# RELEASE OF α-MSH FROM THE *IN VITRO* SUPERFUSED NEURO-INTERMEDIATE LOBE OF THE PITUITARY OF THE RAT BY ANTIDIURETIC HORMONE

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**Summary** : The effect of antidiuretic hormone (ADH) on the release of immunoreactive α-melanocyte stimulating hormone (α-MSH) from the superfused neurointermediate lobe of the pituitary of the normal Wistar and Brattleboro (diabetes insipidus) rat was stiudied *in vitro*. In control experiments, there was usually an initial peak, after which α–MSH release fell exponentially over the course of the perfusion. Follwowing the addition of ADH, the levels of α–MSH in the superfusate showed a significant rise. It is suggested that ADH is normally involved in the secretion of α–MSH by the pars intermedia of the rat, especially in response to osmotic stimuli.

**Key words** : antidiuretic hormone pars intermedia α–MSH secretion

## INTRODUCTION

From studies in normal rats, it is established that α–MSH can be released from the pars intermedia of experimental animals in response to hyper-osmotic stimuli (6 8, 9, 10) and it appears likely that this is mediated by antidiuretic hormone (8). However, it cannot be deduced from such *in vivo* experiments whether the hormone is acting directly at the level of the neurointermediate (NI) lobe or indirectly (e.g. via some brain centre). Therefore, as a first step towards elucidating the mechanism of MSH secretion by hyperosmotic stimuli, it was decided to carry out *in vitro* experiments to see if antidiuretic hormone (ADH) applied directly to the NI lobe could effect the release of α-MSH. For these experiments, the whole organ perifusion system of Edwardson and his colleagues (1,7) was employed.

In the removal and preparation of the NI lobe for perifusion, neural lobe fibers are inevitably damaged and it is likely that some endogenous antidiuretic hormone (ADH) is released into the perifusion medium, particularly at the start of the experiment. It was therefore decided to conduct additional experiments on neurointermediate lobes of the Brattleboro strain of rat, which has no endogenous ADH (12).

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## MATERIAL AND METHODS

A total of 31 adult rats weighing between 150-350 *gms* was used ; they included 19 normal rats (both male and female) of the Wistar strain and 12 homozygous rats of the Brattleboro strain (both male and female) exhibiting marked diabetes insipidus (DI). They were housed under standard conditions of lighting and temperature (14 hr light : 10 hr darkness cycle; 22°±1°C), fed on the usual pellet diet (MRC diet 86) and allowed tap water *ad libitum*.

For the perifusion (the nutrients from the continuous flowing bath diffuse into the isolated organ from the periphery) procedure, a rat was decapitated without anaesthesia, its NI lobe rapidly dissected out and immediately transferred to approximately 1 *ml* of Krebs Ringer solution, pending introduction into the superfusion (perifusion) apparatus. This consisted of a water jacketed perspex block, divided into two sections separated by a fine metal wire gauze (Fig. 1). The neurointermediate lobe was introduced into one of the two sections ; only one NI lobe was superfused at a time.

