Results and discussion. The Table shows that a GVHR was observed except where adequate concentrations of RaMFabS or NRS and RC′ were used. The GVHR of 5×10^6 cells/g body wt. injected was inhibited after incubation either with 12.5% RaMFabS and 5.5% RC′ or with 12.5% NRS and 5.5% RC′ (Figure 1) but not with medium 199 alone. Thus the mechanism of inhibition does not seem to depend on a specific reaction, but on the cytotoxicity of rabbit sera. Indeed, the cytotoxicity of RaMFabS, RaMIgGS, and NRS too, could be demonstrated at a concentration of 12.5% as shown in Figure 2.

Résumé. En recherchant une action des anticorps anti-Fab sur la réaction primaire des cellules immunologiquement compétentes, on a mis en évidence une inhibition de la réaction du greffon contre l'hôte par du sérum de Lapin anti-Fab de Souris: cette inhibition n'est cependant pas liée aux anticorps anti-Fab, mais à la cytotoxicité des sérums de Lapin aussi bien anti-Fab que normal.

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Effect of Hypothalamic Crude Extract on the Utilization of D-[^{14}C]-Glucose by the Anterior Pituitary, in vitro

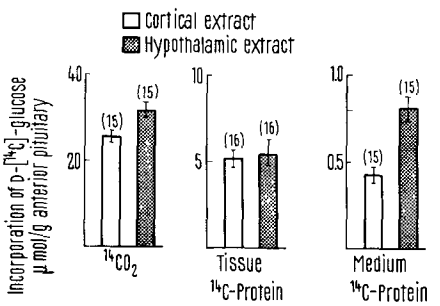
It is a well established fact that the hypothalamic control of the anterior pituitary gland is done by means of chemical substances known as releasing or inhibiting factors[1–3]. These substances are released into the hypophyseal portal vessels and act directly on the adenohypophysis to increase or decrease the release of each trophic hormone.

Evidence afforded by in vivo and in vitro experiments indicates that the hypothalamic extract (HE), in addition to controling the release of the anterior pituitary (AP) hormones, may also influence their synthesis[4–7].

Experiments were undertaken in this laboratory to find out whether the action of the hypothalamic releasing factors is accompanied by changes in the metabolism of energy delivering substracts. The present paper will report the effects of crude HE in the utilization of glucose by AP incubated in vitro, in conditions similar to those in which the HE are already known to effect hormone release[8,9].

Albino male rats weighing 200–250 g were killed by decapitation. Anterior pituitaries were quickly removed, separated from the posterior lobe, weighed, hemisected and placed in the main compartment of incubation flasks provided with a center well for CO_2 collection and with a self-sealing rubber stopper. The incubation medium consisted of Krebs-Henseleit bicarbonate buffer, to which either HE or cerebral cortex (CE) was added. In order to have a paired type experiment, one-half of each pituitary was placed in the experimental flask and the other in the control flask. For preparation of the extracts, hypothalami or cerebral cortex were homogenized in cold 0.1 *N* HCl and centrifuged at 2500 rpm for 30 min at room temperature just before each experiment. The supernatant was separated and the pH adjusted to 7.4 by adding dropwise 1.0 *N* NaOH.

Details of the incubation procedure are indicated in the Figure. At the end of the incubation period 0.3 ml of 30% KOH was injected through the rubber stopper into the center well, and, in a similar manner, 0.3 ml of 3 *M* H_2SO_4 was added to the incubation medium in the main compartment. After allowing at least 30 min for absorption of the $^{14}CO_2$, the contents of the center well were quantitatively transferred to a volumetric flask. An aliquot of this solution was precipitated as $Ba^{14}CO_3$ and its radioactivity measured in a gas flow counter (Model D47, Nuclear Chicago Co.). Corrections for self-absorption were made according to Karnowsky et al.[10]. After washing the AP several times with distilled water, the glands were homogenized in cold 10% trichloroacetic acid. The precipitated ^{14}C-protein was then transferred



Effect of hypothalamic extract on the utilization of D-[^{14}C]-glucose by anterior pituitary gland. The incubation medium consisted of 2 ml of Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 12 µmol of D-[^{14}C]-glucose (0.8 µc/flask) plus 0.2 ml of either hypothalamic or cerebral cortex extract. Anterior pituitaries (4 halves per flask) were incubated for 3 h at 37 °C with constant shaking and with 95% O_2 – 5% CO_2 as the gas phase. Values (mean ± S.E.M.) are expressed as µmoles of glucose incorporated per g of wet tissue per 3 h incubation. The number of experiments is indicated in parentheses.

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quantitatively to a filter-paper disc and washed several times for removal of non-proteic contaminants after the manner described by GOODNER[11]. The radioactivity of the dried disc was measured in the gas flow counter. ^{14}C-protein of the incubation medium was assayed as described for tissue protein.

