

# Hypersensitivity to chironomids (non-biting midges): localization of the antigenic determinants within certain polypeptide sequences of hemoglobins (erythrocruorins) of *Chironomus thummi* *thummi* (Diptera)

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*Ninety-nine individuals who were in contact with larvae of chironomids were investigated. Forty-three developed respiratory, conjunctival, or cutaneous hypersensitivity reactions upon exposure to airborne constituents of these insects. By means of RAST, RAST inhibition, and skin tests, type I sensitization to hemoglobins of chironomids was identified in 33 subjects of the symptomatic group. In one of these cases additional proof for high-grade sensitization was obtained by bronchial provocation with as low as 0.2 µg of one of the isolated hemoglobins. RAST and skin-test investigations with fragments obtained by chemical and enzymatic cleavage of two of the homologous hemoglobins led to localization of the antigenic determinants within the peptide sequences 1-31, 32-90, 91-101, and 109-131 of hemoglobin CTT IV and within the sequence 64-98 of hemoglobin CTT VI of the species *Chironomus thummi thummi*. Our results show that hemoglobins of chironomids are potent allergens in man. Therefore chironomids must be considered in relation to environmental and occupational sensitization contributing to respiratory diseases. (J ALLERGY CLIN IMMUNOL 68:66, 1982.)*

Chironomids, of which more than 6000 species are described, are one of the few insect families known to possess low-molecular weight hemoglobins (erythrocruorins). These hemoglobins show a high degree of polymorphism; in the case of larvae of CTT, 11 hemoglobins with different primary structures (CTT I to X) were identified, which exist as soluble components in the body fluid either in forms of monomers (mol. wt. ~16,000) or homodimers (mol. wt. ~32,000). The different hemoglobins and hemoglo-

bin subunits consist of polypeptide chains of 136 to 151 amino-acid residues, complexed with one heme group. The primary structures of all these hemoglobins have been completely elucidated<sup>1-10</sup>; it could be seen that these proteins may differ in as much as 48% of the amino-acid residues, whereas the smallest difference may be limited to 16% of the amino-acid residues.

## MATERIALS AND METHODS

Crude extracts were prepared from larvae and midges (adults) of different species of the genus *Chironomus* (CTT, *C. annularius*, *C. tentans*, and *C. tepperi*), as well as from a commercially available fish-food product consisting of lyophilized larvae of different chironomid species (mixed larvae). The larvae and midges were suspended in 0.1M NaHCO<sub>3</sub> solution and were homogenized in a motor-driven Teflon glass homogenizer. The homogenates were then stirred in the cold for 2 hr and centrifuged for 10 min at 27,000 × g. The supernatants were filtered, and NaHCO<sub>3</sub> was added to final concentrations of 0.1M; these solutions were used as the crude extracts. Protein contents in these

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| Abbreviations used |                                                         |
|--------------------|---------------------------------------------------------|
| RAST:              | Radioallergosorbent test                                |
| CTT:               | <i>Chironomus thummi thummi</i> (Insecta, Diptera)      |
| CTT I to X:        | Hemoglobins (erythrocruorins), components I to X of CTT |
| DEAE:              | Diethylaminoethyl                                       |

preparations were determined by the method of Lowry et al.<sup>11</sup>

The crude extract of CTT larvae was fractionated by gel filtration on a Sephadex G-75 column (2.6 by 105 cm) and eluted with 0.025M potassium phosphate, pH 6 (Fig. 2, top). Further fractionation into four monomeric and seven dimeric CTT hemoglobins was carried out by chromatography on DEAE cellulose columns (Fig. 1).

Globins were prepared by removing the heme group with ethylmethylketone at pH 2<sup>12</sup> and were cleaved with trypsin, in some cases with cyanogen bromide<sup>13</sup> and BNPS skatole (3-brom-3 methyl-2-(2 nitro-phenylsulphenyl)-3H-indol).<sup>14</sup> Separation of the fragments as listed in Table I was performed by gel filtration on Sephadex G-50 and G-25, Biogel P 6 and P 30, and by chromatography on DEAE cellulose, DEAE Sephacel, CM cellulose, Dowex 1 × 2, and Dowex 50 × 4.<sup>1-10</sup>

The purity of all of the isolated proteins and peptides used in this study was investigated by amino-acid analysis on a Spinco Model 121 C (Beckman Instruments, Inc., Palo Alto, Calif.) and by N-terminal analysis. Amino-acid analysis was used as the only criterion for purity for peptides that did not contain all common amino acids; the purity was checked according to amounts of the "lacking amino acids." By this method no impurities, i.e., no detectable amounts of lacking amino acids, could be found. In other cases the purity was studied by N-terminal analysis. The phenylthiohydantoin (PTH) amino acids were identified by thin-layer chromatography (TLC) in silica gel plates<sup>15, 16</sup> and with high-pressure liquid chromatography (HPLC) on a C-18 reversed-phase column with an acetonitrile gradient.<sup>17</sup>

In the case of TLC no extra spots of PTH-amino acids were visible. In cases of HPLC identification the impurities were under the limit of detection (Table I).

