number of yeast particles present in their cytoplasms. 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. Granulocytosis 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 diagnostic test of marrow granulocyte reserve[9]. Epinephrine causes transient granulocytosis by freeing the marginal pool of peripheral granulocytes[10]. This drug has been used diagnostically[11] but has no role in the management of granulocytopenic patients. Similary, etiocholanolone and endotoxin have diagnostic utility but are fraught with morbidity and cannot be considered for long-term use[12–14].

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[1–3]. It has been shown in model systems[4,5] 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[6], 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[7].

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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Table I. Variations of the increase of L_{50} by addition of plasmas having similar content of unesterified cholesterol but different concentrations of cholesteryl esters and of phosphatides

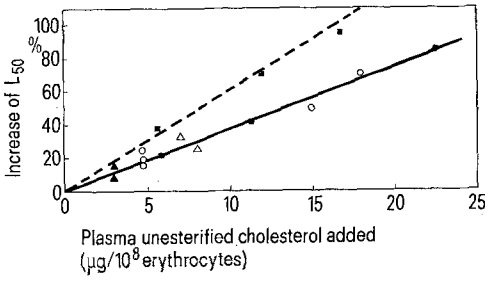
Experiment	Plasma sample	Unesterified cholesterol (mg/100 ml)	Total cholesterol (mg/100 ml)	Cholesteryl esters (mg/100 ml)	Phosphatides (mg/100 ml)	Increase of L_{50} (%)
1	1	24.0	153.0	129.0	202	8
	4	24.0	119.6	95.6	140	15
2	7	56.0	165.7	109.7	N.D.	32
	8	64.0	154.3	90.3	N.D.	25
3	12	39.8	207.5	167.7	135	23
	6	38.7	103.2	64.5	164	17
	9	38.7	129.0	90.3	182	17

All assays were performed with 10^8 erythrocytes in a final volume of 4 ml, which contained, when this was case, 0.5 ml of a 40-fold dilution of the plasma sample indicated. Within each experiment, the same erythrocytes suspension and the same lucensomycin solution were used.

Table II. Effects of lecithin on the ability of added cholesterol to modify the sensitivity of bovine erythrocytes to lysis by lucensomycin and by digitonin

Concentrations in the liposomes (mg/100 ml)		Increase of L_{50} (%)	Increase of D_{50} (%)
Egg lecithin	Cholesterol		
625	0	3.2	0
125	250	180	400
300	250	112	373
625	250	38	345

Liposomes were prepared with lecithin and/or cholesterol at the concentrations indicated. As in Table I, all assays were performed with 10^8 erythrocytes in a final volume of 4 ml, containing, when this was the case, 0.5 ml of a 40-fold dilution of the desired liposomal preparation.



Relation between the increase of L_{50} (expressed as percent of the value in the absence of plasma) and amount of plasma unesterified cholesterol added. Assays were performed, in a final volume of 4 ml and using 10^8 erythrocytes, with 0.5 ml of plasma diluted 1:40; each point corresponds to a different plasma, except those indicated by a full circle (●), for which different amounts (0.25, 0.50 and 1.00 ml) of the same plasma were used. Use of the same symbol indicates that the samples of plasma were assayed in the same experiment, i.e. with the same erythrocytes suspension and the same lucensomycin solution. The upper curve (broken line, full squares) was performed with digitonin instead of lucensomycin.

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Egg lecithin was prepared according to PANGBORN[8]. Cholesterol was recrystallized from hot acetic acid. Cholesteryl stearate and cholesteryl oleate were from BDH, Poole, England, and from Merck, Darmstadt, West Germany, respectively. Egg lecithin liposomes with or without cholesterol and/or cholesteryl esters were formed in NaCl-phosphate buffer according to BANGHAM et al.[9].

For lysis experiments, a known number of fresh bovine erythrocytes (usually around 10^8) was mixed, in a final volume of 4 ml of NaCl-phosphate buffer, with appropriate amounts of plasma (or of liposomes) and scalar concentrations of lucensomycin or digitonin; after 4 h, the degree of hemolysis was evaluated from the concentration of hemoglobin in the supernatant after low-speed centrifugation.

Results and discussion. The concentration of lucensomycin causing 50% hemolysis (L_{50}) of a suspension of bovine erythrocytes becomes significantly higher if human plasma is added to the assay. No significant shift ($< 5\%$) occurs upon use of either purified bovine albumin or of plasma delipidized by extraction with diethyl ether or with isopropanol. Scalar amounts of the same plasma (full circles of the Figure) cause proportional increases of L_{50}. Equal amounts of different plasmas shift L_{50} to an extent roughly proportional to the concentration of unesterified cholesterol (Figure). In some samples, however, with relatively low concentrations of phospholipids and/or high concentrations of cholesteryl esters, deviations from a pattern of strict proportionality are found (Table I).

