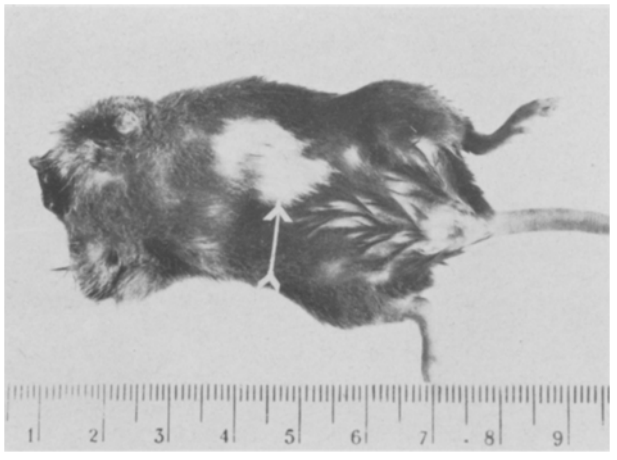

Depigmented area (arrow) in a C57BL female mouse killed 23 months after the first and 21½ months after the last of 5 doses (0.1–0.2 ml) of calcium pantothenate (80–100 mg/ml) injected i.p. into this area.

In recent experiments, T-2 toxin (3α-hydroxy-4β, 15-diacetoxy-8α-(3-methylbutyryloxy)-12,13-epoxy-Δ^9-trichothecene) a secondary metabolite of *Fusarium sporotrichioides* and of certain other species of Fusarium, proved to resemble N-methyl-N-nitrosourethane in being carcinogenic for the digestive tract in rats[3]; and also in inducing depigmentation when applied to the skin of dark mice. Solutions of T-2 toxin, containing 0.2–0.3 mg/ml in 10% aqueous ethanol, when applied to the clipped intrascapular region of C57BL mice (received from the MRC Animal Laboratory Centre, Carshalton, Surrey) caused local irritation, hyperaemia, oedema, ulceration and scab formation; when the scab fell off the healed area remained depilated and in some animals was surrounded by areas of depigmented hair. The necrotising effect of T-2 toxin is caused evidently by higher concentrations than the depigmentation, this may be due to the higher sensitivity of the biochemical reactions involved in the formation of melanins, than those responsible for cell survival.

It has usually been considered that pantothenic acid is an antigreying factor. However, when some of the T-2 toxin-treated mice were concurrently given aqueous solutions of calcium pantothenate, 80–100 mg/ml, by i.p. injections (0.1–0.2 ml/mouse), this did not prevent the local effects of T-2 toxin; moreover at the site of the i.p. injections of calcium pantothenate ulceration sometimes occurred, and after healing the hair in this area became permanently depigmented (as shown in the figure). Similar depigmentation of the hair was seen in mice which were given only calcium pantothenate, by i.p. injections, and no T-2 toxin.

Calcium pantothenate might possibly interract with thiol compounds (and form CoA?); depletion of the intracellular thiols may also be the result of treatment with T-2 toxin, which contains an epoxide ring in its structure. However, further experiments are needed in order to evaluate quantitatively the relation of the agents causing depigmentation, to the various intracellular thiol species, the role of CoA and of other biochemical parameters, which may be involved in the process of melanin formation.

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Effect of food and light schedule on bile flow in the rat

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Summary. In the rat after food intake, whether during the dark or light period, bile flow increases. Food intake seems to be a major factor in the circadian rhythm of bile secretion.

Circadian rhythms have been described in rats for body weight, liver weight, hepatic cell regeneration[1], hepatic glycogen and glycogenolysis, various hepatic enzyme activities as well as enzyme induction[2–4]. Bile flow, biliary concentrations and excretory rates of bile salt, cholesterol and phospholipid also follow a circadian rhythm with a peak at midnight and nadir at noon for bile salts[5]. The bile salt non-dependent fraction of hepatic bile is maximal during the night and early morning and minimal at the end of the light period[6]. Rats eat during the night and sleep during day time. The aim of the present work was to study whether food or darkness were the main stimuli in bile flow increment.

Material and methods. 36 male Wistar rats were housed for 10 days before study in a dark room at constant room temperature and humidity. Rats were lighted with artificial light between 08.00 h and 20.00 h and fed a standard diet from 20.00 h to 08.00 h in group 1 (18 rats), and from 08.00 h to 20.00 h in group 2 (18 rats). Water was given ad libitum in both groups. In each group, experiments were performed either at 08.00 h (9 rats) or 17.00 h (9 rats). The animals were anesthetized with pentobarbital (Nembutal Abbott). Body temperature was maintained between 37.5 and 38.5 °C on heating tables. Renal pedicles were ligated and 5 μCi of ^{14}C erythritol (Amersham) was injected (0.1 ml) via the penis vein. 1 h later, the carotid artery and the bile duct were canulated. Basal bile flow was measured during the first 30 min following bile duct canulation. Arterial blood was sampled (100 μl) at each mid 10 min bile flow period. At the end of the experiment, liver and stomach were weighed. Radioactivity was measured on bile and plasma by liquid scintillation spectrometry (Nuclear Chicago). Quenching was determined by automatic external standardization. The comparison of means was performed with the Student t-test.