Sequence analysis of hemoglobins and isolated peptides was performed with an automatic liquid sequenator (Beckman sequencer, Model 890 C) using the quadrol program<sup>18</sup> and the propyne program.<sup>15</sup>

Crude extracts of chironomid larvae (protein concentration 5 to 7 mg/ml) and midges (protein concentration 4 mg/ml), reconstituted gel-filtration fractions (0.2 mg/ml in 0.1M NaHCO<sub>3</sub>), purified hemoglobins, and hemoglobin fragments (0.2 mg/ml in 0.1M NaHCO<sub>3</sub>) were coupled to cyanogen bromide-activated paper discs; incubation time was 4 hr at room temperature. Further procedures were carried out as usual for the RAST method.<sup>19</sup> RAST results were expressed in units per milliliter as estimated by comparison with commercially available reference standards

TABLE I. Methods used for estimation of purity of the isolated fragments of hemoglobins CTT IV and CTT VI

| Peptides of hemoglobin CTT IV |           | Peptides of hemoglobin CTT VI |           |
|-------------------------------|-----------|-------------------------------|-----------|
| Peptide                       | Methods   | Peptide                       | Methods   |
| 1-31                          | AAA, TLC  | Globin                        | AAA, TLC  |
| 1-90                          | AAA, TLC  | 1-37                          | AAA, TLC  |
| 32-90                         | AAA, TLC  | 31-57                         | AAA, TLC  |
| 69-90                         | AAA, HPLC | 38-140                        | AAA, TLC  |
| 91-101                        | AAA, HPLC | 64-98                         | AAA, HPLC |
| 102-108                       | AAA       | 64-89                         | AAA       |
| 109-131                       | AAA, HPLC | 90-98                         | AAA       |
| 132-136                       | AAA, HPLC | 99-109                        | AAA, HPLC |
|                               |           | 99-147                        | AAA, TLC  |
|                               |           | 118-147                       | AAA, TLC  |

AAA = amino-acid analysis.

TABLE II. Antigenically active fragments of monomeric hemoglobin CTT IV\*

|                                                                 |
|-----------------------------------------------------------------|
| 1                                                               |
| <i>Leu-Thr-Ala-Asp-Gln-Ile-Ser-Thr-Val-Gln-Ser-Ser-Phe-</i>     |
| <i>Ala-Gly-Val-Lys-Gly-Asp-Ala-Val-Gly-Ile-Leu-Tyr-Ala-</i>     |
| 31                                                              |
| <i>Val-Phe-Lys-Ala-Asp</i>                                      |
| 32                                                              |
| <i>Pro-Ser-Ile-Gln-Ala-Lys-Phe-Thr-Gln-Phe-Ala-Gly-Lys-</i>     |
| <i>Asp-Leu-Asp-Ser-Ile-Lys-Gly-Ser-Ala-Asp-Phe-Ser-Ala-</i>     |
| <i>His-Ala-Asn-Lys-Ile-Val-Gly-Phe-Phe-Ser-Lys-Ile-Ile-Gly-</i> |
| <i>Asp-Leu-Pro-Asn-Ile-Asp-Gly-Asp-Val-Thr-Thr-Phe-Val-</i>     |
| 90                                                              |
| <i>Ala-Ser-His-Thr-Pro-Arg</i>                                  |
| 91                                                              |
| <i>Gly-Val-Thr-His-Asp-Gln-Leu-Asn-Asn-Phe-Arg</i>              |
| 109                                                             |
| <i>Lys-Ala-His-Thr-Asp-Phe-Ala-Gly-Ala-Glu-Ala-Ala-Trp-</i>     |
| 131                                                             |
| <i>Gly-Ala-Thr-Leu-Asp-Ala-Phe-Phe-Gly-Met</i>                  |

\*Italics represent amino acids identical to those found in homologous positions in the human hemoglobin α-chain.

(Pharmacia Laboratories, Uppsala, Sweden); values <0.3 U/ml were regarded as negative, since nine nonexposed controls (five asthmatics and four healthy subjects) gave values <0.3 U/ml.

For estimation of the amounts of peptides covalently bound to paper discs, four batches of paper discs carrying different peptides were hydrolyzed. For hydrolysis 15 paper

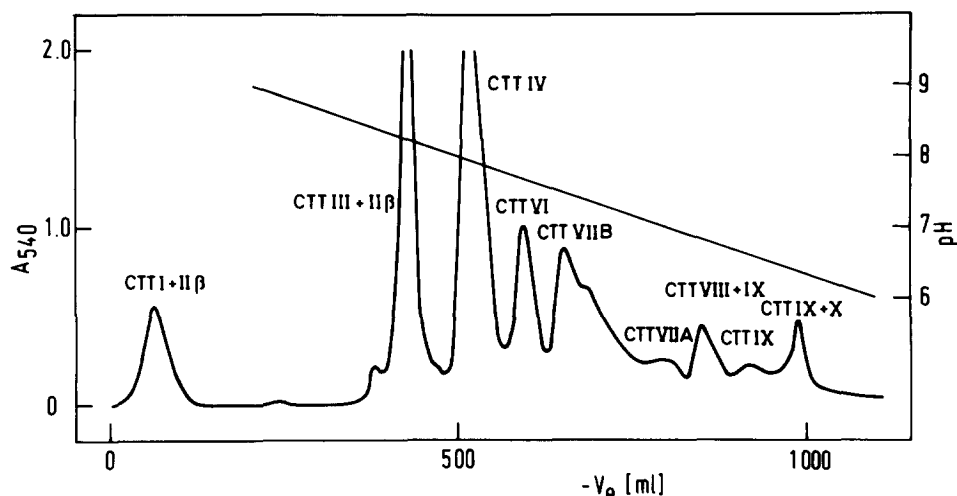


**FIG. 1.** Chromatography of CTT total hemoglobin extract on a DEAE Sephacel column (2.6 by 10 cm).<sup>1</sup> The pH gradients are indicated. Further purification of the different hemoglobins was performed on DEAE cellulose columns with different gradients.<sup>1-10</sup>

discs from each batch were suspended in 1 ml of 6N HCl, incubated in evacuated ampules at 100° C for 24 hr. The hydrolysates were centrifuged and passed through millipore filters and subjected to automatic amino-acid analysis.

RAST inhibition tests were performed by preincubation of positive sera with different allergens (crude extracts, hemoglobins, and hemoglobin fragments) for 12 hr. The remaining unbound amounts of specific IgE in the sera were then measured by RAST and compared with those serum samples from the same patients, which were preincubated only with 0.1M NaHCO<sub>3</sub>.

