Table I. Results of coagulation tests

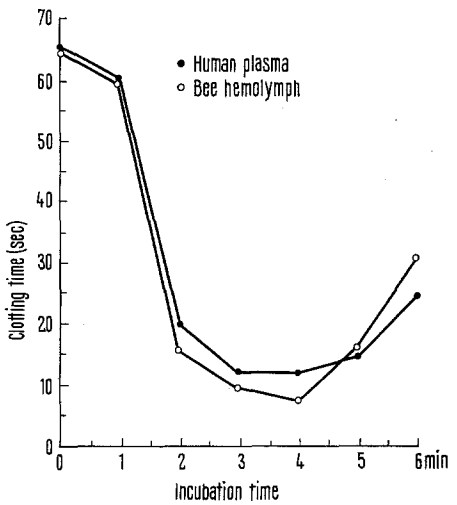
Coagulation test	Coagulation time[a,b] Human plasma	Larval hemolymph
One-stage prothrombin time	18.8	> 2 h
Partial thromboplastin time	53.2	365.8
Thrombin clot time	10.8	> 2 h
Antihemophilic globulin	88.3	150.3
Plasma thromboplastin antecedent	92.5	217.0
Proaccelerin	64.0	203.0
Proconvertin	24.2	> 2 h
Plasma thromboplastin component	83.5	285.0
Hageman factor	186.2	355.3
Stuart factor	20.2	372.4

[a] Average of 3 determinations. [b] In seconds unless otherwise noted.

Table II. Results of recalcification time and nonspecific mixing test for anticoagulants

Substrate	Clotting time[a]
0.5 ml human plasma	3 min
0.5 ml larval hemolymph	> 2 h
0.25 ml human plasma, 0.25 ml larval hemolymph	4 min

[a] Average of 3 determinations.



Results of thromboplastin generation test.

Table II shows that the larval hemolymph lengthened the recalcification time of human plasma. Thus, a circulating anticoagulant and the absence of proconvertin may possibly account for the negative thrombin clot time of bee blood.

The thromboplastin generation time curves of larval hemolymph and human blood (Figure) were similar. Therefore, the hemolymph was capable of generating intrinsic thromboplastin with the aid of human sera and platelets. The concentration of tissue thromboplastin was negligible, but the partial thromboplastin concentration was detectable.

The clotting system for honey bee hemolymph was similar to that of man, except that honey bee hemolymph had no proconvertin and contained lesser amounts of other coagulants; as noted, a circulating anticoagulant probably was present in hemolymph. The coagulants in bee hemolymph might have had a different chemical nature and thus were less suitable substrates, enzymes, or co-factors in the system.

Zusammenfassung. Haemolymphe der Honigbiene *Apis mellifica* wurde auf das Vorhandensein von verschiedenen Koagulantien untersucht, die im menschlichen Blut vorkommen. Die Haemolymphe gerann nicht, weil sie kein Proconvertin enthielt. Zudem konnte ein zirkulierendes Anticoagulans nachgewiesen werden.

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Graft Versus Host Reactivity of Embryonic Lymphoid Cells, Activated by Irradiated Thymus and/or Bursa Cells

The function of the thymus and the bursa of *Fabricius* in chicken was the subject of many investigations up to now. Most of the experiments reported[1-4] were concerned with the loss of certain properties of an immunological nature by chickens thymectomized or bursectomized, either surgically or hormonally.

Our approach[5,6] was based on the 'Simonsen phenomenon', i.e. production of lesions on the chorio allantoic membrane of chick embryos by immunologically competent cells. In a previous work[7] we showed that allogenic bursa cells could cause isologous lymphocytes to give a graft versus host (GVH) reaction on chick embryos. We found the same to be true for thymus cells, which led us to the conclusion that the phenomenon was a result of the action of a humoral factor, contained in bursa and thymus cells of chicken, that activated the lymphocytes[8].

In the present experiment we tried to find out whether this was caused merely by the activation of pre-existing competence or else that the thymus and/or the bursa could also affect the embryonic cells by changing them from inactive cells into active ones[9].

Though it was already stated[10,11] that in chickens neither bursa nor thymus cells can give a GVH reaction, the question was raised whether bursa or thymus cells could not be changed during contact with other cells, prior to inoculation on the chorio allantoic membrane, so as to make them capable of producing lessions.

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