By addition of liposomes of known composition to the hemolytic system, it can be shown that lecithin has, per se, no effect on L_{50}. Inclusion of cholesterol into the liposomes increases the L_{50}, the magnitude of the increase being however strongly dependent not only on the absolute amount of cholesterol but also on the lecithin-to-cholesterol ratio (Table II). Cholesteryl stearate and cholesteryl oleate, even in amounts 3 times higher (on a molar basis) than cholesterol, were unable to substitute for it, or even to modify the effect of high lecithin-to-cholesterol ratios.

Addition of cholesterol-containing plasma (or liposomes) cause also an increase of the concentration of digitonin (D_{50}) required to bring about 50% lysis of a given suspension of erythrocytes. The magnitude of the relative increase of D_{50} is higher as compared with that of L_{50}, and is very strictly dependent upon the amount of unesterified cholesterol added (Figure, dashed line). At variance with the results obtained with lucensomycin, in

the case of digitonin the lecithin-to-cholesterol ratio had very little effect (Table II).

The increase of D_{50} can therefore be utilized, as suggested by SCHMIDT-THOMÉ and AUGUSTIN[10], to obtain a satisfactory estimate of the concentration of unesterified cholesterol in plasma samples. With lucensomycin, the L_{50} concentration is instead affected also by other factors, such as the phospholipid-to-cholesterol ratio. Interference of unesterified cholesterol with lucensomycin-induced lysis occurs only if the sterol is sufficiently free from interactions with phospholipids.

As a matter of fact, in the liposome system, phospholipid-to-cholesterol ratios such as encountered in plasmas would lead to almost full non-availability of the cholesterol present. In plasma, however, unesterified cholesterol is associated only with certain classes of lipoproteins, and therefore only with a fraction of the total phospholipids present. Cholesteryl esters do not apparently exert any effect.

The method described in the present paper allows an evaluation of the plasma concentration of that fraction of unesterified cholesterol, which can not only interact with lucensomycin but is also likely to be most readily 'available' for exchange with the cholesterol of erythrocyte membranes and possibly for interaction with other structures, e.g. the walls of blood vessels.

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Mitigation of Graft-Versus-Host Disease in Rats Treated with Allogeneic and Xenogeneic Anti-lymphocytic Sera[1]

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Summary. The survival of rats receiving a sublethal dose of CY and grafted with allogeneic bone marrow, was prolonged by treatment with either allogeneic or xenogeneic anti-lymphocytic sera. These seem to prevent a fatal GVHD while allowing a temporary 'take' of the graft.

One of the obstacles in a successful clinical bone marrow transplantation is the graft-versus-host disease (GVHD) which accompanies the 'take' of a graft mismatched at the major histocompatibility complex (MHC) and is generally fatal. The GVHD is the result of an immunological attack by transplanted immunocompetent, thymus-dependent cells against allogeneic host tissues. Numerous attempts have been made to destroy or inactivate these cells without destroying the stem cells necessary for haemopoietic reconstitution. Anti-lymphocytic serum seems to be the most promizing agent for the prevention of GVHD in both animals and humans. Xenogenic anti-lymphocytic serum[2-7] and allogenic antilymphocytic serum[8-11] have been used successfully to prevent GVHD in certain cases. The purpose of the present study was to evaluate the therapeutic possibilities of anti-lymphocytic sera in an animal model.

Materials and methods. Induction of GVHD. Recipient rats (inbred Lewis, Ag-B1) were treated with 1 injection i.p. of 225 mg/kg of cyclophosphamide (CY), 24 h before grafting[12]. Bone marrow cells from the femora and tibiae of the donor rats (inbred DA, Ag-B4) were collected in Hank's balanced salt solution (HBSS). 1 ml of the suspension containing 64×10^6 cells was injected i.v. into Lewis rats. The 'take' of the graft was determined by the presence of GVHD symptoms (weight loss, dermatitis, post-mortem weight ratio of the spleen and body) and by typing the peripheral blood lymphocytes with DA anti-Lewis and Lewis anti-DA sera using the microlymphocytotoxicity test with guinea-pig complement.

Preparation of antisera. Allogeneic anti-lymphocytic sera were produced in Lewis and DA rats by cross-immunization with $180-300 \times 10^6$ rat lymph node cells injected once a week for 3 weeks. Xenogeneic anti-lymphocytic sera were prepared by immunizing rabbits in the same way with lymph node cells from DA rats[13]. The animals were exsanguinated and the individual sera were pooled into 2 batches and frozen at -20 °C. 1 ml of pooled antiserum was administered i.p. to the recipient 24 h before grafting. In some experiments, bone marrow cells were incubated at 37 °C for 30 min with xenogeneic antiserum[14,15] then washed once in HBSS and injected i.v. to the recipient rats.

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