Our preliminary findings reported above support the theory that auto-antigen formation through modification by any aggression of the constituents of the venous tissue would result in antibody production indicating the aggression. Increased production of these auto-antibodies (by repeated aggressions and/or dysfunction of the antibody-forming systems) could render these antibodies pathogenic to the vascular tissue, thus possibly perpetuating the course of the venous disease.

Résumé. Dans 70% des cas examinés, les auteurs ont montré l'existence d'anticorps antiveines dans le plasma des malades atteints de thrombophlébithe. Il est à présumer que des facteurs auto-immunes jouent quelque rôle dans la pathogenèse des thrombophlébites.

S. Gero, Judit Székely, Eva Szondy, A. Jobbágy, A. Orosz and Eva Seregélyi

Semmelweis University of Medicine, IIIrd Department of Medicine, Mezo Imre ut. 17, Budapest VIII (Hungary), 9 February 1973.

Effect of Synthetic Luteinizing Hormone Releasing Hormone on Pituitary and Serum Levels of Luteinizing Hormone of Intact and Median Eminence Lesioned Male Rats

Various reports have indicated that the hypothalamus may control both the synthesis and release of pituitary follicle stimulating hormone (FSH)[1-4]. The availability of synthetic luteinizing hormone (LH) releasing hormone (LRF), which is known to stimulate the secretion of both hypophysial FSH and LH[5], provided us with the opportunity to test the hypothesis that this specific hypothalamic neurohormone may regulate both the synthesis and release of pituitary LH.

Methods and materials. Synthetic LRF[6] was injected into the jugular vein of mature male rats (Sprague-Dawley, 225–250 g; 5–10 rats/group), both intact animals and those that had borne ME lesions for approximately 10 days, at which time pituitary LH was significantly depressed. The lesions had been made by applying a direct current of 5 mA/15 sec. A study was carried out to determine optimal post-injection time to detect major changes in pituitary and serum LH in both intact and ME-lesioned animals.

For the dose-response studies, 45 min after injection of LRF blood was withdrawn by cardiac puncture and the animals were immediately decapitated. Anterior pituitaries were removed, pooled, weighed, homogenized and diluted with physiological saline for use in the Parlow ovarian ascorbic acid depletion assay for LH[7]. The standard NIH-LH-S17 was tested at 2 or 3 levels, with the total doses ranging from 0.4 μg to 6.4 μg (4-fold interval). Pituitary homogenates were assayed at total doses of 0.5 mg and 2.0 mg. Five assay rats were employed at each dose level. Estimates of pituitary LH concentration were calculated by the method of Bliss[8]. Results are expressed in terms of NIH-LH-S1. Serum LH levels were determined by the double antibody radioimmunoassay technique of Niswender et al.[9], and are expressed in terms of NIAMD-Rat LH-RP-1.

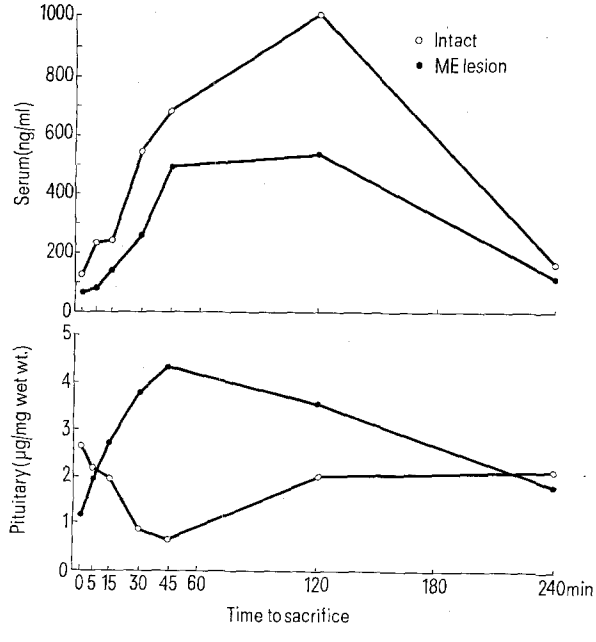

Fig. 1. Time study: Effect of synthetic LRF (100 μg/rat, i.v.) on pituitary and serum LH of intact and ME-lesioned mature male rats.

