(Sigma Chemical Co.) (10 mM), Li carbamoylphosphate (Sigma Chemical Co.) (10 mM), liver homogenate and water to a final volume of 2.00 ml; final pH, 8.1. Reactions were run at 38 °C for 15 min. The citrulline produced was determined spectrophotometrically after reaction with diacetylmonoxime by the method of Archibald[10] as modified by Ratner[11]. The unit of enzyme activity employed is 1.00 μmole citrulline produced per min.

To determine dependence of the reaction on the substrates employed, incubations of homogenate were carried out in the complete system and in the complete system minus either ornithine or carbamoylphosphate. To rule out the possibility of citrulline being produced by a non-enzymic carbamoylation of ornithine by carbamoylphosphate, boiled enzyme was also assayed in the complete system.

Results and discussion. Liver of the 3 marine mammals exhibited high levels of the enzyme: Balaenoptera borealis, 141 units/g liver wet wt; Tursiops truncatus, 344 units/g liver wet wt; Zalophus californianus, 399 units/g liver wet wt. These values are typical of those found for liver of terrestrial mammals[2]. As shown in the table, the reaction was strongly dependent upon ornithine, carbamoylphosphate and undenatured (unboiled) enzyme. Since in the presence of boiled enzyme less than 4% of the activity of that of the untreated enzyme was realized, the possibility that the observed citrulline production was due to significant non-enzymic carbamoylation reactions is ruled out.

These findings, coupled with the observations (see above) on the high percentage of urinary urea-nitrogen in marine mammals studied, support the view that marine mammals, like their terrestrial counterparts, synthesize urea by the ornithine-urea cycle. Further studies on the other enzymes of the cycle are warranted, however. They could provide data on the levels of these other enzymes, on the rate-limiting step of the cycle and on comparative properties of the enzymes from marine mammals and other ureotelic vertebrates.

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Protohemin in bile during primate development[1]

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Summary. Protohemin is excreted in biles of primate fetuses and premature newborns. It also was found in relatively large quantities in the lower ileum and mesenteric lymph-nodes of newborn primates.

Virtually all pigments in normal adult bile are open chain tetrapyrroles, predominantly bilirubins. While bile contains small amounts of different porphyrins[3], metalloporphyrins apparently have not been demonstrated. We observed a brown pigment in the intestinal mucosal cells and lamina propria of newborn monkey[4] and in human fetuses weighing 200 g or more (Blumenthal, Bergstrom and Ruebner, unpublished). In the older German histopathology literature, this pigment had been named meconium corpuscles, the assumption being that it represented absorbed meconium. We also observed this pigment in ileocecal lymph nodes of newborn monkeys, both grossly and histologically[4]. Histochemically, this pigment was not bilirubin. Spectrophotometric studies of this pigment in frozen sections of intestinal mucosal cells and the lamina propria from a newborn monkey (Blumenthal, Bergstrom and Ruebner, unpublished) had an absorption peak at about 410 nm. In pentan-2-one-n-butyl acetate (17:3, v/v) extracts of diazoreactions using ethyl anthranilate, we observed a diazo-negative brown pigment in all monkey and human newborn meconiums. The R_f-value of this pigment was 0.40 with chloroform-methanol-

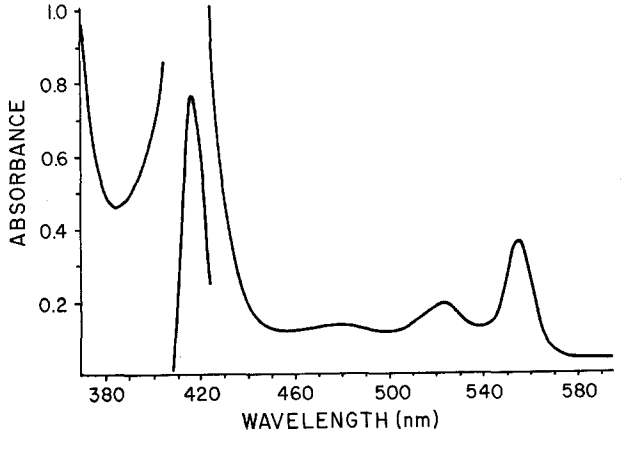

Fig. 1. Electronic absorption spectrum of protoheme-pyridine hemochrome in water-pyridine-1.0 M KOH (4 : 1 : 0.5 v/v).

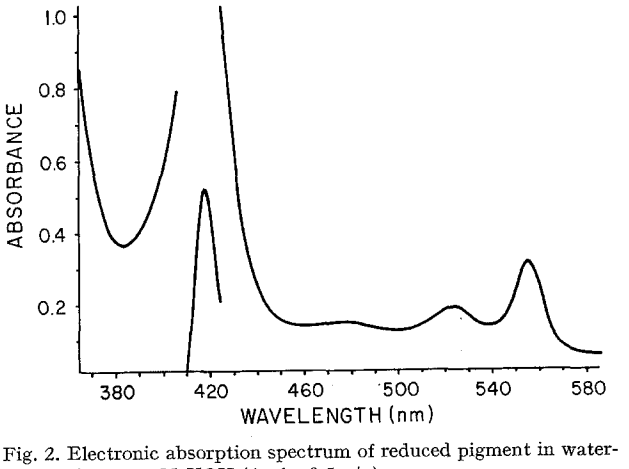

Fig. 2. Electronic absorption spectrum of reduced pigment in water-pyridine-1.0 M KOH (4 : 1 : 0.5 v/v).

water (65:35:3, by volume) on silica gel G plates[5] (Merck, Darmstadt, Federal Republic of Germany). A pigment with chemical characteristics unlike bilirubin and resembling our pigment (diazo-negative) with absorption peak at 410 nm has been isolated from the intestinal wall and urine of fetal dogs and monkey urine and bile[6].

