Chironomid Haemoglobins: Their Detection and Role in Allergy to Midges in the Sudan and Elsewhere.

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ABSTRACT. — Studies on allergic reactions of humans to chironomids, particularly to nuisance midges in the Sudan, are reviewed. Evidence is presented that chironomid haemoglobins are important allergens, a finding which indicates that chironomid midges should be seen as significant environmental allergens.

INTRODUCTION

Massive swarms of non-biting midges (Diptera: Chironomidae) emerge from the Sudanese Nile in the winter months (Lewis, 1956; Cranston et al., 1981), and, according to Lewis (loc. cit.), appear to have done so since the 1920s. The swarms which are a most serious nuisance to man and livestock, restrict outdoor activity and may, in extreme cases, cause asphyxia. These swarms consist largely of *Cladotanytarsus lewisi* (Freeman, 1950), although other species of Chironomidae may also occur in large numbers (Lewis, 1956; Wülker, 1963; Cranston et al., 1981). In order to understand the biology of these nuisance midges, Lewis (1956, 1957) and Lewis et al.,
(1954) studied aspects of Nilotic chironomid life histories and fluctuations in numbers of adult midges attracted to lights. Attempts to control the midges using DDD and DDT as larvicides were reported by Brown et al. (1961), but fish mortality was high and the subsequent reduction in midge numbers cannot be interpreted as effective insecticidal control without detailed knowledge of normal population fluctuations. This need for "thorough baseline studies" was observed by Wilker (1963) when investigating biological control as a possible alternative mechanism for alleviation of the midge problem.

Apart from the nuisance caused by midge swarms, the association of human allergic reactions, such as asthma and allergic rhinitis with seasonal midge emergence has long been suspected (Kirk, 1952, 1953). In more recent years detailed investigations into the nature and extent of chironomid allergy in the Sudan have taken place. Particular emphasis has been given to the relationship between the epidemiological, entomological and immunological aspects of the problem. The nature and results of these multidisciplinary investigations are reviewed below. These studies, together with those of other researchers, indicated that chironomid haemoglobins are important allergens for humans. Therefore, the second part of the paper details our investigations into the role of these haemoglobins in the Sudanese midge allergy and discusses the belief that chironomid midges are more than a local Sudanese problem, being potentially a world-wide cause of allergy.

**BACKGROUND**

Strong evidence for the association between midges and seasonal human allergic reactions was provided by Kay et al. (in press a), who compared the prevalence of allergic symptoms in two Sudanese villages. An epidemiological survey was made of the population of Kalakla, a Nilotic village with midge problems, and contrasted with a similar survey of Umm Dawa Ban, a desert village some 40 kms east of Khartoum, distant from the Nile and without midge problems. The results indicate that allergic rhinitis occurred at a rate of 6.7% in Kalakla and 1.5% in Umm Dawa Ban. The percentage of those surveyed with asthma in addition to allergic rhinitis, was four times greater in Kalakla than in the control village. The sufferers' own assessment of the provoking agents indicate that winter seasonal exposure to chironomid midges was a major aetiological factor in asthma and rhinitis in Kalakla. Kay et al., (in press) concluded that repeated exposure to chironomids results in a very high incidence of allergic rhinitis, as well as increasing significantly the indigenous asthmatic population.
The geographical distribution of midge induced allergy was considered by Lewis (1956) who recorded the problem in Khartoum and Wadi Halfa, and by Satti & Abdel Nur (1974) who suggested that problems of nuisance midges and allergy might occur as far north as Lake Nasser (Lake Nubia or Lake Aswan). In an attempt to obtain further information on the geographic extent of hypersensitivity to chironomid midges, Cranston et al. (in press) performed skin tests with unfractionated ('crude') C. lewisi extracts on asthmatic subjects living close to the Nile in Sudan and Egypt. Hypersensitive individuals were found on the White Nile as far south as Kosti, on the Blue Nile as far east as Sennar, and on the Nile as far north as Aswan, Luxor and Qena in Upper Egypt (Cranston et al., in press). The problem appears not to occur in middle and Upper Egypt (Dr. Soliman Diaa el Din, pers., comm.; ms in prep.).

