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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References and Notes

1. R. C. Skarnes and D. W. Watson, *Bacteriol. Rev.* **21**, 273 (1957); K. F. Austen and Z. A. Cohn, *N. Engl. J. Med.* **268**, 933 (1963).
2. T. F. Dozois, S. Seifter, E. E. Ecker, *J. Immunol.* **47**, 215 (1943).
3. A. C. Wardlaw, *J. Exp. Med.* **115**, 1231 (1962).
4. K. Rother, U. Rother, K. Peterson, D. Gemsa, F. Mitze, *J. Immunol.* **93**, 319 (1964).
5. M. R. Klemperer, H. C. Woodworth, F. S. Rosen, K. F. Austen, *J. Clin. Invest.* **45**, 880 (1966).
6. C. A. Alper, N. Abramson, R. B. Johnston, Jr., J. H. Jandl, F. S. Rosen, *ibid.* **49**, 1975 (1970).
7. K. Inoue, K. Yonemasu, A. Takamizawa, T. Amano, *Biken J.* **11**, 203 (1968); J. N. Goldman, S. Ruddy, K. F. Austen, D. S. Feingold, *J. Immunol.* **102**, 1379 (1969).
8. W. P. Reed and E. L. Albright, *Immunology* **26**, 205 (1974).
9. J. Sterzl, J. Kostka, A. Lane, *Folia Microbiol.* **7**, 162 (1962); D. K. Tachibana, *ibid.* **10**, 145 (1965).
10. J. Pensky, C. F. Hinz, Jr., E. W. Todd, R. Wedgewood, J. T. Bayer, I. H. Lepow, *J. Immunol.* **100**, 142 (1968).
11. O. Götze and H. J. Muller-Eberhard, *J. Exp. Med.* **134** (3), pt. 2, 90s (1971); R. K. Root, L. Ellman, M. M. Frank, *J. Immunol.* **109**, 477 (1972).
12. J. N. Goldman and K. F. Austen, *J. Infect. Dis.* **129**, 444 (1974).
13. C. A. Alper, H. R. Colten, F. S. Rosen, A. R. Rabson, G. M. Macnab, J. S. Gear, *Lancet* **1972-II**, 1179 (1972).
14. Four strains of *Shigella sonnei* and one strain of *Salmonella enteritidis* were obtained as recent clinical isolates from a local hospital. All cultures were maintained on enriched agar slants and transferred to Trypticase soy broth on the day of the experiment. Test inocula (~ 100 bacteria) were prepared by dilution from 4-hour broth cultures. The bactericidal assay is described elsewhere [R. C. Skarnes, *Nature (Lond.)* **225**, 1072 (1970)].
15. Complement components were purchased from Cordis Laboratories, Miami, Fla. Individual C components are functionally purified in terms of immune hemolysis; concentrations are expressed in units of hemolytic activity, as packaged.
16. E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry* (Thomas, Springfield, Ill., 1961), chap. 4.
17. M. M. Frank, J. May, T. Gaither, L. Ellman, *J. Exp. Med.* **134**, 176 (1971).
18. C. A. Daniels, T. Borsos, H. J. Rapp, R. Snyderman, L. Notkins, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 528 (1970).
19. P. G. Klein and H. J. Wellensiek, *Immunology* **8**, 590 (1965).
20. A. Boivin and L. Mesrobeanu, *Rev. Immunol. (Paris)* **1**, 553 (1935).
21. We thank Drs. F. S. Rosen, C. A. Alper, H. R. Colten, and D. R. Bing of the Harvard Medical School for generous donations of complement-deficient human and guinea pig serums and specific antiserums to complement components and properdin. We also thank Dr. Rosen for constructive comments on this manuscript. Supported by NSF grant GB 40990.

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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*

