duce positive results by methods 1 or 2. Other studies (*10*) demonstrate no correlation between the detection of "viruslike" particles by electron microscopy (*1*) and detection of "reverse transcriptase" by methods 1 or 2.

The data of Table 1 show that the occurrence of "reverse transcriptase" was not significantly higher in milk donors with a positive family history of breast cancer or any other cancer than in donors with normal histories. Donors in this study were sampled only once, and few donors with positive family histories had more than one relative with breast cancer. However, the lack of correlation is not due to small numbers of positive determinations but to an equal distribution of positives among the three classes.

Thus, there is no correlation of the "viruslike" enzyme activity observed in human milk with a positive family cancer history of the donor. The enzyme activity being measured may still be virus-associated. If so, then the presence of a viral agent containing this enzyme might be a necessary cause but could not be sufficient cause for the development of breast cancer.

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Milk Carbohydrates of the Echidna and the Platypus

Abstract. *The principal neutral carbohydrate of three samples of echidna milk was identified as a trisaccharide, fucosyllactose. That of a sample of platypus milk was a tetrasaccharide, difucosyllactose. Free lactose was found in small amounts only. The milk carbohydrate of monotremes is distinguished from that of both marsupials and placental mammals by its high fucose content.*

The principal carbohydrate of the milk of placental mammals is lactose (*1*). Marsupial milk contains oligosaccharides that, upon acid hydrolysis, yield mainly galactose (*2*), but the identity of the carbohydrate of the milk of monotremes (egg-laying mammals) has been in doubt (*3*, *4*). We now report on the carbohydrate composition of milk of the echidna (*Tachyglossus aculeatus*) and the platypus (*Ornithorhynchus anatinus*).

Echidna milk sample 1 was from an animal caught in October 1969 on Kangaroo Island, South Australia; its pouch young weighed 300 g. The other two samples were from one animal from Rankin Springs, New South Wales, which was milked in October 1971 (sample 2a) and again in November 1971 (sample 2b), when its pouch young weighed 150 and 312 g, respectively. The platypus milk was from an animal caught in the Bendora Dam, Australian Capital Territory, on 9 February 1972. Milking was done by squeezing the mammary glands immediately after the injection of synthetic oxytocin (*5*).

After the removal of fat and protein (*6*), the samples were analyzed for total carbohydrate (*7*), sialic acid (*8*), and fucose (*9*), and the results were compared with those we obtained with milk from three different species of marsupials (Table 1). Echidna milk was rich in sialic acid, in which respect it resembled marsupial milk; but of the samples examined only those of the monotremes contained detectable amounts of fucose.

For the separation and partial identification of the milk carbohydrates, we used a column of Sephadex G-15 gel that had been calibrated with maltodextrins and other small saccharides. Neutral carbohydrates were eluted in order of decreasing molecular size and could be separated from each other (Fig. 1), but those containing sialic acid appeared earlier than expected, presumably because of the exclusion effect exerted by negative charges in the Sephadex gel (*10*).

Figure 1a shows the elution pattern obtained with echidna milk sample 2a; the patterns obtained with samples 1 and 2b were very similar. The material was resolved into five peaks, of which the first contained some protein and the second contained sialyllactose. The other three peaks contained neutral carbohydrates which were eluted at volumes (V_e/V_0) corresponding to those of tetra-, tri-, and disaccharides, respectively. Peaks 3 and 4 contained fucose. The carbohydrate of platypus milk was also separated into five peaks (Fig. 1b); the major fraction (peak 4) emerged at a volume expected for tetrasaccharides and contained fucose. Peak 5 was a disaccharide.