The following protein solutions were used for skin tests, which were performed according to a modified prick method<sup>20</sup>: (1) crude extract of mixed chironomid larvae (protein concentration 0.1 mg/ml); (2) crude extract of chironomid midges (protein concentration 0.05 mg/ml); (3) gel-filtration peak fractions A, B, C, and D obtained by fractionation of the crude extract of CTT larvae (0.1 mg/ml); (4) the purified hemoglobins CTT III and CTT IV (0.1 mg/ml); and (5) purified CTT VI fragments 1-37, 31-57, 38-140, and 118-147 (0.1 mg/ml).

We evaluated skin-test reactions 20 min after application of the allergens, as recommended by the Northern Society of Allergy<sup>21</sup>; a wheal size equal to the reaction of 1 mg/ml of histamine was recorded as 3+.

### Study population and clinical symptoms

Individuals investigated here had been in contact with larvae of chironomids or with their extracts for periods from 3 mo up to more than 12 yr. According to the conditions under which this contact occurred, the study population was divided into four groups. Group 1 comprised 63 persons engaged in the production of fish food. Among these subjects there was usually low-grade exposure to dusts of the lyophilized larvae because these larvae constituted only a small part of the fish food; heavy exposure, however, took place once every 1 or 2 mo when pure lyophilized larvae

were packed in small doses. Group 2 consisted of 27 of 32 persons who responded to an announcement concerning hypersensitive reactions to chironomid larvae, published by us in a pet-fish journal; these 27 subjects were chosen for the study because they had carefully registered case histories and investigations of their blood samples were possible. Group 3 comprised five fish hobbyists who had consulted our outpatient department because of respiratory complaints, mainly in connection with fish feeding. Group 4 comprised four co-workers of a research laboratory where CTT hemoglobins were investigated.

All together, 99 subjects were included in this study. In all cases physical examinations and evaluation of clinical symptoms with the help of standardized questionnaires were done. Forty-three of these subjects reported immediate-type hypersensitivity reactions upon contact with airborne constituents of chironomid larvae; the symptoms included itching as well as wheal and flare reactions on uncovered skin areas ( $n = 18$ ), conjunctival irritation ( $n = 29$ ), sneezing and rhinorrhea ( $n = 36$ ), cough, wheezing, and shortness of breath ( $n = 28$ ). These 43 symptomatic persons comprised 25 from group 1, 10 from group 2, all five from group 3, and three from group 4.

## RESULTS

### Skin tests

Of the 99 persons investigated, 92 were subjected to the skin test (seven out of the 43 symptomatic persons were not skin tested, but their sera were available for investigation by the RAST method; see next section). The skin tests gave typical type I allergic reactions in 29 of the 36 symptomatic subjects when the crude extracts of mixed chironomid larvae and CTT midges were used; in most of these cases positive skin-test results were also obtained with hemoglobins CTT III and CTT IV and with the fragment 38-140 of CTT VI.

**TABLE III.** Antigenically active fragment of dimeric hemoglobin CTT VI\*

|                                                                        |
|------------------------------------------------------------------------|
| 64                                                                     |
| Ile-Val-Ser-Phe- <i>Leu</i> -Ser-Glu-Val-Ile-Ser-Leu-Ala-Gly-          |
| Ser-Asp-Ala-Asn-Ile- <i>Pro</i> -Ala-Ile-Gln-Asn- <i>Leu</i> -Ala-Lys- |
| 98                                                                     |
| Glu-Leu-Ala-Thr-Ser- <i>His</i> -Lys-Pro-Arg                           |

\*Italics represent amino acids identical to those found in homologous positions in the human hemoglobin  $\alpha$ -chain.

**TABLE IV.** Amounts of hemoglobin peptides covalently bound to each RAST paper disc

| Peptide sequence | Peptide bound per paper disc |         |
|------------------|------------------------------|---------|
|                  | nMoles                       | $\mu$ g |
| CTT IV           |                              |         |
| 32-90            | 0.047                        | 0.34    |
| 69-90            | 0.46                         | 1.25    |
| CTT VI           |                              |         |
| 38-140           | 0.33                         | 0.41    |
| 64-89            | 0.3                          | 0.92    |

Fifty-three of the 56 asymptomatic subjects had negative skin response to all of these extracts. Gel-filtration fractions B (dimeric hemoglobins) and C (monomeric hemoglobins), which were obtained from crude extract of CTT larvae and tested in five symptomatic subjects, produced strong wheal and flare reactions. In none of the 92 subjects was there a significant response to gel-filtration fractions A (high-molecular weight components peak) and D (low-molecular weight components peak), or to the fragments 1-37, 31-57, and 118-147 of CTT VI. Five asthmatics not sensitized to chironomids and five healthy controls gave negative skin-test results with all of the above-mentioned extracts or purified components.

**RAST**

The sera from 33 of the 43 symptomatic individuals gave positive RAST results with crude extracts of mixed larvae, CTT midges, total CTT hemoglobin, hemoglobin CTT VI, and the fragment 38-140 of CTT VI. Ten of the symptomatic and 55 of the 56 asymptomatic subjects showed negative RAST values with these extracts. Further investigations of 12 sensitized patients revealed strong antigenic activities in the crude extract of the CTT larvae as well as in gel-filtration peak fractions B and C, whereas gel-filtration peak fractions A and D gave negative results (Fig. 2).

