tyrosine. Moreover, the magnitude of the effect increases with prolonged sleeplessness particularly in the time period between 72 and 96 h. In a separate experiment, we have noted extremely high levels for plasma corticosterone at these time intervals which correlate with and can explain the high levels of enzyme activity noted. Tyrosine aminotransferase seems to be particularly sensitive to sleep deprivation resulting in extremely high levels of enzyme activity. Similarly, we had previously noted that when mice are exposed to acute cold stress (0–4 °C), there is also a marked effect upon the activity and periodicity of TAT.

Interestingly, hepatic tyrosine concentration is elevated through 84 h of sleep deprivation culminating in a sharp decline at 96 h. In view of the increased activity of tyrosine aminotransferase, it, at first, appears plausible that hepatic and plasma tyrosine levels may decline. However, tyrosine concentrations can be maintained by ingestion, catabolism of protein or conversion from phenylalanine[10], and our work with other enzyme-substrate systems indicates the latter mechanism is probably most responsible for maintaining hepatic and plasma tyrosine.

The method of sleep deprivation used in this study disturbs the periodicity of tyrosine via the induction of its catabolic enzyme during the first 60–72 h of sleeplessness. However, it appears plausible that plasma levels of this amino acid are being maintained most importantly by hepatic repletion (phenylalanine → tyrosine). Further, it is apparent that with prolonged sleep deprivation the stress response intensifies with concommitant increases in plasma corticosterone and enzyme activity. Thus, with limited amino acid availability, enzyme induction may reduce tyrosine concentrations in the liver and plasma, and hence its availability for thyroxin, protein, and catecholamine synthesis. In fact, CURZON and GREEN[15] relate decreases in brain serotonin to an increase in liver tryptophan oxygenase activity in immobilized rats. In a very recent publication, FERNSTROM and WURTMAN[16] confirm these results suggesting that plasma tryptophan concentrations may affect brain serotonin levels. Hence, it appears likely that the precipitous decrease in hepatic and plasma tyrosine concentrations during the final 72–96 h of sleep deprivation may be closely related to the sharp increases in enzyme activity observed during this interval.

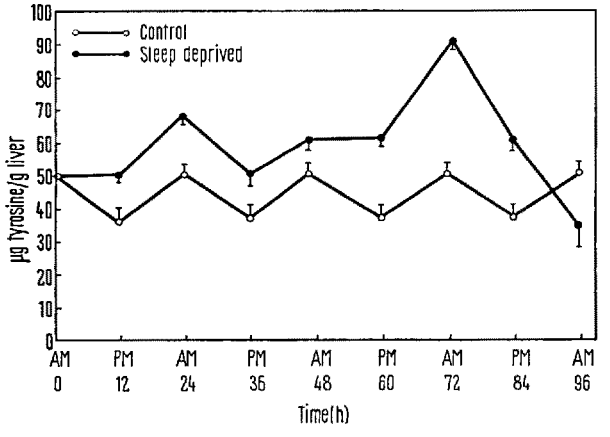

Fig. 2. Effects of sleep deprivation on hepatic levels of tyrosone (µg tyrosine/g liver wet weight). Each point represents the mean of a minimum number of 5 animals, and vertical lines denote ± 1 S.E.M.

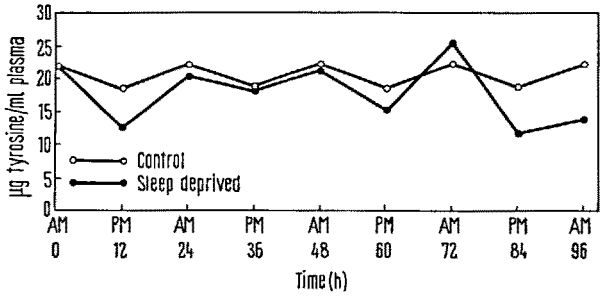

Fig. 3. Effects of sleep deprivation on plasma levels of tyrosine in terms of µg tyrosine/ml plasma. Each point represents the value of pooled plasma from at least 5 animals performed in duplicate.

Résumé. La privation de sommeil pendant 96 h a crée des ruptures dans la périodicité d'enzyme-tyrosine-aminotransférase du foie, à cause de l'induction. Des concentrations hépatiques de tyrosine ont augmenté alors que sa présence dans le plasma fut sans effet. La privation de sommeil affecte non seulement les rhythmes quotidiens naturels, mais, si elle est prolongée, elle intensifie la réponse de «stress» généralisé peut-être au point d'affecter notablement l'effet de l'acide amidé.

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Cumulation of Liver Lipids after Administration of Iron

When peroxidative degradation of polyenic fatty acids in the liver tissue in vivo is activated by divalent iron and ascorbic acid administration, a simultaneous accumulation of neutral liver lipids was found[1]. This effect of iron has not been described sofar. That is the reason why we studied the influence of iron on the cumulation of different liver lipid fractions.

Male white mice (strain H – Konárovice) weighing 20–25 g kept on a standard laboratory diet were used in the experiments.

