combination. This analogy has recently been extended to include three "revertants" of another lethal *Pgd* allele which represent mutations to reduced G6PD levels (*5*).

Although fairly common among prokaryotes and lower eukaryotes, the rescue of lethal mutations by genetic means is relatively undocumented in higher forms. The pentose phosphate shunt pathway of *Drosophila* provides an excellent model system with which to study this type of genetic interaction.

M. BEATRICE HUGHES
JOHN C. LUCCHESI
Department of Zoology, University of North Carolina, Chapel Hill 27514

References and Notes

1. G. C. Bewley and J. C. Lucchesi, *Genetics* **79**, 451 (1975).
2. V. A. Gvosdev, S. A. Gostimsky, T. I. Gerasimova, E. M. Gavzina, *Drosophila Inf. Serv.* **50**, 34 (1973).
3. Low levels of G6PD activity without demonstrable effects on viability have been described in *Drosophila* [B. W. Geer, J. T. Bowman, J. R. Simmons, *J. Exp. Zool.* **187**, 77 (1974)] and man [P. E. Carson, C. L. Flanagan, C. E. Ickes, A. S. Alving, *Science* **124**, 484 (1956)].
4. D. L. Lindsley and E. H. Grell, *Carnegie Inst. Wash. Publ. No. 627* (1968), pp. 1–471.
5. V. A. Gvosdev, T. I. Gerasimova, G. L. Kogan, O. Yu. Braslavskaya, *Fed. Eur. Biol. Soc. Lett.* **64**, 85 (1976).
6. J. C. Lucchesi and J. M. Rawls, Jr., *Biochem. Genet.* **9**, 41 (1973).
7. We thank G. P. Maroni for reading and commenting upon the manuscript. This work was supported by research grant GM-15691 and training grant T01-GM-0685 from the National Institutes of Health.

15 September 1976; revised 28 December 1976

Serum Complement-Like Opsonic Activities in Human, Animal, Vegetable, and Proprietary Milks

Abstract. *Human, animal, proprietary, and soy milks are comparable to human serum C5 in opsonization of baker's yeast. Bovine milk and human serum opsonically reconstitute C5-deficient mouse serum. Such reconstitution is selectively inhibited by antiserum to human C5. Further characterization suggests that bovine milk contains material structurally and functionally similar, but not identical, to human C5.*

The serum complement system is a major participant in the human inflammatory response (*1*). In the course of studies involving one of the complement proteins, that is, the fifth component, or C5, we have observed functionally analagous opsonic activities to serum C5 in human, animal, proprietary, and vegetable milks. In this report we review the steps leading to our findings and compare a number of functional properties of human serum C5 with those of bovine milk.

In 1968, we described a familial opsonic defect (*2*). Clinical improvement of the proband resulted upon the administration of fresh plasma, while stored plasma was ineffective. The plasma factor responsible for the patient's improvement was identified through the use of an assay in vitro of the quantitation of uptake by human polymorphonuclear leukocytes (PMN's) of baker's yeast particles opsonized by human serum. Serums from the proband, her mother, and a number of other family members were deficient in their enhancement of yeast particle uptake by normal human PMN's.

When opsonically deficient serum from the patient was mixed with serum from mice genetically deficient in C5, no improvement in yeast opsonization was observed. However, full reconstitution of yeast opsonization occurred after addition to the patient's serum of either low concentrations of normal mouse serum (not C5 deficient) or normal human serums. Further, full reconstitution of either the patient's serum or C5-deficient mouse serum resulted from the addition of physiologic doses of highly purified human C5. In contrast to the mouse serum, which completely lacks the C5 protein, however, the opsonically abnormal human serums were found to contain normal levels of C5 by immunochemical and hemolytic measurements. Thus, it was hypothesized that the abnormality in the C5 molecule was functional rather than quantitative in the abnormal subjects (*3*).

Table 1. Effects of various antiserums on yeast opsonic activities of human serum and bovine milk. Results are expressed as average numbers of yeast particles ingested per PMN. Each number listed for an individual antiserum is based upon at least four separate measurements. Average values are shown, as no appreciable variation was found among individual measurements for any antiserum.

