Evidences for a Hypocalcemic Factor from Pituitary Gland

Thyrocalcitonin is the hypocalcemic factor found in the thyroid gland[1-3]. So far as we know, there is no convincing evidence that a pituitary control may exist in the production and/or release of hypocalcemic factor. The present study is concerned with the existence of a pituitary substance which stimulates either thyroid or parathyroid or both glands to release hypocalcemic factor.

For this study 111 albino rats, weighing 150–200 g, and 300 guinea-pigs were used. All rats were placed on a low calcium diet for 3 days. Hypophysectomy was performed according to the method of FALCONI et al.[4]. The experiment was started on the day after hypophysectomy. Thyroparathyroidectomy was performed by excision, and 10 min later pituitary extract was injected i.v. All bioassay rats were anaesthetized with pentobarbital. A fine polyethylene catheter was inserted into the heart through the jugular vein for infusion and for extraction of blood. Blood calcium was determined by the method of REHELL[5]. 5 different experiments were carried out in this study.

Experiment 1. Production of hypercalcemia in hypophysectomized rats: hypophysectomized rats were made hypercalcemic by infusion of a solution containing 6 mg of calcium and their urine was collected (1.5–2 ml) and made calcium-free. The procedures of urine collection and precipitation of urinary calcium have been described elsewhere[6]. Calcium-free urine specimens were infused into normal bioassay rats. Repeated blood samples were obtained for a period of 90 min.

Experiment 2. The infusion of pituitary gland extract: the pituitary glands of guinea-pigs were removed immediately after decapitation. They were homogenized in chilled normal saline. The crude homogenates were then subjected to centrifugation at 11,000 *g* for 10 min at 4 °C and only the supernatant was used. The pituitary extract of 10 animals was infused into each normal rat within 1 min. Control animals were injected with 0.9% saline only. Blood samples were obtained after 5, 10, 20 and 30 min.

Experiment 3. Infusion of the pituitary gland extract into thyroparathyroidectomized bioassay rats: the same procedures were used as in experiment 2.

Experiment 4. The infusion of various hormones: rats were divided into 5 groups and were injected i.v. with 10 U of commercial corticotropin, 800 U of chorionic gonadotropin, 3 U of thyrotropin, 0.2 U of vasopressin (pitressin) and 0.15 U of insulin, to stimulate endogenous growth hormone secretion[7], respectively. Repeated blood samples were obtained during a period of 60 min.

Experiment 5. Infusion of heated or digested pituitary gland extract: a part of the pituitary extract was heated for 5 min in boiling water, another part was subjected to tryptic digestion according to the method of LASCOWSKI[8]. The amount of the pituitary extract and the procedures were used as in experiment *2.*

Statistical analysis. The difference between the means were tested by Student's *t*-test[9].

Results and discussion. There was no significant fall in blood calcium level except in the experiment 2 (Table) where the lowest calcium level was reached within 10 min, then there was a gradual increase but the initial level was never attained during the entire period of observation.

Our previous study showed that the urine of the hypercalcemic normal rats contained a blood calcium lowering factor which was not found in the urine of thyro-para-

[1] P. F. HIRSCH, E. F. VOELKEL and P. L. MUNSON, Science *146*, 412 (1964).
[2] G. V. FOSTER, A. BAGDIANTZ, M. A. MUMAR, E. SLACK, H. A. SOLIMAN and I. MACINTYRE, Nature *202*, 1303 (1964).
[3] M. A. KUMAR, E. SLACK, A. EDWARDS, H. A. SOLIMAN, A. BAGDIANTZ, G. V. FOSTER and I. MACINTYRE, J. Endocr. *33*, 469 (1965).
[4] G. FALCONI and G. L. ROSSI, Endocrinology *74*, 301 (1964).
[5] E. REHELL, Scand. J. clin. Lab. Invest. *6*, 355 (1954).
[6] M. S. ZILELI, G. KANRA, G. URUNAY and S. CAGLAR, Turk. J. Pediat., submitted for publication.
[7] J. ROTH, S. M. GLICK, R. S. YALOW and S. A. BERSON, Diabetes *13*, 355 (1964).
[8] M. LASCOWSKI, in *Method in Enzymology* (Ed. S. P. COLOWICK and N. O. KAPLAN; Academic Press, New York 1955).
[9] G. W. SNEDECOR, *Statistical Methods*, 5th edn (Iowa State University Press, Iowa 1956).

