat.

The results are presented in two ways. The raw data are given in Fig. 1. In addition, the raw data were categorized in three classes of latencies, indicative of (i) no avoidance, (ii) incomplete avoidance, or (iii) complete avoidance (*8*). The categorized data are presented in Fig. 2.

None of the doses of Met- or Leu-enkephalin had an effect on entrance latencies on the acquisition trial, that is, before avoidance training had been given (*10*). However, peptide treatment did influence entrance latencies on the retrieval trial. Met-enkephalin diminished amnesia in a dose-dependent manner when given before acquisition (Fig. 1, A and B, panel 1), before retrieval (Fig. 1, A and B, panel 2), or at both times (Fig. 1A, panel 1 + 2). A dose of 0.003 μg produced a slight but significant effect when given before acquisition; effects were seen for preretrieval doses of 0.3 to 30 μg. A dose of 0.03 μg did not affect avoidance behavior of FS-NA animals. The lack of effect of Met-enkephalin on FS-NA rats has been confirmed in other experiments in doses up to 30 μg (*11*).

Leu-enkephalin did not significantly reduce amnesia when injected before acquisition (Fig. 1C, panel 1) but reversed amnesia when given before retrieval (Fig. 1C, panel 2) or at both times (Fig. 1C, panel 1+ 2). A dose of 3 μg did not influence performance of FS-NA rats.

The anti-amnesic effect of Leu-enkephalin is similar to that of ACTH 4-10 and β-LPH in that it is only apparent when the peptide is given shortly before retrieval. In contrast, Met-enkephalin also attenuates amnesia when given before acquisition. A similar pattern of activity has been reported for a vasopressin analog (*2*). In addition to the present results, other data suggest that, apart from potency differences, Leu- and Met-enkephalin may have different pharmacologic properties. The differential influence of sodium on opiate receptor interactions of the two pentapeptides has