Antiserum against	Normal human serum	Bovine milk
	3.5 ± 0.4*	3.4
C3	2.7†	3.3
C4	3.4	3.4
C5	2.0†	1.9†
IgG	3.3	3.6
IgM	3.4	3.3
IgA	3.3	3.3
Whole human serum	1.8†	3.5
α_2-Macroglobulin	3.1	3.4
Ceruloplasmin	3.2	3.2
α_1-Antitrypsin	3.0	3.0
Albumin	3.5	3.5
Fibrinogen	3.4	3.5
Transferrin	3.1	3.0

*The mean value for normal human serum is based upon determination of opsonic activity of 121 individual normal human serums. †The depression of opsonic activity was statistically significant ($P < .001$). For normal human serum, ± 0.4 equals two standard deviations.

The hypothesized defect was verified through the demonstration of functional abnormalities in the C5 isolated from the opsonically deficient human serum (*4*). Studies of the isolated C5 from deficient serum revealed a restricted, primary functional defect analagous to the one suggested in whole serum from the patient. Yeast opsonic activity was absent, but hemolytic function was normal.

As other patients with the disorder were observed, clinical similarities were noted to a syndrome described by Leiner in 1908 (*5*). Of relevance was Leiner's observation that the illness was limited almost exclusively to breast-fed infants (41 of 43 cases). He observed clinical improvement upon placing the infant on bottled (cow) milk feedings or, occasionally, upon changing the wet nurse.

Based upon these observations, we compared yeast opsonic activities in a variety of milks with that of normal human serum.

Suspensions of baker's yeast [1×10^9 yeast particles per milliliter of Earle's balanced salt solution (EBSS)] were incubated with one of the milks or with human serum for 30 minutes at 37°C. The yeast suspensions were then washed three times, resuspended in EBSS and incubated with a suspension of human PMN's (5×10^6 PMN's per milliliter of EBSS). After phagocytosis had occurred, the average numbers of yeast particles ingested per PMN were determined by microscopic examination.

The results of these experiments are shown in Fig. 1. Human milk, bovine milk, goat milk, and proprietary formulas such as Enfamil and Isomil (which contain no animal protein), were comparable to human serum in yeast opsonic activity (*6*). The addition of antiserum to C5 significantly decreased the opsonic activities of the milks and serum. Each of these experiments was performed with three different preparations of antiserum to C5 (*7*).

In order to better assess the specificity of the reaction with antiserum to C5, we conducted similar experiments with a number of other antiserums against a variety of antigens, including C3, C4, IgG, IgM, IgA, whole human serum, α_2-

macroglobulin, ceruloplasmin, α_1-antitrypsin, albumin, fibrinogen, and transferrin (*8*). The effects of these antiserums against opsonic activities of normal human serum and bovine milk are compared in Table 1. Significant depression of opsonic activity of human serum occurred with antiserum to C3 and antiserum to whole human serum, as well as with antiserum to C5. This might be expected since opsonic requirements for yeast uptake in human serum include C3 (*9*). By contrast, opsonic activity of bovine milk was inhibited only by antiserum to C5. The addition of purified C3, C4, IgG, IgM, or IgA to the milk treated with antiserum to C5 had no restorative effect upon opsonic activity. The addition of purified C5, however, restored opsonic activity to normal.

The effects of the same antiserums upon yeast opsonic activity were tested with serums from C5-deficient mice (B10D2 old line) (Table 2). Such serums have previously been shown to be opsonically deficient for yeast but can be reconstituted by the addition of as little as 1 μg/ml (final concentration) of purified C5 (equivalent to 1 to 2 percent of the normal serum concentrations) (*3*). Normal plasma or milk become opsonically deficient when diluted to 1 percent. Such preparations, however, still contain adequate amounts of C5 to restore opsonic activity to C5-deficient mouse serum (*3*). The ability of 1 percent plasma or bovine milk to reconstitute yeast opsonic activity to C5-deficient mouse serum was inhibited by the same antiserums as in the previous experiment; that is, antiserum to C3, antiserum to C5, and antiserum to whole human serum inhibited the opsonic activity of plasma, but *only* antiserum to C5 inhibited the opsonic activity of bovine milk.

