that demonstrate a regulatory role for cyclic nucleotides in lymphocyte function (*1*). A role for cyclic AMP is further implied by the observed synergistic effects of the drugs in all functions tested (*4*). Lithium, on the other hand, appears to be a potent antagonist of the effects mediated by both the phosphodiesterase inhibitor (theophylline) and the β-agonists (salbutamol and isoproterenol). On its own, it was capable of augmenting the lymphocyte proliferative response to PHA and abrogating suppressor cell activity. In contrast, lithium could not prevent the effects of dibutyryl cyclic AMP in any of the assays. The mechanism of lithium action may be through inhibition of adenylate cyclase activation, preventing increases in intracellular levels of cyclic AMP (*3*). An inhibitory effect of lithium at a step beyond the formation of cyclic AMP has also been suggested (*12*). Alternatively, the observations with lithium may be unrelated to adenylate cyclase blockade per se. As an imperfect substitute for other cations involved in a number of cellular processes, lithium effects may be secondary to a more general influence on the cellular microenvironment, particularly the tertiary structure of membrane macromolecules (*3*). Although its precise mechanism of action remains to be defined, the use of lithium as a nonspecific immunologic adjuvant or as an inhibitor of suppressor cell activity warrants further investigation.

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Wax Secretion and Color Phases of the Desert Tenebrionid Beetle *Cryptoglossa verrucosa* (LeConte)

Abstract. *The desert beetle* Cryptoglossa verrucosa *(LeConte) exhibits distinct color phases that range from light blue to jet black when subjected to extremes of low and high humidity, respectively. The color phases are created by "wax filaments" that spread from the tips of miniature tubercles that cover the cuticle surface. The meshwork that accumulates at low humidity reduces transcuticular water loss and may lower the rate at which body temperature rises under a radiation load by increasing reflectance.*

Cryptoglossa verrucosa (LeConte) is a common beetle in the Sonoran Desert of the southwestern United States and adjacent portions of Mexico and Baja California (*1*). It is most abundant during hot summer months and can be collected from the surface from immediately before sunset until shortly after sunrise. Unlike other tenebrionid beetles with which it is sympatric, *C. verrucosa* exhibits distinct color phases that vary from bluish-white to black with intermediate gradations (Fig. 1A).

Initially these color phase changes were thought to reflect hydration state; however, this hypothesis was disproved by simple laboratory experiments. Subsequent tests indicated that relative humidity (RH) was the principal controlling factor. Beetles kept at 25°C and 35 to 40 percent RH in the laboratory retained an intermediate color phase whether fed or starved. When transferred to a desiccator (~ 0 RH), their color gradually changed to the whitish-blue phase (hereafter referred to as blue). Maximum lightening required 7 to 10 days. A second transfer of groups of blue beetles to 0, 20, 32.5, 50, 62.5, 75.5, 85, and 97 percent RH (*2*) produced the following results: (i) beetles at 97 percent RH showed pronounced darkening after 2 hours and were completely black after 24 hours, (ii) beetles at 75.5 and 85 percent RH exhibited limited darkening and some blotching after 24 hours (no further changes were noted), (iii) beetles maintained at 32.5, 50, and 62.5 percent RH assumed an intermediate color phase after $1^1/_2$ to 2 weeks, and (iv) beetles at 0 and 20 percent RH showed no change from the blue phase. More rapid darkening was obtained when beetles were suspended over water in closed containers (100 percent RH). Localized darkening was also achieved by carefully applying a drop of distilled water to the cuticle.

The experiments described above were performed with living intact beetles. When freshly killed beetles were subjected to the same tests, darkening at high humidities proceeded at the rate observed with living beetles. Dead black beetles, however, did not return to the blue phase when transferred to 0 RH even after a 3-week exposure. Irreversible color phase changes were also observed when pieces of elytral cuticle removed from blue beetles were darkened in saturated atmospheres and then transferred to 0 RH. The failure of dead beetles or cuticle segments to lighten from the black phase indicated that the process required energy.

