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Request reprints from: Prof. R. HOIGNÉ, Medical Division, Zieglerspital, CH-3007 Bern (Switzerland)

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Comparative Studies on the Allergens of Horse Dandruff and Horse Serum

G. PONTERIUS, R. BRANDT, E. HULTÉN and L. YMAN

Department of Biochemistry, Pharmacia AB, Uppsala

Abstract. Serum albumin was found to be an important allergen in horse epithelia extracts. The albumin concentration varied considerably, depending on the raw material used for the preparation of the allergen extracts. In addition to serum albumin, two allergenic proteins were demonstrated in horse dandruff. The molecular weights of these allergens were calculated to be about 38,000 and 22,000, respectively. The pI of the latter was 4.2. Fifty sera from allergic patients were studied by means of the radioallergosorbent test (RAST) using horse epithelia extract, horse dandruff extract, a purified horse dandruff allergen and horse serum as test allergens. About 24% of the patient sera studied were found to contain IgE specific to horse serum proteins. IgE specific to horse dandruff proteins was found in about 62% of the patient sera, while the rest, about 14%, apparently contained IgE specific to both kinds of allergens.

Exposure to animal dandruff is known to be a frequent cause of allergy and a large number of different procedures has been used for preparation of allergen extracts since skin testing was introduced about a century ago. These extracts have been, and are still, prepared from raw materials as different as hair, dandruff, epithelia and whole dermis. The resulting products differ considerably. An extract of animal hair, for instance, necessarily contains variable amounts of components originating from the environment of the particular animal, while extraction of chopped dermis yields mainly serum proteins. This variability, of course, also affects the content and specificity of the allergenic material obtained in the extracts.

During the last 50 years, interest has been focused on the horse dandruff antigens. It is well known that serum sickness caused by injection of therapeutic doses of horse serum is closely connected to asthma due to in-

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halation of horse dandruff. Most work, therefore, has been devoted to the establishment of the relationship between the antigens of the two sources. However, the results have been conflicting. RACKEMANN [1918] was unable to show any antigens common to horse serum and dandruff in studies on sensitized guinea pigs. These results were verified by LONGCOPE *et al.* [1925, 1926], who employed uterine muscle strips of sensitized guinea pigs. On the other hand, FORSTER [1928], in a series of cross-precipitation, uterine strip, and anaphylactic reactions, clearly showed the presence of a common antigen in horse serum and dandruff. The concentration of this component was relatively low in the dandruff extract. Similar results were obtained by RATNER and GRUEHL [1929].

From a clinical point of view, COCA *et al.* [1931] stated that individuals sensitive to horse serum are almost invariably sensitive also to horse dandruff, while the reverse relationship is less frequent. TUFT [1934] was able to show in clinical and experimental studies that sensitivity may exist to a varying degree to the dandruff antigen alone, to horse serum alone, or to both of these antigens.

Later results obtained by SQUIRE [1950] from studies which included purified horse serum albumin indicated an immunological relationship between serum albumin and a dandruff allergen rather than the presence of a common antigen. STANWORTH [1957a, b] extended the studies of SQUIRE [1950] and purified an allergen from dandruff. This allergen was entirely different from serum albumin with respect to physicochemical and immunological properties. Both serum albumin and globulins were, however, found in extracts of horse brushings [for a review, see BERRENS, 1971].

CHAN and PORTER [1967] demonstrated the presence of antibodies to horse serum albumin in the sera of patients sensitive to horse serum. The antibodies were partly assigned to the IgE class. IgE-antibodies to animal serum proteins have been demonstrated also in patients allergic to budgerigar [FAUX *et al.*, 1971] and dog [YMAN *et al.*, in press]. These findings, together with recent investigations on serum and epithelia from dog, cat, and horse [HOLFORD-STREVEENS, 1972], again actualize the work done about 40 years ago.

The apparently conflicting results are, in fact, not as confusing as they may seem. The discrepancies can probably be ascribed to a combined effect of the variations of allergen preparations studied, the absence of significant amounts of serum proteins in some extracts, and the limited number of patients employed in many studies.

In this study, radioallergosorbent (RAST) technology was used in combination with other procedures to give a more detailed information on the nature of the allergens of horse allergen extracts.

