Hydrolysis was effected by warming for a few min a solution of the ester in 30% sodium hydroxide solution containing ethanol for solubilization. Acidification gave Wy-14643 in 69% yield: m.p. 150–153°/ethyl acetate; analysis for $C_{14}H_{14}ClN_3O_2S$, Calc: C, 51.93; H, 4.36; N, 12.98. Found: C, 52.01; H, 4.41; N, 12.99.

A structure-activity study has been carried out and will be reported in greater detail in a subsequent paper.

Biological activity. The hypocholesterolemic response of Wy-14643, like that of clofibrate, was linear when plotted against log dose. The dose-response curves for both agents are shown in the Figure. Because of the non-parallel relationship between the two curves, relative potencies were dose dependent, e.g.

Dose (mg/rat/day)	Reduction of cholesterol (%)	Activity vs clofibrate
50	68	1.9×
1	50	60×
0.1	12	180×

A more extensive evaluation of this drug is currently in progress.

Zusammenfassung. Es wird die Synthese von [4-Chlor-6-(2, 3-xylidino)-2-pyrimidinylthio]-essigsäure und seine antihypercholesterolämische Aktivität beschrieben.

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Diurnal Rhythm of Choline-^{14}C Incorporation into Lecithin in Ehrlich-Lettré Ascites Tumour Cells

A diurnal rhythm, especially in the concentration of plasma[1] and liver fatty acids[2], in the level of serum triglycerides[3] and phospholipids[4], and in compounds other than lipids[5,6], has been observed in recent years. Generally, the diurnal rhythm can be looked upon as an expression of the fluctuation of the metabolic activity of a given organism. The present study describes such a rhythm for the choline-^{14}C incorporation into lecithin in Ehrlich-Lettré ascites tumour cells (EAT). A preliminary note has been presented elsewhere[7].

During the course of our studies on choline transport and its conversion into lecithin in ascites cells[8], we observed marked differences of ^{14}C-labelled choline incorporation into lecithin with a few hours time interval between killing the animals. Therefore, we began to follow up the ^{14}C-incorporation over a 24-h period in combination with a concomitant thymidine-^{3}H incorporation into DNA, since it has been argued that the mitotic index might also show a rhythmic pattern.

Methods. For these studies, the glycogen-free strain of the hyperdiploid Ehrlich-Lettré mouse ascites tumour 7 days after transplantation was used. On the day of inoculation, randomly selected mice were put in cages in groups of 10 animals. Lab chow diet was given ad libitum and water renewed at least every other day. In the case of choline-free diet, the feed was given as a slurry mixed with water, which was prepared fresh every day and given at 09.00. Every 3rd day the mice were put into fresh cages. The animals were kept under 12–14 h of daylight from 06.00 to 20.00 h without any artificial lighting or darkening treatment. Usually 3 animals were killed every 4 h for a period of 24 h and radioactivity

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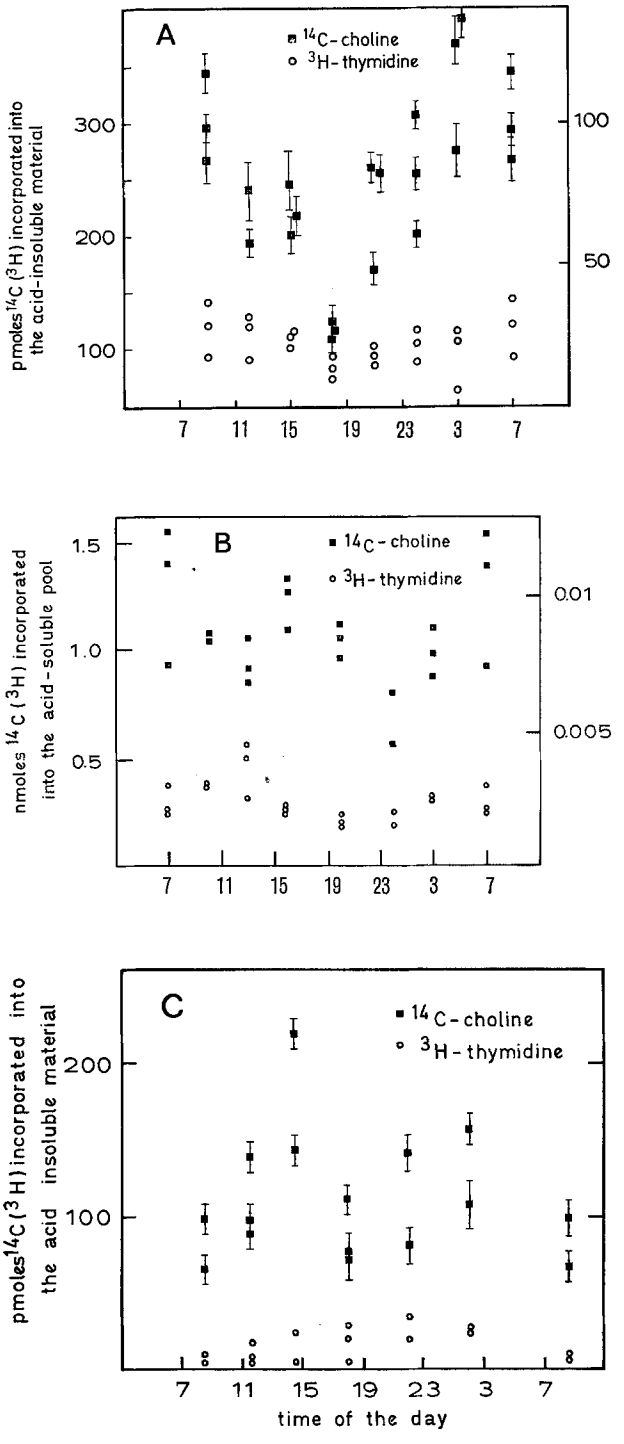
Table I. Analysis of total lipid phosphorus and of lecithin phosphorus after TLC-separation of an aliquot of the lipid extract corresponding to 2×10^6 cells

