nificant red coloration) in run II. Thus the strength of the dynamics essentially controls the redness of a particular region: we therefore hypothesize that the coloration of the Great Red Spot is a natural consequence of strong vertical mixing in this locality, as modeled in run II. Phosphorus particles should also be visible in regions where we suspect the ammonia clouds are largely absent, such as the North Equatorial Belt (*5, 6*). The dynamics also clearly affect the PH_3 concentrations above 80 mbar (Fig. 2). In run I vertical transport times are much greater than PH_3 destruction times and PH_3 is severely depleted in the upper atmosphere.

The results presented here for Jupiter can be extrapolated to the other major planets (Saturn, Uranus, and Neptune), where the phosphorus mixing ratios are expected to be $\gtrsim 10^{-7}$. Phosphine may also conceivably be present in the atmosphere of Titan (a satellite of Saturn) and red phosphorus particles may be responsible for its observed red coloration (*36*). Specific models for these other atmospheres together with a consideration of possible cross products of PH_3, NH_3, and CH_4 photodissociation will appear elsewhere (*37*).

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Human α-Lactalbumin: Measurement in Serum and in Breast Cancer Organ Cultures by Radioimmunoassay

Abstract. *A sensitive and specific radioimmunoassay for human α-lactalbumin, a major milk protein, is described. Some normal men and women have detectable levels of α-lactalbumin in their blood. High values are found in nursing mothers and many patients with galactorrhea. α-Lactalbumin is found in some breast cancer organ cultures. In addition, α-lactalbumin output was stimulated by ovine prolactin in 2 of the 19 tumors studied.*

The physiology of the human breast has not been extensively studied. Current concepts of mammary endocrinology come mostly from animal studies and cannot always be applied to human problems, particularly those of breast cancer, breast feeding, and galactorrhea. Our interest in human breast physiology is a continuation of mouse breast bioassay studies in which we demonstrated that human prolactin was a molecule which was separate from growth hormone and could be measured in plasma (*1*). Prolactin plays a central role in rat breast growth and development (*2*), normal (*3*) and abnormal lactation (*1, 4*), and appears to influence the growth and spread of some experimental rat breast cancers (*5*). To date, the only well-described mechanism by which prolactin acts is its ability to stimulate milk protein synthesis (*6*). To study the effects of prolactin on the human breast, we have developed a sensitive and specific radioimmunoassay for human α-lactalbumin, a major protein of milk. The α-lactalbumin measurements reported here in human serums and human breast cancer organ cultures appear to be the first of this kind.

A highly purified preparation of human α-lactalbumin (supplied by M. L. Groves) was used in the assay. This preparation, used both for iodination and for standards, migrates as a single band on disc gel electrophoresis (*7*). A potent antiserum to human milk, raised in rabbits, is used. The incubation reaction is carried out for 5 days at 4°C; test samples of 25 μl or less, which represent no more than 1/20 the final incubation volume, were used. All samples were tested in duplicate; the mean difference between duplicates was 6.86 percent. A double antibody system with sheep antiserum to rabbit gamma globulin was used to separate bound from free protein. A representative standard curve and measurements of α-lactalbumin in dilutions of serum from an individual galactorrhea patient with high circulating α-lactalbumin are shown in Fig. 1. The curves are superimposable. This assay is capable of detecting 0.05 ng of α-lactalbumin; the sensitivity for serum samples is 1 ng/ml. No cross-reaction was encountered with high concentrations of human preparations of casein, lysozyme, milk serum albumin, lactoferrin, growth hormone, prolactin, or gamma globulin, or with ovine prolactin, porcine insulin, or fetal calf serum. A high degree of cross-reactivity was observed between the α-lactalbumin of humans and that of other primates (*8, 9*), but not with that of ruminants, supporting previous observations (*10*), or with rodents. As expected, human milk contains large quantities of α-lactalbumin. Samples from two nursing mothers had concentrations of 2.0 and 2.6 mg/ml.