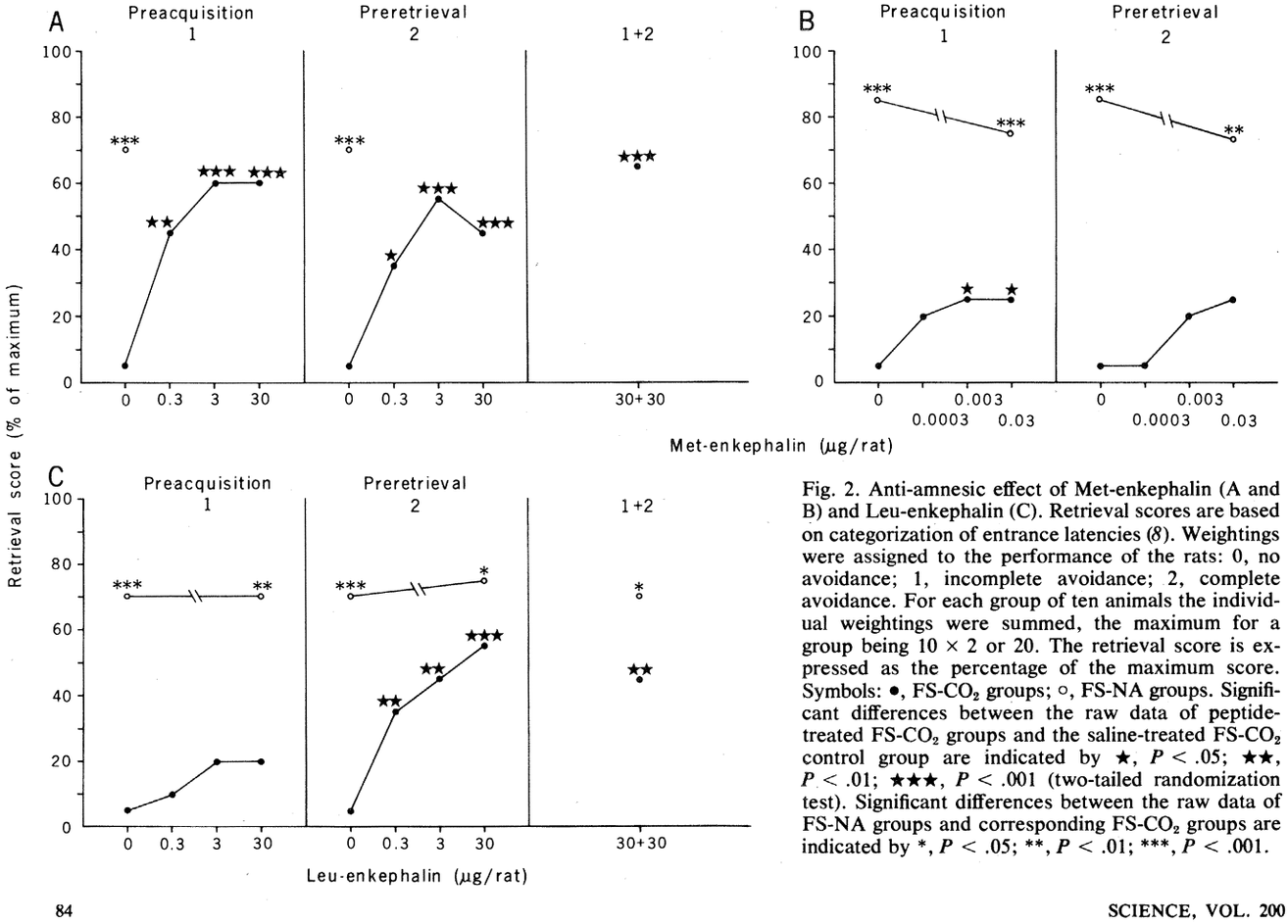

Fig. 2. Anti-amnesic effect of Met-enkephalin (A and B) and Leu-enkephalin (C). Retrieval scores are based on categorization of entrance latencies (*8*). Weightings were assigned to the performance of the rats: 0, no avoidance; 1, incomplete avoidance; 2, complete avoidance. For each group of ten animals the individual weightings were summed, the maximum for a group being 10 × 2 or 20. The retrieval score is expressed as the percentage of the maximum score. Symbols: •, FS-CO_2 groups; ○, FS-NA groups. Significant differences between the raw data of peptide-treated FS-CO_2 groups and the saline-treated FS-CO_2 control group are indicated by ★, $P < .05$; ★★, $P < .01$; ★★★, $P < .001$ (two-tailed randomization test). Significant differences between the raw data of FS-NA groups and corresponding FS-CO_2 groups are indicated by *, $P < .05$; **, $P < .01$; ***, $P < .001$.

been taken as an indication that Leu-enkephalin may be a "purer" agonist than Met-enkephalin (*12*). Intraventricular Met-enkephalin lowered but Leu-enkephalin enhanced pain threshold in a test involving sustained low-intensity pain (*13*). Furthermore, intraventricular administration of a high dose (320 μg) of Met-enkephalin elicited in rats a stuporous immobility that could be prevented by naloxone. The same dose of Leu-enkephalin, on the other hand, induced naloxone-insensitive rotational behavior (*14*). Pharmacologic differences between both peptides are consistent with the suggestion that Met- and Leu-enkephalin may derive from different precursors (*15*).

The anti-amnesic effect of the pentapeptides was observed after systemic administration of low doses. This is in sharp distinction to the analgesic effects of Leu- and Met-enkephalin, which are only seen after intracranial administration of huge quantities of these substances (100 μg or more) (*13, 14, 16*). Behavioral activity of low doses of systemically administered Met-enkephalin has also been shown by Plotnikoff *et al.* (*17*). The anti-amnesic effect of enkephalins is presumably not mediated through opiate receptors. This is suggested by the failure of naloxone to prevent this behavioral effect of the pentapeptides (*18*).

Several explanations are possible to account for the anti-amnesic effect of the enkephalins. Thus, the prevention of CO_2-induced amnesia by Met-enkephalin (the preacquisition effect) may be due either to a facilitation of memory consolidation or to a protection of the animals against the adverse effects of the amnesic agent. The preretrieval effect of the enkephalins may either result from facilitated retrieval of a weak, spared memory trace or from a specific reversal of a CO_2-induced disturbance of memory retrieval. Further studies are necessary to clarify the modes of action of the enkephalins. The present data suggest that the enkephalins may directly or indirectly modulate memory processes but it is premature to postulate a crucial role of these peptides in memory.

HENK RIGTER

Department of Pharmacology, Scientific Development Group, Organon International, Oss, The Netherlands