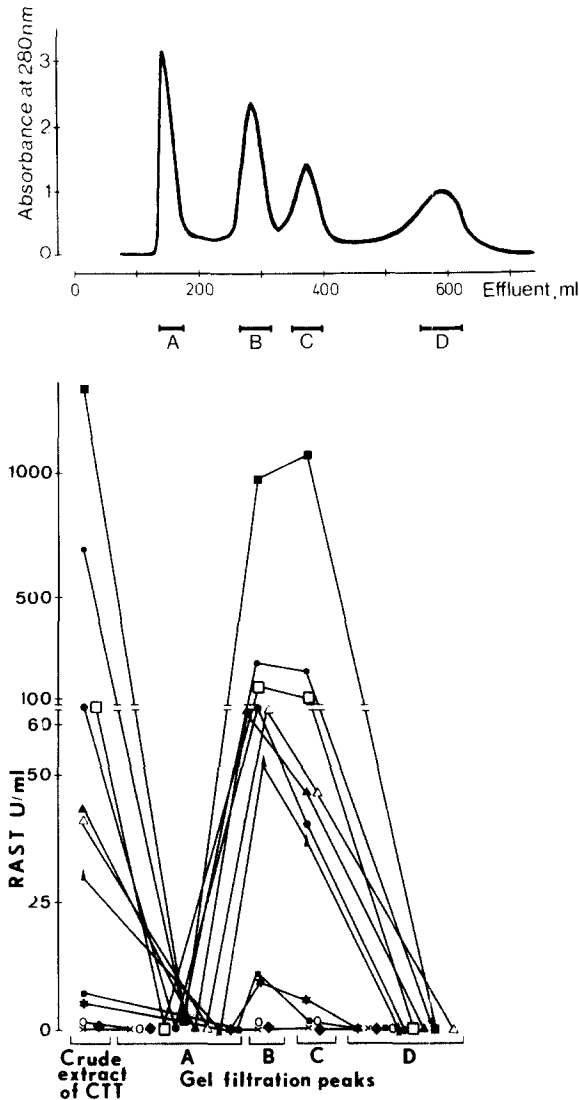


**FIG. 2.** Top, Gel filtration of crude extract of larvae of *C. thummi thummi* on Sephadex G-75. The pooled fractions A (high-molecular weight components peak), B (dimeric hemoglobins), C (monomeric hemoglobins), and D (low-molecular weight components peak) were investigated by RAST for antigenic activity. Bottom, Antigenic activity of crude extract of CTT larvae and of the gel-filtration fractions A to D as measured by RAST with the sera of 12 patients clinically sensitized to larvae of chironomids. Nine control patients who had had no contact with these insect larvae showed RAST values below 0.3 U/ml with the crude extract and peaks A to D. Symbols represent different patients' sera.

All 11 isolated hemoglobins of CTT had antigenic activities. Some of them, e.g., CTT I, CTT III, and CTT X, gave lower RAST values than those of the others; CTT I was negative in two patients with low-grade sensitization (Fig. 3).

Cleavage of CTT IV gave five antigenically active fragments; these were the peptide 1-90, its two cleav-

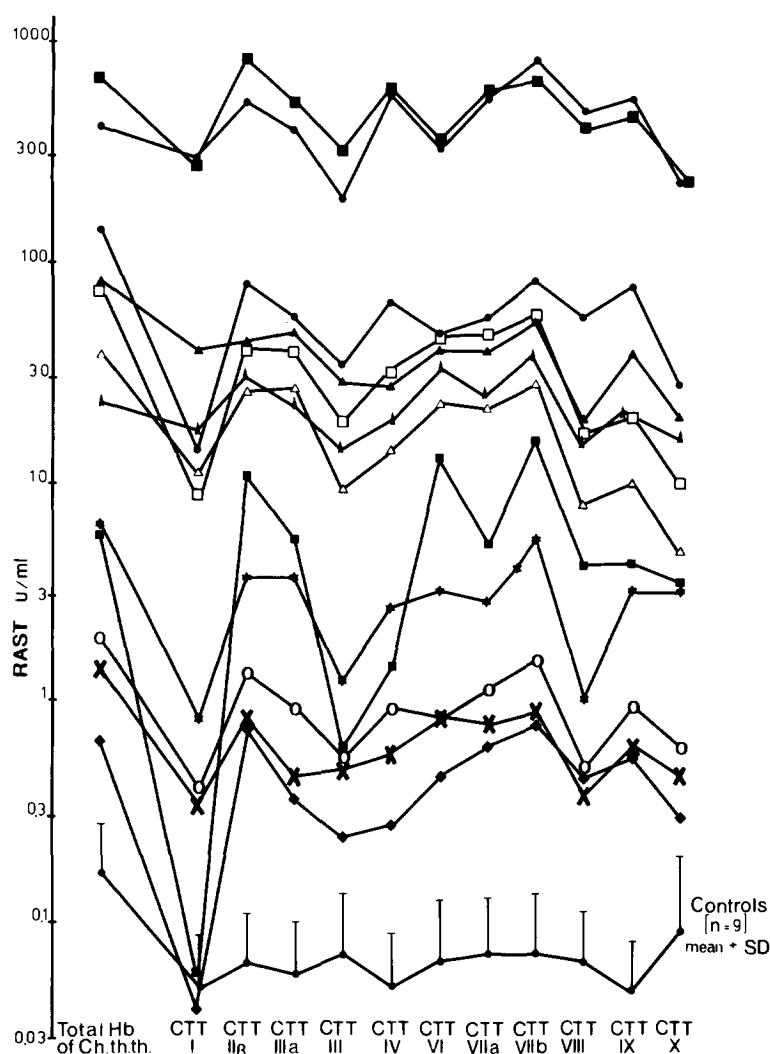


**FIG. 3.** Antigenic activity of total hemoglobin of CTT, of the four monomeric hemoglobins (CTT I, III, III a, IV), and of the seven dimeric hemoglobins (CTT II  $\beta$ , VI, VII a, VII b, VIII, IX, X) as measured by RAST with the sera of 12 patients clinically sensitized to chironomid larvae. Nine persons who had had no contact with these insect larvae served as controls.

age products 1-31 and 32-90, and the peptides 91-101 and 109-131 (Fig. 4, Table II). The regions 69-90, 102-108, and 132-136 showed borderline or negative RAST values.

