effect on fibrinogen-conversion of the split products produced by plasmin and toxins was tested, and it could be proved that snake venoms elicit a greater activity of antithrombin VI within their split products than does plasmin.

In experiments in vivo, sublethal doses of *A. piscivorus* venom (400 μg/100 g) were injected s.c. to white rats. A routine blood coagulation check-up was performed in the time intervals of 30 min, 2 h and 24 h respectively. Except for a slight hypercoagulability of the whole blood in the first 30 min, no significant changes were observed, nor was the fibrinogen content decreased in plasma of experimental animals. This was explained by the fact that snake venom fibrinolysins are inhibited by whole serum (human, bovine, dog, guinea-pig, rabbit and rat).

The lytic activity of plasmin is controlled in the blood through antiplasmins derived from α_1 and α_2 globulin fractions[4,5]. Through the courtesy of Prof. HABERMANN, Pharmakologisches Institut, Giessen and Behringwerke, Marburg-Lahn, we obtained antiplasmin α_1 and macroglobulin α_2 and were able to compare their inhibitory effect on plasmin, trypsin and toxins (Figure 4). Antiplasmin α_1 inhibited fibrinogenolytic and caseinolytic activity of plasmin and trypsin completely, α_2 was effective against plasmin only. None of the isolated plasma antiplasmins effected any of the lytic activities of venoms.

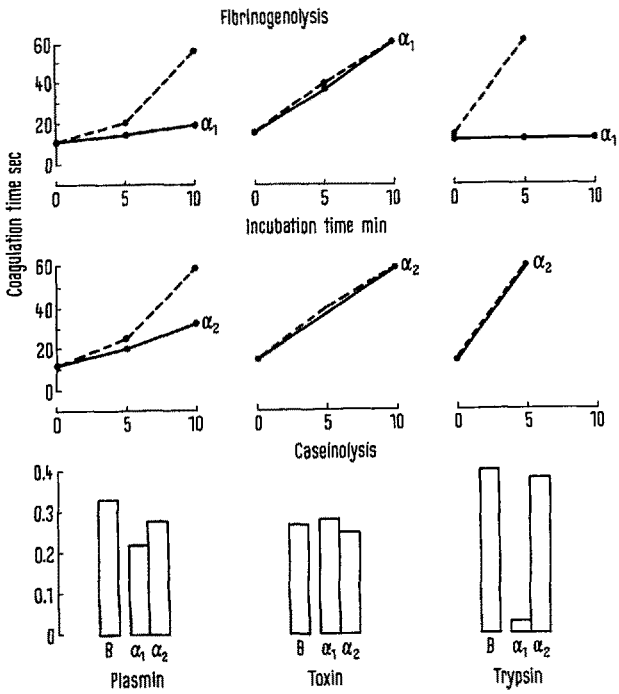


Fig. 4. Inhibition of the fibrogenolytic and caseinolytic properties of plasmin, trypsin and toxin by α_1 and α_2 antiplasmins.

Zusammenfassung. Fibrinolysine zweier Schlangengifte haben — im Gegensatz zu Plasmin und Trypsin — keinen Plasminogen-aktivierenden Effekt, noch sind sie durch Proteasen-Inhibitoren gehemmt. Sowohl in vivo wie auch in vitro sind sie durch Plasmaproteine inaktiviert, was jedoch nicht auf α_1 und α_2-Antiplasmine zurückzuführen ist. Die Spaltprodukte der Toxin-Fibrinogenolyse besitzen einen hohen Gehalt an Antithrombin VI.

F. KORNALIK and Z. STÝBLOVÁ

Institute of Experimental Pythology, Charles University, Prague (Czechoslovakia), 30 May 1967.

[4] A. SHAMASH RIMON, Thromb. Diath. haemorrh. *12*, 119 (1964).
[5] P. S. NORMAN, J. exp. Med. *108*, 53 (1958).

