In order to avoid this possibility we decided to irradiate the thymus and/or bursa cells, prior to their contact with the lymphoid cells[12]. Irradiation of a suspension of bursa and/or thymus cells was performed in petri dishes using a Cobalt 60 tube. The dose employed was 2000 r. After irradiation the cells were washed and resuspended to contain 40,000 c/mm³.

A suspension of Leghorn embryos lymphoid cells, isogenic with the test embryos was prepared in a procedure similar to that used for preparation of tissue culture. Embryos of 9 days were removed from the eggs, and minced into a homogenous suspension in citrate trypsin solution, after first cutting off the head and the extremities.

This suspension was then incubated for 25 min at 37 °C, with repeated shaking every 5 min, then centrifuged for 3 min at 5000 rpm and the sediment resuspended in saline containing 50 U penicillin per cm³. The number of the cells was adjusted to 40,000 c/mm³. Equal volumes of both suspensions were mixed and incubated for 1 h at 37 °C. Each experiment consisted of 3 or 5 groups of embryos innoculated as follows: 1. Embryonic lymphoid cells. 2. Irradiated bursa cells. 3. Irradiated thymus cells. 4. Combination of embryonic cells and irradiated bursa cells. 5. Combination of embryonic cells and irradiated thymus cells. The number of cells innoculated into each embryo was kept constant and was equal among all the groups of this experiment.

Results (see Table) indicate varying degrees of reactivity in groups 4 and 5, as presented by the percentage of embryos showing lesions. In group 4, lesions on the CAM were found in 52% out of 73 inoculated embryos. Group 5 was found to contain 67% positive reactions out of 34 inoculated embryos. At the same time no activity was shown, by either embryonic cells or thymus and bursa cells (groups 1–3). These results seem to indicate that the lesions formed are the product of the embryonic lymphoid cells which must have been transformed into competent cells by means of a factor released from the thymus and/or bursa.

Zusammenfassung. Bestrahlte Zellen von Thymus und Bursa sind fähig, embryonale lymphoide Zellen zu aktivieren, so dass diese auf der Chorionallantoismembran isologer Wirtstiere eine Graft-versus-host-Reaktion ausüben. Die Aktivierung beruht offenbar auf einem Faktor, der von den Thymus- oder Bursazellen abgegeben wird.

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% of embryos with lesions after inoculation with:

1. Embryo lymphoid cells (%)	2. Irradiated bursa cells (%)	3. Irradiated thymus cells (%)	4. Mixture of 1 + 2 (%)	5. Mixture of 1 + 3 (%)
0/15 (0)	0/14 (0)	0/12 (0)	8/14 (60)	12/12 (92)
0/8 (0)	0/10 (0)	0/11 (0)	6/13 (46)	6/11 (54)
0/9 (0)	0/8 (0)	–	11/17 (64)	–
0/0 (0)	0/4 (0)	0/7 (0)	3/9 (30)	5/10 (50)
0/11 (0)	0/4 (0)	–	3/10 (30)	–
0/7 (0)	0/9 (0)	–	7/10 (70)	–

Embryonic lymphocytes were prepared from 9-day-old Leghorn embryos. Thymus and bursa were extracted from giant chicken

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Daunomycin and Skin Allografts Survival in Mice

Daunomycin, an antitumoral antibiotic[1], interfering in nucleic acid synthesis[2], has shown a good immunodepressive activity in vitro on lymphoblastic transformation of lymphocytes[3–5] and in vivo on rat adjuvant arthritis[6,7], while its effects on primary humoral response and on the content of antibody-producing spleen cells in mice immunized with sheep erythrocytes[3,8,9] and on macrophage migration inhibition test[3] are not straightforward.

Daunomycin has also a slight, but not significant protective effect on skin allografts in mice according to BERNARD et al.[3], while the results obtained by CERULLI et al.[10,11] on the same biological system and on corneal xenografts in rabbits seem to show a greater immunodepressive activity of this compound.

Since the discrepancy of these data might be ascribed to the different treatment methods, we thought it would be interesting to investigate the immunodepressive activity of Daunomycin on skin allografts in mice, varying the route of administration, the dose and the treatment schedules.

Materials and methods. C_3H/HeJ and B_6A/F_1 mice (Jackson Laboratories – Bar Harbor Maine) were used, respectively as donors and recipients of skin grafts.

Skin grafts were performed according to the method of BILLINGHAM and MEDAWAR[12]; plaster of Paris casts were removed on day 9 and skin viability scored daily thereafter. The results were statistically analyzed following the method of BLISS[13] and differences between treated animals and controls evaluated by the 't' of DUNNETT.

