lower than that for heat-inactivation). The formation of enzyme-substrate complex may increase the rigidness of the secondary structure of enzyme protein and may suppress the interference between heat and photochemical processes. Therefore, the presence of substrate makes E_1 higher. By applying the theory of absolute reaction rate[5,6] to curves 1, 2, 3′, 4′, 5′, and 6′, the heat of activation (ΔH^*), the free energy of activation (ΔF^*) and the entropy of activation (ΔS^*) were calculated and are given in Table II. These values decrease in the order of heat, visible and UV inactivations, depending on the degree of the interference between heat and photochemical processes. The important feature of the results in Table II is the abnormally high entropies of activation. This explains the abnormally high activation energy for the heat-inactivation. The high value of entropy of activation suggests that a profound structural change is involved. The low value of ΔS^* for the UV-inactivation is due to a strong interference between heat and photochemical processes. The values of thermodynamic quantities for the UV-inactivation are not appreciably changed by the presence of substrate. This means a lack of substrate effect, as stated above. The protective effect of substrate is thought to be attributable to the stabilization of the secondary structure of enzyme protein – the conformation change from disorder to rigid and order – by the formation of enzyme-substrate complex. Such a formation of complex gives rise to the decrease of entropy[7]. This may explain the increase of ΔS^* by the presence of substrate. Not only protection from the heat process but suppression of the interference between heat and photochemical processes by the formation of enzyme-substrate complex inhibit the visible inactivation. For this reason, the difference between ΔS^* in the absence and presence of substrate is large for the visible inactivation.

Table II. Free energies of activation (ΔF^*), heats of activation (ΔH^*) and entropies of activation (ΔS^*) for thermal processes of various inactivations

Inactivation	°C	ΔF^* (Kcal/M)	ΔH^* (Kcal/M)	ΔS^* (cal/M)
Heat	55	24.7	84.5	183
		25.9 (s)	90.5 (s)	197 (s)
Ultraviolet	50	23.7	40.9	53
		23.7 (s)	41.2 (s)	54 (s)
Visible	50	24.9	56.4	99
		25.5 (s)	77.0 (s)	160 (s)

(s) = value in the presence of substrate.

TAA forms enzyme-product complex as well as enzyme-substrate complex, so that the protections observed are considered to be due to the combined effects of substrate and its decomposition products.

Zusammenfassung. Es werden die Bestimmung der thermodynamischen Quantitäten für die Inaktivierungsreaktionen, die Wärme- und Photo-Inaktivierungen der Taka-Amylase A und die Schutzwirkung des Substrats gegen diese Inaktivierungen diskutiert.

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[5] H. EYRING, J. chem. Phys. *3*, 107 (1935).
[6] W. F. K. WYNNE-JONES and H. EYRING, J. chem. Phys. *3*, 492 (1935).
[7] The difference of 14 cal/M between the entropies for the heat-inactivation in the absence and presence of substrate (Table II) is considered to be concerned with the entropy change upon the formation of enzyme-substrate complex.

Direct Aromatization of C_{19}-Steroid Sulphates[1]

In the course of recent investigations on the in vivo biogenesis of 7α-^{3}H-dehydroepiandrosterone (DHEA) ^{35}S-sulphatide and its metabolism, estrogen sulphatides with the original ^{3}H/^{35}S ratio could be demonstrated in peripheral plasma[2]. On theoretical grounds this direct transformation of neutral C_{19}-steroid sulphatide into phenolic C_{18}-steroid sulphatide should involve the dehydrogenation of DHEA sulphatide to the corresponding dienol sulphatide of androst-4-ene-3,17-dione (androstenedione) as the first step in the reaction sequence. In order to substantiate such an hypothesis, the conversion of synthetic androstenedione sulphate into estrogens has been attempted under in vitro conditions.

