contacted discs (figure 2) was similar to aggregation at the original discs, demonstrating that pheromone can be successfully extracted in this medium. This is in agreement with findings for Argas persicus[3].

Highly significant aggregation of female Ap. concolor on nymphal exuviae of that species ($p < 0.001$) occurred within 30 min (figures 3 and 4), and far exceeded the level of aggregation achieved with tick-contacted discs over the same period, although the final aggregation achieved with the disc assays was similar. It is not known whether the exuviae represented a more concentrated source of pheromone than the discs, or whether the more rapid aggregation was due to a different pheromone. Female-contacted discs produced significant aggregation of female ticks of I. holocyclus and Ap. concolor at distances up to 80 cm and 40 cm respectively (table 2). Aggregation under natural conditions may assist both species in their host seeking success. In the case of I. holocyclus, where copulation between unfed adults has been reported[16] and where, presumably, off-host mating may occur under natural conditions, aggregation of males and females could be an important factor in the fertilization of females. The attraction of nymphal cuticles for adults of Ap. concolor would certainly intensify the aggregation. However, there is no information available of the local distribution of adults of either species in an infested site.

16 D. F. Moorehouse, J. med. Ent. *3*, 168 (1966).

Natriuresis after water loading in rats bearing transplants of pars intermedia

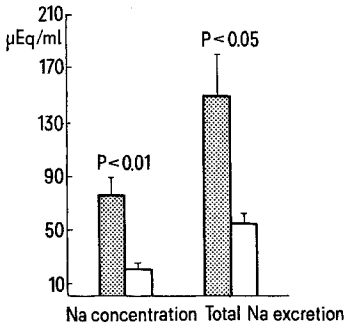
H. P. Levitin, J. San Román and F. C. Iturriza[1]

Laboratorio de Neuroendocrinología, Cátedra 'B' de Histología y Embriología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata, 1900 La Plata (Argentina); and Escuela de Oncología de la Provincia de Buenos Aires, 8–706, 1900 La Plata (Argentina), 10 August 1976

Summary. After water loading, rats bearing ocular grafts of the pars intermedia of the pituitary showed that the content of sodium increased in urine, whereas potassium excretion and diuresis did not vary significatively. These results suggest that in the rat the pars intermedia is involved in the regulation of the electrolyte metabolism.

Orias and McCann[2,3] showed that natriuresis rose in water loaded rats after the injection of melanocyte-stimulating hormone (MSH). Whether the natriuretic effect elicited by the dose level used by these authors evidenced an actual 'physiological' response, or whether it should be assigned to a 'pharmacological' one, is a point under discussion since little is known on the ability of the pars intermedia to secrete MSH at levels similar to the ones reached in blood after the exogenous treatment. Moreover, the question remains if the hormone secreted by the rat pituitary could evoke a natriuretic response in its own species.

Kastin and Ross[4] were the first to show in rats what is nowadays a well accepted fact that the pars intermedia disconnected from the hypothalamus oversecretes MSH. This observation allows us to design a simple model for obtaining animals with continuous high levels of circulating endogenous MSH, offering a condition useful for clarifying the problem.



Urine sodium excretion in grafted and control rats. Values are the mean of 5 rats. Dotted bars correspond to grafted animals and clear bars to controls. p-values are at the top of each bar.

Materials and methods. Male Sprague-Dawley rats weighing 250–260 g at the moment of the transplantation were used. One neurointermediate lobe per receptor animal was grafted into the anterior chamber of the right eye, according to the technique of Olson and Malmfors[5]. Donors were rats of the same age and sex. After 70 days the grafts were observed under a stereomicroscope and those rats showing healthy, well growing transplants were selected together with an equal number of sham operated animals. From the moment of transplantation to the completion of the studies, the rats were maintained in a photoperiod of 12 h of darkness and 12 h of illumination, at a temperature 19–21 °C.

5 grafted rats and 5 controls were placed individually in metabolic cages and at the time of the experiment (09.00 a.m.) the rats were given by stomach tube 10 ml tap water. 1 h later they received a second gavage of the same volume. Urine was collected for 2 h from the moment of the second water loading. After the volume was measured, sodium and potassium content was estimated by flame spectrophotometry and expressed as µEq of cation excretion. During urine collection the animals were deprived of water and food intake.

After completion of the experiment, the rats were decapitated and blood collected in heparinized tubes. Plasma was obtained after centrifugation and it was diluted 2:1 with distilled water. Plasma MSH activity was tested in this material, semiquantitatively, using skins of the toad

1 Professor and Chairman of Histology. Member of the Investigator Career of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

2 R. Orias and S. M. McCann, Proc. Soc. exp. Biol. Med. *133*, 469 (1970).

3 R. Orias and S. M. McCann, Endocrinology *90*, 700 (1972).

4 A. J. Kastin and G. T. Ross, Endocrinology *75*, 187 (1964).

5 L. Olson and T. Malmfors, Acta physiol. scand., suppl. 348 (1970).

Bufo arenarum, according to the reflectometric method of Shizume et al.[6], adapted for using with the Argentinian toad[7].

Observations. In the figure it can be seen that in the grafted rats urine sodium concentration and total excretion of sodium rose, while urine volume and potassium excretion did not change significatively. Plasma of all the transplanted rats showed the ability to darken the toad skins, whereas darkening was not reflectometrically detectable when the plasma of the controls was assayed.