The evidence of the epidemiological survey of Kay et al. (in press) and the geographical distribution studies indicate that the exposed population numbers hundreds of thousands, and the number expected to suffer from midge related allergic problems must be in the tens of thousands.

C. lewisi is always the dominant species of chironomid in samples of midges collected in the areas of Sudan most affected by nuisance midges. Four species, Dicrotendipes fusconotatus (Kieffer), Conchapelopia cygnus (Kieffer), Procladius noctivagus (Kieffer) and Nanocladius vitellinus (Kieffer), were also present in all catches. Although these subdominant species underwent similar daily changes in abundance in light-trap catches as did C. lewisi the greater the total daily catch the greater was the proportion of C. lewisi until in the largest catches of over a quarter of a million individuals per trap per night, the proportion of C. lewisi was over 95% of the total catch (Cranston et al., 1981).

Lewis (1956, 1957) made the only detailed study of the biology of C. lewisi and observed that the midge nuisance seemed to be associated with the construction of dams and the subsequent increase in lacustrine conditions in the Nile. Cranston et al. (1981) confirmed and strengthened this hypothetical relationship and suggested that the summer seasonal rains in the catchment areas of the White and Blue Niles caused a natural eutrophication of the river by washing in of plant nutrients, particularly nitrates, phosphates and silicates. After the period of maximum flow in the river, turbidity decreases in the extensive slow-flowing areas caused by natural and man-made damming of the Nile. As a result, the increased light and high plant nutrient levels allow abundant algal and diatom growth which provides a food source for many chironomid larvae. C. lewisi larvae, shown to be benthic grazers of diatoms and algae and presumed to have a rapid life cycle, make maximum use of this seasonally abundant food
resource. This results in a subsequent large increase in adult midge numbers. Cessation of midge emergence in the Spring seems to coincide quite closely with the crash in algal numbers following nutrient depletion. Further evidence supporting this theory is seen when altered hydrological conditions occur, such as reduced or sporadic rainfall or alterations in the flow regime of the Nile. These factors which occurred in the period 1980-82 were associated with a reduction of the duration and severity of the midge season.

It is significant that the factors which lead to the development of very high populations of midges in the Sudan are not unique to this ecosystem, but occur in many bodies of water throughout the world.

Further evidence for the association between chironomid midges and allergic reactions in Sudanese people has been provided by immunological investigations. Comparison of patients' symptomatology (severity of bronchial asthma and/or allergic rhinitis) with daily numbers of midges assessed by light-trap catches, indicated a relationship. During periods of minimal midge emergence allergic symptoms tended to be reduced, but increased during moderate to large emergences of up to 400,000 midges per trap per night. A massive emergence comprising over 99% C. lewisi during December 1979, not sampled quantitatively but estimated to contain the equivalent of well in excess of half a million midges, was clearly associated with severe signs and symptoms of immediate-type hypersensitivity, including bronchospasm and rhinitis (Kay et al., 1983).

Kirk (1952) showed that a number of Sudanese bronchial asthmatics gave a high proportion of strongly positive responses when skin tested with crude extracts of midges. This skin test reactivity was confirmed by Kay et al. (1978) who also showed that sera from C. lewisi-sensitive Sudanese caused passive sensitisation of lung fragments which led to IgE (immunoglobulin E) - mediated release of histamine and SRS-A (slow reacting substance of anaphylaxis). The development of a radioallergosorbent test (RAST) by Gad El Rab & Kay (1980) allowed quantitative measurement of specific IgE present in serum, and was used to demonstrate a relationship between RAST scores and the severity of patients' symptoms.