The column fractions containing the neutral carbohydrates were lyophilized and studied qualitatively by paper chromatography and quantitatively with the aid of glucose oxidase and galactose dehydrogenase (*11*). The milk disaccharides of both species cochromatographed with lactose and were hydrolyzed to glucose and galactose by β-galactosidase. Hydrolysis with 0.5*M* sulfuric acid (2 hours at 100°C) yielded only glucose and galactose in equimolar amounts. The disaccharides were therefore identified as lactose. The trisaccharide from echidna milk (peak 4 in Fig. 1a) cochromatographed with authentic 2′-fucosyllactose; was resistant to β-galactosidase; and yielded glucose, galactose, and fucose in the molar ratio 1 : 1 : 1 during hydrolysis with sulfuric acid. Partial hydrolysis with 1*M* acetic acid (2 hours at 100°C) resulted in the formation of lactose, fucose, and traces

Table 1. Carbohydrate composition of milk from the echidna, the platypus, and three species of marsupials: gray kangaroo, *Macropus giganteus*; brush-tailed possum, *Trichosurus vulpecula*; and short-nosed bandicoot, *Isoodon macrourus*.

Animal	Carbohydrate (grams per 100 g of milk)		
	Total free carbohydrate*	Sialic acid	Fucose
Echidna			
Sample 1	0.90		
Sample 2a	1.1	0.47	0.18
Sample 2b	1.0	0.43	0.17
Platypus	1.7	0.05	0.91
Kangaroo	5.9	0.43	< 0.01
Possum	6.4	0.33	< 0.01
Bandicoot	4.7	0.55	< 0.01

* Lactose used as standard.

of glucose and galactose; the lactose was identified by its susceptibility to β-galactosidase as well as chromatographically. The trisaccharide was therefore identified as fucosyllactose (*12*). The tetrasaccharides of echidna milk (peak 3 in Fig. 1a) and of platypus milk (peak 4 in Fig. 1b) both had the same chromatographic mobility as authentic 3,2′-difucosyllactose and were resistant to β-galactosidase. In each case, hydrolysis with sulfuric acid yielded glucose, galactose, and fucose in the molar ratio 1 : 1 : 2. Partial hydrolysis with 0.05*M* oxalic acid (10 minutes at 100°C) resulted in the formation of lactose, fucose, and a substance with the same mobility as fucosyllactose. The milk tetrasaccharide of both monotremes was therefore identified as difucosyllactose (*13*).

Although fucosyl oligosaccharides appear to be absent from the milk of marsupials (Table 1) (*2*), small amounts occur in that of some placental mammals. Both fucosyllactose and difucosyllactose are minor components of human milk (*10, 12–14*), and fucosyllactose is also present in the milk of monkeys and dogs (*15*). However, our samples of monotreme milk contained much higher concentrations of these compounds (*16*). From the gel filtration data we calculate that fucosyllactose constituted 29 percent of the carbohydrate of echidna milk (mean of three samples); difucosyllactose was 13 percent of the carbohydrate of echidna milk and 56 percent of that of platypus milk. In contrast, the carbohydrate of human milk contains approximately 0.4 percent fucosyllactose and 0.04 percent difucosyllactose (*12–14*), while the milk of other species contains even smaller amounts (*15*). We conclude that the presence of relatively large amounts of fucosyl oligosaccharides is characteristic of monotreme milk.

Free lactose constituted 8 percent of the total free carbohydrate of echidna milk (mean) and 1 percent of that of the platypus milk, values corresponding to milk lactose concentrations of 0.1 percent or less, compared with 7 percent for human milk. Lactose is also virtually absent from the milk of several species of seals (placental mammals), but in these the milk is devoid of any carbohydrate (*17*). The lactosazone obtained from echidna milk in 1926 by Marston (*3*) was probably formed as a result of hydrolysis of the relatively labile fucosyl–lactose and sialyl–lactose bonds during preparation of the osazone, since this involved heating with acetic acid. However, it is possible that major variations in the amounts of lactose or other milk carbohydrates occur between individual animals or, as has been shown for the fatty acids of echidna milk (*5*), at different stages of lactation.

