To determine whether oral contraceptives induce low erythrocyte COMT activity we studied 9 women before they started taking oral contraceptives, and again after 1 to 3 months of treatment. Results are shown in Table III. Six of the 9 showed reductions from 10 to 44% post-treatment, while the mean decrease in enzyme activity for the group was 15%.

Finally, we studied groups of women in different stages of pregnancy with the results shown in Table IV. Enzyme activity during the 1st trimester is not significantly different to that of non-pregnant women, but a significant reduction occurs during the 2nd and 3rd trimester ($P < 0.05$ and < 0.001, respectively). Erythrocyte COMT activity remains low during the first 7 days post-partum.

As we have been unable to inhibit COMT activity in vitro with natural or synthetic steroid hormones added at $10^{-4}M$ concentration, we feel that hormones act to reduce formation of COMT during erythropoesis. After the first 3 months, there is no correlation between COMT activity and duration of use of oral contraceptives, but there is a progressive decrease in this initial period.

Reduced erythrocyte COMT activity would be excepted to increase the concentration of circulating catecholamines and serum noradrenaline is known to be high in women with depression[5]. If COMT of brain is similarly affected, alterations in catecholamine metabolism will occur and could be of importance in depression.

It seems likely that the high incidence of depression in women taking oral contraceptives, or during the post-partum period, is related to hormonal suppression of erythrocyte COMT activity. As only about half of the women we studied showed this enzyme reduction, determination of the enzyme might be of value in screening women liable to develop depression in response to oral contraceptives or pregnancy.

Table III. Influence of oral contraceptives on erythrocyte COMT

Group of subjects	Erythrocyte COMT activity (nM/h/ml) Pre-treatment	Post-treatment	Change (%)
Women taking oral contraceptives	1.76	0.98	−44
	1.44	1.05	−27
	1.18	1.16	− 2
	1.58	1.62	+ 3
	1.55	1.39	−10
	1.17	0.97	−17
	1.71	1.50	−12
	1.50	1.52	+ 1
	1.66	1.15	−31

Table IV. Erythrocyte COMT activity during pregnancy

Group of subjects	Number in group	Erythrocyte COMT activity (mean nM/h/ml)	S.D.
Normal women	36	1.48	0.33
Pregnant women	35	1.19	0.35
First trimester	7	1.40	0.39
Second trimester	15	1.20	0.40
Third trimester	13	1.08	0.32
Post-partum 7 days)	10	1.15	0.38

Résumé. Dans le cas de beaucoup de femmes prenant des contraceptifs oraux, l'activité du transférase catéchole-*O*-méthyle du globule rouge est réduite à des nivaux analogues à ceux observes dans le cas de femmes souffrant de dépression non-traitée. Le dépôt de contraceptifs à action durable n'affecte pas d'une manière significative l'enzyme, bien qu'elle soit réduite pendant le troisième trimestre de la grossesse.

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A Comparative Study of Nuclear Proteins in Chick Embryo Cells and Their Primary and Secondary Fibroblasts in Culture

Chick embryo cell cultures have been used extensively in many studies. However, the limited quantity of cultured cells constitutes an experimental disadvantage in studies dealing with the isolation of nuclear proteins. The ideal is to use a minimum amount of available radioactively labeled cultured cells which are subsequently mixed with readily available embryo cells serving as a carrier for the isolation of radioactively labeled cultured cell components. Nevertheless, the possible changes of some cellular parameters during the passage of embryo to cultured cells cast some doubt on the methodological validity of using uncultured embryo cells as carrier for components from cultured cells. For these reasons we have carried out a comparison of nuclear proteins of chick embryo cells and of cultured primary and secondary cells derived from chick embryo using polyacrylamide gel electrophoresis.

Materials and methods. Embryos were removed from 10- to 11-day-old fertilized eggs obtained from Spafas Inc. The viscera, limbs and head were discarded. The remaining parts were homogenized at 4 °C in 5 volumes of 0.05 M *Tris* buffer, pH 7.4, containing 0.32 M sucrose and 3 mM $MgCl_2$. Purified nuclei were prepared as described previously[1].

