Nuclear pore flow rates for ribosomal and transfer RNAs per average hepatocyte of 160–190 g albino rats (Wistar) as calculated from the steady state assumption NPFR = synthetic rate per average hepatocyte/mean number of pores per nucleus and synthetic rate = $1n\,2 \times$ (cytoplasmic concentration of product) $\times (t_{1/2} \times \text{min})^{-1}$

Electron microscopic method used for determining nuclear pore frequencies	Mean pore frequency per μm^2 nuclear surface	Mean nuclear diameter (μm)	Total No. of nuclear pores/average nucleus	$NPFR_{N\rightarrow C}$ (10–12 μg rRNA/ pore/min)	$NPFR_{N\rightarrow C}$ (rRNA equivalent to 1 ribosome/pore/ min)	$NPFR_{N\rightarrow C}$ (molecules rRNA/ pore/min) [d]	$NPFR_{N\rightarrow C}$ (molecules tRNA/ pore/min)
Negative staining of isolated nuclear envelope pieces	35.8	8.02 [a]	7.3×10^3	0.45	0.113	0.23	1.5
Glutaraldehyde-OsO_4-fixation, ethanol dehydration, epoxy resin embedding, thin sectioning	16.3	8.04 [b]	3.3×10^3	1.00	0.250	0.51	3.3
Freeze-etching of aldehyde-prestabilized tissue	14.1	8.10 [c]	2.9×10^3	1.14	0.286	0.57	3.8

Average DNA content of the nuclei was 9.1×10^{-12} g. The number of ribosomes per average cell was calculated as 7.6×10^6 from RNA/DNA ratios and the assumption that 90% of the cellular RNA is constituted by rRNA (compare[7–9]). A half-life of rRNA of 106 h was assumed[10–13]. The corresponding data for tRNA were taken from the work of WILSON and QUINCEY[9,13] (see there for further references). rRNA equivalent to one ribosome means the sum of 28s + 18s + 5s RNA present in a ribosome. Index N(ucleus)→C(ytoplasm) gives direction of the flow considered. [a] Values obtained from nuclei isolated with the procedure described elsewhere. [b] Values obtained from 1 μm thick sections through the same blocks as used for EM work. [c] Values obtained from 10 μm freeze-sections (WKF cryotome) of fixed tissue as used for the EM freeze-etch work. [d] Assuming a mean molecular weight of 1.2×10^6 D.

the structural data of the nuclear envelopes in this tissue yielded the NPFR values for tRNA and rRNA as listed in the Table. Since the different electron microscopic preparation techniques result in more or less divergent pore frequency values (FRANKE[3,4]; for detailed discussion of this problem see KARTENBECK et al.[5]) and since no decision on the in vivo state can be made at the moment, all the 3 basic techniques have been considered in parallel. As a consequence of the fact that the mature rat liver represents a mosaic of binucleate and mononucleate cells, as well as different steps in polyploidy, all values listed refer to the abstract but useful term of the 'average hepatocyte'. This describes the cellular portion belonging to an average nucleus which was characterized in our material (rats of 160–190 g body weight) by a mean DNA content of 9.1 picograms. The values for rRNA given in the Table also reflect the total cellular RNA since rRNA constitutes 80–90% of total RNA in rat liver.

It is apparent from these data that the hepatocyte nuclear pores show a RNA transport rate which is slightly less than that of lampbrush stage *Xenopus laevis* oocytes[1,2]. It is also below that of HeLa cells by a factor of about 4 (when comparing with values calculated with data from negative stain preparations of isolated envelope pieces) or 7 (when using the structural data obtained from freeze-etch work). The NPFR values in all these cell types mentioned, however, are much lower, for instance, than that of the macronucleus of the ciliate *Tetrahymena pyriformis* GL during exponential growth. In this cell, the average macronuclear pore conveys 45.8×10^{-12} μg as calculated on the basis of data obtained from negatively stained isolated membranes, and nearly 200×10^{-12} μg as calculated using freeze-etch data given by SPETH and WUNDERLICH[6]. Thus, the RNA transport capacity of a nuclear pore appears to be more than hundredfold higher in *Tetrahymena* than in a hepatocyte.

