with smaller chitin-synthesizing particles, such as chitosomes, as shown by the absence of fluorescence outside the membrane areas in Fig. 1. Both by fluorescence and autoradiography, it appears that the synthetase is present at many different locations on the membrane. Other evidence (*2, 3*) indicates that most of the enzyme is found in the zymogen state if proteolytic activation is prevented. Thus, the initiation of chitin septum at a specific site of the membrane most probably occurs by localized activation of the zymogen, as postulated in our scheme (*2*). The results are not in agreement with an alternative hypothesis, namely, that localization of septum synthesis is attained by delivery of chitin synthetase to specific sites. If this were the case, the enzyme would be found only in a very small zone of the membranes and most of it should already be in the active form, because it would be participating in septum synthesis.

Sloat and Pringle (*9*) have reported that a temperature-sensitive yeast mutant, which is defective in bud initiation, shows, at the nonpermissive temperature, delocalized chitin deposition over the whole cell wall. This behavior sharply contrasts with the precise localization of chitin at the mother cell-bud junction in normal cells. Our working hypothesis (*1, 6*) assumes that during budding initiation, vesicles containing an activator of the chitin synthetase zymogen is directed, by a still unknown apparatus, to a specific site. It seems possible that, when cells are arrested in a prebudding state for a relatively long time, the directional apparatus might become disorganized; the vesicles containing the activating factor would then collide with the plasma membrane at random: activation of chitin synthetase and chitin deposition would occur over the entire surface of the cell as observed by Sloat and Pringle.

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Lithium: A Modulator of Cyclic AMP–Dependent Events in Lymphocytes?

Abstract. *Theophylline, salbutamol, isoproterenol, and dibutyryl cyclic AMP inhibited E-rosette formation by human T lymphocytes and immunoglobulin M secretion from human plaque-forming B cells and augmented T-suppressor cell activity in three patients with agammaglobulinemia. Lithium chloride increased mitogen-induced lymphocyte proliferation and inhibited suppressor cell activity. In the presence of lithium, the effects of all the drugs except dibutyryl cyclic AMP could be prevented. The data suggest a role for lithium in the modulation of cyclic AMP-dependent events in lymphocytes. Its potential role as an inhibitor of suppressor cell activity warrants further attention.*

The regulatory role of cyclic nucleotides on cell function has been demonstrated in a number of experimental systems. Although much of the work has been carried out with nonlymphoid tissues, there is now evidence that these nucleotides are important mediators of hormonal action on a variety of specific lymphocyte functions [reviewed in (*1*)]. Thus, adenosine 3′,5′-monophosphate (cyclic AMP) has been shown to regulate the formation of E rosettes, a marker of T cells, as well as the activation, proliferation, differentiation, and effector function of T lymphocytes. In addition, there is considerable evidence that cyclic AMP regulates the synthesis and release of immunoglobulin from antibody-producing cells. Membrane adenylate cyclase, through the production of cyclic AMP, plays a crucial role in the transduction of regulatory signals in cells following recognition and triggering (*2*). Lithium, probably through its ability to interfere with adenylate cyclase activation, can affect a variety of cyclic nucleotide–dependent events in the kidney, thyroid, and adrenal glands (*3*). We have examined the role of drugs known to involve cyclic nucleotide synthesis or degradation, in modulating the expression of different human lymphocyte functions, and have shown that these drug-induced effects were effectively countered by lithium chloride.

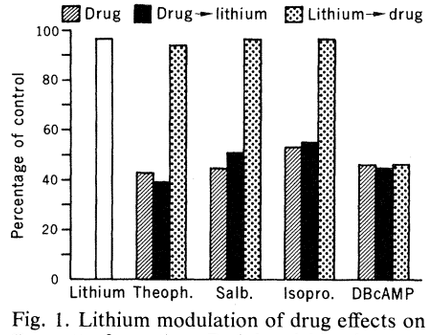

Fig. 1. Lithium modulation of drug effects on E-rosette formation. Peripheral blood mononuclear cells, obtained by Hypaque-Ficoll gradient centrifugation, were incubated with the drugs for 60 minutes at 37°C. In the study shown, lithium was added either 5 minutes before or 60 minutes after the drugs, and the mixtures were incubated for a further 60 minutes at 37°C. Sheep red blood cells were then added, the mixtures were centrifuged (200*g*) for 5 minutes at room temperature, and the pellet was maintained at 4°C for 2 hours. The pellet was then gently resuspended and the number of rosetting cells counted and expressed as a percentage of mononuclear cells. Controls, in the absence of any treatment, ranged from 50 to 62 percent. The means of three experiments, carried out in duplicate, are shown, in which an optimal concentration of lithium ($5 \times 10^{-3}M$) was used; values are expressed as a percentage of the control. Molar concentrations are: lithium, 5×10^{-3}; theophylline, 5×10^{-3}; salbutamol, 1×10^{-4}; isoproterenol, 1×10^{-4}; and dibutyryl cyclic AMP, 1×10^{-4}. Abbreviations: *Theoph.*, theophylline; *Salb.*, salbutamol; *Isopro.*, isoproterenol; and *DBcAMP*, dibutyryl cyclic AMP.