The morphological basis for the humidity-induced color phases was determined by scanning electron microscopy. Pieces of cuticle from the dorsal thorax and abdominal elytra of beetles in the blue and black phases were examined (*3*). The elytra of this species are characterized by rows of large subacute tubercles (*1*). Projecting from the sides of each large tubercle as well as the general cuticle surface are numerous miniature tubercles (1200 per square millimeter) that appear to function in the wax-secreting process. Several of these tubercles in black (high humidity) beetles

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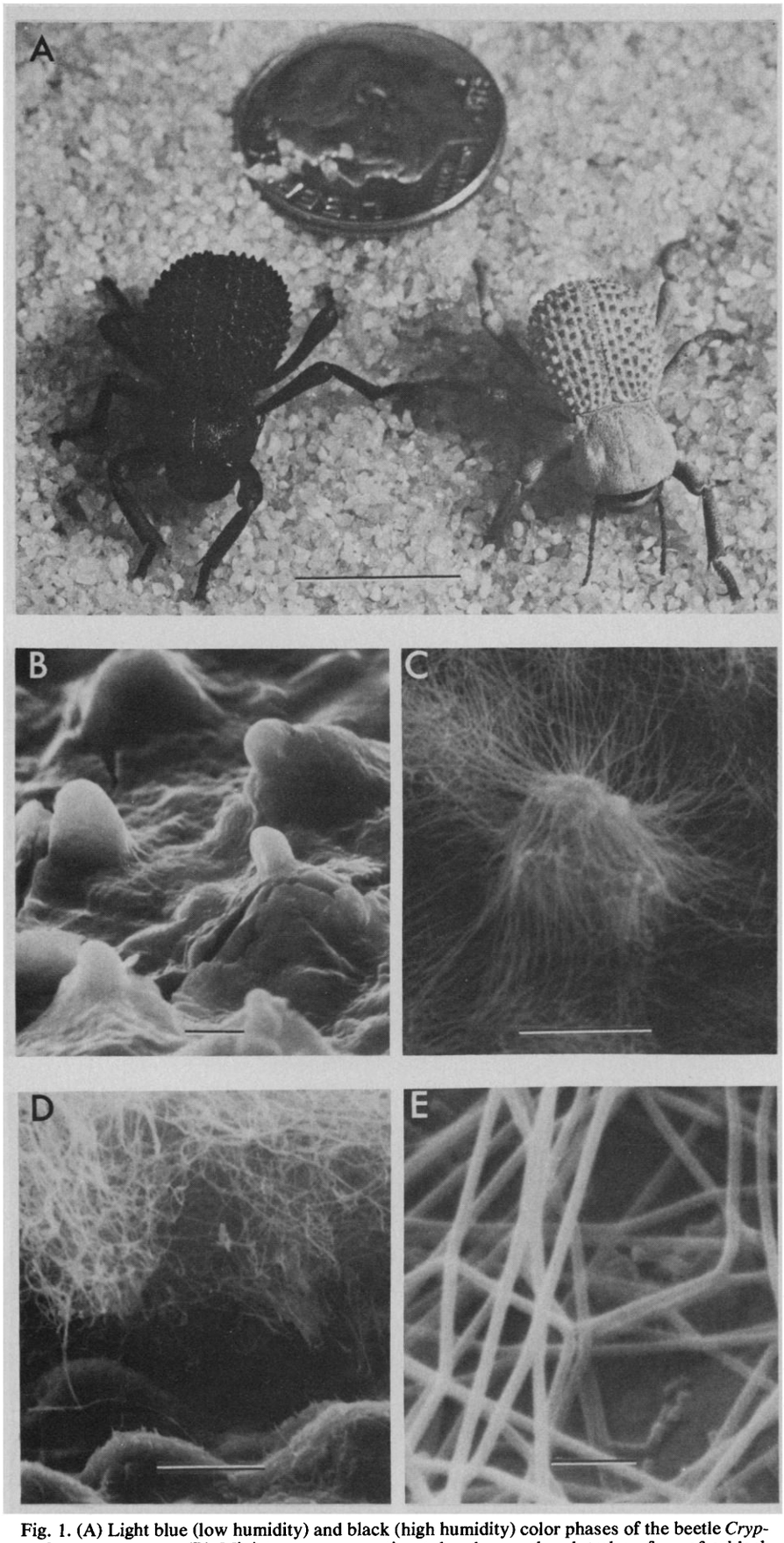

