Table I. Metabolism of ^{14}C-DOPA by *Octopus* brain in vitro

Time (min)	Radioactivity (dpm $\times 10^{-3}$/g brain)			Concentration (μg/g brain)		
	DM	DOPAC	NA	DM	DOPAC	NA
30	11.6 ± 1.1	11.9 ± 1.9	0.92 ± 0.1	8.78 ± 0.64	1.23 ± 0.12	1.95 ± 0.21
60	19.4 ± 2.8	15.3 ± 0.9	1.63 ± 0.2	8.31 ± 0.72	1.96 ± 0.21	1.97 ± 0.11
180	31.6 ± 3.9	30.4 ± 6.1	4.00 ± 0.4	7.52 ± 1.09	2.49 ± 0.60	1.95 ± 0.28

Values are means ± S.E.M. of 4 animals per group. Each brain was incubated with 0.2 μCi ^{14}C-DOPA/ml.

Chromatography of the acid metabolites (Figure 2) showed labelling of DOPAC but very little in the regions where homovanillic acid (HVA) and dihydroxymandelic acid (DOMA) standards ran. Separation of a sample of the medium after incubation (Figure 3) showed that only labelled DOPA was present in significant amounts.

The time-course of the in vitro formation of amines and acid metabolites by *Octopus* ganglia is shown in Table I. The amounts of radioactive DM, DOPA and NA increased steadily from 30 to 180 min although the synthesis of labelled DM and DOPAC was at all times greater than that of NA. The concentrations of the amines in the tissue did not change significantly during the course of the incubation.

After ^{14}C-DOPA had been injected in vivo the formation of radioactive DM, DOPAC and NA was examined at 5, 30, 60 and 180 min (Table II). Substantial labelling of NA and DM was obtained as early as 5 min after injection. The maximal accumulation of radioactive DM was detected at 30 min, lasted about 1 h and was reduced to nearly half its maximal level 3 h after the injection. The highest level of radioactive DOPAC was observed at 30 min coinciding with the labelling of DM. In contrast the level of radioactive NA showed no significant change from 5 to 180 min after precursor administration.

Discussion. The conversion of radioactive tyrosine and DOPA to the catecholamines DM and NA suggests that *Octopus* ganglia contain enzymes with tyrosine hydroxylase, DOPA decarboxylase and dopamine β-hydroxylase activity. Both in vivo and in vitro experiments show that DOPA is rapidly decarboxylated to DM which in turn is metabolized by a monoamine oxidase. Endogenous DOPAC has been detected in the optic lobes of *Octopus* but HVA has not been found[5], even after the administration of 200 mg/kg of l-DOPA (A. V. JUORIO, unpublished observation). The present results confirm that in *Octopus* ganglia radioactive DM is mainly metabolized to DOPAC and not to HVA, suggesting that DOPAC is normally removed from ganglia without methoxylation.

The results obtained in vitro are consistent with the synthesis of NA from DM, assuming that the lower rate of labelling of NA is due to the limiting rate of DM-β-hydroxylation. In vivo, however, the maximum period of DM labelling (30–60 min) is not followed by a corresponding increase in NA. The results suggest that there are 2 pools of DM with different specific activities. One pool of high specific activity in dopaminergic nerves, where DM has a transmitter function and is rapidly metabolized to DOPAC. The other, a low specific acticity pool, in noradrenergic nerves where DM functions as a precursor to NA. Assuming that most of the brain DM is in dopaminergic nerves the higher endogenous level of DM combined with the higher specific activity would imply that the dopaminergic nerves must also have a greater uptake of ^{14}C-DOPA than noradrenergic nerves. When a large exogenous pool of ^{14}C-DOPA is available, as is the case in the in vitro experiments, the synthesis of radioactive NA increases with time as does the synthesis of DM[9].

Table II. Metabolism of ^{14}C-DOPA by *Octopus* brain in vivo

Time (min)	Radioactivity (dpm $\times 10^{-3}$/g brain)		
	DM	DOPAC	NA
5	15.3 ± 2.7	0.93 ± 0.1	2.60 ± 0.4
30	25.5 ± 3.5	15.8 ± 1.4	1.90 ± 0.3
60	23.6 ± 8.2	5.17 ± 1.7	1.71 ± 0.4
180	14.1 ± 5.0	3.43 ± 0.9	1.77 ± 0.5

Values are means ± S.E.M. of 4 animals per group. Each animal was injected with 1 μCi ^{14}C-DOPA.

