

Concentrations of specific dusts in swineries and the humoral response of swinery workers

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The concentrations of swine epithelial antigen (SEA), swine urinary antigen (SUA) and swine feed antigen (SFA) were measured in swineries. SEA seems to be the most prevalent of the constituents measured. In the serum samples of the subjects studied, specific IgG antibodies against the antigens were frequently encountered. However, a difference was seen in the distribution of anti-SUA-IgG antibodies between swinery workers and a control group of students. The influence of the level of specific dust concentrations on specific antibody titers was studied statistically. Positive correlations were observed between stationary site SUA concentrations and anti-SUA-IgG and anti-SEA-IgG titers, respectively, pointing to an association between exposure and humoral response in swinery work. Consequently, our results suggest that the measurement of antibodies against SUA or its components could offer a basis for the estimation of the level of swinery work-related respiratory exposure and for monitoring the quality of the working environment in swineries.

Key words: antibody response; ELISA; exposure; respiratory hypersensitivity; swinery dust

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Respiratory symptoms are common among farmers. Working in livestock production, and particularly, in swineries, seems to be hazardous to the respiratory system (2, 15). Various bronchitis symptoms are the most frequently encountered, but wheezing has also been reported in a significant number of swinery workers (3).

Among the constituents of swinery dust are molds, bacteria, insect parts, pollen, grains, mineral ash, swine feeds and swine-derived materials (4). Because of the complex composition of the dust, it is easy to understand that respiratory symptoms in swinery work can arise through many different mechanisms, such as irritative, toxic or allergic mechanisms. However, too little is known about the relationship between the human IgG responses and exposure to "common" environmental agents, such as

molds, plants or animal-derived materials. If this kind of relationship could be demonstrated it might provide a basis for estimating the level of exposure and for monitoring the quality of the human environment.

The principal aim of this study was to evaluate the influence of respiratory exposure to SEA, SUA and SFA in swineries on the humoral response of swinery workers. In addition, we wanted to determine if any of the specific antibody measurements could be used for differentiating between exposed and unexposed subjects.

MATERIAL AND METHODS

Samples of airborne particulate material were collected in 19 randomly selected swineries from two stationary sites and from the breathing

zones of swinery workers as previously described (13) during the two daily working periods. After collection, filters were weighed and then stored at -70°C . For the preparation of samples, filters were set into beakers and eluted in 5 ml of 0.01 M phosphate-buffered saline (PBS), pH 7.4, containing 0.5% (v/v) nonionic detergent, Nonidet P40 (BDH Chemicals Ltd., England) and 0.02% (w/v) thiomersal (BDH Chemicals Ltd., England) for 2 h at room temperature with an agitation rate of 150 rpm. After centrifugation at 36,600 *g* for 45 min, the supernatant was collected and stored at -70°C .

Three groups of subjects were included in this study: Swinery workers from the swineries studied (Group A, 37 samples), patients from the Department of Pulmonary Diseases at the Kuopio University Central Hospital having various respiratory symptoms when working in swineries (Group B, 9 samples) and medical students from the University of Kuopio (Group C, 44 samples). The mean age of swinery workers was 39 years (range 21–58) and of hospital patients 40 (range 24–51). The students' age range was 20–22 years. Sex distribution was as follows: swinery workers, 14 women and 23 men; hospital patients, 6 women and 3 men; and medical students, 27 women and 17 men. Serum samples were collected from these subjects for antibody measurements.

For preparation of SEA, the powdered raw material composed of swine hair and skin scrapings was purchased from Allergon AB (Ängelholm, Sweden). The antigen was prepared in Coca's solution in essentially the same manner as for the previously described preparation of bovine epithelial antigen (13). Swine urinary antigen (SUA) was prepared essentially as the previously described preparation of bovine urinary antigen (13). Swine feed antigen (SFA) was prepared from the fish powder (Hankkija, Finland) used as a constituent of swine feed in the same manner as SEA. The protein contents of the SEA, SUA and SFA preparations were 4.22 mg/ml, 6.23 mg/ml and 5.33 mg/ml, respectively (7).

Rabbits were immunized with SEA, SUA and SFA every 2 weeks for a 4-month period, each time receiving 2.0 mg of protein. The

antigen solution was emulsified with Freund's incomplete adjuvant. Injections were given subcutaneously. Blood was drawn several times, and the antisera were stored at -70°C .