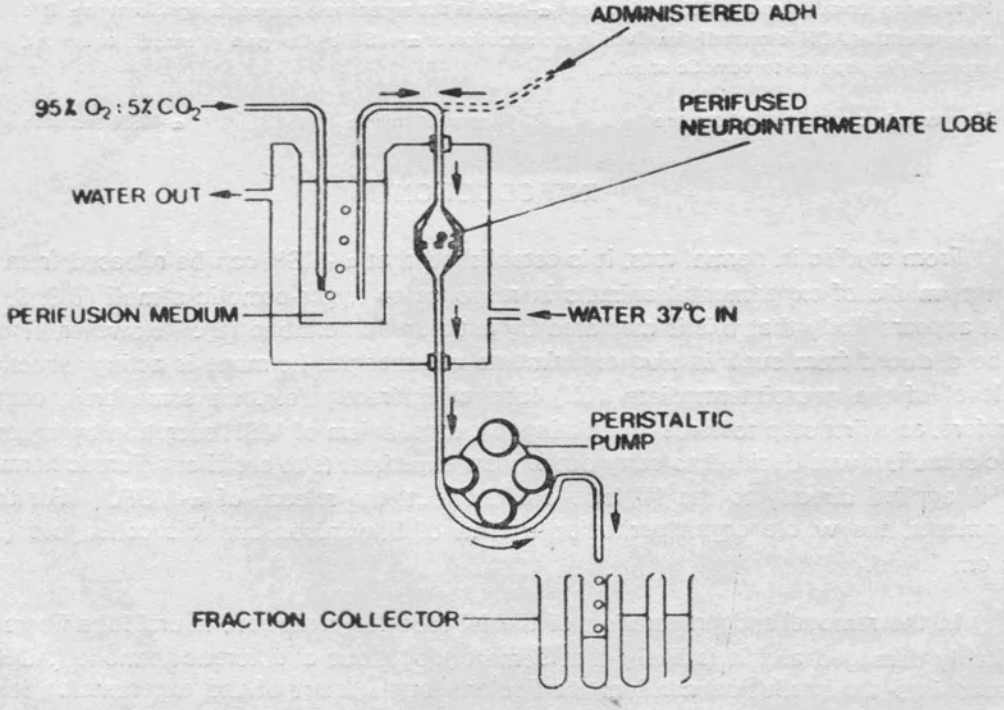


FIG.1 DIAGRAM OF PERIFUSION APPARATUS

One end of the superfusion apparatus was connected by plastic tubing to a water jacketed chamber, maintained at 37°C and charged with Krebs Ringer bicarbonate buffer, containing 0.25% bovine serum albumin, 0.2% glucose and constantly gassed with a 95% oxygen/5% $CO_2$ Mixture The other end of the superfusion apparatus was connected by silastic tubing with an LKB Bromma 1200 vario-perpex peristaltic pump, which sucked the Krebs Ringer fluid through the perpex block and expelled it at a rate of 200 $\mu l\ min^{-1}$.