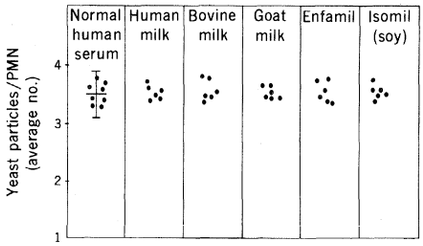

Fig. 1. Comparison of yeast opsonic activities of human serum and a variety of milks. Data are expressed as the average number of yeast particles ingested per polymorphonuclear leukocyte (*PMN*).

These findings might tempt one to conclude that the opsonic activity in bovine milk is due to C5. Not only was the opsonization by milk selectively inhibited by antiserum to human C5, but reconstitution of opsonizing activity in C5-deficient mouse serum by milk was also inhibited by antiserum to C5.

However, there are other observations that suggest the active material is not C5. (i) Unlike human plasma, in which we have previously found the functional opsonic half-life of C5 to be approximately 36 hours (*9*), functional yeast opsonic activity was retained by the milk preparations for prolonged periods of time, often as long as 3 to 4 weeks. Many of the milks tested had, in fact, been on the shelf at room temperature for prolonged periods. (ii) Soy milk (Isomil), which contains no animal protein, had the same degree of opsonic activity as the other milks.

To explore this question further, we compared bovine milk and human serum in a variety of assays reflecting C5 activities.

1) Molecular titration of hemolytically active C5 in plasma or milk by lysis of EAC14^{oxy}23 cells in the presence of a human serum source of C6 through C9 (*10*) was normal. Over a wide range of concentrations, however, no hemolytic C5 activity of milk was demonstrated.

2) The ability of milk or serum to generate chemotactic activity upon the addition of the antigen-antibody complex or endotoxin was examined (*4*).

3) Heat stability (56°C for 1 hour) of opsonically active portions was studied.

4) The *p*H ranges of opsonic activity were determined.

The opsonic activities of serum and bovine milk were each inhibited by antiserum to C5. Serum, however, contained hemolytically active C5, generated chemotactic activity, and contained heat-labile opsonic activity, while bovine milk was heat stable and lacked hemolytic or chemotactic activity. Milk opsonic activity was retained through a *p*H range of 2 to 8, while serum opsonic activity was present through a much narrower range (6.5 to 8).

Bovine milk thus contains material (or more than one material) which shares certain functional activities with C5 and has structural similarity (since it is inhibited by antiserum to C5) but is not identical to C5.

This strongly suggests that bovine milk contains a previously unrecognized mechanism, either a simple substance or an entire cascade, which will opsonize yeast. The nature of this mechanism and how it affects the membranes of the yeast particle have a number of implications.

1) The identification of a substance in milk with functional analogy to a natural component of the human complement system: (i) increases the potential biologic significance of complement, and (ii) provides an aid in the characterization of complement dependent opsonization. Biologically active products of C5 are formed upon normal complement activation. In this process, the native C5 molecule is cleaved into a smaller fragment, C5a, which remains in the fluid phase, and a larger fragment, C5b, which is bound. Each fragment is biologically active. The fragment C5a can mediate chemotaxis, histamine release, smooth muscle contraction, and capillary permeability (*11*). This is a very specific system: C5a, for example, apparently acts on the cell membrane, producing chemotaxis and smooth muscle contraction at a

Table 2. Effects of various antiserums on the reconstitution of yeast opsonic activity to C5-deficient mouse serums (B10D2 old line) by normal human serum (NHS) and bovine milk (BM). The results are expressed as in Table 1. In these experiments, however, the antiserums were tested for their ability to inhibit the yeast opsonic reconstituting activities of serum or bovine milk upon C5-deficient mouse serum. Here, we have used only 1 percent concentrations of milk or plasma (see text for details).