Eight fragments were obtained by cleavage of CTT VI. Of these only the fragments 38-140 and 64-98 had strong antigenic activities, whereas the peptides 1-37, 31-57, 64-89, 90-98, 99-109, 99-147, and 118-147 had weak antigenic activities or were negative (Fig. 5, Table III). Globin CTT VI, which was obtained by removal of the heme prosthetic group, showed lower activity than that of the native hemoglobin CTT VI; this may have been caused by conformational changes after removal of the heme group.

Some of the peptides giving negative RAST results had only few amino groups available for binding to activated paper discs. Therefore the lack of antigen-

icity might have been caused by an impaired binding of these peptides to the solid phase. To test this hypothesis the amounts of peptides bound per paper disc were estimated by acid hydrolysis followed by amino-acid analysis. These investigations showed that the two small RAST-negative peptides 69-90 of CTT IV and 64-89 of CTT VI were more efficiently bound to discs than were their parent RAST-positive peptides 32-90 of CTT IV and 38-140 of CTT VI (Table IV). This higher efficiency of binding may have been due to higher penetrating capacities of the smaller peptides into cavities and pores of the activated paper.

In further studies with the sera of five sensitized persons, IgE antibody reactivity to crude extracts of larvae and midges of the following chironomid species was investigated by the RAST method: *C. annularius*, *C. tentans* (North American and European

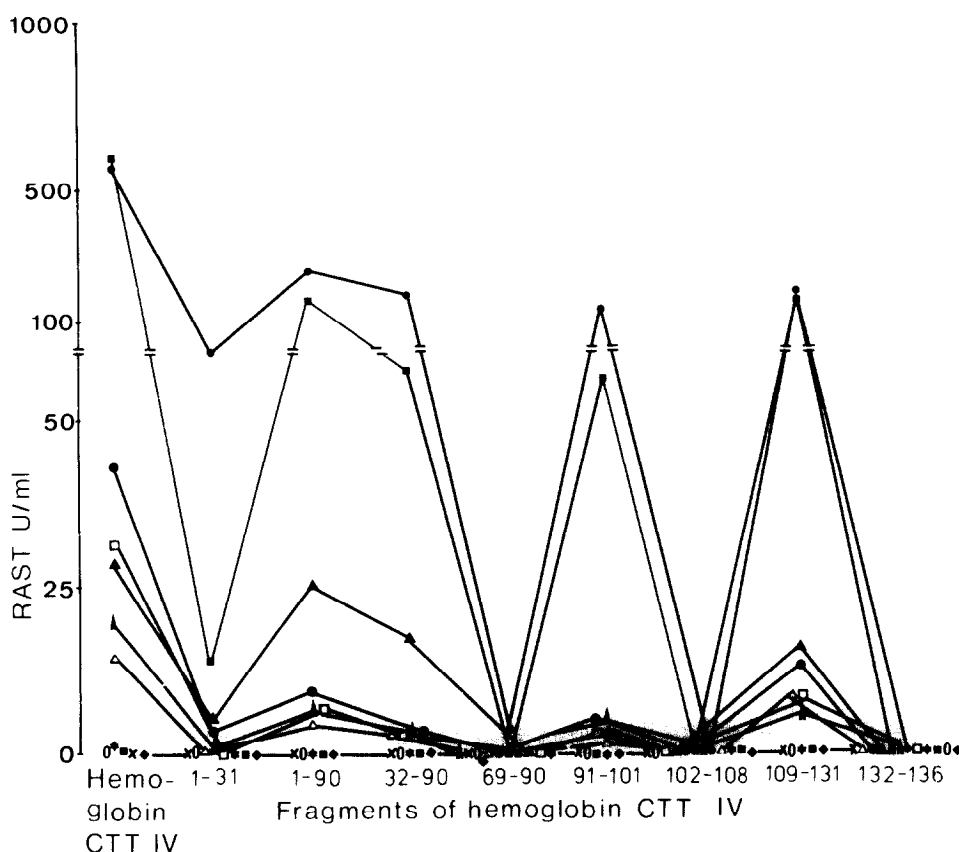


FIG. 4. Antigenic activities of hemoglobin CTT IV and fragments of hemoglobin CTT IV as measured by RAST with sera of 12 patients clinically sensitized to chironomid larvae.

species), and *C. tepperi* (Australian species). In addition, crude extracts of the midges of *Cladotanytarsus lewisi* (Sudanese species; kindly supplied by Prof. A. B. Kay, Edinburgh) was tested; this species belongs to a genus relatively closely related to *Chironomus*.

All five serum samples showed significant IgE binding to components of all these extracts. Sera of two Sudanese people (also supplied by Prof. Kay) hypersensitive to the midge *C. lewisi* exhibited strong positive RAST results in tests with crude extracts of CTT larvae and midges, isolated total hemoglobin fractions of CTT, and fragment 38-140 of CTT VI; the RAST values ranged from 48 to 250 U/ml for one of these two sera and from 8 to 25 U/ml for the other.

### RAST inhibition

Binding of IgE antibodies to all 11 isolated CTT hemoglobins used as solid-phase allergens is nearly totally inhibited by preincubation of the sera with small amounts of fluid-phase hemoglobin CTT VI (Fig. 6). Furthermore, as shown in Table V, antibody reaction with crude extract of CTT midges could be almost completely inhibited by CTT VI as well as by the same crude extract (homologous inhibition).

