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.

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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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Mean values of fibrinolytic components in human seminal plasma (percent of the content of a normal standard consisting of pooled serum from 20 healthy persons)

	No. of samples	Mean ± SD
Fibrinolytic activity on unheated fibrin plates (mm^2)	51	1016 ± 765
Fibrinolytic activity on heated fibrin plates (mm^2)	51	0
Plasminogen (%)	20	traces
Urokinase inhibitors (%)	42	25 ± 10
α_2-macroglobulin (%)	30	15 ± 8
Antiplasmin (%)	20	traces
α_1-antitrypsin (%)	18	1.3 ± 0.8
Total antitrypsin activity (%)	38	8 ± 13
Fibrin/fibrinogen degradation products (µg/ml)	51	0

ent in only low concentrations. The fibrinolytic activity on unheated fibrin plates was high, and no activity was found on heated plates, in agreement with earlier observations[3,4,25]. Hence, the high fibrinolytic activity of human seminal plasma seems to be due to the presence of large amounts of fibrinolytic activators in association with only a low concentration of fibrinolytic inhibitors.

Zusammenfassung. Die menschliche Samenflüssigkeit wurde auf ihre fibrinolytische Aktivität und auf ihre Konzentration von Inhibitoren der Plasminogenaktivierung (Urokinaseinhibitoren), Antiplasmin, α_2-Macroglobulin, α_1-Antitrypsin, totale Antitrypsinaktivität sowie Plasminogen und Antithrombin-III untersucht. Dabei wurden niedrige Konzentrationen der Inhibitoren, die auf die hohe fibrinolytische Aktivität des Seminalplasmas nur eine geringe Hemmwirkung ausüben können, gefunden.

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A Carbohydrate Inhibitor of Cathepsin B Activity Associated with Haptoglobin

Human plasma contains at least 6 different well characterized proteinase inhibitors, recently reviewed by HEIMBURGER, HAUPT and SCHWICK[1]. They called attention to the high content of carbohydrate residues of these inhibitors, mainly hexoses, acetylhexosamines and sialic acid, and they further stressed the unknown role of these components as far as enzymic inhibition was concerned. Turning to the lysosomal cathepsin B1 we reported in 1967 that haptoglobin purified from Cohn's fraction IV-4 showed a marked inhibition of cathepsin B activity[2]. The question was already at that time left open if this was due to haptoglobin as such or an associated component. Data presented below suggest that haptoglobin preparations seem to carry a glucosamine-containing saccharide which has all the proteinase inhibitory activity found in haptoglobin.

Haptoglobin was prepared from clear yellow ascites fluid sampled from mice carrying the Ehrlich-Landschütz tumor (15–20 days after i.p. inoculation of 2×10^6 cells) by means of an acetone precipitation method followed by column chromatography on Sephadex G-200, equilibrated with 0.05 *M* phosphate buffer pH 7.0 and with 0.1 *M* NaCl added. The absorbance at 280 nm was measured on the eluate as well as the inhibitory effect on samples of pure cathepsin B1. The inhibition assays were performed according to the same system as used for activity measurements of the enzyme[3]. The substrate was benzoylarginine-2-naphthylamide (BANA)/ 0.4 mg/ml / in a 0.05 *M* phosphate buffer pH 6.0 containg 5 m*M* EDTA + 4 m*M* cysteine HCl for activation. Incubations were performed for 30 min at 40 °C. The reaction was stopped by added TCA and the released naphtylamine was diazotized and assayed at E_{560} as usual.

The dissociation of the cathepsin B inhibitor from the haptoglobin preparation was shown in the following way. Starting with a purified haptoglobin solution, the total inhibiting effect contained in the solution was not changed following the addition of an excess of haemoglobin. However, when this solution was analyzed on a Sephadex G-200 column at pH 6.0 the well-defined haptoglobin peak had disappeared. A new component appeared near the elution front (Figure, tube No. 16–22) representing the haemoglobin-haptoglobin complex, and this was lacking all cathepsin inhibitor activity. All the inhibitor activity previously associated with the original haptoglobin moiety, appeared instead later at the end of the elution (Figure, tube No. 30–40); This new component

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