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.

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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

Intensity and duration of behavioral excitation during shock-avoidance responding in rats with septal lesions or controls after administration of tetrabenazine (2 mg/kg) given 18 h following iproniazid (50 mg/kg) pretreatment

Treatment	Pre-surgery response rate (mean r/min)	Pre-injection response rate (mean r/min)	Excitation response rate (mean r/min)	Duration of excitation [a] (min)
Septal lesions	10.2	9.4	24.0	23.0 ± 5.3 (14)
Control	8.7	8.7	20.6	50.4 ± 9.4 (14)
				$p < 0.01$

[a] Mean ± S.E.M. A response rate at least 50% greater than control rates was defined as behavioral excitation. Value in parenthesis refers to number of rats. The probability was determined by *t*-test.

with bilateral septal lesions were given iproniazid-TBZ treatment, the resultant periods of behavioral excitation were significantly shorter than controls.

Adult, male Wistar rats, weighing approximately 300 g, were trained on an operant shock-avoidance schedule (RS20:SS10; 1.6 mA; 0.5 sec duration) in a standard lever pressing apparatus[1,4]. After stable responding was obtained, the rats were divided into 2 groups: those receiving bilateral septal lesions and controls. For both the lesioned animals and the controls, the electrode was stereotaxically placed in the septal area of the brain, using coordinates given in the PELLEGRINO and CUSHMAN atlas[8]. The anode was attached to the skin flap from the surgical incision. A direct cathodal flow of 3 mA was applied for 45 sec only in the case of the experimental group of rats and not the controls.

After a recovery period, both groups were returned to daily sessions on shock-avoidance schedules. Approximately 3 weeks following surgery, the rats received 2 mg/kg TBZ (s.c.) 1 h after the beginning of a daily session and 18 h after iproniazid pretreatment (50 mg/kg s.c.). Subsequent periods of avoidance responding were then quantitatively determined for both lesion and surgical-control groups.

The duration of behavioral excitation exhibited by rats with bilateral septal lesions was less than 50% excitation period shown by control animals (Table). There was no significant difference in the degree of excitation (approximately 2.5 times greater than baseline rates) between the lesion and control groups; only the length of the period of excitation differed. In addition, no differences were noted in the post-surgery response rates prior to the iproniazid-TBZ treatment session, nor in sessions subsequent to drug session. Following the determination of all necessary behavioral measures, the correct placement and size of the lesions was confirmed by histological examination.

Since rats with septal lesions were previously shown to have lowered brain ACh levels[6,7], the shorter periods of excitation in the lesioned rats of the present study support the suggestion that ACh plays an important role in this type of behavioral excitation. The fact that PEPEU et al.[6] found the lowest levels of ACh in areas of the telencephalon, where ACh levels were most closely correlated with increased avoidance responding by our animals[1], adds further weight to this hypothesis. Since our explanation relates the excitation noted in these rats to the release of AC hat key cholinergic synapses, the apparent smaller amount of ACh available for release in the septal-lesioned rats could account for the shorter periods of behavioral excitation observed under these conditions. However, the exact role of ACh in relation to its possible interactions with serotonin and/or norepinephrine levels is not yet known[2,9], and a more definitive explanation of the interaction of these putative transmitters (or modulators) in excitation awaits the completion of analytical studies involving the measurement of the content and turnover of their functional pools[10].

Resumen. En las ratas, la reacción elevada de la respuesta evasiva, después de darles iproniazida y tetrabenazina aparentemente está relacionada con el suelto acrecentado de acetylcholina en al parte frontal del cerebro. Cuando las ratas con lesiones septales recibieron estas dos drogas, sus periódos resultantes de excitacion de conducta eran significantamente mas cortos que los de los controles. Como las lesiones septales producen disminución en los niveles de la acetylcholina en el cerebro, estos datos implicaron más el sistema cholinergica en la excitación producida por la iproniziada-tetrabenazina.

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