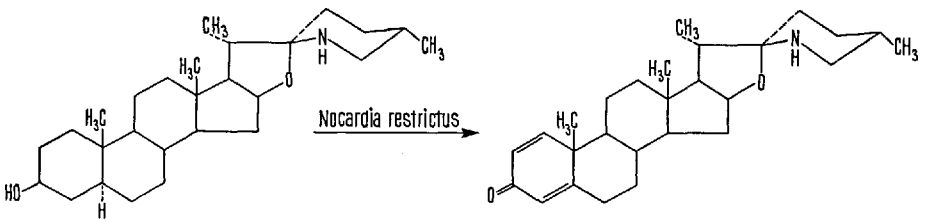
Microbiological Dehydrogenation of Tomatidine

Although the microbiological dehydrogenation of steroids has already become a well-known procedure, such dehydrogenation is scarcely known to occur with steroidal sapogenins and steroidal alkaloids[1]. Diosgenin is dehydrogenated by *Fusarium solani*; the yield, however, is high only if diosgenin is previously transformed to diosgenone[2]. Conessine can be dehydrogenated by *Gloesporium cyclaminis*[3] or *Stachybotrys parvispora*[4] to 4-conenin-3-one. Otherwise only hydroxylation of conessine[5–7], tomatidine[8] and solasodine[9] have been observed.

Our attempts at dehydrogenation of tomatidine and tomatidone by *Fusarium solani* have not been successful[10]. On the other hand, dehydrogenation of tomatidine by *Nocardia restrictus* has been achieved yielding 60% of 1,4-tomatadiene-3-one.

Incubation of tomatidine with *Nocardia restrictus* yielded, as the main metabolite, a crystalline substance:



Tomatidine 1,4-Tomatadiene-3-one

mp 242–245°; TLC homogeneous with the solvent system cyclohexane-ethylacetate (1:2); visualized by spraying with 50% sulfuric acid. The mass spectrum showed a M^+ ion 409, corresponding to a loss of 6 hydrogen atoms from the tomatidine molecule (calc. for $C_{27}H_{39}O_2N$: 409) and, in addition, intense peaks at *m/e* 138 and *m/e* 114, typical for the unchanged tomatidine rings E and F. The presence of the peak at *m/e* 288 corresponding to M-121 points to the 1,4-diene-3-one structure of ring A[11]. The absorption maximum at λ_{max}^{EtOH} 244 nm (ε 15,400)[12] and the IR-spectrum showing absorption bands at 1660 cm^{-1} (3 C=O), 1622 cm^{-1} (1:2 C=C) and 1605 cm^{-1} (4:5 C=C)[13] are in agreement with the above structure of ring A. Therefore, the structure of the metabolite is that of 1,4-tomatadiene-3-one.

Zusammenfassung. Mit Hilfe von *Nocardia restrictus* wurde aus Tomatidin in 60% Ausbeute 1,4-Tomatadien-3-on erhalten.

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Lecithin-Chloroform Interaction as a Model for the Action of General Anesthetics

Water has been proposed by PAULING[1] and MILLER[2] as the primary reactant with non-hydrogen-bonding anesthetic molecules. The formation of anesthetic-water clathrates has been thought possible under physiological conditions if stabilizing agents other than the anesthetic molecule were also operating. The charged side chains of proteins were considered to act as stabilizers on the basis of their analogy with alkylammonium derivatives, which are known to form clathrates with water.

We thought[3] that lecithin would have been a more suitable stabilizer, and here we present some data on the in vitro interaction between chloroform ($CHCl_3$) and synthetic lecithin, and on the stabilizing power of a number of compounds in respect to $CHCl_3$ hydrates.

The escaping tendency of $^{14}CHCl_3$ (GMBH Products) was studied in the presence of DL-α-dipalmitoyl-lecithin, glutathione, choline, phosphocholine, palmitic acid and mixtures of palmitic acid and phosphocholine (Table I). All reagents were Fluka, AG, Buchs products.

The effect of lecithin concentration (Table II) and temperature (Table III) on $^{14}CHCl_3$ evaporation were

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also studied by measuring the residual radioactivity by means of an Ansitron liquid scintillation spectrometer.

$CHCl_3$ hydrates were prepared according to CHANCEL and PARMENTIER[4] and their stability was measured in the presence of different compounds (Table IV).