Fig. 1. (A) Light blue (low humidity) and black (high humidity) color phases of the beetle *Cryptoglossa verrucosa*. (B) Miniature wax-secreting tubercles on the elytral surface of a black phase beetle. (C) Spreading of wax filaments from tip of single tubercle in response to low humidity. (D) Scraped portion of cuticle surface showing boundary layer created by the wax meshwork. (E) High magnification of individual wax filaments. Reference bars: (A) 1 cm; (B to D) 10 μm; (E) 1 μm.

are shown in Fig. 1B. The tip of each tubercle is open; however, it is not known whether tubercles contain a single canal similar in diameter to the aperture or consist of several smaller canals that merge as they approach the tip. Amorphous material, similar in appearance to toothpaste gently squeezed from a tube, has been exuded from the tip of each tubercle. This condition is typical of cuticular surfaces of all beetles maintained at or near saturation and results in reflection of incident light producing a dark or black coloration. As long as the humidity remains at these levels, there is no spreading or further change in the morphology of this secretion.

Dramatic changes result when black beetles are transferred to low humidity. The amorphous secretion exuding from the tip of each tubercle organizes into numerous slender (0.14 μm) filaments that radiate from the tip. Eventually these spread and connect with filaments from other tubercles to form a basketlike mesh that covers the entire surface of the beetle. This phenomenon is illustrated in Fig. 1C; individual filaments are shown in Fig. 1E. The longer the exposure at the low humidity, the greater the mesh buildup. This surface network now refracts incident light to produce the characteristic blue phase. Maximum development of this meshwork is illustrated in Fig. 1D, where a portion of the surface covering was removed by scraping. Average thickness of the mesh is estimated as 20 μm; an air layer is also created between the lower surface of the meshwork and the cuticle surface. The fate of this surface accumulation after the return to high humidity or the application of moisture is not definitely known. Micrographs of the surface after such treatment indicate that the filaments simply "dissolve" into the epicuticular wax and contribute to its thickness rather than retracting into the tubercle tip.

Preliminary chemical analysis of filaments gently brushed from the surface of blue beetles indicated the presence of lipid (primary component) and some protein. Hydrocarbons constituted the bulk of the lipid fraction; however, cholesterol, alcohols, free fatty acids, and triacylglycerols were all detected. Hydrocarbons were all saturated molecules of even and odd chain lengths containing 20 to 36 carbon atoms (*4*). Straight-chain (*n*-alkane) hydrocarbons accounted for 88 percent and branched components 12 percent of the total. Although the ratio of *n*-alkanes to branched components was slightly higher than that observed in surface lipids from beetles in the intermediate color phase (*5*), the overall lipid

compositions in the two groups were very similar; this strongly suggests that the tubercle exudate is the wax material that eventually covers the surface to waterproof the beetle. The type of protein present and the chemical nature of the lipid-protein interaction were not determined. The protein fraction is probably essential for the morphological changes observed at the various humidity extremes, in view of the hydrophobic nature of the hydrocarbons and most of the other components of the lipid fraction.

The adaptive value of the color phases exhibited by *C. verrucosa* in response to humidity is uncertain. The wax filament network that accumulates at low humidity occurs at a time when the potential for transpiration is high. The boundary layer of air between the cuticle surface and the inner surface of the wax filaments, plus the meshwork of filaments itself, will increase the resistance to diffusion of water. A similar accumulation of wax filaments on sorghum leaves is believed to be responsible for this species' tolerance of drought (*6*). Increased diffusion resistance was tested for in *C. verrucosa* by comparing the rates of water loss of blue and black beetles at 40°C and 0 RH over a 6-hour period (*7*). The mean water loss of blue beetles (0.109 ± 0.032 mg cm^{-2} $hour^{-1}$) was slightly lower than that of black beetles (0.140 ± 0.026 mg cm^{-2} $hour^{-1}$) ($P < .01$; Student $t = 4.43$; 18 d.f.). This difference can be attributed largely to evaporation of adsorbed cuticular moisture in black beetles (*8*).