The Citrate Cleavage Enzyme Activity in Chick Embryo and Chicken Liver during Development

The cleavage of citrate might be regarded as a pathway for the supply of both acetyl CoA and oxaloacetate to the cytoplasm (SRERE[1,2]). The concentration of citrate cleavage enzyme (E.C. 4.1.3.6.) varies in accordance with its presumed role as a major source of acetyl units in lipid biosynthesis. While its level is depressed in the livers of diabetic and starved rats, it increases either after refeeding the animals with a rich carbohydrate diet (KORNACKER and LOWENSTEIN[3,4]) or after administration of insulin (SHRAGO and LARDY[5]). Moreover, a large increase in the activity of the citrate cleavage enzyme occurs in the rat mammary gland from the onset of lactation up to weaning (SPENCER and LOWENSTEIN[6]). In suckling rats the citrate cleavage enzyme decreases after birth from higher prenatal levels in both liver and adipose tissue, and increases slowly with weaning reaching adult levels (HAHN and DRAHOTA[7]).

No extensive investigation has been done on the relationship between the level of citrate cleavage enzyme and the carbohydrate metabolism, so that it is still doubtful if the enzyme plays a role in gluconeogenesis. The amount of phosphoenolpyruvate synthesized from citrate under normal conditions is quite significant for the rate of gluconeogenesis, even though the citrate is a poor substrate for phosphoenolpyruvate formation in rat liver if compared with other metabolites such as aspartate, fumarate or malate (SHRAGO and LARDY[5]).

To elucidate the role of citrate cleavage enzyme in the gluconeogenesis, its level has been measured in high speed supernatants of liver of both chick embryo and chicken. The results are shown in the Figure.

Material and Methods. In the liver of chick embryo, the gluconeogenesis rate is high, reaching a maximum on the sixteenth day of incubation and falling towards hatching

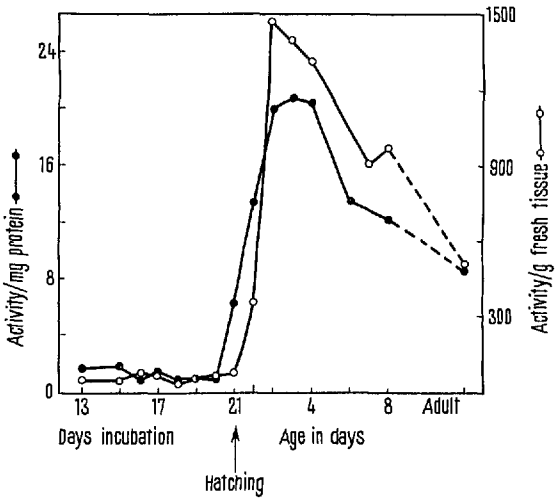
[1] P. A. SRERE, J. biol. Chem. *234*, 2544 (1959).
[2] P. A. SRERE, Nature *205*, 766 (1965).
[3] M. S. KORNACKER and J. M. LOWENSTEIN, Biochim. biophys. Acta *84*, 490 (1964).
[4] M. S. KORNACKER and J. M. LOWENSTEIN, Biochem. J. *94*, 209 (1964).
[5] E. SHRAGO and H. A. LARDY, J. biol. Chem. *241*, 663 (1964).
[6] A. F. SPENCER and J. M. LOWENSTEIN, Biochem. J. *99*, 760 (1966).
[7] P. HAHN and Z. DRAHOTA, Experientia *22*, 706 (1966).

(BALLARD and OLIVER[8]), while the high concentration of total fatty acids presumably accounts for a low rate of lipogenesis in the liver during the incubation period (DUMM and LEVY[9]). On the contrary, after hatching there is a high rate of lipogenesis (ENTENMAN et al.[10]) and a low rate of gluconeogenesis.

Livers were excised and quickly homogenized in ice-cold 0.25 *M* sucrose. The homogenate was centrifuged at 105,000 *g* for 60 min at 0 °C in the Spinco model L centrifuge. The clear supernatant was used for enzyme determinations. Protein concentration was determined by the microbiuret method. The activity assays were carried out according to KORNACKER and LOWENSTEIN[4]. A chromatographically pure acethydroxamic acid, m.p. 58–59 °C, synthesized according to HANTZSCH[11] was used as standard.