α-Lactalbumin was found in the blood of both lactating and nonlactating men and women (Fig. 2). Of 22 normal males aged 7

to 73 years, 9 had more than 1 ng/ml, the limit of detectability. The mean of the measurable values was 5.83 ng/ml. Studies in nonlactating normal female subjects from 5 to 75 years of age (mean 33.3) revealed that α-lactalbumin was present in 15 of 33 subjects (mean, 6.42 ng/ml). In contrast, significantly higher levels of α-lactalbumin were found in the serums of lactating women. α-Lactalbumin was detectable in all nursing mothers so far tested (17 serum samples from 15 nursing mothers, 1 day to 4 months postpartum). In this group, the mean serum α-lactalbumin was 188 ng/ml, with individual values from 45 to 640 ng/ml. α-Lactalbumin was measured in a group of 35 patients with nonpuerperal galactorrhea of varying etiology; 5 had undetectable levels and the remaining 30 had a mean α-lactalbumin of 55 ng/ml, the range being from the value observed in normals to 400 ng/ml. Mean α-lactalbumin was significantly higher in nursing mothers than in patients with galactorrhea ($P < .001$) although individual values frequently overlapped. In a group of 44 patients with metastatic carcinoma of the breast, 5 had detectable levels of α-lactalbumin ranging from 1.0 to 12 ng/ml. The lower incidence of detectable α-lactalbumin in the cancer patients might in part be explained by the group's age (mean, 55 years, range 38 to 86). In a group of 31 normal women ranging in age from 35 to 76 years (mean, 54.9 years), not all represented in Fig. 2, only 6 had measurable serum α-lactalbumin. Hendrick and Franchimont have reported that casein (a protein present in human milk in concentrations similar in magnitude to those of α-lactalbumin) is found in the serums of 80 percent of patients with metastatic breast cancer (*11*), in concentrations 1000 times greater than those of α-lactalbumin in the cancer patients reported here. They also detected casein in patients with lung and gastrointestinal cancers. The significance of their very high values for serum casein in breast cancer patients and the relationship to the much lower ones we have found for α-lactalbumin remain unclear. Of possible relevance is the comparatively lower sensitivity of their casein radioimmunoassay, values of less than 100 ng/ml being undetectable.

The findings reported here suggest that the α-lactalbumin in the serum of lactating women is produced by the breast. Circulating milk proteins might also be produced in breast tissue of nonlactating people, including some patients with metastatic breast cancer. It is somewhat more difficult, however, to accept that α-lactalbumin in men without gynecomastia originates in impalpable breast tissue. At least in this situation an extramammary source of α-lactalbumin must be considered. Small amounts of α-lactalbumin have been found in two of six homogenates of histologically normal breast tissue from nonlactating adults with breast cancer but not in homogenates of normal colon, muscle, stomach, thyroid, or pituitary.

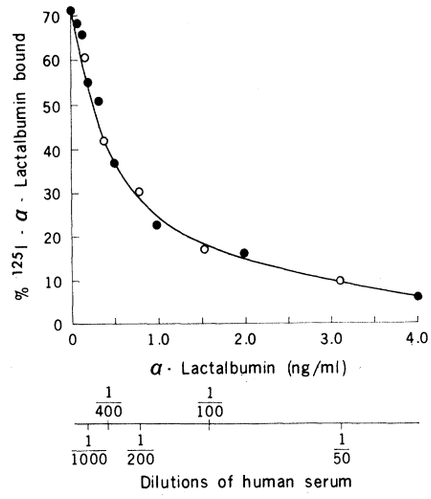

Fig. 1. Representative standard curve of human α-lactalbumin radioimmunoassay. Closed circles (●) represent standard concentrations of purified α-lactalbumin. The open circles (○) represent α-lactalbumin found in dilutions of serum from a patient with high circulating levels of α-lactalbumin.

