Tissue samples for enzyme extraction were collected the 12th day after each transfer, washed twice in distilled water and stored for 24 h at 18°C. Extraction was carried out essentially according to WOLFF[9]. The tissue extracts containing RNases were used for electrophoresis on 7% polyacrylamide gels according to DAVIS[10]. Conditions during the run were the same in WOLFF[9]. Gels were stained according to WILSON[11].

Results and discussion. In Figures 1 and 2 the electrophoretic patterns of the 5 stocks at acid (5.5) and alkaline (7.5) pH are reported.

The results obtained with *N. glauca* seem particularly interesting. Tissue extracts from this species showed 5 identical RNase bands in both normal and habituated tissues, when the test was carried out at pH 5.5; on the other hand, habituated tissue showed 5 bands at pH 7.5, 4 of which were found also in test carried on normal callus and 1 seems to be specific to the autonomous strain: it is worth noting that band 17 also occurs in tumorous hybrid *Nicotiana glauca* × *Nicotiana langsdorffii*[4]. *Nicotiana bigelovii* habituated tissue, showed 6 bands at pH 5.5 and 4 at pH 7.5. Control experiments, carried on habituated tissue grown on hormone supplemented media, gave the same results. The situation was more confuse in the case of *Haplopappus gracilis*: habituation in this case seemed to lead to drastic changes involving the whole RNase complement as judged from the electrophoretic behaviour. No single band specific to the transformation to autotrophy could be found.

The data so far reported seem to confirm the differences in RNase complements and activity, between normal and tumorous tissues reported by other authors in plant material[3,4], pointing out the considerable complexity of the problem in some cases as in the *Haplopappus gracilis* situation.

The results obtained with *Nicotiana glauca* tissues, moreover, suggest that isolation and characterization of the specific RNase band observed on habituated material may give some better clue to the problem of RNase involvement in the process of transformation to autotrophy.

Riassunto. É stata fatta un'indagine elettroforetica delle RNasi di tessuti vegetali normali ed abituati di *Haplopappus gracilis*, di *Nicotiana glauca* e di *Nicotiana bigelovii*, coltivati in vitro. Sono state notate delle differenze nei complementi RNasici dei tessuti abituati rispetto a quelli dei tessuti normali. I risultati sono discussi brevemente in relazione alle loro implicazioni col problema dei tumori nelle piante.

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Failure of Carbon Tetrachloride (CCl_4) and Trichlorobromo-methane (CCl_3Br) to Alter Polyribosomal Profiles in the Rat Brain

Studies on CCl_4 and CCl_3Br toxicity are mainly concerned with liver toxicity[1]. The homolytic cleavage of halomethanes to free radicals takes place in the drug metabolizing enzyme system (DMES) in the liver[1]. Drugs inducing or depressing liver DMES increase or decrease CCl_4 toxicity, respectively[2,3], therefore liver toxicity is considered to be secondary to the formation of free radicals. Conversely, toxicity by CCl_4 in a given organ might be considered a sensitive index of the presence of DMES in that organ. RECKNAGEL and GOSHAL[4] found neither in the kidney nor in the brain the changes (proxidant effects) that were observed in the liver of CCl_4-treated animals. Morevor, these authors demonstrated that CCl_4 is not metabolized by isolated rat liver microsomes unless the liver supernatant fraction is added[4].

The present experiments were carried out to study the effect of 2 chlorinated hydrocarbons, CCl_4 and CCl_3Br, on polyribosome profiles of brain homogenates. In fact polyribosomal breakdown is an early effect produced by CCl_4 and CCl_3Br in the liver and is a very sensitive index of the formation of free radical[5].

Materials and methods. Wistar rats of both sexes, weighing 250–300 g, were used. They were fed with a semisynthetic diet (Vogt-Möller, Ditta Piccioni, Brescia, Italy). The animals were starved 12 h before sacrifice, water was given ad libitum. Phenobarbital (PB), as sodium salt, dissolved in 0.9% NaCl, was administered i.p. at the dose of 80 mg/kg of body wt daily for 3 days. The last dose was given 24 h before intoxication; CCl_4 and CCl_3Br were administered at the dose of 0.25 ml/100 g, by stomach tube to untreated and PB-treated animals. All rats were killed by bleeding 2 h after intoxication. Brain polysomal profiles were obtained according to the method of WEISS et al.[6], modified as follows: after killing, the brains were taken and homogenized in 2 volumes of medium S_5 (50 m*M* *Tris*/HCl; 80 m*M* KCl; 5 m*M* $MgCl_2$; 5 m*M* mercaptoethanol and 250 m*M* sucrose). The homogenate was then centrifuged at 13,000 × *g* for 20 min. Aliquots of the postmitochondrial supernatant, treated with sodium deoxycholate to a final concentration of 1%, was layered on to a linear 15%–50% (w/v) sucrose gradient and centrifuged at 2000,00 × *g* for 40 min in a Spinco SW50 rotor at 0°C. The extinction profiles at 260 nm were recorded automatically with a Unicam model SP1800 spectrophotometer, by pumping the gradient trough a flow cell at constant flow rate. Liver polysomal profiles were obtained as follows: after killing, the liver was taken and homogenized in 2 volumes of medium S_5. The homogenate was then centrifuged at 20,000 × *g* for 20 min. Aliquots of the postmitochondrial supernatant, treated with sodium deoxycholate to a final concentration of 1%, were layered on to a linear 15%–50% (w/v) sucrose