	Time of the day	Lipid phosphorus[a] (nmoles)	Lecithin phosphorus[a] (nmoles)	Difference probability for lecithin (P)
Experiment 1	24.00–09.00	164.1 ± 80.5	78.7 ± 28.1	< 0.01
	12.00–21.00	173.1 ± 78.0	49.1 ± 18.9	
Experiment 2	24.00–09.00	200.6 ± 29.8	75.4 ± 7.1	< 0.01
	12.00–21.00	214.0 ± 51.1	46.3 ± 23.4	

[a] Mean values plus SD from 12 (experiment 1) and 9 (experiment 2) animals for each time interval. Student's t-test gave t-values of 3.03 (experiment 1) and of 3.37 (experiment 2).



Simultaneous incorporation of 15 μM choline-^{14}C (0.25 μCi/6 nmoles) and of 0.5 μM thymidine-^{3}H (4 μCi/0.2 nmole) into the glycogen-free EAT cells beginning 7 days after transplantation. Every 4 h, at the times indicated on the graph, 3 animals were killed, the ascites cells collected, the ascites fluid removed by centrifugation and the cells incubated to 2×10^6 cells in 200 μl of Eagle's medium at 37 °C for 1 h after addition of the tracer compounds. The subsequent treatment, including the extraction procedure, is described under methods. A) shows the radioactivity distribution in pmoles of ^{14}C (■) and ^{3}H (○) in the acid-insoluble material; B) in the acid-soluble fraction and C) in the acid-insoluble material after a 24 h starvation treatment of the animals. (Scale on the left ordinate refers to ^{14}C-, and on the right ordinate to ^{3}H-radioactivity). A) Difference probability between the high phase 298.2 $\pm$ 85.6 pmoles (24.00–09.00 h) and the low phase 199.1 $\pm$ 54.3 pmoles (12.00–21.00 h) $P < 0.001$.

Table II. Mitotic rates of the glycogen-free EAT cells at various times of the day

Mouse No.	Clock time 09.00 h	15.00 h	21.00 h	03.00 h
1	18	22	21	26
2	—	17	16	17
3	23	24	20	26
4	29	19	24	16
5	19	—	21	23
Average value	23	21	20	22

The tumours were obtained from male albino mice 7 to 8 days after transplantation. The data represent average values obtained by counting 1000 cells per mouse.

incorporation was followed by incubating 15 μM choline-^{14}C (0.25 μCi/6 nmole), 0.5 μM thymidine-^{3}H (4 μCi/0.2 nmole), and 2×10^6 cells at 37 °C for 1 h in a total volume of 200 μl of Eagle's medium, pH 7.4. The cells were collected by centrifugation, the upper phase removed, the cells washed once with Ringer solution, pH 7.4 and treated with 5% TCA to obtain the acid-soluble and insoluble material. ^{14}C- and ^{3}H incorporation was followed using a Packard Tricarb liquid scintillation counter equipped with Model 544 Absolute Activity Analyzer. Analysis of total phosphorus and lecithin phosphorus was performed by the BARTLETT[9] procedure after separating the latter on Merck silica gel plates using Chloroform-Methanol-water (60-30-4) as solvent system. The mitotic rates were determined by counting the number of mitotic phases per 1000 cells which had been stained with Hemalaun.

Results and discussion. The incorporation of choline-^{14}C and thymidine-^{3}H into the acid-insoluble material of EAT in relation to the time of day, is shown in Figure A. Each time of killing the animals is represented by 3 points including standard deviations for the choline incorporation. As can be seen, the ^{14}C activity decreased from 07.00 h with a minimum at 18.00 h, and then increased again. No significant changes of thymidine incorporation into the acid-insoluble material were observed. Similar results were obtained when the mice were put on a choline-deficient diet on the day of inoculation, indicating that this diet does not seem to play a role in the rhythm[5], and that the choline requirement of the cell must have been fulfilled by some other means, probably through methylation of ethanolamine. When the ^{14}C (^{3}H) distribution was measured in the intracellular soluble pool (Figure B), no rhythmic pattern was found. From studies in our laboratory[8] it was observed that choline at 15 μM concentrations is incorporated into the cell via a transport reaction. Figure C shows the ^{14}C and ^{3}H incorporation in the acid-insoluble material after the animals had been kept without food for 24 h. As can be seen, there was no rhythmic pattern of choline incorporation into lecithin, suggesting that food ingestion by the mice might be involved in this phenomenon (see also ref. [3]). The tracer studies are supported by the data on the extractable amounts of lecithin present in 2×10^6 cells over the 24 h period (Table I). Whereas total lipid phosphorus exhibits only insignificant variations, the values on lecithin phosphorus show significant differences between the high (24.00–09.00 h), and the low (12.00–21.00 h) phase. It might also be pointed out that the fluctuations (100 to

