ASP-I (I) shows the similar mobility of TLC as holothurin A, and forms prisms from acetone, mp 181–185°, and crystalline powder from ethanol, mp 200–205° (decomp.). The IR-spectrum displays its glycosidic nature [intense absorptions at 3400 cm^{-1} (OH), and 1050 cm^{-1} C-O], and the presence of a sulphate group (1220 cm^{-1}). Elementary analysis gave C, 54.02%; H, 8.29% and S, 2.06%. Assuming the presence of 1 sulphate group in the molecule, the molecular weight, ca. 1500, was speculated. The substance is a sodium salt of a sulphate ester, and upon treatment with cold HCl, it liberated a free sulphate compound (II), needles mp 105–110° (decomp.), KBr_{max} 3400 (OH), 1200 cm^{-1} (sulphate), which showed the same mobility on partition type TLC as I. I was hydrolyzed with 2*N* HCl at 80° for 2 h to the aglycone and sugar fraction. Examination of the sugar fraction with various solvent systems shows that it consists mainly of one kind of sugar (III) which has a very similar Rf value to 3-*O*-methylglucose, and a trace of quinovose.

The aglycone (IV), needles (from MeOH and water), mp 202–210° shows strong absorptions for hydroxyl groups at 3400 cm^{-1} and lacks absorptions for carbonyl groups in the IR-spectrum. High resolution mass spectrum of IV gave the molecular ion at *m/e* 434.33852, from which the molecular formula $C_{27}H_{46}O_4$ (Calcd. 434.33961) was computed. The NMR-spectrum showed a methyl signal pattern similar to that of cholesterol (a composite of methyl signals around 9.10 and a signal for 18-methyl group at 9.35)[6]. In the mass spectrum, peaks arisen by dehydration of up to 4 molecules of water and several demethylated fragments were observed. On the basis of these data, ASP-I is considered to be a hydroxylated cholestene derivative conjugated with 5–6 sugar molecules and a sulphate group probably on the sugar part. Recently, MACKIE et al.[7] also have reported a cholestene derivative as a constituent of the avoidance substance excreted by starfish, *Marthasterias glacialis*. The aglycones of holothurins from sea-cucumbers are known to be triterpenoids having lanostane skeleton[8]. YASUMOTO and HASHIMOTO reported asterogenin I and II for the aglycones of Asterosaponins from *Asterias amurensis*. The same empirical formula, $C_{22}H_{34}O_3$, was postulated for both aglycones[9]. They also claimed the steroidal nature of the compounds from color reactions. The variation of the aglycones in the same phylum Echinodermata arouses considerable interst[10].

Zusammenfassung. Eine Gruppe von teilweise chemisch charakterisierten Verbindungen, welche die Vermehrung des Influenza-Virus in Huhnembryo-Test verhindern, wurde aus den Seesternen *Asterias forbesi*, *Asterias pectinifera* und *Acanthaster planci* isoliert.

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Effect of Cerium on Drug Metabolizing Activity in Rat Liver

Earlier investigations have shown that after i.v. injection of light lanthanons, including cerium, there are changes in the liver endoplasmic reticulum, which manifest primarily as dilatations of the cisternae and dissociation of ribosomes[1].

The liver microsomes, which consist of the fragments of the endoplasmic reticulum in vitro, contain enzymes which detoxicate foreign compounds and metabolize drugs. We were interested in finding out if and to what extent the morphological changes induced by cerium injection are accompanied by an impairment of the drug metabolizing capacity of rat liver.

Material and methods. In the present study, male Sprague-Dawley rats weighing 180–270 g were used. They were obtained from Orion Oy, Finland. The animals were given normal laboratory food and water ad libitum. Following a single i.v. injection of 2 mg cerium/kg of body weight as chloride in physiological saline solution of pH 3.5–4.0, the rats were decapitated after 1, 2, 3, 6 and 16 days always at the same time of the day.

The 20% liver homogenates were prepared in 1.15% ice-cold KCl with a motor driven Potter-Elvehjem type homogenizer. The 15,000 × *g* supernatant was used as enzyme preparation. The microsome fraction for cytochrome P-450 determinations was obtained by centrifuging the supernatant at 105,000 × *g* for 1 h and resuspending the pellet in 0.1 *M* phosphate buffer, pH 7.4.

The incubation mixtures and other procedures for determining the activities of oxidative enzymes, which hydroxylate hexobarbital and demethylate N-methylaniline, were similar to those previously described[2]. Cytochrome P-450 content was determined according to the method described by OMURA and SATO[3]. The blood glucose was determined by the glucose-oxidase method and the free fatty acids were titrated according to TROUT et al.[4]. Protein concentrations were assayed by the biuret procedure. Student's *t*-test was used in calculating the significance of the results.

Results. The cerium treated animals behaved normally. No differences in food or water consumption or in weight increase could be seen, compared with controls. Macroscopically the livers of cerium treated animals showed a typical occurrence of fatty degeneration already on the first day after injection.

In vitro studies demonstrated that both hexobarbital and N-methylaniline metabolism in liver had already decreased significantly on the 1st day after cerium injection. On the 2nd day these metabolic rates declined further and the lowest levels were reached on the 3rd day after injection when the hexobarbital oxidation and

N-methylaniline demethylation activities were 31% and 47% respectively from the corresponding control values. After 6 days only the hexobarbital metabolism was still at a significantly lower level, but both enzyme activities had completely normalized within 16 days (Table).

