ether anesthesia one blood sample (0.5 ml) is withdrawn from the jugular vein, and the material to be tested is immediately injected in the same vein in a total volume of 1.0 ml. 2 h later, a new blood sample (0.5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.

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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

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strength of 1.2–1.3 T. On this background of cortical activity, the threshold for inhibition of the MR was never found below 1.5–1.7 T, while the ipsilateral flexion reflex usually occurred at thresholds of 2–3 T.

(2) During *drowsiness or synchronized sleep*, the threshold for the MR was only slightly higher than the values obtained during wakefulness. In some cases, however, there was no change in threshold of the MR, but occasionally a slight decrease in intensity of the muscular reflex response could be detected when stimulus intensities only slightly suprathreshold for the MR were used. The threshold for the ipsilateral flexion reflex also was equal to or slightly higher than the values obtained in the awake animal. Similar values (usually ranging from 3 to 4 T) were also found to be liminal for an arousal reaction. This fact incidentally gives further support to previous findings indicating that the group I*a* afferent volleys do not exert any influence on the ascending activating system, at least as far as it is possible to decide on the basis of the electroencephalographic test[3,4].

(3) During the episodes of *deep, desynchronized sleep* (see [5]), stimulation of the medial gastrocnemius nerve at 1.2–1.4 T did not cause any muscular reflex response even by increasing the duration of the stimulus train (at 100/sec) up to 10 sec, or the rate of stimulation up to 500/sec. This last condition greatly potentiated the MR in the awake cat. On the other hand, when the animal roused spontaneously from sleep or awoke following acoustic stimuli, the MR gradually reappeared. Stimulation of the medial gastrocnemius nerve at 5.8 T, 100/sec, when applied during deep sleep, caused behavioral arousal, which was accompanied by a generalized contraction of both extensor and flexor muscles.

The present experiments clearly show that spinal reflexes are only very slightly affected in the synchronized sleep compared with relaxed wakefulness. On the contrary, during deep sleep there is a complete abolition of the MR and an increase in threshold of the flexion reflex. This response actually comes to light only as a symptom of a generalized motor response occurring during the arousal elicited by stimulating flexion reflex afferents[3,4]. The striking changes of spinal reflexes occurring during the deep stage of sleep may be due to (i) decrease of a facilitatory influence exerted by the brain stem on spinal cord, (ii) descending inhibitory volleys.

Riassunto. Nel gatto integro non anestetizzato il riflesso monosinaptico estensorio e il riflesso polisinaptico flessorio subiscono soltanto lievissime, a volte impercettibili, modificazioni nel passaggio dallo stato di veglia al sonno sincronizzato. Per contro, nel sonno profondo desincronizzato si osserva l'abolizione completa del riflesso monosinaptico e un'elevazione della soglia per il riflesso ipsilaterale flessorio. Per stimoli efficaci a produrre nel sonno profondo questa contrazione flessoria si osserva anche una risposta motoria generalizzata, che si accompagna ad una reazione di risveglio.

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The Effect of Adrenalectomy on the Norepinephrine and Serotonin Content of the Brain and on Reserpine Action in Rats

MONTANARI and STOCKHAM[1] established that reserpine is more toxic 6 days after adrenalectomy than in intact animals. The present experiments were undertaken to investigate the effect of adrenalectomy on the convulsion-facilitating and biogen amine-depleting action of reserpine.

Methods. The experiments were made on Wistar rats. Adrenalectomy was performed under ether anaesthesia and the animals were given saline for drinking. The experiments were carried out the seventh day after the operation. Reserpine was given intravenously and the rats were used for experiment after 4 h. Electroshock (ES) was elicited with bitemporal electrodes and the threshold stimulus was determined on each rat, namely the minimum amount of current which produced tonic extensor seizures. The norepinephrine (NE) determination was carried out according to PAASONEN and KRAYER[2] by blood pressure determination in cats. Serotonin (5-HT) was extracted with acetone[3] and determined on the rat's stomach[4].

Results. The experiments show that adrenalectomy facilitates the effect of reserpine. The convulsion threshold does not differ in rats 7 days after adrenalectomy related to intact animals. 0.15 mg/kg reserpine i.v. has no considerable influence on the seizure threshold in intact animals, but in adrenalectomized rats significant decrease can be observed (Table I).

The augmented effect of reserpine in adrenalectomized rats may be explained by the experiments, which show that the 5-HT content of the brain is significantly lower in adrenalectomized than in intact animals. NE content does not change. The 5-HT content is decreased to 45% of the intact animals and this change can be detected already on the third day after adrenalectomy.

Reserpine has a more marked influence on the 5-HT content of the brain in adrenalectomized than in intact rats. After administration of 0.25 mg/kg reserpine i.v. the

Table I. Convulsive threshold in mA

Treatment	Intact	Adrenalectomized	No. of rats
—	14.5	15.4	10
0.15 mg/kg reserpine i.v.	13.3	11.3	10

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