

*flaccidifolia* was the only species which did not have such glands and PADOLINA<sup>15</sup> later showed that this species did not elaborate sesquiterpene lactones.

Trichomes and their secretory products are recognized as defensive mechanisms against herbivores<sup>16</sup>, and since at least one sesquiterpene lactone is known to be an insect feeding deterrent<sup>17</sup>, parthenin and ambrosin in the trichomes of *P. hysterothorus* may also function as feeding deterrents.

Allergic contact dermatitis in man results from rupture of the glands and deposition of parthenin and ambrosin on the exposed skin. In India many of the cases of dermatitis from *P. hysterothorus* involve people who do not come in direct contact with the plant (LONKAR, personal

communication). The abundance of trichomes on wind-disseminated dried plant parts suggests a mechanism by which city dwellers may develop contact dermatitis from *P. hysterothorus*.

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The Effect of Lithium Carbonate on the Granulocyte Phagocytic Index<sup>1</sup>

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Summary. Preincubation in lithium carbonate of granulocytes from normal human male volunteer subjects did not alter the ability of these cells to perform properly the ingestion phase of phagocytosis in vitro.

Lithium carbonate (LC) has emerged as effective therapy for patients with manic depressive psychosis<sup>3</sup>. With regularity, patients given this drug develop leukocytosis, characterized by absolute granulocytosis<sup>4-7</sup>. The purpose of this study was to determine the phagocytic indices (PI) of polymorphonuclear neutrophils (PMNs) incubated in vitro for varying periods of time with concentrations of LC covering the therapeutic range and extending into toxic levels (0-5.0 meq/l).

Materials and methods. The technique of PI determination was a modification of the procedure as described by BERG and BRANDT<sup>8</sup>.

Heparinized blood was collected by venipuncture in plastic syringes from 4 healthy male volunteer subjects on 6 occasions. The whole blood was spun at 800 × g for 15 min in plastic centrifuge tubes. The buffy coat was removed by suction. Total white blood cell count and differential were determined by hand on an aliquot. The buffy coat was then diluted to 10<sup>4</sup> PMNs/μl with the donor's own plasma. 0.25 ml of the white blood cell suspension was added to LC in phosphate-buffered saline (PBS) to yield a final volume of 0.5 ml with LC concentrations of 0, 0.5, 1.0, 2.0 and 5.0 meq/l. These specimens were prepared in triplicate and incubated for 1, 2 and 4 h at 37°C in a water bath.

Upon completion of this incubation, 0.5 ml of heat-killed *Torulopsis glabrata* at a concentration of 4 × 10<sup>4</sup>/μl was added to the PMN suspension. This dilution provided an 8:1 ratio of organisms to PMNs. This mixture was incubated at 37°C for an additional 30 min. The specimens were then spun for 3 min in a small clinical centrifuge. The supernatant was removed and the button was spread and air-dried on a clean glass microscope slide, which was then stained with Wright's stain. 100 granulocytes were counted under oil immersion and scored according to the

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Mean PIs ± 1 SD of PMNs after incubation with varying concentrations of LC (0-5.0 meq/l) for intervals of 1, 2 and 4 hours

Lithium carbonate (meq/l)	Incubation period 1 h	2 h	4 h	Overall mean
0	4.13 ± 1.77	4.04 ± 1.53	4.45 ± 1.49	4.21
0.5	4.32 ± 1.69	4.11 ± 1.58	4.34 ± 1.50	4.26
1.0	3.94 ± 1.56	4.22 ± 1.42	4.15 ± 1.70	4.10
2.0	4.10 ± 1.39	4.14 ± 1.41	4.14 ± 1.52	4.13
5.0	4.31 ± 1.81	4.36 ± 1.58	4.16 ± 1.13	4.28
Overall mean	4.16	4.17	4.25	

Each value represents 6 experiments performed on PMNs from 4 normal healthy male volunteers. The SD are reported for descriptive purposes only and were not used in the statistical testing.

number of yeast particles present in their cytoplasm. The PI is defined as the mean number of ingested yeast per PMN.

In addition to the PMNs incubated in PBS without LC, another control value was obtained for each sample by determining the PI of PMNs exposed only to the heat-killed yeast without prior incubation in the salt solution.