Formation of E rosettes by human T lymphocytes is inhibited by drugs that increase the intracellular levels of cyclic AMP via different mechanisms (*4*). Theophylline, salbutamol, isoproterenol, and dibutyryl cyclic AMP lead to a dose-dependent inhibition of E-rosette formation. As shown in Fig. 1, incubation with $5 \times 10^{-3}M$ lithium chloride, for as little as 5 minutes prior to the addition of the drugs, could abrogate drug-related inhibition of rosette formation in all instances except for inhibition induced by dibutyryl cyclic AMP. Moreover, lith-

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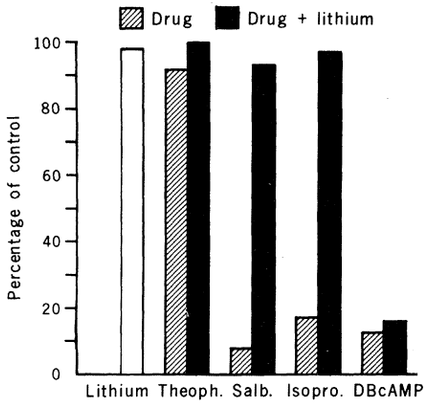

Fig. 2. Inhibition of the effect of drugs on immunoglobulin secretion. The drugs or lithium, added 5 minutes prior to the drugs, were added to a constant number of PFC specific for ovalbumin. Plaque-forming cells were generated in culture after 5 days of incubation with antigen, and the residual PFC activity was assayed. Control tubes contained 50 to 60 PFC per well. Results, expressed as a percentage of control, represent the means of three experiments carried out in triplicate. The optimal concentration of lithium in these studies was $1.5 \times 10^{-3}M$. Molar concentrations are: lithium, 1.5×10^{-3}; theophylline, 1×10^{-3}; salbutamol, 1×10^{-4}; isoproterenol, 1×10^{-4}; and dibutyryl cyclic AMP, 1×10^{-5}.

ium could prevent the inhibitory effects of synergistic combinations of theophylline, isoproterenol, and salbutamol; lithium alone had no effect. If the drugs were added to the cell suspension prior to the addition of lithium, their inhibitory effects were not reversible.

The generation of hemolytic plaque-forming cells (PFC) and the effector function of these antibody-producing B cells are also regulated by intracellular levels of cyclic AMP (*5, 6*). Activators of adenylate cyclase, inhibitors of phosphodiesterase, and dibutyryl cyclic AMP were able to inhibit plaque formation by murine spleen cells, sensitized in vivo, and the inhibitory effects were correlated with an elevation of lymphocyte levels of cyclic AMP (*6*). Using a human hemolytic PFC assay system (*7*), we were able to assess the role of these drugs in the release of specific antibody from antibody-forming cells. Salbutamol, isoproterenol, and dibutyryl cyclic AMP were inhibitory to the secretion of immunoglobulin M (IgM) antibody to ovalbumin from human lymphocytes (Fig. 2) (*8*). Theophylline was only marginally effective but, in combination with suboptimal concentrations of the other agents, synergism was observed with inhibition of PFC activity. The addition of lithium to the incubation mixtures permitted the full expression of PFC activity with all combinations of drugs, but it was ineffective in preventing the inhibitory activity contributed by dibutyryl cyclic AMP.

These data imply that cyclic AMP exerts a negative control over the synthesis or release of antibody from antibody-forming cells. The findings of inhibition of IgM secretion by the drugs were similar to those observed if peripheral blood lymphocytes (or purified T cells), freshly obtained from certain patients with humoral immunodeficiency, were mixed with PFC generated in culture (*9*). That is, significant suppression of PFC activity was demonstrated when patient cells were added to generated PFC just prior to the plaque assay; the addition of normal cells did not alter PFC activity (Fig. 3). In an attempt to ascertain whether this suppression could be regulated or influenced by intracellular levels of cyclic AMP, we studied the effect of the drugs on suppressor cell activity. Figure 3 illustrates one such study in which theophylline was shown to augment suppressor cell activity in three patients with congenital agammaglobulinemia. Significant augmentation of suppressor cell activity could also be observed in the presence of salbutamol, isoproterenol, and dibutyryl cyclic AMP alone or in combination. In contrast, the addition of lithium virtually eliminated the expression