Discussion. Plasma of the rats bearing ocular transplants darkened the toad skins. Since the plasma of intact normal rats has no darkening activity when tested in skins of B. arenarum[8], it can be assumed that the grafted rats showed raised levels of MSH or MSH-like substances in their plasma. After water loading the rats displaying high levels of circulating MSH excreted significant by larger amounts of sodium when compared with the controls. Urine volume and potassium excretion did not vary, indicating that the natriuresis found was absolute.

These observations allow us to realize that the effect formerly described by Orias and McCann[2,3] could be assigned to a 'physiological' mechanism. This assumption had been previously suggested by us after observing that the MSH-releasing drug, fluphenazine, induced natriuresis[9]. The results herein reported lead us to speculate that the physiological role of MSH shifts to a new function, parallel with phylogenetic evolution. In lower vertebrates, the hormone mediates the well-known mechanisms involving tegumentary colour changes, whereas in mammals it seems to participate in the electrolyte balance. Previous studies showing that the morphology of the pars intermedia varies according to the availability of water[10], and that the injection of hypertonic saline elicits release of MSH[11], support the hypothesis expressed above.

6 K. Shizume, A. B. Lerner and T. B. Fitzpatrick, Endocrinology *54*, 553 (1954).

7 F. C. Iturriza, C. G. Ferese and R. Raña, Acta physiol. latinoam. *22*, 140 (1972).

8 M. E. Celis, R. Macagno and S. Taleisnik, Endocrinology *93*, 1229 (1973).

9 H. P. Levitin and F. C. Iturriza, Acta physiol. latinoam. *24*, 275 (1974).

10 H. Legait and F. Legait, in: Neurosecretion, p. 165. Ed. Heller and Clark. Academic Press, New York–London 1962.

11 A. J. Kastin, Fed. Proc. *26*, 255 (1967).

Comparison of the effectiveness of intramuscular and intraperitoneal ACTH in the rat[1]

N. Yasuda, K. Takebe and M. A. Greer

Division of Endocrinology, Department of Medicine, University of Oregon Medical School, Portland (Oregon 97201, USA), 11 October 1976

Summary. Injection of 1–24 ACTH is more effective by the i.m. than i.p. route. Large doses are required to induce consistent maximal adrenal corticosterone secretion.

ACTH is usually given either i.v. or i.p. to experimental animals[2,3]. In evaluating the adrenal response to ACTH in intact rats, i.v. ACTH may not be appropriate, since it requires stressful maneuvers, such as a skin incision or a tourniquet placed on the root of the tail, which may induce secretion of endogenous ACTH to add to the effect of exogenously administered ACTH. In the present study, seeking the most appropriate route for injection of ACTH in intact rats, we compared the effect of i.m. or i.p. injection of synthetic 1–24 ACTH.

Materials and methods. Male Sprague-Dawley rats weighing 250–300 g were housed 2/cage with controlled lighting (lights on 06.00–18.00 h) and temperature (24 ± 1 °C). Purina Laboratory Chow and tap water were allowed ad lib. The animal quarters were not entered 10 h prior to the experiment to standardize the experimental conditions. 30 ng to 30 μg/0.1 ml 0.9% saline/100 g b.wt of synthetic 1–24 ACTH (Cortrosyn, Organon), or an equivalent volume of saline, was given i.p. or i.m. in the thigh. At various times after injection, 0.3 ml heparinized blood samples for corticosterone measurement were obtained from the subclavian vein via percutaneous venipuncture, under < 3 min ether anesthesia. In some experiments, plasma ACTH as well as corticosterone concentration was measured. In such experiments, dexamethasone phosphate (Decadron, Merck, Sharp & Dohme) 100 μg/0.1 ml/100 g b.wt was given i.p. at 07.00–07.30 h to inhibit stimulation of endogenous ACTH secretion at the time of sampling, and pentobarbital (Nembutal, Abbott) 4 mg/0.5 ml/100 g b.wt was given i.p. 4 h later. 5 min after pentobarbital injection, synthetic 1–24 ACTH or saline was injected. Heparinized blood (1.5 ml) was obtained for both ACTH and corticosterone measurement at various times after ACTH injection. All blood samples were collected between 10.00 and 13.00 h. ACTH was measured by radioimmunoassay[4]; corticosterone was measured by

Table 1. Plasma corticosterone 30 or 45 min after 100 or 300 ng/100 g b.wt i.m. or i.p. 1–24 ACTH injection

Dose and route of ACTH injection	Plasma corticosterone (μg/100 ml) 30 min	45 min
100 ng i.m.	10.1 ± 2.2	13.2 ± 10.1
100 ng i.p.	13.4 ± 6.5	5.6 ± 0.9
300 ng i.m.	14.4 ± 2.2	9.5 ± 2.8
300 ng i.p.	35.4 ± 18.0	5.1 ± 0.3

Mean and SE of 5 rats are shown for each time point.

1 Supported by grants from the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland, USA.

2 A. M. L. Van Delft, J. Kaplanski and P. G. Smelik, J. Endocr. *59*, 465 (1973).

3 E. Haus, Ann. N. Y. Acad. Sci. *117*, 292 (1964).

4 L. H. Rees, D. M. Cook, J. W. Kendall, C. F. Allen, R. M. Kramer, J. G. Ratcliffe and R. A. Knight, Endocrinology *89*, 254 (1971).

5 M. A. Greer, C. F. Allen, P. Panton and J. P. Allen, Endocrinology *96*, 718 (1975).

6 B. J. Winer, in: Principles in Experimental Design. McGraw-Hill, New York 1962.