Conversion of β-Sitosterol to Steroid Hormones by Rat Testes in vitro

It has been well established that plant sterols are absorbed to considerable extent by both man[1] and experimental animals[2–4], and have been detected in concentrations of 3–5% in rat adrenals[5] and in tumours of ovary and breast of man[6]. Furthermore, the plant sterols have been shown to become converted into bile acids[7–9] and cortisol[10] which have been claimed[9,10] to take place via cholesterol as intermediate.

The present study reports on the direct conversion of β-sitosterol to steroid hormones by a mitochondrial enzyme system of the rat testes which have been shown[11] to cleave the side chain of cholesterol at C_{20} to yield pregnenolone and isocaproic aldehyde.

Materials and methods. Standard progesterone, pregnenolone, 17α-progesterone, and testosterone were obtained from Sigma Chemical Co., Saint Louis, Mo. β-sitosterol-4-^{14}C (spec. act. 61 mCi/m*M*) was obtained from New England Nuclear Corp., Boston, Mass. Cholesterol-4-^{14}C (spec. act. 61 mCi/m*M*) was purchased from the Radiochemical Centre, Amersham, England. Lecithin emulsions of the sterols were prepared according to the method of HOYES and SAUNDERS[12]. Mitochondria of rat testes were prepared as described by TOREN et al.[11] and a fraction representing 1 g of wet testes per ml was used at a time. The incubations were done as suggested by MENON et al.[13] for cholesterol oxidation. The steroids were resolved by thin-layer chromatography[14]. Radio-gas chromatography of the steroids was done as previously described[7].

Results and discussion. Table I shows the distribution of radioactivity among the oxidation products of β-sitosterol-4-C^{14} and cholesterol-4-C^{14}. The extent of total oxidation achieved varied from 2–3% of the original sterol, which was sufficient to obtain significant amounts of radioactivity in the steroid fractions corresponding to progesterone, pregnenolone and testosterone. The identification of the major oxidation product of β-sitosterol as pregnenolone was confirmed by the similarity in behaviour to standard pregnenolone on TLC in solvent systems ethyl: ether chloroform (9:1, v/v) and ethyl acetate: methanol (99:1, v/v) (Rf values 0.31 and 0.80, respectively) and by crystallization to constant specific activity in the presence of carrier pregnenolone (specific activity of the crystals in 4 successive crystallizations was 825, 882, 861 and 877, respectively). By similar means it was possible to demonstrate that the radioactivity associated with the steroid spot corresponding to progesterone was indeed progesterone (Rf value of 0.52 and 0.78 in solvent systems ethyl ether:chloroform (9:1, v/v) and ethyl acetate:methanol (99:1, v/v) which corresponded to authentic progesterone). The radioactivity in this fraction was then crystallized to constant specific activity with carrier progesterone (specific activity of the crystals during 4 successive

Table I. Yield of steroid hormones from β-sitosterol and cholesterol in rat testes mitochondria[a]

Steroid Hormone[b]	Substrate total radioactivity (%)			
	β-Sitosterol-4-^{14}C		Cholesterol-4-^{14}C	
	I	II	I	II
Progesterone	0.6	0.5	0.8	1.0
Pregnenolone	1.0	1.1	1.0	0.89
Testosterone plus 17α-progesterone	0.7	0.6	0.5	0.6
Polar steroids	0.6	0.5	0.8	0.67

[a] The total yield of steroid products averaged 2–3% of the dose per g of wet testes. [b] Assayed after elution from thin-layer plates, which were developed in diethyl ether-chloroform and ethyl acetate-methanol as described in the text.

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Table II. Effect of NADPH on the oxidation of β-sitosterol and cholesterol by rat testes mitochondria[a]

NADPH added (mg)	β-Sitosterol-4-^{14}C		Cholesterol-4-^{14}C	
	Steroid products (cpm)	Stimulation (%)	Steroid products (cpm)	Stimulation (%)
0.0	450	0	644	0
0.1	2,065	458	1,757	272
0.5	12,801	2,844	11,506	1,786
1.0	19,507	4,334	21,500	3,338
2.0	25,000	5,555	24,507	3,805

[a] Each incubation contained 1×10^6 dpm of β-sitosterol or cholesterol and mitochondria equivalent to 1 g wet testes in 2 ml 0.033 *M* phosphate buffer, pH 7.2. After incubation for 3 h, carriers were added and the reaction products extracted and separated from the parent sterols by thin-layer chromatography using 2 solvent systems. The appropriate sections of the gel were eluted and counted.

crystallization was 156, 162, 171 and 183 respectively). The radioactivity corresponding to testosterone could not be recrystallized to constant specific activity. This was probably due to the presence of 17α-hydroxyprogesterone, which also migrated to the same place in the two thin-layer chromatographic systems employed in this study. The proportions of the individual oxidation products varied from one incubation to the next and differed somewhat from those reported by MENON et al.[13] for the oxidation products of cholesterol.

It is possible that the differences in the proportions of the products were due to the presence of variable amounts of extramitochondrial steroid dehydrogenases and isomerases in the mitochondrial preparations[15].