Results. Results concerning bile flow (Bf), b.wt, stomach weight (s.wt), liver weight (l.wt) and bile over plasma erythritol ratio (B/P) are given in the table. In group 1 at the end of the feeding period, that is to say in the morning, s.wt, l.wt and Bf whether expressed per 100 g b.wt^{-1} or per g l.wt^{-1} are significantly higher than at the end of the fasting period. In group 2 these values are significantly higher at the end of the feeding period, that is to say at 17.00 h. However Bf when expressed per g l.wt^{-1} is not significantly different. This is due to a very high increase in l.wt and can be appreciated by a significant increase in the l.wt/b.wt ratio. Erythritol B/P ratios are not significantly different in each group.

	Group 1 08.00 h (9 rats)	p values	Group 1 17.00 h (9 rats)	Group 2 08.00 h (9 rats)	p values	Group 2 17.00 h (9 rats)
Body weight (g)	229 ± 23	NS	218 ± 15	209 ± 17	NS	220 ± 21
Stomach weight (g)	9.6 ± 2.2	$p<0.001$	4.0 ± 1.6	3.2 ± 1.7	$p<0.001$	10.0 ± 2.0
Liver weight (g)	10.01 ± 1.48	$p<0.02$	8.76 ± 1.48	8.46 ± 1.22	$p<0.001$	10.89 ± 1.51
Liver weight/body weight	0.043 ± 0.004	NS	0.040 ± 0.006	0.041 ± 0.006	$p<0.01$	0.049 ± 0.007
Bile flow µl min^{-1} 100 g $b.wt^{-1}$	9.0 ± 1.5	$p<0.01$	7.5 ± 1.2	8.6 ± 1.0	$p<0.01$	9.6 ± 1.1
Bile flow µl min^{-1} g $l.wt^{-1}$	2.2 ± 0.5	$p<0.01$	1.8 ± 0.3	2.1 ± 0.4	NS	1.9 ± 0.3
Erythritol B/P ratio	1.14 ± 0.20	NS	1.18 ± 0.13	1.13 ± 0.22	NS	1.10 ± 0.14

* Values are means ± 1 SD.

Discussion. Normally rats eat during the night and sleep during the day[7]. When the food schedule is reversed they are perfectly able to eat during the light period, as shown in group 2 by the increase in s.wt, l.wt and l.wt/b.wt ratio at the end of the light period.

As shown before, bile flow, in the normal food and light schedule, is higher in the morning than in the evening whether expressed per 100 g $b.wt^{-1}$ or g $l.wt^{-1}$ [6]. When food is only available during the light period, bile flow is higher at the end of the feeding period but only when expressed per 100 g $b.wt^{-1}$. Therefore food seems to be a major stimulus for bile secretion. Rats do not have a gallbladder; therefore bile cannot be stored. The increment in bile flow was of canalicular origin as suggested by the constant B/P erythritol ratio. In these experiments we have not measured the bile salt secretory rate, therefore it is difficult to speculate whether the bile salt dependent or non-dependent fraction is responsible for the increment. The bile salt secretory rate follows a circadian rhythm[5] with a peak at midnight. The bile salt non-dependent fraction is also increased during the feeding period compared to the fasting period[6]. The reasons for the bile flow increment in relation to food are unknown. During digestion, hepatic blood flow increases. In the isolated perfused rat liver, bile flow was shown to be largely independent of hepatic blood flow[8]. Blood levels of several hormones rise during digestion. Insulin and glucagon increase the bile acid independent fraction[9,10]. Secretin increases mainly canalar bile flow when injected at very high dose via the portal vein or the hepatic artery[11]. Cholectystokinin seems to increase both canalicular and ductular bile flow[12]. Gastrin does not increase bile flow[12]. It is difficult to know whether those hormones play a physiological role.

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The influence of a new antitumor agent on hemopoietic colony forming cells

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Summary. The cytostatic and immunsuppressive agent N′-methyl-N′-β-chloroethylbenzaldehyde hydrazone (B1) in in-vitro experiments has a stimulating effect on colony-forming culture (CFUc) of bone marrow from C57BL mice. This unusual behaviour, which is in contrast to other cytostatics, could also be observed in vitro with CFUc obtained from mice treated with therapeutic doses of B1 for 2 weeks. This stimulation is not a particular effect of B1 alone but seems to depend on a synergistic effect of the combination of B1 and the colony-stimulating activity (CSA) present in the serum from endotoxin-treated mice (MP) in the testing system. The results suggest that the described effect of B1 is due to an interference at the cell membrane of CFUc or their precursor cells.

In previous papers we have reported that N′-methyl-N′-β-chloroethylbenzaldehyde hydrazone (B1), whose structure is similar to that of cytostatic mustards and methylhydrazine derivatives, has cytostatic[1,2] fungistatic[3,4] and powerful immunosuppressive[5] qualities. In mice (NMRI) a high dose of B1 (150 mg/kg) caused a depression of leucocytes which affected lymphocytes more strongly than the granulocytes. After 2 weeks with a daily dose of 65 mg/kg of B1, there was an unexpected increase in the number of neutrophils[6]. For this reason we studied the influence of B1 on the colony-forming cells (CFUc) of the bone marrow from C57BL mice in vitro and from mice which were treated with a daily dose of 15 mg/kg and 75 mg/kg for 2 weeks. Furthermore, the colony-forming capacity of bone marrow from mice treated with a single dose of 400 mg/kg B1 was tested.

Methods. In the in-vitro experiments, colony-forming cells (CFUc) were tested in an agar system described by Pluznik