radioactivity in the medium. The concentration of NGF necessary to affect the degradation of chondromucoprotein is much higher than those necessary to stimulate the growth of sympathetic and sensory ganglia. Rather high concentration of NGF was found in granulation tissue[6] and therefore NGF may play some role on the involution of granulation tissue by degrading the extracellular matrix. But the true physiological significance of this unique and specific effect of NGF in stimulating the degradation of cartilage chondromucoprotein is unknown at present.

Table 2. Effect of NGF on the uptake of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-leucine into TCA insoluble fraction in 9 day chick embryo femur cultivated in vitro

	Incorporated radioactivity (cpm)		
	^{3}H-thymidine	^{3}H-uridine	^{3}H-leucine
Control	26507±2647*	24517±995	15614±722
NGF (10 µg/ml)	23425±861	24441±988	12702±1205
% of control	88±6	101±4	83±7

The femora precultivated for 2 days were exposed to [methyl-^{3}H] thymidine, [6-^{3}H] uridine and L-[4,5-^{3}H] leucine containing medium for 12 h with or without NGF to determine the DNA, RNA and protein synthesis respectively. After the incubation, the femur was homogenized in 1 ml of cold 5% of trichloroacetic acid by the use of a glass homogenizer and the radioactivity of the trichloroacetic acid insoluble fraction was determined. * Mean ± SE (n = 6).

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Lead metabolism in lactation[1]

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Summary. A 2-fold increase in lead absorption was observed in lactating animals which received 2 mg Pb/l in drinking water. About one-half of the absorbed lead was transferred to the litters.

Lead is an environmental poison which can be transferred from mother to offspring through milk[3-5]. Various degrees of brain damage were produced in suckling rats with doses tolerated by their mothers, indicating that infants are a high risk group in relation to lead exposure[6-8]. A previous report from this laboratory showed an increase in the absorption of trace amounts of lead in lactating animals[9]. Considering that the gastrointestinal tract is the main route of entry of environmental lead into the body[3,10], the aim of this experiment was to investigate the effect of lactation on the absorption of lead from drinking water. A dose 20 times higher than the tentative limit of 0.1 mg Pb/l[11] was chosen because such an amount can be found in human diet[12].

Methods and results. The experimental group consisted of 10 lactating female rats each with a litter of 6. The control group consisted of an equal number of virgin rats of the same age. The animals were fed a stock laboratory diet (1.2% Ca and 0.8% P) and drank distilled water ad libitum. On the 12th day of lactation, 2 mg Pb/l were added to the drinking water marked with radioactive lead-203 (0.17 µCi/ml). After 2 days the lead-containing drinking water was discontinued and the animals were given distilled water for one more day. Individual urine samples were collected until the rats were killed on the 3rd day. The gastrointestinal tract of adult animals was removed to avoid the interference of nonabsorbed lead-203. Gamma-ray activity of the rat's carcass and urine, and, in the case of lactating rats, the whole body activity of their litters were determined in a single channel, twin crystal scintillation counter (Tobor, Nuclear Chicago, USA).

The apparent absorption expressed as the percentage retention of lead-203 in the carcass and urine, and, in the case of lactating rats, the whole body retention of their litters were the same in control and lactating rats (table). Lead-203 retention of the litters was almost 30% higher than the percentage dose retained in the carcasses of their mothers. When the results were corrected for a 2-fold increase in water consumption of lactating rats (58.4±2.0 vs 24.8±1.4 ml/day/rat) the absorption of stable lead was twice as high in lactating (3.6 µg Pb) as in control animals (1.5 µg Pb). Litter lead retentions (1.7 µg Pb) were as high as those for control animals.

Discussion. Increased lead absorption in lactating animals is most likely to be attributed to the well-documented morphological augmentation of the gastrointestinal tract during the lactation period[13,14]. However, the complexity due to

Administered dose of lead and lead-203, retention in carcass and litter and urinary excretion*

	Dose ^{203}Pb (%)	µg Pb
Controls		
Oral intake from drinking water	100	100±6
Carcass	1.0±0.1	1.0±0.3
Urine	0.5±0.1	0.4±0.4
Apparent absorption	1.5±0.6	1.5±0.6
Lactating		
Oral intake from drinking water	100	240±10
Carcass	0.5±0.03	1.2±0.3
Litter	0.7±0.2	1.7±1.1
Urine	0.3±0.1	0.6±0.6
Apparent absorption	1.6±0.7	3.6±1.4

* Each figure represents the mean of 10 animals ± SD.

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