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300 pmoles) measured by the tracer studies represent only a small portion of the changes within the entire lecithin fraction (46 to 93 nmoles) suggesting a much higher lecithin metabolism under in vivo conditions. As Figures A and C indicates, no rhythmic changes were found for the thymidine incorporation into DNA. These results are also in accordance with the mitotic rates shown in Table II.

[10] Acknowledgments. The author wishes to thank Dr. C. GRANZOW from this institute for providing the data on the mitotic index. The excellent technical assistance of O. GILCH is greatly appreciated.

Zusammenfassung. Es wird über den diurnalen Rhythmus des Cholin-Einbaues sowie des Gesamt-Lecithin-Gehaltes des glykogenfreien Ehrlich-Lettré Mäuse-Ascites-Tumors berichtet. 24stündige Hungerbehandlung der Mäuse löscht diesen Effekt, während cholinfreie Diät keinen Einfluss auf den rhythmischen Verlauf hat. Thymidin-Einbau in DNA und Mitoserate zeigen keinen diurnalen Rhythmus.

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Fibrinolytic Inhibitors in Human Seminal Plasma

The fibrinolytic activity in the blood and body fluids is a result of their concentration of fibrinolytic enzymes and inhibitors respectively. Seminal plasma possesses a high fibrinolytic activity[1]. Activators of plasminogen have been demonstrated in this fluid[2–5]. Concerning the inhibitors of fibrinolysis it has been shown that seminal plasma contains inhibitors of trypsin[6–9], but extensive studies of the other fibrinolytic inhibitors are lacking. We therefore thought it legitimate to study seminal plasma with respect to its fibrinolytic activity and its content of all known inhibitors of such activity. The samples were examined also for antithrombin III and plasminogen.

Material and methods. The material consisted of 51 males, aged 18–41 years, from the Fertility Clinic, Malmö General Hospital. Their sperm counts were all within normal limits ($\geqslant$ 20 milj/ml). Semen samples were obtained after 3–5 days of sexual abstinence. 1 h after the sample had been delivered, it was centrifuged at 2,000 *g* for 15 min. The supernatant was then decanted and stored at −20 °C until analysed.

The fibrinolytic activity of the seminal fluid was determined on unheated and heated plates, and the results were expressed in mm² of lysis[10].

Plasminogen. Immunological method by GANROT and NILÉHN[11], as slightly modified by EKELUND et al.[12]. Blood collected with epsilon-aminocaproic acid (EACA). Inhibitors of plasminogen activation by urokinase (urokinase inhibitors). Clot method[13].

Antiplasmin. Caseinolytic method by SHAMASH and RIMON[14], as modified by EKELUND et al.[12]. α_2-macroglobulin. Esterolytic method[15]. Total antitrypsin activity (TAT). Esterolytic method[16]. α_1-antitrypsin was determined immunologically. Rocket method by LAURELL[17].

Fibrin/fibrinogen degradation products (FDP) were determined according to the immunochemical method by NILÉHN[18] in which LAURELL's rocket method[17] was used. EACA[19] and thrombin was added. Total protein was determined by the method of KJELDAHL. The inhibitors of which there were hardly detectable amounts, were checked by OUCHTERLONY technique[20].

Results and discussion. The results are given in the Table. The total protein content ranged from 3.3 to 5.9 g/100 ml. Human seminal plasma has an inhibitory effect on the fibrinolytic activity of trypsin[4, 6, 8, 9, 21]. These trypsin inhibitors have been thought to inhibit sperm acrosomal proteases[22–24]. In the present study we found the concentration of inhibitors of trypsin to be low. They cannot inhibit the fibrinolytic activity of seminal plasma to any appreciable extent, but they might be sufficient to have a possible effect on sperm acrosomal proteases.

Plasmin inhibition by human seminal plasma has been found by HAENDLE et al.[6], but could not be confirmed by HIRSCHHÄUSER and KIONKE[9]. We found only an exceedingly small inhibitory effect of seminal plasma on plasmin. The finding of low concentrations of α_2-macroglobulin and α_1-antitrypsin had no demonstrable effect in our caseinolytic antiplasmin test.

As far as we know, no earlier investigations are available on the inhibitory effect of seminal plasma on plasminogen activation. In this study we found the inhibitors of urokinase-induced plasminogen activation to be pres-

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