Table II. Effect of splenectomy on the anaphylactic response to EA in *Calotes versicolor*

Group treatment[a]	Time of shocking dose	No. animals	−	+	D[b]
Ie splenectomy	14 days after sensitization	10	10	0	0
Ic sham	14 days after sensitization	10	2	8	8
IIe splenectomy	21 days after sensitization	5	5	0	0
IIc sham	21 days after sensitization	5	2	3	1

[a] Animals were sensitized 7 days after operation. [b] Symbols as in Table I.

antibody-mediated and 2. splenectomy deprives the animal of the ability to synthesize antibodies that are responsible for the manifestation of anaphylaxis. In higher animals, spleen plays a definite role in humoral antibody synthesis, especially when small doses of antigen and i.v. routes are employed[15]. It is probable, therefore, that in the lizard the spleen plays a definite role in the production of antibodies that mediate anaphylaxis, since small dose of antigen and intra-cardiac route have been employed.

That the maximum mortality occurs at the end of the 2nd week after sensitization (Figure) may be due to the persistence of more antibodies in the tissues. This is in correlation with the maximum fatal sensitivity observed in birds on 14th day[16]. The specificity of the antigen-antibody interaction is revealed by the unresponsiveness of EA sensitized lizards to the shocking dose of bovine serum albumen (BSA) as reported in fishes[3] and in other species[5]. Further studies are underway to characterize the nature of antibody, mediators and the immunological mechanisms involved in anaphylaxis.

Summary. Anaphylaxis was induced in the lizard, *Calotes versicolor* by egg albumen. The symptoms were similar to those found in other species. The maximum mortality occurred 14 days after sensitization and the reaction was specific. Splenectomy before sensitization abrogated the manifestations of anaphylaxis.

S. Jayaraman[17] and
Vr. Muthukkaruppan[18]

Department of Biological Sciences, Madurai University, Madurai-625021 (India), 21 March 1975.

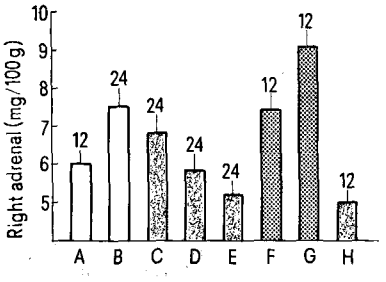
[1] N. Cohen, Am. Vet. med. Ass. *159*, 1662 (1971).
[2] P. G. H. Gell and R. R. A. Coombs in *Clinical Aspects of Immunology* (Eds. P. G. H. Gell and R. R. A. Coombs; Blackwell, Oxford 1968), p. 575.
[3] N. B. Dreyer and J. W. King, J. Immun. *60*, 277 (1948).
[4] T. Makinodan, H. R. Wolfe, M. Goodman and R. Ruth, J. Immun. *68*, 219 (1952).
[5] F. R. Aronson, N. M. Bilstad and H. R. Wolfe, Poultry Sci. *40*, 319 (1960).
[6] S. G. Cohen, T. M. Sapp and J. R. Shaskas, J. Allergy *47*, 121 (1971).
[7] T. C. Fletcher and B. A. Baldo, Science *185*, 360 (1974).
[8] C. M. Downs, J. Immun. *15*, 77 (1928).
[9] P. Kanakambika and Vr. Muthukkaruppan, J. Immun. *109*, 415 (1972).
[10] V. Manickavel and Vr. Muthukkaruppan, Transplantation *8*, 307 (1969).
[11] P. Kanakambika and Vr. Muthukkaruppan, Experientia *28*, 1225 (1972).
[12] Vr. Muthukkaruppan, P. Kanakambika, V. Manickavel and K. Veeraraghavan, J. Morph. *130*, 479 (1970).
[13] B. C. Seegal in *Agents of Disease and Host Resistance* (Charles C. Thomas, Springfield, USA 1935), p. 54.
[14] R. J. Turner, J. exp. Zool. *183*, 35 (1973).
[15] J. W. Uhr and M. S. Finkelstein, Progr. Allergy *10*, 37 (1966).
[16] J. E. Gahringer, J. Immun. *12*, 477 (1926).
[17] Grateful to the Madurai University for the award of studentship during the tenure of the work.
[18] This work was supported in part by a PL-480 (NIH) research grant, No. 01-077.