Zusammenfassung. Aus den Fliessgleichgewichts-Werten (mittlere Kernporenzahl und RNA-Synthesegeschwindigkeiten) der ausdifferenzierten Rattenleberzelle wurde die Kernporen-Durchflussrate (NPFR) für ribosomale und transfer RNA berechnet. Diese Hepatocytenwerte werden mit den entsprechenden RNA-Transportleistungen der Kernporenkomplexe anderer Zelltypen verglichen.

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[3] W. W. FRANKE, Z. Zellforsch. mikrosk. Anat. *105*, 405 (1970).
[4] W. W. FRANKE, J. Cell Biol. *31*, 619 (1966).
[5] J. KARTENBECK, H. ZENTGRAF, U. SCHEER and W. W. FRANKE, Z. Zellforsch., in press.
[6] V. SPETH and F. WUNDERLICH, J. Cell Biol., *147*, 772 (1970).
[7] G. BLOBEL and V. R. POTTER, J. molec. Biol. *26*, 279 (1967).
[8] E. R. WEIBEL, W. STÄUBLI, H. R. GNÄGI and F. A. HESS, J. Cell Biol. *42*, 68 (1969).
[9] S. H. WILSON and R. QUINCEY, J. biol. Chem. *244*, 1092 (1969).
[10] J. N. LOEB, R. R. HOWELL and G. M. TOMKINS, Science *149*, 1093 (1965).
[11] C. A. HIRSCH, J. biol. Chem. *242*, 2822 (1967).
[12] S. H. WILSON and M. B. HOAGLAND, J. Biochem. *103*, 556 (1967).
[13] R. QUINCEY and S. H. WILSON, Proc. natn. Acad. Sci., USA *64*, 981 (1969).

The Influence of Several Sterols on the Conversion of β-Sitosterol into Cholesterol in the Cockroach

Although sterols are important for them, insects do not possess the enzymic system necessary for the synthesis of sterols from acetate or mevalonate. Therefore, sterols are indispensable components in the insects' food. Carnivorous insects obtain cholesterol from their food and they are able to convert it into the moulting hormones α- and β-ecdysone. Plant-eating insects obtain β-sitosterol from their food and they can convert it into cholesterol,

which implies a dealkylation of the ethyl group containing C-28 and C-29.

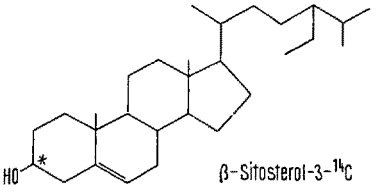
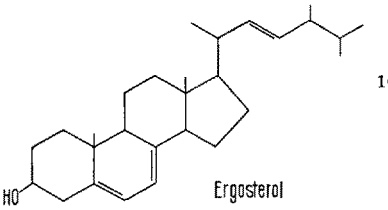
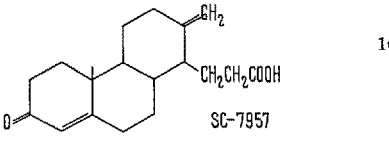
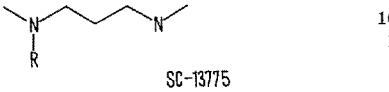
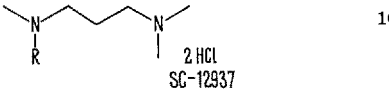
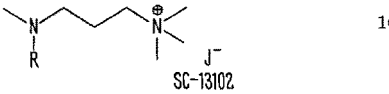
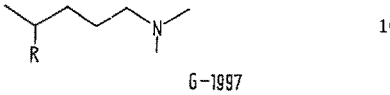
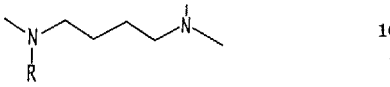
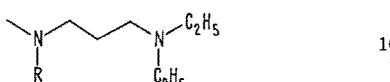
For strictly phytophagous insects, whose natural source of nutrition does not contain cholesterol, this ability to dealkylate is of vital importance. Inhibition of this reaction, resulting in a depletion of cholesterol, will disturb their development[1-3]. Since knowledge in this field may help in developing selective ways of insect control, we decided to try to learn more about this dealkylation[4].