Rabbit IgG was purified according to the method of McKinney & Parkinson (8). First, the rabbit serum was diluted 1:5 in acetate buffer (60 mM, pH 4.0), and the pH was adjusted to 4.5 with NaOH. Caprylic acid (Sigma, C-2875; USA), 25 $\mu\text{l}/\text{ml}$ of diluted sample, was added dropwise and the solution was stirred for 30 min. Insoluble material (albumin and other non-IgG proteins) was then removed by centrifugation at 10,000 *g* for 30 min. 10 parts of supernatant were mixed with one part of a 10-fold concentrated PBS containing 20 ml of 100 mM EDTA (Merck, FRG)/1 l of concentrated PBS. The pH was adjusted to 7.4 with NaOH. In the next step, the supernatant was cooled to 4°C (at which temperature the following steps were also carried out) and fractionated with ammonium sulfate (0.277 g/ml to give 45% saturation). The sample was then stirred for 30 min, after which the precipitated IgG was collected by centrifugation at 5,000 *g* for 15 min. The IgG pellet was then suspended in PBS in the original volume of the serum sample. The resuspended IgG was dialysed overnight against a vast excess of PBS. On the next day, the sample was heated to 55°C for 20 min and centrifuged once more at 5,000 *g* for 20 min, and the IgG preparation was stored at -70°C .

Biotinylation of rabbit IgG was carried out as follows (17): the IgG preparation was diluted to a concentration of 1 mg/ml of protein in 0.06 M carbonate buffer (pH 9.0) and dialysed in an excess of the same buffer overnight at 4°C . d-Biotin-N-hydroxysuccinimide ester (Sigma, H-1759; USA) diluted in dimethyl sulfoxide (Fluka AG, Switzerland) to a concentration of 1 mg/ml was added to the IgG preparation at a biotin/IgG molar ratio of 10:1. The mixture was incubated at 25°C for 3 h. Finally, the unreacted biotin was removed by extensive dialysis against PBS, and the biotinylated IgG preparation was stored at -70°C .

After preliminary tests, the following procedures were used for double antibody ELISAs. A microelisa plate (Nunc-Immuno Plate I,

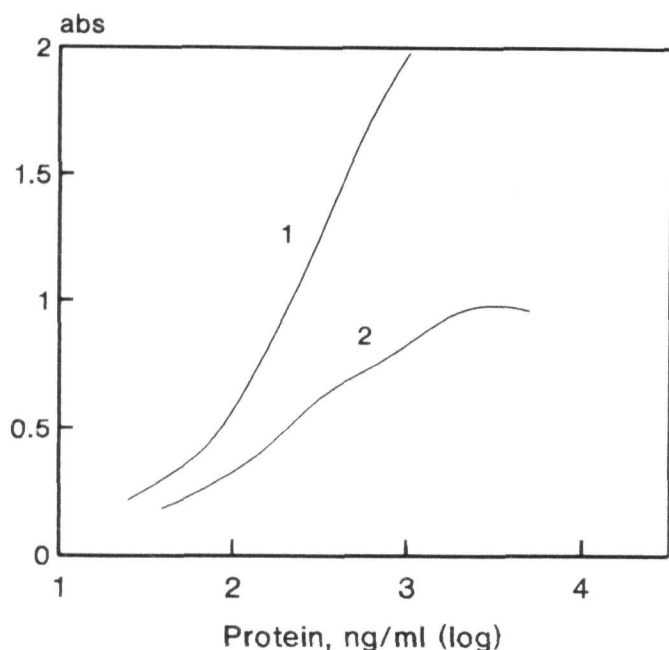


Fig. 1. Standard curve of SEA double antibody ELISA (1) with a SUA standard (2) curve.