Opposite Effects of Vasotocin Injected Intrapituitarly and Intraventricularly on Corticotropin Release in Mice

We have previously reported that the mammalian pineal, including that of man, contains[1,2] and synthesizes[3] the specific octapeptide arginine vasotocin (AVT), and that minute amounts of AVT injected into the 3rd ventricle of the mouse inhibit gonadotropin[4] and corticotropin[5] release. Since in vitro AVT has ACTH-releasing activity[6], and when injected into the 3rd ventricle, on the contrary, inhibits ACTH release[5], the present study compares the effects of AVT injected intrapituitarly and intraventricularly on the compensatory adrenal hypertrophy (CAH) in unilaterally adrenalectomized mice. Male RAP (Rockland for All Purposes) mice weighing 18–22 g were used. The mice were unilaterally adrenalectomized under Evipan anesthesia. The uninjected controls were sham adrenalectomized under the same anesthesia. 1 h later, synthetic AVT in a volume of 2 µl was injected into the left lobe of the pituitary or into the 3rd ventricle. Controls received 2 µl saline. The substances were injected via a 26 gauge needle with a micrometer syringe attached to a Horsley-Clark rat stereotactic apparatus, as described[5]. All animals were killed 3 days postoperatively and the remaining (right) adrenal was cleaned and weighed fresh to the nearest 0.1 mg on a torsion balance. Adrenal weights were expressed as mg/100 body weight. The data were evaluated statistically by the Student *t*-test. As shown in the Figure, a single injection of 10 pg AVT/µl into the anterior pituitary of the mouse on the day of surgery, significantly potentiated CAH measured 3 days later, whereas the same concentration of AVT injected into the 3rd ventricle produced adrenal atrophy. Lower concentrations of AVT between 0.001 and 0.00001 pg/µl injected into the pituitary were

[1] S. Pavel, Endocrinology *77*, 812 (1965).
[2] S. Pavel and S. Petrescu, Nature, Lond. *212*, 1054 (1966).
[3] S. Pavel, M. Dorcescu, R. Petrescu-Holban and E. Ghinea, Science *181*, 1252 (1973).
[4] S. Pavel, M. Petrescu and N. Vicoleanu, Neuroendocrinology *11*, 370 (1973).
[5] S. Pavel, L. Mătrescu and M. Petrescu, Neuroendocrinology *12*, 371 (1973).
[6] M. Saffran, Br. med. Bull. *18*, 122 (1962).

without effect on CAH. However, when injected into the 3rd ventricle, the concentration of 0.00001 pg AVT/µl, corresponding to only 6000 molecules AVT/µl, completely prevented CAH. Since the total volume of injections was 2 µl, this corresponds to 12,000 molecules AVT/mouse. To our knowledge, this represents the lowest concentration so far reported for an active biological substance able to produce an endocrine effect. In the range tested, the effect of AVT appears to be dose-dependent since 0.000001 pg AVT/µl only partially inhibited CAH, whereas 0.001 pg AVT/µl produced adrenal atrophy. Excepting the mice from group C (Figure), statistical analysis indicates a highly significant difference between the adrenal weights of mice injected with AVT intrapituitarly and intraventricularly ($p < 0.001$). There is now convincing evidence that unilateral adrenalectomy induces CAH by increasing ACTH secretion[7], and that the pineal exerts an inhibitory influence on adrenal cortex through the suppression of ACTH[8–11]. Since AVT injected into the pituitary is without effect on CAH or, on the contrary, potentiates CAH, and since the presumed physiological site for AVT release from the pineal is the cerebrospinal fluid[12,13], the present results, although indirect, strongly suggest that AVT injected into the 3rd cerebral ventricle of the mouse reversed CAH by inhibiting synthesis and/or release of ACTH-releasing hormone. CAH inhibition by AVT is highly specific, because neither oxytocin, nor arginine vasopressin are able to inhibit CAH when injected into the 3rd ventricle of the mouse[5]. Since in vitro AVT has ACTH-releasing activity[6], the potentiation of CAH by AVT injected into the pituitary probably represents an unspecific ACTH-releasing activity common to all basic octapeptides[14]. Indeed (GREIDANUS and DE WIED, personal communication), AVT given i.v. in chlorpromazine pentobarbital blocked rats, releases ACTH, suggesting that preventing AVT from exerting its inhibitory effects on ACTH-releasing hormone, it retained only its unspecific ACTH-releasing activity. Although the mechanism of action of such a small number of molecules cannot be explained at present, our results clearly demonstrate that the mammalian brain contains the most sensitive receptor for AVT so far described.



