cells formed in the presence of insulin and hydrocortisone induces casein synthesis[9,10], and this action of prolactin was not abolished by the presence of lithium at concentrations which prevent DNA synthesis. The 'baseline' level of casein synthesis, as measured in the initial period and maintained by medium containing insulin and hydrocortisone, appears to represent the biosynthetic function of differentiated (and presumably non-dividing) cells formed in vivo, and this function was not diminished by the presence of lithium ions. This inhibition of cell differentiation by the inhibition of DNA synthesis and subsequent cell proliferation confirms previous results indicating that new cell formation is necessary for the expression of new differentiated function in this system[5,9,10]. By these criteria, the effect of lithium ion is not a non-specific toxic effect, but rather appears to represent inhibition of the induction of DNA synthesis in cells preparing for proliferation.

These studies demonstrate that lithium and ammonium ions can act in G_1 to delay the onset of DNA synthesis in mouse mammary epithelial cells in vitro. Their action emphasizes the importance of mechanisms which may be operative in G_1 to regulate the rate of cell proliferation and thus govern the expression of new differentiated function in progeny cells. Grain counts overlying labeled cells indicated that the rate of DNA replication, once replication was initiated, was not detectably altered, suggesting that similar control mechanisms may not be operative during the S phase[11].

Zusammenfassung. Lithium- und Ammoniumionen verhindern den Anfang der DNA-Synthese im Milchdrüsenepithel in vitro. Die Replikation von DNA, nach dem Anfang der S-Periode, wird jedoch nicht verhindert. Diese spezifische Verhinderung der Dauer der G_1-Periode durch die ionale Umgebung zeigt, dass Mechanismen, die für die Regulierung der Zellproduktion wichtig sind, während der G_1-Periode intervenieren.

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The Specificity of Histones in Nucleated Erythrocytes

Due to their localization in the cell nucleus and due to their close association with DNA, histones are regarded as potential repressors of the genetic loci on DNA. Findings that histones function as potent inhibitors of the enzymatic synthesis of RNA in vitro or of the in vitro DNA replication support this possibility. Since histones have been shown to lack the tissue and species specificity once thought to be essential for their gene-regulatory function, other mechanisms such as interaction with acidic proteins, RNA, hormones, or direct chemical modification of the individual histone fractions by acetylation, phosphorylation, methylation, or thiolation were suggested to modify the histone-DNA interaction at sites involved in genetic transcription[1-5].

If histones function as gene repressors, a substantial deviation from the rather constant composition of somatic histones in vertebrata can be expected in cells with permanently arrested DNA and RNA synthesis. One of such changes can be seen during the spermatogenesis in fish and other animals. The transition of histone to protamine first observed by MIESCHER[6] and confirmed by many investigators is a natural example of a major involvement of histones in cellular differentiation.

Another example of a profound change in the composition of histones associated with a specialization of cellular functions appears again in cells with permanently arrested RNA and DNA synthesis – in chicken erythrocytes. Occurrence of a histone fraction specific for nucleated erythrocytes in domestic fowl has been reported by several authors[7-9]. The histone isolated by NEELIN et al.[10] as histone fraction V and by HNILICA[11] as histone fraction F2c, differs in its amino acid composition from the other histones known to occur in the vertebratae nuclei. It is relatively rich in lysine (21%), alanine (15%), serine (12%), and arginine (11%). In starch gel electrophoresis it migrates as a homogeneous band of a slightly slower mobility than the very lysine-rich histone F1. Because of its absence in other tissues of the chick, it appears that the F2c histone may serve as a permanent genetic repressor.

To investigate this possibility, histones from the erythrocytes of chicken, bull frog (*Rana catesbeiana*) and of the common jack (*Caranx hippos*) were isolated, fractionated, and analyzed. Blood from the sacrificed animals freshly collected with anti-coagulant (sodium citrate) was quickly frozen over pulverized solid CO_2. After thawing in an ice bath in the laboratory, the hemolyzed blood cells were washed several times in 0.14 M NaCl containing 0.01 M trisodium citrate. Nuclei, collected by differential centrifugation, were washed with isotonic saline, 0.1 M *Tris* buffer pH 7.6, and 95% ethanol[11]. The

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Amino acid composition of histones from nucleated erythrocytes

