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Exposure to Protein Aeroallergens in Egg Processing Facilities

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Proteinaceous materials in the air can be highly allergenic and result in a range of immunologically mediated respiratory effects, including asthma. We report on the largest evaluation of exposure to date of airborne egg protein concentrations in an egg breaking and processing plant that had cases of occupational asthma. Personal air samples for egg protein were analyzed in duplicate on each PTFE filter using two analytical methods: (1) a commercial assay for non-specific total protein, and (2) indirect competitive inhibition assay using an ELISA method to quantify specific egg protein components. The highest concentrations were found in the egg washing room (mean exposure 644 $\mu g/m^3$) and breaking room (255 $\mu g/m^3$), which were also the areas where the risk of being sensitized was the greatest. There was excellent quantitative agreement between the airborne concentrations of total protein and sum of the specific protein antigens (ovalbumin, ovomucoid, and lysozyme). The correlation coefficient of the log-transformed data from the two methods was 0.88 ($p < 0.0001$). Size-selective sampling also indicated that most of the aerosol was capable of reaching the small airways. The methods described can be utilized to evaluate employee exposure to egg proteins. Exposure documentation, coupled with recommended exposure reduction strategies, could facilitate prevention of future employee sensitization and allergic respiratory responses by identifying high-exposure jobs and evaluating control measures.

Keywords Egg, Protein, Occupational Exposure, Occupational Asthma, Sensitization, Allergy

Aerosolized plant, animal, and microbial proteins are important causes of human allergic disease and asthma.[1] These allergens often comprise compounds in the 15 to 70 Kilodalton (Kd) range. In previously sensitized individuals such compounds may elicit allergic respiratory symptoms ranging from rhinitis to asthma. Although asthma in the United States affects an estimated 10 million individuals, the contribution of occupational causes to the overall prevalence is not known. Estimates from different countries range from 2 to 15 percent.[2] In the United Kingdom, newly diagnosed cases of occupational asthma account for 26 percent of all occupational respiratory diseases, with an estimated incidence rate of 22 cases per million workers.[3] However, due to deficiencies in ascertainment and reporting, it has been suggested that the true incidence of occupational asthma may be three times the reported rate.[2]

Specific occupations with highly elevated prevalence of occupational asthma attributed to natural, proteinaceous materials include flour processing workers and bakers, animal handlers, those handling seafood and meat products, and work involving potential exposure to insects and other arthropoda.[4–8] For example, among 30,000 persons exposed to laboratory animals, the prevalence of allergic responses to protein allergens may be as high as 19 percent, with 8 percent having asthma.[9]

Two occupational groups known to be exposed to high molecular weight (HMW) proteins are egg processing workers and workers using egg products in prepared foods.[10–13] Egg products can be processed and sold to the end users in either liquid or powder form. It is estimated that there may be 10,000 workers employed in 81 egg processing plants in the United States.[14–15] It is not known how many additional people are frequently exposed to egg protein powders and liquid aerosols in the food preparation industry. In previous studies, egg processing workers exposed to egg proteins in four facilities had an overall asthma prevalence of 10 percent. When analyzed according to job categories, the prevalence ranged up to 33 percent, the highest occurring in egg candlers.[7]

Egg protein comprises several specific proteins, each with a unique molecular weight and structure. The primary specific proteins and percent contributions for each protein are: ovalbumin (54%), conalbumin (ovotransferrin) (13%), ovomucoid (11%), and lysozyme (G1 globulin) (3.5%).[16] The respective

molecular weights of these proteins are 45, 80, 28, and 14 Kd. There are two distinct parts to the edible portion of an egg: the yellow or yolk, and the white or "albumen." About 13 percent of the egg is protein, with roughly 41 percent of the total protein in the albumen and 59 percent in the yolk.[16]

There are no occupational exposure standards or recommendations specific for egg protein aerosols. The only criteria for workplace exposure to another protein allergen—subtilisins, a protolytic enzyme of *Bacillus subtilis*—has been given a recommended ceiling limit of 0.06 $\mu g/m^3$ by the American Conference of Governmental Industrial Hygienists® (ACGIH)® (1974). Although this exposure limit is intended to prevent sensitization and occupational asthma, it was actually based on analytical limitations, not clinical observations.[17]

The purpose of this article is to provide a description of the methods used, summarize the survey results found to date in egg breaking plants, and provide some suggestions regarding prevention of exposures and sensitization of workers in this industry.