Prolactin was measured in the patients by radioimmunoassay (*12*) with reagents supplied by the National Pituitary Agency. Mean prolactin concentrations for each of the groups are shown in Fig. 2. As was anticipated (*13*), prolactin values in the normal women were slightly but significantly higher than in the males. The prolactin measurements in patients with metastatic breast cancer do not significantly differ from those of normal women (*14*). Prolactin was elevated (> 25 ng/ml) in all but two of the nursing mothers and in one-half of the galactorrhea patients reported in this series. Values ranged from 12.5 to 150 ng/ml in the nursing women and varied from 1.2 to 730 ng/ml in the galactorrhea patients. In one patient with galactorrhea and amenorrhea, serum α-lactalbumin was 90 ng/ml and 102 ng/ml on two occasions prior to therapy with CB-154, an ergot preparation that lowers prolactin. Four weeks after starting treatment, prolactin fell from an initial 100 to 5.4 ng/ml, and galactorrhea disappeared. Simultaneously, α-lactalbumin fell to 45 ng/ml. From these studies it appears that circulating α-lactalbumin is higher in subjects in whom milk production is greatest. The correlation between serum prolactin and α-lactalbumin in the lactating women in this series is not significant ($r = .060$). Although many patients with elevated prolactin have high α-lactalbumin as well, some patients with galactorrhea and normal prolactin have high circulating α-lactalbumin. In contrast, some patients without galactorrhea who have very high prolactins have been found to have unmeasurable serum α-lactalbumins.

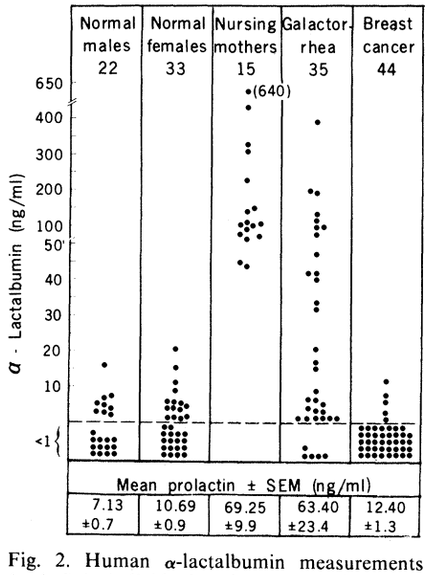

Fig. 2. Human α-lactalbumin measurements (ng/ml) in individual patients. Each dot represents a single value. Mean prolactin (± S.E.M.) for each group of patients is represented below individual columns.

Early studies of the effect of prolactin on human breast cancer tissue in organ culture indicate that some of these tumors are capable of producing α-lactalbumin (*9*). Fragments of breast cancer tissue (approximately 100 mg per culture dish) were maintained for 3 days in medium 199 with added insulin and hydrocortisone at 37°C in an atmosphere of 95 percent O_2 and 5 percent CO_2. Of 19 breast cancers grown in this control medium, α-lactalbumin was detected in the medium of 4 tumors at concentrations ranging from 0.9 to 3.3 ng/ml and was undetectable in the others. The addition of prolactin did not increase α-lactalbumin output in these 4 tumors or 13 others. However, two other tumors appeared to be sensitive to the action of prolactin. When these tumors were maintained in organ culture, α-lactalbumin was not detected in the control medium bathing the tissues. The addition of human female serum in concentrations of 20 percent resulted in detectable α-lactalbumin, which we attribute to the serum α-lactalbumin content. When ovine prolactin in concentrations (greater than physiologic) of 100 ng/ml was added to the medium in addition to the human female serum, α-lactalbumin was detectable at 4.9 ng/ml and 18.2 ng/ml, representing greater than two- and threefold increases, respectively, of α-lactalbumin over that attributable to the

serum additive. Ovine prolactin alone does not contain immunoassayable α-lactalbumin or affect the assay in any way. A greater than tenfold increase in α-lactalbumin was found when medroxyprogesterone acetate (100 ng/ml) was added to the culture medium of these same tumors, There was no increase in α-lactalbumin production when medroxyprogesterone acetate was added to the other breast tumor cultures. If, indeed, the major effect of prolactin on breast epithelium is stimulation of milk protein synthesis, the data described here would indicate a much lower incidence of prolactin sensitivity than reported by Hobbs *et al.* (*15*), who used activity of glucose-6-phosphate dehydrogenase as an endpoint of prolactin activity. These studies indicate that milk proteins circulate in human blood and that measurements of α-lactalbumin in serum, milk, and organ cultures of breast tissue provide additional means of studying human breast physiology.

