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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ard lighting schedule, fed and watered ad libitum. Highly purified H-TSH suitable for iodination purposes, was used for immunization procedure. The antigen was thoroughly mixed with Freund's Adjuvant in a standard fashion and injected into the rabbit's popliteal lymph node[2] and intradermally at multiple sites[3]. The schedule of injection, amount of antigen and time of bleeding are shown in the figure. The animals were fasted overnight (14 h) for blood drawing for the determinations of cholesterol, triglyceride and percent binding of the antibody to Human TSH^{125}I.

To measure the binding of antibody to H-TRH^{125}I in a standard fashion for comparison purposes, all the antisera were diluted to 1:1000 using phosphosaline buffer containing bovine serum albumin and all the samples were incubated with H-TSH^{125}I at 4 °C for 4 days and then suitable concentrations of goat-anti-rabbit serum globulin was added and an additional 48-h-incubation was done. At the end of the incubation period, the tubes were centrifuged at 2500 × g for 20 min. The supernatent was sucked out and the precipitate counted, using a Packard Auto Gamma Scintillation Counter, percent binding was calculated and used as a criteria for comparing the 2 rabbit's antibody producing ability. The serum lipids were extracted with isopropanol, phospholipids removed with activated zeolite, and cholesterol and triglycerides determined according to the Manual of Laboratory Operations of the Lipid Research Clinics Program[4].

Results. The first injection of 25 μg H-TSH per rabbit was given as described in the methodology section. The second injection of 50 μg/rabbit was given after 72 days (from the first injection), and the rabbits were bled 20 days later for cholesterol, triglyceride and percent binding to H-TSH^{125}I. Rabbit No. S-543 had a cholesterol concentration of 240 mg/dl, triglyceride of 122 mg/dl and binding of 57.8%. Rabbit No. S-544 had a cholesterol concentration of 76 mg/dl, triglycerides of 90 mg/dl and binding of 7.1%. Another blood sample was drawn 76 days after the last injection (day 148). Rabbit No. S-543 had a cholesterol concentration of 58 mg/dl, triglycerides of 30 mg/dl, and rabbit No. S-544 had cholesterol concentration of 49 mg/dl, triglycerides of 53 mg/dl.

93 days after the last immunization (day 169) another booster of 50 μg H-TSH was given and blood was collected 13 days later for lipid profile and percent binding to H-TSH^{125}I. Rabbit No. S-543 had elevated cholesterol value of 190 mg/dl and triglyceride of 108 mg/dl and binding of 57.4%. Rabbit No. S-544 had an increase in cholesterol to 81 mg/dl, but no significant change was noted in triglyceride concentration (45 mg/dl) or in binding (12.4%).

10 days later, i.e., 23 days from the day of the last booster (day 192) another sample was drawn. The cholesterol of rabbit No. S-543 fell to 80 mg/dl and triglyceride to 53 mg/dl and binding to 54.3%. The same trend was found in rabbit No. S-544, cholesterol of 64 mg/dl, triglycerides of 45 mg/dl and binding 10%. A final sample was drawn on these rabbits 31 days (day 200) from the last booster. In rabbit No. S-543 there was a further drop in cholesterol value to 48 mg/dl and triglycerides to 36 mg/dl and TSH binding to 50%. In rabbit No. S-544 the cholesterol levels fell to 49 mg/dl, but the triglyceride concentration remained at 59 mg/dl, and the binding was 8.00%.

10 normal rabbits of the same age and weight were housed and fed under similar conditions as the experimental animals. These animals were also bled under similar conditions. The plasma cholesterol concentration was 79.12 ± 38.67 mg/dl ($\bar{x}$ ± SD) and triglycerides 38.87 ± 15.76 mg/dl ($\bar{x}$ ± SD). Percent binding of normal rabbit serum to TSH^{125}I varied 1–3%.

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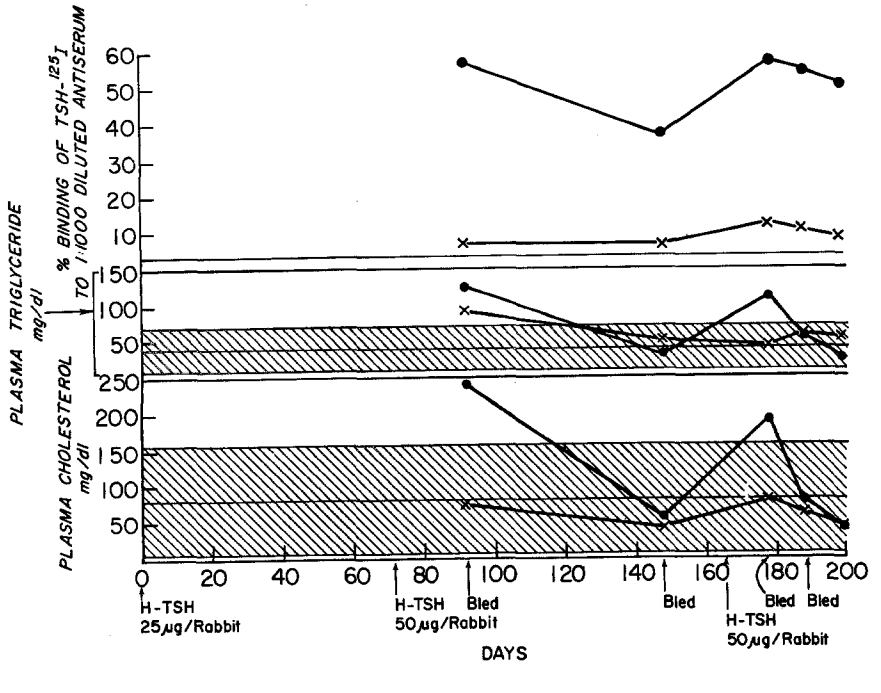

·—·—·, Rabbit No. S-543; X—X—X, rabbit No. S-544. Shaded area + mean ± 2 SD.