Results and discussion. Figure 1 represents the effect of a single i.v. injection of 100 μg LRF on pituitary and serum LH of intact and ME-lesioned rats in relation to time. In the intact rat, as pituitary LH was being significantly depleted ($P \leq 0.05$ vs. intact + saline), serum LH rose significantly ($P \leq 0.05$ vs. intact + saline). Maximal pituitary LH depletion occurred at 45 min whereas serum LH continued to rise, reaching its apex at 120 min post-injection. Animals with ME lesions, whose pituitary and serum LH levels were significantly depressed ($P \leq 0.05$ and $P \leq 0.01$ vs. sham lesion, respectively), exhibited maximal and significant pituitary LH repletion at 45 min ($P \leq 0.01$ vs. ME lesion + saline) associated with corresponding maximal and significant increments in serum LH ($P \leq 0.01$ vs. ME lesion + saline.) Both pituitary and serum LH levels in both types of recipients returned to baseline values by 4 h after LRF administration.

Figure 2 represents combined means of LRF stimulation studies performed individually but in triplicate and clearly

[1] J. S. Evans and M. B. Nikitovitch-Winer, Neuroendocrinology *4*, 83 (1969).
[2] A. Corbin, J. E. Milmore and E. L. Daniels, Experientia *26*, 1010 (1970).
[3] A. Corbin and J. E. Milmore, Endocrinology *89*, 426 (1971).
[4] A. Corbin, in *The Regulation of Mammalian Reproduction* (Eds. S. J. Segal, R. Crozier, P. A. Corfman and P. G. Condliffe; C. C. Thomas, Springfield 1973), p. 45.
[5] A. V. Schally, A. Arimura and A. J. Kastin, Science *179*, 341 (1973).
[6] Wyeth Compound No. 16,558.
[7] A. F. Parlow, in *Human Pituitary Gonadotropins* (Ed. A. Albert; C. C. Thomas, Springfield 1961), p. 300.
[8] C. I. Bliss, *The Statistics of Bioassay* (Academic Press, New York 1952).
[9] G. N. Niswender, A. R. Midgley, jr., S. E. Monroe and L. E. Reichert jr., Proc. Soc. exp. Biol. Med. *128*, 807 (1968).

demonstrates the log dose related alterations in both hypophysial and serum LH values. Intact animals showed a prominent depletion of pituitary LH with a concomitant rise in serum LH; in contrast, ME-lesioned recipients exhibited marked repletion of pituitary LH with simultaneous increments in serum LH.

These data provide strong evidence supporting the concept that a specific hypothalamic hormone can control the synthesis and release of pituitary LH. Initial substantiation was provided by the fact that ME lesions not only reduced pituitary LH stores, but also concomitantly lowered serum levels of this gonadotropic hormone. Further substantiation was obtained from the effect of synthetic LRF employed as the stimulus. The present results agree with those reported by us[2-4] concerning the effect of crude hypothalamic extracts on pituitary FSH, and support other reports[10-13] pertaining to a dual effect of hypothalamic neurohormones on the synthesis and release of pituitary tropic hormones. Furthermore, the present data clearly demonstrate that both synthesis of LH and its release into the blood can occur simultaneously[14].

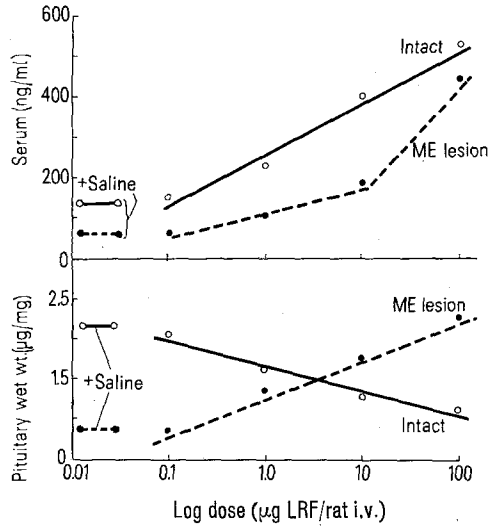

Fig. 2. Dose-response study: Effect of various doses of synthetic LRF on pituitary and serum LH of intact and ME-lesioned mature male rats.