Antiserum against	B10D2 old line	1% NHS	1% BM	B10D2 + 1% NHS	B10D2 + 1% BM
	1.4	1.4	1.6	3.3	3.4
C3	1.3	1.4	1.5	2.4*	3.2
C4	1.4	1.3	1.5	3.4	3.4
C5	1.4	1.4	1.4	1.7*	1.5*
IgG	1.3	1.4	1.2	3.2	3.1
IgM	1.5	1.2	1.3	3.6	3.5
IgA	1.6	1.4	1.5	3.4	3.5
Whole human serum	1.3	1.4	1.7	2.2*	3.2
α_2-Macroglobulin	1.4	1.4	1.7	3.1	3.2
Ceruloplasmin	1.4	1.5	1.6	3.3	3.5
α_1-Antitrypsin	1.3	1.3	1.5	3.0	3.1
Albumin	1.4	1.5	1.6	3.4	3.3
Fibrinogen	1.6	1.5	1.4	3.4	3.4
Transferrin	1.2	1.4	1.4	3.5	3.4

*The depression of opsonic activity was statistically significant ($P < .001$).

different site from that of C3a, a similarly derived cleavage product of C3 (*12*). Fragment C5b can also participate in the chemotaxis of PMN's when it is complexed with C6 and C7 (*13*). A role for a C5-derived product, possibly C5b, has also been demonstrated in the opsonization of baker's yeast particles (*9*). Various functional activities of C5 products probably reflect different individual sites on the C5 molecule (*9, 14*). Characterization of the biologically active material in the milks may, therefore, contribute significantly to the characterization of the opsonically active fragment of human C5.

2) The identification of a biologic activity in milks with the potential for enhancing the inflammatory response has significance in a variety of human nutritional deficiency states. Preliminary characterization of the opsonically active bovine milk fraction by Sephadex G-200 chromatography showed no activity in the casein micelle complex (molecular weight 10^8). Heat-stable opsonic activity was found between the lactalbumin and lactoglobulin peaks. Lactalbumin lost opsonic activity and was denatured in highly acid *p*H ranges, suggesting that the opsonically active fraction resides in the lactoglobulin fraction, with a molecular weight of approximately 36,000. Effects of the various antiserums upon this partially purified isolated product are identical to those shown with whole bovine milk.

3) The identification of the material in nonanimal milks (that is, soy) suggests a wide biologic role for this molecule. Precedents exist for such relationships, for example, with the glycoprotein blood group antigens which are found throughout the animal and plant kingdoms. Complete characterization of the opsonically active milk fraction should permit exploration of this point.

MICHAEL E. MILLER
ROLAND G. GANGES

Department of Pediatrics, University of California, Los Angeles, School of Medicine, Harbor General Hospital, Torrance, California 90509

References and Notes

1. S. Ruddy, I. Gigli, K. F. Austen, *N. Engl. J. Med.* **287**, 498, 545, 592, and 642 (1972).
2. M. E. Miller, J. Seals, R. Kaye, L. C. Levitsky, *Lancet* **1968-II**, 60 (1968).
3. M. E. Miller and U. R. Nilsson, *N. Engl. J. Med.* **282**, 354 (1970).
4. U. R. Nilsson, M. E. Miller, S. Wyman, *J. Immunol.* **112**, 1164 (1974).
5. C. Leiner, *Arch. Dermatol. Syph.* **89**, 163 (1908).
6. Previous data show that maximum yeast opsonic activity is present in serum concentrations of 10 percent or greater. When serum is used in concentrations greater than 50 percent in the yeast assay, one often finds inhibitory activities. We, therefore, use 10 percent serum as the standard positive control. Milk preparations at 90 to 100 percent had opsonic activities equivalent to 10 percent serum. Further studies will be necessary to determine the nature of these differences.
7. Three entirely different antiserums were used in these studies. (i) Antiserum to highly purified human C5 was raised in C5-deficient mice (strain A/HeJ) as described by U. R. Nilsson and H. J. Müller-Eberhard [*J. Exp. Med.* **125**, 1 (1967)]. (ii) Antiserum to human C5 raised in goats was obtained from Meloy Laboratories. (iii) Goat antiserum to human C5 was also obtained from Behring Diagnostics. Prior to use in the opsonic assays, each antiserum was shown to have a single immunodiffusion band directed against either highly purified human C5 or whole human serum.
8. These antiserums were obtained commercially from Meloy Laboratories, Behring Diagnostics, or Cordis Laboratories. In most cases, two sources of antiserums were used for each experiment. Each of the antiserums was added to milk in a range of final concentrations from 25 to 80 percent.
9. M. E. Miller and U. R. Nilsson, *Clin. Immunol. Immunopathol.* **2**, 246 (1974).
10. N. R. Cooper and H. J. Müller-Eberhard, *J. Exp. Med.* **132**, 775 (1970).
11. H. S. Shin, R. Snyderman, E. Friedman, A. Mellors, M. M. Mayer, *Science* **162**, 361 (1968).
12. C. G. Cochrane and H. J. Müller-Eberhard, *J. Exp. Med.* **127**, 371 (1968).
13. P. A. Ward, C. G. Cochrane, H. J. Müller-Eberhard, *ibid.* **122**, 327 (1965).
14. U. R. Nilsson, R. J. Mandle, Jr., J. A. McConnell-Mapes, *J. Immunol.* **114**, 815 (1975).