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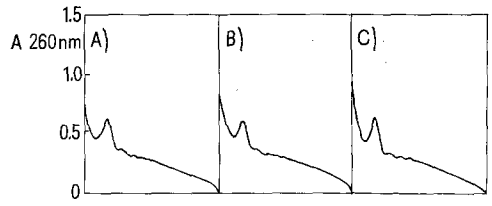

Fig. 1. Sedimentation profiles from the postmitochondrial supernatant of rat brain homogenates. A) normal rat; B) CCl_4-treated rat; C) CCl_3Br-treated rat. Top of the gradient is to the left.

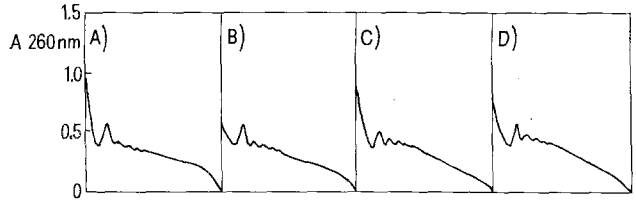

Fig. 2. Sedimentation profiles from the postmitochondrial supernatant of rat brain homogenates. A) CCl_4-treated rat; B) CCl_3Br-treated rat; C) PB + CCl_4-treated rat; D) PB + CCl_3Br-treated rat. Top of the gradient is to the left.

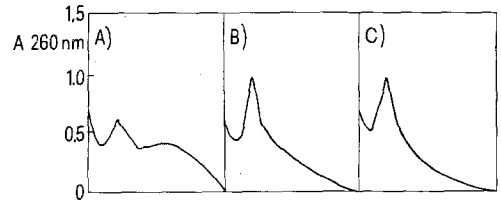

Fig. 3. Sedimentation profiles from the postmitochondrial supernatant of rat liver homogenates. A) normal rat; B) CCl_4-treated rat; C) CCl_3Br-treated rat. Top of the gradient is to the left.

gradient and centrifuged at 200,000 $\times g$ for 40 min in a Spinco SW50 rotor at 0 °C° The extinction profiles at 260 nm were recorded as previously described.

Results. After 2 h of intoxication with CCl_4, as well as with the more toxic CCl_3Br[5], the brain polysomal profile was not different from that of the control group (Figure 1). In addition, these halomethanes failed to modify the polysomal pattern in the brain of animals pretreated with PB (Figure 2). On the other hand, as expected, CCl_4 and CCl_3Br produce polysomal breakdown in the liver, both in normal rats and in PB-treated rats (Figure 3).

Our data show that CCl_4 and CCl_3Br not cause in the brain one of the very typical alteration which they produce in the liver, that is polysomal dissociation. This observation can be explained with the absence in brain tissue of a microsomal system capable of metabolizing foreign drugs. Conversely, since CCl_4 reaches, in brain, a concentration higher than in liver[7], and since polysomal breakdown is a sensitive index of the toxicity by free radicals, our data provide strong evidence that DMES is not present in the rat brain. Finally, our results indicate that neither CCl_4 nor CCl_3Br inhibit protein synthesis per se.

Riassunto. La disaggregazione dei polisomi di fegato di ratto trattato con CCl_4 o CCl_3Br non é riproducibile nei polisomi di cervello, neppure in animali pretrattati con fenobarbital.

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Inhibition of Phosphodiesterase by Dihydroergotamine and Hydergine in Various Organs of the Cat in vitro

The classical phosphodiesterase inhibitors (PEase-I), caffeine and theophylline, are roughly equipotent when tested in vitro on homogenates of various organs[1–3]. However, this is not the case with all substances which reduce phosphodiesterase (PEase) activity.

Lately, numerous papers have appeared reporting different levels of inhibitory activity or organ-specific activity for apomorphine[1], papaverine[1,4,5], 3,4-dihydroxyphenyl-acetic acid[1], quazodine[6], a pyrazolo-pyridine derivative[2] and the bronchodilator 3-acetamido-6-methyl-8-n-propyl-5-triazolo-4, 3-pyrazine[7]. Pichard et al.[8] recently demonstrated in human tissues that the coronary vasodilator dipyridamol inhibits blood platelet PEase 4.5 times more efficiently than the brain enzyme. These authors found that the situation was reversed with tricyclic antidepressants, e.g. nortriptyline, these compounds inhibiting the PEase activity of the brain 3 times more strongly than that of the blood platelets. All these findings suggest that a relationship exists between inhibition of the specific PEase of an organ and pharmacological effect on the organ in question.

Some ergot alkaloids and lysergic acid derivatives are known as PEase-inhibitors. Thus, Kukovetz and Pöch[9] showed that bromo-LSD reduces myocardial PEase activity. Ward and Fain[10] have demonstrated that dihydroergotamine (DHE) inhibits PEase in adipose tissue. We[2,11] noted PEase inhibition in cerebral grey matter with DHE and a large group of analogous compounds. DHE and Hydergine ® (an equimolecular mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine – PEase-I belonging to the group of compounds mentioned) exert pharmacological effects on the

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