Discussion. Immunization of the rabbits with human TSH is associated with transient elevations of serum lipids. The exact mechanism for this observation is not answered by this study. The apparent correlation of the lipid levels with the H-TSH antibody titers suggest that H-TSH antibodies may be cross reacting with the rabbit TSH, thus leading to hypothyroidism secondary to TSH insufficiency. Hypothyroidism is well-known to result in hypercholesterolemia[5] and hypertriglyceridemia[6,7]. The mechanism suggested by our data may not, however, entirely explain the hyperlipidemia. It has been stated that hypothyroidism secondary to pituitary insufficiency does not lead to hypercholesterolemia[8]. Further work is therefore necessary to understand the pathogenesis of this somewhat unusual form of experimental hyperlipidemia.

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Induction of phycoerythrin in Anabaena ambigua Rao and its strains

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Summary. Phycoerythrin was not present in Anabaena ambigua Rao, and in its strains, Lh- and Ha-forms under normal culture conditions, but it developed in parent and Lh-form when treated with green light and nitrate. However, Ha-form appeared to be unable to form phycoerythrin.

In many of the blue-green algae, the red pigment phycoerythrin may not be present under general growth conditions, but its formation can be induced by a special treatment. However, some algae may not have the ability at all to synthesize the red pigment, due to the absence or loss of genes concerned with its formation. The present work describes for the first time induction of phycoerythrin in strains of blue-green alga Anabaena ambigua.

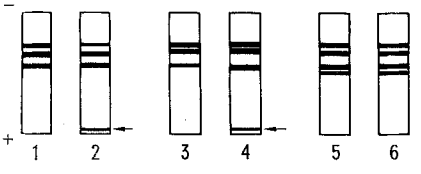

Fig. 1. Polyacrylamide gel electrophoresis of the phycobilin extracts of untreated strains, parent strain (1); Lh-form (3); Ha-form (5), and treated parent strain (2); Lh-form (4); and Ha-form (6) for the formation of phycoerythrin; → phycoerythrin band.

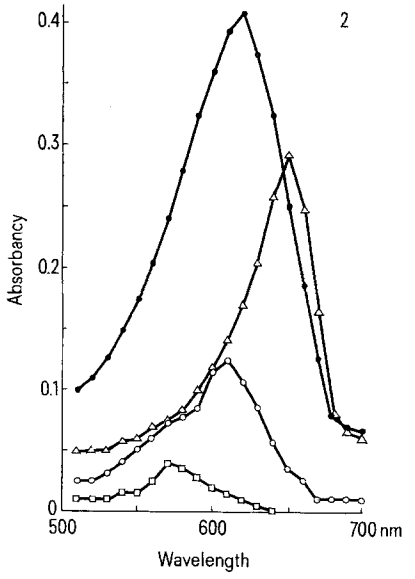

Fig. 2. The pigment bands obtained on gels (after the induction of phycoerythrin in A. ambigua) were cut separately and were eluted. The absorption spectra for individual fractions were determined; △—△ band No. 1; ○—○ band No. 2; ●—● band No. 3; and □—□ band No. 4.

Both phycoerythrin and phycocyanin are synthesized from a common precursor and this process is dependent on light in the presence of available nitrogen (nitrate) as shown in the case of Tolypothrix tenuis[2]. Phycoerythrin synthesis was strongly induced by blue-green light (541 nm), whereas synthesis of phycocyanin by red light (641 nm). These studies indicated that the common precursor can be switched towards the synthesis of either pigment by illumination with a wavelength close to its absorption maximum.

The ability to synthesize phycoerythrin was tested by giving a treatment as described by Fujita and Hattori[2] in A. ambigua strains, parent form, Lh-form and Ha-form, which do not contain phycoerythrin (Lh-form was originally obtained as natural mutant of A. ambigua, and Ha-form was obtained by treating the parent strain with hydroxylamine hydrochloride[3].)

The exponentially growing algal material, washed with sterile double-distilled water, was preincubated in Allen and Arnon[4] medium (without combined nitrogen) for 20 h in fluorescent tube light, and then it was treated with green light (541 nm) for 60 min and subsequently incubated in darkness for 24 h in the presence of potassium nitrate (0.4 mg per ml). In the parent and as well as in Lh-form, phycoerythrin formation was induced which can be seen as a distinct band on the polyacrylamide gels during the separation by electrophoresis (figure 1). However, Ha-strain did not show phycoerythrin formation. Evidently, the Ha-strain is a biochemical mutant that has lost the ability to synthesize the red pigment. The red band phycoerythrin was further characterized by determining its absorption spectrum, and showing the absorption peak at 570 nm (figure 2), which is consistent with absorption peak of diluted phycoerythrin[5].

1 My thanks are due to Atomic Energy Commission, India, for the financial assistance.
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