Gel filtration chromatography gave 2 active peaks with molecular weights of 27,000 and 52,000 daltons (figures 4 and 5). We also observed that in early cultures mainly the high molecular KA was released, and in late cultures mainly the low molecular KA. No other mol.wt determinations of preparations from kidney cultures are on record. For urinary preparations, various mol. wts have been reported. Lesuk et al.[3] prepared urokinase with a mol.wt of 54,000. White et al.[4] found 2 fractions with mol.wts of 31,500 and 54,700, respectively, and Kok and Astrup[17] 2 fractions of about 43,000 and 54,000. Using Sephadex separation Doleschel and Auerswald[18] found 3 active peaks for fractions with the mol.wts of 27,000, 54,000 and 104,000. Recently Ogawa et al.[5] reported the mol.wt to be 33,000, Johnson et al.[19] 2 forms *viz* 33,500 and 47,000, and Holmberg et al.[15] 2 forms *viz* 33,000 and 54,000. This indicates the existence of 2 molecular forms of about 30,000 and 50,000 daltons in urinary preparations. In our study, in which the KA was obtained from organ cultures, i.e. with the kidney cells in their histotypical arrangement, the 2 mol.wt fractions thus seem to correspond to those found in the urinary preparations.

17 P. Kok and T. Astrup, Biochemistry *8*, 79 (1969).
18 W. Doleschel and W. Auerswald, Med. Pharmac. exp. *16*, 225 (1967).
19 A. J. Johnson, M. E. Soberano and E. B. Ong, in: Serano Symposia, p. 21. Milan.

On urea formation in marine mammals[1]

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Summary. Ornithine carbamoyltransferase (EC 2.1.3.3) has been determined in homogenates of liver of the sei whale (Balaenoptera borealis), the bottle-nose dolphin (porpoise) (Tursiops truncatus) and California sea lion (Zalophus californianus). These marine mammals show levels of this ornithine-urea cycle enzyme which are typical of terrestrial mammals.

All terrestrial mammals studied possess in liver the enzymes for the biosynthesis of urea via the ornithine-urea cycle[2]. It is established that terrestrial mammals excrete urea in the urine as the principal nitrogenous waste product[3]. Only a few reports are available on the urinary nitrogen components of marine mammals, however. In some early work, Schmidt-Nielsen and Holmsen[4] made determinations on nitrogenous components of the urine of several sei whales (Balaenoptera borealis) and found that urea was the major nitrogenous material. In one specimen, for example, urea-N constituted 93% of the total urinary nitrogen, while lesser amounts were found partitioned among ammonia-N (3.3%), protein-N (1.9%), uric acid-N (0.58%) and creatine-N (0.45%) (calculated from the original data by Brown[5]). Most of the urinary nitrogen in harbor seals (Phoca vitulina)[6] and in northern fur seals (Callorhinus ursinus)[7] is accounted for by urea. The urea content of the urine of the fasting humpback whale (Megaptera nodosa) reported by Bentley[8] suggests that this marine mammal is also primarily ureotelic.

Ornithine carbamoyltransferase of marine mammals (liver, 38 °C)

System	μmoles citrulline produced in 15 min		
	Balaenoptera borealis*	Tursiops truncatus**	Zalophus californianus***
Complete	6.34 ± 0	2.58	2.99
Less ornithine	0.25	0.01	0.01
Less carbamoylphosphate	0.14	0.07	0
Boiled homogenate	0.21	0.03	0.02

*Homogenate in cetyltrimethylammonium bromide representing 3.0 mg liver; triplicate determination. Male specimen; 14 m body length. **Homogenate in water representing 0.5 mg liver. Male specimen, 114 kg; 3 m body length. ***Homogenate in water representing 0.5 mg of liver. Adult specimen.

One of the 5 enzymes responsible for the biosynthesis of urea by the ornithine-urea cycle is ornithine carbamoyltransferase (EC 2.1.3.3) which catalyzes the following reaction:

Ornithine + carbamoylphosphate = citrulline + P_i.

