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Milk-Like Fluid in a Mammary Adenocarcinoma: Biochemical Characterization

Abstract. *The milk-like accumulation in the R3230AC mammary adenocarcinoma that follows treatment with estrogen contains lactose, fatty acids, and proteins with electrophoretic properties similar to those of casein and whey of rat milk. This mammary tumor retains the biochemical capacity of the mammary gland in its lactational response to administration of hormone.*

Studies in our laboratory of the hormone-responsive, transplantable, R3230AC mammary adenocarcinoma have demonstrated that estrogen treatment of the tumor-bearing host results in many striking morphologic and biochemical changes. Microscopic examination revealed extensive secretory activity and vacuolization, and histochemical procedures employing oil red O clearly demonstrated the presence of sizable quantities of lipids. It has been reported that administration of estrogen increased: glucose-6-phosphate dehydrogenase, malate dehydrogenase (decarboxylating), and phosphoglucomutase activities; concentrations of free fatty acids and triglycerides; and the RNA:DNA ratio (*1*). The fact that these changes can be prevented by concomitant administration of actinomycin D or cycloheximide suggests that these hormone-induced alterations occur by way of *de novo* synthesis of protein (*2*). Following excision and section of the tumors, a white fluid was readily expressed from the neoplasm. It was of interest, therefore, to examine this milk-like fluid for lactose, fatty acids, and casein, substances that characterize milk.

Tumor fluid was obtained by direct aspiration with a hypodermic needle and syringe from neoplasms of animals that had received subcutaneously estradiol valerate at 10 mg kg^{-1} $week^{-1}$ for 3 weeks; the yield was usually 3 to 5 ml of a whitish, viscous fluid. Rat milk was obtained from actively lactating Fischer rats (*3*). Fluid from tumors and milk were kept at −20°C pending analysis; both fluids were always treated identically.

Lactose was determined on the deproteinized filtrate by paper chromatography by the procedure of Roberts *et al.* (*4*). Under these conditions the tumor fluid contained a sugar identical in R_F with the lactose present in rat milk, as well as with a crystalline lactose standard. However, the content of lactose in the tumor fluid was approximately 0.06 percent, considerably below the 3 percent reported for rat milk (*5*). Shatten *et al.* also investigated the amount of lactose in this neoplastic fluid by a colorimetric procedure, finding 0.05 percent (*6*). It is of interest that Shatten, in her studies of galactose-synthesizing enzymes, observed that the activities of uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose epimerase were lower in the neoplasm than in the lactating mammary gland, an observation that would account for the low level of lactose found in the tumor fluid of these estrogen-treated animals.

The fatty-acid composition of this tumor fluid was examined and compared with that of rat milk. Samples were extracted with Folch reagent and

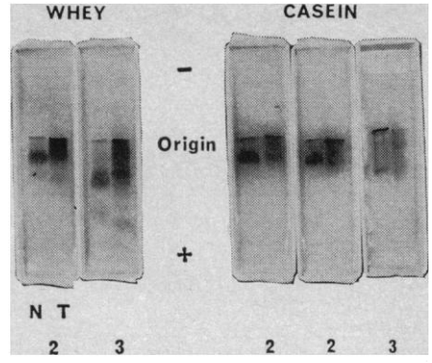

Fig. 1. Starch-gel electrophoresis of casein and whey proteins from rat milk and R323OAC mammary-tumor fluid. Samples were electrophoresed for 2 or 3 hours as indicated. Origin, anode, and cathode are indicated. Normal (*N*) rat milk is located at the left of each cup; tumor fluid (*T*), at the right.

the lipids were methylated directly, before separation by gas-liquid chromatography (*7*). The areas under the peaks were calculated by triangulation, and the data are presented as percentages, the total area being set at 100 (Table 1). The major components of rat milk analyzed in this manner were palmitic (16:0), lauric (12:0), oleic (18:1), myristic (14:0), linoleic (18:2), and stearic (18:0), in descending order of magnitude; they comprised 87 percent of the total. (The fatty acid ratios are of the numbers of carbons to numbers of unsaturated bonds.)

Analyses of fatty acids in the tumor fluid by this procedure revealed the following, in descending order of

Table 1. Fatty acids (percentages of totals) in rat milk and in tumor fluid from R3230AC mammary adenocarcinomas of rats being treated with estrogen. The fatty acids were extracted, methylated directly, and separated by gas-liquid chromatography. Areas under peaks were calculated by triangulation; total area was set at 100.