The results obtained are shown in the Figure. It can be seen that the production of $^{14}CO_2$ from labelled glucose was enhanced by the crude hypothalamic extract. Although the increase was only of about 20%, this effect was very uniform, being obtained in all experimental flasks. The utilization of labeled carbon from glucose for protein synthesis by anterior pituitary was very active, but no influence of the HE was observed. The amount of radioactivity recovered in protein from the incubation medium was much lower than that incorporated in both CO_2 and tissue protein. The addition of hypothalamic extract to the incubation medium significantly increased the incorporation of ^{14}C-glucose into medium protein. It is attractive to suppose that this protein represents hormones liberated from the gland during the incubation, but further experiments are needed to clarify this point.

The stimulation by HE of the amount of ^{14}C from glucose recovered as CO_2 was obtained in the same conditions in which the extracts are known to induce the release of hormones to the incubation medium. This suggests that the hormone-releasing action of the HE on the anterior pituitary is a process that requires energy, which is provided, at least in part, by increased glucose utilization. Such a view is corroborated by recent findings[12] showing that crude extracts of median eminence induces a depletion of anterior pituitary glycogen[13].

Resumen. Los efectos de extractos crudos de hipotalamo en la utilización de D-[^{14}C]-glucosa por hipófisis anteriores fueram estudiados in vitro en condiciones identicas a aquelas en las cuales los extractos inducen la liberación de hormonas al medio de incubación. Los resultados indican que los extractos hipotalámicos aumentan la producción de $^{14}CO_2$ por la hipófisis a partir de glucose y la incorporación de ^{14}C en proteinas del medio de incubación. Los extractos no modificaron la cantidad de ^{14}C glucose utilizada para sintesis de proteinas tissulares.

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C_{19}- and C_{18}-Steroids in Cerebrospinal Fluid, Plasma and Urine after Intravenous Administration of 7α-^{3}H-DHEA ^{35}S-Sulphate

In a previous communication[1], the isolation of free and conjugated C_{21}-, C_{19}- and C_{18}-steroids from human cerebrospinal fluid (CSF) was reported, the majority of steroids occurring in the fraction of lipophile steroid sulphatides[2]. In order to gain further information on the passage of such sulphoconjugates from blood to CSF, 20.2 μg 7α-^{3}H-3β-hydroxy-5-androstene-17-one (dehydroepiandrosterone, DHEA) ^{35}S-sulphate with 44.2×10^6 cpm ^{3}H and 2.99×10^6 cpm ^{35}S ($^3H/^{35}S = R = 14.8$) were injected i.v. into a 35-year-old male subject and CSF, peripheral plasma and 24-h urine assayed for labelled free and conjugated C_{19}- and C_{18}-steroids. Samples of CSF, collected by lumbar punction 30 and 165 min after administration of the substrate, were processed like the 6 plasma samples obtained after 5, 30, 60, 90, 120 and 165 min. Extraction of free and conjugated steroids and separation of the latter into steroid sulphatides, sulphates and glucuronosides were achieved by techniques described in a recent publication[3]. Individual C_{19}- and C_{18}-steroids in the fractions of free or conjugated steroids from CSF, plasma or urine, were isolated by standard procedures, including derivative formation and multiple chromatography[4,5]. For final identification of isolated steroids, the chromatographic purification to constant specific activity, after reverse isotope dilution with authentic compounds, was considered adequate.

As can be derived from the Table, both CSF samples contained double-labelled steroid sulphoconjugates, but practically no labelled free steroids or steroid glucuronosides. Since only minute amounts of steroid sulphates were detected in CSF, it may be assumed that lipophile properties of steroid sulphatides promote their transport from blood to CSF. The isotope ratio R of sulphoconjugates in the first sample of CSF corresponded to that of the substrate or of steroid sulphatides in the first plasma samples, thus indicating a fairly rapid passage of the latter compounds into CSF. On the other hand, only a minor proportion of circulating steroid sulphatides appears to reach the CSF, as evidenced by a distinctly lower specific ^{3}H-activity of DHEA or its metabolites in CSF. The specific ^{3}H-activity of DHEA or 5-androstene-3β,17β-diol, for instance, amounted to 3950 cpm/μg or 2100 cpm/μg respectively in the first CSF sample and 22,700 cpm/μg or 13,400 cpm/μg respectively in the combined plasma samples 1–3. In addition to 5-androstene-3β,17β-diol also 4-androstene-3,17-dione, 17β-hydroxy-4-androstene-3-one, 3α-hydroxy-5α-androstan-17-one and 3α-hydroxy-5β-androstan-17-one could be identified in CSF, their quantitative distribution resembling that found in steroid sulphatides from peripheral plasma. The ^{3}H-activity in the fraction of phenolic steroids from CSF did not suffice for isolation of individual estrogens. From 530 ml urine, collected over 24 h in 3 portions, a total of 4,277,000 cpm ^{3}H or 9.65% of injected ^{3}H-activity were recovered. The ratio of free steroids to steroid glucuronosides to steroid

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