animal. Comparison of the percentage change from the control of the total EEG activity following administration of the higher dose of all 3 drugs (Table I) showed decreases which are not significantly different from those found by alerting the animal. If the drug-induced EEG patterns obtained at these dose levels did not differ from those in an arousal situation then the percentage change in the different frequency bands when compared with the control values would not have differed from those found by alerting the animal. In Table II it can be seen that the percentage changes in a number of EEG frequency bands following D-amphetamine (0.5 mg/kg) and caffeine (50 mg/kg) administration were significantly different from those of an alert state. The EEG following nicotine administration (5 µg/kg/min) did not differ significantly in percentage change in any frequency band from the alert state.

Discussion. These results raise a number of interesting questions. The possibility that nicotine produces an EEG arousal state which is closer to a normal arousal than either caffeine or D-amphetamine is in accord with present knowledge of drug interactions on the central nervous system. Nicotine type cholinergic neurons exist[3] and there is evidence for their presence in the central nervous system[4]. The results of KAWAMURA and DOMINO[5] suggest that nicotine may act primarily in the mid brain reticular formation. This structure plays an important role in the arousal response[6], and nicotine may act primarily by stimulating the normal arousal pathways. No such receptors or mechanisms have been suggested for amphetamine or caffeine. The suggestion that nicotine induces a 'normal' arousal state whilst other centrally acting stimulants do not is well fitted by these results. It could be however, that the effect is general for central nervous system stimulants, but dose-related, though the results to date do not suggest this. It is fully realised that the results are based on EEG's from only one area of the cortex using relatively few dose levels. It is intended to conduct more studies along lines to test the validity of this hypothesis further.

Résumé. Les auteurs ont établi qu'après des doses de nicotine, telles qu'on en absorbe en fumant une cigarette, le tracé de l'EEG ressemble plus à celui qui se présente après une réaction d'alerte qu'après l'administration d'amphétamine ou de caféine.

P. J. CHESHIRE, D. N. KELLETT and G. L. WILLEY

Huntindon Research Centre, Huntingdon PE18 6ES. (England), 23 May 1972.

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The Effect of Intoxication Induced in Rat Liver by Carbon Tetrachloride, Ethionine and White Phosphorus on the Level of Microsomal Cytochromes b_5 and P_{-450}

The analysis of the microsomal cytochromes b_5 and P_{-450} may represent an useful tool in the study of the pathogenesis of hepatic fat accumulation induced by administration of carbon tetrachloride (CCl_4), ethionine or white phosphorus.

The action of CCl_4, which involves a lipoperoxidative mechanism by the liberation of $CCl_3^{\bullet}$ free radicals, appears to be primarily concerned with the membranes of the endoplasmic reticulum. This lesion is accompanyed by an early accumulation of triglycerides, followed by a mitochondrial injury (ref.[1] for review). A decrease of cytochrome P_{-450} has also been reported by SMUCKLER[2] in the liver microsomal fraction of rats treated orally for 2 h with CCl_4. Ethionine is supposed to interfere with ATP synthesis, through the formation of S-adenosyl ethionine, a competitive analog of S-adenosyl methionine[3]. As a consequence, there is a reduced availability of ATP and a decrease in the intracellular concentration of K^+[4]. The steatogenic effect of white phosphorus would also imply a lipoperoxidative mechanism similar to that of CCl_4[5].

The aim of the present work is to establish whether the morphological damage induced by CCl_4, ethionine or white phosphorus poisoning in liver is accompanied by changes in the level of the microsomal respiratory pigments, which may be correlated to a lipoperoxidative mechanism. Moreover, since in vitro experiments[6,7] have shown the conversion of cytochrome P_{-450} into a solubilized form, called cytochrome P_{-420}, owing to the modification of the lipoproteic shell around the haemoprotein molecule, we attempted to establish whether a similar modification could happen in vivo by the lipoperoxidative action of CCl_4.

The results obtained indicate that the pathogenic mechanisms of the steatogenic agents employed are different as far as the spectral determination of the content of microsomal pigments is concerned. A preliminary account of this work has been presented[8].

Materials and methods. Female albino rats of the Wistar strain, weighing 200–250 g, fasted for 16 h, were treated with CCl_4 (0.25 ml/100 g body weight, i.p. or by gastric intubation). with ethionine (100 mg/100 g body weight, i.p.) or white phosphorus (0.75 mg/100 g body weight, by gastric intubation), and sacrified according to the scheme shown in the Table. At the times of treatment chosen for the different drugs a pronounced hepatic fat accumulation and reduction in the level of circulating lipoproteins is observed[9–11]. For CCl_4 intoxication assays have also been performed at the early stages of treatment.

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The microsomal fraction was prepared by the method of OMURA and SATO[5], with slight modifications, and suspended at a protein concentration of about 15 mg/ml in 0.15 M KCl, containing 0.05 M, pH 7.5 *Tris*-HCl buffer. Proteins were determined by the biuret method[12].

Difference absorption spectra were performed in the Dual wavelength/split beam Aminco-Chance spectrophotometer. For the calculation of the cytochromes content, the following millimolar extinction coefficients have been used: cytochrome b_5, $\Delta\varepsilon$ (424–409 nm) = 165 $cm^{-1} \times mM^{-1}$ (ref.[13]) and cytochrome P_{-450}, $\Delta\varepsilon$ (450–490 nm) = 91 $cm^{-1} \times mM^{-1}$ (ref.[14]).