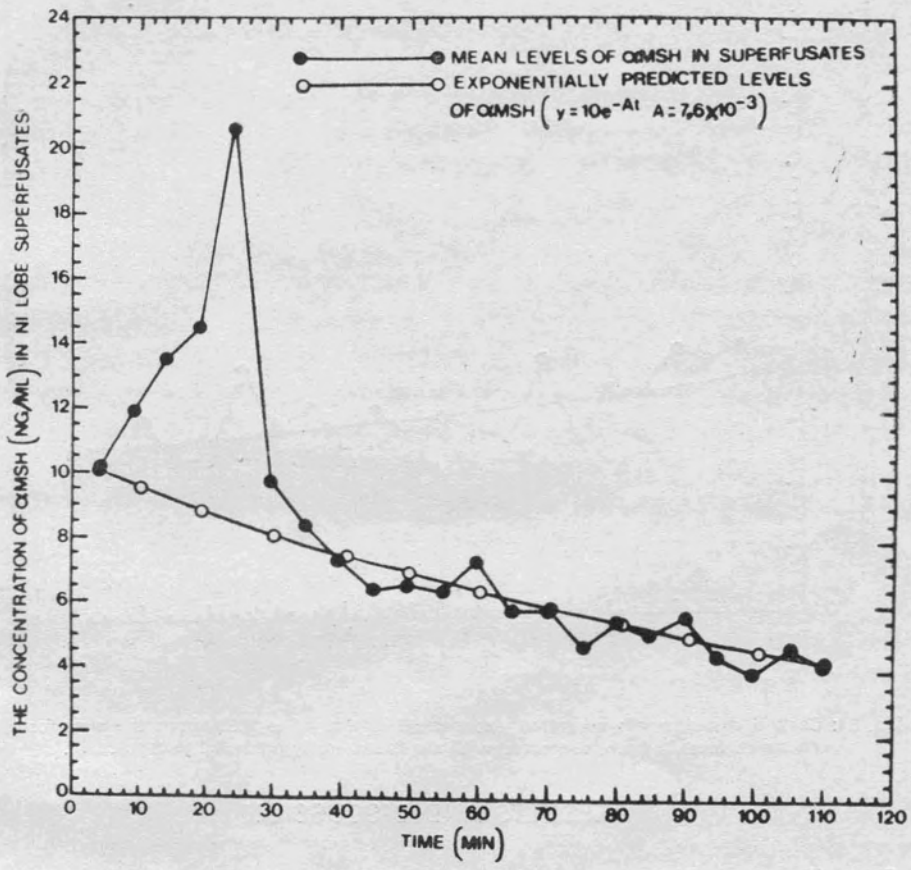


Fig. 2 : Control experiment : measured exponential release of α-MSH from the superfused neurointermediate lobe, compared with the theoretically predicted release curve. This figure also illustrates an example of the initial peak of MSH released at the begining of the perifusion.

The superfusate was collected as 1 *ml* fractions every 5 min for 110 min, using an LKB Bromma 7000 Ultrorac Fraction Collector ; these were snap frozen with dry ice-methanol mixture and stored at –20°C until assayed. The α–MSH content of the superfusates was assayed by a radioimmunoassay method (11). At various times (as indicated in the Figs. 4, 5 and 6) after the start of the perifusion (50 or 100 mins), a pulse of 50 or 500 mU lysine ADH (in 1 *ml* of Krebs Ringer solution) was introduced into the system Control tissues were similarly perifused, without the addition of ADH (Figs. 2 and 3).

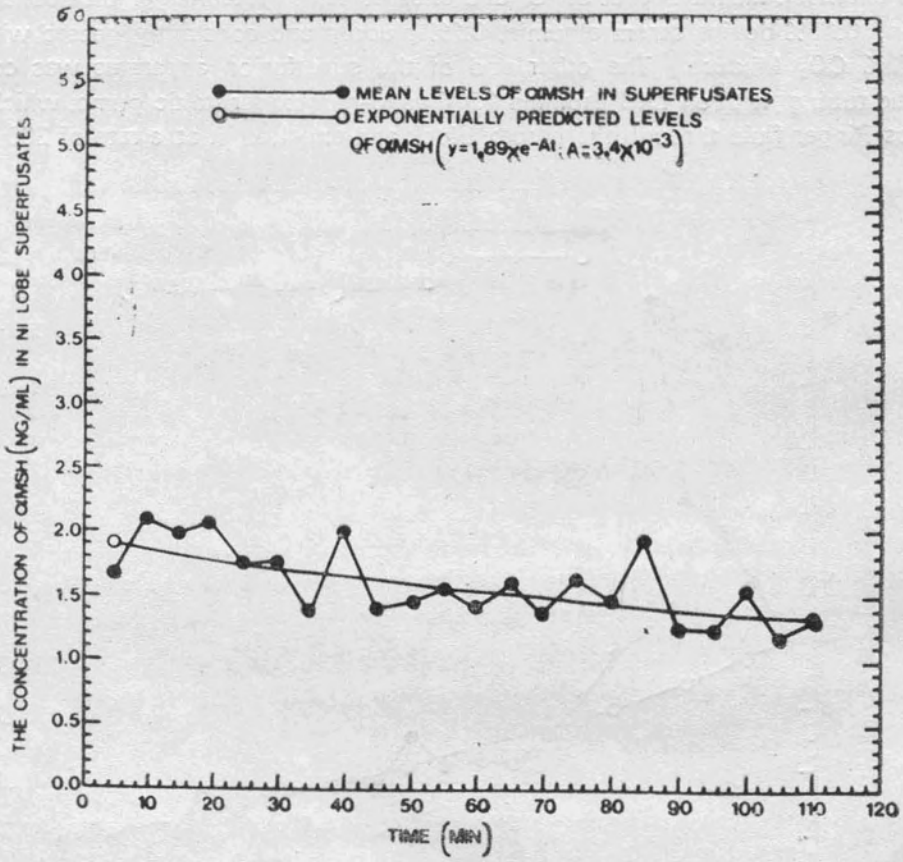


Fig. 3 : Control experiment : measured exponential release of α–MSH from the superfused neurointermediate lobe, compared with the theoretically predicted release curve.