Fatty acids*	Content (%)	
	Rat milk	Tumor fluid
10:0	0.9	0.5
12:0	16.3	20.9
13:0	0.3	0.3
14:0	14.5	21.1
14:1	1.2	0.3
16:0	27.0	21.3
16:1	3.6	2.0
18:0	5.3	3.8
18:1	15.8	15.2
18:2	8.2	8.4
18:3	0.9	0.4
20:0	.3	.6
20:4	5.0	5.3
21:0	0.9	
22:0	.5	

* Expressed as the ratio of carbon atoms to the number of unsaturated bonds.

magnitude: palmitic, myristic, lauric, oleic, and linoleic; they also comprised 87 percent of the total. The results indicated that the relative concentrations of the shorter-chain fatty acids in the tumor fluid were higher than those of milk. A recent finding by Rees *et al.* was similar (*8*); they analyzed the fatty acids in the dimethylbenzanthracene-induced mammary carcinomas of animals being treated with estrogen, finding that the lipids from those neoplasms had a higher proportion of C_{10}-C_{14} fatty acids than had milk.

One of the most characteristic components of milk is casein, and its presence in milk and tumor fluid was determined. Whole rat milk was treated with acetate buffer, *p*H 4.6, to precipitate casein. The supernatant was collected, since it contains the whey proteins, and lyophilized. Both preparations were solubilized in 7*M* urea and subjected to starch-gel electrophoresis at *p*H 8.6 for 2 or 3 hours (*9*). The proteins were localized by staining with Ponceau S at 0.2 percent in a 3-percent solution of trichloroacetic acid. The tumor fluid was handled similarly, and both fluids in one cup were simultaneously subjected to electrophoresis (Fig. 1). A protein component in the tumor fluid proved similar in electrophoretic mobility to casein from rat milk, and two protein components in the whey fraction of the tumor fluid apparently showed electrophoretic properties similar to those of the whey proteins of rat milk. One should note that there were many more protein components in the tumor fluid than in milk.

These studies indicate that the milk-like accumulation in the R3230AC adenocarcinoma after treatment with estrogen contained several substances that occur in rat milk. I should point out that quantitative measurements of these components in the tumor fluid are subject to error, since the tumor fluid undoubtedly contained tissue and cell components—contaminants that could not be completely avoided because of the lack of a duct system in the neoplasm and because of the method used for collecting this fluid. However, my data demonstrate that this adenocarcinoma of the breast can produce a milk-like fluid under the influence of estrogen.

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Visual Accommodation in the Green Turtle

Abstract. *Ophthalmological and anatomical studies indicate that the Atlantic green turtle is extremely myopic when its eyes are out of water. This finding bears on current theories of visual orientation and navigation in these animals.*

Celestial navigation has been considered as a possible guidance mechanism underlying the impressive homing feats of the Atlantic green turtle (*Chelonia mydas*) (*1*), yet nothing is known about the visual acuity of these marine animals. The freshwater turtles, because they have exceptionally well-developed ciliary and iris musculature and a highly flexible lens, are able to accommodate over a wide dioptric range, and they have a high visual acuity in both air and water (*2*). However, the loggerhead turtle, *Caretta (Thalassochelys) caretta,* the only marine turtle whose vision has been examined, was found by Beer (*3*) to be highly myopic on land; this finding was confirmed by Walls (*4*).

Errors in refraction in two immature green turtles, weighing 1 kg and 17 kg, were measured retinoscopically. Measurements were made in air and also while the turtles were under water in a flat-sided, glass aquarium. For purposes of comparison, a freshwater turtle (*Clemmys insculpta*) and two land tortoises (*Gopherus polyphemus*) were also examined (Table 1).

Table 1. Refractive errors (in diopters) of three species of turtle measured in air and water.

	Air	Water
Chelonia mydas	40	0
	40	0
Clemmys insculpta	0	−1
Gopherus polyphemus	−5.5	−45 to −50
	−5.5	−45 to −50

The green turtles were approximately emmetropic when submerged in an aquarium, but extremely myopic while in air. In contrast, the tortoises were somewhat hyperopic in air and, like humans, were unable to overcome the loss of corneal refraction under water. As previously reported in the literature, the freshwater turtle adapted rapidly to both media.

Gross and microscopic examination of sections of the eyes of an adult green turtle and the eyes of a member of another species of marine turtle, the hawksbill (*Eretmocheyls imbricata*), extended and supported the conclusions of Walls (*4*) that marine turtles lack some of the efficient mechanisms of accommodation found in freshwater turtles. The ciliary processes did not impinge directly upon the body of the lens, and there was no apparent expansion of the sphincter iridis muscle into a powerful muscle of accommodation.

Except when surfacing to breathe and possibly to orient, the only time green turtles may need aerial vision is when the mature females go ashore to nest and when the hatchlings emerge from the nest and head for the ocean. Ehrenfeld and Carr (*5*) studied the sea-finding orientation of the green turtle and found this to be largely a visual process. They observed, however, no significant reduction in the ability of mature females to find the sea when they wore spectacles that contained light-diffusing filters. This is not surprising, since the retinoscopic measurements and anatomical evidence indicate that these animals could hardly have been using an orientation mechanism that depended upon the formation of sharp retinal images when on land.

The implications of the green turtle's limited visual acuity in air also extend to considerations of guidance mechanisms during their lengthy, open-sea migrations. Bi-coordinate star navigation no longer seems to be a realistic hypothesis. It is extremely unlikely that turtles can perceive stars or star con-