Résumé. Des rats mâles intacts ou ayant une lésion du ME ont reçu une seule injection intrajugulaire de LRF synthétique. Les variations de taux de LH hypophysaire et sérique (en fonction du temps et de la dose réponse) montrent que le LRF contrôle la sécrétion (la décharge et la synthèse) du LH hypophysaire.

A. CORBIN and G. VIRGINIA UPTON

Wyeth Laboratories, Research Division, Endocrinology Section, Box 8299, Philadelphia (Pennsylvania 19101, USA); and Endocrine and Polypeptide Laboratories, Veterans Administration Hospital, West Haven (Connecticut 06516, USA), 5 March 1973.

[10] Y. C. LIN, M. TAKAHASHI and Y. SUZUKI, Endocrinologia jap. *19*, 145 (1972).
[11] W. C. WORTHINGTON JR., S. E. FOLSOM JR. and M. G. BUSE, Endocrinology *90*, 1664 (1972).
[12] T. W. REDDING, A. V. SCHALLY, A. ARIMURA and H. MATSUO, Endocrinology *90*, 764 (1972).
[13] H. STEINER, M. MOTTA, F. PIVA, D. GILLESSEN and R. O. STUDER, Acta endocrin. Copenh. Suppl. *155*, 5 (1971).
[14] The technical assistance of K. KNUDSEN, J. BELL, C. CAVALCANTO, K. KOCH and J. TRACY is gratefully acknowledged.

Sensitivity to Mutagens of *Rumex acetosa* Chromosomes

Data on the radiosensitivity of several species of the genus *Rumex* have been reported[1]. Some of these species were found to be highly sensitive due especially to their large nucleus and chromosomes. Few data on the effects of chemicals on such chromosomes have been reported so far. Only a difunctional alkylating agent, diepoxybutane, was tested[2].

On the other hand, several authors described the ability of some chemicals to break preferentially the sex chromosomes of mammals. Since the occurrence of a $X\ Y_1\ Y_2$ mechanism of sex determination has been demonstrated in *Rumex acetosa*[3], it was worthwile getting information on the sensitivity of such chromosomes to mutagens. For this reason, we selected two chemicals well known for their chromosome-breaking ability, methyl-methane-sulfonate and methyl-nitroso-urea. Their effects will be compared with those of ionizing radiations.

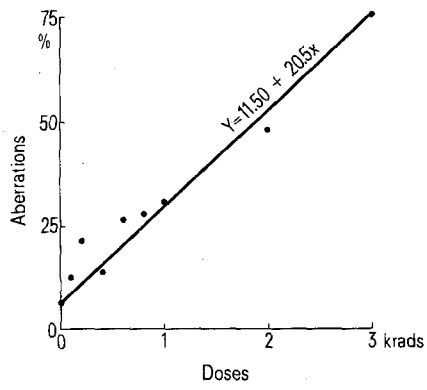

Fig. 1. Effects on ^{60}Co γ-rays on *Rumex* chromosomes (400 metaphases analyzed at each exposure).

Material and methods. *Rumex acetosa* L. cultivar 'Large de Belleville' was chosen for the present investigation. Karyotype of this species, as well as some karyological particularities, were previously described[4]. Dry seeds were irradiated by ^{60}Co γ-rays (25 °C, dose-rate 300 krads/h) at doses ranging from 100 to 3000 rads. Methyl-methane-sulfonate (MMS, Eastman Kodak) was used at concentrations ranging from 1×10^{-3} *M* to 1×10^{-2} *M* for 3 h and methyl-nitroso-urea (MNU, synthesized by the Biochemical Institute of Stockholm) at concentrations ranging from 5×10^{-4} *M* to 5×10^{-3} *M*/3 h. All the solutions were prepared extemporaneously. After this, seeds were abundantly washed, then sown on moisted filter paper in Petri dishes and incubated at 21 °C. After 2 days, primary

[1] S. ICHIKAWA and A. SPARROW, Genetics *54*, 341 (1966).
[2] J. ZUK, Heredity *24*, 69 (1969).
[3] H. KIHARA and T. ONO, Bot. Mag., Tokyo *37*, 84 (1923).
[4] J. MOUTSCHEN, N. DEGRAEVE and B. MONFORT, Cytologia *37*, 119 (1972).