During the sampling and the sample preparation, it only came into contact with plastic surfaces which had been cleaned with nitric acid and bidistilled water.

The determination of selenium was carried out by means of instrumental neutron activation analysis. The analytical procedure has been described in detail elsewhere[3]. It included the irradiation of 25 mg of dried serum, sealed in highly pure silica ampoules, with thermal reactor neutrons and the measurement of the gamma rays of ^{75}Se by means of a Ge(Li) detector. The selenium content was calculated for the dry weight of the serum sample.

Results. The serum selenium content was significantly lower ($p < 0.001$) in pregnant rats on d 20, whereas for the animals, which had been hysterectomized on d 10, no difference from the nulliparous control group was found (table 1).

From the results of the 2nd experiment in the figure, it can be seen that the serum selenium content in pregnant animals did not deviate from that of the ovariectomized control group up to d 12, but then continuously decreased, and shortly before term, at 70% of the content before pregnancy, reached its lowest point. On d 24 (d 2 p.p.) it had risen again to the level of the ovariectomized control group, and no difference between the lactating and the ovariectomized control animals was found on d 36 (d 14 p.p.) either. Nursing did not influence the serum selenium content (table 2).

Changes in the protein/water ratio in serum were observed during pregnancy and lactation. Compared with the values of the ovariectomized controls, the amount of dry matter in the serum was significantly lower on d 12 ($p < 0.01$) and on d 36 ($p < 0.001$).

Discussion. As nearly all of the selenium in serum is bound to proteins, changes in the water content may simulate changes in the serum selenium level when calculated for the wet weight or for the volume of the sample[4]. It was therefore necessary here to relate the selenium content to the dry weight of the serum sample. In accordance with the earlier study[1], the serum selenium content of pregnant rats was found in these 2 different experiments to be reduced substantially at term. Since it began to decrease the day when the hormonal control of the corpora lutea was transferred from the pituitary to the placenta[2] and returned to the original level within 2 days after delivery, and since hysterectomy on d 10 prevented this drop, placental secretions could have been involved in these changes.

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Somatostatin inhibits the mitogenic effect of thyroliberin[1]

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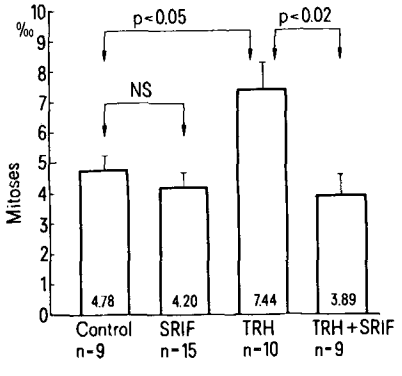
Summary. The effect of somatostatin and of thyroliberin on the mitotic incidence in the organ-cultured anterior pituitary lobe of the rat was investigated, using the colchicine metaphase-arrest technique. It was found that somatostatin added to the culture medium, together with thyroliberin, blocked the mitogenic effect of the latter.

It was previously shown in our laboratory that thyroliberin (thyrotropin releasing hormone, TRH), stimulated the rat adenohypophysial cell proliferation in vitro[2-4] as well as in vivo[5]. It was therefore of interest to see whether somatostatin (SRIF), the first hypothalamic inhibiting hormone available in a synthetic form, influences the adenohypophysial cell proliferation. The present report deals with the effects of somatostatin alone, and somatostatin together with TRH, on the mitotic incidence in the organ-cultured anterior pituitary of the rat.



The mitotic incidence in the organ-cultured adenohypophysial explants: the control, and exposed to TRH, SRIF and to TRH plus SRIF. The bars indicate mean values ± SEM. n = number of investigated explants in each group.

Male Sprague-Dawley rats, weighing about 160 g each, were used as donors of the pituitaries. The anterior lobes, each divided into 4 parts, were cultured in a medium composed of TC 199 (95%) and calf serum (5%) at 37 °C, in the atmosphere of 95% of oxygen and 5% of carbon dioxide. Additionally, some of the media contained TRH (Calbiochem) and SRIF (Calbiochem), or the 2 latter compounds together. TRH was added at the beginning of the incubation in a concentration of 3×10^{-6} M. SRIF was added twice: at the beginning and on the 6th h of incubation, in a concentration of 10^{-7} M. To all the media, colchicin (Fluka AG) was added in a dose of 0.05 µg/ml, twice, on the 4th and 9th h of incubation. After 14 h of incubation, the explants were fixed on formol-sublimate.

