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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Survival of Lewis rats grafted with DA marrow and treated with allogeneic or xenogeneic anti-lymphocytic sera[a]

Treatment	No. of rats	Cumulative survival at		
		15 days	45 days	90 days
Control				
CY only (no graft)	10	1[b]	0	0
CY + graft without antiserum	39	30 (29)[c]	5 (5)	0
Allogeneic anti-donor serum (Lewis anti-DA)				
Pool No. 1	18	15 (0)	12 (0)	10 (0)
Pool No. 2	15	14 (12)	4 (2)	2 (2)
Allogeneic anti-recipient serum (DA anti-Lewis)				
Pool No. 1	36	34 (19)	13 (5)	10 (3)
Pool No. 2	8	8 (8)	8 (8)	6 (6)
Xenogeneic anti-serum (rabbit anti-rat)				
Pool No. 1	10	10 (3)	10 (3)	5 (0)
Pool No. 2 (incubated with bone marrow)	30	28 (3)	15 (1)	9 (0)

[a] Rats received 225 mg of CY per kg body weight followed by injection of 64×10^6 DA bone marrow cells. [b] Number of rats surviving. [c] Number of rats with a 'take' of the graft.

Results. (Table). In the control group of rats which were given only CY without graft, mortality from severe aplasia was 9/10 at 15 days and 10/10 before 45 days. In the control group of rats which received a graft and no antiserum, a 'take' of the graft was observed in all but one animal (29/30). These 29 rats died from GVHD before 90 days.

Allogenic anti-donor serum. With pool No 1, more than half of the rats survived at 90 days, but none had a 'take' of its graft. With pool No 2, almost all rats had a 'take' of their graft, but only 2/15 survived at 90 days.

Allogenic anti-recipient serum. With pool No 1, 10/36 rats survived at 90 days, but only 3 of them had a 'take' of their graft. Pool No 2 allowed all rats to have a 'take' of their graft and 6/8 survived at 90 days.

Xenogenic anti-rat serum. With pool No 1, half of the rats (5/10) survived at 90 days, but without 'take' of the graft. With pool No 2, where bone marrow was incubated with antiserum before injection, 9/30 animals survived at 90 days, but without 'take' of their graft.

Discussion. The results presented in this study indicate that, under certain conditions, allogeneic and xenogeneic anti-lymphocytic sera may induce a prolongation of survival in animals grafted with allogeneic bone marrow cells. However, this increase in survival varies considerably from one pool to the other. Some allogeneic sera may be rich in blocking or 'enhancing' antibodies while others appear to be toxic or inactive, failing to protect the animals against the effects of GVHD and even sometimes by shortening their survival[8]. The results obtained with pool No 2 of anti-recipient sera, which allowed a prolonged survival and a protection against a lethal GVHD, without preventing a 'take', are probably due to the fact that this pool contained blocking, non-cytotoxic antibodies which protected the animals against the immunocompetent cells of the graft. The in vitro incubation of bone marrow cells with xenogenic antiserum is also based upon its potential cytotoxic or blocking effect on lymphoid cells. Our experiments show that, despite the risk of graft destruction, this procedure could be useful and merits further attention, as recommended recently[16].

In most experiments, the antisera, although preventing a 'take' of the graft, cause a prolongation of survival of the treated animals, compared to those receiving no serum and dying from GVHD. This suggests that anti-lymphocytic sera may act in two possible ways: 1. Through their immunosuppressive and cytotoxic properties, they inhibit the proliferation of the donor's immunocompetent and haemopoietic cells, but they allow them nevertheless to protect the recipient against the toxicity of CY. 2. By an unknown mechanism, they stimulate the repopulation of bone marrow by recipient's own haemopoietic cells[17].

These observations may have a most interesting clinical application: in some patients with aplastic anaemia of either toxic or idiopathic origin, a temporary bone marrow graft, even mismatched at the major histocompatibility complex (MHC), could protect them during a period of severe lifethreatening bone marrow insufficiency without risk of GVHD. It has recently been observed in rabbit[7] and also in humans[4] that histo-incompatible haemopoietic grafts performed in recipients conditioned with anti-lymphocytic serum resulted in split chimaeric states, with prolonged survival without signs of GVHD. Recent clinical trials[18] illustrate this possible application: 3 patients with severe aplastic anaemia were given anti-lymphocytic globulin followed by an HL-A semi-incompatible bone marrow graft. No clear-cut engraftment could be established and no GVHD was seen, but all patients showed a remission lasting 1 to 2 years.

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