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PROCESS DESCRIPTION

In the study facility, whole raw eggs were received from various farms in the region on plastic trays stacked on pallets. Approximately three million eggs per day were delivered to the facility for processing. Processing essentially entails washing, cracking, separation of albumen from the yolk, pasteurization, and often drying.[18] The company employed about 95 employees in production, predominantly working during two shifts.

From a refrigerated storage room, egg carts were first pushed into the transfer area. In the transfer area, operators loaded the plastic egg trays onto one of several transfer machines; the eggs were automatically removed from the trays via suction cups and placed on a conveyer leading to the egg washer. In the washer, the eggs were mechanically brushed and sanitized with heated (49°C) alkaline sodium hypochlorite solution. This alkaline wash solution had a pH of between 11–12 and a total chlorine concentration of 120–130 ppm to reduce bacterial contamination.

Immediately after washing, the eggs continued on the conveyer belt to the candling station. At this station eggs passed over a bright light to alert an employee to cracked or otherwise unacceptable eggs. The candler employee stood alongside the conveyor where the eggs exited from the washer. Upon seeing a defective egg, this person would pick up the egg and might crush the egg before dropping it into a bucket. In this facility, workers in the transfer department rotate their job responsibilities several times during the work shift.

Upon leaving the candling station by conveyor, the eggs immediately enter the breaking room where they were mechanically broken into metal containers with a small hole in the bottom. This process separately the yolk from the albumen by allowing the egg white to drain out. An employee was stationed at each egg breaking machine to check for unacceptable eggs which were rejected. For the acceptable eggs, the employee determined if the egg was properly separated; if so, gravity removed the drained egg white from the bottom container, and a concentrated stream of air removed the egg yolk from the upper container. The separated egg parts were pumped to separate storage tanks.

In the egg breaking room about 250 eggs were processed per minute by each employee. This facility had four egg breaking machines: one had a double deck that processed roughly twice as many eggs as the other three machines.

After breaking, the shells from all machines were air-blown into a chute and removed via a paddle conveyer, where they were later ground for use as fertilizer. The paddle conveyer was in the breaking room, near the floor beside the wall separating the transfer and breaking rooms.

The liquid egg products were pasteurized in large stainless steel vessels at a temperature of approximately 65°C. Egg products were dried in one of two spray driers, one for egg whites, the other for yolks or whole eggs. In the dryers, liquid egg products were sprayed into a hot air stream. The dried egg product that falls to the floor of the chamber was transferred into an auger-type conveyer.

After spray drying, egg product powder was conveyed to one of two packaging areas, one for yellow and one for white egg powder. There was very little visible egg product dust in the air, although settled dust on surfaces was apparent. It has been reported elsewhere that a typical median particle diameter for spray-dried egg was 24 microns.[16] The dried egg products were loaded into 50 pound capacity cardboard boxes lined with plastic bags. The empty bags were filled by placing them under one of two loading chutes, then secured with an elastic band. A vacuum line removed dust-laden air that was displaced from the container during filling.

Each night during the third shift, a sanitation process called cleaning in place (CIP) took place. This was a full eight-hour job, requiring the employees to sanitize the egg breaking machines, pipes and all associated equipment. CIP entailed flushing the equipment with water and injecting a sodium hypochlorite solution, pumping that back out, flushing with city water, followed by injecting a phosphoric acid-based commercial sanitizing product. CIP was performed also in the pasteurization tanks and plumbing during the day shifts as needed.

METHODS

Sampling Procedures

Personal Sampling

Personal breathing zone air sampling for total and specific egg proteins were performed on randomly chosen workers on three consecutive days. All air samples were collected on 0.45 μm pore size Teflon filters in 37-mm closed-face cassettes (SKC Catalog #225-17-04, Eighty Four, PA) using constant flow air pumps calibrated to operate at 2 Lpm. Each day different workers were selected from each department for sampling, when practical, without any prior knowledge about their individual

characteristics or work practices. The percentage of samples collected in each department was inversely proportional to the number of employees in each department in accordance with a statistical approach that achieves representative sampling of exposed workers in a given job.[19] For example, daily sampling included 40 percent of the workers in the transfer department, which had the largest number of workers, and virtually everyone in each of the other smaller departments. Only first shift employees were monitored. All samples essentially reflect the full shift in duration (eight hours). Shortly after sample collection each day, air filter cassettes were stored intact at −20°C until eluted and processed for analysis in the laboratory.