## RESULTS AND DISCUSSION

From the start of the superfusion, there was usually a sharp increase in the levels of α–MSH released by the neurointermediate lobes, reaching a peak by some 20-25 mins (Figs. 2 and 4); thereafter, the levels of MSH released fell steadily. This pattern of release was exhibited by pituitaries of both Wistar and Brattleboro animals. Sometimes, this initial peak was absent, the release of MSH simply following a steady exponential decline throughout the period of the perifusion (Figs. 3, 5, 6). Since from control experiments it was found that the titer of α-MSH in the perfusate effluent did not quickly reach a plateau level, but continued to fall exponentially for the duration of the perfusion (110-175 min), we decided not to wait an unduly long period for the preparation to settle to a stable baseline, as this would run the risk of tissue deterioration and possible unrespon-

siveness. Rather, we decided to test the effect of ADH on the NI lobe after a suitable period of perfusion (some 50-100 min was adopted), sufficient to avoid, the initial peak of MSH output that sometimes follows *in vitro* incubation. However, the lack of a flat

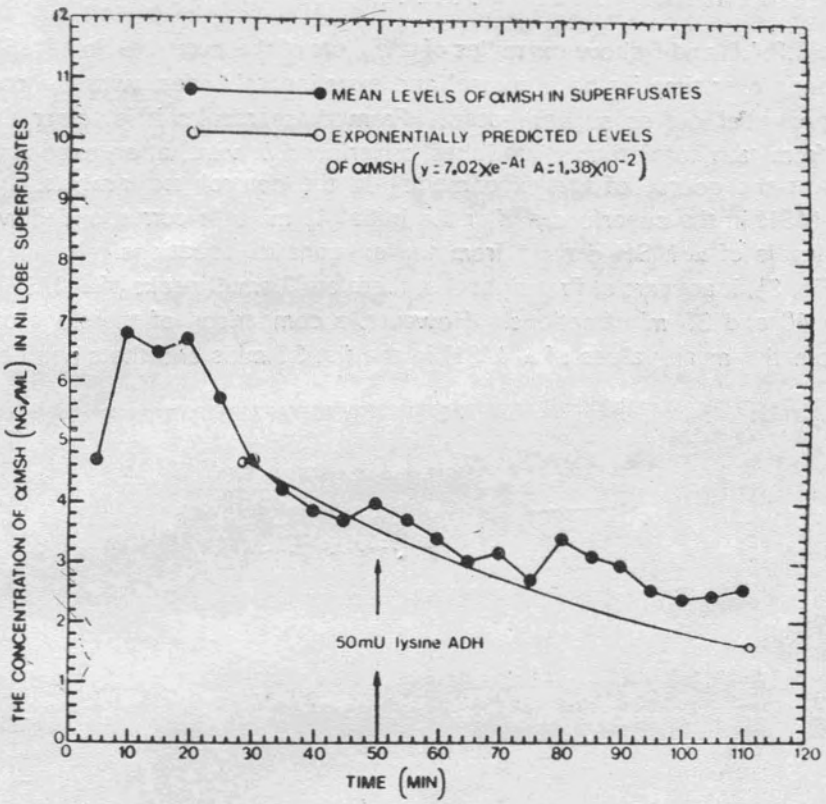


Fig. 4 : The effect of additing 50 mU lysine ADH to the perfusion medium on the release of α-MSH from the superfused neurointermediate lobe of Normal (Wistar) rat, compared with the theoretically predicted release curve in the absence of ADH. (This figure also illustrates the initial peak of MSH release at the beginning of perifusion, prior to addition of ADH ; vide also Fig. 2.).