Resumen. Los ganglios cerebrales del pulpo (*Octopus vulgaris*) sintetizan la dopamina y la noradrenalina radioactivas luego de la administración in vivo o la incubación in vitro con sus precursores marcados. La dopamina es luego metabolizada a ácido dihidroxifenilacético mientras que el correspondiente derivado de la noradrenalina no fué encontrado.

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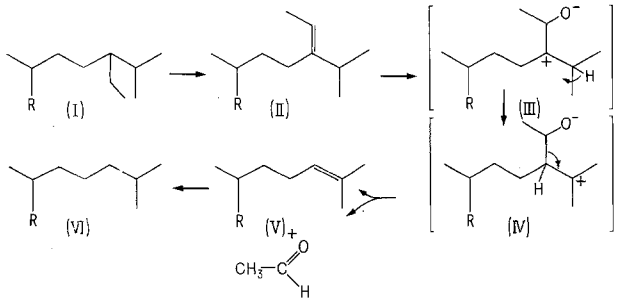
Fucosterol-24,28 Epoxide and 28-Oxo-β-Sitosterol as Possible Intermediates in the Conversion of β-Sitosterol into Cholesterol in the Locust *Locusta migratoria* L.

Phytophagous insects transform β-sitosterol (I) into cholesterol (VI)[1–4]. In the locust *Locusta migratoria* L., using 3-^{3}H precursors, the conversion of (I) has been shown to proceed through fucosterol (II) and desmosterol(V)[4,5]. This seems to indicate the simultaneous elimination of carbon atoms 28 and 29. Such a reaction should start with an oxidation, but it is rather difficult to predict at what carbon atom the attack would begin.

Recently, a new reaction between fucosterol-24, 28 epoxide and BF_3 has been published[6]; (V) and 28-oxo-β-sitosterol are formed ,the simultaneous elimination of carbon atoms 28 and 29 appearing as a type of biomimetic degradation of fucosterol to a C_{27} sterol. In order to demonstrate the possibility of a biological conversion $C_{29} \rightarrow C_{27}$ from a 24, 28-epoxide an incorporation of 3-^{3}H-fucosterol-24, 28 epoxide was performed in the Locust. Meanwhile, the results of parallel experiments with *Bombyx mori*[7] have been published. In both cases radioactive cholesterol was isolated. We wish to describe here the results of our own experiments, as well as those of a second series using 3-^{3}H-28-oxo-β-sitosterol. In connection with the results so far obtained in our laboratory, a degradation scheme is proposed as hypothesis.



R = 3 β–hydroxy Δ_5–steroid nucleus

Conversion of fucosterol-24, 28 epoxide into cholesterol. 8 mg of 3-^{3}H-fucosterol-24, 28 epoxide propionate[5] (specific activity $8{,}6 \times 10^5$ dpm/mg[8]) are suspended in 1 ml of Ringer solution with 1 drop of Tween 80. The solution of the precursor is injected into the abdomen of 40 larvae (5th instar) of the locust *Locusta migratoria*. After 8 days, the insects are extracted as usual with alcohol and ether. The acetone-soluble lipids are then fractionated on a SiO_2 column. The free sterols (40 mg, radioactivity 1.25×10^4 dpm/mg) are propionylated; 2 mg of fucosterol propionate and 2 mg of desmosterol propionate are added for dilution. The mixture is further submitted to a $Al_2O_3/AgNO_3$ 3:1 preparative TLC[9] (hexane-ethyl acetate 25:1). About 95% of the total radioactivity is recovered in the Δ_5 mono-ene sterol propionates fraction (Rf 0.78). Separation of the propionates is carried out by preparative GLC[9] (gas chrom Q, OV 101 1.3%, 250 °C). All the radioactivity is found in cholesterol propionate (18% of the lipids total radioactivity). It is noticeable that no labelling could be detected in the C_{28} and C_{29} sterols. Traces of radioactivity are found in desmosterol propionate.

