after the embryonic differentiation of the pituitary structure is completed. It is probable, therefore, that the time of its appearance in blood coincides with the process of maturation of the hypothalamo-hypophysial system.

Till now an attempt to characterize the physiological role of the enzyme has been undertaken only in the work on plasma cystine aminopeptidase activity during oviposition in the hen[13]. A great decrease in aminopeptidase activity (from 30 min before to 1 h after oviposition) has been found. Similarly, periodical changes in the hormonal content of the neurohypophysis of hens during the laying cycle were observed[14,15]. It has been concluded that neurohormones together with the enzymatic inactivating system affect the process of oviposition, i.e. the decrease of aminopeptidase activity in the plasma makes possible an influence of the neurohormones on the process[13].

According to the facts reported in literature, and to the results obtained in the present experiment, it may be suggested that in the chick there is a functional relationship between oxytocin and its inactivating enzyme similar to the oxytocin-oxytocinase system found in the pregnant women.

Zusammenfassung. In Hühnerembryonen wurde der Blutplasmaspiegel der Cystinaminopeptidase (Oxytocinase) bestimmt. Das Ferment konnte erst nach 19 Tagen Inkubationszeit nachgewiesen werden. Die Oxytocinasemenge steigt mit dem Alter der Embryonen an und erreicht nach 3 Tagen die Blutplasmawerte der adulten Hühner.

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Disturbed Release of Lipoprotein from Ethanol-Induced Fatty Liver

In some kinds of fatty liver, disturbed lipid release from the liver was observed as a result of impaired lipoprotein synthesis[1-3]. Concerning ethanol-induced fatty liver, SEAKINS et al.[4] reported that the incorporation of ^{14}C-leucine into liver and plasma proteins, including low and high density lipoprotein, was not affected in vivo. On the basis of hepatic perfusion studies, ISSELBACHER et al.[5] obtained the data suggesting that ethanol impaired the release of lipoprotein from the liver. This communication reports evidence to support the theory of disturbance of the release of lipoprotein from ethanol-induced fatty liver.

Albino rats of Wistar strain, maintained on standard laboratory chow, were used. Female rats weighing about 150 g were given ethanol (6.0 g/kg body weight) as a 50% solution or an isocaloric amount of glucose by stomach tube after 8 h fast. The animals were killed under light ether anesthesia by exsanguination from the heart at 8 or 16 h after the treatment. The livers were rapidly removed and chilled. From a portion of the liver, slices were prepared for the incorporation study.

The rat liver slices (500 mg) were incubated with 10 μC of ^{14}C-lysine for 2 h in 5.0 ml of the incubation medium[6] at 37°C under an atmosphere of 95% of O_2 and 5% of CO_2. The incubated slices were homogenized with the incubation medium using a glass homogenizer. The homogenate was centrifuged at 12,000 *g* for 10 min at 0°C to remove mitochondria and larger cell fragments. The supernatant was further centrifuged at 105,000 *g* for 60 min at 0°C to obtain the microsomal fraction, which was washed twice with iced 0.15 *M* NaCl.

From a portion of the microsomal fraction, the proteins were precipitated with 5% perchloric acid. The other portion of microsome was treated to 3 times freezing and thawing to obtain microsomal proteins[7]. From the eluted protein fraction of microsome, the albumin and high density lipoprotein (HDL) were isolated by a precipitation with the specific antiserum. To lessen coprecipitation of non-specific proteins and other labelled materials, all samples were previously treated with egg albumin and anti-egg albumin serum[7-9]. For HDL isolation, 0.2 ml of 10 times diluted pooled normal rat serum was added as carrier protein followed by the precipitation with the anti-rat HDL serum. The specific radioactivity of each isolated protein was measured[10].

The specific antisera against rat serum albumin and HDL were obtained from the rabbits immunized with the antigens isolated from freshly pooled rat serum[9]. The antiserum against low density lipoprotein (LDL) was also prepared, but the specificity of anti-LDL serum was not sufficient, probably due to the incomplete isolation of LDL from rat serum.

The liver lipid content was determined as described by FILLIOS et al.[11], the concentration of serum total lipids by DE LA HUERGA et al.[12], serum cholesterol by SEARCY et al.[13], serum HDL by quantitative precipitin reaction[14] and serum albumin by electrophoresis on cellulose acetate film.

