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*.

Table 2. Effects of antiserum to human complement components on bactericidal activity and immune hemolysis.

Specific antiserum	Bactericidal action of normal human serum versus		Immune hemolysis‡	Interpretation of results		
	*S. sonnei**	*S. enteritidis*†		Category§	C requirements to kill	
					S. sonnei	*S. enteritidis*
Cl_q	Blocked	Blocked	Blocked	1	C1 required	C1 required
Cl_s	Blocked	Blocked	Blocked	1	C1 required	C1 required
C4	Blocked	Unimpaired	Unimpaired	2	C4 required	(See text)
C3	Unimpaired	Blocked	Blocked	3	C3 not required	C3 required
C5	Unimpaired	Blocked	Blocked	3	C5 not required	C5 required
C6	Unimpaired	Blocked	Blocked	3	C6 not required	C6 required

*Incubation conditions: 0.02 ml of serum, ~ 100 cells, antiserum dilutions. †Incubation conditions: 0.15 ml of serum, ~ 100 cells, antiserum dilutions. ‡Incubation conditions: 0.02 ml of serum, 0.25 percent sensitized erythrocytes, antiserum dilutions (*16*). §Category 1, killing of bacterial strains and immune hemolysis fully blocked at equivalent antiserum dilutions (1 : 10→1 : 160). Category 2, killing of *Shigella* fully blocked at antiserum dilutions that do not impair killing of *Salmonella* or immune hemolysis (for example, 1 : 80). Lower dilutions of antiserum to C4 block both immune hemolysis and killing of *Salmonella* (see text). Category 3, killing of *Shigella* unimpaired at antiserum dilutions required to block killing of *Salmonella* and immune hemolysis (1 : 20→1 : 40).

tain components of complement could be involved. For example, heating normal serum at 50° or 52°C abolished bactericidal activity, as did treatment with the chelating agent ethylenediaminetetraacetate. Treatment of normal serum with hydrazine or cobra venom factor resulted in loss of bactericidal activity. However, recombinations of treated samples of serum did not give clear indications of complement component requirements. Apparently such treatments affect unidentified components, in addition to complement, that are essential in order to kill *S. sonnei.*

Normal serum treated with ammonia to destroy C4 was inactive in immune hemolysis (*16*) and in killing of the shigella and salmonella strains; these activities were restored in full to the treated serum by addition of 2000 to 4000 units of functionally purified C4 per milliliter. A requirement for C2 was demonstrated by the use of serum from a patient with hereditary C2 deficiency (*5*). This serum possessed about 15 percent of normal capacity to kill *S. sonnei*, but full bactericidal activity was achieved on addition of 2000 units of functionally purified C2 per milliliter. Representative results of bacterial killing by normal and complement-deficient serums are shown in Table 1.

In other experiments, the shigellacidal activity of normal human serum was blocked by specific antiserums to C1 and C4. By contrast, dilutions of specific antiserum to C3, which blocked immune hemolysis and the killing of *S. enteritidis* by normal serum, failed to block the killing of *S. sonnei* by either normal or C3-deficient serum. Dilutions of specific antiserums to C5 and C6 that blocked immune hemolysis and the killing of *S. enteritidis* were likewise without effect on the shigellacidal activity of normal serum. Specific antiserum to properdin failed to block killing of *S. sonnei*, even at concentrations sufficient to produce a partial block of immune hemolysis.

The use of specific antiserums to individual complement components may provide only presumptive information on requirements for bactericidal activity (see Table 2). The fact that antiserum to C1 blocked bacterial killing and immune hemolysis at equivalent dilutions (Table 2, category 1) is not necessarily a demonstration of a C1 requirement in the shigellacidal system since a complement-fixation reaction unrelated to a specific block of C1 could account for the observed result. In this case, however, additional evidence was obtained to implicate C1 in the bactericidal reaction. When a minimal concentration of antiserum to C1 was used, just sufficient to block the killing of *S. sonnei*, addition of small amounts of functionally purified C1 effectively reversed the inhibition of bactericidal activity.

Interpretations of results in categories 2 and 3 (Table 2) are less equivocal and, in these instances, clear-cut information is obtainable on recognition of complement component requirements. A requirement for C4 in the shigellacidal system was shown by the finding that dilutions of antiserum to C4 that blocked killing of approximately 100 shigella organisms did not block immune hemolysis or the killing of approximately 100 salmonella organisms (category 2). Killing of the salmonella strain was blocked by antiserum to C4 at dilutions proportional to those required to block immune hemolysis, that is, taking into account differences in volume of serum employed in each of these test systems.

Serum from guinea pigs with hereditary C4 deficiency (*17*) was only slightly less active than serum from normal guinea pigs in killing *S. sonnei*, which suggests that *S. sonnei* is sensitive to the alternative pathway of complement activation in guinea pig serum. The results in Tables 1 and 2 implicate the conventional pathway of complement activation in the killing of *S. enteritidis* by human serum.