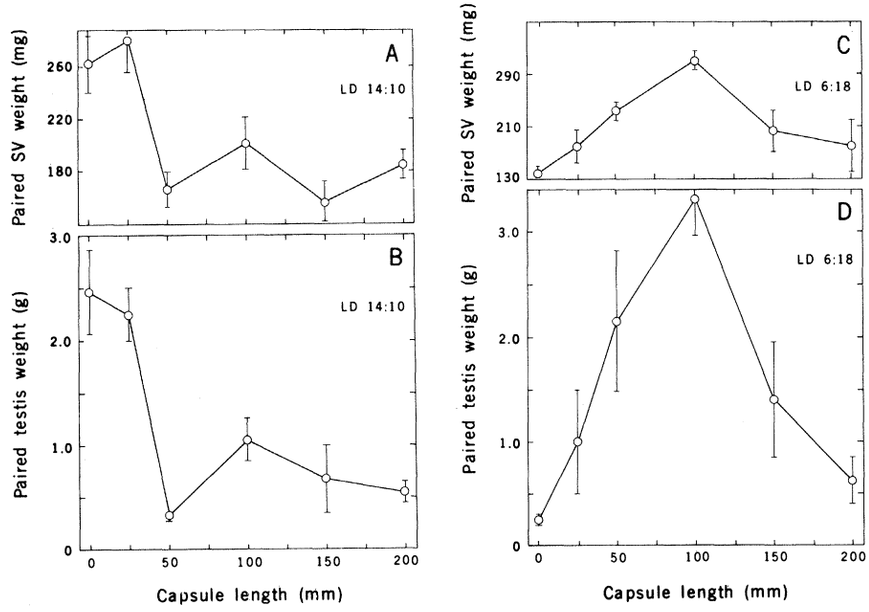

Fig. 1. Average weight of seminal vesicles (*SV*) (A) and testes (B) of hamsters maintained on an LD 14 : 10 photoperiod and implanted with melatonin-filled capsules of various sizes for 60 days. Average weight of seminal vesicles (C) and testes (D) of hamsters implanted with melatonin-filled capsules 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.

auratus) were reared in our laboratory under an LD 14 : 10 light-dark cycle (14 hours of light followed by 10 hours of darkness) from animals originally purchased from Lakeview Hamster Colony, Newfield, New Jersey. Polydimethylsiloxane (PDS) capsules containing melatonin were placed subcutaneously along the dorsal midline of anesthetized hamsters (*4*). The amount of melatonin released from each capsule was determined by weighing the capsules before and after 60 days subcutaneous placement in situ (*5*). The rate at which melatonin was released from PDS capsules was relatively constant and directly proportional to capsule length (*6*).

In one experiment, adult male hamsters were maintained on a photostimulatory LD 14 : 10 light cycle and divided into six groups (six animals per group) (*7*). Animals in one group received an empty PDS capsule, while the animals in the other five groups received melatonin-filled capsules that were 25, 50, 100, 150, or 200 mm long. Animals were killed 60 days after the capsules were implanted. The testes and seminal vesicles of eight initial control animals averaged 2642 ± 212 mg and 254 ± 12 mg, respectively. Gonadal and seminal vesicle weights were maintained within the normal range in animals receiving empty capsules and 25-mm capsules filled with melatonin (Fig. 1, A and B). In contrast, marked regression of the testes and seminal vesicles occurred in hamsters receiving 50- or 200-mm capsules of melatonin. Determination of the relative number of germ cells at stage VII of the cycle of the seminiferous epithelium indicated that implants of 50- or 200-mm capsules of melatonin did not affect the number of type A spermatogonia but caused marked reductions in the number of spermatocytes and spermatids (Table 1). Moreover, no spermatids beyond step 11 of spermiogenesis were seen in tubular cross sections. This evidence represents the first demonstration of an antigonadal effect of melatonin in golden hamsters, and to our knowledge the most dramatic antigonadal response attributable to melatonin.

A second experiment, performed at the same time as the first, utilized the same initial controls and six groups of adult males (five animals per group). The animals were implanted with melatonin-filled capsules of various lengths and were immediately transferred to a nonstimulatory LD 6 : 18 light cycle. After 60 days, testicular and seminal vesicle regression occurred in control animals receiving empty capsules (Fig. 1, C and D) (*7*). Hamsters that received 25- or 50-mm capsules of melatonin had partially regressed testes, while those receiving 100-mm capsules of melatonin had testes, seminal vesicles, and germ cell numbers that were comparable to those of initial control animals. However, when hamsters received implants of 150- and 200-mm capsules of melatonin, testicular and seminal vesicle regression occurred. Although melatonin has been shown to exert progonadal effects in hamsters exposed to nonstimulatory photoperiods (*2, 3*), the present findings represent the first demonstration of a dose-dependent differential effect of melatonin on the testis (*8*).

Table 1. Relative number of germ cell nuclei per cross section of seminiferous tubule at stage VII of the cycle of the seminiferous epithelium. Each value represents the mean ± S.E. of testes from five hamsters. The indicated germ cell nuclei were counted in 25 "round" tubular cross sections at stage VII of the cycle of the seminiferous epithelium. All nuclear counts were corrected for differences in nuclear diameter by Abercrombie's formula and for tubular shrinkage by a Sertoli cell correction factor (*11*).

