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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As with most antigen extracts used in allergology, mite extracts are usually complex, unstandardized mixtures variable both in their composition and potency. Knowledge of the individual allergenic components present in, or released by, the mites is limited and little progress has been made in isolating and characterizing pure mite allergens. Attempts to prepare such allergens must overcome two main problems. Firstly, it is difficult to obtain enough mites and mite components for biochemical investigation. This is especially true for *D. pteronyssinus* which is more difficult to culture than *D. farinae*[5]. Secondly, once mites have been grown in sufficient numbers they must be obtained as free from medium contaminants as possible. In practice this is difficult to achieve[5]. The little that has been done on the composition of house dust mite allergens reveals that the main allergenic fractions from *D. farinae* consists of glycoproteins containing 5 main sugars, ribose, xylose, mannose, glucose and galactose[6].

In this paper we describe our attempt to selectively isolate saccharide-containing components from mite extracts by using tridacnin, the naturally-occurring anti-carbohydrate reagent or lectin[7] from the clam *Tridacna maxima* (Roding).

Materials and methods. Clams obtained from Israel were dissected with a knife and the pooled haemolymph dialyzed against distilled water and lyophilized. Tridacnin was isolated by affinity chromatography on a column of insoluble polyleucyl larch arabinogalactan[8]. Lyophilized haemolymph dissolved in saline containing 0.01 *M* Ca^{++}[9] was added to the column and subsequently eluted with a solution of saline-Ca^{++} containing 0.025 *M* N-acetyl-D-galactosamine[8]. Tridacnin has combining sites which are complementary to this amino sugar and to β-linked sugars with the D-galactopyranosyl configuration[9,10]. *D. farinae* extracts were obtained from Bencard, Brentford, England or grown on Gaine's dog meal and prepared for use as described by HOLFORD-STREVENS et al.[11]. The quantitative precipitin, immunodiffusion, immunoelectrophoresis (IEP) and radioallergosorbent test (RAST) methods used have all been described[10,12–15].

Results and discussion. Screening experiments with over 40 different lectin preparations revealed that haemolymph from *T. maxima* precipitates strongly with both *D. farinae* and *D. pteronyssinus* extracts (Figure 1). 6 different *D. farinae* extracts precipitated with tridacnin both in gel and in solution. Upon IEP in agar at pH 8.6 the tridacnin-precipitating component migrated with a mobility equivalent to that of a slow γ-globulin.

In isolation experiments, 100 mg of *D. farinae* extract was mixed with approximately 5 mg of tridacnin after the proportion of mite extract to lectin had previously been determined in microquantitative precipitin experiments[10,12]. After 5 days at 4 °C, the precipitate was washed 5 times with physiological saline containing 0.01 *M* Ca^{++} and then dissolved in 3 ml of 0.1 *M tris* HCl with 0.1 *M* EDTA, pH 6.5. This is an effective way of dissolving tridacnin precipitates since the lectin requires Ca^{++} for its precipitating and haemagglutinating (HA) actions[9]. Separation of the mite component from tridacnin was achieved on a 2×32 cm column of DEAE-Sephadex A50 (Pharmacia, Uppsala) in the above buffer. Negatively charged tridacnin adsorbed to the ion exchange column while the house dust mite component which is positively charged, eluted in the first peak off the column. Recovery of the lectin was achieved by the addition of 0.3 *M* NaCl to the elution buffer. Carbohydrate determinations using phenol-H_2SO_4 reagents showed positive reactions in the fractions comprizing the first peak. After dialysis and lyophilization, material from this peak was found to precipitate in gel with *T. maxima* extract and this precipitin line was confluent with the line formed from the reaction between *D. pteronyssinus* extract and *T. maxima* (Figure 2a). Like unfractionated *D. farinae* extract, the isolated material also gave an arc in the γ-region on IEP at pH 8.6 (Figure 2b). Unfractionated *D. farinae* extract and the tridacnin-reacting component were examined in haemagglutination inhibition experiments with 8 full HA doses of tridacnin. Inhibition of the agglutination of human group 0 erythrocytes occurred with 49.5–99 μg/ml of whole extract but only 5.2 μg/ml of the isolated mite material was needed for complete inhibition. On 5% polyacrylamide disc gels at pH 8.9, the isolated material showed one band near the anode end of the gel when stained for protein with Coomassie blue and for carbohydrate with Schiff's reagent.