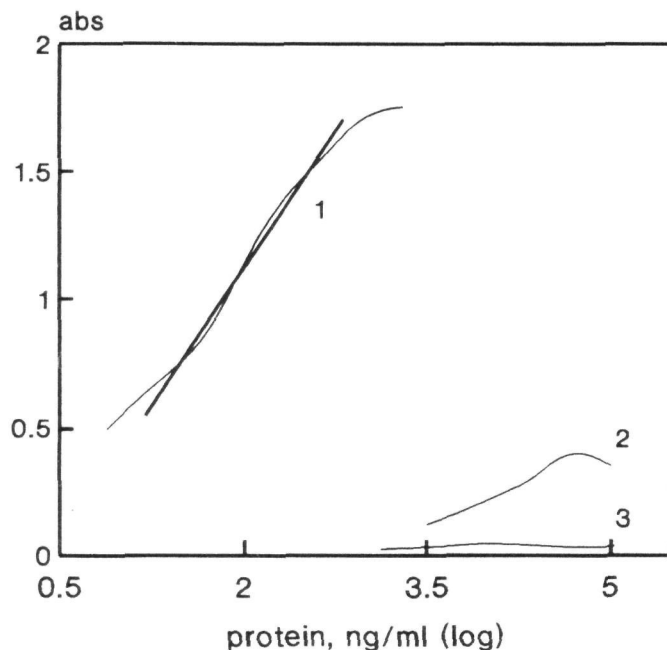


Fig. 2. Standard curve of SUA double antibody ELISA (1) with SEA standard (2) and SFA standard (3) curves.

Denmark) was coated for 2 h with rabbit anti-SEA-, anti-SUA- or anti-SFA-IgG at a concentration of 0.1 mg/ml of protein in a coating buffer (0.05 M carbonate buffer, pH 9.6) in volumes of 200 μ l/well at 37°C. The plate was washed and incubated for 2 h at 37°C with a diluent (PBS containing 0.5% (v/v) Nonidet P40 (BDH Chemicals Ltd, England) and casein (BDH Chemicals Ltd, England) 0.1% (w/v)). After incubation, the plate was stored at -70°C. Before use, the plate was allowed to reach room temperature and was then washed. The standard antigen and sample dilutions in 200 μ l volumes were added in duplicate, and the plate was incubated at 37°C for 1.5 h (SEA) or 18 h (SUA and SFA). After washing and incubation with diluent for 1 h at 37°C, biotinylated rabbit anti-SEA-, anti-SUA- or anti-SFA-IgG, at a dilution of 1:100, 200 μ l/well, was added. The plate was incubated for 1.5 h (SEA) or 2 h (SUA and SFA) at 37°C. After another wash, Vestastain ABC Standard Kit (Vector laboratories, PK-4000; USA) reagents (avidin DH and horseradish peroxidase H) were added at a dilution of 1:500 and left at room temperature for 30 min. After a further wash, the substrate solution (tetramethylbenzidine, 0.1 mg/ml; Merck, FRG) and urea hydrogen peroxide (BDH Chemicals Ltd., England), correspond-

ing to 0.05% H_2O_2 in 0.1 M sodium acetate buffer (pH 6.0), were added. The reaction was stopped (2 M H_2SO_4), and the absorbance was measured at 450 nm using a Titertek Multiskan spectrophotometer (Flow Laboratories, Finland). For the determination of antigen concentrations in swinery samples, the standard curves of absorbance against the log concentrations of the standard antigens were plotted. The amount of antigens in the samples were then calculated from the linear part of this curve.

For IgG antibody measurements against SEA, SUA and SFA, microtiter plates (Nunc-Immuno Plate I, Denmark) were coated for 2 h at 37°C in volumes of 100 μ l/well at concentrations of 0.5 μ g/ml of protein. The microtiter plates were then washed and incubated with diluent for 2 h at 37°C, after which the plates were stored at -70°C. Before use, the plates were allowed to reach room temperature and were then washed. Serum samples were added in duplicate in volumes of 100 μ l/well at dilutions of 1:100 (SEA), 1:200 (SUA) and 1:50 (SFA). After incubation at 37°C for 1 h, the plates were washed and incubated with diluent at 37°C for 30 min. Peroxidase-conjugated anti-human IgG (Dako-immunoglobulins a/s, P214; Denmark) was added at a dilution of 1:1,000 and incubated for 1 h at 37°C. After washing,

