Note

Neutral carbohydrates in the milk of the Bottlenose dolphin (*Tursiops truncatus*)

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Reports on the milk carbohydrates of marine-mammals are comparatively rare, though *inter alia*, the chemical composition of the milks from Harp seal (*Pagophilus groenlandicus*)[1], Polar bear (*Thalarctos maritimus*)[2], Dolphin (*Tursiops truncatus* and *Prodelphinus plagioden*)[3], Fin whale (*Balaenoptera physalus*)[4], and the California sea lion (*Zalophus californianus*)[5] have been reported. In all cases except the last, no attempt was made to define the individual sugars besides lactose. The work on the California sea lion reported that, in addition to lactose, a component (at a concentration of 0.025%) which had about the same chromatographic mobility as D-glucose (in an undefined solvent system) was present in the natural milk. General chemical results indicate that, compared to bovine milk, sea-mammal milk tends to be much richer in fat and protein, and contains considerably less lactose. White[6] has suggested that this is characteristic of mammals whose young have a rapid rate of growth.

The only previous results on the dolphin (*Tursiops truncatus*) studied in this paper, were those of Eichelberger *et al.*[3], who found in 1940, that the sugar content of the milk from two individual dolphins was 0.773 and 0.388 g/100 ml; the latter dolphin, however, was nearing the end of lactation, and the results are not comparable. The fat contents of the milk of three dolphins were 16.7, 14.8, and 10.8 g/100 ml. The sugar and fat contents of human milk are 6.29 g and 3.76 g/100 ml, respectively. The work reported in this paper was designed primarily to investigate the non-lactose carbohydrates in the milk of marine mammals, to complement the extensive work which has already been done on the minor sugar components of bovine and human milk, and to observe similarities in carbohydrate patterns in mammalian milks of terrestrial and marine origin.

EXPERIMENTAL

Methods and materials. — The milk was obtained within 30 min of death, and immediately deep-frozen to minimize enzymic or bacterial breakdown. The sample was collected, by expression, from a dolphin 289.2 cm long and weighing 279 kg, which was captured on February 24th, 1969 in a position Lat 28° 11′ N, Long

79° 21′ W. Estimates based on the length of an accompanying calf indicated that the milk was collected during a late stage of lactation[7].

Pre-treatment of a portion of the milk to remove the lipid and fatty acid material was undertaken at the Fisheries Research Board of Canada Station at Halifax, N.S., using a modification[8] of the Bligh and Dyer extraction procedure[9]. This frozen, defatted milk (250 ml), as received in the author's laboratory, had been considerably diluted with water during the extraction procedure; it had originated from 16.5 g of milk.

Paper chromatography was performed on Whatman No. 1 paper with ethyl acetate–pyridine–water (10:4:3 v/v). Carbohydrates were revealed by the *p*-anisidine hydrochloride reagent[10], or the alkaline silver nitrate dip[11]. Mobilities are given relative to that of D-glucose. Gas–liquid chromatography was performed on a Pye Series 104 Model 64B gas chromatograph (with flame-ionization detector) used as a single-column instrument. Peak areas were calculated by triangulation and converted, taking into account previously calculated minor differences in the response of standard compounds to the flame-ionization detector. Trimethylsilyl ethers of the isolated carbohydrates were analyzed isothermally[12] at 230°, on a column [5 ft (1.52 m) × 0.25 in (3.16 mm) internal diameter] of 10% SE-30 on non-acid washed Chromosorb W with a helium flow-rate of 100 ml/min. Acetylated alditols were obtained and chromatographed as previously described[13]. Retention times for the trimethylsilyl ethers and the acetylated alditols were measured relative to trimethyl tetra-*O*-(trimethylsilyl)-α-D-glucopyranoside, and hexa-*O*-acetyl-D-glucitol, respectively. A standard mixture of *myo*- and *scyllo*-inositol was prepared by sodium borohydride reduction of 2,4,6/3,5-pentahydroxycyclohexanone (*myo*-inosose-2)[14].

Isolation of the neutral carbohydrates. — The frozen, defatted, and diluted milk (250 ml) was thawed, and centrifuged at 6500 *g* for 30 min. The solids, which contained no carbohydrate, were re-extracted with water (20 ml) and discarded. Acetone was added to the combined supernatants to a concentration of 50% and the mixture was centrifuged. The supernatant was evaporated to a syrup, which was redissolved in water, and the solution was passed successively through columns of Rexyn 101 (H^+) and Duolite (OH^-). The eluate was freeze-dried, to give a white powder (297 mg).