base-line makes it difficult to judge visually whether any deviations in the release curve observed after the administration of ADH are to be interpreted as a direct result of the action of the hormone. Further mathematical treatment of the results was therefore necessary and it was decided to superimpose best fit exponential curves of the mean values of MSH in the superfusates, over the whole period of time for the control experiments and over the period of perifusion before the addition of ADH in the case of experimental series. In both cases, these derived curves were plotted over the remainder of the perifusion period in order to determine any deviations in α-MSH from the mean levels following the addition of ADH, the exponential fits were of the form $y = Ke^{-At}$ where :

y = the coordinates of y axis (i.e. the unknown or the theoretical α–MSH level)
K = constant
A = slop of the straight line
t = time of each fraction (i.e. 5, 10 . . . . . ., 105, 110 mins)

Figures 2,3,4,5 and 6 show examples of the plots of the mean levels of MSH in superfusate fractions over time for both control and experinental series, with the corresponding best fit exponential curves superimposed. From these results it is clear that α–MSH is released from rat neurointermediate lobes, superfused *in vitro*, at an exponentially decaying rate over the course of the experiment. In the control experiments, the measured levels of α–MSH in the superfusate after the initial 40 minutes corresponded well with the theoretical levels of α–MSH derived from the exponential equations. In one control experiment (Fig. 3), it appears at first sight that there are 2 small peaks of α–MSH, corresponding to the 40 and 85 min fractions. However, a comparison of the slope of the points obtained from the mean values of α–MSH in the fist 35 mins and the slope obtained from

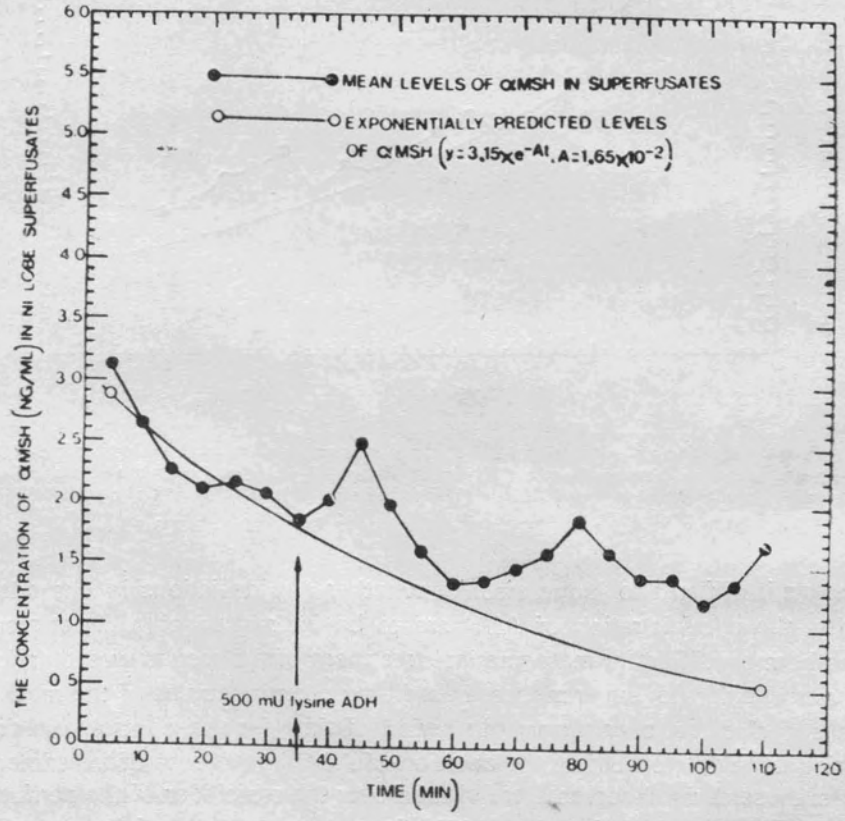


Fig. 5 : The effect of adding 500 mU lysine ADH to the perfusion medium on the release of α–MSH from the superfused neurointermediate lobe of Normal (Wistar) rat, compared with the theoretically predicted release curve in the absence of ADH.