Daunomycin was administered by various routes and with different schedules as shown in the Table. The number of animals for each group varied from 10–20 mice in the different experiments.

Results. The results obtained and reported in the Table show that Daunomycin increases skin allografts survival time. The differences observed between controls and treated animals are statistically significant at a level of 5% or even lower, whatever the doses and the treatment schedules employed.

The best results were obtained when the i.v. route was used with doses of 5 and 3.3 mg/kg 3 days before skin grafting or the i.p. route with a dose of 1.25 mg/kg for 8 days. However, the i.v. treatment is preferable, since it is non-toxic as compared to the i.p. treatment which at equivalent or lower cumulative doses resulted in

Immunodepressive activity of Daunomycin on skin allografts in mice

Drug	Treatment route	Schedules at days[a]	Doses (mg/kg/day)	Mean survival time	Probability level (P) (comparison with control group)
–	–		–	12.4	
Daunomycin	i.p.	−3, −2, −1, 0	1.25	13.1	$\leqslant 0.05$
Daunomycin	i.p.	−1, 0, +1, +2	1.25	14.1	$\leqslant 0.05$
Daunomycin	i.p.	−8, −7, −6, −5, −4, −3, −2, −1	1.25	15.0	$\leqslant 0.05$
Daunomycin	i.p.	−4, −3, −2, −1, +1, +2, +3, +4	1.25	13.7	$\leqslant 0.05$
Daunomycin	i.p.	+1, +2, +3, +4, +5, +6, +7, +8	1.25	14.1	$\leqslant 0.05$
–	–		–	13.2	
Daunomycin	i.v.	−3, −2, −1	5	15.4	$\leqslant 0.05$
Daunomycin	i.v.	−3, −2, −1	3.3	14.3	$\leqslant 0.05$
Daunomycin	i.p.	−8, −7, −6, −5, −4, −3, −2, −1	1.25	17[b]	

[a] 0 = day of grafting. [b] Some animals are dead before the end of the experiment.

delayed death of some animals in a group of younger mice. In fact it is well known that the lethal dose of Daunomycin is higher when this drug is administered i.v. as compared to the i.p. route.

Discussion and conclusions. According to our data Daunomycin determines an increase of skin allografts survival time in mice. This increase correlates well with the cumulative doses used, since the 5 mg/kg/day i.v. treatment gave the longest survival time, without any toxic side-effects, which points to the importance of the administration route. In fact the i.v. route allowed us to reach much higher cumulative doses of Daunomycin, moreover this route is currently used in human practice. Also, Daunomycin i.p. causes a marked local inflammatory reaction.

The mechanism of action of Daunomycin as immunodepressant is not completely understood, this drug is an antimitotic agent, thus it destroys mainly immature and actively proliferating cells i.e. bone marrow cells, gastrointestinal epithelium and neoplastic cells. Unpublished observations[14] from this laboratory have shown that it has no activity upon the reticulo-endothelial system and upon macrophages. The effect displayed against delayed hypersensitivity reactions is definite and well documented[3, 6, 7, 10, 11].

On the other hand, clinical studies by KOULINSKY et al.[15] have shown that delayed hypersensitivity reactions to 4 different antigens in a group of 21 patients with acute leukemia were completely abolished by Daunomycin in 6 subjects, who presented severe, irreversible bone marrow aplasia. It would thus seem that immunodepressive activity of Daunomycin is non-specific, probably related both to its antimitotic properties and to the destruction of immature cells (stem cells).

Riassunto. L'effetto immunodepressivo della Daunomicina è stato studiato sulla sopravvivenza del trapianto cutaneo nel topo. La Daunomicina determina un aumento del tempo di sopravvivenza del trapianto cutaneo, statisticamente significativo. Gli AA. discutono l'importanza che possono assumere le dosi, la via di somministrazione e lo schema di trattamento impiegato.

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Long Term Replacement Therapies with Testosterone Propionate and Human Chorionic Gonadotrophin in Hypophysectomized Adult Male Rats[1]

Daily testosterone propionate (TP) injections commencing immediately after hypophysectomy obviously prevented substantial testicular weight losses, sustained spermatogenesis, as judged by histological criteria, during a three weeks period in adult rats[2]. Longer replacement therapies revealed that testosterone did not maintain the germ cell numbers at the normal level[3]. Questions arose for how long a period testosterone, HCG could give replacement for the rat's testicle when started with the injections immediately after hypophysectomy.