Stationary Respirable and Total Aerosol Sampling

Simultaneously operating stationary air sampling trains were paired for determining size selective respirable protein aerosol and total aerosol concentrations, respectively. To perform respirable sampling, 10-mm nylon Dorr-Oliver type cyclones (SKC, Eighty Four, PA) with a 50 percent aerodynamic cut-off collection efficiency of 3.5 μm were used at a flow rate of 1.7 Lpm to collect egg aerosols and these results were compared to the total aerosol collected by close face cassette samplers at the same flow rate.[20] Sample pairs were collected each day over a period of three days at the same locations in the transfer and breaking departments (eight pairs) where liquid aerosols were likely and in the white packing departments (three pairs) where only dry aerosol existed.

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Elution of Filters

Filter cassettes containing air samples were stored intact at −20°C prior to laboratory processing of filters. In the laboratory, filters were removed from cassettes. Filters were placed in 15 ml polypropylene screw-cap centrifuge tubes and covered with 2 ml T-PBS (0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4, 0.05% Tween 20). Tubes were vortexed for one minute, placed in a refrigerator at 4°C overnight, re-vortexed for one minute, then the filter eluates were removed and aliquoted. Aliquots that were not used immediately were stored at −80°C. The aliquots from each filter were assayed for total protein or for specific proteins using the following techniques.

Total Protein Assay

Total non-specific protein in filter eluates was quantified by the BCA (bicinchoninic acid) Protein Assay (Pierce Chemical Co., Rockford, IL), following the manufacturer's instructions. Standard solutions of protein were prepared at dilutions ranging from 10–2000 μg/ml, using crystallized ovalbumin (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Response over this range was linear. The mean value for each sample was calculated from duplicate determinations by interpolation from the ovalbumin standard curve. Filter eluates were initially analyzed by the BCA standard test procedure (37°C incubation). Samples showing protein concentrations less than 200 μg/ml were retested by the enhanced test procedure (60°C incubation), which provides greater accuracy at lower protein concentrations. The lower limit of detection (LLD) of the enhanced method was 0.01 μg/ml. Total micrograms protein per air sample was calculated by multiplying results in μg/ml by 2.0 ml. Recovery of a pure ovalbumin standard solution which was liquid spiked onto sample filters at 10, 100, and 1000 μg/ml was 83% ± 29, 99% ± 5 and 75% ± 3, respectively.

Immunoassay

Antisera

Rabbits were immunized with purified ovalbumin, ovomucoid, or hen egg white lysozyme (Sigma Chemical Co., St. Louis, MO) in complete Freund's adjuvant (Difco Laboratories, Detroit, MI), by intramuscular injection of 10 mg protein, and re-stimulated 6 weeks later with 10 mg protein in incomplete Freund's adjuvant, then bled for antiserum two weeks later. The anti-ovalbumin rabbit serum and the anti-lysozyme rabbit serum each produced a single precipitin band against whole egg white using gel diffusion analysis, demonstrating specificity for the homologous egg antigens. The ovomucoid antiserum contained antibody to ovomucoid and also a lesser amount of antibody reactive with ovalbumin, which did not cross-react with ovomucoid at the anti-serum dilution used. Mouse monoclonal antibody to ovalbumin was an IgG_1 immunoglobulin, clone OVA-14 (Sigma Chemical Co., St. Louis, MO).

Immunochemical Quantitation of Egg Antigens

Filter eluates were analyzed for three specific egg proteins by modified standard immunochemical test procedures that included both an indirect competitive ELISA assay and a double antibody "sandwich" ELISA.[21] Ovalbumin, ovomucoid, and lysozyme were quantitated by indirect competitive inhibition enzyme-linked immuno sorbent assay (ELISA), using specific rabbit antisera at dilutions of anti-ovalbumin (1:40,000), anti-ovomucoid (1:40,000), or anti-lysozyme (1:10,000) and the corresponding antigen standards consisting of purified, crystallized proteins (ICN Pharmaceuticals, Inc., Costa Mesa, CA). The competitive inhibition assay was performed in T-PBSA (T-PBS containing 1% bovine serum albumin [Bayer Corp., Pittsburgh, PA]). Antibody-inhibitor solutions were prepared by mixing 50 μl diluted antiserum with 50 μl of antigen standard at three dilutions (0.1 μg/ml–2000 μg/ml) or filter eluates, followed by overnight incubation at room temperature.