Amino acid	Chicken erythrocyte histones				F2c histones		
	F3	F2a	F2b	F1	Chick	Fish	Frog
Lysine	9.0	10.9	15.4	25.1	24.9	23.9	28.7
Histidine	2.4	1.9	2.5	0.2	1.8	0.9	0.5
Arginine	12.6	12.6	7.7	2.6	11.4	7.1	9.9
Aspartic acid	5.9	5.4	5.2	4.0	1.7	3.9	3.1
Threonine	6.2	5.1	6.4	3.5	3.1	4.9	4.2
Serine	5.2	4.3	9.4	6.5	13.1	8.8	7.8
Glutamic acid	10.9	7.4	8.2	4.3	3.7	3.9	1.7
Proline	4.4	3.0	4.4	7.7	6.9	6.2	7.0
Glycine	6.1	11.5	6.8	7.7	4.7	5.6	4.7
Alanine	11.5	10.8	10.3	27.1	15.2	16.2	17.0
Cystine-half	0.2	–	–	–	–	–	–
Valine	5.0	7.0	7.1	5.4	4.0	6.2	5.8
Methionine	1.7	0.9	1.3	–	0.5	0.7	0.1
Isoleucine	4.6	5.0	4.9	1.1	3.0	3.4	2.8
Leucine	9.2	9.7	6.0	4.3	4.0	5.8	5.3
Tyrosine	2.3	2.8	3.2	0.5	1.4	1.5	0.6
Phenylalanine	3.0	1.7	1.4	0.5	0.5	1.0	0.6

All values are expressed as % of total moles of amino acids observed. Serine values were corrected (10%) for hydrolytic losses. Fish = *Caranx hippos* (common jack). Frog = *Rana catesbeiana* (bullfrog).

arginine-rich histones were removed by 3 successive extractions with a mixture of absolute ethanol and 1.25*N* HCl (4:1, v/v). The residue was then treated with 0.2*N* HCl to solubilize the lysine-rich histones which were fractionated by chromatography on carboxymethyl cellulose; the F2c fraction was separated from F2b histone by gel filtration on Sephadex G-75[11]. After the preliminary experiments established that frog and fish erythrocytes lack the very lysine-rich histone F1, the fish and frog lysine-rich histones were fractionated by gel filtration on Sephadex G-75 only. The fractionation was monitored by analyzing the amino acid composition of the fractions and by determining their homogeneity in starch gel electrophoresis[12]. In several preparations the C-terminal amino acid content of the F2c histones was determined by digestion first with carboxypeptidase B followed by additional treatment with carboxypeptidase A. Both enzymes were Worthington DFP-treated preparations. Attempts to determine the NH_2-terminal amino acids in the F2c histones resulted in very low yields of threonine and other amino acids, indicating that the NH_2-terminal amino acid of the F2c histones may be acetylated.

The C-terminal amino acid of the F2c histone is lysine. As the results of amino acid analysis shown in the Table indicate, histones similar to the F2c fraction of chicken erythrocytes are also present in nucleated erythrocytes of other genera. Also shown in this Table is the amino acid composition of the 4 major histone fractions present in the chicken erythrocytes (i.e., F1, F2a, F3 and F2b). These 4 fractions are present in all somatic tissues of the chicken and their composition resembles strongly that of the corresponding histone fractions in calf thymus[1–5,13]. Even though the amino acid composition of the F2c histones in chick, frog and fish is not identical, there is sufficient similarity to justify a conclusion that this fraction appears in cells with the same biological function, i.e. to produce large quantities of protein, namely hemoglobin. No F2c-like proteins were found in the bone marrow of chickens with phenylhydrazine produced anemia, or in the livers of fish and frog. Similarly, PALAU and BUTLER who analyzed histones from the livers of another species of fish, the European trout, did not report any fractions resembling the F2c histone[14].

The absence of F2c histones in liver, spleen, kidney and other tissues in chicken and its presence in nucleated erythrocytes of animals as distant as chick, frog, and fish indicates that this histone may function as a permanent repressor turning off the DNA replication and RNA synthesis in mature erythrocytes of species which retain the nucleus in their red blood cells. The observation that F2c histones are potent inhibitors of the in vitro RNA synthesis and DNA replication further supports this possibility[15,16].

Riassunto. Dagli eritrociti del pollo, della *Rana cateasbeiana* e del pesce *Caranx hippos* sono stati isolati gli istoni nella ipotesi che gli eritrociti forniti di nucleo contengano una frazione di istone funzionante come repressore permanente della sintesi degli acidi nucleici. Detti istoni sono stati frazionati ed analizzati nella loro composizione in amino-acidi. In tutte e tre le speci su menzionate è stata trovata una frazione relativamente ricca in lisina, arginina, alanina, e serina che è stata denominata F2c. L'assenza di detta frazione negli altri tessuti incluso il midollo osseo indica che la frazione F2c può costituire un repressore genetico permanente.

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