Results and discussion. As the Figure shows, there is little or no change in the activity of the citrate cleavage enzyme during the incubation period and the activity is constantly lower than in adult chickens. After hatching, the activity increases, reaching, values 3–4 times as high as the normal adult value in 2 days, and 15–16 times as high as the normal embryo value. After the maximum peak, the activity decreases, tending to the adult value.

The level of activity of the citrate cleavage enzyme in the liver of chick embryo is the lowest among those assayed by SRERE[1] and by ourselves in different tissues and species, yet the rate of phosphoenolpyruvate synthesis of the chick embryo liver is 20 times higher than that of the adult rat liver (BALLARD and OLIVER[8]), which needs for the rate of gluconeogenesis 1 μmole/min/g of fresh tissue (SOLOMON et al.[12]). As shown in the Figure, the citrate cleavage enzyme produces in the chick embryo liver less than 0.1 μmole of oxaloacetate/min/g of fresh tissue. These data suggest that the citrate cleavage enzyme gives a very little, if any, contribution to the chick embryo liver gluconeogenesis, and are well in agreement with its role in fatty acids synthesis in the liver of new born chickens[13,14].



The citrate cleavage enzyme activity in chick embryo and chicken liver. The activity is given as nmoles of citrate cleaved/min. Each point is the mean of 3 separate experiments on different chick embryo and chicken liver preparations. In each assay 3 protein dilutions were tested to ensure proportionality of initial reaction rates to enzyme amount.

Riassunto. La relazione tra le misure sperimentali dei livelli di attività del «Citrate cleavage enzyme» (E.C. 4.1.3.6.) e lo andamento dei processi neoglicogenetico e lipogenetico nel fegato di embrione di pollo e di pollo a vari tempi di sviluppo esclude che la scissione enzimatica del citrato partecipi alla neoglicogenesi e conferma il ruolo di questa reazione nella lipogenesi.

R. A. FELICIOLI and F. GABRIELLI[15]

Institute of Biological Chemistry, University of Pisa (Italy), 17 July 1967.

[8] F. J. BALLARD and I. T. OLIVER, Biochim. biophys. Acta *77*, 578 (1963).
[9] M. E. DUMM and M. LEVY, J. cell. comp. Physiol. *33*, 373 (1949).
[10] C. ENTENMAN, F. W. LORENZ and I. L. CHAIKOFF, J. biol. Chem. *133*, 231 (1940).
[11] A. HANTZSCH, Berdt. chem. ges. *27*, 799 (1894).
[12] A. K. SOLOMON, B. VENNESLAND, F. W. KLEMPERER, J. M. BUCHANAN and A. B. HASTINGS, J. biol. Chem. *140*, 171 (1941).
[13] The authors are indebted to Prof. C. A. ROSSI for his suggestions and fruitful discussions. They thank Miss I. NARDI for technical assistance in some of the experiments.
[14] This work was supported by grants from the Impresa Enzimologia of the Italian C.N.R.
[15] Post-doctoral fellow of the Scuola Normale Superiore, Pisa.

Impairments of RNA Synthesis in Ehrlich Ascites Tumour by Luteoskyrin, a Hepatotoxic Pigment of *Penicillium islandicum* Sopp

Luteoskyrin, a hepatotoxic metabolite of *Penicillium islandicum* Sopp, is a yellow pigment of *bis*-anthraquinone[1]. In the long-term feeding experiments with luteoskyrin, mice and rats produced liver diseases including hepatoma[2,3]. In the biochemical examinations on the rat liver, the alternations in the mitochondrial function and morphology were demonstrated in vitro and in vivo[4–6].

As for the mode of action on a nucleus metabolism, the studies on the interaction of the pigment with nucleic acids in vitro have revealed the facts that the pigment complexed with DNA and DNH (deoxyribonucleohistone) in the presence of magnesium ion[7–8], and that it modified the activity of DNA-dependent RNA-polymerase[10]. Physicochemical approaches on the complex (DNA-Mg^{++}-luteoskyrin) have proved that the pigment stacked beside the pyrimidine-bases of a helical DNA and that the planar chromophore was oriented in parallel with the axis of DNA molecule[11].

Based on these findings, the authors investigated the effect of the pigment on the synthesis of nucleic acids and