Similarly, IgE antibody binding to globin CTT VI was inhibited by hemoglobin CTT VI, crude extract of CTT midges, and globin CTT VI (Table V). The high inhibitions demonstrated in these studies must be considered to have been due to competition between the antigenic determinants of chironomid crude extracts, hemoglobins, globins, and fragments used and not due to nonspecific effects, since these extracts or hemoglobins failed to inhibit to any significant extent the binding of IgE to grass pollen (Table V).

Binding of IgE antibodies to the solid-phase peptide 32-90 of CTT IV was not inhibited by its RAST-negative fragment 69-90, whereas up to 78% inhibition was seen in the homologous RAST inhibition, i.e., by preincubation of the serum with the fragment 32-90 of CTT IV (Fig. 7, left). Similar results were found with the solid-phase peptide 64-98 of CTT VI, whose affinity to IgE was not impaired by its RAST-negative fragments 64-89 or 90-98 (Fig. 7, right).

### Bronchial provocation test

Bronchial challenge was performed in one of the sensitized fish hobbyists; inhalation of only 0.2 µg of hemoglobin CTT VI resulted in a moderate immediate and weak, late asthmatic reaction (Fig. 8).

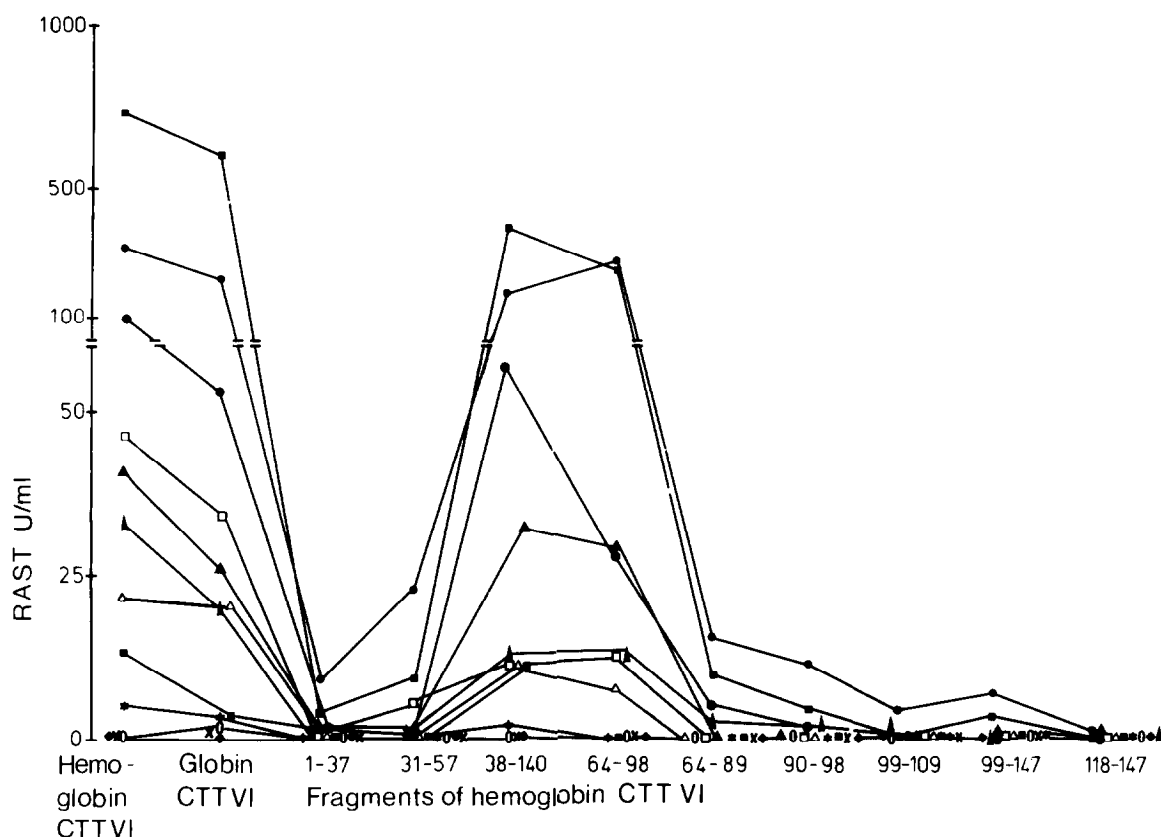


FIG. 5. Antigenic activity of hemoglobin CTT VI, globin CTT VI, and fragments of hemoglobin CTT VI as measured by RAST with the sera of 12 patients clinically sensitized to chironomid larvae.

## DISCUSSION

Thirty-three out of the 43 persons complaining of respiratory, conjunctival, or cutaneous symptoms when exposed to airborne constituents of chironomid larvae exhibited in skin testing and RAST strong evidence for IgE-mediated sensitization to components of both larvae and midges of the chironomid species CTT. Furthermore, the presence of specific antibodies was confirmed by RAST in one and by skin test in three of the 56 asymptomatic individuals. This, as well as the finding of our latest RAST experiments showing sera of hypersensitive persons to react with crude extracts of other chironomid larvae and midges (also of those to which they may have not been exposed), suggest that immunologic cross-reactivities exist between different chironomid species.

Cross-reactivity between components of these insects in different developmental stages is confirmed by the results of RAST inhibition demonstrating the mutual inhibition capacities of larval hemoglobin, globin, and crude extracts of CTT midges.

Fractionation of the larval extract and use of the different fractions for skin and RAST tests and for

bronchial provocation revealed hemoglobins of CTT larvae to be the potent antigenic components, causing IgE-mediated sensitization in a large number of the exposed subjects. Clinically this is manifested especially in forms of immediate-type bronchial and nasal reactions. No evidence for other clinically relevant allergenic components of chironomid larvae and midges was found.

