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CHEMICAL ANALYSES OF AMERICAN OPOSSUM (*DIDELPHYS VIRGINIANA*) MILK*

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Abstract—1. American opossum milk contains more solids, fat and protein, and less carbohydrate, than eutherian milks. This finding agrees with values reported for several other marsupial species.

2. Analysis of the carbohydrate fraction shows free galactose but no free glucose. The ratio of bound galactose to bound glucose is 5 : 1.

INTRODUCTION

MARSUPIAL milk appears to differ from eutherian milk in the percentage composition and the character of several components. Analyses of milks obtained from the kangaroo (*Macropus robustus*) and the common Australian opossum (*Trichosurus vulpecula*) were reported by Bolliger & Pascoe (1953), Gross & Bolliger (1958, 1959) and Bolliger & Gross (1960). Their data showed that the milks of both marsupial species contained higher percentages of solids, fat and protein, and lower percentages of carbohydrate, than are found in the milks of most eutherians. In addition, these authors reported that the major carbohydrate component of the marsupial milks was galactose rather than glucose. Jenness *et al.* (1964) examined the dialyzable carbohydrates obtained from the milks of a variety of mammals including several marsupial species (*Didelphys virginiana*, *Macropus rufus* and *Setonix brachyurus*). The authors concluded that lactose was not the predominant milk sugar and that free glucose, galactose and oligosaccharides yielding glucose and galactose on hydrolysis were present in the marsupial milks. Summaries of data on the chemical composition of eutherian milks are available in the literature (Spector, 1956; Dittmer, 1961).

This paper is a report of data obtained on the percentage composition of various ingredients and on the type of carbohydrates obtained with milk from the American opossum (*D. virginiana*).

MATERIALS AND METHODS

During the early stages of lactation, milk was expressed into capillary collection tubes by gentle manipulation of the opossum teat. In the later stages of lactation,

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the mothers were injected subcutaneously with 10 U.S.P. units of oxytocin; and the milk was withdrawn into an inverted glass dropper (connected by polyethylene tubing to a 30-ml syringe) placed over an engorged teat. A slight negative pressure was applied along with gentle manual expression. About 4–5 ml of milk could be withdrawn in this manner. The milk was stored frozen until use.

The sheep milk for the analyses reported in this paper was collected from one lactating ewe of the animal colony at 8 and 17 days *post partum*.

Total solids were determined on a weighed sample of milk by dehydration at 100°C to constant weight. Milk lipids were measured by a modified Babcock test. Protein was quantitated by the biuret method. Protein-free filtrates were prepared by tungstic acid precipitation for the carbohydrate determinations. Hydrolysis of the carbohydrate determinations was performed by heating the protein-free filtrates in N sulfuric acid to 100°C for 30 min and neutralizing with barium hydroxide. Reducing sugars were estimated with the Folin–Wu method, glucose with the glucose oxidase reaction, and galactose with the galactose oxidase reaction. The pH was measured with a pH meter. Osmolality was determined with a freezing-point type of osmometer.

Paper chromatograms for carbohydrate detection were prepared with a solvent system of *n*-propanol and water (4 : 1) and a spray of aniline hydrogen phthalate.

An ultrafiltrate from the pooled blood serums of four male opossums was obtained by suction through a dialysis membrane stretched over a nylon frame. The apparatus was kept at 6°C during the filtration.

RESULTS AND DISCUSSION

Table 1 is a summary of data for various components in twenty-one milk samples taken from ten opossums; nineteen samples were collected between 18 and 110 days *post partum* and two samples at 3 and 5 days *post partum*. Because the volume of some samples was small, all of the analyses could not be performed with each sample. The range of values appears to be rather broad, an effect that may be due partially to the *post-partum* collection time of the milk. Bolliger & Gross (1960), for example, found that fluctuation in the protein content of *Trichosurus vulpecula* milks occurred in relationship to stages of lactation, which were grouped as transitional, mature and weaning. In the analyses reported herein, most of the samples tested were considered to be mature milks. Note that, in Table 1, the sum of the means of the major ingredients (23·0 per cent) apparently accounts for nearly all of the solids content (23·2 per cent).

Two comparisons were made with the data obtained. Table 2 is a comparison of the results for three marsupial species, and Table 3, for sheep and opossum milk analyses. From Table 2, it is evident that there is good agreement among the three marsupial species in the percentages of the various ingredients of the milks. The similarity of the marsupial data is even more striking when compared with the data for sheep milk shown in Table 3. The percentage of solids, protein, fat and ash is lower, and the carbohydrate higher, for the sheep milk than for the opossum milk. The data for sheep milk agree with the published data for sheep and related eutherians (Spector, 1956; Dittmer, 1961).