We administered β-sitosterol-3-^{14}C[5], added to a sterol-free diet[6,7], to 3 species of aseptic[8] cockroaches, viz. *Blattella germanica* L., *Periplaneta americana* L. and *Eurycotis floridana* Walker. Although the cockroach is not a strictly phytophagous insect, the said dealkylation can also be studied in this omnivorous test animal. Besides cholesterol, several radioactive minor metabolites were found. One of these had the same gas-chromatographic behaviour as $\Delta^{24(28)}$-sitosterol. However, the amounts of the metabolites were too small for elucidation of their structures.

The present paper reports on the influence of a variety of sterols with unnatural side-chains on the conversion of sitosterol into cholesterol. 10 mg of sterol-free diet[6,7], to which 10 µg of β-sitosterol-3-^{14}C (0.12 µCi; spec. act. 5 mCi/mM)[5] and 1, 10 or 100 µg of the potential antimetabolite[9] had been added, was administered to 6 nymphs of *Blattella germanica* per test, after they had been starved for 3 days. This radioactive diet was eaten within 1 or 2 days. Next, the insects obtained 200 mg of sterol-free diet, were killed after 2 days and extracted with hot ethanol and acetone. The extract was saponified with 10% (w/v) KOH in 80% (v/v) ethanol at room temperature overnight. The reaction mixture was separated by means of centrifugal column chromatography[10]. Sorbent: Al_2O_3 H (for thin-layer chromatography, Merck); speed: 1900g; fraction 1: 25 ml benzene, fraction 2: 25 ml ethyl acetate-benzene (10 + 90) and fraction 3: ethyl acetate-benzene (30 + 70).

Radioactivity measurements were carried out by means of a Packard Tri-Carb liquid scintillation spectrometer, model 3380. Fractions 2 which contained the free sterols, were analyzed by means of radio gaschromatography[11] with a flow reactor RGC 170 (Laboratory Prof. Dr. Berthold, Wildbad, Germany, and Bodenseewerk Perkin-Elmer & Co. GmbH, Überlingen, Germany). Stationary phase 3% OV-17 on Chromosorb G.AW.DMCS; argon ionization detection; column temperature 250 °C; reactor filling CuO; reactor temperature 680 °C; flow-proportional counter 80 ml. In some cases also 5% DEGS on Chromosorb W.AW was used with discontinuous radioactivity measurements of methyl ether derivatives[12].

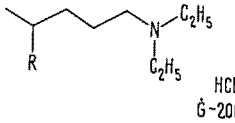
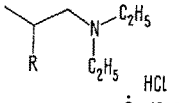
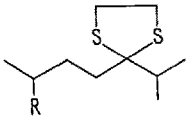
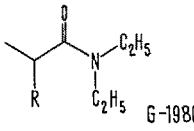
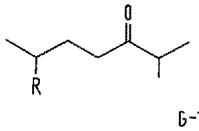
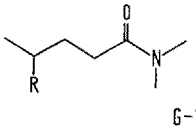
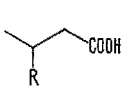
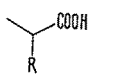
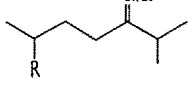
Relative composition of the sterols in the cockroaches, to which β-sitosterol-3-^{14}C plus several sterols with unnatural side-chains in different concentrations were administered

