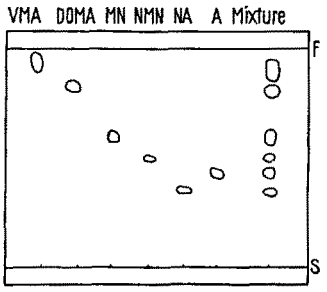
Quantitative estimations were performed by double spotting a known standard mixture of the substances studied. After development of the chromatogram one series of spots is sprayed for identification purposes whereas the corresponding spots of the other series are brought (1 cm² areas) into a centrifuge tube, to which are added 1 ml of 0.1 *N* HCl as well as the various reagents for the fluorimetric assay of NA and A at pH 5 according to SHORE and OLIN[5], and of NMN and MN according to BERTLER et al.[6]. After centrifugation for 15 min at 4000 rpm, the supernatant is removed for measurement of the fluorescence, the excitation and fluorescence wavelength maxima being respectively for NA and A 395 mμ and 525 mμ, for NMN 385 mμ and 490 mμ, and for MN 395 mμ and 515 mμ.

Reproducibility and linearity were found to be quite satisfactory in the 0.2–1 μg range, whereas the recovery from the chromatoplates was of 97–100% for NA, A and MN, and of 75% for NMN[7].



Thin layer chromatogram (cellulose powder) using *n*-butanol saturated with 3 *N* HCl as solvent. See text for abbreviations.

Substance (μg of pure base)	$K_3Fe(CN)_6$	Ethylene-diamine	*p*-Nitro-aniline
NA	0.008	0.008	0.050
A	0.010	0.008	0.050
NMN	–	0.416	0.080
MN	–	0.160	0.080
DOMA	–	0.080	0.100
VMA	–	0.500	0.080

Zusammenfassung. Eine einfache, schnelle und empfindliche Methode zur dünnschichtchromatographischen Trennung von 0,2–1 μg Menge von Noradrenalin, Adrenalin, Normetanephrin, Metanephrin, 3,4-Dihydroxymandelsäure und 3-Methoxy-4-hydroxymandelsäure wird beschrieben.

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J. F. and C. Heymans Institute of Pharmacology, University of Ghent (Belgium), December 27, 1964.

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An in vivo Method for Evaluating the Hypothalamic Follicle Stimulating Hormone Releasing Factor

It has recently been suggested that, in addition to the releasing factors for ACTH, TSH, LH and GH[1], the median eminence (ME) region of the hypothalamus might also contain a neurohumoral agent which specifically stimulates the release of follicle stimulating hormone (FSH). IGARASHI and MCCANN[2] have shown that crude acidic extracts of rat or bovine ME elevate plasma FSH after intravenous injection into ovariectomized rats, in which the secretion of FSH has been blocked by lesions in the ME, or by large doses of ovarian steroids. KUROSHIMA et al.[3], and MITTLER and MEITES[4] have confirmed these results and reported in addition that rat pituitaries cultured in vitro with hypothalamic exctracts (from sheep, beef or ovariectomized rats) release significant amounts of FSH.

The methods so far employed for evaluating the hypothalamic FSH-releasing activity do not, however, seem completely adequate for assessing the physiological role of this new releasing substance. As far as IGARASHI's[2] procedure is concerned, no data have been reported so far on the amounts of FSH available in the pituitary of the animals (brain-lesioned or treated with ovarian steroids) receiving the injections of the hypothalamic extracts: differences in the amounts of hormones stored in the pituitary may obviously greatly influence the reactivity of the gland. In addition to this, the specificity of the FSH assay method in the mouse used by IGARASHI et al.[2] also requires further study.

On the other hand, doubts have been raised as to the physiological significance of in vitro incubation methods as used by KUROSHIMA et al.[3] and by MITTLER and MEITES[4]; release of FSH in the medium might simply reflect passive leakage of preformed hormone rather than activation of true secretory processes[5].

A new procedure for the quantitative evaluation of the FSH-releasing factor (FSH-RF) has recently been developed in this Department as will be reported in this preliminary note.

This method, which is based on the ability of hypothalamic extracts to deplete pituitary FSH stores in vivo,

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meets the requirements of using normal rats with presumably a physiological pituitary FSH-content as test animals, and, for the measurement of FSH, of using a recognized and highly specific method.