References and Notes

1. H. Rigter, S. Shuster, A. J. Thody, *J. Pharm. Pharmacol.* **29**, 110 (1977).
2. H. Rigter, H. van Riezen, D. de Wied, *Physiol. Behav.* **13**, 381 (1974).
3. W. H. Gispen, J. M. van Ree, D. de Wied, *Int. Rev. Neurobiol.*, in press.
4. J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature (London)* **258**, 577 (1975).
5. H. Rigter and H. van Riezen, in *Current Developments in Psychopharmacology*, W. B. Essman and L. Valzelli, Eds. (Spectrum, New York, 1977), vol. 5, in press.
6. R. Ader, J. A. W. M. Weijnen, P. Moleman, *Psychonom. Sci.* **26**, 125 (1972).
7. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, London, 1956), pp. 152–156. Group differences associated with probability values of $P < .05$ were accepted as statistically significant.
8. No avoidance: entrance latencies of 0 to 10 seconds. This category is based on my experience that virtually all day 4 entrance latencies of nonshocked rats fall in this range (*2*). Incomplete avoidance: entrance latencies of 11 to 179 seconds. Complete avoidance: failure of an animal to enter within 180 seconds. The validity of the present categorization is apparent from the fact that an analysis of the categorized data yields essentially the same pattern of statistical significance as the analysis of the raw data (*7*). The categorized data can be analyzed with the Yates test [F. Yates, *Biometrika* **35**, 178 (1948)].
9. Met-enkephalin and Leu-enkephalin were synthesized by H. M. Gfeven, Organon.
10. Mean entrance latencies at the acquisition trial were as follows. Experiment 1: saline-treated animals (N = 50), 1.1 seconds; Met-enkephalin, 0.3 μg (N = 10), 1.3 seconds; 3 μg (N = 10), 1.0 second; 30 μg (N = 20), 1.1 seconds. Experiment 2: saline (N = 60), 1.2 seconds; Met-enkephalin, 0.0003 μg (N = 10), 1.0 second; 0.003 μg (N = 10), 1.0 second; 0.03 μg (N = 20), 1.3 seconds. Experiment 3: saline (N = 60), 1.6 seconds; Leu-enkephalin, 0.03 μg (N = 10), 1.5 seconds; 3 μg (N = 10), 1.8 seconds; 30 μg (N = 40), 1.3 seconds.
11. H. Rigter, unpublished data.
12. R. Simantov and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2515 (1976).
13. L. Leybin, C. Pinsky, F. S. LaBella, V. Havlicek, M. Rezek, *Nature (London)* **264**, 458 (1976).
14. J.-K. Chang, B. T. W. Fong, A. Pert, C. B. Pert, *Life Sci.* **18**, 1473 (1976).
15. A. Goldstein, *Science* **193**, 1081 (1976).
16. J. D. Belluzi, N. Grant, V. Garsky, D. Sarantakis, C. D. Wise, L. Stein, *Nature (London)* **260**, 625 (1976); A. Z. Rónai, J. I. Székely, L. Graf, Z. Dunai-Kovács, S. Bajusz, *Life Sci.* **19**, 733 (1976).
17. N. P. Plotnikoff *et al.*, *Life Sci.* **19**, 1283 (1976)
18. H. Rigter, H. Greven, H. van Riezen, *Neuropharmacology* **16**, 545 (1977).
19. I thank R. Janssens-Elbertse for skillful assistance and J. Crabbe, H. Greven, H. van Riezen, and D. de Wied for stimulating interest and advice.

23 April 1977; revised 23 November 1977

Polyribosomes Associated with Forming Acrosome Membranes in Guinea Pig Spermatids

Abstract. *Ribosomes, some of which are arranged in polyribosomal configurations, are attached to specialized regions of the acrosomal membrane in guinea pig spermatids. This finding indicates a new functional dimension for the acrosomal membrane, that of protein synthesis, and suggests that during acrosome formation, proteins of the acrosomal membrane or acrosomal contents need not be synthesized before or during passage through the Golgi apparatus.*

Protein synthesis is generally thought to be restricted to polyribosomes or polyribosome-rich regions of the cell. For the most part, two classes of polyribosomes are recognized: those bound to rough endoplasmic reticulum and those that occur free in the cytoplasm (*1*). Possible exceptions to this classification are the Golgi apparatus polyribosomes first reported by Franke *et al.* (*2–4*). These polyribosomes are not membrane-bound in the strict sense; rather they represent a special class of free polyribosomes found within the Golgi apparatus zone of exclusion (*5*). Golgi apparatus polyribosomes have been isolated from rat liver and are functional in protein synthesis (*4*).

We report here particles resembling ribosomes that are associated with parts of the acrosomal membrane of developing spermatids (Fig. 1, A and B). The particles are confined to the part of the acrosomal membrane covering the acrosomal vesicle (headcap); no particles are found on any part of the acrosomal membrane in contact with the acrosomal granule. These ribosome-like particles (RLP's) react to fixative and stain like ribosomes. For example, RLP's are preserved after glutaraldehyde–osmium tetroxide fixation but are lost after potassium permanganate fixation. Also, when the method of Bernhard (*6*) is used to distinguish between RNA and DNA, the glutaraldehyde-fixed RLP's have characteristics of RNA; that is, they stain positive with uranyl acetate and lead citrate and do not destain after treatment with EDTA.

Most RLP's are dispersed over the surface of the acrosomal membrane (Fig. 1, A to D). Aggregates of three or more RLP's are common (Fig. 1D). These aggregates of RLP's often extend outward from the membrane surface (Fig. 1D). The RLP's attached to the membrane appear loosely bound and do not seem to penetrate the membrane surface (Fig. 1, C and D). In many instances, the RLP's appear attached to the acrosomal membrane by short segments of filamentous material (Fig. 1C).

Ribosome-like particles are present on the acrosomal membrane beginning with the clear-cut differentiation of the headcap (stage IV to stage V) up to the beginning of nuclear condensation. At this stage of development, the endoplasmic reticulum becomes closely aligned along the surface of the acrosome and the RLP's are no longer evident. The number of RLP's associated with the acrosomal membrane seems to increase during

0036-8075/78/0407-0085$00.50/0