In order to test if C. lewisi alone was responsible for midge hypersensitivity in the Sudanese, patients previously shown to be hypersensitive to extracts of pure C. lewisi were skin 'prick' tested with extracts of seven of the subdominant species of Nilotic Chironomidae. The results indicated that C. lewisi was the most important species, but that there was limited cross-reactivity with some other species, particularly Dicrotendipes fusconotatus, Procladius noctivagus and Conchapelopia cygnus (Cranston et al., in press).
Confirmation of the aetiological role of chironomid midges, particularly *C. lewisi*, in allergic problems in the Sudan, led to attempts to characterise the major allergens involved (Gad El Rab, Thatcher & Kay, 1980; Tee et al., in press). Fractions from Sephadex G100 gel filtration and ion exchange chromatography were assayed for antigenicity by skin testing hypersensitive individuals. These techniques indicated that a major proportion of the allergenic material was associated with a molecular weight of 15-20,000 daltons and a pI of 4.3. More definitive techniques, still using Sephadex G100 gel filtration but including RAST inhibition and autoradiography with 125I-anti-IgE, showed that the 'major peak' of allergenicity was associated with molecules of approximately 17,000 daltons and with a pI range of 3.5 to 5.5. The major allergens from *C. lewisi* therefore appear to be a group of closely related acidic peptides.

Contemporaneously with the revival of interest in midge allergy in the Sudan, Baur and his colleagues in West Germany were investigating the incidence of hypersensitivity and respiratory allergy amongst workers occupationally exposed to freeze-dried larvae of *Chironomus riparius* (cited as *Chironomus thummi thummi* (CTT), but see synonymy by Credland, 1973). Using RAST and RAST inhibition, Baur et al. (1982) showed that the antigenic determinants were sited within peptide sequences in some of the 11 polymorphic forms of haemoglobin present in CTT, and that specific antibody against haemoglobins accounted for a proportion of the total IgE of hypersensitive individuals. The first indications that there might be antigens common to both the German occupational allergy and to the Sudanese environmental allergy came from Baur (1982) and Baur et al. (1982). They found that the sera of Sudanese hypersensitive to *C. lewisi* gave positive RAST results in tests with CTT larvae and adults, with isolated total CTT haemoglobin and with one of the antigenic peptide sequences.

The implications of an allergen or group of allergens common to two genera of chironomids which are phylogenetically distantly related, are considerable, in view of the ubiquity and abundance of the Chironomidae. Thus it was important to establish whether haemoglobin was one of the major allergens in the Sudanese midge allergy. It became necessary to extend the range of diagnostic skin tests on *C. lewisi* sensitive individuals to try to test these ideas. Extracts were prepared from larvae and pupae of *C. lewisi* and from larvae and adults of *Ch. riparius* (= CTT) and used in skin testing together with a haemoglobin extract from CTT provided by Dr. X. Baur, and with four allergenic fractions derived from Sephacryl S200 by gel filtration and rechromatography from *C. lewisi* extract. The methods of preparation of the extracts, results of skin tests and interpretation of the results are presented below.
Since the results are dependent on quantification of skin 'prick' test responses, it was necessary to establish the relationship between these responses and the actual levels of specific anti-C. lewisi IgE present in patients' sera. RASTs were performed on the serum from each patient following a skin test with unfractionated C. lewisi extract and the relationship examined.

**Materials and Methods**

**Radioallergosorbent test (RAST).** — A relationship between the percentage binding of $^{125}$I-anti-IgE to allergen polymer complex (the RAST value) and the severity of clinical symptoms of C. lewisi hypersensitive individuals was demonstrated by Gad El Rab & Kay (1980). In order to demonstrate whether there was a similar relationship between the severity of skin test response and the specific IgE directed against C. lewisi antigen(s), RAST tests were performed on the sera of 40 Sudanese individuals previously shown to be skin test sensitive to unfractionated C. lewisi. A group of 24 skin test negative individuals from the United Kingdom, who had not been exposed to C. lewisi, were selected as controls, and their sera were similarly subject to RAST testing. The basis for this method of allergen determination is given by Wide et al. (1967).

(i) **Preparation of Allergen Polymer Complex (APC).** — Ten mg of lyophilised, unfractionated C. lewisi extract was coupled with each gram of CNBr-activated Sepharose 4B (Pharmacia).