Possible allergenic activity of the separated mite component was investigated by coupling the material to cyanogen bromide activated paper discs[13,14], and performing RAST experiments[15] with sera from newborn babies (cord serum), non-allergic individuals and house dust mite sensitive patients. With each of 6 sera from mite allergic individuals, strong positive reactions were observed indicating that each serum contained IgE antibodies specific for the carbohydrate-containing component isolated from *D. farinae* extracts. By contrast,

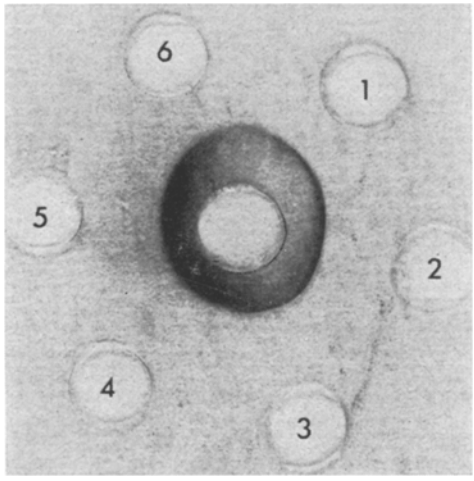

Fig. 1. Agar gel diffusion pattern formed from the reaction of *Tridacna maxima* haemolymph extract with house dust mite extracts. Centre well, *T. maxima* extract 5 mg/ml; peripheral wells, 1 and 6, *D. pteronyssinus* extract (Commonwealth Serum Laboratories, 150 × 'D' strength); 2 and 3, *D. farinae* extract (Bencard) number 5, 30 mg/ml; 4 and 5, *D. farinae* extract (Bencard) number 1, 50 mg/ml.

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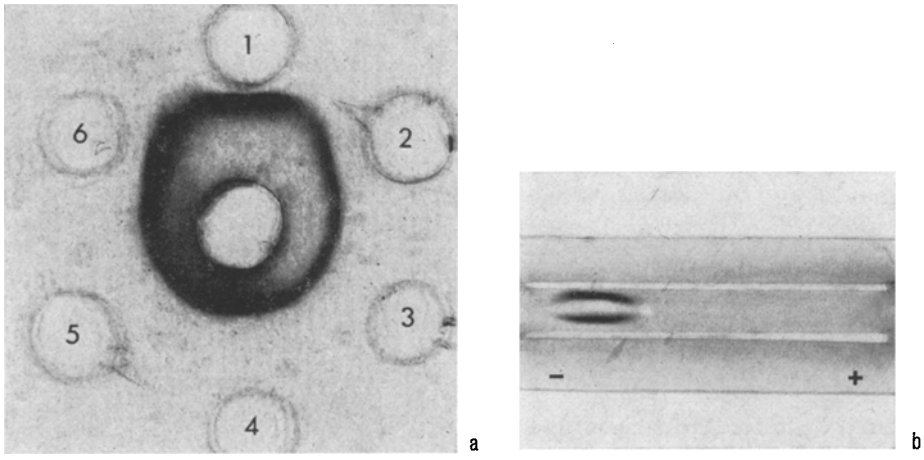

Fig. 2. a) Agar gel diffusion pattern formed from the reaction of *T. maxima* with *D. pteronyssinus* extract and the glycoprotein isolated from *D. farinae* extract. Centre well, *T. maxima* extract 5 mg/ml; peripheral wells, 1 and 6, *D. pteronyssinus* extract (Commonwealth Serum Laboratories, 150 × 'D' strength); 2, tridacnin-reacting component from *D. farinae* extract (Bencard) 0.9 mg/ml; 3 and 4, *D. farinae* extract (Bencard) number 1 50 mg/ml; 5, tridacnin-reacting component from *D. farinae* extract (Bencard) 1.7 mg/ml. b) Immunoelectrophoretic examination of the glycoprotein isolated from *D. farinae* extract. Well, tridacnin-reacting component from *D. farinae* extract (Bencard) 2.6 mg/ml; troughs, *T. maxima* extract 5 mg/ml.