3 September 1976; revised 7 December 1976

Cleptoparasitism and Odor Mimetism in Bees: Do *Nomada* Males Imitate the Odor of *Andrena* Females?

Abstract. *Identical chemical compounds are present in the Dufour gland secretion of female* Andrena *bees and in the cephalic secretion of many male* Nomada *bees. Females of* Nomada *parasitize the nests of* Andrena. *Many* Nomada *species confine their attacks to a single host species. In two such host-parasite pairs,* Andrena haemorrhoa–Nomada bifida *and* Andrena carantonica–Nomada marshamella, *all*-trans *farnesyl hexanoate was found to be the totally dominant component in respective secretions. In two other pairs,* Andrena helvola–Nomada panzeri *and* Andrena clarkella–Nomada leucophthalma, *geranyl octanoate is the major component. This pairwise odor correspondence is discussed in relation to critical points of contact in the life cycles of host and parasite, male and female.*

In an investigation of biologically active volatile chemical compounds in bees of the genus *Andrena* F. we have found all-*trans* farnesyl hexanoate or geranyl octanoate to be the dominant component in the female Dufour gland secretion. These compounds are included in the nest odor. In the cephalic secretion of many male *Nomada* bees, which are cleptoparasites (nest parasites) mainly of *Andrena*, the same chemicals were found to be dominant. We believe that this finding will be of some importance for understanding the evolution of strategies that cleptoparasites use to gain entry into host nest.

The bee genus *Andrena* is found in the Holarctic and African regions and is often represented by populations rich in individuals. Most species of *Andrena* are solitary, some are communal (*1*). The females build their nests, consisting of a main burrow with short lateral tunnels, in the ground. At the end of each lateral tunnel is a nest cell coated on the inside with a hydrophobic lining. This coating is secreted from the Dufour gland located in the abdomen of the female (*2*). After the female has stocked the cell with a food supply of pollen and nectar and has laid an egg on top of it, the cell is sealed.

Species of *Nomada* parasitize mainly species of *Andrena* but also species in other genera of bees (*3*). Each species confines its attacks predominantly to a single host species or a group of closely related species. The *Nomada* female first locates *Andrena* nests from visual and chemical cues (*4*), and later, she lays an egg in a nest cell prepared by the host. The *Nomada* larva kills the host egg and consumes its food supply (*4*). In spite of the harm caused by the *Nomada* larva in the nest of the *Andrena*, an encounter between females of the two species in or just outside the nest causes no aggressiveness (*5*). The two females show no resemblance in general appearance in either color or pubescence (Fig. 1).

Members of the two populations of the host-parasite pair also meet in another situation. *Andrena* males make route flights (patrolling flights) (*6*) in habitats that are specific for different *Andrena* species. The males aggregate in a certain part of this habitat, where the flight paths are marked by odor points perfumed with volatile secretion from the cephalic glands (*7*). In species nesting in aggregations, the nest areas are patrolled by the males (*8*). The *Nomada* males make route flights in the same localities as the males of their host species. We have often observed *Nomada* males to join groups (often the more numerous ones) of *Andrena* males following certain flight paths.