Only lecithin is able to slow down the evaporation of $^{14}CHCl_3$ and this suggests the existence of some kind of interaction (Table I) which is supported by the data obtained with different concentrations of lecithin (Table II) at different temperatures (Table III). In fact from both types of experiments a certain degree of negative deviation from Raoult's law can be recognized, indicating that a strong interaction between solute and solvent does occur. The formation of a $^{14}CHCl_3$-lecithin complex should lower the activity and hence the vapor pressure of the free $^{14}CHCl_3$ and lecithin in solution.

The data obtained at different temperatures were plotted according to ARRHENIUS and 2 straight lines were obtained yielding a negative enthalpy change of -9 Kcal/mole from $-20\,°C$ to $+5\,°C$ and a positive enthalpy change of $+6$ Kcal/mole from $+5\,°C$ to $+40\,°C$. The ARRHENIUS plot was constructed by putting $\log (p - p_o)$ on the y axis and $1/T$ on the x axis; p_o was the residual radioactivity of $^{14}CHCl_3$ alone after 10 min and p was its residual radioactivity in the presence of lecithin. The temperature effect therefore allows one to distinguish 2 processes taking place, below and above the temperature of melting of clathrates. The apparent optimum of temperature for the interaction appears in a zone around $+5\,°C$ which corresponds to the temperature of maximal anesthesizing potency of $CHCl_3$ as measured by CHERKIN and CATCHPOOL[5].

A double reciprocal plot of Table II data allows us to calculate an apparent dissociation constant ($K_s = 1.67\,M$) for the $CHCl_3$-lecithin complex which corresponds roughly to a free energy change of -300 cal/mole.

The water content of lecithin was measured by dry weight with a Cahn Electrobalance. An amount of 3 molecules of water per 4 molecules of lecithin was found. This water is organized mainly around the alkylammo-

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Table I. Evaporation of $^{14}CHCl_3$ alone or in the presence of other compounds (lecithin, cephalin, glutathione, choline, phosphocholine, palmitic acid and palmitic acid + phosphocholine)

Time (min)	$^{14}CHCl_3$ (125 μM) (cpm)	$^{14}CHCl_3$ + lecithin (5 μM) (cpm)
0	10,000	10,000
5	2,000	6,320
10	300	3,980
20	280	1,410
30	140	500

Evaporation was carried out in a thermostated room at 26 °C and p.a. in 2 containers placed in a $20 \times 50 \times 50$ box hermetically closed. It was stopped at different times by adding 5 ml of scintillation liquid POPOP/PPO to both containers. The residual radioactivity was measured (count time 4 min) and the data are reported after having subtracted the background radioactivity. Each assay was run in triplicate. The residual radioactivity of the samples with $^{14}CHCl_3$ alone was the same as the one of the samples with $^{14}CHCl_3$ plus each of the compounds other than lecithin.

Table II. Effect of lecithin concentration on the escaping tendency of $^{14}CHCl_3$ (125 μM)

Lecithin (μM)	Residual radio-activity/min (cpm)
1.5	121
3	252
6	440
12	641
16	823
18	830

The residual radioactivity of each sample was measured after 10 min at 26 °C and experimental conditions as given in Table I. The values are average of 5 experiments.

Table III. Effect of temperature on the escaping tendency of $^{14}CHCl_3$ in the presence of lecithin

Residual radioactivity (cpm)	Temperature (°C)
2435	−20
2938	−10
4658	0
6680	5
6149	10
5085	20
4029	30
854	40

125 μM of $^{14}CHCl_3$ were mixed with 5 μM of lecithin at a.p. and the residual radioactivity was measured after 10 min by subtracting from each sample the radioactivity of the $^{14}CHCl_3$ alone. Different temperatures were obtained by thermostating the box with an Ultra Kryostat UK 20 L (Messgeräte Werke Lauda). The values are average of 5 experiments.

Table IV. Stabilization of $CHCl_3$ hydrates by different compounds

Compound	Melting point (°C)	Stabilization period (min)
None	1.6	3.30
Cephalin	1.6	1.75
Phosphocholine	2.1	2.41
Palmitic acid	1.8	6.25
Glutathione	2.1	4.10
Choline	2.0	10.75
Lecithin	4.1	25.08

10 mM of $CHCl_3$ were mixed with 400 mM of bidistilled water in the presence of 0.4 mM of each compound. The mixture was solidified slowly by solid CO_2-aceton (−77 °C) and then put at 0.8 °C in the Ultra Kryostat. After the excess water was removed, the melting point of the hydrates and the period of their permanence at 26 °C were determined. The stabilization period was computed on the basis of the disappearence of the last crystal of hydrate.

nium head of lecithin and its features will be discussed in a separate paper.