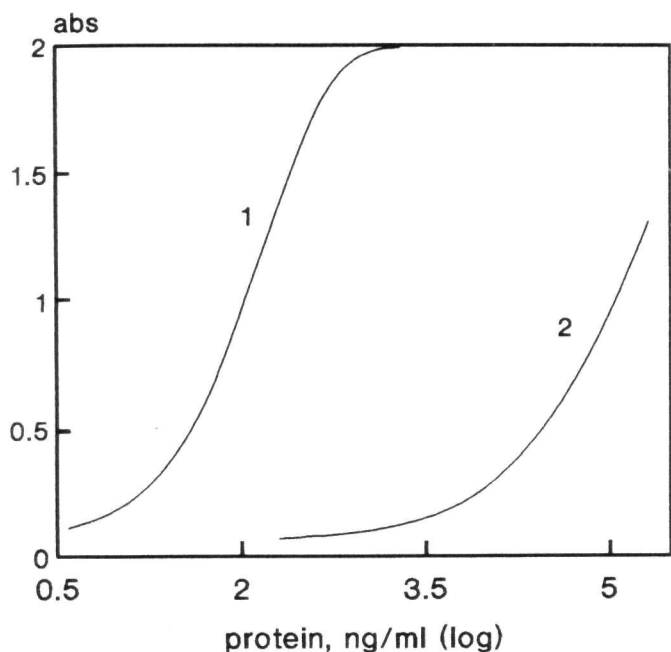


Fig.3. Standard curve of SFA double antibody ELISA (1) with a SEA standard (2) curve.

the color reaction was developed as described in the procedures for double antibody ELISAs.

Variations in the results between plates in the antigen specific antibody measurement were corrected as follows. A coefficient for the plate was calculated by dividing the mean absorbance of the positive control wells of all the plates by the respective mean of an individual plate (four wells/plate). The results for the plate were then multiplied by this coefficient and the corrected absorbances were considered to be the antibody titers.

RESULTS

Concentrations of total dust

In the breathing zone, total dust concentration was 12.8 ± 11.9 mg/m³ (mean $\pm$ SD; *n* 31). At

stationary sites, total dust concentration was 6.9 ± 4.7 mg/m³ (mean $\pm$ SD; *n* 19). The stationary site results were calculated as a mean of two simultaneously collected samples from each swinery. The difference between breathing zone and stationary site concentrations was statistically significant (Wilcoxon matched pairs test, *p* < 0.001).

Specificity of assays for antigen quantitation

In the double antibody ELISA for SEA, cross-reactivity was not observed with SFA (14). However, SUA caused a clear cross-reaction in the linear range of the SEA standard curve (Fig. 1). Interestingly, in the double antibody ELISA for SUA, SEA caused only a minor cross-reaction (Fig. 2). SFA showed no cross-reactivity in the SUA double antibody ELISA (Fig. 2). However, SEA caused a clear cross-reaction in the SFA double antibody ELISA (Fig. 3) but according to the preliminary tests with shorter incubation times, SUA was not at all cross-reactive in the ELISA (data not shown).

Concentrations of specific dusts

In the breathing zone samples, SEA concentrations were measurable in two samples where total dust concentrations were too low to be measured. On the other hand, SUA concentrations were at a measurable level in only seven samples, and SFA concentrations in 30 samples. In the stationary site samples, concentrations of SEA and SFA were high enough to be measured from each swinery. However, SUA concentrations were at a measurable level in only 10 of them.

Concentrations of SEA, SUA and SFA in the breathing zones and at the stationary sites are

Table 1
Specific antigen concentrations in swineries $\mu\text{g}/\text{m}^3$ (mean $\pm$ SD)

	SEA	SUA	SFA
BZ	271 $\pm$ 278 (<i>n</i> 33)	0.93 $\pm$ 1 (<i>n</i> 7)	5.63 $\pm$ 5.22 (<i>n</i> 30)
SS	285 $\pm$ 208 (<i>n</i> 19)	0.18 $\pm$ 0.2 (<i>n</i> 10)	1.61 $\pm$ 0.85 (<i>n</i> 19)

