Table II. Weights (mg) of lymphoid organs (means ± SE) in non-grafted rats and in rats bearing a GVHR

Group	Balanced diet				Protein-free diet (9 weeks)			
	Body wt. (g)	Thymus	Spleen	Cervical lymph node[a]	Body wt. (g)	Thymus	Spleen	Cervical lymph node[a]
Non-grafted rats	343.7 ± 7.7 (11)[b]	547.6 ± 25.9	796.5 ± 74.3	17.3 ± 2.8	114.1 ± 1.2 (15)[b]	33.5 ± 3.6	166.3 ± 13.0	4.9 ± 0.9
Grafted rats	357.1 ± 4.0 (16)	379.0 ± 13.2	603.8 ± 12.3	11.8 ± 0.7	112.1 ± 1.3 (8)	20.8 ± 2.5	142.8 ± 7.0	3.6 ± 0.3
Difference (%)		− 37.7[c]	− 28.3[e]	− 31.8 ns		− 37.9[d]	− 14.2 ns	− 26.5 ns

[a] Means obtained from the largest 4 lymph nodes; [b] No. of animals; [c] $p < 0.001$; [d] $p < 0.01$; [e] $p < 0.02$; ns, not significant.

Discussion. The finding of a significant correlation between the weight of the lymphoid organs and the strength of GVHR agrees with the hypothesis that the involution of these organs plays a major role in the dietary inhibition of the host response to the graft, by reducing the supply of T cells involved in the struggle against the grafted cells.

The fact that the GVHR-initiated reduction of the lymphoid organs is less pronounced in PD rats than in normally nourished ones, could be ascribed first to the 'stress' effect of GVHR. Indeed, protein deficiency is by itself a stressing factor as suggested by the increased adrenal and plasma levels of corticosterone in PD rats[8], and the high plasma levels of cortisol in human protein malnutrition[9–11]. This should reduce the sensitivity of the surviving PD lymphocytes to the GVHR stress, since these cells are predominantly cortisone-resistant[12]. However, the decrease in the weight of the thymus and the spleen, observed in grafted rats, is very probably also the consequence of a prolonged transfer of T lymphocytes from the lymphoid organs to the GVHR area in order to compensate the destruction of these cells by the graft. Such a transfer should be, of course, more important with a balanced diet than with a diet deficient in protein.

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Alpha-Tocopherol: Its Inhibition on Human Platelet Aggregation

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Summary. Alpha-tocopherol inhibits human platelet aggregation induced by arachidonate sodium, collagen, epinephrine, adenosine diphosphate or thrombin – arachidonate sodium being the most susceptible. The second phase of the biphasic platelet aggregation induced by epinephrine or adenosine diphosphate is preferentially inhibited.

The generalized Shwartzman reaction (GSR), an experimental model of disseminated intravascular coagulation, can be induced in pregnant rats by means of an α-tocopherol (aT) deficient diet[2]. Conversely, this pathologic condition can be induced in non-pregnant rats by a high lipid diet[3]. These results suggest the possibility of a synergistic mechanism between diets with either a high lipid content or a deficiency of aT in the pathogenesis of GSR in rats. Supplementation with aT to rats on a high lipid diet regularly protects the animals[4].

This report deals with in vitro studies on the inhibitory effect of aT on human platelet function. 9 ml samples of venous blood from healthy volunteers were collected in polystyrene tubes containing 1 ml of 0.1 *M* citrate buffer (pH 6.5) in 2.5% (w/v) of dextrose. Platelet-poor plasma (PPP) was prepared by centrifugation of blood at 1000 *g* for 20 min at 4 °C. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 300 *g* for 20 min at room temperature. Platelet aggregometry was performed as previously described using a Chrono-log platelet aggregometer coupled to a Fisher Recordall recorder[5]. Briefly, 0.45 ml of PRP was added to a cuvette with a magnetic stirring bar. This was placed in the aggregometer and the light transmission was adjusted to about 10%; similarly that for PPP was adjusted to about 90%. At zero time, 50 µl of normal saline or aT (Arlington Laboratories, Montreal, Que.) were added. 1 min later 50 µl of an aggregating agent were added. Platelet aggregation resulted in an increased light transmission and thus an upward deflection of the recording pen. The aggregating

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Kinetic analysis of α-tocopherol inhibition on human platelet aggregation