Some of the CTT hemoglobins were further cleaved into discrete peptides, which were then investigated for their antigenic activities. At least five antigenic determinants could be localized. For hemoglobin CTT IV (136 amino-acid residues) antigenic determinants were found to be situated within the polypeptide sequences 1-31, 32-90, 91-101, and 109-131. Considering the helical structure of the native molecule, these active fragments comprise the regions NA1-B11, B12-FG2, FG3-G9, and G17-H16. For CTT VI (147 amino-acid residues) at least one antigenic determinant is located within the sequences 38-140 and 64-98. These fragments comprise C6-H18 and E11-FG2 of CTT VI, respectively.

We cannot exclude the possibility that the real

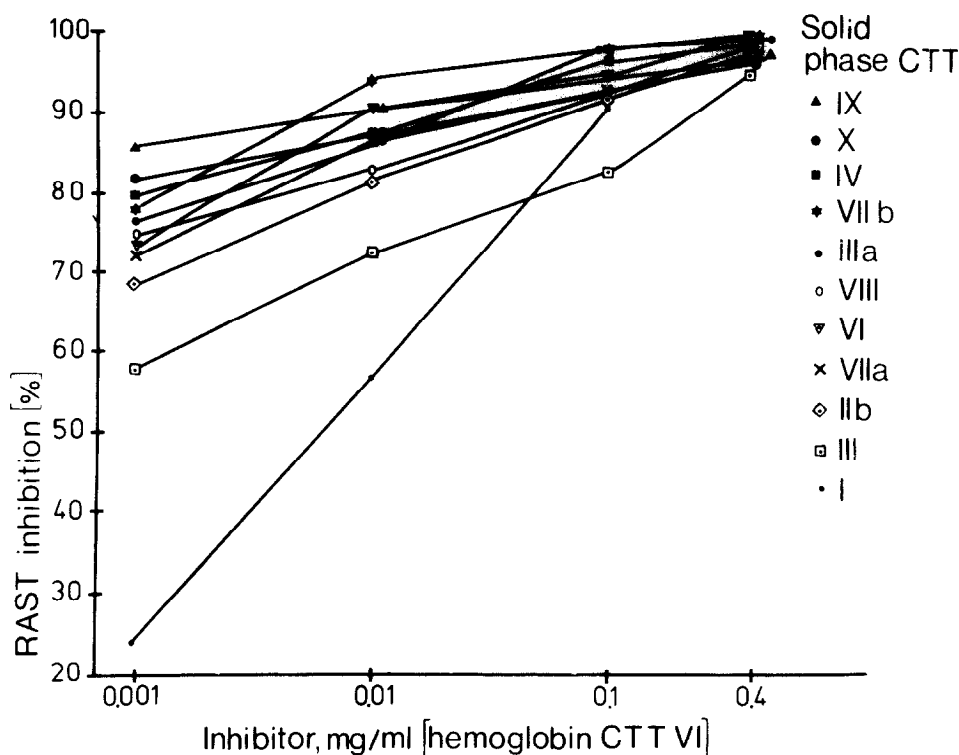


FIG. 6. RAST inhibition data. IgE binding to solid-phase CTT hemoglobins I to X by fluid-phase hemoglobin CTT VI.

number of antigenic determinants is greater than the numbers given here. The cleavage methods could split within some antigenic determinants and destroy antibody binding sites, making it impossible to detect the antigenicity of some hemoglobin regions with the methods employed here. In addition, alteration of the tertiary structure, necessary for antibody binding as well as loss of some quaternary determinants caused by cleavage-induced destruction of native conformation, cannot be excluded. Significant decrease of antigenicity by cleavage is shown for the active peptide 64-98 of CTT VI; the two fragments 64-89 and 90-98 exhibit only little antigenic activity.

The RAST negativity of some of the hemoglobin fragments assayed cannot have been the result of an impaired binding of these peptides to paper discs because the amounts of these peptides bound to each paper disc were even higher than those of larger RAST-positive peptides.

We must emphasize that the antigenicity of different peptides mentioned here are mainly those detected by the RAST method, i.e., their positive or negative RAST values, since it is known that under RAST conditions, the amounts of antigens employed are not always necessarily saturating. Because of these limitations of the RAST method, peptides shown here to be RAST-negative may prove to be antigenic by other

TABLE V. RAST inhibition—fluid-phase inhibitors of IgE binding to discs

| Coupled with:                                | % Inhibition |
|----------------------------------------------|--------------|
| Crude extract of CTT midges (solid phase)    |              |
| Inhibitors:                                  |              |
| Hemoglobin CTT VI (2 mg/ml)                  | 97           |
| Crude extract of CTT midges (2 mg/ml)        | 100          |
| Globin of CTT VI (solid phase)               |              |
| Inhibitors:                                  |              |
| Hemoglobin CTT VI (0.1 mg/ml)                | 90           |
| Crude extract of CTT midges (2 mg/ml)        | 89           |
| Globin of CTT VI (0.5 mg/ml)                 | 99           |
| Grass-pollen extract (solid phase)           |              |
| Inhibitors:                                  |              |
| Hemoglobin CTT VI (2 mg/ml)                  | 2            |
| Crude extract of larvae of <i>C. tepperi</i> |              |
| 0.7 mg/ml                                    | 0            |
| 3.5 mg/ml                                    | 12           |

detection methods. One other method used here was the skin test, although the RAST results for all peptides investigated were confirmed by this method. Furthermore, the RAST negativity of some fragments (69-90 of CTT IV, 64-89 and 90-98 of CTT VI) is also confirmed by their inability to inhibit to any sig-