Materials and Methods

Allergen preparations. (1) Horse epithelia extract. A commercial extract obtained from Dome Laboratories, 10,000 PNU/ml. (2) Horse dandruff extract. A dialyzed extract prepared from horse dandruff (horse brushings obtained from Allergen AB, Engelholm) by extraction with Evans solution (1/10, w/v). The raw material was defatted prior to extraction. (3) Purified horse dandruff allergen. A main allergen of the horse dandruff extract was purified by gel chromatography as described below. (4) Horse serum. (5) Purified horse serum allergen (albumin) was prepared by polyacrylamide-gel electrophoresis (7% gel) of horse serum on an analytical scale as described below. The albumin zone carrying bromphenol blue was cut out and the gel slice eluted with 0.1 M phosphate buffer, pH 6.8. The preparation was homogeneous, as judged by repeated electrophoresis.

Estimation of allergen concentration. The relative concentration of the allergen preparations and the fractions separated by gel chromatography (see below) was estimated by the radioimmunosorbent assay previously described [CESKA *et al.*, 1972; YMAN *et al.*, in press]. Two patient sera were chosen for these studies. Patient serum A contained IgE apparently specific only to horse serum proteins, while patient serum B reacted only with a main dandruff allergen.

Gel chromatography. 5 ml of the horse dandruff extract (preparation No. 2) was applied to a Sephadex G-75 column (2.5 cm $\times$ 88 cm) equilibrated with 0.05 M phosphate buffer, pH 7.4, containing 0.02% sodium azide. 2.5-ml fractions were collected. The flow rate was about 30 ml/h. The relative allergen concentration was estimated for every fifth fraction as described above and calculated as percent of the concentration of the extract applied to the column. Approximate molecular weights were calculated from the relation between K_{D1} and the logarithm of the molecular weight. Ovalbumin, cytochrome C, chymotrypsinogen A and myoglobin were used as standards.

Polyacrylamide gel electrophoresis. This procedure was carried out essentially as described by DAVIS [1964]. Only the small pore gel was used and the samples (5–200 μ l) were applied directly on top of the gel rods. The acrylamide concentration was raised to 10.5% while the ratio acrylamide: bisacrylamide was unmodified. After the electrophoresis, the gel rods were cut longitudinally into two halves. One part was used for staining of proteins [DAVIS, 1964] and glycoproteins [CLARKE, 1964], and the other one was cut transversely into thin slices. Each slice was eluted with 0.5 ml 0.1 M phosphate buffer, pH 6.8. The allergen activity, i.e. the ability to bind specific IgE, of the eluates was detected as previously described [CESKA *et al.*, 1972; YMAN *et al.*, in press] by covalent coupling of the allergens to paper discs and subsequent incubation of the discs with patient serum and ^{125} I-labelled anti-IgE. The radioactivity uptake of the discs reflects, within certain limits, the allergen con-

centration of the eluates tested. The same patient sera (A and B) as described above were used. For some experiments, also a third serum (patient serum C) was used. The IgE of this serum is specific to another dandruff allergen than the IgE of serum B. *Isoelectrofocusing*. These experiments were performed essentially as described by WRIGLEY [1968].

Immunoelectrophoresis. A 200- μ l sample of the horse epithelia extract was subjected to polyacrylamide-gel electrophoresis as described above in a gel containing 7% acrylamide. The gel was then cut longitudinally into two parts. One part was used for the detection of allergen activity as described above. The other part was cut in the same way once more. One quarter of the gel was stained with amido black, while the other quarter was applied to the surface of a 1.5-percent agar gel (1-mm layer on a microscope slide), permitting the separated components to diffuse into the agar gel. A trough was cut out on each side of the polyacrylamide-gel slice. One of the troughs was filled with rabbit antihorse plasma antiserum (Behringwerke AG) and the other one with the purified horse serum albumin preparation described above (preparation No. 5).