Primary and secondary cell cultures were prepared from embryos according to the method described by VOGT[2]. Approximately 4×10^7 cells were added to Roux bottles and incubated at 37 °C. The cells usually growing to confluency within 3 to 5 days were designated as primary cell culture. Secondary cells were produced by the passage of primary cells[2]. Isolation of nuclei from primary and secondary cells (5×10^9 cells per experiment) was accomplished as described above for embryo nuclei.

[1] R. A. JUNGMANN, J. S. SCHWEPPE and F. A. LESTINA, J. biol. Chem. *245*, 4321 (1970).

[2] P. VOGT, in *Fundamental Techniques in Virology* (Ed. H. SALZMAN; Academic Press, New York and London 1969), p. 198.

Table I. Relative quantitative distribution of histones from various cell types

Cell type	Histones		
	F1[a] (%)	F2a2 + F2b + F3[a] (%)	F2a1[a] (%)
Chick embryo	17.2 ± 1.1[b]	58.5 ± 1.1	24.3 ± 0.9
Primary chick fibroblasts	16.7 ± 1.7	56.7 ± 1.8	26.5 ± 3.3
Secondary chick fibroblasts	16.7 ± 1.3	55.5 ± 2.6	27.8 ± 3.7
Rat liver	17.0 ± 0.5	60.6 ± 5.5	22.3 ± 5.0
Rat brain	16.3 ± 0.5	61.5 ± 0.9	22.2 ± 0.9

[a] Percent of F1, F2a2 + F2b + F3 and F2a1 of the histone fraction calculated on the basis of the paper weight[9] of the profiles of polycrylamide gels. [b] Arithmetic mean of 3 determinations ± S.D. F1 + F2a1 + F2a2 + F2b + F3 is considered as 100% of histones.

Table II. Relative quantity of fast moving and slow moving nuclear acidic proteins in chick embryo, primary, and secondary chick embryo fibroblast cells

Cell Type	Acidic nuclear proteins	
	Fast moving proteins (%) (peak A)[a]	Slow moving protein (%) (peak B)[a]
Chick embryo	4.9 ± 0.5[b]	34.0 ± 0.7
Primary chick fibroblasts	27.6 ± 2.7	13.7 ± 1.0
Secondary chick fibroblasts	21.4 ± 1.6	14.0 ± 1.1

[a] Percent of fast moving or slow moving protein of the total nuclear acidic proteins calculated on the basis of the paper weight[9] of the profiles of polyacrylamide gels. [b] Atrihetic mean of 3 determinations ± S.D.

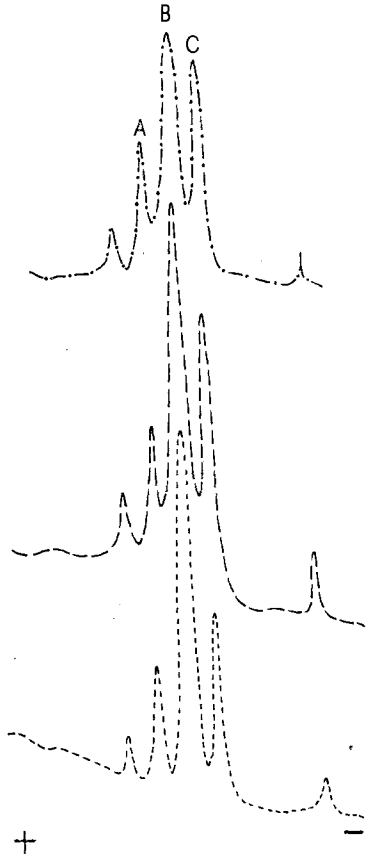

Fig. 1. Densitometer scan of electrophoretically separated histones from chick embryo (----), primary (·–·–·), and secondary (–––) chick embryo fibroblasts. Histones were fractionated by electrophoresis in 7.5% polyacrylamide gels. The gels were stained with Amido black 10 B to indicate the positions and relative concentrations of individual protein bands. Peaks: A) histone F1; B) histones F2a2 + F2b + F3; C) histone F2a1.