Plasma calcium levels before and after injection of different solutions

Experiments	No. of rats	Operations	Infused solutions	Plasma calcium control values means ± S.E.	Level, mg% lowest values means ± S.E.	Significance
1	23	Hypophysectomy	6 mg of calcium in 5% glucose solution	–	–	–
	23	–	Urine	10.456 ± 0.343	10.182 ± 0.343	> 0.05
2	10	–	Pituitary extract	10.760 ± 0.215	7.830 ± 0.215	< 0.001[a]
	10	–	physiological saline	10.260 ± 0.145	9.810 ± 0.145	
3	10	Thyropara-thyroidectomy	Pituitary extract	10.780 ± 0.306	11.190 ± 0.306	> 0.05
4	5	–	10 U of corticotropin	11.200 ± 0.161	10.900 ± 0.089	> 0.05
	5	–	800 U of chorionic gonadotropin	11.400 ± 0.202	10.440 ± 0.200	> 0.05
	5	–	0.2 U of pitressin	10.240 ± 0.148	9.700 ± 0.275	> 0.05
	3	–	3 U of thyrotropin	10.733 ± 0.040	10.300 ± 0.149	> 0.05
	5	–	0.15 U of insulin[b]	11.080 ± 0.312	10.840 ± 0.312	> 0.05
5	5	–	Boiled pituitary extract	11.380 ± 0.157	11.040 ± 0.157	> 0.05
	5	–	Digested pituitary extract	10.780 ± 0.459	10.340 ± 0.459	> 0.05

[a] As compared with saline infused control. [b] Infusion of insulin produced a significant fall in blood glucose levels (8–24 mg%).

thyroidectomized hypercalcemic rats[6]. It was suggested that the origin of the urinary hypocalcemic factor was thyroid or parathyroid or from both glands. The present study (experiment 1) revealed that the urine specimens of hypophysectomized hypercalcemic rats contains no hypocalcemic factor. The above experiments indicate that, after the removal of the pituitary gland, the functional capacity of the thyro-parathyroid glands to produce the hypocalcemic factor is lost. The results also indicate that hypercalcemia causes the release of a pituitary factor which stimulates a hypocalcemic factor from thyro-parathyroid. Furthermore, experiment 2 shows the presence of a calcium lowering substance in the pituitary gland. This substance is found to be effective only in the presence of thyro-parathyroid glands (experiment 3). The pituitary hypocalcemic factor is different from known pituitary hormones, as suggested by NATELSON et al.[10] and as observed in experiment 4. The hypocalcemic effect of the guinea-pig pituitary extract, under the present experimental conditions, is much faster than that observed by NATELSON et al. This discrepancy may be due to the species difference of animals used and/or to the mode of administration of pituitary extract. Loss of its activity by boiling or by tryptic digestion may suggest that this pituitary factor is a protein or a polypeptide.

Zusammenfassung. In der Hypophyse wurde ein Faktor festgestellt, welcher die Ausschüttung von Thyreocalcitonin fördert. Die Ausschüttung dieses Kalzium-senkenden Hormones scheint einer hypophysären Regulation unterstellt zu sein.

M. S. ZILELI, G. KANRA, G. ÜRÜNAY, T. GÜNER and S. CAGLAR

Metabolic Division, Hacettepe Medical School, Ankara (Turkey), 30 October 1967.

[10] S. NATELSON, J. B. PINCUS and G. RANNAZZISI, Fedn Proc. Fedn Am. Socs exp. Biol. *22*, 420 (1963).