With respect to complement requirements, the killing of *S. sonnei* bears a superficial resemblance to the neutralization of herpesvirus by early complement components (*18*). However, an important difference from viral neutralization was observed. Varying proportions of functionally purified C1, C4, and C2 together with dilutions of antiserum to group D *Shigella* did not constitute a bactericidal system, nor did the addition of C3 produce any noticeable effect. This finding indicated that additional serum components were required to kill *S. sonnei.*

Our initial approach to the characterization of essential components began with the preparation by dialysis of a euglobulin fraction from normal human serum (*16*). The euglobulin precipitate from 10 ml of serum was solubilized in 10 ml of physiologic saline containing 5 m*M* Veronal buffer (*p*H 7.4) and 1 m*M* concentrations of Ca^{2+} and Mg^{2+}. The bactericidal activity of this fraction was examined in the presence of functionally purified complement components. The single addition of 200 or 400 units of C4 to the euglobulin fraction was sufficient to initiate the killing of *S. sonnei*, whereas similar amounts of C2 were without effect. By limiting the concentration of C4 to 100 or 200 units, a marked enhancement of bactericidal activity was observed upon addition of 200 to 400 units of C2. This result accords with the established sequence of activation of early complement components and also indicates a more critical need for C4. The latter point is reinforced by the present data which show that the shigellacidal system is more sensitive to inhibition by antiserum to C4. The euglobulin fraction plus combinations of C4 and C2 exerted no bactericidal action against *S. enteritidis.*

Evidence against a requirement for C3 in the bactericidal action of the euglobulin fraction was obtained as follows. A sample of human serum was rendered nonhemolytic by treatment with cobra venom factor (*19*) prior to isolation of the eu-

globulin fraction. This fraction was found to be as active against the shigella strain as the euglobulin fraction prepared from an untreated sample of the serum. Chromatographic fractionations of the human serum euglobulins indicate the presence of two heat-labile components, in addition to C1, which are essential for shigellacidal activity.

If antibody is required in the shigellacidal system, only a minute amount would appear to be necessary. The addition of varying dilutions of specific antiserum failed to affect killing by normal serum or by the euglobulin-C4,C2 preparation. Concentrations from 1 to 150 μg of a Boivin preparation of endotoxin (*20*) isolated from *S. sonnei* did not block killing of the shigella strain by normal serum or by the euglobulin-C4,C2 preparation, indicating that antibody to endotoxin is not required.

The present observations suggest that (i) the initial phase of complement activation in the shigellacidal system is similar to that which occurs via the conventional pathway and (ii) activated C4,C2 complexes act on serum proteins other than late complement components, thereby evoking an attack phase quite different from any previously envisaged.

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Melatonin: Antigonadal and Progonadal Effects in Male Golden Hamsters

Abstract. *Melatonin induced marked testicular regression in hamsters maintained on photostimulatory long days (light-dark 14 : 10). In animals maintained on nonstimulatory short days (light-dark 6 : 18), small amounts of melatonin (50 micrograms per day; 100 millimeters capsule length) prevented testicular regression; but testicular atrophy occurred in hamsters that received larger amounts of melatonin (75 to 100 micrograms per day; 150 to 200 millimeters capsule length) and in control hamsters that received none. The results demonstrate that melatonin can exert either pro- or antigonadal effects and emphasize that the effects of melatonin on the testis cannot be properly assessed unless account is taken of the dosage and mode of melatonin administration and the photoperiod on which experimental animals are maintained.*

The pineal hormone, melatonin, has been reported to exert antigonadal, progonadal, or no effect upon the mammalian reproductive system (*1–3*). Because the reported effects of melatonin on the neuroendocrine-gonadal axis are inconsistent, no consensus has emerged regarding the role of the pineal gland in the control of reproduction. We reexamined the effects of melatonin on the reproductive system of male golden hamsters after discovering a novel mode for administering melatonin over a wide range of concentrations at relatively constant rates for protracted time periods. The results suggest that melatonin and hence the pineal gland may play a primary role in controlling mammalian reproduction, and they potentially clarify previous conflicting reports concerning the effect of melatonin on the male reproductive system.

Male golden hamsters (*Mesocricetus*

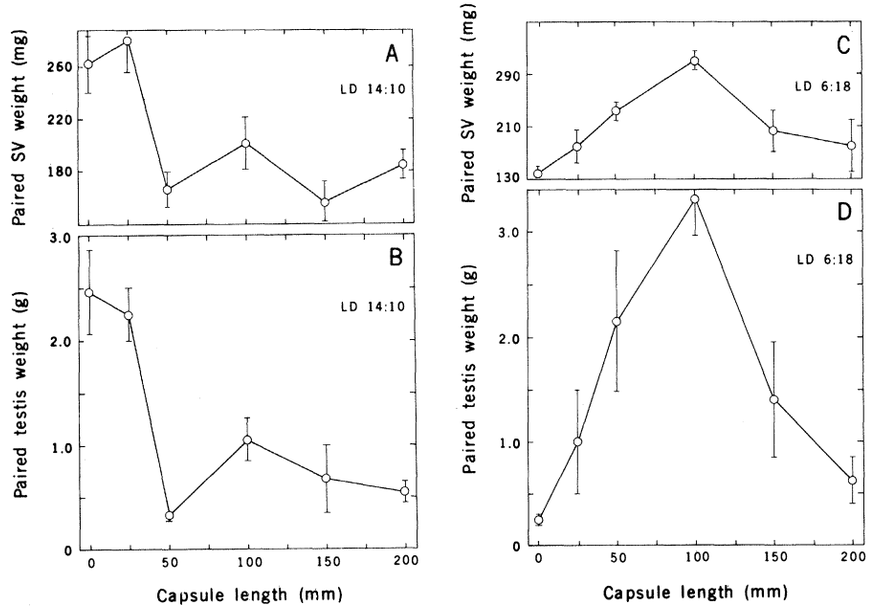

Fig. 1. Average weight of seminal vesicles (*SV*) (A) and testes (B) of hamsters maintained on an LD 14 : 10 photoperiod and implanted with melatonin-filled capsules of various sizes for 60 days. Average weight of seminal vesicles (C) and testes (D) of hamsters implanted with melatonin-filled capsules of various sizes for 60 days and transferred from LD 14 : 10 to LD 6 : 18 on the day the capsules were implanted. Control animals (at zero on the abscissa) received an empty 50-mm capsule. Each point represents the mean ± S.E. (vertical bars) of five to six hamsters.