Known amounts of crude hypothalamic extracts (pooled or individual) or of purified hypothalamic fractions are injected into the exposed carotid artery of normal, sexually mature male rats (body weight 180 ± 10 g) anaesthetized with pentobarbital (2.5 mg/100 g body weight). Control animals are injected with comparable amounts of cerebral cortex extracts. Female animals are as suitable as males as recipients of the hypothalamus; male rats are however preferred in order to avoid cyclic variations of pituitary FSH stores; in addition they have much higher pituitary concentrations of FSH than female animals[6]. The intracarotid (i.c.) mode of administration is used because previous work in this laboratory on ACTH[7,8] and GH[9] releasing factors had shown that, when compared to the intravenous mode of administration, it permits the use of much smaller quantities of releasing principles. The animals receiving hypothalamic or cerebral cortex preparations are decapitated with a guillotine 20 min after treatment. The pituitary is taken out, cleaned and homogenized in cold saline solution. The FSH content of the pituitary homogenates so obtained is measured by using the ovarian augmentation test of STEELMAN and POHLEY[10] as modified by PARLOW[11], with a 4 point design (4 animals per dose) and a purified FSH preparation (NIH) as reference standard.

The i.c. injection of cerebral cortex preparations does not modify pituitary FSH stores; the content in FSH of the pituitaries of animals injected with cerebral cortex extracts may then be taken as the control level.

Significant depletions of pituitary FSH stores are induced by the i.c. injection of hypothalamic extracts containing FSH-RF. The depletion of pituitary FSH stores induced by hypothalamic extracts is strictly related to the dose of the FSH-RF activity which has been administered. An example of the results which are obtained with this method is given in the Figure. It is clear that a significant depletion of FSH is obtained with an i.c. injection of half a hypothalamus; a greater reduction is obtained when 3/4 of a hypothalamus is injected. This method is more sensitive than the one proposed by IGARASHI and MCCANN[2] in which the injection of 2 hypothalami is needed to induce a clear-cut response.

Résumé. L'injection intracarotidienne d'extraits hypothalamiques est suivie chez le rat d'une chute significative des taux de FSH à niveau hypophysaire. Ce phénomène se vérifie déjà 20 min après l'injection et est proportionnel à la dose d'hypothalamus injecté.

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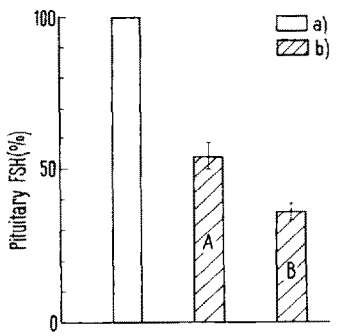

a) Animals injected with cerebral cortex extracts.
b) Animals injected with 0.5 (A) and 0.75 (B) of a hypothalamus obtained from castrated male rats.

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Spezielle Überlebensbedingungen für isolierte Netzhäute verschiedener Warmblüter

Um eine isolierte menschliche Netzhaut überlebend zu halten und von diesem Präparat ein normales Elektroretinogramm (ERG) wie in situ bei Gleichspannungsverstärkung[1] abzuleiten, müssen mehrere Bedingungen eingehalten werden[2]. Dem mit grosser Geschwindigkeit an der Netzhaut vorbeiströmenden Gemisch aus Plasma und modifizierter Tyrode-Lösung kommt für die normale Konfiguration und die Stabilität des ERG eine besondere Bedeutung zu. Da die Blutgruppeneigenschaften des Plasma unberücksichtigt bleiben können[3], erhebt sich die Frage, ob der im Plasma enthaltene wirksame Faktor überhaupt artspezifisch ist. So kann das ERG isolierter Froschnetzhäute auf das Doppelte vergrössert werden, wenn der Umströmungsflüssigkeit Schweineplasma zugesetzt wird.

Wird eine isolierte Kaninchennetzhaut in entsprechender Weise wie die menschliche Netzhaut präpariert und in der gleichen Apparatur bei Raumtemperatur (ca. 20°C) mit modifizierter Tyrode-Lösung umströmt, so antwortet sie auf Lichtreize mit einem abnormen ERG: Belichtung verursacht eine negative Potentialschwankung, die der Komponente P III von *Granit* entspricht (Figur a). Durch Zusatz von menschlichem Plasma wird diese Komponente

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