(ii) **RAST assay.** — Optimum conditions for the RAST assay were determined as two 16 hour incubations with 50 μl of serum and 100 μl of APC (6.25% concentration) in the first incubation, and 50 μl $^{125}$I-anti-IgE (Pharmacia) in the second. Cord blood sera with no demonstrable IgE were used in all assays as negative controls.

**Skin ‘prick’ testing.** — Patients shown previously to be hypersensitive to unfractionated C. lewisi extracts by skin test or RAST test, or both, and who had been recruited for clinical trials in Khartoum, Soba or Kalakla clinics, were selected for two further series of tests. In one group 16 patients were tested with unfractionated extracts of C. lewisi adults, pupae and larvae. In another group, 26 different patients were tested with unfractionated C. lewisi adult extract, four S200 fractions of adult C. lewisi, three unfractionated extracts of Chironomus riparius and with Ch. riparius haemoglobin. Skin 'prick' tests were performed on the volar region of the forearm, and maximum and minimum diameters of any resultant weals were measured 15 minutes after the test. All patients selected gave a
response of at least 4mm by 4mm to the histamine control and of at least 2mm by 2mm to the unfractionated *C. lewisi*. Patients who gave any significant response to the negative control were not selected for further tests.

All skin 'prick' test responses were calculated as a weal 'area' by multiplying the maximum and minimum diameters of each weal and subtracting any weal 'area' provoked by the negative control. In 23 cases from the group of 26, skin 'prick' tests were duplicated in the reverse sequence on the opposite arm; in these cases the average response to each antigen was calculated. Antigenic material for the skin tests was obtained as follows:

(i) *Collection of midges.* — Adult midges were collected by light-trap at Kalakla in the Sudan and identified as comprising over 99% *C. lewisi*. These were dried at 26°C and stored at 4°C in sealed plastic bags. Larvae and pupae of Nilotic Chironomidae were collected by aquatic drift nets partially immersed in the White Nile at Khartoum. The samples, comprising up to 75% *C. lewisi*, were dried on filter paper at 26°C and stored at 4°C.

Larvae and adults of *Chironomus riparius* were collected from cultures maintained by Dr. P. Credland and dried as before. A commercially available tropical fish food containing predominantly *Ch. riparius* larvae, killed by ultraviolet radiation, packed by "Gamma Foods", was purchased from an aquarists' shop and dried as above. The provenance of these larvae is unknown.

(ii) *Preparation of extracts.* — A known weight of each sample of adult, pupal or larval midge was defatted in three changes of ether over 24 hours. Extractions were performed in Coca's solution (5g sodium chloride, 2.75g sodium bicarbonate and 4g phenol, made up to 1 litre with distilled water) for 48 hours at room temperature. The midges were removed by filtration and the filtrate centrifuged at 18,000g for 40 minutes. The supernatant was passed through a 0.45μ Millipore filter (Millipore Ltd., Bedford, U.S.A.), dialysed against six changes of distilled water over two days and lyophilised.

The material used in skin testing was prepared at 1 mg ml⁻¹ in 50% Coca's solution: 50% glycerol, and passed through a 0.45μ Millipore filter into a glass bottle with plastic cap and applicator (Bencard Ltd, Worthing, England). Histamine at 1 mg ml⁻¹ was used as a positive control solution and 50% Coca's solution: 50% glycerol as a negative control.

Crude *Ch. riparius* (CTT) total haemoglobin sent by Dr. X. Baur, had been extracted from larvae following the technique of Baur et al. (1982), and was prepared at 0.1 mg ml⁻¹.

(iii) *Preparation of allergenic fractions.* — Lyophilised *C. lewisi* extract, prepared according to the method outlined above, was applied in four separate batches of 200 mg to a Sephacryl S200 (Pharmacia, Uppsala, Sweden) column (90cm x 2.6cm diameter, in 0.05M NH₄HCO₃, at 29.5 ml
Fig. 1. Optical densities of C. lewisi extracts before and after rechromatography of fractions: a) 200 mg of unfractionated C. lewisi extract; (b), (c), (d) & (e) rechromatography of fractions 1, 2, 3 & 4 respectively from four fractionations of (a). Numbers in parentheses show relative magnitude of skin test weal response as indicated in the text. [Sephacryl S200 gel filtration, column calibrated with markers of known molecular weight from 1.4K to 10^6 daltons.]