the mean values of α–MSH over the subsequent 40 to 110 mins shows that they had, in fact, an identical value (0.003), confirming the exponential nature of the release curve. After the addition of ADH (50 or 500 mU), the levels of α–MSH in the superfusate fractions were consistently higher than those predicted theoretically from the exponential curves (see Figs. 4, 5 and 6). Hence, in both Wistar and Brattleboro rats, an increase in the rate of release of α–MSH from the NI lobe *in vitro* follows the application of ADH.

From such experiments, however, it cannot be deduced whether the release of α–MSH is due to the direct action of ADH *per se* on pars intermedia cells or is a secondary effect, perhaps via the mediation of some posterior lobe factor, such as MSH-releasing factor (MRF). It has been proposed that MRF is formed by the enzymatic cleavage of oxytocin (2,4), in which case one might expect the neural lobe of both Wistar and Brattleboro rats to contain both MRF and oxytocin. While MRF is a possible candidate, it may be that other yet unidentified releasing factors are involved. Since we have little information concerning the viability of the tissue, diffusion characteristics or the levels of cellular respiration in this *in vitro* situation, and no idea of the proportion of pars intermedia cells that may be reached by the ADH or able to react, further discussion on the mechanism of release must remain speculative for the present.

The initial peak of α–MSH, which sometimes appears in he superfusates shortly after the neurointermediate lobes are removed from the animals and placed in the perifusion apparatus (see Figs. 2 and 4), suggests that, early on, there is an increase in the release processes of the glands. This could be interpreted as support for the well known

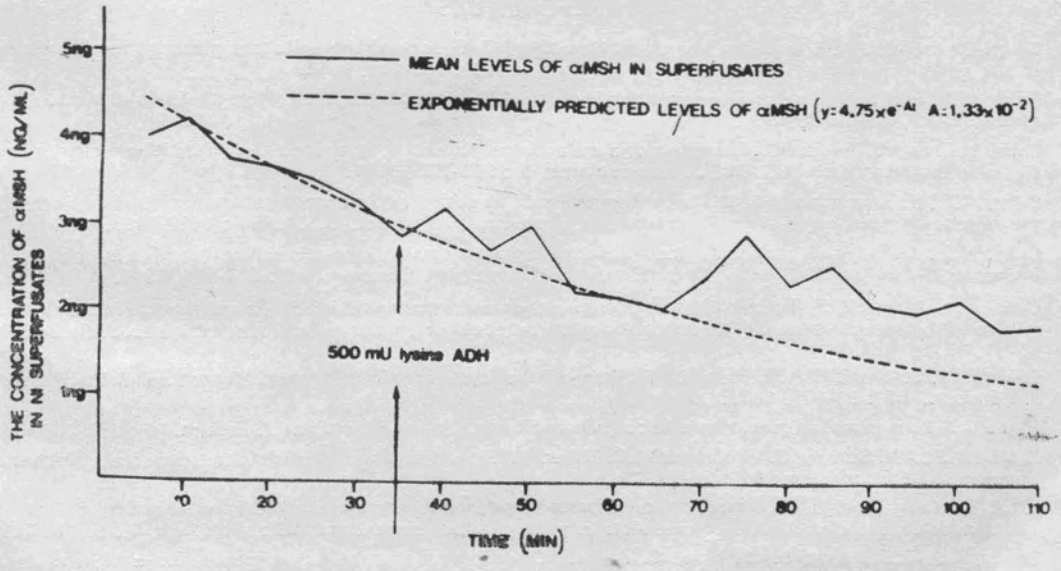


Fig. 6 : The effect of adding 500 mU lysine ADH to the perfusion medium on the release of α–MSH from the superfused neurointermediate lobe of Brattleboro (DI) rat, compared with the theoretically predicted release curve in the absence of ADH.

concept of a central nervous 'tonic' inhibitory control, normally operating on the secretion of α–MSH from the parsintermedia *in vivo* (8), which is lost in these *in vitro* conditions when the connections between brain and gland are disrupted. Moreover, since such peaks usually occur some 10 to 25 mins after the start of the superfusion, this might indicate the presence of some inhibitory substance(s) in the neurointermediate lobe itself which are progressively eluted by the Krebs Ringer fluid over that period (3,5).

