tents per g of liver of the phenobarbital-treated rats, the tumour-bearing rats or the tumour-bearing phenobarbital-treated rats is not significantly changed.

These results indicate that a treatment with phenobarbital produces a marked liver weight increase in only 48 h. This enlargement is a real hypertrophy, since at the same time there is an increase of total proteins, DNA and RNA.

The phenobarbital activity on tumour-bearing rats can be compared with that observed in weanling rats. It is probable that some growth-promoting factor(s) from the tumour is responsible for the greater sensitivity toward the phenobarbital action on the liver. Our previous results show that there is an increased sensitivity toward phenobarbital in partial hepatectomized or in pregnant rats, and in both cases the presence of growth-promoting factor(s) has been reported [10–12].

Riassunto. Gli autori hanno dimostrato che nei ratti portatori di tumore di Walker (carcinosarcoma 256), un trattamento eseguito 48 h prima con phenobarbital produce un aumento del rapporto peso fegato/peso corporeo pari al 26%, mentre esso è soltanto del 6% dei ratti normali. Tale fatto si accompagna ad aumento di contenuto totale di proteine, di RNA e di DNA.

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Decreased Activities of Liver Microsomal Drug-Metabolizing Enzymes in the Rats Bearing Walker Carcinosarcoma

There are many reports concerning systemic effects of malignant tumours on host animals[1]. NAKAHARA and FUKUOKA, NAKAGAWA et al. and KAMPSCHMIDT and McCOY reported a marked decrease of liver catalase activity in the tumour-bearing animals, and also that injection of the tumour extracts produced a depression of liver catalase activity[2–4]. On the other hand, GREENE and HAVEN have shown an increase in cytochrome oxidase activity in liver homogenates or mitochondria from livers of rats bearing Walker carcinosarcoma 256[5].

In recent years, the importance of oxidative processes in liver microsomes for drug metabolism have been widely recognized. The microsomal enzymes have a common requirement of reduced triphosphopyridinenucleotide (TPNH) and oxygen. The oxidation of TPNH in the microsomes by TPNH oxidase and oxygen may involve a formation of organic peroxide, but the mechanism of action is not yet clear[6].

In the present work, evidence is given that rats bearing Walker carcinosarcoma 256 show a depressed activity of the liver microsomal enzymes responsible for metabolism of hexobarbital, strychnine and meprobamate.

Male rats of the Sprague-Dawley strain, weighing about 200 g were used. Walker carcinosarcoma 256 were inoculated subcutaneously in the lateral abdominal region. The animals were killed 22 days after the inoculation.

The enzyme activities were determined by measuring the metabolized hexobarbital, strychnine and meprobamate by a liver microsomal preparation during incubation for 1 h. The liver was immediately removed and homogenized in 3 parts of isotonic KCl (1.15%) with a Potter-Elvehjem type homogenizer. The nuclei and mitochondria were sedimented by centrifugation of the homogenate at 8500 *g* for 15 min. The incubation mixture (5.0 ml) contained 3 ml of the microsome-containing supernatant, 0.1 ml of 20 μmole glucose-6-phosphate, 0.4 μmole TPN, 50 μmole nicotinamide and 75 μmole $MgCl_2$ and 2 *M* KCl and more 1.3 ml of 0.2 *M* sodium phosphate buffer pH 7.4 (pH 8.2 for strychnine) and 0.2 ml of the substrate. Final concentrations of hexobarbital, strychnine and meprobamate were 6×10^{-4}, 2×10^{-4} and $3 \times 10^{-4} M$ respectively.

The determinations of hexobarbital, strychnine and meprobamate were carried out according to the methods of AXELROD et al., KATO et al., and HOFFMAN and LUDWIG respectively with little modification[7–9]. The results are shown in the Table.

The metabolisms of hexobarbital, strychnine and meprobamate were markedly decreased in liver of Walker carcinosarcoma-bearing rats. Hexobarbital metabolism was decreased 53%. In preliminary experiments, we could not demonstrate the presence of an inhibitor in the liver of the tumour-bearing rats. Similarly, decreased enzyme activity was also observed by us in rats bearing Flexner-Jobling carcinosarcoma even of less intense degree.