Demonstration of specific IgE in patient sera. 50 patient sera were studied by a modification of RAST, originally described by WIDE *et al.* [1967]. Four batches of immobilized allergen preparations were produced by coupling of the allergens of horse epithelia extract, horse dandruff extract, a purified horse dandruff preparation and horse serum, respectively, to BrCN-activated paper discs. The procedure was essentially the same as previously described [CESKA *et al.*, 1972; CESKA and LUNDKVIST, 1972] and represents an upscaling of the coupling method used for the estimation of allergens. The allergens were, in all cases, present in a vast excess during the coupling process in order to ensure maximal and reproducible capacity of the allergen-cellulose complex. The patient sera studied were obtained from several hospitals over a period of several years. The horse-specific IgE content of these sera ranges from extremely low to very high, as judged by prior RAST screening [LUNDKVIST and BERGLUND, personal commun.]. Several commercial horse epithelia extracts of varying composition have been used during the course of this screening. The specific IgE content of a patient serum, represented by the radioactivity uptake of the paper disc used in the test, can at present be calculated only in arbitrary units. We have, in this case, chosen to express the result of each test as the specific anti-IgE uptake of the disc, calculated as a percentage of the total amount of immunoreactive anti-IgE added in the last step of the test procedure. A 5-percent uptake is considered as a significantly positive response.

Results and Discussion

A. Some Properties of Three Allergens of Horse Epithelia, Horse Dandruff (Brushings), and Horse Serum

The specific detection of allergens constitutes the main difficulty in investigations of the kind reviewed in the introduction. If experimental ani-

Table 1. Relative concentration of the allergen preparations studied

Allergen preparation	Serum allergen (patient serum A)	Dandruff allergen (patient serum B)
Horse epithelia extract (No. 1)	1	1
Horse dandruff extract (No. 2)	< 10 ⁻⁶	110
Purified dandruff allergen (No. 3)	0	5
Horse serum (No. 4)	150	0

mals or antisera raised in animals are used, the results have to be verified on allergic subjects to ensure that the antigen detected is an allergen. This verification, however, is associated with numerous problems. The variability and heterogeneity of the allergen preparations in combination with genetic variations of the patients and the different possible ways of their prior exposure to the allergens, e.g. inhalation of dandruff, eating of horse meat or injection of horse serum, make the results difficult to interpret. Three patient sera were chosen for the investigations reported here, one serum containing IgE specific to horse serum allergens (patient serum A) and two specific to horse dandruff allergens (patient sera B and C). The properties of these and other patient sera are further discussed in section B below.

The relative concentration of the allergen preparations No. 1-4 was estimated using the patient sera A and B (table 1). The values obtained are restricted to the particular allergens to which IgE was present in these sera. Furthermore, the values obtained for one allergen preparation using different patient sera are not directly comparable due to uncontrolled differences in concentration and/or avidity of the IgE antibodies. However, the results clearly show that the horse dandruff extract was about 100 times more active than the commercial horse epithelia extract with respect to dandruff-specific allergen activity. Concerning the serum-specific allergen, the situation was the reverse and the difference in activity even more marked.

Two allergens were detected after polyacrylamide-gel electrophoresis of the extracts of horse epithelia (preparation No. 1) and horse dandruff (preparation No. 2), employing patient sera A and B (fig. 1). One of these components apparently originated from the dandruff, while the other one was found also in horse serum (fig. 2). The 'serum allergen', having an electrophoretic mobility corresponding to that of serum albumin, was pre-

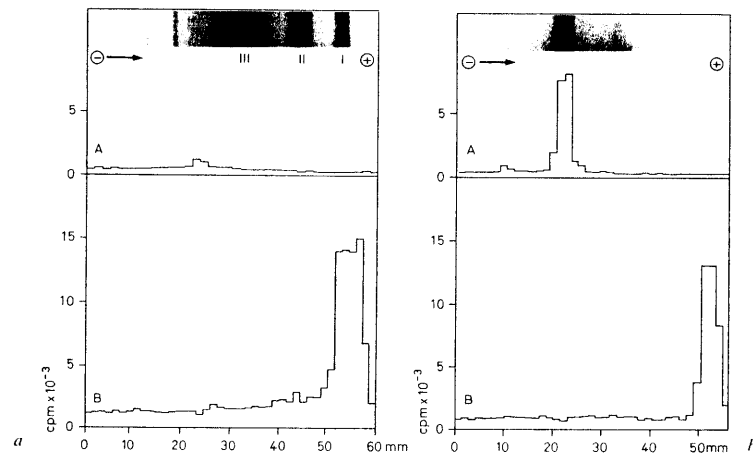


Fig. 1. Polyacrylamide-gel electrophoresis of horse dandruff extract (a) and horse epithelia extract (b). The upper parts of the graphs show the distribution of proteins and the lower parts the distribution of allergen activity detectable with patient sera A and B. For further details, see under 'Materials and Methods'.