Light-dark cycle (hours)	Length of melatonin capsule (mm)	Relative number of germ cells			
		Type A spermatogonia	Preleptotene spermatocytes	Pachytene spermatocytes	Step 7 spermatids
14 : 10	None	0.93 ± 0.15	27.9 ± 1.2	26.9 ± 1.0	103.4 ± 2.4
14 : 10	50 (empty)	0.98 ± 0.11	28.2 ± 0.9	27.8 ± 1.3	107.2 ± 3.1
14 : 10	50	0.95 ± 0.10	7.2 ± 1.4	1.8 ± 1.2	0.5 ± 1.1
14 : 10	200	1.04 ± 0.13	13.5 ± 2.1	2.5 ± 1.4	2.1 ± 1.1
6 : 18	50 (empty)	0.88 ± 0.14	12.9 ± 1.6	5.3 ± 0.8	1.5 ± 1.2
6 : 18	100	0.96 ± 0.12	27.4 ± 1.7	26.5 ± 1.5	101.9 ± 2.1
6 : 18	200	1.00 ± 0.18	10.7 ± 1.8	3.3 ± 1.1	3.0 ± 1.0

Antigonadal effects of melatonin have been reported in some species (*1*), but Reiter *et al.* (*3*) have repeatedly failed to inhibit gonadal activity in golden hamsters. We suggest that it was possible to demonstrate a pronounced antigonadal effect of melatonin in the present study because the animals were exposed to exogenous melatonin administered at relatively constant rates for prolonged periods. The failure of previous investigators to record this effect of melatonin on the testis may be due to the limitations inherent in previous modes of melatonin administration.

Many investigators have speculated that peptides produced by the pineal gland may be responsible for the antigonadal activity ascribed to this organ (*9*). Within this framework, it has been hypothesized that melatonin may exert its effects upon the neuroendocrine-gonadal system by affecting the release of other substances by the pineal gland (*10*). We feel it to be just as likely that the presumed antigonadal pineal peptides may exert their regulatory effects on the gonads by modulating pineal melatonin release. Although the mechanism (or mechanisms) by which melatonin influences the reproductive system has yet to be established, the present experiments suggest that melatonin (or one of its metabolites) may influence the neuroendocrine-gonadal axis at more than one locus, since differential responses were observed in the testes of hamsters exposed to nonstimulatory light cycles.

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References and Notes

1. R. J. Wurtman, J. Axelrod, D. E. Kelly, *The Pineal* (Academic Press, New York, 1968); I. A. Kamberi, R. S. Mical, J. C. Porter, *Endocrinology* **88**, 1288 (1971); A. Moszkowska, C. Kordon, I. Ebels, in *Ciba Foundation Symposium on The Pineal Gland*, G. E. W. Wolstenholme and J. Knight, Eds. (Churchill, London, 1971), pp. 241–258; C. Liu and G. A. Kinson, *Contraception* **7**, 153 (1973); R. J. Reiter, *Annu. Rev. Physiol.* **35**, 305 (1973).
2. K. Hoffman, *Naturwissenschaften* **61**, 364 (1974).
3. R. J. Reiter, M. K. Vaughan, D. E. Blask, L. Y. Johnson, *Science* **185**, 1169 (1974); *Endocrinology* **96**, 206 (1975).
4. Melatonin was obtained from Regis Chemical Co., Morton Grove, Illinois, and checked for purity according to the method of R. K. Raj and O. Hutzinges [*J. Chromatogr.* **44**, 199 (1969)]. Polydimethylsiloxane capsules were prepared from medical grade Silastic tubing (1.47 mm inside diameter and 1.96 mm outside diameter) by sealing one end of a segment of tubing with type A Silastic medical adhesive (Dow-Corning, Medical Products Div., Midland, Michigan). Twenty-four hours after sealing one end, tubes of the desired length were packed with crystalline melatonin (chromatographically pure) and the remaining end was filled with Silastic adhesive.
5. All capsules were dried for 24 hours at 60°C prior to weighing. Less than 2 percent of the melatonin was released after capsules were placed under the skin of hamsters for 60 days. No mortality or evidence of adhesions or lesions was apparent in animals receiving melatonin implants. Moreover, no significant changes ($P > .50$) were observed in the body weight of control and melatonin-treated hamsters. Silastic capsules have previously been used to administer steroid hormones [L. G. Stratton, L. L. Ewing, C. Desjardins, *J. Reprod. Fertil.* **35**, 235 (1973)].
6. The melatonin release rates in capsules that were 25, 50, 100, 150, or 200 mm long were, respectively, 14 ± 1.0, 26 ± 1.1, 50 ± 1.5, 73 ± 1.3, and 101 ± 1.6 μg/day.
7. Hamsters maintained on LD 14 : 10 have normal testes, while a 60-day exposure to short days (for example, LD 6 : 18) results in gonadal regression. See R. A. Hoffman and R. J. Reiter, *Science* **148**, 1609 (19[illegible] S. Gaston and M. Menaker, *ibid.* **158**, 925 (1967); W. E. Berndtson and C. Desjardins, *Endocrinology* **95**, 195 (1974); F. W. Turek, J. A. Elliott, J. A. Alvis, M. Menaker, *ibid.* **96**, 854 (1975).