The mechanism which produced the decreased activities of the microsomal drug metabolizing enzymes is not yet clear. It is probable that as toxohormone produces a decrease in the activity of liver catalase, some factors elaborated by the tumour cell may act directly or indirectly on the liver microsomes to produce a decrease of the oxidative processes. We also observed a decreased activity of microsomal TPNH oxidase in the tumour-bearing rats, but according to GILLETTE et al.[6], the production of H_2O_2 and its decomposition by catalase are not connected with the process of drug metabolisms in liver

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microsomes[10]. The results reported here therefore indicate that tumour-bearing rats are somewhat more sensitive towards some drugs than normal rats. This fact must be taken into consideration in cancer chemotherapy.

In vitro metabolism of hexobarbital, strychnine and meprobamate by microsome-containing supernatant fraction obtained from normal and tumour-bearing rats

	Controls	Tumour-bearing rats	Variations %	*P*
Number of rats	8	8		
Hexobarbital metabolism (μg/g/h)	363 ± 10.9	179 ± 8.7	−53%	<0.001
Strychnine metabolism (μg/g/h)	240 ± 8.8	163 ± 7.2	−32%	<0.001
Meprobamate metabolism (μg/g/h)	52 ± 3.5	28 ± 2.7	−46%	<0.001
Body weight (g)	313 ± 6.9	307 ± 7.2		
Tumour weight (g)	—	25.2 ± 3.4		
Tumor weight / Body weight ×100	—	8.3 ± 0.61		

Enzyme activity expressed as metabolized drug after 1 h incubation with microsome-containing supernatant obtained from 1 g of rat liver.

Riassunto. Ratti portatori di carcinosarcoma di Walker, presentano una notevole diminuzione di attività di quel gruppo di enzimi microsomici epatici da cui dipende il metabolismo di una numerosa serie di farmaci. I nostri rilievi sono stati fatti prendendo in considerazione il metabolismo dell'esobarbital, della stricnina e del meprobamato.

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[10] A decreased activity of the gonads in male rats also produces a decrease in the activity of the enzymes responsible for metabolisms of hexobarbital and strychnine[8,11,12]. Therefore the possibility arises that some factors produced by the tumour cell may interfere with the male sex gland activity.

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On the Possibility of Spontaneous Tuberculous Infection in the Course of Long-Term Trials in Mice

The possibility of transmission of tubercle bacilli from one mouse to another in a group of animals living in the same cage during long-term chemotherapeutic or immunologic trials is still poorly understood. It was shown by KIRCHHEIMER et al.[1] that isolation of virulent tubercle bacilli could be accomplished from the faeces of intravenously infected mice. Moreover, virulent tubercle bacilli have also been found in gastric contents of such mice. It may therefore be suggested that inter-animal transmission of tubercle bacilli is possible, since the environment in the cage must contain a great number of virulent mycobacteria. We had, by chance, the opportunity to investigate such a possibility in the course of a chemotherapeutic trial.

Twenty white mice of the H strain, allegedly femals, were infected intravenously with 0.25 mg of semi-dry weight of the H37Rv-M strain of *M. tuberculosis* and maintained in a glass cylinder. Therapy with perorally administered isoniazid (5 mg/kg of weight, added to Larsen diet) was started the day after infection. The animals were observed daily, and in dead mice the advancement of the disease was assessed by microscopy and, in some cases, by enumeration of viable tubercle bacilli recovered from lungs and spleens.

On the 40th day of duration of the experiment, one female mouse littered 5 young mice. These were identified by a mark and maintained in the original cylinder up to the end of the experiment (2nd generation). Approximately a month later another female mouse littered again 4 young mice which were treated in the same way as mentioned above (3rd generation). A month later another litter of 4 young mice was observed in still another mouse (4th generation), followed by the last litter of 3 young mice again in another mouse (5th generation). The young mice were proceeded in the exactly same way as these of the 2nd generation. The time interval for which the individual generations lived in close contact with the originally infected animals is registered in Table I.

Ten days after the last litter, all mice born in this cylinder were killed with ether. All animals were autopsied and their lungs and spleens were removed and weighed under sterile precautions. Average weight of both organs obtained from animals of individual generations is presented in Table II.

Tab. I. Duration of contact with the tuberculous environment

2nd generation	107 days
3rd generation	72 days
4th generation	45 days
5th generation	10 days

Tab. II

Average organ weights observed in animals of different generations

Generation	Lungs (mg)	Spleen (mg)
2nd	563.7	282.2
3rd	341.3	212.0
4th	169.4	128.7
5th	100.1	39.9

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