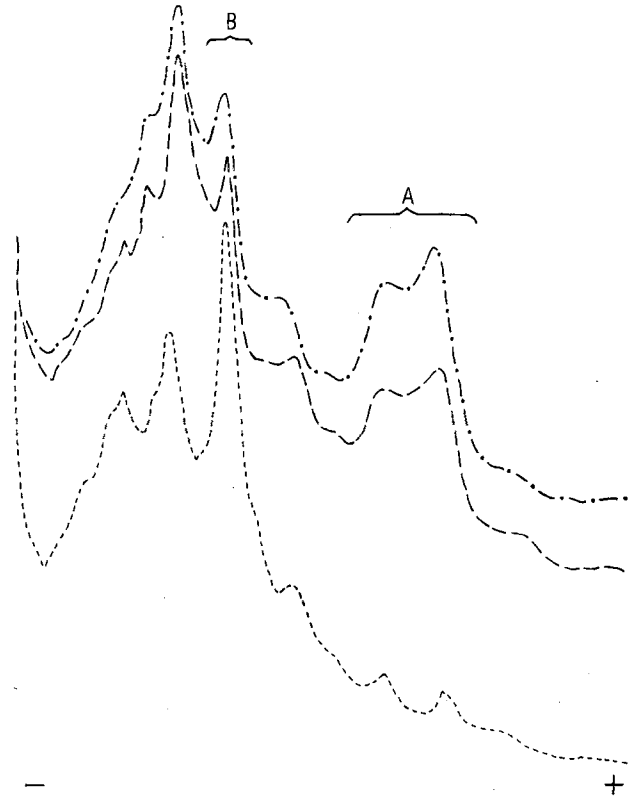

Fig. 2. Densitometer scan of electrophoretically separated acidic proteins from chick embryo (----), primary (·–·–·–), and secondary (–––) chick embryo fibroblasts. Nuclear acidic proteins were fractionated by electrophoresis in 10% polyacrylamide gels containing 0.1% SDS at pH 7.4. The gels were stained with Amido black 10 B to indicate the positions and relative concentrations of individual protein bands. Peaks: A) fast moving protein; B) slow moving protein.

Histones and nuclear acidic proteins were isolated by acid and phenol extraction according to TENG et al.[3]. Characterization of histones and nuclear acidic proteins by polyacrylamide gel electrophoresis was described previously[1,3].

Results and discussion. Histones F1, F2a1, F2a2, F2b, and F3 of chick embryo, primary and secondary cells were identified with the aid of standard calf thymus histones. As Figure 1 indicates no qualitative difference is found between histones of chick embryo, primary, and secondary cells when their electrophoretic patterns are compared. There are 3 major peaks corresponding to the

[3] C. S. TENG, C. T. TENG and V. G. ALLFREY, J. biol. Chem. *246*, 3597 (1971).

known histone fractions. Table I compares the quantitative distribution of the 3 major histone fractions of embryo, primary and secondary cells along with histones of rat liver and brain. No significant quantitative differences of histone distribution are found in the various cell types examined. Thus, the data indicate a regular quantitative distribution and qualitative similarity of nuclear histones from chick embryos and their primary and secondary cell cultures. The observed similarity of histones derived from various cell types confirms the known characteristic of histones which in general have been found to be neither tissue nor species specific[4-6].

Similarly, there are no electrophoretically discernible qualitative differences of nuclear acidic proteins from chick embryo, and from primary and secondary chick embryo cell cultures (Figure 2). However, the quantitative distribution of several acidic proteins from embryo, primary and secondary cells differs. The differences are illustrated in Table II where the relative quantitation of the proteins in peak A and peak B is given. There is a significant increase of electrophoretically faster moving protein and a decrease of slower moving protein from both primary and secondary cells when compared to chick embryo nuclear proteins. Recently it has been found that proliferation of chick fibroblasts is stimulated by folic acid or serum factors in a plasma containing medium[7]. The stimulation of proliferation seemed to require early protein synthesis, possibly nuclear acidic protein biosynthesis[8]. Since the major difference between embryo and primary (or secondary) cells is the culturing of the latter in a growth medium, it could be this nutritional treatment of the cultured cell which causes the observed effects.