BZ: breathing zone
SS: stationary site

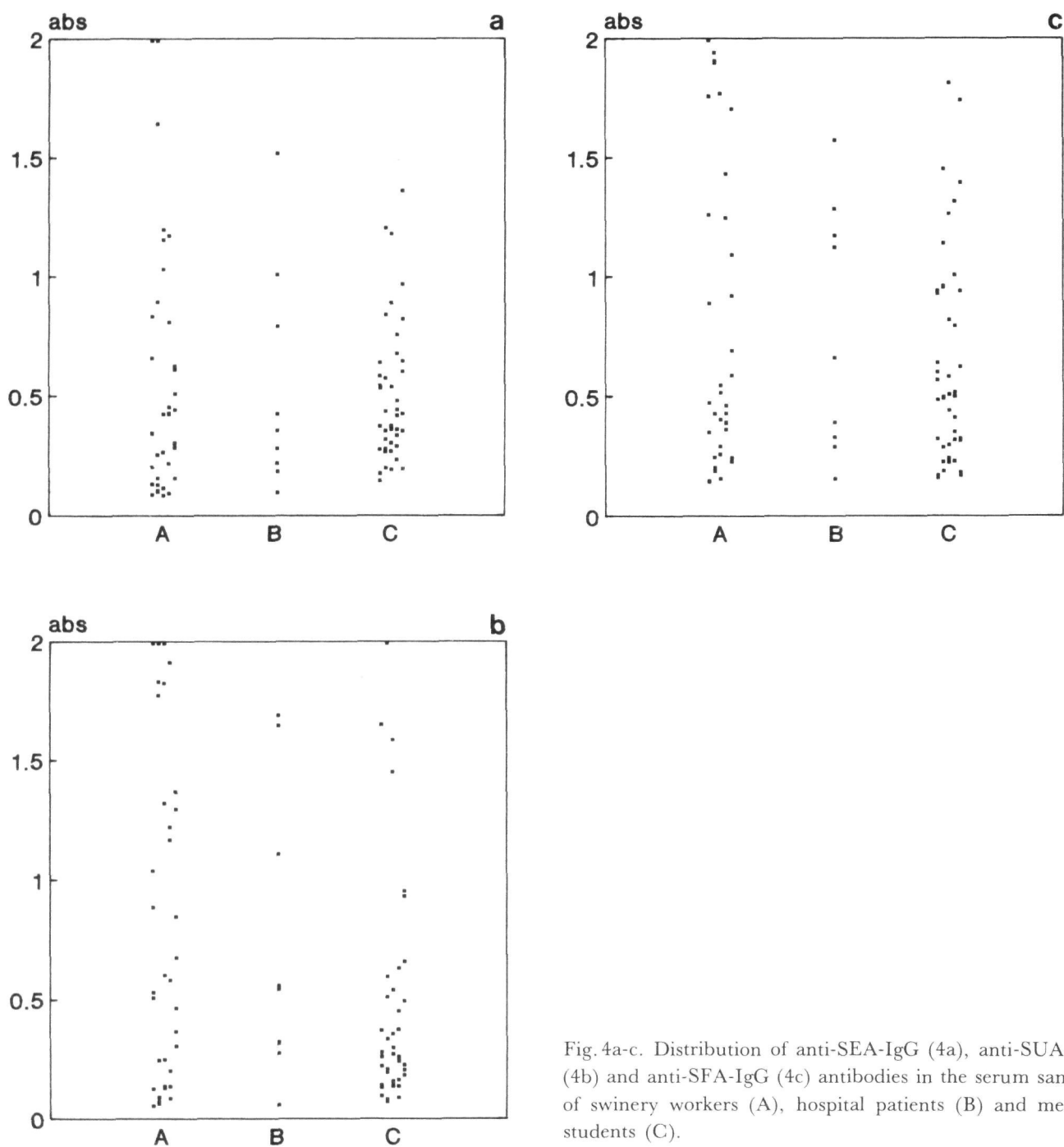


Fig. 4a-c. Distribution of anti-SEA-IgG (4a), anti-SUA-IgG (4b) and anti-SFA-IgG (4c) antibodies in the serum samples of swinery workers (A), hospital patients (B) and medical students (C).

shown in Table 1. Concentrations of SUA and SFA in the breathing zones were significantly higher than at stationary sites (Wilcoxon matched pairs test, $P = 0.028$ and $P < 0.001$, respectively). However, no difference was found in SEA concentrations ($P > 0.05$). The results between samples showed wide variation both in the breathing zones and at stationary sites.

Antibody titers

The highest anti-SEA-IgG titers were found in the group of swinery workers (Fig. 4a), but high

titers were also met in the other two groups. The distribution of anti-SUA-IgG titers is plotted in Fig. 4b. In contrast to the anti-SEA-IgG results, a greater proportion of high titers could be seen in the group of swinery workers than in the control group of medical students. However, anti-SFA-IgG titers were evenly distributed in all groups (Fig. 4c). Table 2 shows the levels of specific antibodies against the three antigens.

The specific IgG titers of all groups were compared statistically using the Mann-Whitney U test. Generally, no statistically significant dif-

Table 2
Specific antibody titers against SEA, SUA and SFA

	anti-SEA-IgG	anti-SUA-IgG Titer (mean±SD)	anti-SFA-IgG
Group A (n 37)	0.56±0.51	0.81±0.70	0.76±0.62
B (n 9)	0.54±0.44	0.72±0.57	0.77±0.49
C (n 44)	0.49±0.29	0.43±0.46	0.64±0.43

Group A: swinery workers
B: hospital patients
C: students

ferences were seen between the groups. However, in the comparison of anti-SUA-IgG results between swinery workers and a control group of medical students a result approaching the limit of significance was achieved ($P = 0.06$).

