wet liver. Protein content was measured by the method of LOWRY et al.[6].

Results. 5 of the 15 mice inoculated with leukaemic spleen cells died spontaneously 8 to 10 days after inoculation and the 10 surviving animals were sacrificed on day 10 for biochemical studies. The livers and spleens of these animals were enlarged and showed almost complete obliteration of normal structures by leukaemia cells. 15 mice inoculated with 10^6 leukaemic spleen cells and treated for 9 days with Ftorafur (90 mg/kg) survived for 10 days. 10 animals from this group were sacrificed on day 10 after inoculation; very few leukaemia cells could be detected in the portal areas of their spleens and livers. The remaining 5 animals received 90 mg/kg Ftorafur until death which occurred between 40 and 48 days, with hemorrhage into the gut and brain, but without morphological evidence of leukaemia in livers and spleens. 15 mice were treated with Ftorafur (90 mg/kg) for 9 days. 10 animals of this group were sacrificed on day 9 and the remaining 5 animals received the same dose of Ftorafur until death, which occured between day 47 and 58, with hemorrhage into the gut or brain. The livers and spleens of sacrificed mice were histologically normal (Table II).

The significance of the difference between various parameters are calculeted by Student's t-test. Liver weight, as percent of body weight, significantly ($P < 0.05$) increased in leukaemic mice (Group 2). This was paralleled by a decrease in protein content ($P < 0.01$) and in total acid phosphatase activity ($P < 0.01$) and by enhancement of unsedimentable activity ($P < 0.01$) in whole liver homogenates. Similar changes were observed in our previous study on 8 pooled livers[2]. In leukaemic and non-leukaemic mice treated with Ftorafur (Groups 3 and 4) liver weight and protein content remained essentially normal. On the other hand, total acid phosphatase activity was evidently higher ($P < 0.01$) in the livers of leukaemic animals treated with Ftorafur (Group 3) than in leukaemic mice without the drug (Group 2), but still did not reach the control value (Group 1). However, no significant difference in this parameter was established between leukaemic and non-leukaemic mice after treatment with Ftorafur (Groups 3 and 4). Unsedimentable activity was somewhat lower ($P < 0.05$) both in leukaemic and in normal animals treated with Ftorafur (Groups 3 and 4) as compared with controls (Group 1) but related to leukaemic mice (Group 2) the difference was almost 3-fold.

Discussion. Prolonged survival of leukaemic mice treated with Ftorafur, and reduced infiltration of their spleens and liver by leukaemia cells indicate an antileukaemic effect of Ftorafur. The drug produced no toxicity until sacrifice of mice on day 10 post inoculation. Prolonged administration of Ftorafur caused haemopoietic toxicity and death. Further discussion will be based on the assumption that the activity of liver acid phosphatase, a lysosomal marker enzyme, actually reflected response of liver reticuloendothelium to the invasion of the liver by leukaemia cells[2]. Decreased unsedimentable acid phosphatase activity in leukaemic animals after treatment with Ftorafur may be explained by a) an inhibitory effect of Ftorafur on growth of leukaemic cells in the liver, b) by decreased fragility of lysosomes caused by Ftorafur in vivo or c) by both effects. The fact that unsedimentable acid phosphatase activity significantly decreased in normal mice after treatment with Ftorafur supports the idea that this drug indeed decreased the fragility of lysosomes. Many authors observed an increased number of lysosomes and release of their enzymes after treatment with antineoplastic drugs or X-rays. They considered this autophagic vacuole response to be a prerequisite for the action of drugs on tumors[7–9]. Our results indicate, in the contrary, stabilization of lysosomes by Ftorafur. We envisage that upon contact with malignant cells lysosomes of liver reticuloendothelium become labilized and release their enzymes into cytoplasm of reacting cells[2]. If lysosomal vesicles remained stable, when reticuloendothelium encountered malignant cells, they would be available for fusion with phagocytic vacuoles. In that case malignant cells could be digested and destroyed intravacuolarly. The present results are in favour of the idea that antileukaemic effect of Ftorafur would be assisted by its ability to stabilize lysosomes.

Zusammenfassung. Nachweis, dass Ftorafur, N_1-(2'-Furanidyl)-5-Fluorouracil das Überleben leukämischer Mäuse deutlich steigert und ausserdem als Lysosomen-Stabilisator wirken könnte, wobei die in vivo Stabilisierung der Lysosomen den antileukämischen Effekt dieses Zytostatikums unterstützen könnte.

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Cholesterol Oxidation by Rat Liver Preparations: Effect of Age

Cholesterol synthesis is significantly reduced in aging rats[1–3]. YAMAMOTO and YAMAMURA[4] confirmed the fact that 5- or 8-month-old rats convert less acetate to cholesterol than do 2-month-old rats. They also reported that the older rats excreted fewer acidic steroids in their bile and feces. We have compared the activity of the two hepatic enzyme systems involved in bile acid synthesis from cholesterol in young (2 month) and old (18 month) rats.