sent in extremely low concentration in the extract of horse dandruff (table 1). The epithelia extract contained a substantially higher amount of this protein. Immunoelectrophoresis clearly showed that the 'serum allergen' of the epithelia extract was immunologically identical to horse serum albumin (fig. 3). The dandruff extract yielded three major protein zones, I–III (fig. 1), each containing at least two glycoproteins. None of these zones was detected in horse serum. Only zone I possessed allergen activity detectable with patient serum B. The isoelectric point of this highly potent allergen (component I) was found to be 4.2 by isoelectrofocusing in polyacrylamide gel. However, also zone III, constituting the main part of the dandruff proteins, was found to contain an allergen (component III) when employing another patient serum (patient serum C). As seen in figure 4, the IgE of this serum was specific to the allergen of zone III and did not react with the allergen of zone I. No activity was detected in zone II. The allergen of zone I was partly purified by gel chromatography of the dandruff extract on Sephadex G-75 (fig. 5). Testing the fractions with patient serum B, again only one active component could be detected. Fig-

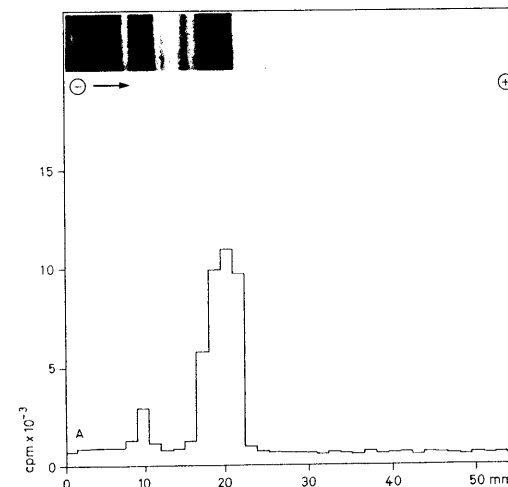


Fig. 2. Polyacrylamide-gel electrophoresis of horse serum. The upper part shows the distribution of proteins and the lower part the distribution of allergen activity detectable with patient serum A. No allergens could be detected using patient sera B or C.

ure 6a shows polyacrylamide-gel electrophoresis of the pooled fractions 94–95 which were used for further studies, reported in section B below. The allergen (component I) was contaminated by other proteins, but was free of allergens detectable by patient sera A and C. The main protein peak of the chromatogram, corresponding to the fraction electrophoretically characterized as zone III, contained the other dandruff allergen detectable by patient serum C (fig. 6b). The molecular weights of the proteins eluted in peaks I and III were calculated to be around 22,000 and 38,000, respectively.

B. Comparative Studies on Crude and Purified Horse Allergen Preparations by Means of RAST

RAST has proved to be a valuable tool in allergy diagnosis, showing good correlation with other methods, such as skin test, provocation test,

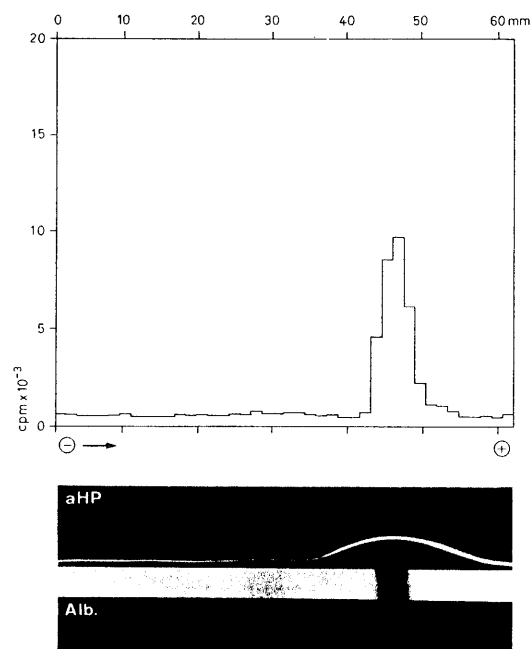


Fig. 3. Polyacrylamide-gel/agar-gel immunoelectrophoresis of horse epithelia extract. The upper part shows the distribution of allergen activity (patient serum A) and the lower part the distribution of antigens detectable by immunodiffusion against rabbit antihorse plasma antiserum (aHP). Electrophoretically homogeneous horse serum albumin (Alb.) was applied to one of the troughs. A stained slice of the polyacrylamide gel is shown in the position occupied by the polyacrylamide-gel rod during immunodiffusion.