**Results.** A three-factor analysis of variance procedure was used to analyze all data except the controls. The three factors were concentration, incubation period and sample. The sources of interest were differences among concentration means, among incubation period means, and the interaction of concentration and incubation periods. No significant interaction ( $p > 0.10$ ) was detected. The overall concentration means did not differ significantly ( $p > 0.10$ ) nor did the overall incubation period means. These data are summarized in the Table.

The unincubated control data were then compared to the data of LC = 0 meq/l by a two factor (sample, incubation period) analysis of variance procedure. This revealed no significant difference between the control mean ( $PI = 4.25 \pm 0.96$ , one SD) and any one of the other incubation period means ( $p > 0.10$ ). (See the 3 incubation means for LC = 0 in the Table). In addition, the mean of all the incubation data from each sample was then determined and the paired *t*-test was performed on these mean values and the associated control data which again showed no significant difference ( $p > 0.10$ ). The overall incubation mean is 4.20.

**Discussion.** Granulocytopenia is a troublesome and frequently life-threatening cyclic or chronic phenomenon in many patients. Few therapeutic agents reliably cause elevation of the absolute PMN count. Granulocytopenia can be induced with corticosteroid therapy but the long-term consequences of such therapy reduce the utility of these agents to the temporary situation or as a diagnos-

tic test of marrow granulocyte reserve<sup>9</sup>. Epinephrine causes transient granulocytosis by freeing the marginal pool of peripheral granulocytes<sup>10</sup>. This drug has been used diagnostically<sup>11</sup> but has no role in the management of granulocytopenic patients. Similarly, etiocholanolone and endotoxin have diagnostic utility but are fraught with morbidity and cannot be considered for long-term use<sup>12-14</sup>.

What is needed for management of these patients is an inexpensive, easily administered non-toxic agent that will increase the absolute PMN count without disturbing the PMNs' functional integrity. LC may fit this need. LC has been given to thousands of patients with great safety. Toxicity of LC is a direct function of serum  $Li^+$  concentration and this can be measured readily in most clinical laboratories allowing careful monitoring of patient dosage. These features of LC imply a possible role for this agent in the management of granulocytopenic states or in the preparation of granulocyte donors. Our data indicate that one parameter of PMN function, the ingestion phase of phagocytosis, is not perturbed by LC at therapeutic or toxic concentrations.

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## Concentration of 'Available' Unesterified Cholesterol in Human Plasma as Evaluated from Inhibition of Hemolysis by Lucensomycin

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**Summary.** The unesterified cholesterol content of plasma samples can be evaluated from the extent of inhibition of lucensomycin-induced hemolysis. The test measures, however, only the fraction of cholesterol which is available for interaction with lucensomycin, this availability being adversely affected by high phospholipid-cholesterol ratios.

Polyenic antibiotics are known to increase cell membrane permeability through specific interaction with membrane cholesterol<sup>1-3</sup>. It has been shown in model systems<sup>4,5</sup> that complex formation occurs with free cholesterol, cholesteryl esters being completely ineffective. Since polyene-induced lysis of mammalian erythrocytes is prevented by the presence of serum<sup>6</sup>, it seemed of interest to verify whether this effect was somehow related to the presence, in the serum, of free cholesterol and, if so, whether other factors contributed to it.

Most experiments were performed with the tetraenic macrolide antibiotic lucensomycin; in some cases the results were compared to those obtained with digitonin, a saponin of steroidal nature which is also specific for cholesterol<sup>7</sup>.

**Materials and methods.** Lucensomycin®, a kind gift of Prof. M. GHIONE, Farmitalia, Milan, Italy, was dissolved,

immediately before use, in a minimal amount of dimethylsulfoxide, and then diluted in 0.140 M NaCl, 0.015 M phosphate buffer, pH 7.0. Digitonin (Merck, Darmstadt, West Germany) was similarly dissolved in a minimal amount of methanol, and then diluted in the same buffer. In both cases, the organic solvent had been diluted to such an extent ( $\leq 1:200$ , v/v) that it had no undesirable effects by itself.

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