The 3,5-dienol sulphate of androstenedione was prepared by routine methods[3], using chlorosulphonic acid or its methyl ester in absolute pyridine. The sodium salt of the sulphoconjugate, purified by thin layer chromatography on silica gel G in chloroform-methanol-ammonia (20:5:0.2 v/v) (Rf = 0.20), on DEAE-cellulose in isopropanol-water-formic acid (65:33:2 v/v) (Rf = 0.21) and paper chromatography on Whatman DE-20 in 1.0M acetate buffer of pH 4.7[4] (Rf = 0.36), had a melting point of 194–200 °C (uncorr.). The UV-spectrum in methanol exhibited an absorption maximum at 238 nm, the IR-spectrum strong absorption bands near 1742 cm^{-1} (17-keto group), 1638 cm^{-1} (3,5-dienol ester group), 1245 cm^{-1} and 1048 cm^{-1} (assymetric and symmetric S–O vibration), while the characteristic absorption band of Δ^4-3-keto-steroids near 1618 cm^{-1} had disappeared.

When 1.95 μg (5 nmMol) of 7α-^{3}H-androstenedione ^{35}S-sulphate-Na with 310,000 dpm ^{3}H and 161,000 dpm ^{35}S

[1] This investigation was carried out with the support of the Deutschen Forschungsgemeinschaft, Bad Godesberg.
[2] G. W. OERTEL, P. KNAPSTEIN, and L. TREIBER, Hoppe-Seyler's Z. physiol. Chem., in press (1966).
[3] A. B. ROY, Biochem. J. *62*, 41 (1956).
[4] G. W. OERTEL and E. KAISER, Biochem. Z. *336*, 10 (1962).

($^3H/^{35}S = 1.92$) and 1.96 μg (5 nmMol) 7α-^{3}H-DHEA ^{35}S-sulphate-Na with 528,000 dpm ^{3}H and 271,000 dpm ^{35}S ($^3H/^{35}S = 1.95$) were incubated in duplicate with placental microsomes[5], equivalent to 250 mg of wet tissue, in 0.1 *M* phosphate buffer of pH 7.2 and in the presence of 1.5 mg $NADPH_2$, 22.6% and 17.2% of ^{3}H-activity were found in the fraction of free and conjugated phenolic steroids. Following the ion exchange chromatography of steroid conjugates on DEAE-Sephadex A-50[6] and thin layer chromatography of the steroid sulphates on silica gel G in chloroform-methanol-ammonia (20:5:0.2 v/v), on DEAE-cellulose in isopropanol-water-formic acid (65:33:2 v/v), and paper chromatography in isopropyl ether-ligroin-t-butanol-ammonia-water (5:2:3:1:9 v/v)[7], the radioactive compound with the mobility of authentic estrone sulphate (Rf = 0.17; Rf = 0.12; RT = 1.05) represented 15.9% ^{3}H of incubated androstenedione sulphate and 10.4% ^{3}H of incubated DHEA sulphate. The corresponding $^3H/^{35}S$ ratio of the isolated fractions amounted to 2.03, 2.14 and 1.98 or 2.10, 2.04 and 2.01 respectively. After cleavage of estrone sulphate by solvolysis in ethyl acetate/sulphuric acid the liberated estrone was isolated and characterized by reverse isotope dilution and purification to constant specific activity.

From these findings it becomes evident indeed that the 3,5-dienol sulphate of androstenedione can be converted biosynthetically into estrone sulphate. The yields of this biotransformation apparently exceeded those obtained by incubation of DHEA sulphate[8,9], thus favouring the concept that the biosynthesis of estrogens from DHEA sulphate may proceed via androstenedione sulphate.