Table I summarizes the liver lipid content and the result of incorporation study. Single oral administration of ethanol induced lipid deposition in the liver after 8 h of

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Table I. Effect of ethanol on lipid content and protein synthesis in the liver[a]

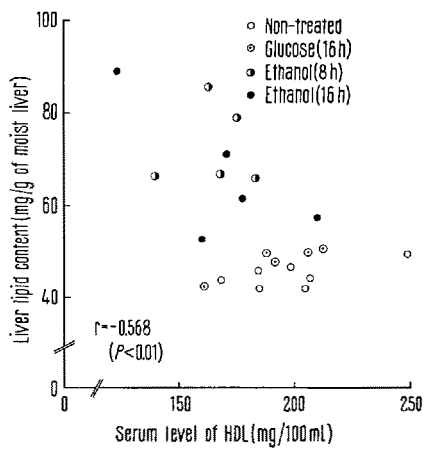
Treatment (time after treatment)	No. of animals	Lipid content (mg/g of moist liver)	Incorporation of ^{14}C-lysine		
			Total microsomal protein	Albumin	HDL[c]
			(dpm × 10^{-2}/mg of protein)		
Glucose (16 h)	5	47.3 ± 3.4	346 ± 71	4737 ± 1228	25.8 ± 5.6
Ethanol (8 h)	5	72.4 ± 8.7[b]	330 ± 69	3607 ± 653	30.9 ± 7.5
Ethanol (16 h)	5	66.0 ± 14.4	311 ± 53	3638 ± 1144	30.6 ± 5.0
Non-treated	7	44.3 ± 2.7	303 ± 59	4200 ± 1449	24.4 ± 7.9

[a] Values are expressed as mean ± standard deviation. [b] The difference to glucose group is statistically significant ($P < 0.05$). [c] Carrier rat serum was added for isolation and the specific activity was expressed as dpm × 10^{-2}/mg of immune precipitate.

Table II. Effect of ethanol on serum lipids and proteins[a]

Treatment (time after treatment)	No. of animals	Total lipids (mg/100 ml)	Cholesterol (mg/100 ml)	HDL[c] (mg/100 ml)	Albumin) (g/100 ml
Glucose (16 h)	5	390 ± 33	90 ± 16	192 ± 17	3.4 ± 0.2
Ethanol (8 h)	5	407 ± 53	87 ± 18	164 ± 36	3.6 ± 0.1
Ethanol (16 h)	5	316 ± 59[b]	78 ± 17	165 ± 28	3.3 ± 0.2
Non-treated	7	...	92 ± 45	195 ± 45	3.4 ± 0.2

[a] Values are expressed as mean ± standard deviation. [b] The difference to glucose group is statistically significant ($P < 0.05$). [c] Protein moiety.



Correlation between liver lipid content and serum HDL levels.

the treatment but not significantly after 16 h. The specific activity of each protein fraction, however, did not change significantly from those of control animals fed with glucose or non-treated rats. The specific activity of the free amino acids in the cell sap and medium in glucose (16 h)-, ethanol (8 h)- and ethanol (16 h)-treated rats were 1475 ± 138, 1568 ± 227 and 1492 ± 289 dpm × 10^{-2}/μmole amino acid, respectively (mean of 5 experiments ± standard deviation).

Effect of ethanol treatment on the serum level of lipids and proteins are shown in Table II. After ethanol administration the concentration of cholesterol, HDL and albumin did not change significantly but the concentration of total lipids decreased after 16 h of ethanol feeding.

Triglycerides are considered to be released from the liver as a form of very low density lipoprotein (VLDL). In man, 3 different proteins are found in the protein moiety of VLDL[15] and they are generally designated as A (or α), B (or β) and C protein. The A protein is the only protein found in HDL and the B protein in LDL. Studies on patients with abetalipoproteinemia demonstrated that B protein probably has a function in transport of glyceride from cells, and patients with α lipoprotein deficiency provided the data that A protein is not essential in the transport of glyceride[16,17]. In rat serum, however, LDL occupies only the smaller portion of lipoprotein and HDL occupies the part about 3 times of LDL[9,18], suggesting some participation of HDL in lipid metabolism.

Since triglycerides are released from the liver in the form of lipoprotein, impaired hepatic lipoprotein synthesis must be one of the pathogenic factors for the development of fatty liver. In ethanol-induced fatty liver, however, no decreased lipoprotein synthesis was obserbed in our experiment and by others[4]. The Figure shows that the serum level of HDL was inversely proportional to the liver lipid content. It was suggested by these data that release of HDL from the liver was disturbed by ethanol-feeding, having no relation to HDL synthesis.

Zusammenfassung. Nachweis, dass in der Ethanol-induzierten Fettleber der Ratte die «High Density Lipoprotein»-Synthese nicht beeinflusst und eine gestörte Freisetzung von Lipoproteinen aus der Leber angedeutet war.

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