Observations on the Ultrastructure of the Non-Articulated Laticifers of *Jatropha podagrica* (Euphorbiaceae)

One of the most enigmatic cell types in the Angiospermae is the non-articulated laticifer. Despite a wealth of light microscope information on the morphology of adult laticifers and considerable biochemical information on the synthesis of rubber, an understanding of the role of this system in the living plant is lacking. In addition, no description of the fine structure of non-articulated laticifers has been published other than MOOR's detailed analysis of the walls of laticifers of *Euphorbia splendens*[1]. Information on the ultrastructure of the cytoplasm of laticifers might give some clue to their function. This communication contains some of the early findings of an electron microscope investigation of non-articulated laticifers.

Immature 2.0 mm embryos of *Jatropha podagrica* were dissected from seeds and fixed for 4 h in cold 6% glutaraldehyde in 0.06 *M* Sorensen's phosphate buffer at pH 6.9. After fixation they were washed with several changes of the same buffer. Embryos for light microscopy were dehydrated with a graded acetone series and embedded in epon. Embryos for electron microscopy were post-fixed for 30 min in unbuffered 2% OsO_4 and dehydrated with acetone. These embryos remained overnight in 70% acetone containing 1% uranyl nitrate before final dehydration and embedding. Thick $1^1/_2$ μ sections for light microscopy and thin sections for electron microscopy were cut with Dupont diamond knives. The thin sections were stained on grids for $3^1/_2$ min with REYNOLDS lead citrate[2] before being examined with a Zeiss EM 9a.

Laticifer distribution at this stage of embryogeny consists of a ring of elongate cells surrounding the provascular cylinder in the hypocotyl with extensive cortical ramification in the nodal region and a continuous cotyledonary system. In both the hypocotyl and cotyledons there is an intimate relationship between the developing vascular tissue and the major laticifer branches. The laticifers already have a thickened wall, a prominent central vacuole, and are multinucleate. Laticifer nuclei in this species assume the form of elongated spindles and are generally oriented with their longitudinal axes parallel to the longitudinal axis of the laticifer. Light microscopy has shown that laticifer nuclei frequently occur in closely packed groups of 2 or more. Electron micrographs of such groups of nuclei demonstrate that the outer nuclear membranes of adjacent nuclei are connected by short segments of rough endoplasmic reticulum (Figure 1). Such interconnections would explain the linear arrays of nuclei

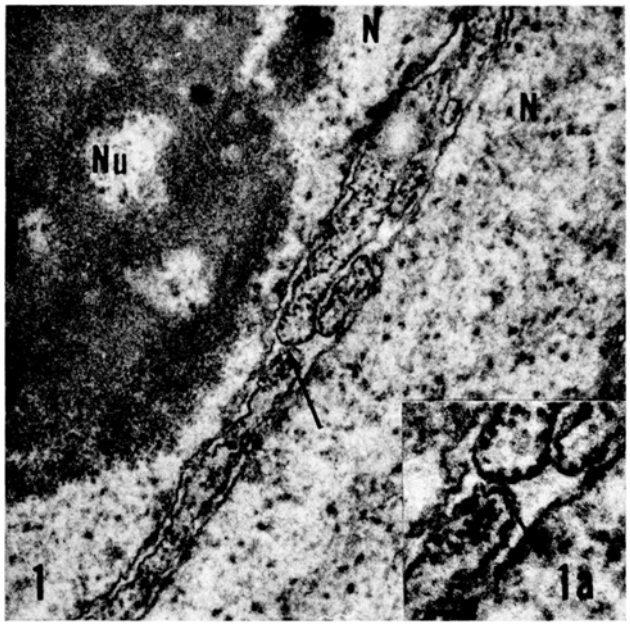

Fig. 1. Electron micrograph of 2 laticifer nuclei showing a bridge of rough endoplasmic reticulum (arrow) between the nuclei. N, nucleus; Nu, nucleolus. × 32,500. 1a. Higher magnification of the same nuclear interconnection (arrow). × 61,750.

[1] H. MOOR, J. Ultrastruct. Res. *2*, 393 (1959).
[2] G. S. REYNOLDS, J. Cell Biol. *17*, 208 (1963).