8. In hamsters implanted with melatonin-filled capsules of various lengths and maintained under an LD 14 : 10 light cycle, exposure to a 50-mm melatonin capsule resulted in the maximum antigonadal effect on the testis. The mean testicular weight of animals implanted with a 50-mm capsule was significantly lower ($P < .001$) than that of animals implanted with a 100-mm capsule. Whether this also represents a dose-dependent differential effect of melatonin on the testis remains to be determined.
9. B. Benson, M. J. Matthews, A. E. Rodin, *Life Sci.* **10**, 607 (1971); I. Ebels, B. Benson, M. J. Matthews, *Anal. Biochem.* **56**, 546 (1973); S. Pavel, M. Petrescu, N. Vicoleanu, *Neuroendocrinology* **11**, 370 (1973); R. J. Orts, B. Benson, B. F. Cook, *Life Sci.* **14**, 1501 (1974); *Acta Endocrinol.* **76**, 438 (1974); M. K. Vaughan, G. M. Vaughan, D. C. Klein, *Science* **186**, 938 (1974).
10. W. B. Quay, *Pharmacol. Rev.* **17**, 321 (1965); S. Pavel, *Nature (Lond.)* **246**, 183 (1973).
11. Y. Clermont, *Rev. Can. Biol.* **13**, 208 (1954); ——— and S. C. Harvey, *Ciba Found. Colloq. Endocrinol.* **16**, 173 (1967).
12. This research was supported by PHS research grants HD-03803, HD-00268, HD-07727, and HD-09327 from the National Institute of Child Health and Human Development. We thank Don W. Carroll, Anne Reynolds, and Sam Siddiqui for their assistance.
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Human Interferon Production: Superinduction by 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole

Abstract. *Polyinosinic·polycytidylic acid [poly(I·C)] induced production of interferon by a strain of diploid human fibroblasts (FS-4), measured between 5 and 24 hours from induction, is enhanced up to 128-fold by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), a reversible inhibitor of nuclear heterogeneous RNA synthesis. A normalized dose-effect plot shows a close correlation between the superinducing effect of DRB and inhibition of RNA synthesis. Cultures that contained DRB continue to produce interferon for up to 4 days. Removal of the drug at any time during this period leads to a prompt shutoff of interferon production.*

Tamm *et al.* (*1*) reported in 1954 that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) was inhibitory for the multiplication of influenza virus. Subsequently mumps virus, vaccinia virus, poliovirus, and adenovirus have also been shown to be inhibited by DRB (*2, 3*). Furthermore, it was observed that DRB inhibits cellular RNA synthesis (*3, 4*). The latter observation has been extended recently by Egyházi *et al.* to the inhibition of RNA synthesis in the salivary gland cells of *Chironomus tentans*, by Tamm *et al.* to L and HeLa cells, and by Granick to chick embryo fibroblasts (*5*). The new evidence indicates that DRB is a selective and reversible inhibitor of nuclear heterogeneous RNA synthesis.