The rats used were males of the Wistar strain. The two enzyme systems assayed were the mitochondrial system described by WHITEHOUSE, STAPLE and GUREN[5] which is involved in the oxidation of the cholesterol side chain, the final step in bile acid synthesis[6]; and the microsomal 7α-hydroxylase, the first and rate-limiting step in bile acid synthesis from cholesterol[7].

The oxidation of [26-^{14}C] cholesterol to $^{14}CO_2$ was carried out according to published procedures[5,8]. Incubations were carried out in stoppered 125 ml Erlenmeyer flasks containing center wells. The incubation mixture consisted of 1 ml of mitochondrial preparation; 1 ml of a solution containing adenosine triphosphate

Influence of age on cholesterol oxidation by rat liver preparations[a]

	Age	
	Young (2 months)	Old (18 months)
Oxidation of [26-^{14}C] cholesterol to $^{14}CO_2$ (*p*moles/mg N)		
Cytosol present	6.65 ± 1.71	2.03 ± 0.73[a]
Cytosol absent	0.92 ± 0.11	0.37 ± 0.13[b]
7α-Hydroxylation of [1,2-^{3}H] cholesterol		
*p*moles/liver	401.9 ± 37.1	1068.7 ± 204.5[a]
*p*moles/mg/min	7.08 ± 0.77	4.83 ± 0.84[c]

[a] Average of 6 experiments ± S. E.; [a] $p < 0.05$. [b] $p < 0.01$; [c] $p < 0.10$.

(ATP, 25 mg), nicotinamide adenine dinucleotide (NAD, 5 mg), adenosine monophosphate (AMP, 8 mg), reduced glutathione (15 mg), sodium citrate monohydrate (30 mg), magnesium nitrate hexahydrate (10 mg), potassium penicillin G (2000 units) and streptomycin sulfate (1 mg); 5 ml of labeled substrate in 0.25 *M tris* (hydroxymethyl) aminomethane HCl, pH 8.5; and 5 ml of boiled supernatant.

Incubations were carried out at 37 °C for 18 h. At the end of this period 2.5 ml of a 1 *M* methanolic solution of Hyamine 10X (*p*-diisobutyl-cresoxyethoxyethyl) dimethylbenzylammonium hydroxide was injected into the center well. The solution was acidified with 1 *N* H_2SO_4 (2.5 ml) and the flasks were shaken for 3 h at 37 °C to displace $^{14}CO_2$. The Hyamine solution was removed from the center well and a sample was taken for radioactive assay by liquid scintillation spectrometry.

The assay of cholesterol 7α-hydroxylation was carried out using the method described by SHEFER et al.[9] Incubation was carried out in a 25 ml Erlenmeyer flask containing [1,2-^{3}H] cholesterol (0.5 μmole, 8.25×10^5 cpm/μmole) solubilized with 6.7 mg Tween 20; potassium phosphate buffer, pH 7.4, 0.167 μmole; $MgCl_2$, 11 μmoles; $NADP^+$, 3.0 μmoles; glucose-6-phosphate, 6.0 μmoles; glucose-6-phosphate dehydrogenase, 1 IU; and 1 ml microsomal suspension. The final volume was 2.3 ml. Incubation was carried out at 37 °C with shaking. The reaction was stopped by the addition of 7.5 ml methylene dichloride-ethanol (5:1) to a 0.5 ml aliquot of the reaction mixture. Steroids were separated by thin layer chromatography on silica gel G with ethyl acetate-hexane (8:2). The bands were visualized with iodine vapor, scraped from the plates, and assayed for radioactivity by liquid scintillation spectrometry.

All radioactive substrates were purchased from New England Nuclear Corporation, Boston, Mass., and the cholesterol was purified by thin layer chromatography prior to use.

Our results are presented in the Table. It is evident that particulate preparations of young rat liver are significantly more active than those from older rats. The oxidation of [26-^{14}C] cholesterol to $^{14}CO_2$ by mitochondrial preparations from young rat livers is greater in either the presence or absence of the boiled cytosol. In the presence of cytosol the extent of oxidation by young rat liver mitochondria is 3.3 times that of old and in the absence of cytosol it is 2.5 times greater. The data suggest little role for the cytosol in the decrease in oxidative activity. Cholesterol hydroxylation is greater in old rats when calculated on the basis of total liver weight, but when compared on a rate basis (*p*moles hydroxylated/mg liver/min) the hydroxylation is 1.5 times more rapid in young rat liver preparations. These experiments extend the findings of earlier workers who reported diminution of other phases of cholesterol metabolism in aging rats. The decreased excretion of bile acids[4] has been shown to be mediated at the level of both 7α-hydroxylation and side chain oxidation of cholesterol[10].

Résumé. On a constaté que les vieux rats synthétisent moins de cholestérine et qu'ils excrètent moins de stéroides acides que les jeunes. Nous avons comparé l'activité de deux systèmes d'enzymes hépatiques chez les jeunes et les vieux rats. Ces systèmes sont la préparation microsomale qui comprend l'activité de la cholestérine 7α-hydroxylase, et la préparation mitochondrique qui parvient à oxyder [26-^{14}C] de cholestérine à $^{14}CO_2$. Les deux systèmes qui entrent en jeu dans la synthèse des acides de la bile sont significativement plus actifs dans le foie des jeunes rats.

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