Table IV shows that lecithin is indeed the best stabilizer of $CHCl_3$ hydrates, and it is interesting to note that it is also the only one capable of influencing the evaporation of $^{14}CHCl_3$ strongly interacting with it.

If we look again Table II we can calculate a saturation value which corresponds to a ratio of about 8 between lecithin and $CHCl_3$. This means a ratio of 6 between water and $CHCl_3$ which represents the ideal ratio for $CHCl_3$ hydrates formation[6].

All the data presented in this note therefore strongly suggest that the interaction $CHCl_3$-lecithin depends on a stabilization of the lecithin-bound water.

The interaction between lecithin and $CHCl_3$ is conceivably occurring also in vivo at the neural membrane level, and the end plate region of the synapse should be an ideal target for $CHCl_3$ molecules owing to its physical characteristics[7]. The water phase which is involved in the formation of the clathrate structures might be the hydration water of lecithin[8] existing in a peculiar liquid state as 'paracrystals' at the membrane level. The energy required to rearrange tetrahedrically coordinated water within the membrane has been calculated as 350 cal/mole[9]. This value happens to be very close to the free energy change we found for the $CHCl_3$-lecithin interaction, so that the possibility of water state-transitions in our in vitro system, analogous to the ones occurring in membrane systems, can be suggested.

In the normal membrane, water is present as columns permeable to both organic and inorganic substances and it is clear that all mechanisms of neurotransmitter liberation, ion translocation and depolarization are dependant on such a normal state. The presence of $CHCl_3$ or other general anesthetics able to form microcrystal hydrates, certainly modifies the features of these channels and to this very first change the block of the neural transmission can be related[10].

Riassunto. Gli idrati di cloroformio sono stabilizzati in vitro dalle lecitine. Si possono così riconciliare la teoria dei clatrati e quella della fase lipidica per spiegare l'azione degli anestetici generali.

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The Effect of Sound, Light and Vibratory Stimuli on Serum Lipid Levels and Liver Fatty Acid Content of Old and Adult Rats

There is a discrepancy in the opinions of different investigators regarding the alteration of plasma and liver lipids in old rats. CARLSON and FRÖBERG[1] found an elevated serum lipid level in old rats as compared to younger ones. TAYLOR et al.[2] failed to find this alteration; however, they did find an elevated lipid level in the liver cells of old rats. According to the opinion of ROCKSTEIN and HRACHOVECZ[3], there is no difference between the liver lipid levels of old and younger animals.

The elevation of serum long chain free fatty acid level caused by epinephrine administration was smaller[4], the hyperlipaemia caused by pathogenic diet was higher[5], the alteration of serum cholesterol level caused by sound, light and other stimuli was equal[6] in the old animals as compared to the younger ones. The effect of the latter agents causes an alteration in the serum lipids, the extent of which is equal in both the old or younger animals. Since there is a close connection between the alterations of serum lipids and liver long chain free fatty acid, or acyl-coenzyme A content, it also seemed worth investigating the effect of sound and light stimuli on these liver lipid components of old and adult rats.

Methods. Experiments were made on a total of 82 Wistar inbred male rats. The 4–6, or 24–26-month-old animals were fed a standardized semisynthetic diet, 15 g a day. Excitation by auditory, photic and vibratory stimuli was undertaken according to a fixed scheduled program in a sound-proof chamber (see Figure) for a period of 8 days. The duration of excitatory periods was twice a day for $1^1/_2$ h. The rats were subjected to periodical and continuous sound stimuli of a frequency of 2000, or 3300 cps and a linear total level of 101, or 118 db. The vibratory stimuli had an amplitude of 8 mm and a frequency of 3, or 6 cps. The intensity of light stimuli was 1100 lux. Parameters of sound and vibratory stimuli were controlled by a Brüll-Kjer precision sound level meter, while those of the light stimuli by a Zeiss apparatus. The animals were bled to death at the end of the fixed scheduled program. Total cholesterol, free fatty acid, phospholipoid and total lipoid level were determined in the blood serum using the method of BLOOR[7], TROUT et al.[8], BAGINSKY and ZAK[9], and ZÖLLNER and KIRSCH[10], respectively. The long chain free fatty acid and acyl-coenzyme A content of liver cells were measured by the methods of DOLE and MEINERTZ[11] and

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