Antibody-inhibitor solutions were added to micro-titer plates coated with 1 μg egg antigen/well, and plates were incubated for 1 hr. at room temperature. After washing with T-PBS three times, mouse monoclonal anti-rabbit IgG conjugated with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) was added to plates for 1 hr. at room temperature. After washing, substrate (0.1 ml of 1 mg/ml p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8) was added. Reactions were stopped with 0.1 ml of 1.0 N NaOH when antibody uninhibited control wells showed an $O.D._{405}$ nm of 0.7and the sample wells were read.

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Standard curves spanning concentrations between 0.1 microgram/ml and 2000 microgram/ml, were done in duplicate on each ELISA plate. Antigen concentrations were calculated from linear regression calibration curves of log-transformed data for each ELISA plate analyzed. The analytical lower limit of detection (LLD) was estimated as the concentration associated with the lowest O.D. above background on the standard curve. Samples with optical density readings above the range of the standard curve were re-analyzed at higher dilution. Exposure measurements below the analytical limit of detection were divided by the square route of two, for data analysis.[22] Recovery from filters spiked with pure albumin at concentrations of 10, 100, and 1000 μg/ml was between 82 percent $\pm$ 7.6 and 130 percent $\pm$ 33. The lower limit of detection (LLD) of the competitive inhibition ELISA for each of the three specific proteins was 1 μg/ml.

Results of low levels of ovalbumin (0–100 μg/ml) in field samples were obtained from a antibody-sandwich (two-site) quantitative assay, that showed a LLD of less than 0.1 μg/ml. Briefly, micro-titer plates were coated with 800 ng/well of mouse monoclonal anti-chicken ovalbumin antibody. Plates were incubated 1 hr. at room temperature with blocking buffer (T-PBSA diluent). Then, 0.1 ml ovalbumin standard solutions, T-PBSA diluent control, or unknowns (filter eluates) were added to duplicate wells for 1 hr. at room temperature. Ovalbumin standards consisted of 3-fold dilutions in T-PBSA (0.002–120 μg/ml). Plates were washed with T-PBS, and incubated one hour at room temperature with 0.1 ml of polyclonal rabbit antiserum to ovalbumin (1:40,000 dilution), followed by mouse monoclonal anti-rabbit IgG, alkaline-phosphatase conjugate, and finally para-nitrophenol substrate. Recovery of spiked filters in the laboratory at the 10 and 100 μg/ml concentrations was 114 percent $\pm$ 3 and 102 percent $\pm$ 23, respectively.

Comparison to Other Surveys

Four small-scale surveys of egg processing facilities have previously been conducted. They described airborne concentrations of total protein, and three specific egg proteins: ovalbumin, ovomucoid, and lysozyme. These data were summarized in order to compare the air concentrations found at those facilities with the current facility, considering differences in both process and engineering controls when possible. These four facilities are hereafter referred to as A through D.

Photomicrographs

Aerosols collected by MCS were collected in the breaking room in facility A. The samples were collected on Teflon filters mounted in a five-stage Andersen size-selective impactor (model no. GMW 65000) using a flow rate of 300 Lpm. Scanning electron photomicrographs were taken at 500$\times$ magnification.

Blank Samples and Referent Plant

Multiple field blanks were collected during the survey by uncapping the cassette briefly, but not drawing air through. A referent plant was also sampled for airborne proteins. This was located within a half-mile of the egg processing facility. This plant manufactured box spring sections for beds and used no known products containing egg proteins. The analytical laboratory was "blinded" as to the identity of these samples.

RESULTS

Total Protein

Fifty-three personal sampling results were used in the analysis of total protein. Five results were below the detection limit and were associated with the low relative exposures in the pasteurization department and sanitation activities. These data are depicted in Figure 1, showing the arithmetic means and variability of the results for each department. Arithmetic means, rather than geometric means, are presented to allow comparison with earlier survey report results.

At this plant, the highest average concentrations existed in the transfer (644 $\pm$ 355 μg/m^3) and breaking departments (255 $\pm$ 147 μg/m^3). High concentrations were also found in the white packing room (426 $\pm$ 177 μg/m^3), where one person was exposed per shift in this job. All other departments and jobs appeared to have appreciably lower exposure potentials (mean 9 to 91 μg/m^3).

