dietary components[15], availability of biological ligands[16] and various transport mechanisms[17,18] may also be involved, since the mechanism of lead absorption is still not well understood. Indeed, a higher lead absorption was observed in a previous experiment when only trace amounts of lead were available[9]. It is reasonable to assume that the lead dose level as used in this experiment saturated or inhibited the carrier-mediated transport process of lead absorption or affected some low-molecular weight lead-binding protein[19]. The accumulation of lead in the litters is indicative of the high capacity of the mammary gland of lactating rats to excrete increased levels of absorbed lead to the offspring. Considering that lead is preferentially deposited in the brain of suckling rats[20,21], a high risk of lead exposure to infants is clearly indicated. Therefore the availability of safe drinking water to all segments of population is of essential importance, especially in relation to the pollution of the environment with heavy metals.

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Effects of cycloheximide on protein synthesis in human lung tumors, regenerating rat liver and hepatomas

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Summary. 10^{-4} M cycloheximide (CHM) inhibits leucine incorporation to about the same degree in slices of human lung tumors, rat hepatomas, regenerating livers and normal tissues. At 10^{-6} M, CHM has a more pronounced effect on tumor tissue and regenerating liver than on normal tissues. 10^{-8} M CHM stimulates protein synthesis in normal rat liver slices.

The inhibitors of protein synthesis in eukaryotes are considered useful as tools for research in cell biology, since, in some cases, it is possible to demarcate a step (or steps) in protein synthesis that is affected by a given drug[1]. As there can be little doubt that protein synthesis plays an important role in tumor development, it would appear interesting to observe whether certain tumors differ from controls in regard to some inhibitors. The results reported here concern the in vitro effects of 10^{-8} M, 10^{-6} M and 10^{-4} M cycloheximide (CHM) on leucine incorporation into proteins of human lung tumors and rat hepatomas caused by 4-dimethylaminoazobenzene (DAB) as compared to those obtained in controls. The use of this antibiotic appears advantageous since it influences, in various types of cells, all the phases of protein synthesis (initiation, elongation and termination), and the primary sensitive step affected varies with the concentration of drug from between 10^{-9} M to 10^{-3} M[2,3]. In order to observe whether the possible difference in response to CHM is present not only in tumoral growth but also in nonmalignant growing cells, the effects of the above concentrations of this drug on protein synthesis of regenerating liver were also investigated. Furthermore, in an attempt to discriminate between changes related to the development of liver tumors and those due to other factors, such as the action of DAB as a foreign compound, kidneys of DAB-fed rats were included as they do not form malignant growth in response to this chemical.

Materials and methods. Human pulmonary neoplasms and the uninvolved tissues (used as controls) removed at surgery were placed in ice-cold 0.9% NaCl. (We are greatly indebted to Prof. G. Pellegrini, Head of the Istituto di Patologia Chirurgica II, Università di Milano, Milan, Italy, for making available portions of pulmonary neoplasms and of uninvolved lung tissues.) Once in the laboratory, the tumor and nontumor sites were isolated and processed separately. For the induction of liver tumors, female Wistar albino rats, weighing 150–200 g, were fed on a pelletted diet containing 0.06% DAB[4] for 7–9 months. Control rats were fed on a pelletted complete commercial diet (Piccioni, Brescia, Italy). Regenerating livers were used 48 h after partial hepatectomy[5] in female rats of the same experimental stock as were used for liver tumor induction. To study protein synthesis, 8–10 slices of livers or kidneys were incubated, as previously described[6], in the presence of radioactive leucine for 1 h. 1 or 2 slices of each human lung tumor were incubated (100–150 mg wet weight) simultaneously with 1 or 2 slices of the uninvolved lung of the same patient, under the conditions used for rat organs. After incubation, the slices were homogenized in water and the radioactivity of purified proteins determined[7]. The amount of leucine incorporated by slices of lung tumors and of pulmonary uninvolved areas was calculated on the dry weight basis, since the presence of anthracosis, mainly located in the uninvolved areas, interferes very strongly with the reactions generally used for the determination of the reference substances. The dry weight was determined by drying portions of each homogenate at 100 °C to constant weight. The radioactivity of liver and kidney proteins was referred to the protein content determined by the biuret reagent[8]. Addition of the alkaline copper reagent

Effects of cycloheximide on L-leucine $^{14}C(U)$ incorporation into protein of different tissues