The column was calibrated with blue dextran (> 10^6K), bovine serum albumin (67K), ovalbumin (43K), chymotrypsin (25K), cytochrome C (12.4K) and vitamin B12 (1.4K). Four fraction areas were identified by O.D. profile at 280 nm (Fig. 1a), which demonstrated that Sephacryl S200 gave better resolution than Sephadex G100. The Kav of the two median fraction peaks (O.D. at 280 nm) (labelled 2 and 3 in Fig. 1) were 0.307 and
0.418 respectively, equivalent to molecular weights of approximately 32,000 and 17,000 daltons. Each of the four fraction areas from each of the four fractionations of extract were pooled individually and purified further by rechromatography on the S200 column as shown in Fig. 1b, c, d, & e respectively. After harvesting, these four fractions were lyophilised and used for skin testing.

**RESULTS**

**Radioallergosorbent test (RAST).** — After establishing the optimum conditions for the maximal binding of IgE in the RAST, the percentage of $^{125}$I-anti-IgE binding was determined for the serum of each of the 40 hypersensitive Sudanese and 24 United Kingdom controls. The relationship
between this percentage of isotope binding and the skin test reactivity of each individual, with the skin test response aggregated into three size categories, is illustrated in Figure 2. Twenty-four United Kingdom skin test negative controls gave between 0.6 and 2.6% isotope binding. Fifteen skin test reactive Sudanese with weal 'areas' (maximum × minimum weal diameters) of 1 - 20 mm² gave RAST values from 4 to 21% binding, and patients with stronger skin test responses of 21 - 80 mm² had RAST values of between 17 and 27% binding. Eight individuals with very strong skin test reactions from 81 to 180 mm² did not give RAST values higher than those individuals giving intermediate (21 - 80 mm²) size weals. All values were statistically highly significant: 1 - 20 mm² weals versus 0 mm² weals (p<0.005), 1-20 mm² weals versus 21-80 mm² weals (p<0.025), 21 - 80 mm² weals versus 81 - 180 mm² weals (not significant).

Skin 'prick' tests. — The skin 'prick' test responses, as weal 'areas' of each of 16 Sudanese patients in the group tested with adult, pupal and larval extracts of C. lewisi, Coca’s glycerol negative control and histamine positive control, are illustrated in Figure 3.

The skin test responses, as weal 'areas', of the other group of 26 Sudanese patients tested with the four rechromatographed S200 fractions of C. lewisi are shown in Figure 4, and the responses of the same individuals to four extracts of Ch. riparius (= CTT) are shown in Figure 5. The responses of these 26 individuals to the Coca’s glycerol negative control and to the
histamine positive control were similar to those of the 16 patients of the first group shown in Figure 3. The range of responses to the negative control is shown in Figure 5, but omitted entirely from Figure 4. Each skin 'prick' response to these eight antigens (as measured in 26 patients) was standardized by conversion to a proportion of the response to unfraccionated *C. lewisi* adult extract. In order to investigate possible differences between responses to antigens tested, a Fisher-Yates analysis of variance test was applied to these data. The results, ranked from high to low response, are as follows: The response to allergen fraction 2 is significantly greater than to allergen fraction 3 (p < 0.0001), which is significantly greater (p < 0.0001) than to *Ch. riparius* adult, laboratory and commercial larval extracts, none of which differ significantly from each other. *Ch. riparius* responses are significantly greater than to the high molecular weight allergen fraction 1 (p < 0.005). The response to the low molecular weight fraction 4 is significantly lower than to any other allergen tested (p < 0.0001).

In another analysis the weal data from *C. lewisi* extract and the four rechromatographed S200 allergen fractions were ranked and mean 'scores' rescaled to a mean value of 50. The Fisher-Yates analysis was applied where, with rescaled data, a difference of 10 is significant at the 0.1% level. The 'score' for unfraccionated *C. lewisi* was 62.3, and the scores for S200 rechromatographed fractions 1, 2, 3, & 4 (Fig. 1) were 37.1, 67.6, 59.3 and 27.1 respectively (shown in Fig. 1 in parentheses under each peak).