Specific Egg Proteins

The mean results of the individual proteins (ovalbumin, ovomucoid and lysozyme) along with the sum of these three proteins for each department, are depicted in Figure 2. There was good agreement between the specific proteins in Figure 2 and the total non-specific protein measurement data in Figure 1, with the possible exception of a relative concentration difference in egg white packaging. Lysozyme, while found at concentrations

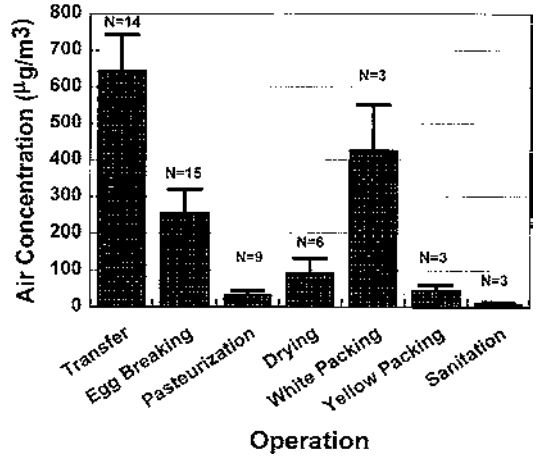

FIGURE 1

Total protein assay results for each department of egg breaking facility (site E). Arithmetic mean air concentrations with standard error bars and number of samples shown.

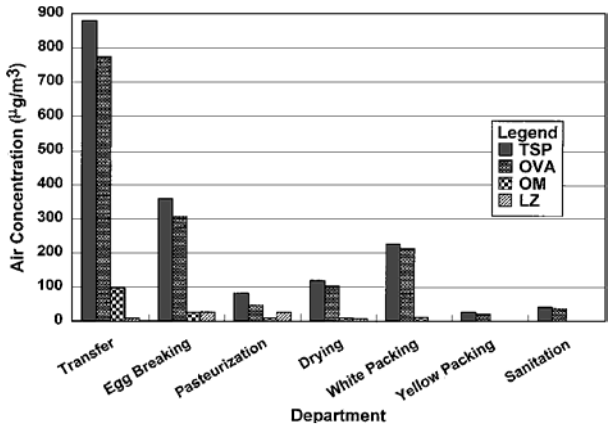

FIGURE 2
Arithmetic mean air concentrations of four protein measures. Sum of specific proteins = TSP; ovalbumin = OVA; ovomucoid = OM; lysozyme = LZ.

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that were a fraction of OVA and OM even in the transfer and egg breaking departments, appears to be conspicuously low or undetectable in the egg white packaging department as measured by the immunoassay.

The sum of the three specific egg proteins was related to the total protein analysis by linear regression analysis after normal log transformation of the data. Using the same filter eluent in both analytical techniques eliminates sample-to-sample variation and the results of the two methods can be better compared. The results are graphically depicted in Figure 3. The explained variance (r^2) of the regression equation was 0.77, indicating a moderately strong association between the two analytical techniques. The model according to the analysis of variance is highly significant ($p < 0.0001$). The equation for the fitted model is LN3PROT = 0.61 + 0.922∗LNTOTPROT, where LN3PROT is the natural log of the sum of three specific proteins and LNTOTPROT is the natural log of the total protein measurements.

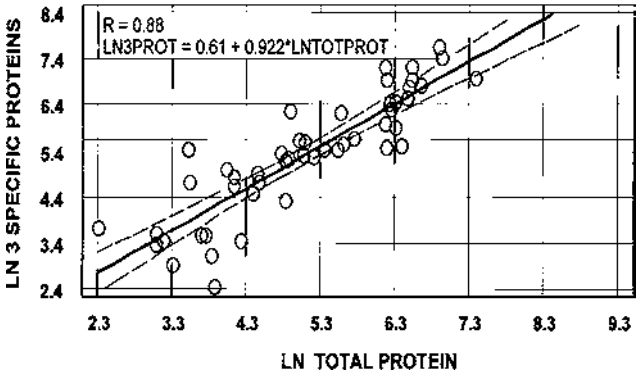

FIGURE 3
Regression of air sampling results using sum of three specific egg proteins and total protein results ($\mu g/m^3$).