and the test for histamine release from passively sensitized chopped human lung [WIDE *et al.*, 1967; FAGERBERG and WIDE, 1970; BERG *et al.*, 1971; AAS and JOHANSSON, 1971; FOUCARD, 1972]. The results obtained with the methods mentioned are, in all cases, dependent on the potency and specificity of the allergen preparations used.

Fifty patient sera were tested with RAST using a horse epithelia extract (preparation No. 1), a horse dandruff extract (preparation No. 2), a

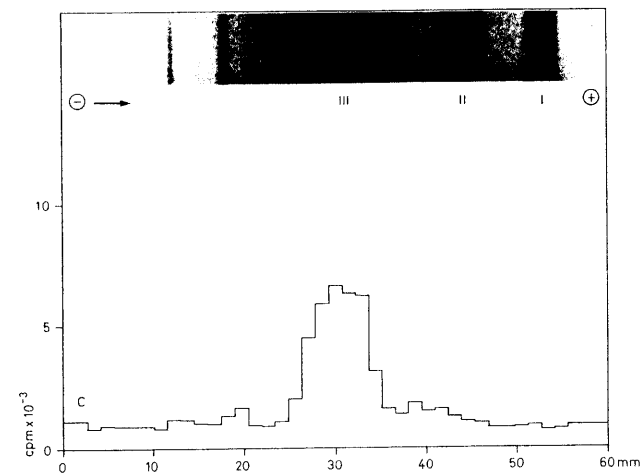


Fig. 4. Polyacrylamide-gel electrophoresis of horse dandruff extract. Patient serum C was used for detection of allergen activity. See also figure 1a.

partially purified dandruff allergen (preparation No. 3) and horse serum as test allergens. The correlation or the lack of correlation between the results obtained is illustrated in figure 7. The patient sera may be divided into three groups on the basis of figure 7a and b. Twelve sera were found to contain IgE specific to horse serum proteins; 31 of the patient sera gave positive results if the allergen preparation contained dandruff allergens; and seven sera contained IgE to both serum and dandruff allergens. Figure 7b also clearly shows that the dandruff extract contained allergens not present in the purified preparation. This was to be expected, as only one allergen (component I) could be detected in the latter preparation (fig. 6a), while the crude extract contained also the allergen denoted as component III (fig. 4) and traces of serum albumin (fig. 1a, table I). As shown above (fig. 1b, table I), the epithelia extract (preparation No. 1) contained a high amount of serum albumin. This resulted in a partial agreement between the results obtained with epithelia extract and horse serum (fig. 7c) and those obtained with epithelia extract and dandruff extract (fig. 7d), respectively.

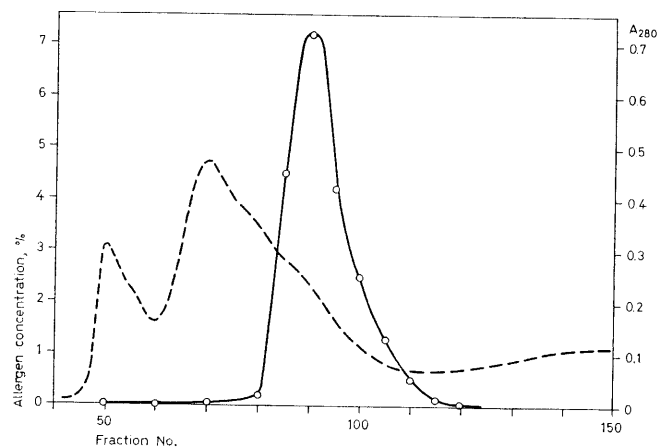


Fig. 5. Gel chromatography on Sephadex G-75 of horse dandruff extract. Patient serum B was used for the detection of allergen activity. The allergen concentration of the fractions is expressed as percent of the concentration of the extract applied to the column. ----- A_{280} ; ○ allergen concentration.

