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<sub>3</sub> hydrates, and it is interesting to note that it is also the only one capable of influencing the evaporation of <sup>14</sup>CHCl<sub>3</sub> 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<sub>3</sub>. This means a ratio of 6 between water and CHCl<sub>3</sub> which represents the ideal ratio for CHCl<sub>3</sub> hydrates formation <sup>6</sup>.

All the data presented in this note therefore strongly suggest that the interaction CHCl<sub>3</sub>-lecithin depends on a stabilization of the lecithin-bound water.

The interaction between lecithin and CHCl<sub>3</sub> 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<sub>3</sub> molecules owing to its physical characteristics? The water phase which is involved in the formation of the clathrate structures might be the hydration water of lecithin<sup>8</sup> 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<sup>9</sup>. This value happens to be very close to the free energy change we found for the CHCl<sub>3</sub>-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<sub>3</sub> 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 <sup>10</sup>.

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.

L. Galzigna<sup>11</sup> and J. Méry

University of Nairobi, Medical Biochemistry, P.O. Box 30197, Nairobi (Kenya), and Département de Biochimie Macromoleculaire, CNRS, B.P. 1018, F-34 Montpellier (France), 7 December 1970.

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- <sup>11</sup> Present address CNRS, F-34 Montpellier (France).

## 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 Fröberg¹ 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 Hrachovecz³, 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<sup>4</sup>, the hyperlipaemia caused by pathogenic diet was higher<sup>5</sup>, the alteration of serum cholesterol level caused by sound, light and other stimuli was equal<sup>6</sup> 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.

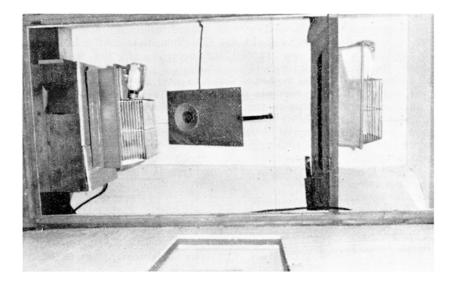
Methods. Experiments were made on a total of 82 Wistar inbred male rats. The 4-6, or 24-26-month-old animals were fed a standardized semisynthetic diet, 15 g a day. Excitation by auditory, photic and vibratory stimuli was undertaken according to a fixed scheduled program in a sound-proof chamber (see 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, Trout et al., Baginsky and Zak, and Zöllner and Kirsch, respectively. The long chain free fatty acid and acyl-coenzyme A content of liver cells were measured by the methods of Dole and Meinertz<sup>11</sup> and

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Effect of excitation on serum lipid levels and liver long chain fatty acid content of rats

Rat-groups	Old control (mg/100 ml)	Old hyper- lipaemic (mg/100 ml)	Statistical probability	Adult control (mg/100 ml)	Adult hyper- lipaemic (mg/100 ml)	Statis- tical proba- bility
Serum						
Total cholesterol	$102 \pm 14$	$161 \pm 44$	0.001 > P	96 $\pm 11$	151 ± 33	0.001 > P
Total lipoid	$270 \pm 21$	$390 \pm 24$	0.001 > P	$270 \pm 14$	$400 \pm 66$	0.001 > P
Phospholipoid	99 ± 18	$159 \pm 28$	0.001 > P	97 $\pm 14$	$153 \pm 34$	0.001 > P
Free fatty acid	$15.3 \pm 0.9$	$23.8 \pm 2.8$	0.001 > P	$15.3\pm2$	$24.6 \pm 2.6$	0.001 > P
Liver						
Long chain free fatty acid	$95.7 \pm 14.9$ naequ/mgN	227 ± 41.8 naequ/mgN	0.01 > P > 0.001	$65.5 \pm 12$ naequ/mgN	140 ± 23.9 naequ/mgN	0.001 > P
Long chain acyl- coenzyme A	$0.703 \pm 0.155$ nmoles/mgN	$1.24 \pm 0.131$ nmoles/mgN	0.05 > P > 0.020	$0.462 \pm 0.113$ nmoles/mgN	1.645 ± 0.227 nmoles/mgN	0.001 > P
No. of rats	14	14`		32	22	



The sound-proof chamber. The cage is on top of the vibratory machine. A loud-speaker is on the wall of the chamber.

BORTZ and LYNEN<sup>12</sup>, respectively. The nitrogen content was measured by the Kjeldahl method.

The controls consisted of 4-6, and 24-26-month-old rats fed on the same diet. Spectrophotometric measurements were undertaken by a Spektromom-360 equipment.

Results. There was no significant difference between any serum lipid values of the 4-6 and 24-26-month-old rats. The excitation caused an elevation in all serum lipid values, the extent of which was similar in both age groups as compared to the age-matched controls.

The long chain free fatty acid content was higher in the old control animals as compared to the 4-6-month-old rats. Changes in connection with long chain acyl-coenzyme A content were not found.

After excitation there was an elevation in the long chain free fatty acid and acyl-coenzyme A content of liver cells in both age groups, but the extent of the increase was higher in the case of free fatty acids and smaller in the case of acyl-coenzyme A content. The results are demonstrated in the Table.

Discussion and conclusions. GEBER et al.<sup>6</sup> found that there are no age differences between the alterations of serum cholesterol levels caused by sound, and light stimuli. Similarly, we failed to find any discrepancy according to age in the changes of different serum lipid levels caused by sound, light and vibratory stimuli; therefore the hyperlipaemia caused by these stimuli is different from serum lipid changes caused by other agents (fatty diet, epinephrine) in respect to age.

The higher extent of the increase of long chain free fatty acid content and the smaller extent of the rise of long chain acyl-coenzyme A content in the liver cells of old rats may show a weakened fatty acid activating capacity. The cause of this phenomenon might be the decrease of the coenzyme A content in the old animal cells described by Nikitin and Martinyenko 13 and Oeriu 14, or the inhibition of activating enzymes. Further investigations are necessary to answer this question.

Zusammenfassung. Der Einfluss von Schall-, Licht- und Schwingungsreizen auf den Serumlipoid- und Leberfettsäurespiegel von 4-6 bzw. 24-26 Monate alten Ratten ergab, dass der Serumlipoidspiegel, die langkettigen freien Fettsäuren und das Acyl-Coenzym A der Leberzellen sowohl in erwachsenen als auch in älteren Tieren zugenommen hatte.

T. Szamosi

Institute of Biochemistry, Semmelweiss Medical University, XIII. Nepfürdö u. 37, Budapest (Hungary), 14 December 1970.

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