Thus the response to fraction area 2 is significantly greater than to unfraccionated *C. lewisi* and fraction area 3 (p < 0.005), which are significantly greater than to the high molecular weight fraction 1 (p < 0.0001), which in tum is significantly greater than to the molecular weight fraction 4 (p < 0.0001).

**DISCUSSION**

The responses of Sudanese individuals hypersensitive to *C. lewisi*, when tested with ten different chironomid antigenic extracts, give a strong indication that haemoglobins are major allergens in chironomid allergy in the Sudan. The four rechromatographed antigenic fractions elicited different responses which help to elucidate the nature of these allergens. The allergen area 2, with a molecular weight of approximately 32,000 daltons, elicited a significantly greater response than did the unfraccionated extract. The allergen area 3, equivalent to a molecular weight of about 17,000, provoked a response equivalent to that of the unfraccionated extract. The major allergenic activity is clearly associated with these two fractions. The higher molecular weight fraction 1 (molecular weight 66,000 and above) and the lower molecular weight fraction each elicited smaller responses, none higher.
than the response to unfractionated extract. However, in some individuals, there were weak but positive responses, particularly to the higher molecular weight fraction. Although the precise natures of fractions 2 and 3 are not fully elucidated, it is significant that the molecular weights of these fractions coincide quite closely with those reported for dimeric and monomeric
Skin prick tests to unfractionated *C. lewisi* and extracts from *Ch. riparius*. Abbreviations: lab.-laboratory culture; comm.-commercial, "Gamma Foods"; pp.-partially purified total haemoglobin (from Baur). Note: Haemoglobin is 1/10 dilution of other extracts. The interrupted lines delimit the range of responses to the Coca's negative control.

Haemoglobins. The high molecular weight fraction contains some molecules with a molecular weight similar to that of tetrameric haemoglobin. Biochemical studies by Tee *et al.* (1983, in press) on the structure of the allergens are not in conflict with this identification.

Further indications that haemoglobins are major antigens in the *C. lewisi* allergy come from skin tests performed with extracts of *Ch. riparius* and of the immature stages of *C. lewisi*. *Ch. riparius* has been shown to contain potent allergens for people occupationally exposed to freeze dried larvae used by aquarists. The antigenic activity has been identified as belonging to specific peptide sequences within the different haemoglobins present in the haemolymph of the larvae, and apparently present also in the adult midges (Baur *et al.*, 1982). Our tests reported in this paper indicate that there is an
appreciable degree of cross-reactivity between the antigens of *Ch. riparius* and those of *C. lewisi*, evidenced by skin reactivity to extracts of both larval and adult *Ch. riparius*. Since *Ch. riparius* is a species unknown in the Afrotropical region and it is inconceivable that Sudanese sensitized to *C. lewisi* could have encountered *Ch. riparius* prior to skin testing, their skin test reactivity to *Ch. riparius* extracts is most likely to be due to the presence of similar antigens in these distantly related species. The presence of one or more antigens common to unrelated species of Chironomidae may be the explanation for the cross-reactivity observed between subdominant species of Nilotic Chironomidae reported by Cranston *et al.* (in press). That these antigens include haemoglobins is confirmed by the response of *C. lewisi* sensitised Sudanese to tests with the *Ch. riparius* haemoglobin supplied by Dr. X. Baur. Although this antigen was prepared at 1/10 the dilution of all others tested, a strong positive response was elicited in several of the patients tested, and weaker responses in many others.

Since the occupational sensitisation reported by Baur was to antigens shown to be present in larval Chironomidae, it was important to test whether the immature stages of *C. lewisi* were antigenic. That numerous individuals did show reactivity to extracts of larvae, and to a lesser extent to the pupae, can be explained most parsimoniously as being due to the presence of similar antigens in all stages of this holometabolous insect. This conclusion is strengthened by Baur’s (1982) discovery that both larvae and adults of *Ch. riparius* contain similar antigenic determinants, namely haemoglobins.