Relationship between antigen concentrations and antibody titers

The influence of specific antigen concentrations on specific antibody titers was examined statistically using Spearman correlation analyses. The

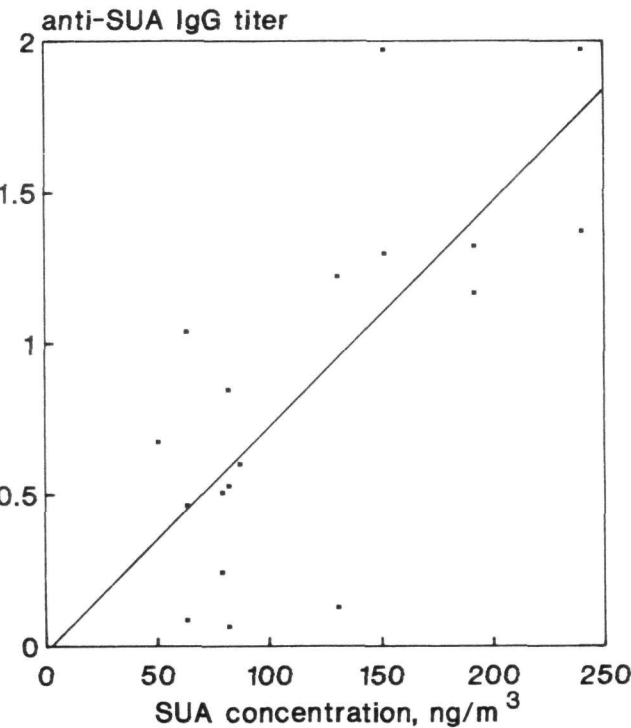


Fig. 5. Correlation of anti-SUA-IgG titers and stationary site SUA concentrations (with a regression line) when two persons working in the same swinery with an exceptionally high SUA concentration were excluded.

titers were compared with the antigen concentrations both in breathing zones and stationary sites. Only in the analyses between stationary site SUA concentrations and anti-SUA-IgG and anti-SEA-IgG titers were significant results achieved. In the former case, the correlation coefficient was 0.48 ($P = 0.03$), and in the latter 0.45 ($P = 0.04$). In one swinery, an exceptionally high SUA result was obtained in one of the two stationary sites (stationary site 1: 1448 ng/m³ and stationary site 2: 68 ng/m³) resulting in a high mean SUA concentration (758 ng/m³) compared with the respective results obtained from other swineries (maximum concentration being 240 ng/m³). If the two persons working in this swinery are excluded from the analysis, the correlation coefficients are 0.73 ($P < 0.001$) and 0.67 ($P = 0.002$), respectively. The former result is plotted in Fig. 5.

DISCUSSION

We have developed immunochemical methods to measure concentrations of specific dusts (11, 14). These methods were applied in the present study for the measurement of swine epithelial antigen (SEA), swine urinary antigen (SUA) and swine feed antigen (SFA) in swineries.

The double antibody ELISAs showed various kinds of cross-reactions between the antigens. The SEA double antibody ELISA showed clear reactivity with SUA (Fig. 1) but not with SFA (14). Thus, the method measured swine-derived antigens, and probably to all practical purposes, SEA, since the levels of SUA proved to be low in swineries. The SUA double antibody ELISA

appeared to be specific showing only minor reactivity with SEA (Fig. 2). Most likely, this minor cross-reactivity did not affect the SUA results and thus the method measured SUA concentrations. However, SEA showed clear cross-reactivity in the SFA double antibody ELISA (Fig. 3). Thus, it is possible that part of the reactivity detected by the SFA double antibody ELISA was due to SEA.

Although quantitative comparisons between different antigens measured by immunochemical methods should be made cautiously, it seems that SEA concentrations exceed those of other antigens. Common to all measurements was the wide range of results. This kind of phenomenon seems typical in the measuring of agricultural dusts (6, 12).

