l'Énergie Atomique, France, and stock solutions in 0.01 *N* HCl were stored at −30°.

Results and Discussion. In each experiment one reserpine-treated and one control animal were used. In each experiment the amount of incorporated C^{14}-amines after reserpine injection is given in % of the control value, and amine levels are given in µg per pair of adrenals.

Reserpine caused a pronounced blockade of the uptake of adrenaline or noradrenaline by the storage granules (Figure). The effect lasted 12 to 24 h. There was no detectable difference between results obtained with labelled adrenaline and noradrenaline. After about 48 h the incorporation was restored while the level of catecholamines was still very low in the adrenal medulla.

It is known that after a single injection of a large dose of reserpine (2–5 mg/kg intravenously) to rabbits, the pharmacological effects (sedation, miosis, ptosis) disappear within the first two days whereas the concentrations of tissue catecholamines still remain low. The return to normal values of catecholamines requires about 2 weeks[3].

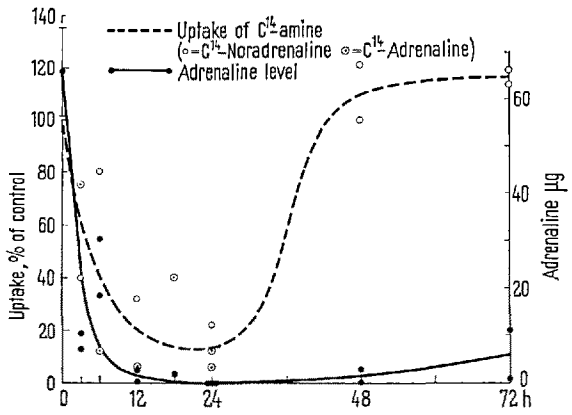

Fig. 1. Adrenaline level of adrenal medulla and uptake of C^{14}-catecholamines by adrenal medullary granules *in vitro* at various intervals following injection of reserpine (5 mg/kg i.v.) to rabbits.

Thus there is a much better correlation between the pharmacological effects of reserpine and storage function than between these effects and amine levels, which indicates that the actual content of amines of the tissues is of only minor importance for the function.

HILLARP[4] has shown that there are different fractions of amines in the adrenal medulla. It may be that the smaller labile fraction will be restored rather soon after the depletion achieved by reserpine and that only this fraction is necessary for function, while the larger stable fraction may still be completely depleted of amines[5].

Zusammenfassung. Der Speichermechanismus der Amingranula des Nebennierenmarkes von Kaninchen wurde in verschiedenen Intervallen nach Injektion einer einzigen Dosis Reserpin (5 mg/kg intravenös) untersucht.

Der Speichermechanismus war 12 bis 24 h nach der Injektion blockiert, aber nach 48 h wieder normal, während der Catecholamingehalt der Amingranula immer noch vollständig depletiert war.

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[5] *Acknowledgments.* This work has been supported by a grant (-2854) from the United States Public Health Service. – I wish to express my thanks to Mr. B. WALDECK for the C^{14}-determinations and other technical assistance.

An *in vivo* Bioassay for TSH-Releasing Factor (TRF)

When it was proposed in this laboratory to engage in a program studying the hypothalamic control of the secretion of TSH, a bioassay for the postulated TRF (TSH-releasing factor) had to be chosen. Since none was available in the literature which would adequately meet our criteria, it was decided that a new method should be set up which would fulfill the various requirements outlined below.

(1) It was proposed to accept as a working hypothesis that thyroxine inhibits the secretion of endogenous TSH at least in part through a negative feedback mechanism acting directly at the level of the adenohypophysis. (2) The method should be reasonably sensitive for TSH. (3) The method should be simple enough to allow in a day's work the testing of at least 10 unknowns (fractions of the effluent of some separation method). (4) It should utilize an animal in which hypophysectomy is a simple procedure so that the various fractions could be tested for direct (TSH) activity or transhypophysial (TRF) activity. (5) The method should allow the testing of crude extracts —which precluded considering an *in vitro* assay[1] for routine studies.

On the basis of these premises it was decided to use a modification of the method of MCKENZIE[2]. Because of the difficulty of routine performance of hypophysectomy in mice (the animal species used in the original method of MCKENZIE), it was decided to use the rat. In keeping with the concept that thyroxine inhibits TSH release at least in part by acting at the pituitary level, it was proposed to maintain the animals under a low load of thyroxine (rather than without thyroxine at all), so as to increase their (peripheral) sensitivity to TSH while at the same time retaining the ability of the pituitary to respond to TRF be secreting TSH.

The proposed bioassay for TRF-activity is as follows:

Rats, males, body weight 50 g when received in the laboratory, are kept in a constant temperature room (22°C ± 2), on a low iodine diet (Nutritional Biochemicals Co., Cleveland, USA) and double (glass) distilled water as drinking fluid for 10 days. They are given one i.p. injection of 3.0 µc I^{131}, carrier free, followed 5 h later by one s.c. injection of 5 µg *l*-thyroxine. They are used in the test 65–70 h after injection of *l*-thyroxine: under

[1] R. GUILLEMIN and A. V. SCHALLY, Endocrinology *65*, 555 (1959).