In conclusion, nuclear proteins from chick embryos, and from primary and secondary chick embryo fibroblasts exhibit identical electrophoretic mobilities. Based on this similarity, chick embryo nuclear proteins can serve as carrier proteins for the isolation of pulse-labeled nuclear proteins from primary and secondary chick embryo fibroblasts[10].

Zusammenfassung. Das elektrophoretische Muster von Histonen und sauren Zellkernproteinen von Hühnerembryonen und deren primären und sekundären Fibroblastzellen wurde qualitativ und quantitativ verglichen. Die Zellkernproteine der verschiedenen Zelltypen erwiesen sich qualitativ als identisch, die relative quantitative Verteilung der Kernproteine war jedoch leicht verschieden.

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Riboflavin Enhancement of Bilirubin Photocatabolism in vivo[1]

Phototherapy has become a common therapeutic procedure in neonatal hyperbilirubinemia[2-6]. Despite the well-established efficacy of light therapy in reducing bilirubin levels, there have been reservations expressed with respect to exposing infants to light intensities of 300 to 500 foot candles, often continuously for periods of several days[7]. We have been examining the possibility of administering agents which would promote the photochemical decomposition of bilirubin in vivo, and might thus reduce either the exposure time or the light intensity necessary to reduce plasma bilirubin levels.

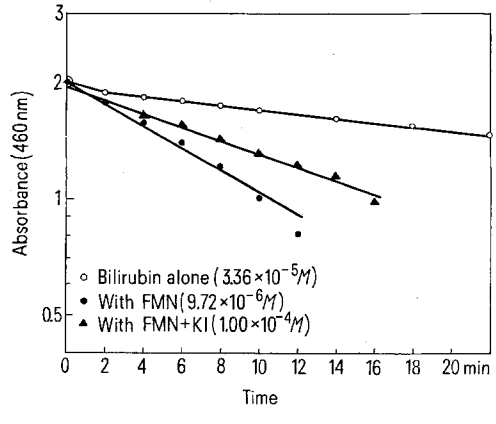

Fig. 1. Plot showing effect of FMN on rate of bilirubin photodecomposition by visible light in aqueous solution of 1% BSA, pH 7.4, under aerobic conditions.

Experimental. Preliminary in vitro experiments indicated that monosodium salt of riboflavin 5′-phosphate (FMN) increases the rate of photochemical decomposition of bilirubin. Bilirubin solutions ($3.36 \times 10^{-5} M$) were prepared in 0.05 M phosphate buffer, pH 7.4, containing 1% w/v bovine serum albumin (Armour fraction V), with and without 0.97×10^{-5} M FMN. These solutions were irradiated intermittantly in 1 cm quartz spectrophotometer cells located 24 in. from a projector equipped with a 500 W tungsten lamp. Absorbance spectra were recorded, versus a buffer blank, on a Cary 15 spectrophotometer at appropriate time intervals. Studies were conducted at room temperature of 22–25 °C, with solutions exposed to air. Initial bilirubin absorbance at 460 nm was approximately 40 times that of FMN, and the enhanced initial rate of decline in absorbance at 460 nm in presence of FMN, as illustrated in Figure 1, indicates acceleration of bilirubin photodecomposition by FMN.

Potassium iodide at a concentration of 10^{-4} M reduced the FMN enhancement of bilirubin photodecomposition, while showing no effect on rate of bilirubin photodecomposition in absence of FMN. This observation implicates the FMN triplet in the enhanced bilirubin photodecomposition. A further observation that the photofading of FMN is inhibited by the presence of bilirubin is compatible with