The principal aim of this study was to evaluate the influence of respiratory exposure to SEA, SUA and SFA in swineries on the humoral response of swinery workers. Information about the level of humoral response can be helpful for a physician in making a correct diagnosis in cases of work-related respiratory disorders. However, to show the association between a disease and humoral response, causality between them must be verified; humoral sensitization might only reflect an exposure to agents in the workplace. On the other hand, if a relationship between the antibody response and exposure to common environmental agents could be demonstrated, it might offer a basis for estimating the level of exposure and for monitoring the quality of the human environment. In addition, in some work-related disorders, demonstration of the level of exposure alone can provide sufficient information to corroborate the etiology of a patient's symptoms.

As shown in Fig. 4a-c, specific IgG antibodies against SEA, SUA and SFA are widely present in the sera of swinery workers. Also, in the study of Brouwer et al. (1), antibodies of the IgG4 class to swine urine, hair and feed antigens were commonly found in the sera of swinery workers. Katila et al. (5) demonstrated IgG antibodies against swine epithelial and serum antigens in about a third of those exposed. Interestingly, although SUA concentrations were at measurable levels in only seven breath-

ing zone samples, several serum samples contained anti-SUA-IgG antibodies.

Antibodies to the three antigens were frequently found also in the serum samples of hospital patients presenting various respiratory symptoms when working in swineries. The even distribution of titers without any predominance of high values probably reflects the multitude of factors inducing respiratory symptoms in swineries.

In the serum samples of medical students, specific IgG antibodies were widely found against all the antigens. The finding is interesting because of the lack of obvious exposure. The rural origin of some of the students may partly account for the finding. It should be noted that Donham (3) also reported that anti-swine antibodies were encountered in the samples of control subjects. Similarly, Platts-Mills et al. (9) identified some individuals among unexposed control subjects who had anti-rat urinary allergen IgG in their sera. Cross-reactions may explain these phenomena. They have been found between animal species (10, 16). The appearance of antibodies against SFA may also be explained by cross-reactions. It is made of fish powder, and common components can probably be found in the environment. Katila et al. (5) reported that they also found antibodies against swine feed antigens in the serum samples of occupationally unexposed controls. Thus, it seems that the development of antibody response to various environmental agents is not unusual. The problem is to find a suitable antigen for the measurement of antibodies, thus making it possible to separate exposed and unexposed persons.

In Fig. 4b, a conspicuous feature is that the group of swinery workers have a larger proportion of high anti-SUA-IgG titers than the control group of medical students. Statistically, the difference in antibody levels between the groups (0.43 vs. 0.81) was not significant, but did approach it (Mann-Whitney U test, $P = 0.06$). The result suggests that the measurement of antibodies against a suitable antigen, e.g., SUA or its components, could offer a way for separating persons exposed and unexposed to swine antigens. The fact that the difference was ob-

served particularly in anti-SUA-IgG levels may be explained by the greater specificity associated with urinary proteins than with epithelial antigens (10). Accordingly, in a previous study of ours (13), it was shown that dairy farmers with high IgG levels against bovine urinary antigens had higher levels of bovine epithelial antigen in their cowsheds than farmers with low IgG levels. Also, the study of Platts-Mills et al. (9) indicated that in laboratory animal allergy the quantity of IgG antibodies to rat urinary allergens correlated with the degree of exposure to animals. It is possible that if the control group of students had been selected more strictly, including only students who did not have any previous contact with pets or farm animals, the difference in anti-SUA-IgG levels between swinery workers and students might have been even more pronounced. Thus, it seems possible that urinary proteins have properties which make them suitable for estimating exposure to animal-derived materials.

However, not much is known about the possible association between dust concentrations and humoral response in agriculture. We studied the issue by statistically comparing SEA, SUA and SFA concentrations with the specific antibody titers. It was observed that stationary site SUA concentrations correlated with anti-SUA-IgG and anti-SEA-IgG titers. This finding suggests that there is an association between exposure and humoral response in swineries to swine-derived antigens, especially to SUA.

We have studied dust exposure and humoral response in the swinery environment. Our results indicate that swine epithelial, urinary and feed antigens are commonly encountered in the air. In addition, antibodies against these antigens are detected not only in the sera of swinery workers, but also in the samples of other subjects. Interestingly, a difference in the distribution of anti-SUA-IgG antibodies between swinery workers and medical students was seen. Our results suggest an association between swine urinary antigen concentrations in the air and humoral response. However, further studies are needed to assess the significance of anti-SUA-IgG measurements for individual swinery workers and of swine-derived antigens on their respiratory symptoms.

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