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.

R. H. MIGLIORINI and J. ANTUNES-RODRIGUES

Department of Physiology, School of Medicine, Ribeirão Prêto (S.P., Brazil), 21 August 1969.

[11] C. J. GOODNER, Endocrinology *75*, 846 (1964).
[12] T. HIROSHIGE, I. WAKABAYASHI, K. YOSHIMURA and S. ITOH, Endocr. jap. *15*, 499 (1968).
[13] The authors are indebted to Miss MARIA A. RISSATO and Mr. José R. OLIVEIRA for expert technical assistance.

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

[1] G. W. OERTEL and P. BRÜHL, Z. klin. Chem. *4*, 66 (1966).
[2] G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *343*, 276 (1966).
[3] G. W. OERTEL, P. MENZEL and D. WENZEL, J. Steroid Biochem., in press 1969.
[4] G. W. OERTEL, P. KNAPSTEIN and L. TREIBER, Hoppe-Seyler's Z. physiol. Chem. *345*, 221 (1966).
[5] G. W. OERTEL and L. TREIBER, Europ. J. Biochem. *7*, 234 (1969).

Free and conjugated steroids in CSF, plasma and urine

Material	Time	ml	cpm $^3H/^{35}S$ per 10 ml CSF or plasma and 100 ml urine							
			Free steroids	R	Sulphatides	R	Sulphates	R	Glucurono-sides	R
CSF										
(1)	30 min	8.9	6	>100	1,370	13.2	32	16.0	2	>100
			0		105		2		0	
(2)	165 min	6.0	0		227	16.2	10		0	
			0		14		0		0	
Plasma										
(1)	5 min	5.8	189	>100	85,200	14.6	11,900	14.7	32	>100
			0		5,830		810		0	
(2)	30 min	18.5	923	>100	62,000	14.6	2,840	14.8	51	>100
			0		4,240		191		0	
(3)	60 min	5.0	133	>100	52,700	14.9	1,130	15.1	28	>100
			0		3,530		75		0	
(4)	90 min	6.0	40	>100	45,700	15.2	962	15.3		
			0		3,010		63			
(5)	120 min	21.0	18	>100	40,500	15.6	733	15.9		
			0		2,590		46			
(6)	165 min	9.8	11	>100	28,600	16.9	680	16.2		
			0		1,690		42			
Urine										
(1)	0–4 h	260	19,600	>100			620,000	22.0	32,800	>100
			32				28,200		63	
(2)	4–12 h	180	15,100	>100			230,000	30.4	43,600	>100
			0				7,630		14	
(3)	12–24 h	90	12,600	>100			289,000	38.3	69,400	>100
			102				7,550		31	

sulphates approximated 1:2:22. A steady increase in the isotope ratio R of urinary steroid sulphates from 22.0 in the first portion to 30.4 in the second and 38.3 in the third portion reflects a substantial (22.7%, 51.3% and 61.4%) hydrolysis of sulphoconjugates and resulphurylation of liberated steroids in the course of the experiment. Such findings, as well as the quantitative distribution of metabolites in the different fractions from urine, were in close agreement with previous results, obtained after i.v. administration of double-labelled DHEA sulphate[4,5].

From the data presented it was concluded that only lipophile steroid sulphoconjugates, e.g. steroid sulphatides, may pass from blood to CSF within a reasonable period of time. However, a certain blood/CSF barrier seems to exist even for these compounds.

Zusammenfassung. Nach i.v. Injektion von 7α-H-DHEA-^{35}S-sulfat wurden Liquor, Plasma und 24-Stundenharn eines Mannes auf freie und konjugierte, markierte C_{19}- und C_{18}-Steroide untersucht. Es zeigte sich, dass schon 30 Minuten nach Versuchsbeginn im Liquor fast nur doppelt-markierte Steroid-sulfatide mit praktisch unverändertem Isotopenverhältnis $^3H/^{35}S$ enthalten waren. Da weiterhin DHEA und seine Metaboliten im Liquor eine weitaus niedrigere spezifische ^3H-Aktivität besassen als die entsprechenden Verbindungen im Plasma, ist anzunehmen, dass der Übertritt von lipophilen Steroidsulfatiden zwar verhältnismässig rasch, aber nur in begrenztem Umfang erfolgte.

G. W. Oertel, H. Gumpert, P. Knapstein und D. Vogt

Abteilung für Experimentelle Endokrinologie, Universitäts-Frauenklinik und Neurochirurgische Universitätsklinik, D-65 Mainz (Germany), 15 October 1969.

The Effect of Castration and Hypophysectomy on the Content of Noradrenaline and Serotonin in the Hypothalamus of the Rat

Recently it has been shown that the castration of male rats has an influence on the formaldehyde induced fluorescence of the primary catecholamines of the hypothalamus[1]. Castration increases the turn-over rate of noradrenaline (NA) in the hypothalamus[2–4]. The hypophysectomy of short duration (5 days) has no effect on the catecholamine fluorescence in the hypothalamus[1]. In the present work, quantitative confirmation has been tried by estimating chemically NA and serotonin (5-HT) of the hypothalamus and cerebral cortex in male and female rats after castration or the hypophysectomy of long

[1] M. Hyyppä, Z. Zellforsch. *98*, 550 (1969).
[2] F. Anton-Tay, R. W. Pelham and R. Wurtman, Endocrinology *84*, 1489 (1969).
[3] A. O. Donoso, M. B. de Guitierrez Moyano and R. C. Santolaya, Neuroendocrinology *4*, 12 (1969).
[4] J. A. Coppola, J. Reprod. Fert., Suppl. *4*, 35 (1968).