[2] J. M. MCKENZIE, Endocrinology *63*, 372 (1958).

ether anesthesia one blood sample (0.5 ml) is withdrawn from the jugular vein, and the material to be tested is immediately injected in the same vein in a total volume of 1.0 ml. 2 h later, a new blood sample (0.5 ml) is taken under ether anesthesia from the same or the contralateral jugular vein. Total blood radioactivity is measured in a well scintillation counter on both samples. There is a linear relation between the log-dose of injected TRF and the difference in radioactivity of both blood samples[3]. The complete mathematical analysis of the data based on covariance analysis has been described[3,4].

Hypophysectomies are performed in similarly prepared animals by the parapharyngial route 68 h after the injection of *l*-thyroxine. Animals are used for the i.v. injections 2 h later. Under these conditions, the response to TSH of the animals after hypophysectomy is similar to that of the normal animals[3]. The sensitivity to TSH of both preparations (normal or hypophysectomized) is of the order of 1.0 mU USP TSH Standard. We make a routine study of the response to 1.5 mU and 4.5 mU TSH in every experiment to assess the sensitivity of the animals to TSH.

With this assay combining the use of hypophysectomized and non-hypophysectomized animals, we were able to demonstrate the presence of TRF in crude acetic acid extracts of hypothalamic tissues of ovine origin[3]. The method has been used successfully to follow purification procedures for TRF[5]. Within the limits of sensitivity of this assay, at the doses tested, lys-vasopressin, oxytocin, α-MSH, β-MSH, are completely inactive in modifying the adenohypophysial secretion of TSH[3]. It is pertinent to introduce here results which were obtained while we were in the early stages of devising the methodology for this TRF assay, i.e. the observation that highly purified α-MSH or β-MSH can have a TSH-like effect in mice[6]. Such an effect was never seen in rats[3,6]. As α-MSH and β-MSH are known as constituents of acetic extracts of hypothalamic tissues[7], it is obvious that the choice of the rat as an assay animal vs. the mouse has the considerable advantage of permitting us to differentiate between TRF and α- or β-MSH in their thyroid stimulating abilities. Had we used the mouse as an assay animal, we should have had considerable difficulty in separating the thyroid stimulating activity due to TRF and α- or β-MSH, especially since the 3 materials have closely related mobility coefficients in the early purification stages we have used so far[3]. A differentiation between TRF and α- or β-MSH would have been possible on the basis of the observed difference in slopes of the log-dose/response function[3,6]; it should be pointed out, however, that this statement is part of an *a posteriori* reasoning. The point is that we should have had considerable difficulty in assessing the presence of a specific TRF, had we used the mouse as a bioassay.

We have described in detail elsewhere an *in vitro* method of pituitary incubation which can be used also as an assay for purified TRF[8]. We have clearly established, however, that such a method is not a choice procedure for routine studies[1,8,9].

Résumé. Une méthode de bioétalonnage *in vivo* pour la mise en évidence de l'activité TRF (TSH-releasing factor) est décrite. Cette méthode est basée sur la mesure de la radioactivité totale du sang chez le rat normal (non-hypophysectomisé) prétraité avec I^{131} et une dose liminaire de L-thyroxine. La spécificité de ce test est discutée; Vasopressine, ocytocine, α-MSH, β-MSH sont inactifs dans ce test.

E. Yamazaki,
E. Sakiz, and R. Guillemin

Laboratoire de Morphologie expérimentale et Endocrinologie, Collège de France, Paris (France), June 18, 1963.

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Reflex Activity of Extensor and Flexor Muscles Following Muscular Afferent Excitation during Sleep and Wakefulness[1]

The present experiments are concerned with the changes of spinal reflexes occurring during sleep and wakefulness in the intact, unanaesthetized cat. Both monosynaptic and polysynaptic reflexes were produced by graded stimulation of extensor muscle afferent fibres.

Using barbiturate anaesthesia, 6 screw-type cortical recording electrodes and 1 EMG electrode for cervical muscles were chronically implanted in each cat. A stimulating collar-type electrode[2] was also applied to the medial gastrocnemius nerve, which was tied distally to the electrode in order to prevent peripheral but not central conduction of the volleys. The plantar nerve was carefully dissected and removed, and precautions were taken to prevent spread of the stimulus to the lateral gastrocnemius nerve, which was left intact. In order to record the monosynaptic (MR) and polysynaptic (PR) reflexes produced respectively by stimulating group I*a* and group I*b*, II, III fibres of the medial gastrocnemius nerve, EMG electrodes were placed respectively in the lateral gastrocnemius and the tibialis anterior muscles. No recordings were taken until 24–48 h after the operation. The medial gastrocnemius nerve was usually stimulated with 2 sec trains at 100/sec, 0.05 msec pulse duration. The duration of the trains as well as the rate of stimulation were occasionally changed. The stimulus strengths were expressed in terms of times threshold (T) for the monosynaptic extensor reflex. These values were slightly higher than the threshold for group I*a* muscle afferents. The following results were obtained:

(1) During *relaxed wakefulness* with desynchronized EEG activity, the threshold for the MR was quite constant and a strong response was obtained at a stimulus

[1] This investigation was supported by PHS research grant B-2990 from the National Institute of Neurological Diseases and Blindness, N.I.H., Public Health Service (U.S.A.).
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