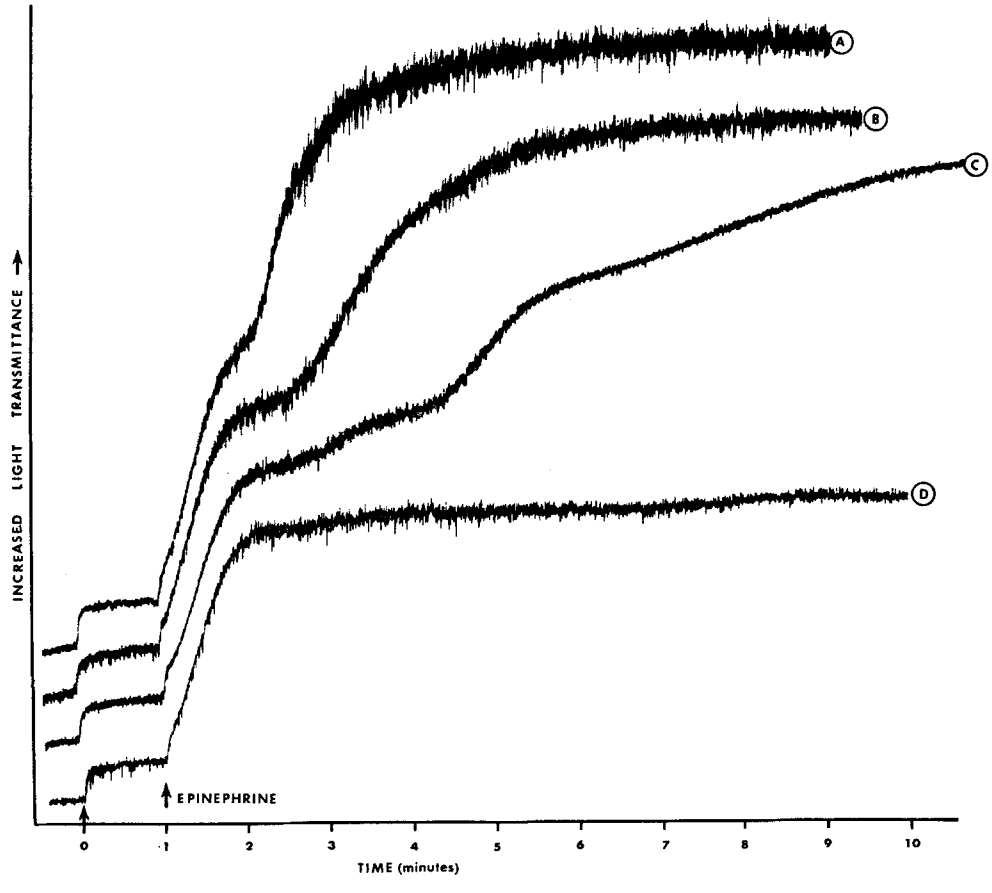
α-tocopherol in saline (μg/ml)	Aggregation agents							
	Collagen		ADP		Thrombin		Arachidonate sodium	
	S (%)	dT (%)	S (%)	dT (%)	S (%)	dT (%)	S (%)	dT (%)
0	11	77	20	59	19	55	14	98
50	9	65	16	45	19	55	4	8
250	1	16	15	39	15	31	0	0
500	0.5	15	10	30	14	30	0	0

To 0.45 ml of platelet-rich plasma, 50 μl of α-tocopherol in normal saline were added at zero time and the same volume of aggregating agent at 1 min. Concentration of aggregating agents used was as follows: collagen at a minimal dose that gave maximal aggregation, adenosine diphosphate (ADP) at 1×10^{-5} m*M*/ml, thrombin at 10 units/ml and arachidonate sodium at 2 μ*M*/ml. Slope (S) represents the maximal change in percent of light transmission over a 12 sec period. Delta-T (dT) is the maximal change in percent of light transmission as a result of platelet aggregation.

agents used were adenosine diphosphate (ADP), arachidonate sodium, and collagen (Sigma Chemical Co., St. Louis, Mo.), epinephrine (Parke, Davis and Co. Ltd., Brockville, Ont.) and thrombin (Upjohn Co., Kalamazoo, Mich.).

A classical normal response was noted when 50 μl of epinephrine (0.1 mg per ml) were added to 0.45 ml of PRP (Figure; tracing A). The biphasic curve reflects an immediate phase of platelet aggregation when epinephrine is added and a second wave of platelet aggregation induced by the endogenous release of ADP from platelets. When aT was added to the PRP prior to the introduction of epinephrine, a dose related inhibition of platelet aggregation occurred. The immediate or first phase of aggregation induced by epinephrine was not appreciably altered by the different amounts of aT added, however the second phase was markedly inhibited (Figure). Similarly the second rather than the first wave of platelet aggregation caused by ADP was extremely sensitive to aT inhibition.