Effects of synthetic AVT injected into the pituitary and into the 3rd ventricle of the brain, on adrenal hypertrophy produced in male mice by unilateral adrenalectomy (UA). A, sham operated controls; B, UA + 2 µl saline, 3rd ventricle; C, UA + 0.000001 pg AVT/µl, 3rd ventricle; D, UA + 0.00001 pg AVT/µl, 3rd ventricle; E, UA + 0.001 pg AVT/µl, 3rd ventricle; F, UA + 0.001 pg AVT/µl, anterior pituitary; G, UA + 10 pg AVT/µl, anterior pituitary; H, UA + 10 pg AVT/µl, 3rd ventricle. Numbers above bars indicate number of animals; vertical lines represent standard error of the mean.

Summary. A single injection of 10 pg synthetic arginine vasotocin into the pituitary significantly potentiated adrenal hypertrophy produced in male mice by unilateral adrenalectomy, whereas the same concentration injected into the 3rd ventricle produced adrenal atrophy.

S. PAVEL[15]

Institute of Endocrinology, Bucuresti (Romania), 8 March 1975.

[7] W. F. GANONG, in *The Hypothalamus* (Eds. L. MARTINI, M. MOTTA and E. FRASCHINI; Academic Press, New York 1970), p. 313.
[8] G. A. KINSON, B. SINGER and L. GRANT, Gen. comp. Endocr. *10*, 447 (1968).
[9] M. MOTTA, O. SCHIAFFINI, F. PIVA and L. MARTINI in *The Pineal Gland* (Eds. G. E. W. WOLSTENHOLME and J. KNIGHT; Churchill, Livingstone, London 1971), p. 279.
[10] M. K. VAUGHAN, G. M. VAUGHAN, R. J. REITER and B. BENSON, Neuroendocrinology *10*, 139 (1972).
[11] K. L. DICKSON and D. L. HASTY, Acta endocr., Copenh. *70*, 438 (1972).
[12] S. PAVEL, Endocrinology *89*, 613 (1971).
[13] S. PAVEL, Nature, Lond. *246*, 183 (1973).
[14] W. DOEPFNER, E. STÜRMER and B. BERDE, Endocrinology *72*, 897 (1963).
[15] I should like to thank Prof. M. BODANSZKY, Case Western Reserve University, Cleveland, Ohio, for the synthetic AVT.

Effets de micro-doses de testostérone et de 5α-DHT sur l'épididyme de lézard castré (*Lacerta vivipara* Jacquin) en culture organotypique

Effects of Microdoses of Testosterone and 5α-DHT on Castrated Lizard (*Lacerta vivipara* Jacquin) Epididymis Cultivated in vitro

Lors de précédentes études nous avons montré que l'épididyme de lézard castré pouvait être maintenu en culture organotypique sans changement appréciable pendant au moins 10 jours[1]. L'addition de testostérone, de 5 α-DHT (17 β-hydroxy-5-α-androstane 3one), de 3 α-et de 3 β-androstanediol (5α-androstane-3 α, 17 β-diol et 5 α-androstane-3 β, 17 β-diol) au milieu de culture a conduit à une reprise d'activité de l'organe[2,3]; cependant les réponses obtenues (hypertrophie de l'épithélium et activité sécrétoire) avec chacune de ces hormones aux doses de 5 µg/ml (20 µM/ml) de milieu montrent des degrés différents: le 3 β-androstanediol et la 5 α-DHT sont les plus efficaces puis vient le 3 α-androstanediol et enfin la testostérone. Nous nous sommes demandé à quoi pouvait tenir cet effet moindre de la testostérone.

La testostérone n'est-elle pas l'androgène actif chez cette espèce, ou bien la testostérone se métabolise-t-elle difficilement dans nos conditions expérimentales?

Pour répondre à la première question, nous avons éprouvé par la même méthode et aux mêmes doses un autre androgène également présent dans le testicule de lézard: l'androstène dione[4]. Cette hormone induit une

[1] A. GIGON, C. GATHIER et J. P. DUFAURE, C. r. Acad. Sci., Paris *276*, 2391 (1973).
[2] A. GIGON, C. r. Acad. Sci., Paris *278*, 2815 (1974).
[3] J. P. DUFAURE et A. GIGON, Gen. comp. Endocr., accepté pour publication.
[4] C. LUPO DI PRISCO, G. CHIEFFI et G. DELRIO, Experientia *23*, 73 (1967).