It has been suggested that a disaccharide (lactose) may have been favored during the evolution of placental mammals because a given weight of lactose exerts only about half of the osmotic pressure of the same weight of a monosaccharide, such as glucose (*1*). Since milk appears to be necessarily isotonic with plasma, this may be advantageous both in conserving energy during the secretion of milk and in ensuring an adequate supply of water to the young (*18*). The tri- and tetrasaccharides found in monotreme milk would similarly be even more advantageous, but this explanation fails to account for their remarkably high fucose content. Conceivably, fucose may be essential for the growth and development of the suckling monotreme.

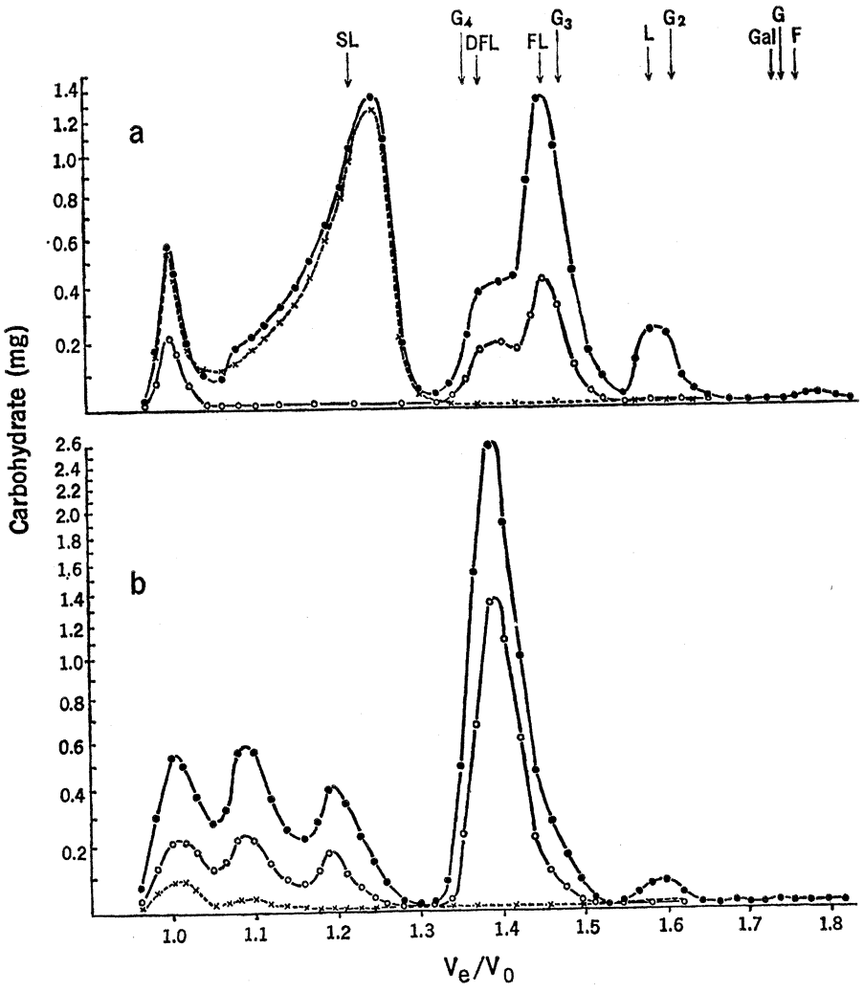

Fig. 1. Gel filtration of milk carbohydrates of the echidna (a) and the platypus (b). In each case, 15 mg of carbohydrate (lactose equivalents) were filtered through two columns of Sephadex G-15 (2.2 by 90 cm) connected in series. Elution was done with water at a flow rate of 15 ml/hour. Fractions of 4 to 5 ml were analyzed for total carbohydrate (●—●), sialic acid (X - - X), and fucose (○—○); V_e is the elution volume and V_o is the void volume, determined with blue dextran. Arrows show the V_e/V_o values of the oligo- and monosaccharides used for calibration of the column; G_4, maltotetraose; G_3, maltotriose; G_2, maltose; *G*, glucose; *SL*, sialyllactose; *DFL*, difucosyllactose; *FL*, fucosyllactose; *L*, lactose; *Gal*, galactose; and *F*, fucose.