Kinetic data on aT inhibition of platelet aggregation mediated by collagen, ADP, thrombin and arachidonate sodium are presented in the Table. Generally, a dose related inhibition was observed. In decreasing order of their susceptibility to aT inhibition of platelet aggregation were arachidonate sodium, collagen, ADP and thrombin.



α-tocopherol inhibition of in vitro human platelet aggregation induced by epinephrine. At zero time, 50 μl of α-tocopherol were added to 0.45 ml of platelet-rich plasma. The final concentration of α-tocopherol was A=0, B=50, C=100 and D=250 μg per ml respectively. At 1 min, platelet aggregation was induced with the addition of 50 μl of epinephrine achieving a final concentration of 10 μg/ml.

Thus aT is a potent in vitro inhibitor of human platelet aggregation. While the efficiency of inhibition against a diversified group of aggregating agents varies it is particularly active against the second wave of biphasic platelet aggregation induced by epinephrine or ADP. At a concentration of 250 µg of aT/ml of PRP, the first phase of aggregation was reduced by about 25% and 20% of control values respectively, when the second wave of aggregation was completely inhibited .Thus the response to exogenously introduced ADP or epinephrine as represented by the first wave of platelet aggregation was only minimally reduced. The second wave of platelet aggregation, mediated through endogenously released ADP, was completely inhibited. This finding of preferential inhibition by aT of the second phase of platelet aggregation is similar to that induced by other inhibitors such as acetylsalicylate sodium, indomethacin and 5,8,11,14-eicosatetraynoic acid (TYA)[6].

One of the possible mechanisms of this aT inhibition is suggested by its characteristic pattern of inhibition of platelet aggregation, namely it inhibits the second phase of platelet aggregation induced by ADP or epinephrine and platelet aggregation induced by arachidonate sodium very effectively. Arachidonate sodium is a precursor for the synthesis of prostaglandins which have been shown to play a role in platelet aggregation[7]. The addition of arachidonate sodium in vitro to human or rabbit PRP caused rapid aggregation of platelets; when arachidonate sodium was injected i.v. into rabbits platelet thrombi were formed rapidly[8]. Chemicals, such as acetylsalicylate sodium, indomethacin or TYA, that interfere with the arachidonic acid-prostaglandins metabolism are potent inhibitors of platelet aggregation[8,9]. The present study shows that the inhibition by aT of platelet aggregation induced by ADP, collagen and epinephrine bears similarity to that by acetylsalicylate sodium, indomethacin or TYA. Thus the mechanism of inhibition by aT may rest on its ability to interfere with the metabolism of arachidonic acid-prostaglandins system[10]. This ability to interfere may be due to the stoichiometric structure of aT that favours its interaction with the arachidonic acid[11].

Another possible mechanism for aT inhibition of platelet aggregation is through its lipid antioxidant activity – inhibiting the preoxidation of polyunsaturated lipids[12]. Specifically, platelets may be induced to aggregate with hydrogen peroxide, and this aggregation can regularly be prevented with tocopherols[13]. It appears that aT is necessary to inhibit the peroxidation of unsaturated fatty acids which form an integral part of membrane structures[14].

An earlier study reported minimal inhibitory activities of aT on the first phase of human platelet aggregation induced by ADP. Storage of platelets at 4 °C and prolonged incubation periods might account for the lack of the second wave of platelet aggregation even in control samples[15]. While the present report shows the inhibitory activities of aT on human platelets, prolonged feeding of rats with an aT deficient diet was associated with an increased response of platelet aggregation towards collagen[16]. Preliminary studies in this laboratory on 3 adult human volunteers ingesting huge doses of aT support the present in vitro findings.

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Isolation of a Potent Allergen from House Dust Mite by Interaction with the Lectin Tridacnin

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Summary. Tridacnin, the lectin from the clam *Tridacna maxima*, precipitates with house dust mite extracts and provides a simple procedure for obtaining a potent, purified mite allergen. Allergenic activity was investigated using the radioallergosorbent test (RAST).

Hypersensitivity to house dust is of considerable importance in the aetiology of respiratory allergic disease in both temperate and tropical localities. In 1964 it was suggested that common house dust mites of the acarine family Pyroglyphidae were an important source of allergens in many house dust samples[3]. Since then, independent work in a number of countries has confirmed these observations[4]. Material from the 2 most common mite species found in house dust, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* provide some of the most potent and troublesome allergens involved in respiratory allergic diseases.

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