There may, however, be alternative explanations. One possibility is that the initial low levels of α–MSH in the first fractions of the superfusates are artefacts, due to the lowering of biological (enzymatic) activities by the fall in temperature of the tissue, since the freshly exercised neuro-intermediate lobes were kept briefly at room temperature before the start of the superfusion experiments. Hence, the time lag in α–MSH release might be an expression of the time taken for the return of such activities as the tissue regains its normal physiological (37°C) temperature in the perifusion apparatus.

Thus, from *in vitro* experiments, using normal and DI rat neuro-intermediate lobes, it appears that directly administered ADH can cause the release of α-MSH. Whether ADH is a normal integrel part of the release mechanism for MSH *in vivo*, for example under conditions of hyperosmotic stress, requires further investigation.

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## REFERENCES

1. Beloff-Chain, A., J.A. Edwardson and Janet Hawthorn. Influence of the pituitary gland on insulin secretion in the genetically obese (ob/ob) mouse. *J. Endocr.*, **65** : 109-116, 1975.
2. Celis, M.E. and S. Taleisnik. *In vitro* formation of a MSH releasing agent by hypothalamic extracts. *Experientia*, **27** : 1481-1482, 1971.
3. Celis, M.E. and S. Taleisnik. Formation of a melanocyte stimulating hormone release inbibiting factor by hypothalamic extracts from rats. *Int. J. Neuroscience*, **1** : 223-230, 1971.
4. Celis, M.E., S. Taleisnik and R. Walter. Regulation of formation and proposed structure of the factor inhibiting the release of melanocyte-stimulating hormone, *Proc. natn. Acad. Sci., U.S.A.*, **68** : 1428-1433, 1971.
5. Celis, M.E., S. Taleisnik and R. Walter. Release of pituitary melanocyte-stimulating hormone by the oxytocin fragment, H-Cys-Tye-Ile-Gln-Asn-OH. *Biochem. biophys. Res. Commun.*, **45** : 564-569, 1971.
6. Cyrkowicz, A. and W.Z. Traczyk. Effect of intravenous and intracarotid injections of hypertonic fluids on the release of melanocyte-stimulating hormone from the rat posterior pituitary lobe incubated *in situ*. *J. Endocr.*, **66** : 85-91, 1975.
7. Edwardson, J.A. and C.A.M. Hough. Pituitary-adrenal system of the genetically obese (ob/ob) mouse. *J. Endocr.*, **65** : 99-107, 1975.
8. Howe, A. The mammalian pars intermedia : a review of its structure and function. *J. Endocr.*, **59** : 385-409, 1973.
9. Kastin, A.J., A.V. Schally, S. Viosca and M.C. Miller. MSH activity in plasma and pituitaries of rats after various treatments. *Endocrinology*, **87** : 20-27, 1969.
10. Orias, R. Cambics en la concentracion de hormona melanocitoestimulante (MSH) en hipofisis de rata por deshidratacion soluciones hipertonicas. *Acta physiologica latinoamericana*, **16** : suppl. vol. 1., p. 95, 1966.
11. Thody, A.J., R.J. Penny, D. Clark and C. Taylor. Development of a radioimmunoassay for α-MSH in the rat. *J. Endocr.*, **67** : 385-395, 1975.
12. Valtin, H., J. Steward and H.W. Sokol. Genetic control of the production of posterior pituitary principles Handbook of Physiology. *Endocrinology Section*, **7** : Vol IV, 131-171, 1974.