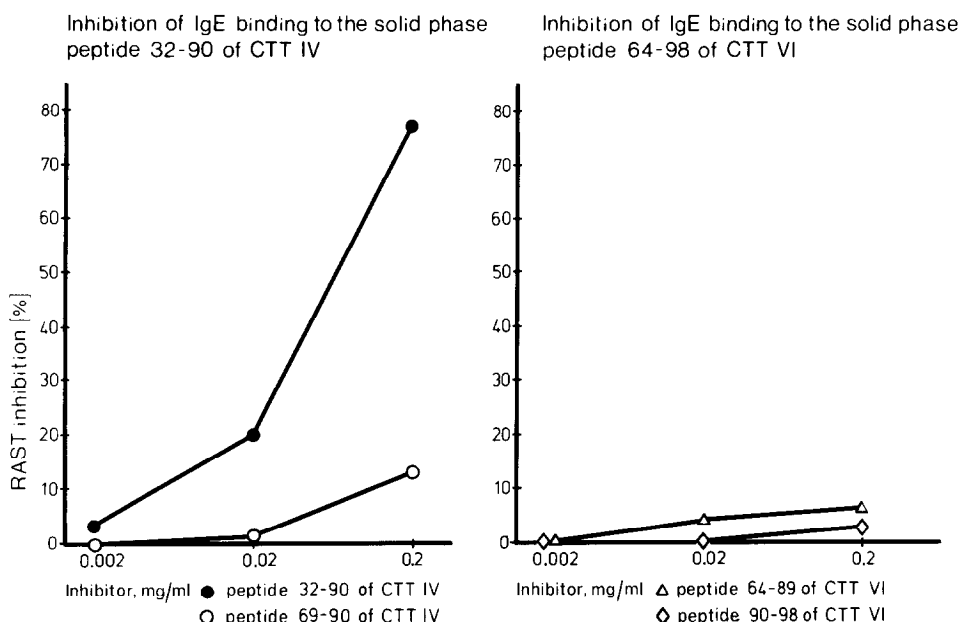


**FIG. 7.** RAST inhibition experiments with peptides of hemoglobin CTT IV (left) and of hemoglobin CTT VI (right).

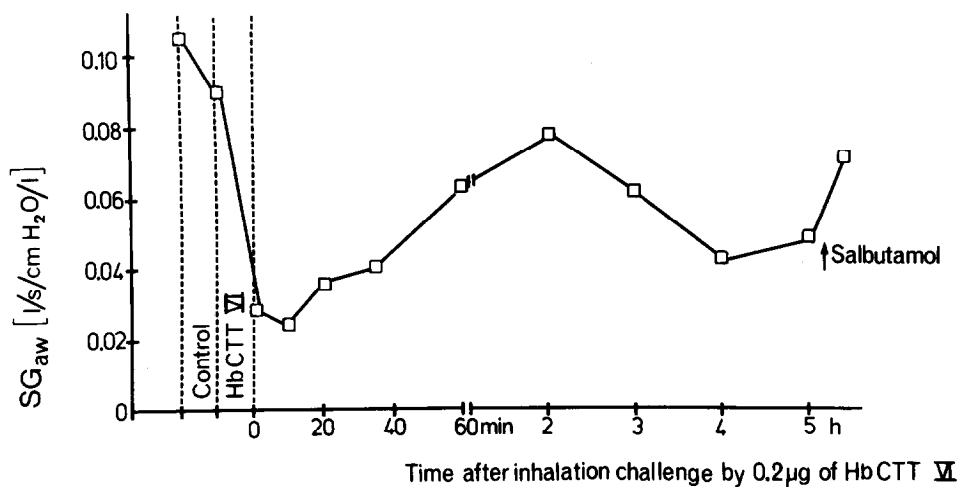


**FIG. 8.** Immediate and late asthmatic reaction after inhalation challenge by 0.2 µg of hemoglobin CTT VI dissolved in 1 ml of 0.9% NaCl and 0.4% phenol. Control was inhalation of 1 ml of 0.9% NaCl and 0.4% phenol. SG<sub>aw</sub>, Specific airway conductance.

nificant extent the binding of IgE to their parent peptides in the RAST inhibition.

Because IgE binding to all of the 11 CTT hemoglobins could be almost totally inhibited by addition of hemoglobin CTT VI, identical or very similar antigenic determinants must be present in the different hemoglobin species.

Due to the world-wide distribution of chironomids, these insects may play an important role as sources of potent environmental allergens. We suggest that the increase in asthmatic diseases reported by several authors<sup>22-28</sup> in water-rich regions such as river basins

during the chironomid season is predominantly caused by sensitization to hemoglobin molecules of this insect family. This is supported by the finding of strong IgE binding of sera of sensitized Sudanese people in RAST investigations with isolated CTT hemoglobin.

The possibility remains, however, that some of the respiratory complaints made by persons exposed to these insects may be not caused by immunologic sensitization, since some of our symptomatic subjects ( $n = 11$ ) gave negative skin tests and negative RAST results. These patients (group 1) were periodically

exposed to large amounts of larval dust when engaged in packing freeze-dried larvae. Such high exposure may also lead to irritative effects of the respiratory mucosa not immunologically mediated.

In an excellent research study using consecutive overlapping fragments and synthetic peptides as immunoadsorbents Atassi and Twining<sup>29, 30</sup> were able to identify the complete antigenic structure of a protein of the same family, sperm-whale myoglobin. It is worth noting that in the three-dimensional structure, the five antigenically active sites of this polypeptide predominantly occupy bends between helices and the end of helices, i.e., regions of highly accessible locations on the native molecule. In similar studies, five antigenic determinants within the  $\beta$ -chain of human hemoglobin have been detected.<sup>31</sup> In connection with these results, it should be mentioned that in spite of the great differences in primary structures, human and chironomid hemoglobins (maximum ~20% sequence homology) as well as hemoglobins and myoglobins of different taxa are quite similar in their tertiary structures.<sup>32, 33</sup>

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