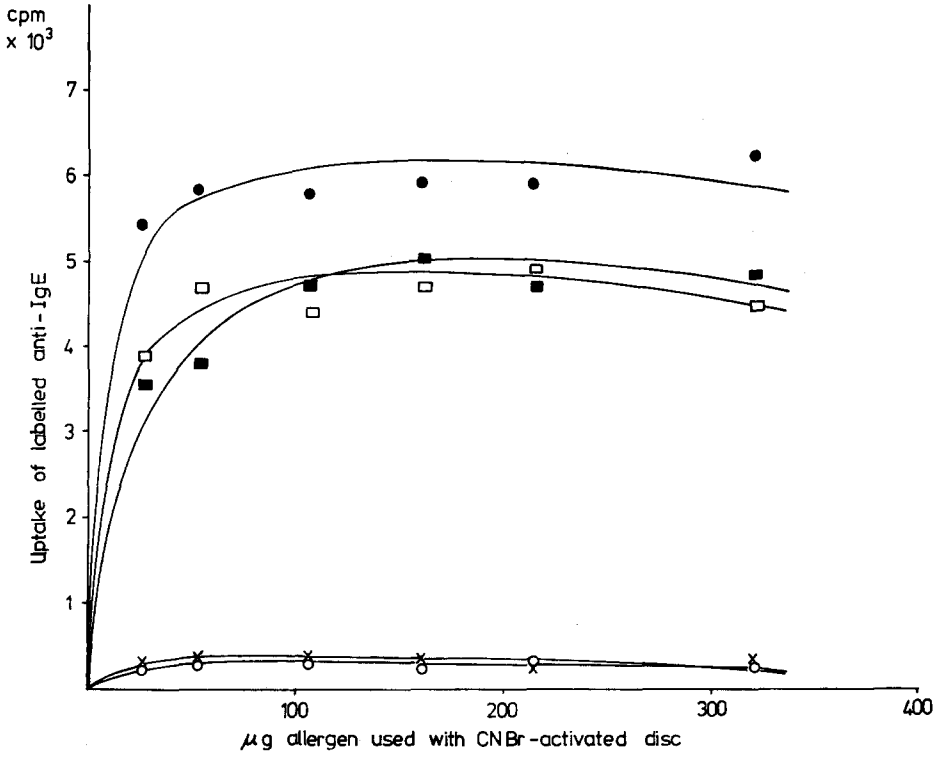

Fig. 3. Curves showing uptake of ^{125}I-labelled anti-human igE after incubating human sera with paper discs coupled to increasing amounts of tridacnin-reacting *D. farinae* glycoprotein. Each cyanogen bromide activated paper disc was coupled to the required amount of *D. farinae* glycoprotein before incubating for 3 h with sera from house dust mite sensitive patients (Sera Egg (●), Fit pool (□) and Cro (■)), normal serum (×) or cord serum (○). Unbound serum proteins were removed by washing, Pharmacia ^{125}I-labelled anti-human IgE (23,800 cpm/tube) was added and tubes were incubated for a further 15 h. After washing to remove unbound radioactivity, activity remaining on each disc was estimated by counting each tube for 5 min in a Packard Auto-Gamma Sepctrometer.

uptake of label by the discs incubated with cord and non-allergic serum samples was equal to the background counts usually observed with sera which, in our hands, react negatively in paper disc RAST experiments. Figure 3 shows the uptake of ^{125}I-labelled anti-IgE with 3 allergic sera, a typical non-allergic serum sample and a sample of pooled cord sera. Depending on the amount of mite allergen used with activated paper discs, the percent uptake of radioactivity in the tubes which contained the allergic sera varied from 14.3 to 25.3%. With cord and non-allergic sera, radioactive uptake was in the range of 0.9 to 1.4%.To test that house dust mite media components were not responsible for the reactions observed, the usual media used to grow the mite, namely, Winalot medium, Gaine's dog meal and dried brewer's yeast and human γ-globulin and serum albumin were each tested in precipitation and RAST experiments. Results showed that contaminants from the media were not responsible for the lectin-reacting and allergenic activities detected in D. *farinae* extracts.