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Hepatitis B Virus Antigen: Validation and Immunologic Characterization of Low-Titer Serums with ^{125}I-Antibody

Abstract. *Radioimmunoassay was used to determine the serologic subspecificities of 85 blood donor serums positive for hepatitis B virus–associated antigen. There was complete agreement with results obtained by immunoprecipitation of 43 serums. The remaining 42 serums were negative by immunoprecipitation but followed type-specific immunology by radioimmunoassay, and this served as a validation for authentic hepatitis B virus antigen.*

The use of highly sensitive testing procedures, such as radioimmunoassay, has established that the hepatitis B virus antigen occurs much more frequently among blood donors and hepatitis patients than has been found by less sensitive immunologic methods (*1*, *2*). Study of antigenic subspecificities as epidemiological markers is limited because most of the low-titered serums cannot be analyzed by immunoprecipitation in agar gels. Some difficulty is also encountered in assessing a serum that is positive by radioimmunoassay but cannot be confirmed by any other method. Both of these difficulties can be minimized if radioimmunoassay procedures are used to determine antigen subtype specificities. We now report that immunologic specificities established by immunoprecipitation were correctly identified by radioimmunoassay. In addition, many serums which were negative by immunoprecipitation were successfully subtyped by radioimmunoassay.

Soon after the discovery of Australia antigen and its association with hepatitis type B, Levine and Blumberg (*3*) showed evidence of the possible serologic heterogeneity of this antigen. Later, the nature of the heterogeneity was clarified by LeBouvier (*4*, *5*) and by Kim and Tilles (*6*). LeBouvier's characterizations suggest that three antigenic determinants are carried on antigen particles. One of these, designated a, is group-specific and is common to all antigenic types. Two others, designated d and y, are type-specific and are mutually exclusive. Although it was not well characterized, LeBouvier also indicated that a fourth determinant, x, was unequally shared by the antigenic subsets. Further classification of the determinants has been provided by Bancroft *et al.* (*7*), with the discovery of two new mutually exclusive determinants, w and r. Thus, three patterns emerge, ayw, adr, and adw. A putative ayr was not discovered by Bancroft, but has since been reported by Holland *et al.* and LeBouvier *et al.* (*8*).

The above immunologic characterizations were performed by immunoprecipitation with the micro-Ouchterlony technique. We have shown that ^{125}I-labeled specific immunoglobulin can be used in a highly specific and highly sensitive radioimmune procedure (*1*). The test takes advantage of the multiple combining sites on each viruslike particle. Briefly, a mixture of antibodies specific for a, d, and y determinants was fixed to a solid surface, usually polypropylene or polystyrene (test tubes). A test serum (0.1 ml) was pipetted into the tube and incubated at room temperature for 16 hours. During this period, antigen in the serum was fixed to the solid-phase antibody at the liquid-surface interface. The contents of the tube were then aspirated, the tube was washed, and ^{125}I-labeled antibody was then added. During the subsequent 90-minute incubation period at room temperature the purified radioactive antibodies were fixed at the available free combining sites on the antigen particles. The unreacted radioactive antibody was removed, and radioactivity in the tubes was counted. The count rate was in direct proportion to the amount of antigen in the original sample. For general detection of the antigen in an unknown sample by radioimmunoassay we used a mixture of antibodies to the known antigenic determinants. The antibodies generally were in unequal proportion, and the test showed a slightly greater sensitivity for ad subtypes as compared to the ay subtype. Ginsberg *et al.* (*9*) have used this differential feature of the test to correctly identify a number of antigen subtypes in serums previously characterized by agar gel diffusion. It is obvious that great specificity can be obtained if the labeled antibody consists of only a single serologic type, antibody to a, d, or y.

We have now purified and radiolabeled the group-specific antibody to a and the two type-specific antibodies to d and y. When these were individually used in our direct radioimmunoassay (as in Table 1), antigen-positive serums fell into the y or d subsets. This provided a method for the detection and immunologic characterization of antigen samples not amenable to characterization by immunoprecipitation; also it served to validate samples found