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Paraffin sections were stained according to Herlant's tetrachrome method. Mitoses (metaphases) were counted in 1000 cells of each explant. Statistical significance was evaluated by Student's t-test. The adenohypophysial explants exposed to TRH alone showed significantly higher mitotic incidence ($7.44 \pm 0.85‰$) if compared with the control ($4.78 \pm 0.66‰$). SRIF alone had no significant influence on the mitotic incidence of the adenohypophysial explants ($4.2 \pm 0.47‰$). However, SRIF added together with TRH totally blocked the mitogenic effect of the latter ($3.89 \pm 0.74‰$). Vale et al.[6] reported that SRIF blocks thyroropin release induced by TRH. Thus, the effect of SRIF on the cell proliferation seems to be connected with its effect on hormone release. It has been postulated that SRIF acts on the pituitary by lowering the cyclic AMP[7]. Since the cyclic AMP is known to be an inhibitor of mitoses in certain cells, some authors presumed rather a stimulatory than an inhibitory influence of SRIF on the adenohypophysial cell replication[8,9]. However, we demonstrated previously that dibutyryl cyclic AMP does not inhibit, but stimulates adenohypophysial cell proloferation in vitro[3]. It was also shown that estradiol, which is known to stimulate adenohypophysial cell proliferation, increased cyclic AMP concentration in the anterior pituitary[10]. Thus, the inhibitory effect of SRIF on TRH-stimulated adenohypophysial mitotic activity is not in opposition with the effect of SRIF on adenohypophysial cyclic AMP.

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PRO EXPERIMENTIS

A new method of measuring functional recovery after crushing the peripheral nerves in unanesthetized and unrestrained rats

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Summary. The distances between the first and fifth digits and between the second and fourth digits of the rat's hind paw were measured after crushing the sciatic nerve. The distances between the digits recovered significantly faster in weak nerve crushing than in strong crushing, and faster in distal nerve crushing than in proximal crushing. These results suggest that this method is available for evaluating the functional recovery after nerve crushing.

A simple method for evaluating the degrees of nerve regeneration after crushing the peripheral nerve in the unanesthetized and unrestrained animal has been required in pharmacological, physiological and biochemical studies. For example, it is much needed in investigating the time course of functional recovery in the same animal after nerve injuries or in measuring both the biochemical changes of muscle or of nerve and the degrees of the nerve regeneration. This report describes a new method.

Methods. Male Wistar-Imamichi rats (6 weeks after birth) were anesthetized with pentobarbital sodium (40 mg/kg, i.p.). The left sciatic nerve was crushed over a length of 2 mm under constant pressure at the level of the hips ('proximal crush') or the thigh ('distal crush') for 5 min with Péan's hemostatic forceps whose contact surfaces had been flattened. The nerve was crushed at a position 10 mm from the tip of the forceps. The forceps have 3 levels (refered to here as the weakest step, the middle step and the strongest step) of compressive strength. In this experiments the middle step ('weak crush') and strongest step ('strong crush') of the forceps were used. The sciatic nerve which is approximately 0.8 mm thick was crushed to approximately 0.3 mm thick in the weak crush and to approximately 0.2 mm thick in the strong crush.

Fig. 1. The rat's hind paw on the day after strong nerve crushing. The 5 digits of the rat's hind paw are spread apart on the intact side, but not on the injured side.

Maximum distances between the digits (DBD) of the rat's hind paw were measured with callipers at the tips by holding the rat's hips from behind and by pushing the paw slightly to the floor. When the first and fifth digits approached and their tips became invisible from the dorsal side, the DBD were gauged from the sole side. The distances between the first and fifth digits (DBD · 1–5) and between the second and fourth digits (DBD · 2–4) were measured 3 times or more and the values were averaged.

Student's t-test was used for statistical comparison between different treatment groups.

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