Whole *D. farinae* extract was used in coupling experiments with CNBr-activated paper discs and then examined in the RAST with the same normal and allergic sera used in the experiments described above. On a weight basis the isolated allergen had approximately the same activity as the unfractionated extract. This result was not unexpected since house dust mite extract, like many natural products which provoke allergic responses in humans, almost certainly contains more than one allergenic component. Although the mite allergen isolated with tridacnin reacted strongly in the RAST with sera from mite patients, it seems likely that IgE antibodies with specifities for other allergens from *D. farinae* are also present in these sera. This highlights the problem of

allergen standardization, a problem which has always been particularly difficult in relation to house dust mite and mite extracts.

The component isolated from *D. farinae* in this study should prove valuable in skin testing, for house dust mite allergen standardization and for use in in vitro tests such as the RAST. RAST studies with mite extracts have been found to correlate well with the results of skin tests and positive clinical diagnoses of house dust mite hypersensitivity[16,17]. The above results therefore, lead us to conclude that we have isolated an important allergen from extracts of *D. farinae*. *D. farinae* and *D. pteronyssinus* show antigenic and allergenic cross-reactivity[5,17] and the *D. farinae* component isolated by precipitation with tridacnin, cross reacts with a constituent in *D. pteronyssinus* extract. Tridacnin should therefore be equally useful for allergen extraction studies with the latter species. Studies are in progress in an attempt to characterize the tridacnin-reacting mite component and to further examine its role in clinical hypersensitivity to mites.

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Effect of Dipyridamole on Human Platelet Phospholipids

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Summary. The effect of dipyridamole on platelet phospholipids has been studied. After the platelet incubation with dipyridamole, a 38% reduction in the concentration of the sphingomyelin and a 21% increase in phosphatidyl choline were produced.

Dipyridamole seems to have an antithrombotic effect as proved in vitro[1] as well as in vivo[2]. The dipyridamole action mechanism may be related to the maintainance of a high level of plasmatic adenosin[3], either by hindering the circulating adenosine deaminase action[4] or because its incorporation into the platelets is altered[5-7]. However, ROZENBERG and WALKER[8] suggest that adenosine-induced inhibition is not mediated extracellularily or intracellularily, but by a process within the platelet membrane.

On the other hand, we have observed in our laboratory that, when the platelets are incubated in vitro with dipyridamole, an alteration of their membrane electrokinetic potential is produced[9]. As the phospholipids participate in the electrokinetic potential of the platelet through their negatively charged phosphate groups[10], we have thought it interesting to evaluate the action of dipyridamole on the platelet phospholipids so as to verify if after platelet incubation with dipyridamole, any phospholipid alteration is obtained that could be related to the variations of the electrokinetic potential or that could influence the ionic transport through the platelet membranes.

Material and methods. All the methods applied have been described in an earlier paper[11] and they are, in principle, as follows: The blood sample to be used is drawn with trisodium citrate solution 3.8% (9/1). It is centrifuged at 900 rpn ($\simeq$ 100 *g*) and 4 °C for 15 min, thus obtaining a platelet-rich plasma (PRP). Subsequently, a platelet precipitate is obtained by differential centrifuga-

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Effect of dipyridamol on the platelet phospholipids

	Major phospholipids (%)					
	Platelet rich plasma (0.9 ml) plus saline (0.1 ml)		Platelet rich plasma (0.9 ml) plus dipyridamol (final concentration 4 µg/ml)			
	$\bar{x}$ ($n = 17$)	SD	$\bar{x}$ ($n = 16$)	SD	t	P
Sphyngomyelin	14.85	2.56	11.12	2.59	3.96	< 0.01
Phosphatidyl-choline	41.40	4.79	44.75	5.54	1.77	< 0.05
Phosphatidyl inositol	5.77	2.50	5.02	3.42	0.68	> 0.1
Phosphatidyl serine	10.89	3.29	10.56	3.98	0.24	> 0.1
Phosphatidyl ethanolimine	25.98	3.32	28.46	5.32	1.53	> 0.1