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.

WAN-BANG LO, R. A. JUNGMANN, J. S. SCHWEPPE, B. S. BERLIN and M. GROVER

Department of Biochemistry and Medicine, Northwestern University Medical School, 303 East Chicago-Avenue, Chicago (Illinois 60611, USA), 4 August 1972.

[4] L. HNILICA, E. W. JOHNS and J. A. V. BUTLER, Biochem. J. *82*, 123 (1962).
[5] R. H. STELLWAGEN and R. D. COLE, A. Rev. Biochem. *38*, 951 (1969).
[6] J. MOHBERG and H. P. RUSCH, Arch. Biochem. Biophys. *134*, 577 (1969).
[7] S. D. BALK, Proc. natn. Acad. Sci. USA *68*, 1689 (1971).
[8] G. ROVER, J. FARBER and R. BASERGA, Proc. natn. Acad. Sci. USA *68*, 1725 (1971).
[9] T. KUROKI and C. HEIDELBERGER, Biochemistry *11*, 2116 (1972).
[10] This investigation was supported by Steroid Hormone Research Fund, Chicago Wesley Memorial Hospital and American Cancer Society Grant No. BC-37.

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.

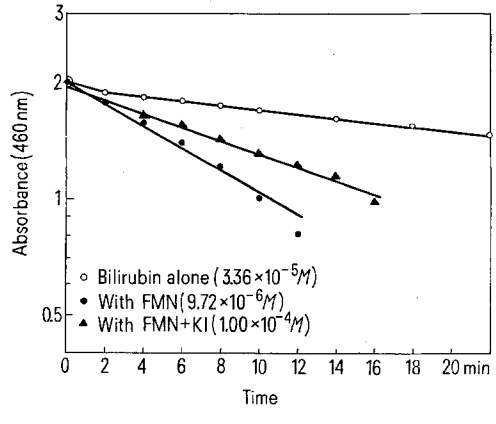

Fig. 1. Plot showing effect of FMN on rate of bilirubin photodecomposition by visible light in aqueous solution of 1% BSA, pH 7.4, under aerobic conditions.

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

a mechanism whereby bilirubin quenches the FMN triplet. Detailed studies of this photochemical process will be considered in a separate report[8].

Adult male jaundiced Gunn rats[9] weighing 275–400 g were dosed with 75 mg/kg of FMN administered in 0.7 to 1.0 ml of normal saline solution via the penis vein every 9 h up to 36 h and then every 12 h for the duration of the experiment. Control animals were injected at the same time intervals with normal saline solution. The backs of both the control and the FMN treated rats were shaved and the animals were placed in individual cages under a light canopy containing four 60 W daylight fluorescent lamps (Sylvania). Constant light intensity was maintained throughout the experiment by daily monitoring of light intensity with a light meter and adjustment of the lamp-to-animal distance as required. Blood sampling was performed prior to and during the exposure period by a technique similar to that described by HURWITZ[10]. Plasma bilirubin concentration was determined by a modified Malloy-Evelyn colorimetric method[11]. Riboflavin plasma levels encountered at the selected sampling times did not interfere with the assay for bilirubin.

Results and discussion. Both the albino and the hooded strains of Gunn rats responded to phototherapy. Preliminary studies conducted with light of 750–1100 foot candles intensity showed a rapid decline in plasma bilirubin levels, with no difference detectable for control and FMN treated rats. However, when rats were exposed to light of 300 foot candles, marked differences in rate of decline of plasma bilirubin levels were observed for the control and FMN treated animals, as illustrated in Figure 2. No change in plasma bilirubin was observed for FMN treated animals kept in the dark. In vitro studies have shown no competitive displacement of albumin-bound bilirubin by FMN at FMN concentrations up to 50 mg/100 ml.