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Complement-Mediated Bactericidal System: Evidence for a New Pathway of Complement Action

Abstract. *The early components of human complement (C1, C4, and C2) plus certain serum euglobulins will kill pathogenic strains of* Shigella sonnei. *Serum from patients with hereditary C3 deficiencies and specific antiserums to C3, C5, and C6 were utilized to demonstrate the absence of requirements for late-acting complement components in this unusual bactericidal system.*

The participation of heat-labile components in the killing of enteric bacteria by mammalian serum was established before the end of the last century and a requirement for antibody has been demonstrated repeatedly since that time (*1*). Despite an extensive accumulation of literature, however, only recent studies have succeeded in delineating certain of the essential humoral components that function in this important bactericidal milieu.

In 1943, Dozois and colleagues (*2*) reported that the four classical components of human complement (C) recognized at the time were required to kill a laboratory strain of *Vibrio comma*. This observation was extended subsequently (*3*) to include a common strain of *Escherichia coli*. During the past decade, it has been shown that C6 and presumably C1, C4, and C2 are required for killing *Salmonella typhi* O-901 (*4*). Hereditary complement deficiencies in C2 (*5*) and C3 (*6*) have clearly implicated these components in the serum bactericidal reaction against pathogenic strains of *Salmonella*. Other studies indicate that all major components of the hemolytic complement system (C1 through C9) together with antibody are required to kill *E. coli* (*7*) and several strains of *Shigella* (*8*). Considered together, these studies substantiate the view that the conventional pathway of complement action functions with antibody to kill many enteric bacteria. Some studies, however, indicate that complement can effect the killing of nonpathogenic strains without participation of antibody (*9*).

The alternative pathway of complement action (C3 through C9, plus properdin factors) appears to function in killing laboratory strains of *Shigella dysenteriae* (*10*) and *E. coli* (*11*); there is some question regarding the necessity for antibody in this system. A recent article (*12*) describes a weak bactericidal effect of C5 through C9 complexes and a pseudoglobulin factor against a common *E. coli* strain in the absence of antibody.

In the present studies on human complement requirements for killing pathogenic strains of enteric bacteria, we observed that serum from a patient with a hereditary deficiency in C3 (*13*) showed a marked inability to kill *Salmonella enteritidis* but a normal capacity to kill smooth strains of *Shigella sonnei*. The serum from another patient with a hereditary deficiency in C3-inactivator (*6*), a defect that leads to consumption of alternative pathway proteins, exhibited a similar pattern in killing these bacterial strains (*14*). The addition of functionally purified C3 (*15*) to the serum of the patient with C3 deficiency initiated immune hemolysis and bactericidal action against *S. enteritidis* but did not alter the already potent killing action against *S. sonnei*.

Although these experiments afforded a persuasive argument against a role for complement (C3) in killing shigella organisms, other evidence suggested that cer-

Table 1. Bactericidal activity of normal and complement-deficient serums.

Bacterial strain	Serum (ml)	Percent of bacteria killed by following human serums				
		Normal	C2 deficient	C3 deficient*	C3 deficient†	C4 deficient‡
Shigella sonnei	0.10	100	100	100	100	0
	0.06	100	32	100	100	0
	0.02	100	0	100	100	0
	0.01	0	0	0	0	0
Salmonella enteritidis	0.50	100		0	0	0
	0.20	97		0	0	0
	0.10	86		0	0	0
	0.04	0		0	0	0

*From a patient with C3 deficiency; contains < 0.1 percent of normal C3 concentration (*13*). †From a patient with C3-inactivator deficiency; contains approximately 10 percent of normal C3 concentration (*6*). ‡Normal bactericidal activity against both strains was achieved in C2- and C4-deficient serums upon addition of the missing complement component, that is, 2000 units of C2 per milliliter or 4000 units of C4 per milliliter. Addition of C3 to either of the C3-deficient serums did not increase shigellacidal activity which was already equivalent to that of normal serum. The addition of 2000 units of C3 per milliliter to serum from the patient with C3 deficiency resulted in normalization of bactericidal activity against *S. enteritidis*.