This report provides evidence for the occurrence of ornithine carbamoyltransferase in the liver of 3 marine mammals: the sei whale, Balaenoptera borealis; the bottle-nose dolphin (porpoise), Tursiops truncatus; and the California sea lion, Zalophus californianus.

Materials and methods. Homogenates of liver (10% w/v in water or in 0.1% cetyltrimethylammonium bromide) were assayed for ornithine carbamoyltransferase as described elsewhere[9]. The complete incubation system contained Na glycylglycine buffer (45 mM), L-ornithine

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2 G. W. Brown, Jr, and P. P. Cohen, Biochem. J. *75*, 82 (1960).
3 P. L. Altman and D. S. Dittmer (Ed.), Biology Data Book, vol. 3, tables 185 and 186. Fed. Am. Soc. exp. Biol., Bethesda, Maryland 1974.
4 S. Schmidt-Nielsen and J. Holmsen, Arch int. Physiol. *18*, 128 (1921).
5 G. W. Brown, Jr, in: Biochemical and Biophysical Perspectives in Marine Biology, vol. 3, p. 319. Ed. D. C. Malins and J. R. Sargent. Academic Press, New York and London 1976.
6 H. W. Smith, J. cell. comp. Physiol. *7*, 465 (1936).
7 M. C. Keyes, E. J. Barron and J. Ross, J. Am. Vet Med. Ass. *159*, 567 (1971).
8 P. J. Bently, Comp. Biochem. Physiol. *10*, 257 (1963).
9 G. W. Brown, Jr, and P. P. Cohen, J. biol. Chem. *234*, 1769 (1959).

(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.

10 R. M. Archibald, J. biol. Chem. *156*, 121 (1945).
11 S. Ratner, in: Methods in Enzymology, vol. 1, p. 356. Ed. S.P. Colowick and N. O. Kaplan. Academic Press, New York 1955.

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-

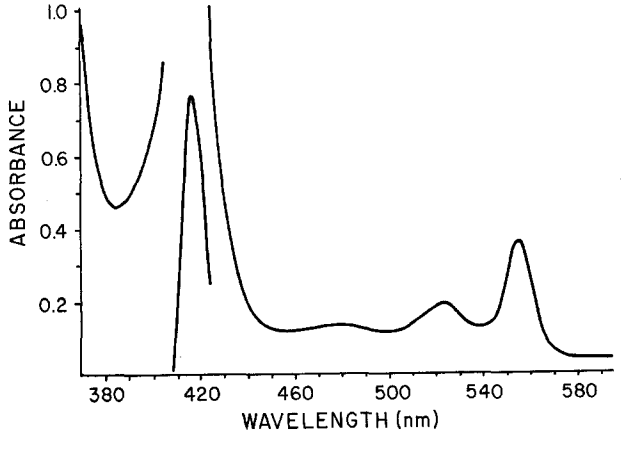

Fig. 1. Electronic absorption spectrum of protoheme-pyridine hemochrome in water-pyridine-1.0 M KOH (4 : 1 : 0.5 v/v).

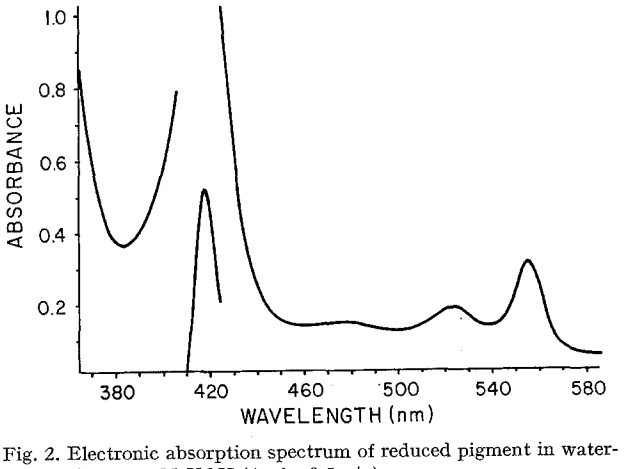

Fig. 2. Electronic absorption spectrum of reduced pigment in water-pyridine-1.0 M KOH (4 : 1 : 0.5 v/v).