The blue (light) color created by the wax meshwork should also increase reflection of radiation and thus reduce the heat load on individuals directly exposed to sunlight. Namib Desert tenebrionid beetles with white elytra exhibited higher reflectance and lower body temperatures than closely related all-black species (*9*); similar results were obtained when *Eleodes armata* (black) from the Sonoran Desert were compared with individuals with their elytra painted white (*10*). Attempts to experimentally verify a similar relationship in *C. verrucosa* by comparing heating rates of blue and black beetles under artificial radiation were unsuccessful. Evaporation of water from the cuticle of black beetles cooled them sufficiently to keep their heating rates slightly below those of blue beetles in each test run. These results, however, are rather meaningless in view of the nature of the black phase, which is unlikely to occur under actual desert conditions. Blue phase beetles, which are commonly encountered in the field, probably derive some thermoregulatory benefits from increased reflection of radiation, but this is also of questionable significance because of the species' predominantly nocturnal surface activity. In any case, these adaptive benefits are secondary to the low cuticular transpiration rates that result from the production and deposition of surface lipids by this unique wax-secreting system.

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Methylazoxymethanol Treatment of Fetal Rats Results in Abnormally Dense Noradrenergic Innervation of Neocortex

Abstract. *A single injection of methylazoxymethanol in pregnant rats at 15 days of gestation results in severe cortical atrophy in the offspring. In the adult offspring, the neurochemical markers for the cortical γ-aminobutyric acid–containing neurons are severely reduced, whereas the noradrenergic markers are minimally altered. Immunohistofluorescence microscopy demonstrates a marked increase in the density of noradrenergic axons which have an abnormal pattern of distribution in the atrophic cortex. The results suggest that the central noradrenergic neurons determine the number of axons to be formed early in brain development, but local factors in the terminal field regulate the ultimate distribution of the noradrenergic axons.*

Cell divisions of functionally and morphologically distinct neuronal groups in the central nervous system occur in temporally discrete bursts (*1*). When two neuronal types differ significantly with respect to their time of "birth," the selective destruction of one group with sparing of the other may follow brief fetal or neonatal exposure to agents toxic to dividing cells (*2*). After this type of developmentally incurred brain damage, novel synaptic relationships among the remaining neurons probably account for some neurologic and behavioral abnormalities (*3*). In this report, we demonstrate that a single treatment of the rat fetus with methylazoxymethanol, a nucleic acid alkylating agent, results in an abnormally dense noradrenergic innervation of the atrophic neocortex in adulthood.

The cerebral cortex receives widespread innervation from noradrenergic neurons with cell bodies localized in the locus coeruleus. The cells that form the locus coeruleus undergo a period of intense mitotic activity between 11 and 13 days of gestation in the rat, after which cell division ceases; neurochemical and histofluorescence microscopic studies indicate that noradrenergic axons reach the primordial neocortex by 15 days of gestation (*4, 5*). In contrast, the neurons with cell bodies intrinsic to the rat neocortex undergo terminal cell divisions during days 14 to 20 of gestation with the smaller and more superficially located neurons dividing last (*6*).

In the present experiments we destroyed selectively, in fetal rats, the cortical neurons which divide on day 15 of gestation by injecting pregnant dams with a single dose of methylazoxymethanol acetate (MAM), the aglycone of cycasin. This compound is a potent alkylating agent that kills dividing cells by methylating the purine bases of the nucleic acids; the cytotoxic effects of MAM are confined to a brief period from 2 to 24 hours after injection, with maximum effects occurring at 12 hours (*7*). Previous studies have shown that a single injection of MAM to rats during the last week of gestation results in offspring with microcephaly (*8*).

Pregnant Sprague-Dawley rats that were sperm-positive on a definite date were housed in separate cages after 13 days of gestation. On day 15 of gestation, the rats received intraperitoneal injections of either MAM (20 mg/kg, diluted to a concentration of 10 mg/ml in 0.9

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