		C	S	A	B
 β-Sitosterol-3-^{14}C		94	6		
 Ergosterol	10:1	97	3		
	1:1	96	4		
	1:10	84	16		
 SC-7957	10:1	95	5		
	1:1	92	8		
	1:10	93	7		
 SC-13775	10:1	90	10		
	1:1	93	7		
	1:10	75	17	8	
 2 HCl SC-12937	10:1	91	9		
	1:1	91	9		
	1:10	91	9		
 SC-13102	10:1	90	10		
	1:1	86	14		
	1:10	83	17		
 G-1997	10:1	96	4		
	1:1	68	23	9	
	1:10	64	31	5	
 SC-13817	10:1	94	6		
	1:1	76	16	8	
	1:10	85	9	6	
 2 HCl SC-13058	10:1	93	7		
	1:1	90	10		
	1:10	92	8		

[1] N. W. EARLE, E. N. LAMBREMONT, M. L. BURKS, B. H. SLATTEN and A. F. BENNETT, J. econ. Ent. *60*, 291 (1967).
[2] J. A. SVOBODA and W. E. ROBBINS, Science *156*, 1637 (1967).
[3] J. A. SVOBODA and W. E. ROBBINS, Experientia *24*, 1131 (1968).
[4] F. J. RITTER and W. H. J. M. WIENTJENS, T.N.O.-Nieuws *22*, 381 (1967).
[5] P. PH. H. L. OTTO, A. C. BESEMER and W. H. J. M. WIENTJENS, J. Labelled Comds *6*, 111 (1970).
[6] J. L. NOLAND, Arch. Biochem. Biophys. *48*, 370 (1954).
[7] A. J. CLARK, Thesis Harvard (1958), p. 5.
[8] R. B. CLAYTON, Nature *184*, 1166 (1959).
[9] 3 β-Hydroxy-24-norchol-5-en-23-oic acid was a gift of Dr. J. A. SVOBODA, Insect Physiology Laboratory, Entomology Research Division, ARS, USDA, Beltsville, Maryland, USA. The sterols encoded with SC were kindly supplied by G. D. Searle & Co., Chicago, Illinois, USA, and those encoded with G by Laboratory of Organic Chemistry, State University Groningen, Netherlands. 28-Chloro-$\Delta^{5,24(28)}$-ergostadienol was synthesized by Dr. P. PH. H. L. OTTO, Mr. A. C. BESEMER and Mr. TH. J. VAN VEEN (Central Laboratory T.N.O., Delft, Netherlands) from 24-keto cholesterol, which was a gift of Dr. B. RIEGEL, G. D. Searle & Co., 3 β-Hydroxy-23,24-dinorchol-5-en-22-oic acid was from Nutritional Biochemical Corporation, Cleveland, Ohio, USA, and ergosterol from Chemed Inc., Odenton, Maryland, USA.
[10] J. W. A. MEYER, H. I. A. EID and P. A. VERDUIN, Analyt. Chem. *236*, 426 (1968).
[11] R. TYKVA and H. SIMON, Chromatographia *2*, 5 (1969).
[12] R. B. CLAYTON, Biochemistry *1*, 357 (1962).

The results are summarized in the Table. The sum of these values was put at 100. Values of radioactive metabolites of less than 3% were not taken into account. The figures in the second column show the ratio of radioactive sitosterol to sterol under investigation, as administered.

Administration of sitosterol alone gave an average ratio of 94 to 6 for cholesterol to sitosterol in the insects.

