An Attempt to Determine the Time of Cystine Aminopeptidase (Oxytocinase) Appearance in the Chick Embryo Plasma

Studies on the inactivating system of oxytocin in animals revealed the presence of cystine aminopeptidase in hen and cock serum[1,2]. This enzyme acts upon the specific substrate L-cystine-di-β-naphthylamide and in this respect is similar to the oxytocinase of pregnant women's serum. The oxytocin inactivating properties of enzyme was also confirmed by the biological method[2]. This finding is of great interest in view of the fact that the serum cystine aminopeptidase can be found only in pregnant women and anthropoid apes[3,4].

The pharmacological activities in the chicken neurohypophysis correspond to 2 active compounds: 8-arginine-oxytocin and oxytocin[5–7]. However, the concentration of oxytocin in the dried fowl neurohypophysis is about 10 times less than that of 8-arginine-oxytocin[5,8].

The physiological significance of the presence of serum cystine aminopeptidase is not known and is difficult to explain in view of this very low oxytocic activity of bird pituitary. It seems most probable, however, that there is causal and time relationship between oxytocin and its inactivating enzyme system in blood.

The neural lobe of the bird hypophysis becomes separated by connective tissue from the derivatives of RATHKE's pouch at about the eleventh day of incubation. The basophilic and acidophilic cells of adenohypophysis do not differentiate until the tenth day of incubation[9]. The time at which these tissue differences appear does not necessarily correspond to the time at which hormones are produced. In our study, however, the possibility was investigated that, during the embryonic growth of the chicken, the level of cystine aminopeptidase activity follows the process of maturation of the neuro-hypophysial system and therefore the time of cystine aminopeptidase appearance can be determined.

In the present experiment, the levels of serum cystine aminopeptidase were determined at various stages of chick embryo development until the time of hatching and during the first few days of the post-embryonic life.

Blood samples were drawn directly from the heart. Cystine aminopeptidase in blood plasma was estimated according to the chemical method of TUPPY and NESVADBA[10] with L-cystine-di-β-naphthylamide as substrate. The results were presented in mg of β-naphthylamine liberated from substrate by 100 ml of plasma after 1 h incubation or in mU of enzyme/1 ml of plasma.

It has been found that the level of cystine aminopeptidase in chick embryos, until the eighteenth day of incubation, was on the border line of sensitivity of the chemical determination. From the nineteenth day of incubation, the amount occurred in measurable quantities. The level increased thereafter from the nineteenth day to the twenty-first day of incubation (Table).

The greatest increase in the level of cystine aminopeptidase was seen when measurements were taken daily during the first few days of the chicken's life. Shortly after hatching the level was 1.11 but it increased to 1.79 on the third day. The results expressed in mU of enzyme per ml of plasma are presented in the Figure.

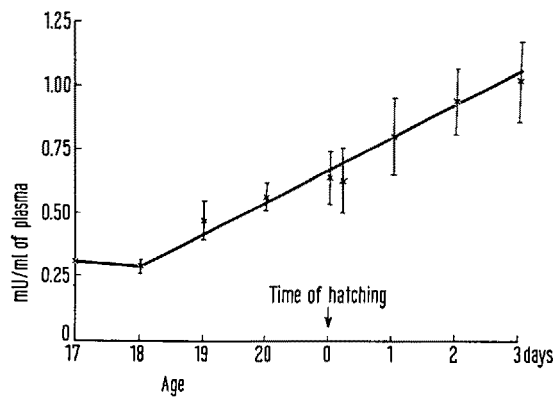
The data show that the cystine aminopeptidase increases with advancing age and that on the third day after hatching it is equal to that recorded in the adult fowl[2]. This level of cystine aminopeptidase (1.04 mU/ml of plasma) corresponds to that found in women in the 8 lunar month of normal pregnancy[11,12].

It is particularly interesting that the cystine aminopeptidase in the chick appears in plasma at about 1 week

The level of cystine aminopeptidase in plasma of embryos and chicken

Age	No. of animals	mg β-naphthylamine liberated by 100 ml of plasma/h		
		Mean	S.E.	Range
Embryos (day of incubation) days				
17	5	0.54[a]	± 0.058	0.40–0.65
18	5	0.51[a]	± 0.012	0.40–0.65
19	5	0.83	± 0.064	0.70–1.07
20	8	1.00	± 0.038	0.85–1.15
0 Hatching	7	1.13	± 0.089	0.85–1.50
Chicken days				
0–4 h	7	1.11	± 0.108	0.80–1.35
1	7	1.40	± 0.113	1.03–1.97
2	5	1.64	± 0.128	1.20–1.90
3	6	1.79	± 0.163	1.45–2.57

[a] Amount on the border of sensitivity of the method.



Changes in plasma cystine aminopeptidase concentration in embryos and chicken with age

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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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