The cytochrome P-450 content of the liver was determined only on the 3rd day after cerium injection when the enzyme activities were at lowest level. Its content had fallen to 53% of the corresponding control level.

The fate of blood glucose was similar to that of the enzyme activities. It was already significantly lower on the 1st day and had declined to 52% of the corresponding controls on the 3rd day after injection. On the 6th day, the control level was again reached (Figure).

On the 2nd day after cerium injection, there was a sudden increase of 252% in the plasma free fatty acid level. This high level had subsided on the 3rd day and the value remained at control level throughout the observation period (Figure).

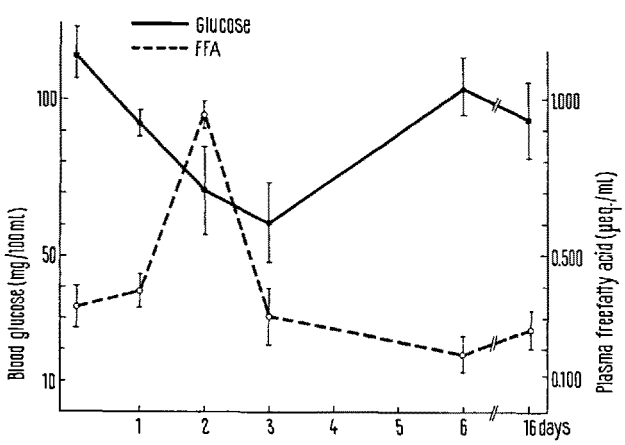
In the control animals, the mean protein content of the liver was 100 mg/g of tissue. In the cerium treated animals the protein content was measured 1, 2, 3, 6 and 16 days after injection, but no significant changes were observed in any of those instances.

Discussion. Cerium dose was chosen according to earlier morphological studies[1] and our pilot experiments, which showed that 2 mg of cerium/kg was an effective sublethal dose for rats. Our studies show that, after a single i.v. injection of 2 mg cerium/kg, there is a marked decrease in the rates of oxidative hexobarbital and N-methylaniline metabolisms in liver tissue.

Effect of cerium on drug metabolizing capacity of rat liver

Days after Ce-inj.	No. of animals	μmol metabolized/1 h/g tissue Hexobarbital	p	N-Methylaniline	p
Control	13	4.42 ± 0.55		0.280 ± 0.028	
1 day	6	2.95 ± 0.72	< 0.001	0.232 ± 0.035	< 0.01
2 day	7	2.04 ± 0.65	< 0.001	0.210 ± 0.029	< 0.001
3 day	7	1.38 ± 0.38	< 0.001	0.159 ± 0.024	< 0.001
6 day	7	1.95 ± 0.38	< 0.001	0.214 ± 0.094	N.S.
16 day	7	4.17 ± 0.55	N.S.	0.27 ± 0.011	N.S.

Rats were given i.v. 2 mg of cerium/kg of body weight. The mean values ± S.D. of hexobarbital oxidation and N-methylaniline demethylation activities are given as μmoles/h/g of tissue.



Effect of cerium on blood glucose and plasma free fatty acid levels in rat. Animals were given i.v. 2 mg of cerium/kg of body weight. Vertical bars represent standard deviations of the means.

The low level of cytochrome P-450, which acts as oxygen-activating enzyme, reflects the impairment of the whole drug oxidating capacity of the liver. In which way cerium impairs drug metabolism is not clear. According to SNYDER et al.[5], 70% of the injected dose is taken up by liver tissue where it is retained for at least 4 h and up to 12 days. Why most toxic symptoms have disappeared in 6 days, although cerium concentration in the liver is still high, remains obscure.

The properties of cytochrome P-450 depend largely on its association with microsomal phospholipids[6]. A parallelism has also been observed between the drug-induced enhancement of the activity of microsomal enzymes and an increase in the microsomal phospholipid content[7]. Because the rare earths are known to react especially with phospholipids, it may be postulated that cerium impairs the oxidative drug metabolism through interaction with these functionally important components.

Another possible explanation for the effect of cerium could be its antagonism with some divalent cations[8]. It has been shown that Mg- and Ca-ions enhance the metabolism of some drugs by hepatic microsomal enzymes in vitro[9]. Lanthanons, on the other hand, are able to antagonize the Ca^{++} induced activation of respiratory chains in mitochondria possibly by affecting the membrane permeability[10]. Accordingly, it seems likely that cerium may in the same manner inhibit the oxidative metabolism in liver microsomes.

Our findings concerning the blood glucose and free fatty acid levels in plasma are in good accordance with the earlier studies made with female rats[5]. In many investigations the male rats have been more resistant against the cerium-induced changes in lipid metabolism than we have found in our studies. It has been shown that this inverse relationship between blood glucose and plasma free fatty acid level can be prevented by conditions similar to those which prevent the cerium induced fatty liver[10–12].

Zusammenfassung. Es wird gezeigt, dass i.v. injiziertes Cerium (2 mg/kg) eine hemmende Wirkung auf den Stoffwechsel von Arzneien in der Rattenleber hat.

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