		C	S	A	B
 G-2005	10:1	89	11		
	1:1	83	14	3	
	1:10	71	22	7	
 G-1998	10:1	95	5		
	1:1	96	4		
	1:10	94	6		
 G-1962	10:1	94	6		
	1:1	84	16		
	1:10	79	21		
 G-1980	10:1	92	8		
	1:1	92	8		
	1:10	90	10		
 G-1627	10:1	93	7		
	1:1	96	4		
	1:10	85	15		
 G-1996	10:1	90	10		
	1:1	84	16		
	1:10	75	15	10	
	100:1	95	5		
	10:1	96	4		
	1:1	92	8		
	1:10	95	5		
	1:100	93	7		
	100:1	95	5		
	10:1	90	10		
	1:1	95	5		
	1:10	97	3		
	1:100	93	7		
	10:1	89	11		
	1:1	91	9		
	1:10	67	26		7

R = cholesterol nucleus. C, S, A and B are respectively: radioactive cholesterol, sitosterol and 2 conversion products of β-sitosterol-3-^{14}C, as found in the insects. The figures in the second column show the ratio of radioactive sitosterol to sterol under investigation, as administered.

SC-7957 and ergosterol were used as a sort of blank in the same concentrations as those of the potential anti-metabolites, and only ergosterol with the highest concentration gave a slight decrease of cholesterol and a slight increase of sitosterol. With use of SC-13775, the cholesterol over sitosterol ratio was not only somewhat smaller, but, moreover, 8% of a radioactive compound, A, was found. SC-12937 had no influence, but when, as in G-1997, carbon atom 20 was not replaced by a nitrogen atom, there was an evident influence: a decrease of cholesterol, an increase of sitosterol and the presence of radioactive A, also with the middle concentration. Also SC-13817, with a side-chain which is one C-atom longer than those of the previous sterols, did have an evident influence. With SC-13058 in the highest concentration a trace of A was found. Again, when carbon atom 20 was not replaced by nitrogen (G-2005), an accumulation of A occurred. The same results were obtained with the free nitrogen compound. Of the 3 anti-metabolites with a keto function in the side-chain, only G-1996 caused accumulation of A in the cockroaches.

The gas chromatographic behaviour of compound A is the same as that of desmosterol. SVOBODA et al.[2,13] found desmosterol as an intermediate of the conversion of β-sitosterol into cholesterol in the tobacco hornworm under influence of triparanol and 22,25-diazacholesterol. Desmosterol was also found by SVOBODA et al.[3] as a conversion product of several phytosterols in the tobacco hornworm under influence of 20,25-diazacholesterol and as conversion product of sitosterol under influence of 3β-hydroxy-24-norchol-5-en-23-oic acid and 3β-hydroxy-23,24-dinorchol-5-en-22-oic acid[14]. We also tested these 2 compounds with the cockroaches, but, performing the usual tests, did not find other conversion products of sitosterol than cholesterol. Only when we worked with some hundreds of cockroaches, about 2 or 3% of compound A could be detected.

Administration of 28-chloro-$\Delta^{5,24(28)}$-ergostadienol in the highest concentration resulted not only in a change of the cholesterol over sitosterol ratio, but also in the presence of 7% of radioactive metabolite B, which has another gas-chromatographic behaviour on OV-17 and DEGS than metabolite A. This would suggest a possibility to inhibit, in a more selective way, the dealkylation. Inhibition of the dealkylation by a method which involves an inhibition of the conversion of desmosterol into cholesterol is not selective, since this conversion is also important in vertebrates. However, continuous administration of the chloro compound in long-term experiments did not cause any disturbance in the development of the cockroaches[15].

Zusammenfassung. Die Umwandlung von β-Sitosterol in Cholesterol in der Schabe *Blattella germanica* L. wird durch bestimmte Sterine mit unnatürlichen Seitenketten gehemmt. Anhäufung von zwei verschiedenen Metaboliten wurde festgestellt.

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Central Laboratory TNO, P.O.B. 217, Delft (Netherlands), 22 October 1970.

[13] J. A. SVOBODA, M. J. THOMPSON and W. E. ROBBINS, Life Sci. *6*, 395 (1967).

[14] J. A. SVOBODA, M. J. THOMPSON and W. E. ROBBINS, Steroids *12*, 559 (1968).

[15] This work was supported by a grant from the National Council for Agricultural Research TNO.