Iron was administered in solution with ascorbic acid (Fe-AA complex); containing 0.1*M* $FeCl_2$ and 0.2*M* ascorbic acid neutralized by sodium hydroxide solution to pH 7.4. The dose was 0.5 mmol Fe^{2+}/kg. The animals

Analyses in liver tissue after administration of iron-ascorbic-acid complex

Estimations	Controls	Iron complex	Percent changes
1. Neutral lipids Silic acid fractionation (mg/g)	7.9 ± 1.78 (8)[b]	15.8 ± 2.41 (8)[c,d]	+ 102
Heptane fraction (mg/g)	11.7 ± 0.77 (8)	22.3 ± 2.71 (12)[c]	+ 91
Radioactivity in glycerides (dpm/mg)	589 ± 115 (8)	917 ± 100 (8)[d]	+ 56
2. Polar lipids			
Lipoid P (mg/g)	2.24 ± 0.09 (5)	2.25 ± 0.09 (5)	± 0
Polar fraction (mg/g)	20.7 ± 0.40 (9)	21.9 ± 0.52 (7)	+ 6
Radioactivity in phospholipids (dpm/mg)	685 ± 101 (8)	879 ± 43 (8)	+ 28
3. Total lipids			
Gravimetrically (mg/g)	56.4 ± 2.99 (9)	65.5 ± 4.80 (9)	+ 16
Radioactivity (dpm/mg)	1279 ± 102 (8)	1796 ± 91 (8)[d]	+ 41
4. Cholesterol (mg/g)	13.7 ± 0.36 (5)	18.1 ± 0.75 (5)[f]	+ 33
5. Protein (mg/g)	193 ± 5.7 (6)	198 ± 3.1 (7)	+ 3
6. Iron (μg/g)	9.8 ± 1.70 (6)	108.0 ± 5.34 (7)[f]	+1002
7. Ascorbic acid (mg/g)	1.2 ± 0.11 (6)	2.2 ± 0.12 (7)[f]	+ 90

[a] 100% equals the values of controls. [b] Figures in parentheses give the number of analyzed animals. [c] Statistical evaluation comparing appropriate controls to Fe-AA complex treated animals (Student's *t*-test); statistically significant on the level of [d] 5, [e] 1 and [f] 0.1%, respectively.

were sacrified 3 h after i.p. administration of the solution and a 10% homogenate was prepared either in chloroform-methanol (2 + 1 v/v) for the gravimetrical estimation of total lipids, lipoid phosphorus[2] and cholesterol[3], or in heptane-isopropanol (1 + 1 v/v) for the estimation of neutral and polar lipids[1], or else a 25% homogenate in 0.9% NaCl for the estimation of proteins[4], iron[5] and ascorbic acid[6]. Neutral lipids were estimated as esterified fatty acids[7] either after removing the polar lipids by means of absorption on silicic acid[8] or by separating the lipids into the heptane layer for neutral lipids estimation (in the isopropanol-water layer polar lipids were assessed[7]).

5μCi of palmitate-1-^{14}C in an albumin complex was administered i.v. 120 mins after Fe-AA complex administration. At 5 and 120 min intervals after palmitate administration, the radioactivity in glycerides and phospholipids (separated by thin layer chromatography[9]) and total lipids was measured by a liquid scintillation counter ABAC – 40L Intertechnique (Paris). The amount of palmitate-1-^{14}C trapped in the liver 5 min after administration did not differ: controls 2646 ± 240 dpm/mg, Fe-AA complex 2455 ± 630 dpm/mg.

3 h after i.p. administration of the iron-ascorbic acid complex, an increased concentration of neutral lipids was found by all 3 types of assessment. In the total lipids, the increase of the neutral lipid fraction is masked by phospholipids which are not affected by this treatment. The concentration of divalent iron reaches enormously enhanced concentrations, while the concentration of ascorbic acid is increased only twice. The iron-ascorbic acid complex causes a transient, metabolic accumulation of liver lipids, which after a single dose returns to normal levels within 24 h.

The mechanism of the divalent iron effect (in combination with ascorbic acid) has not yet been followed as regards oxidoreductive systems of the liver cell in connection with impaired lipid metabolism. However, from the experimental conditions and assembled results, it may be concluded that the lipid cumulation is not affected by an increased lipid intake (short-time induction period, the same radioactivity trapped in liver of control and treated animals). This is why we assume that a reduced output of lipids from the liver is involved. We must also take into account that divalent iron, being a lipid peroxidation activating compound[1], may induce appropriate functional or structural impairment of the hepatocyte, which in turn affects the lipid metabolism, as is presumed in other types of liver damage associated with fatty liver formation[10–12].

Zusammenfassung. Die vorübergehende Kumulation der Leberlipide nach Verabreichung von zweiwertigem Eisen und Askorbinsäure führte zur Annahme, dass der durch Eisen induzierte peroxidative Abbau der ungesättigten Fettsäuren am anomalen Stoffwechsel teilnimmt.

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