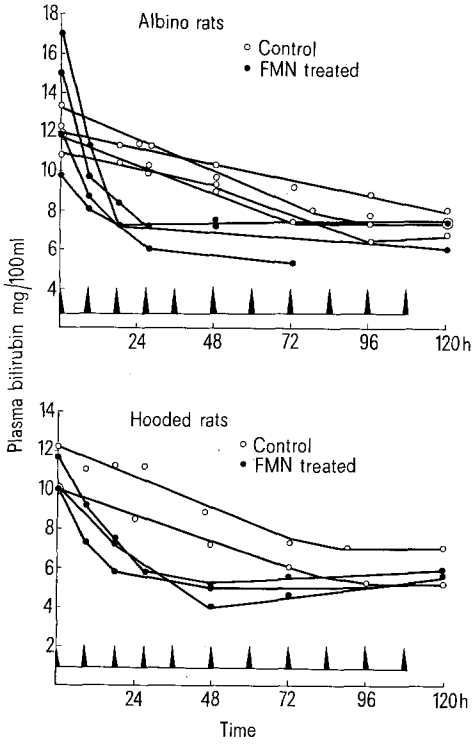

Fig. 2. FMN enhancement of rate of plasma bilirubin decline in both albino and hooded Gunn rats exposed to daylight fluorescent lights at 300 foot candles intensity. FMN dosing of 75 mg/kg i.v., indicated by markings on the time axis.

Riboflavin injected i.v. at a dose of 75 mg/kg can be observed to color the skin of either jaundiced or normal rats, suggesting that at this dosage level riboflavin, like bilirubin, is distributed to superficial tissues in appreciable concentration.

As shown in Figure 2, plasma bilirubin levels in jaundiced Gunn rats exposed to light of 300 foot candles intensity, in absence of FMN administration, decline at an apparent zero order rate to a new plateau level. Animals treated with FMN and exposed to the same light intensity exhibit virtually the same plateau plasma bilirubin as non-FMN-treated animals, but the plateau is achieved more rapidly in the FMN treated animals and at an apparent first order rate.

The results of these studies suggest that there is potential for improving the efficiency of bilirubin phototherapy through systemic administration of an appropriate agent. An improved efficiency of phototherapy which would either decrease the exposure time of infants to bilirubin lights or permit use of lower intensity light (perhaps even normal room lighting) would eliminate reservations with respect to some of the potential hazards cited for phototherapy[7]. It is not suggested that FMN at the admittedly extraordinary dose level used in this study, nor at any other dose level, is suitable for this purpose on the basis of present knowledge. Currently an FMN dose-response study is being conducted in the Gunn rat model, and studies are in progress to determine whether FMN administration results in photo-products which differ from those produced by light exposure without FMN treatment.

Zusammenfassung. Intravenös verabreichtes Riboflavin-5′-Monosodium-Phosphat-Salz (FMN) bewirkt eine beschleunigte Senkung des Plasma-Bilirubin-Spiegels bei Gunn-Ratten, wenn diese vorher fluoreszierendem Tageslicht (300 Kerzen) ausgesetzt waren. FMN beschleunigt ausserdem den Photozerfall in vitro. Die Bilirubin-Phototherapie durch Injektion eines geeigneten Katalysators wird diskutiert.

H. B. KOSTENBAUDER and D. R. SANVORDEKER

College of Pharmacy, Albert B. Chandler Medical Center, University of Kentucky, Lexington (Kentucky 40506, USA), 21 August 1972

[1] Albino Gunn rat breeding stock was obtained through the courtesy of Dr. LOIS JOHNSON, Pennsylvania Hospital, Philadelphia, Pennsylvania, and hooded Gunn rat breeding stock through the courtesy of Mr. RICHARD L. PIERSON, Rodent and Rabbit Production Section, National Institutes of Health, Bethesda, Maryland. This investigation was supported in part by research grant HD 05745-01 from the National Institute of Child Health and Human Development.

[2] R. J. CREMER, P. W. PERRYMAN and D. H. RICHARDS, Lancet, *1*, 1094 (1958).

[3] J. F. LUCEY, Pediatrics, *25*, 690 (1960).

[4] J. F. LUCEY, M. FERREIRO and J. HEWITT, Pediatrics, *41*, 1047 (1968).

[5] S. O. PORTO, R. S. PILDES and H. GOODMAN, J. Pediat. *75*, 1045 (1969).

[6] J. E. HODGMAN and A. SCHWARTZ, Am. J. Dis. Child. *119*, 473 (1970).

[7] G. ENTE and S. W. KLEIN, New Engl. J. Med. *283*, 544 (1970).

[8] To be published.

[9] C. H. GUNN, J. Hered. *29*, 137 (1938).

[10] A. HURWITZ, J. Lab. clin. Med. *78*, 172 (1971).

[11] H. MATTENHEIMER, *Micro Methods for the Clinical and Biochemical Laboratory*, (Ann Arbor Scientific Publishers, Inc., Ann Arbor 1970), p. 47.