Conversion of 28-oxo-β-sitosterol into cholesterol. 2.6 mg of 28-oxo-β-sitosterol propionate[5] (specific activity 1.9×10^6 dpm/mg) suspended as above in Ringer solution are injected into the abdomen of 29 larvae (5th instar) of the locust. After an 8-day incubation, the sterols are isolated as described previously. 25 mg of sterol propionates (3.1×10^2 dpm/mg) were obtained. About 98% of this radioactivity was recovered in the Δ_5 mono-ene sterol propionates (0.2% of the total lipids radioactivity). After fractionation through GLC, radioactive cholesterol, campesterol and β-sitosterol were isolated.

Discussion. The present experiments indicate that fucosterol-24, 28 epoxide is a possible precursor of cholesterol in the locust, *Locusta migratoria*. It may thus be an intermediate in the dealkylation of (I).

The 28-oxo-β-sitosterol seems not to be a precursor of cholesterol for the following reasons; 1. Compared to the transformation of fucosterol-24, 28 epoxide, the 28-oxo-β-sitosterol degradation into cholesterol shows a much poorer yield. 2. The observed transformation is unspecific. 3. The sterol composition after injection of 28-oxo-β-sitosterol is modified; the total cholesterol decreases from 90–92% to 80–82% and the $C_{28}+C_{29}$ sterols increase from 10 to 20% (values verified through different repetitions). It may be that 28-oxo-β-sitosterol is an inhibitor of the C_{24} dealkylation in the locust. Our results are in agreement with those of IKEKAWA et al.[7] with *Bombyx mori* and also with the observations of RANDALL et al.[10] concerning the migration of the hydrogen atom from position C_{25} to C_{24} during the transformation of isofucosterol into (VI) in *Tenebrio molitor* larvae. The poor and non-specific conversion of 28-oxo-β-sitosterol and its possible inhibitory effect on dealkylation show that the scheme 2 proposed by RANDALL et al.[10] (in which 28-oxo-β-sitosterol is an intermediate) cannot be retained for the locust. We propose the following scheme for the conversion of β-sitosterol into cholesterol in this insect.

In this scheme, (III) and (IV) which are in brackets may correspond to the products of the opening of a 24, 28-epoxide of fucosterol, but in fact the latter has never been isolated from an insect. The intermediates (III) and (IV) would rather be expected to exist in an enzyme-substrate complex in which a dipole $\overset{-}{O}\text{-}C_{28}\text{-}\overset{+}{C}_{24}$ would appear. This complex would break down with liberation of (V) plus one mole of acetaldehyde or its equivalent. The observed inhibitory effect of 28-oxo-β-sitosterol can be explained by preventing the enzyme-substrate complex formation or the dipole formation[11].

Résumé. Le Criquet *Locusta migratoria* L. transforme l'époxyde-24, 28 du fucostérol en cholestérol; le desmostérol est un intermédiaire. Ce même Insecte ne transforme pas spécifiquement le céto-28 β-sitostérol en cholestérol; on observe dans ce cas une inhibition de la désalkylation en 24 du β-sitostérol. Un schèma de dégradation du β-sitostérol par *Locusta migratoria* est présenté.

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[1] J. A. SVOBODA and W. E. ROBBINS, Experientia *24*, 1131 (1968).
[2] J. A. SVOBODA, M. J. THOMSON and W. E. ROBBINS, Nature, New Biol. *230*, 57 (1971).
[3] J. P. ALLAIS, J. PAIN and M. BARBIER, C.r. Acad. Sci., Ser. D *272*, 877 (1971).
[4] J. P. ALLAIS and M. BARBIER, Experientia *27*, 506 (1971).
[5] Unpublished results. The synthesis of the 3-^{3}H-sterol precursors used in this work will be described elsewhere.
[6] N. IKEKAWA, M. MORISAKI, H. OTAKA and Y. CHIYODA, Chem. Commun. *1971*, 1498.
[7] M. MORISAKI, H. OHTAKA, M. OKUBAYASHI, N. IKEKAWA, Y. HORIE and S. NAKASONE, Chem. Commun. *1972*, 1275.
[8] The radioactivities have been measured on a Nuclear Chicago Mark I scintillator.
[9] TLC, thin layer chromatography; GLC, gas liquid chromatography.
[10] P. J. RANDALL, J. G. LLOYD-JONES, I. F. COOK, H. H. REES and T. W. GOODWIN, Chem. Commun. *1972*, 1296.
[11] Acknowledgments. We thank Professors E. LEDERER and J. BERGERARD for their interest.
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