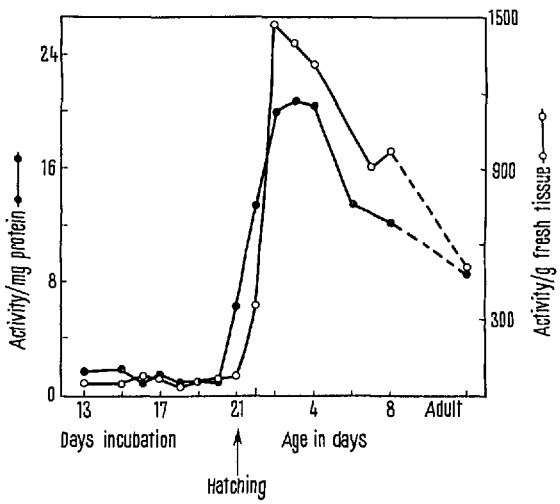
(BALLARD and OLIVER[8]), while the high concentration of total fatty acids presumably accounts for a low rate of lipogenesis in the liver during the incubation period (DUMM and LEVY[9]). On the contrary, after hatching there is a high rate of lipogenesis (ENTENMAN et al.[10]) and a low rate of gluconeogenesis.

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.

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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[7–9], 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

protein in a Ehrlich ascites tumour cells with results which indicated that luteoskyrin inhibited the synthesis of a nuclear RNA of the cell.

Ehrlich ascites tumour cells, strain 4 *N*, were maintained in *dd*-male mouse and harvested 8 days after i.p. inoculation. As for the uptake of radioactive precurcors into macromolecules, tumour cells (4×10^7) were preincubated in 2 ml of 0.9% NaCl solution with shaking at 37 °C for 10 min in the presence or absence of luteoskyrin. At the end of this preincubation, Locke-Ringer's solution containing 0.5 μC of 2-C^{14}-uracil, 2-C^{14}-thymidine or L-C^{14}-leucine was added to the cell suspension, making a final volume of 8.0 ml, to continue the incubation. After 40 min of the incubation, the incubated solution was mixed with an equal volume of 10% PCA to fractionate acid-insoluble precipitates into RNA, DNA and protein, according to the method of REICH et al.[12]. For the characterization of the cellular RNAs, the method of ROBERT[13] was adopted for the fractionation of RNAs into a nuclear and cytoplasmic RNA. As in the case of a glycolytic metabolism, the tumour cells (8×10^6) were preincubated with shaking at 37 °C for 10 min, aerobically or anaerobically, in 1.0 ml of 0.9% NaCl solution in the presence or absence of the pigment. At the end of the preincubation, Locke-Ringer's solution was added to the cell suspension, making a final volume of 3.0 ml, and the incubation continued for 50 min.

In preliminary experiments carried out with the tumour cells in the absence of luteoskyrin, C^{14}-uracil, C^{14}-thymidine and C^{14}-leucine were incorporated into RNA, DNA and protein of the cell, respectively, linearly with the incubation time up to 60 min. In the presence of luteoskyrin, as shown in Table I, the toxic pigment at the concentration of $1 \times 10^{-6} M$ markedly inhibited the uptake of C^{14}-uracil without affecting significantly the uptakes of C^{14}-thymidine and C^{14}-leucine. The aerobic and anaerobic production of lactic acid was also proved to be indifferent to luteoskyrin, as shown in Table II. These results indicate that the toxic pigment specifically inhibits the synthesis of RNA without interferring with the energy metabolism of the tumour cells.

Further experiments conducted with the phenol method revealed that luteoskyrin at the concentration of 1.2×10^{-6}–$1.2 \times 10^{-5} M$ inhibited the incorporation of C^{14}-uracil into RNA of 'nuclear fraction' of the cell, as shown in Table III. According to ROBERT[13], over 90% of the rapidly-labelled RNA of a tumour cell was found in the nuclei. In this respect, the impairment of RNA synthesis by luteoskyrin represents the depressed activity of the synthesis of rapidly-labelled RNA in the nuclei.

Summing up the above discussion, it is concluded that the cartinogenic luteoskyrin interferes with the synthesis of the nuclear RNA of Ehrlich ascites tumour cells, and this inhibitory effect of the toxic pigment is presumably caused by the binding of luteoskyrin to DNA[7,8] and also by the inhibition of RNA-polymerase[10].

Table I. Effects of luteoskyrin on the synthesis of RNA, DNA and protein in tumour cells

Luteoskyrin (*M*)	Incorporation (counts/min)		
	2-C^{14}-uracil into RNA	2-C^{14}-thymidine into DNA	1-C^{14}-leucine into protein
0	560 (100) [a]	10532 (100)	2998 (100)
1×10^{-6}	260 (51)	9780 (98)	2511 (85)
1×10^{-5}	84 (15)	8162 (78)	1874 (62)

[a] Parentheses indicate the % activities of the control.

Table II. Effects of luteoskyrin on the glycolysis in tumour cells

Luteoskyrin (*M*)	Lactic acid (μ moles)	
	aerobic	anaerobic
0	3.2	3.4
1×10^{-6}	3.3	3.6
1×10^{-5}	3.7	4.1

Table III. Inhibition of nuclear RNA synthesis by luteoskyrin

Luteoskyrin (*M*)	Incorporation (counts/min/mg RNA)	
	nucleic RNA	cytoplasmic RNA
0	4250 (100) [a]	760 (100)
1.2×10^{-6}	2640 (58)	690 (91)
3.0×10^{-6}	1770 (42)	780 (103)
6.0×10^{-6}	1200 (28)	670 (88)
1.2×10^{-5}	1040 (24)	690 (91)

[a] Parentheses indicate the % activities of the control.

Résumé. La lutéoskyrine, pigment cartinogène synthétisé par le *Penicillium islandicum* Sopp, inhibe la synthèse du RNA nucléaire dans la cellule de la tumeur Ehrlich.

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