The presence of a brownish pigment in intestinal mucosal cells, lamina propria and mesenteric lymph nodes of newborn monkeys may indicate absorption of this pigment from the intestinal lumen. The present investigation was undertaken to isolate and identify the brown pigment from human and monkey meconiums and compare it with the pigments in the intestinal mucosa and lymph nodes of newborn monkeys. The brown pigment was extracted from human meconium with acidified pentan-2-one-n-butyl acetate (17:3, v/v). Thin layer chromatography on silica gel G glass plates[7] and polyamide microlayer plates[8] (Analyt. Tech. Inc.) was used for the purification of the pigment. The electronic spectrum of the purified pigment was recorded with a Cary 17 spectrophotometer. Absorption maxima and minima are given with relative absorption calculated as based on a band at 396 nm: λ_{max} 595 (0.11) and 396 (1.00), λ_{min} 545 (0.10) and 300 nm (0.61). This spectrum was characteristic of a metalloporphyrin and we assumed it to be probably a ferroporphyrin. The electronic spectra of pyridine complexes of ferroporphyrins are characteristic of the various porphyrins[9,10]. Therefore, the electronic absorption spectrum of protoheme-pyridine hemochrome was compared with that of reduced pigment pyridine hemochrome. Sodium dithionite was used to reduce Fe(III) to Fe(II) immediately prior to recording the spectrum[9] (figures 1 and 2). The absorptions of both complexes were calculated, based on the 418 nm bands. Protohemepyridine hemochrome gave the following spectrum: λ_{max} 550 (0.16), 532 (0.09), 447 (0.07) and 418 nm (1.00), λ_{min} 537 (0.06), 500 (0.06), 455 (0.07) and 384 nm (0.19). Reduced pigment-pyridine hemochrome gave the following spectrum: λ_{max} 550 (0.20), 523 (0.10), 477 (0.07) and 418 nm (1.00), λ_{min} 537 (0.06), 500 (0.05), 455 (0.05) and 384 nm (0.25). These data strongly suggested that the brown pigment was protohemin.

Paramagnetically shifted proton magnetic resonance spectra of porphyrin iron(III) cyanide complexes are particularly useful in the identification and location of the porphyrin β-substituents[11,12]. Consequently, the methylated pigment[10] in a solution of NaCN in methanol-d[6], was subjected to FTNMR analysis on a 100 MHz instrument (JEOL PS-100 HR NMR Spectrometer). The resulting spectrum was identical with that obtained from a known sample of Fe(III) protoporphyrin-IX dimethylester dicyanide (Dr G. N. La Mar and D. Viscio, unpublished), but differed significantly from spectra obtained for a large number of other related Fe(III) porphyrin dicyanide complexes. The 4 methyl groups showed resonances at 1658.2, 1620.1, 1425.8 and 1361.3 Hz downfield from internal tetramethylsilane.

Extracts of brown pigment were prepared from the following: Biles, meconiums and from human fetuses of 10–11 weeks up to 33 weeks, biles and meconiums of premature babies, small intestine and mesenteric lymph nodes from human and monkey term newborns. The brown pigment from all these extracts was separated on tlc plates using different solvent systems. R_f-values of the brown pigment from all these sources and of commercial bovine hemin were: 0.04 in chloroform-ethanol (94:6, v/v), 0.38–0.40 in chloroform-methanol-water (65 : 35 : 3, by vol.), 0.54 in methanol-glycine buffer-pH2.7 (95 : 5, v/v) and 0.77 in methanol-acetic acid (95 : 5, v/v).

We also found protohemin in the sera of primate fetuses. It occurs in biles of normal fetuses to about 32 weeks. This suggests that up to the age of 32 weeks protoheme is excreted unchanged by the liver into the bile and is absorbed by the small intestinal mucosa into the blood streams and lymphatics.

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Induction of hyperlipidemia by human thyroid stimulating hormone immunization in rabbits

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Summary. In our study we are able to demonstrate that with TSH booster and bleeding animals 10–20 days post immunization, when rabbits have increased in antibody titer, there is an increase in cholesterol and triglyceride. These findings are suggestive that most probably we are rendering these rabbits hypothyroid.

During the course of immunization of rabbits with human thyroid stimulating hormone (H-TSH) to produce a TSH antisera, we measured the cholesterol and triglyceride levels and found them to be elevated. To document the effects of booster injections of rabbit plasma lipids in relation to the titer of antibodies, we did a time course study.

Materials and methods. 2 New Zealand albino female rabbits weighing approximately 2.5 kg were housed in environmentally controlled animal facilities under stand-

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