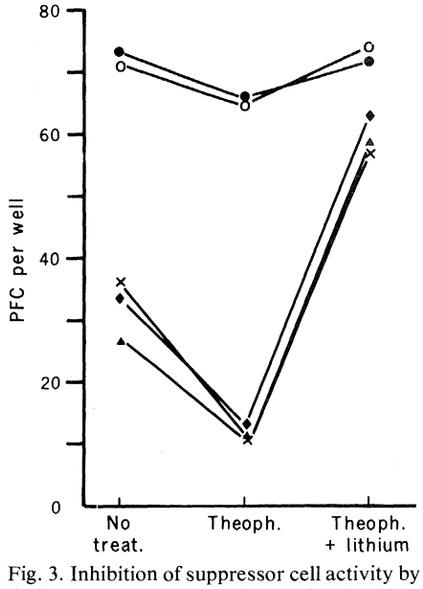

Fig. 3. Inhibition of suppressor cell activity by lithium. Equal numbers of normal cells (○) or cells from three patients (x, ◆, ▲) with congenital agammaglobulinemia were added to a constant number of PFC specific for ovalbumin (●). Residual PFC were then assayed in the presence or absence (*No treat.*) of theophylline (*Theoph.*) ($1 \times 10^{-3}M$) or theophylline plus lithium ($1.5 \times 10^{-3}M$). Lithium was added 5 minutes prior to the drug. Results are expressed as the number of residual PFC per well and represent the means of three experiments carried out in triplicate.

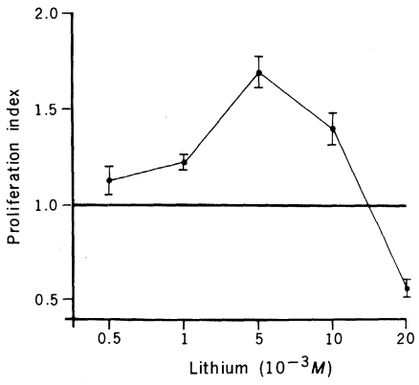

Fig. 4. Augmentation of PHA-response in the presence of lithium. Peripheral blood mononuclear cells were incubated in the presence or absence of varying concentrations of lithium for 30 minutes at 37°C, prior to the addition of 10 μg per milliliter of PHA. The degree of proliferation at 72 hours was assessed by uptake of [^{3}H]thymidine. The proliferation index was calculated by counts per minute (experimental tube)/counts per minute (control tube), where an index of 1.0 represents the control values in the absence of lithium. The means of two experiments (± 1 standard deviation), carried out in triplicate, are shown.

of suppressor cell activity by these patients' cells and prevented the expression of drug-augmented suppressor cell activity in all instances except for that induced by dibutyryl cyclic AMP.

Since increased levels of intracellular cyclic nucleotides and activation of adenylate cyclase have been demonstrated in lymphocytes after exposure to phytohemagglutinin (PHA) (*10*), we determined the effect of lithium on PHA-induced proliferation (Fig. 4). The addition of lithium at the same time or up to 1 hour prior to the addition of PHA resulted in a dose-dependent enhancement of [^{3}H]thymidine uptake with a peak response at $5 \times 10^{-3}M$ lithium. Higher concentrations of lithium ($\geq 10^{-2}M$) were inhibitory in this 72-hour assay. The addition of theophylline to the incubation mixture resulted in a dose-dependent inhibition of proliferation, which could be reduced or eliminated by the inclusion of lithium in the incubation mixtures, and the degree of reversibility was dependent on the concentration of theophylline. The inhibitory effects of $10^{-3}M$ theophylline were completely reversible by $5 \times 10^{-3}M$ lithium.

These experiments indicate that drugs associated with the induction of increased intracellular levels of cyclic AMP can modulate E-rosette formation, PHA-induced proliferation, IgM secretion, and suppressor cell activity in three patients with congenital agammaglobulinemia. Although cyclic AMP levels were not measured (*11*), our findings are analogous to a number of other studies

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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11. Since a number of the functions measured represent the activities of a small proportion of the cells in a given, but heterogeneous, cell population, the measurements of cyclic AMP and adenylate cyclase may not accurately correlate with functional effects. For example, the PFC frequency in the cell populations tested was < 0.1 percent (*7*) and frequency of suppressor cells in the patients, < 3 percent (*9*).
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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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