Previous workers had demonstrated an absolute requirement for pyridine nucleotide, preferably NADPH, for the oxidation of cholesterol by the mitochondria of rat testes[11] and adrenals[16]. Table II compares the yields of oxidation products obtained for β-sitosterol and cholesterol in response to the addition of increasing amounts of NADPH to the incubation medium. There is sufficient similarity in the extent of oxidation of cholesterol and β-sitosterol to conclude that the two sterols are affected by the same enzyme systems.

Although no attempt was made to recover the cleaved-off portion in this study, on the basis of the reported characteristics of the enzyme system[15], it may be assumed that the fragment was released in one piece. Furthermore, radio-gas chromatography failed to reveal the conversion of β-sitosterol-4-^{14}C into cholesterol or any other C_{27} sterol[7].

The above demonstrated mechanism of plant sterol oxidation would be expected to operate also in the adrenal tissue, as it is known to convert cholesterol to progesterone via a comparable pathway[15]. Hence the observed conversion of ^{3}H-sitosterol into cortisol in the guinea-pig[10] may also have taken place without the formation of cholesterol as an intermediate. Because of the limited mass of the tissue in the gonads and in the adrenals, these organs would not be expected to contribute much to the balance of the plant sterol in the animal body[17].

Résumé. Les mitochondries des cellules testiculaires de rat convertissent le β-sitostérol-4-C^{14} en prégnénolone et progestérone. Comme celle du cholestérol, l'oxydation du β-sitostérol en composés stéroidiens est stimulée par le NADPH. La radio-chromatographie en phase gazeuse des stéroides n'indique aucune conversion du β-sitostérol en cholestérol ou en stéroides à 27 carbones dans les conditions étudiées.

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Antibiotisch wirksame Tripeptide der Sequenz L-Arg-D-X-L-Phe

Antibiotically Active Tripeptides of the Sequence L-Arg-D-X-L-Phe

Nachdem LÖFFLER et al.[1] ein antibiotisch aktives Tripeptid der Sequenz L-Arg-D-allo-Thr-L-Phe aus dem Kulturmedium des Dermatophyten *Keratinophyton terreum* isoliert hatten, stellte sich die Frage nach dem Wirkungsmechanismus dieses Antibiotikums. Als Hilfsmittel zur Klärung dieser Frage wurde eine Sequenzvariation durchgeführt. Dabei wurde das D-allo-Threonin unter Beibehaltung der Restsequenz durch andere D-Aminosäuren ersetzt.

Material und Methoden. Die Peptidsynthesen wurden anhand der Merrifield-Synthesetechnik durchgeführt. Die Reinigung der Peptide erfolgte durch Säulenchromatographie an Kieselgel bei 4 °C. Das Laufmittel war *n*-Butanol: H_2O: Eisessig = 4:2:1.

Alle t-Boc-Aminosäuren wurden nach SCHNABEL[2] und Z_3-L-Arginin nach WÜNSCH et al.[3] hergestellt. Die Aktivitätsmessungen wurden an den von LÖFFLER et al.[1] verwendeten Testorganismen mit Hilfe des Plattendiffusionstests[4] durchgeführt. Folgende Tripeptide wurden synthetisiert und getestet:

1. L-Arg-D-Ala-L-Phe;
2. L-Arg-D-Asn-L-Phe;
3. L-Arg-D-Glu-L-Phe;
4. L-Arg-D-Leu-L-Phe;
5. L-Arg-D-Phe-L-Phe;
6. L-Arg-D-Ser-L-Phe;
7. L-Arg-D-Tyr-L-Phe;
8. L-Arg-D-Val-L-Phe.

Ergebnisse und Diskussion. Von den 8 synthetisierten Tripeptiden mit variierter Mittelposition waren 5 antibiotisch aktiv. Ihre Aktivität erstreckte sich jedoch nur auf Fungi und einige pathogene Pilze. Sowohl gram-positive als auch gram-negative Bakterien wurden von diesen Peptiden nicht angegriffen. Dieses Verhalten der synthetischen Peptide stimmt mit dem des von LÖFFLER et al.[1] isolierten Peptids überein.

Die nachfolgenden Testergebnisse enthalten die Werte für den Pilz *Paecilomyces varioti*. Die Tests wurden durchweg auf Komplexmedium (Malzextrakt[5]) durchgeführt. Für die 5 aktiven Tripeptide konnte bei *Paecilomyces varioti* die folgenden minimalen Hemmkonzentrationen ermittelt werden:

L-Arg-D-Ala-L-Phe	1,7 mg/ml;
L-Arg-D-Tyr-L-Phe	0,9 mg/ml;
L-Arg-D-Val-L-Phe	1,5 mg/ml;
L-Arg-D-Phe-L-Phe	0,12 mg/ml;
L-Arg-D-Leu-L-Phe	1,2 mg/ml.

Die Tripeptide 2, 3 und 6 zeigten keinerlei antibiotische Aktivität.

Im Kreuztest[6] zeigte sich, dass die Hemmwirkung der Peptide nur dann aufgehoben wird, wenn man die der im Peptid in Mittelposition befindlichen D-Aminosäure ent-