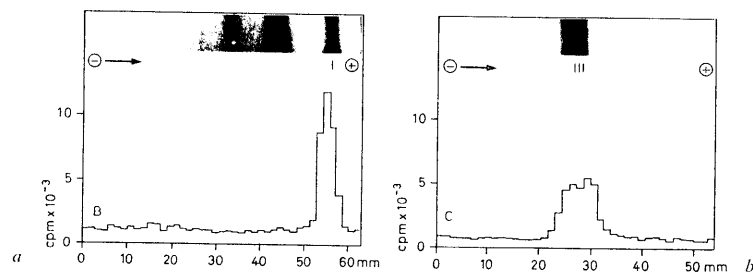


Fig. 6. Polyacrylamide-gel electrophoresis of the pooled fractions 94-95 (a) and fraction 70 (b) obtained by gel chromatography of horse dandruff extract (fig. 5). The patient sera B(a) and C(b) were used for detection of allergen activity. No allergens could be detected in fractions 94-95 using sera A and C or in fraction 70 with sera A and B.

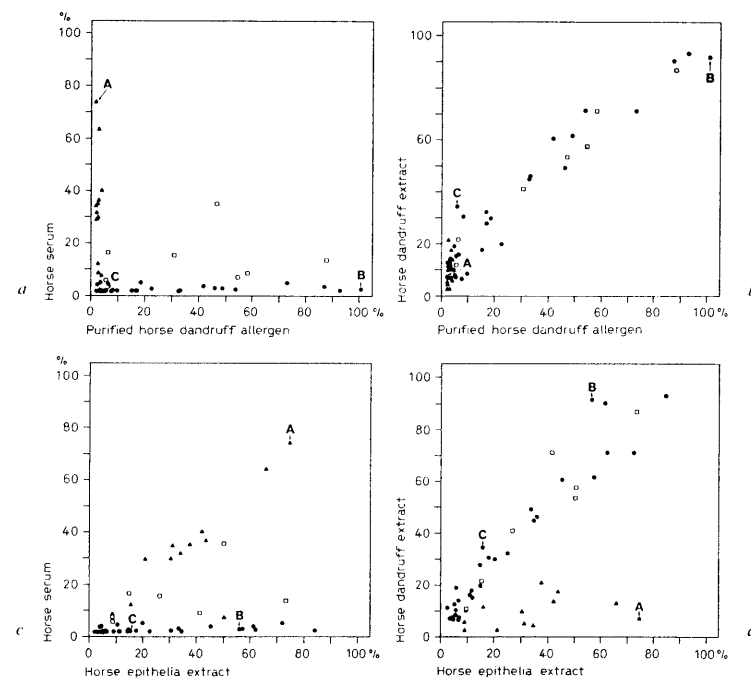


Fig. 7. Correlation between RAST results obtained with four different horse allergen preparations. 50 patient sera were tested. The patient sera contained IgE specific either for serum proteins (▲) or for dandruff proteins (●) or for proteins of both kinds (□). A, B, and C denotes the sera used for the studies reported in section A. (For further details, see under 'Materials and Methods'.)

All the dandruff-sensitive patients seemed to produce IgE specific to either one or both of the dandruff allergens detected. It, therefore, seems probable that most horse dandruff allergies will be detected if these two allergens are present in proper concentration in the extracts used for testing. However, it is still possible that detailed studies employing a larger number of carefully chosen patient sera will show an even more complex pattern [WIDE *et al.*, 1972] than is indicated by the results of this in-

vestigation. It is obvious that the value of a diagnostic test based on a complex preparation like the horse dandruff extract is highly dependent not only on the qualitative composition of the extract, but also on the relative concentration of the active constituents. This is of special importance in the cases when the patients have to be desensitized. As judged by the results reported here, separate preparations containing either horse serum allergens or dandruff allergens would mean great diagnostic and therapeutic advantages. However, thorough studies will be needed to elucidate the clinical importance of the individual allergens.

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Request reprints from: Dr. L. YMAN, Pharmacia AB, Department of Biochemistry, Box 604, S-75125 Uppsala (Sweden)