Zusammenfassung. Bei Bebrütung von synthetischem 7α-^{3}H-Androst-4-en-3,17-dion-^{35}S-sulfat mit Mikrosomen aus menschlicher Placenta in Gegenwart von $NADPH_2$ wurden 15.9% des Substrats in doppelt-markiertes Östron-sulfat mit unverändertem $^3H/^{35}S$-Verhältnis umgewandelt. Da die Ausbeute vergleichsweise höher lag als bei Verwendung von 7α-^{3}H-Dehydroepiandrosteron-^{35}S-sulfat, wird angenommen, dass die Biosynthese von Östron-sulfat aus Dehydroepiandrosteron-sulfat über ein dem Androst-4-en-dion entsprechendes 3,5-Dienol-sulfat verläuft.

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[5] K. J. RYAN, J. biol. Chem. *234*, 268 (1959).
[6] G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *339*, 125 (1963).
[7] E. E. BAULIEU, C. CORPECHOT, and R. EMILIOZZI, Steroids *2*, 429 (1963).
[8] T. MORATO, A. E. LEMUS, and C. GUAL, Steroids, Suppl. *1*, 59 (1965).
[9] E. BOLTE, S. MANCUSO, G. ERIKSSON, N. WIQUIST, and E. DICZFALUSY, Acta endocr., Copenh. *45*, 535, 560, 576 (1964).

Studies on the Regional Biosynthesis and Metabolism of Catecholamines in the Central Nervous System of the Monkey

Recently it was shown that norepinephrine-H^3 injected into the lateral ventricle of the rat can accumulate in the brain, and that exogenous norepinephrine introduced in this way mixes with the endogenous stores[1]. In the present study the biosynthesis and metabolism of catecholamines was investigated in specific regions of the central nervous system (CNS) of the monkey following intraventricular injection of tyrosine-C^{14} and of dopamine-H^3. Also, the tyrosine hydroxylase activity was determined in the specific regions of the CNS.

In all experiments green monkeys (*Cercopithecus sabaeus*) weighing 2.0–3.5 kg were used. The animals were injected with dopamine-1-H^3 (50 μc, 5 μg) or with tyrosine-C^{14} (25 μc, 11 μg) into both lateral ventricles of the brain by a stereotaxic technique. In experiments with dopamine-H^3 the animals were pretreated with pheniprazine (10 mg/kg i.p.) 4 h before the intraventricular injection of the labeled amine. 2 h after administration of the labeled compounds the animals were killed and the brains were removed. The labeled amines and their metabolites were isolated and determined by previously described procedures[2]. The catecholamines were absorbed on alumina and determined fluorimetrically[3,4]. Tyrosine hydroxylase activity was determined by the procedure of NAGATSU et al.[5].

Studies with tyrosine-C^{14}. Following intraventricular injection of tyrosine-C^{14} the catechols represented only a

Table I. The in vivo and in vitro formation of catecholamines from tyrosine-C^{14} in different regions of the CNS

	Catecholamines formed cpm/g tissue[a]	
	in vivo experiments	in vitro experiments[b]
Caudate nucleus	8500 ± 600	25,000 ± 1500
Putamen	400 ± 50	32,000 ± 2000
Hypothalamus	4000 ± 500	4800 ± 600
Brainstem	1500 ± 150	N.E.
Cerebellum	1050 ± 100	N.E.
Spinal cord	1000 ± 100	N.E.

[a] Each value is the mean from 3 experiments ± S.E.M. [b] The tissue homogenates were incubated with tyrosine-C^{14} for 30 min at 37 °C. N.E. = not estimated.

[1] J. GLOWINSKI, I. KOPIN, and J. AXELROD, J. Neurochem. *12*, 25 (1965).
[2] M. GOLDSTEIN, Int. J. Neuropharmac. *3*, 37 (1964).
[3] A. H. ANTON and D. F. SAYRE, J. Pharmac. exp. Ther. *138*, 360 (1962).
[4] A. H. ANTON and D. F. SAYRE, J. Pharmacol. exp. Ther. *145*, 326 (1964).
[5] T. NAGATSU, M. LEVITT, and S. UDENFRIEND, J. biol. Chem. *239*, 2910 (1964).