Further evidence for the close similarity between the antigens present in *Ch. riparius* and *C. lewisi* comes from RAST tests. Baur (1982) reported that the sera of *C. lewisi* sensitised patients contained high titres of *Ch. riparius* haemoglobin specific antibodies. Conversely, the serum of one *Ch. riparius* sensitised individual contained antibodies specific to extract of *C. lewisi*. However, Tee (R.D. Tee, unpubl. obs.) found that there was variable inhibition of the *C. lewisi* RAST by *Ch. riparius* total haemoglobin provided by Baur, indicating that although haemoglobins are clearly significant in the problem, they may not account for the total allergenicity in all *C. lewisi* sensitive individuals. The considerable amount of variability between individual responses to skin tests may similarly indicate that the antigens of *C. lewisi* may not be completely identical to those of *Ch. riparius*. Whether one would expect total inhibition of the RAST by haemoglobins from distantly related species of Chironomidae is an open question. Although high immunological cross-reactivity between haemoglobins of different species of *Chironomus* was demonstrated by Baur *et al.* (1982) and Tichy *et al.* (1982), the species examined belong to the same genus, while *C. lewisi*
and Ch. riparius are in different tribes, although belonging to the same subfamily.

If, as seems likely, chironomid haemoglobins are important allergens in the Sudanese midge allergy, as in the Ch. riparius occupational allergy, questions remain unanswered concerning the mechanism by which the haemoglobin is available as a rapid acting allergen associated with adult C. lewisi. Susceptible Sudanese individuals may have an asthma attack triggered by contact with a single midge and the onset of the attack may be immediately on contact with the whole fly. The limited amount of information available, based on studies of one or two species of the genus Chironomus, suggests that the larval haemoglobin which is present in the haemolymph of many Chironomidae, may not be fully broken down during metamorphosis, but may persist in the pupa and, to a lesser extent, in the adult (Schin et al., 1974). Laufer & Poluhowich (1971), studying Chironomus pallidivittatus, showed that haemoglobin, and what appeared to be breakdown products of haemoglobin metabolism, were present in the meconium of the newly emerged adult midge. Further investigations are required to establish whether haemoglobins and related antigenic molecules are present in the meconia of the newly emerged (and short-lived) adult C. lewisi and whether this might be a significant mechanism for the dispersal of the antigens.

Although haemoglobins are believed to be present in many Chironomidae, particularly in the subfamilies Chironominae and Tanypodinae, precise information on the distribution within the family is missing. Detailed understanding of the structure of chironomid haemoglobins is restricted almost entirely to the genus Chironomus. The inadequacy of our knowledge concerning the importance and prevalence of these antigenic molecules restricts our ability to speculate on the extent of the problem of chironomid allergy. However, in reporting their findings on the cross-reactivity between Ch. riparius and C. lewisi, Baur and his colleagues (1982) suggest that “the increase in asthmatic diseases reported by several authors in water-rich regions such as river basins during the chironomid season is predominantly caused by sensitisation to haemoglobin molecules in this insect family.” Our investigations tend to support this suggestion, and, in view of our field-based studies, indicate the potentially world-wide nature of this environmental allergen. We have suggested that the environmental conditions which allow the development of huge populations of adult midges along the River Nile are not unique to this region. Indeed, Ali (1980), in documenting nuisance midge outbreaks and their control, lists eight countries which have been afflicted with midge outbreaks serious enough to have been documented in the literature. Our own obser-
vations, together with those of colleagues studying chironomids, show that problems of nuisance chironomids are steadily increasing as waters become more eutrophic, and man lives closer to such habitats. Ali reviews in some detail the midge nuisance in central Florida, and now, for the first time, there is evidence (through responses to skin 'prick' test) that some inhabitants of this region have become hypersensitive to chironomid midges (Cranston, unpubl. obs.).}

In view of these findings, chironomids must be seen as more than a source of world-wide nuisance causing economic problems through the defacing of buildings and paintwork, traffic hazards and restriction of outdoor activity, but should be seen as significant environmental allergens.

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