TABLE 1—ANALYSES OF MILK FROM TEN OPOSSUMS
(*D. virginiana*)

Constituent	Units	No. of samples	Range	Mean content	± Standard error of mean
Solids	g/100 g	14	9·4–31·3	23·2	0·174
Protein	g/100 ml	13	6·7–11·8	8·40	0·430
Fat	g/100 ml	13	4·9–17·9	11·3	1·12
Ash	g/100 ml	7	1·00–2·28	1·72	0·184
Carbohydrate	g/100 ml	13	0·73–3·15	1·59	0·195
pH		8	6·47–7·04	6·74	0·115
Osmolality	m-osmol/kg	3*	290; 308; 314	—	—

* Six other samples gelled on freezing; reliable readings not obtainable.

TABLE 2—COMPARISON OF AVERAGE MILK COMPOSITION FOR THREE MARSUPIAL SPECIES

Source of data	Marsupial species	Solids (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)
This report	*Didelphys virginiana*	23·2	8·4	11·3	1·6	1·7
Bolliger & Pascoe (1953)	*Macropus robustus*	23·5	9·8	8·1	1·4	1·6
Gross & Bolliger (1959)	*Trichosurus vulpecula*	24·5	9·2	6·1	3·2	1·6

TABLE 3—COMPARISON OF SHEEP AND OPOSSUM (*D. virginiana*) MILK ANALYSES

Animal	Lactation (days *post partum*)	Solids (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	pH	Osmolality (m-osmol/kg)
Sheep	8	20·1	5·45	7·05	4·50	0·67	6·63	297
Sheep	17	20·0	5·09	6·95	4·50	0·59	—	—
Opossum	5–110	23·2	8·40	11·3	1·59	1·72	6·74	290; 308; 314

Reports on the distribution and kind of carbohydrates indicate that lactose is not the principal sugar present in the milk of several marsupial species. Gross & Bolliger (1958, 1959) have identified lactose, galactose and a galactan in *T. vulpecula* milk; the lactose constitutes about 30 per cent of the carbohydrate fraction. Jenness *et al.* (1964) investigated by chromatography the carbohydrates in the milks of fifty-two mammalian species, including three marsupial species.

TABLE 4—ANALYSIS OF SEVERAL CARBOHYDRATE COMPONENTS OF MILK FROM ONE OPOSSUM (*D. virginiana*)

Lactation (days *post partum*)	Reducing sugars (%)		Glucose (%)		Galactose (%)	
	Free	After hydrolysis	Free	After hydrolysis	Free	After hydrolysis
110	1·38	2·52	0	0·16	0·69	1·80
111	0·62	3·35	0	0·20	0·43	2·25
112	0·80	3·04	0	0·25	0·69	1·85
114	1·34	3·17	0	0·29	0·61	1·74
115	0·96	3·39	0	0·45	0·43	1·96
Mean ± S.E.M.*	1·02 ± 0·148	3·09 ± 0·157	0	0·27 ± 0·051	0·57 ± 0·059	1·92 ± 0·090

* Standard error of the mean.

These authors found that the usual carbohydrate components in the milk of eutherians are lactose, a trisaccharide, glucose, galactose and various oligosaccharides. In contrast, the three marsupial species (*Didelphys virginiana*, *Macropus rufus*, *Setonyx brachyurus*) produced milks containing free glucose and galactose, and oligosaccharides yielding both glucose and galactose on hydrolysis; some lactose appeared to be present.

In this study, the paper chromatograms, which were prepared from the unhydrolyzed protein-free filtrates of *D. virginiana* milks, indicated only galactose and three spots of oligosaccharides. No lactose, glucose or pentose spots were evident. The protein-free filtrates were further investigated with specific enzyme procedures to quantitate the glucose and galactose contents. Data obtained with five serial collections of milk from one opossum are given in Table 4. Free galactose, but no free glucose, was detected in the unhydrolyzed filtrates. Approximately 62 per cent of the reducing sugars after hydrolysis were galactose and only 8·7 per cent were glucose. The galactose and glucose accounted for about 71 per cent of the reducing substances. It should be recalled that the Folin–Wu test also responds to hexuronic acids, uric acid, glutathione and other reducing compounds that may be present in milk. Calculation of the data showed that the average ratio of the bound galactose to the bound glucose was approximately 5 : 1.

A clue was sought concerning the origin of the galactose in the carbohydrate fraction of the opossum milk. If the blood was a normal carrier of galactose to the mammary glands, galactose ought to be present as a compound of opossum blood. An ultrafiltrate was prepared from the pooled serums of four male opossums. This filtrate was tested for galactose and glucose by their specific enzyme reactions. The analyses showed the presence of 0·73 per cent glucose but no galactose. It was concluded that galactose of the opossum milk presumably originates in the mammary gland cells.

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