Results and discussion. In the Table is shown the kinetics of cytochrome b_5 and P_{-450} in the liver microsomal fractions of rats treated i.p. with CCl_4. The decrease of cytochrome P_{-450} is about 40% after 1 h and reaches about 50% after 4 h of treatment with the poison. Cytochrome b_5 decreases by about 40% after 4 h. The same effect of CCl_4 on the amount of cytochromes b_5 and P_{-450} is observed when the drug is given orally. The lack of difference in the behaviour of the microsomal pigments during intoxication by CCl_4, administered either i.p. or orally, rules out the possibility that the effect of the drug might be somehow dependent on its mode of administration.

Effect of CCl_4, ethionine and white phosphorus upon the level of microsomal pigments

Treatment	Time (h)	Pigment concentration (nmoles/mg protein)	
		Cytochrome b_5	Cytochrome P_{-450}
None		0.492 ± 0.017 (20)	0.627 ± 0.032 (16)
CCl_4 (i.p.)	1	0.392 ± 0.040 (3)	0.379 ± 0.030 (5)
	2	0.390 ± 0.010 (4)	0.374 ± 0.030 (6)
	4	0.298 ± 0.021 (11)	0.301 ± 0.018 (16)
CCl_4 (g.i.)	4	0.259 ± 0.015 (6)	0.281 ± 0.018 (6)
Ethionine (i.p.)	6	0.423 ± 0.013 (4) [a]	0.540 ± 0.050 (4) [b]
Phosphorus (g.i.)	24	0.515 ± 0.017 (8)	0.579 ± 0.021 (8)

Rats have been treated with CCl_4 i.p. or by gastric intubation (g.i.), with ethionine i.p. or with white phosphorus by g.i. and killed at different time intervals. Cytochrome b_5 has been measured in difference absorption spectra recorded between NADPH-treated minus untreated aerobic microsomes. Cytochrome P_{-450} has been recorded from (CO + $Na_2S_2O_4$)-treated minus CO-treated microsomes. The microsomal fraction was suspended in 0.15 M KCl, containing 0.05 M *Tris*-HCl buffer, pH 7.5 at the concentration of 1.5 mg protein/ml. NADPH was added at the final concentration of 330 μM. Values are given as means ± S.E. with the number of animals in parentheses. [a] Not significantly different from value without ethionine, by Student's t-test, $P > 0.05$. [b] Not significantly different from value without ethionine, by Student's t-test, $P > 0.20$.

An attempt has been made to show the presence of cytochrome P_{-420}, eventually produced in vivo by lipoperoxidation of the lipid shell of cytochrome P_{-450}, in the high-speed supernatant of the homogenate after CCl_4 poisoning. Spectra have been performed between (CO + $Na_2S_2O_4$)-treated minus CO-treated samples. However, the contamination of the supernatant with even small amounts of haemoglobin made difficult the essay of cytochrome P_{-420}, which ,on the other hand, did not seem to be present in the microsomal fraction.

Intoxication with ethionine did not cause significant changes in the amount of microsomal pigments (Table). At the concentration used, ethionine induces an 80% decrease of intracellular ATP[15]. As shown in the Table, white phosphorus has a behaviour similar to that of ethionine, since no effect has been observed after 24 h of treatment with this substance.

The data obtained indicate that, among the 3 poisons employed at concentrations able to cause fatty liver degeneration, only CCl_4 influences the level of the microsomal respiratory pigments. The change in the content of such pigments induced by CCl_4, which is not observed when the drug is added to isolated microsomes[2], may be related to the lipoperoxidation of phospholipids occuring in vivo at the level of the endoplasmic membranes. The same conclusion cannot be drawn for ethionine and white phosphorus, probably because their different mechanism of action, even though a lipoperoxidative action has been suggested for white phosphorus by other authors[5], on the basis of different kind of experiments[16].

Riassunto. L'intossicazine di ratti Wistar con tetracloruro di carbonio (CCl_4) causa nel fegato una marcata diminuzione dei citocromi microsomiali b_5 e P_{-450}. L'etionina e il fosforo bianco, somministrati anch'essi ad una dose capace di determinare degenerazione grassa del fegato, non influenzano significativamente il livello dei due citocromi microsomiali.

C. BARONE, A. CITTADINI, T. GALEOTTI and T. TERRANOVA

Institute of General Pathology, Catholic University, Via Pineta Sacchetti 644, I-00168 Roma (Italy), 5 July 1972.

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[16] Acknowledgments. The work was supported by a grant from Consiglio Nazionale delle Ricerche, Italy.

Shortened Duration of Drug-Induced Behavioral Excitation in Rats with Septal Lesions

A recent series of studies[1–5] suggests a major role for acetylcholine (ACh) in the behavioral excitation observed following administration of tetrabenazine (TBZ) 18 h after iproniazid pretreatment to rats trained on operant shock-avoidance schedules. Excitatory responding was temporally correlated with lowered levels of total ACh (but not serotonin or norepinephrine) in the telencephalon[1]. Pretreatment with low doses of atropine, a known anticholinergic drug, enhanced the duration of behavioral excitation, whereas larger atropine doses completely blocked excitation[3].

If neuronal pathways utilizing ACh as a transmitter are involved in this excited responding, septal lesions should either reduce or eliminate the excitation, since this type of lesion results in a 20–37% reduction of total ACh content in the rat brain[6,7]. In the present study, when rats