We have used DRB as a probe to study the regulation of interferon production in a strain of diploid human fibroblasts designated FS-4 (*6*). The focus of our studies is the mechanism of the enhancement of interferon production induced by polyinosinic·polycytidylic acid [poly(I·C)] in the presence of inhibitors of RNA or protein synthesis (*7*). Paradoxical enhancement of this kind, usually termed superinduction, has been observed in numerous other systems (*8*). We report that treatment of FS-4 cells with DRB at concentrations that inhibit RNA synthesis leads to an enhanced and prolonged production of poly(I·C)-induced interferon. In contrast to control cultures in which interferon production stops 6 to 8 hours after poly(I·C) induction, cultures treated with 30 μM DRB continue to produce interferon for up to 4 days. Removal of the drug at any time during this period leads to a prompt shutoff of interferon production. The results demonstrate the usefulness of DRB as a tool in the investigation of the regulation of biosynthetic processes and indicate that the interferon superinduction mechanism is closely linked to the inhibition of RNA synthesis.

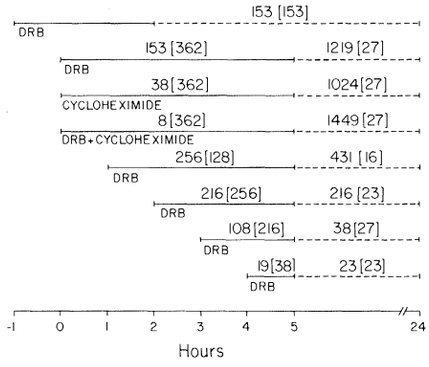

Fig. 1. Temporal characteristics of interferon superinduction by DRB. Cultures (13 days old) in 60-mm dishes were induced with poly(I·C), (100 μg/ml; 2 ml per dish) in Eagle's medium for 1 hour beginning at zero hour; DRB (12.8 μg/ml, 40 μM) or cycloheximide (50 μg/ml, 178 μM) was present during the periods indicated by the solid lines. At the ends of the periods of drug treatment the medium was harvested, and the cultures were washed and replenished with inhibitor-free maintenance medium, which was kept in the cultures during the periods indicated by the interrupted lines. Each number in the figure represents the geometric mean interferon yield in reference units per milliliter from two cultures, where each sample was titrated in duplicate. Interferon yields given outside brackets refer to cultures treated with drugs; those in brackets represent interferon produced in control cultures that were free of inhibitors measured in parallel for the periods indicated. In the groups treated with drugs for 0 to 5 hours, interferon production was measured between 1 and 5 hours.

FS-4 cells were grown at 37°C in Falcon petri dishes (60 mm or 35 mm) in Eagle's medium (*9*) containing heat-inactivated (56°C; 0.5 hour) fetal bovine serum, 10 percent (Grand Island Biological). The cultures were usually used between 9 and 15 days after plating. Poly(I·C) induction was carried out by exposing the cultures to a solution of 100 μg of poly(I·C) per milliliter (obtained from the Antiviral Substances Program, National Institute of Allergy and Infectious Diseases, Bethesda, Md.) in Eagle's medium for 1 hour, after which the cells were washed four times with warm phosphate-buffered saline (PBS) (*10*). Interferon produced by cells and released into medium was monitored at 37°C in 2 ml (60-mm dish) or 1 ml (35-mm dish) of maintenance medium (Eagle's medium containing 2 percent heat-inactivated fetal bovine serum). Interferon was assayed by a semimicro method (*6*), with FS-4 cells and vesicular stomatitis virus. Interferon titers are expressed in terms of the 69/19 reference standard for human interferon (obtained from the Antiviral Substances Program). Samples that contained inhibitor were dialyzed prior to titration. In all experiments zero time refers to the time when poly(I·C) was added to cultures.

The superinducing effect of DRB on interferon production is illustrated in Fig. 1. In this experiment cultures were treated with 40 μM DRB for varying intervals and at different times with respect to induction with poly(I·C). At 2 or 5 hours, from zero time as defined above, the medium was harvested, the cells were washed four times with PBS, 2 ml of inhibitor-free maintenance medium was added to the cultures, and incubation continued to 24 hours. At this time, the supernatants were again harvested. Control cultures and those containing cycloheximide [50 μg/ml (Polysciences)] were employed in parallel as indicated.

The results show that, whereas control cells produced the bulk of their interferon prior to 5 hours, cells treated with DRB from 0 to 5 hours made a large additional amount of interferon in the 5- to 24-hour period. A reduction in the time of exposure to DRB reduced the amount of interferon made after 5 hours. It is noteworthy that exposure to DRB from 1 to 5 hours was only one-third as effective as exposure from 0 to 5 hours. This suggests that the process, the inhibition of which is responsible for superinduction, may already be occurring during the first hour of poly(I·C) induction. Superinduction by DRB was not additive with that produced