A portion (100 mg) was hydrolyzed in 0.5M sulfuric acid for 6 h at 100°. The solution was neutralized with barium carbonate, and, after filtration, evaporated. The resulting syrup (88 mg) was separated into four fractions on a cellulose powder (Whatman) column (30 × 2 cm), using butyl alcohol–water mixtures for elution. The first three fractions were compared with standards, both on paper and gas–liquid chromatography, and shown to be identical with glucose (30 mg), an equal mixture of glucose and galactose (20 mg), and galactose (26 mg). The fourth fraction (5 mg) had an R_{Glc} value of 0.48 on paper chromatography, gave a slightly elongated spot, positive to ammoniacal silver nitrate, but not to *p*-anisidine hydrochloride, and appeared to be optically inactive. Gas–liquid chromatography of the trimethylsilyl ethers of fraction 4 afforded two peaks with retention times 1.59 and 1.96, relative to the standard. G.l.c. of the acetylated fraction 4 gave one peak only, with a retention time of 1.19 relative to hexa-*O*-acetyl-D-glucitol. An identical, single peak was obtained when the fraction was reduced with sodium borohydride prior to acetylation and analysis.

RESULTS AND DISCUSSION

Initial quantitative and qualitative analyses of the milk sugars, both by g.l.c. of the trimethylsilyl ethers and by paper chromatography, showed that the identity of the major component was lactose, and that one of the minor components was glucose. In addition to these two components, there was also present an additional compound which gave two definitive g.l.c. peaks as the trimethylsilyl ethers (Table I), but no galactose was detected. Acid hydrolysis left the unidentified component(s) unchanged. D-Glucose and D-galactose were released in the amounts to be expected from the breakdown of lactose. In all probability, the unknown component, which on reduction and acetylation afforded one g.l.c. peak corresponding to 6.4% of the total neutral carbohydrate, is the same component which, as a trimethylsilyl derivative, gives two peaks, totalling 6.8% of the total carbohydrate (Table I). The evidence thus strongly indicates that the component was either an alditol or cyclitol.

TABLE I

QUANTITATIVE ANALYSIS OF THE DOLPHIN MILK SUGARS PRIOR TO, AND AFTER ACID HYDROLYSIS[a]

Sugar	*Before hydrolysis*	*After hydrolysis*[b]
Glucose	3.7	48.2
Galactose	—	45.1
Lactose	89.5	—
scyllo-Inositol (R_{std} 1.59)	2.0	2.3
myo-Inositol (R_{std} 1.96)	4.8	4.4

[a] By g.l.c. of the trimethylsilyl ethers. Results expressed in % of total neutral carbohydrates. [b] Comparable results obtained from g.l.c. of the acetylated alditols after hydrolysis showed glucose (48.0%), galactose (45.5%), and the two unknown peaks combined (6.4%).

Partition chromatography of the hydrolyzate, allowed the separation and identification of glucose and galactose, but the unknown component was eluted as a single band (Fraction 4), essentially homogeneous on paper chromatography and showing an R_{Glc} 0.48 similar to that of the heptitols and of most of the inositols. The g.l.c. data of the acetylated fraction did not agree with those of acetylated heptitols[15], whereas comparison of the trimethylsilyl derivative with trimethylsilylinositols[16–18] suggested that the unknown peaks were due to *myo*-, *scyllo*-, or *epi*-inositol. Gas–liquid chromatography of the trimethylsilyl ethers of an authentic *scyllo*- and *myo*-inositol mixture gave peaks at R_{std} 1.59 and 1.96, respectively. The order of elution is in agreement with previously published data[18], as are their retention times relative to each other on SE-30 (0.81). Paper chromatography of fraction 4 and g.l.c. of the acetylated fraction did not distinguish between *myo*- and *scyllo*-inositol. The neutral carbohydrates present in the dolphin milk are thus (by quantitative analysis of the trimethylsilyl ethers on g.l.c.): Lactose (89.5%), glucose (3.7%), *myo*-inositol (4.8%), and *scyllo*-inositol (2.0%).

Although *myo*- and *scyllo*-inositol have on many occasions been found together in tissues, and are regarded as metabolically related, the ratio of *myo*- to *scyllo*-inositol in dolphin milk (2.4:1) is considerably different from that reported for rabbit tissues[19] which varied from 6.6 to >70:1, with most tissues having a ratio greater than 20:1. The presence of free D-glucose in the milk does indicate the possibility that the *myo*-inositol may be synthesized in a way similar to that of rat testicular *myo*-inositol[20]. *scyllo*-Inositol on the other hand, although it can be formed from *myo*-inositol[21], is more likely to be of dietary origin. Dietary intake of *scyllo*-inositol into biological systems is widely accepted, and small cartilagenous fish, a logical source of food for the dolphin, are known to have *scyllo*-inositol as the principal cyclitol[22].

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