Cycloheximide concentration in media	Human uninvolved lung[a]	Human lung tumors[a]	Normal rat livers[b]	Rat hepatomas[b]	Regenerating rat livers[b]	Normal rat kidneys[b]	Kidneys of DAB-fed rats[b]
0	24.8 ±4.75 (10)	46.0 ±5.13 (8)	9.3±3.48 (7)	18.9±11.41 (7)	92.1±22.12 (5)	48.5±20.53 (8)	15.8±5.01 (8)
10^{-4} M	5.35±1.34[e] (10)	6.28±1.75[d] (10)	2.6±0.85[d] (7)	2.0±0.94[e] (8)	5.6±0.28[e] (5)	5.4±0.41[d] (8)	3.3±0.66[d] (8)
Deviation (%)[c]	−78	−87	−73	−89	−94	−89	−79
10^{-6} M	17.4±3.40 (8)	6.16±2.5[d] (9)	6.4±1.48 (8)	5.7±2.18[e] (8)	44.1±12.02[d] (5)	8.9±1.42[d] (8)	8.6±1.78[d] (8)
Deviation (%)[c]	−30	−87	−31	−70	−52	−82	−46
10^{-8} M	31.8±8.37 (10)	22.0±9.70 (10)	15.4±4.78[e] (8)	11.5±5.90 (7)	65.4±10.11[d] (5)	21.1±4.09 (8)	18.5±4.09 (8)
Deviation (%)[c]	28	−52	66	−39	−29	−56	17

Values are given as: [a] pmoles of leucine incorporated/min mg dry tissue; [b] pmoles of leucine incorporated/min mg protein. The figures are the mean ± SE of the number of the determinations given in parentheses. [c] Deviation (%), [d] $p < 0.05$, [e] $p < 0.01$ from appropriate control.

often resulted in a persistent cloudiness which could be eliminated by mixing with diethyl ether. The mixtures were centrifuged and the lower phase transferred by Pasteur pipettes to cuvettes and their adsorbance determined. When neoplasms contained a soft centre, this was rejected. The statistical significance was evaluated by Student's t-test. When the comparison was made with material from the same source, the significance was evaluated by applying the t-test to the mean difference of a series of paired samples[9]. No statistical significance was attached to differences with a probability value $p > 0.05$.

Results. The results are summarized in the table. Leucine incorporation into proteins of lung tumors, hepatomas and regenerating livers is inhibited by 10^{-4} M CHM to about the same extent as in the controls. The inhibition by 10^{-6} M CHM in controls is not statistically significant and is of a notably lesser magnitude than that observed in lung tumors, hepatomas and regenerating livers. 10^{-8} M CHM increases leucine incorporation into proteins of normal rat livers. Also the uninvolved pulmonary tissues show an increase, although to a lesser extent than that found in the liver: it is not statistically significant. In tumors and regenerating livers, protein synthesis is never increased by 10^{-8} M CHM and indeed appears to be generally depressed, although not to a statistically significant degree. In comparison with controls, regenerating livers show a very high rate of leucine incorporation ($p < 0.01$). Renal protein synthesis of DAB-fed rats is more resistant to CHM than that of normal ones and, in the absence of this drug, synthesis is lower in DAB-fed animals than in controls ($p < 0.02$).

Discussion. The study of total protein synthesis by lung is complicated by various factors such as the 40 different cell types, the choice of labelled amino acids, the reference parameter and so on[10]. Furthermore the so-called uninvolved tissue may be infiltrated by malignant cells, inflammatory or lymphoid tissue and, therefore, could not be expected to behave as a completely normal tissue. However, the data here reported seem to be of interest since they do not generally differ consistently from those found for the rat liver as regards the effects of CHM on protein synthesis of both control and tumoral tissues. Previous results demonstrate that CHM inhibits protein synthesis approximatively to the same extent in liver as in lung of normal rats[11]. As compared to controls, the greater sensitivity of tumor tissues to low CHM concentrations could indicate a higher sensitivity, in tumors, of the steps involved in protein synthesis initiation. Namely, it has been shown that at low concentrations CHM mainly inhibits protein synthesis initiation in *Saccharomyces cerevisiae*[2] and in growing cells[3].

In vitro the activity of initiation factors of Morris hepatomas has been found to be increased as compared to that of host livers[12]. From our data it appears that also protein synthesis of regenerating livers is more sensitive than that of normal ones to lower concentrations of CHM and, therefore, at least as far as the liver is concerned, this increased sensitivity to CHM arises not only in the case of cancer growth but also in nontumoral growth. A reduced initiation efficiency has been observed in resting as compared to proliferating cultures of human diploid fibroblasts[13]. The increase in leucine incorporation found in controls with 10^{-8} M CHM is not very understandable. However, it is worthwhile recalling that the administration of low doses of CHM to rats causes an increase of tyrosine aminotransferase in the liver[14]. Furthermore, it has been reported that in vivo the reversal of CHM inhibition of protein synthesis is associated with an anomalously rapid rate of synthesis of proteins[15] and of ribonucleic acid[16]. The increase at 10^{-8} M CHM in leucine incorporation has not been found in tumoral tissues and regenerating liver and this seems to constitute another difference between controls and growing tissues. The kidneys of DAB-fed rats respond in a different way to the liver to CHM. Namely these organs are more resistant to this drug than controls. Since the kidneys do not show a tumoral growth, the effects observed in the liver do not seem attributable to an action of DAB as a foreign compound. The decreased renal protein synthesis observed in DAB-fed rats, in the absence of CHM, in comparison with controls, could be due to some changes occurring in these kidneys[17].

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