Respirable and Total Protein

Overall, the average concentration of respirable protein (RNSP) and total protein (TNSP) in the paired data set is 322 and 607 $\mu g/m^3$, respectively. Thus, of all the samples collected throughout the facility, 53 percent of the total aerosol mass was respirable (≤ 3.5 μm). The mean difference between RNSP-TNSP was −284.5 (std. dev = 330). It is perhaps worth noting that the two concentrations are less dissimilar in the wet departments (transfer and breaking area means were 440 and 768 for respirable and total aerosol, respectively, i.e., 57% respirable) than in the white packing department where dried egg protein is present (10 and 179, respectively, i.e., 6% respirable) suggesting that the airborne aerosol size is smaller in the wet processing areas than is the particle size of the dried egg. Since the differences of RNSP-TNSP were normally distributed, a matched-paired t-test was performed on the non-transformed data. The RNSP-TNSP difference between the two matched data sets were significantly less than zero ($p = 0.009$, 1-tailed test).

Referent Plant Samples

Total protein and three specific egg proteins were also sampled in the referent plant. These were collected in various locations inside the plant and outdoors. It was not expected to find egg-specific proteins in this environment, but low levels of total protein could be present from natural environmental sources. Low levels of total protein were found in the referent plant (ND −41 $\mu g/m^3$, n = 5) while only a relatively minute amount of ovomucoid (2.7 $\mu g/m^3$) was detected in one of these samples.

Comparison to Other Surveys

Table I provides a summary of the total protein and ovalbumin results of personal sampling from other studies that have previously been performed in egg breaking facilities. Some of the procedures used to collect and analyze the protein samples in these previous studies were different from methods used in the survey reported here. However, we believe that these different methods provided comparable results. Facility E represents the present survey and the results are included in part in Table I to facilitate comparison to the other studies.

Photomicrographs

Scanning electron photomicrographs of aerosols in the breaking room in facility A are shown in Figure 4. The drawn line is indicative of a distance of 10 μm. The photomicrographs show globular shaped particles on the largest size stage but mostly spherical shapes among the smaller particles. This might be expected as typical of an aerosol initially generated as a liquid. The corresponding size-selective air concentrations at each stage for ovalbumin are shown in Table II.

TABLE I
Egg protein concentrations obtained at other egg processing facilities—personal monitoring results

Facility/Date/ Work area	Total Protein			Ovalbumin	
	Number of samples	Average ($\mu g/m^3$)	Standard deviation	Number of samples	Average ($\mu g/m^3$)
Facility A-3/87[39]					
Transfer Rm.	5	580	174	1	107
Breaking Rm.	3	167	11	1	7
Dryer	1	390	—	1	132
Packaging	2	1615	—	1	140
Facility B-3/87[40]					
Transfer Rm.	7	404	62	2	150
Breaking Rm.	3	267	106	1	420
Yellow dryer	2	545	—	2	555
White dryer	1	10000	—	1	9800
Blend packaging	3	57000	63720	2	34500
Facility C-3/87[41]					
Transfer Rm	6	665	291	2	119
Breaking Rm	4	710	168	1	360
Facility D-8/93[42]					
Transfer Rm.				6	1022
Breaking Rm.				6	2518
Facility E-10/95					
Transfer Rm.	14	644	377	14	774
Breaking Rm.	15	255	501	15	307
Dryer Rm.	6	91	84	6	102
White packing Rm.	3	426	25	3	213

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Quality Control Samples

Field blank and laboratory blank samples are expected to contain non-detectable amounts of analyte. None of the field blanks, or laboratory blanks, from the referent plant survey contained specific egg proteins, but 6 μg per sample of total protein were detected in one field blank and one laboratory blank (n = 5). None of the field blank samples taken at the egg breaking facility had detectable quantities of total protein, ovalbumin or ovomucoid proteins. However, there were relatively low amounts of lysozyme protein (0.6 to 4.2 μg per sample) on three of four field blank samples from the egg breaking facility. Due to the typically non-detectable results from the blank samples, and inconsistent nature of some positive blank samples, the reported data were not blank corrected.

TABLE II
Size selective ovalbumin aerosol concentrations in egg breaking room of facility[A]

50% Cut-Off diameter (μm)	Air concentration ($\mu g/m^3$)	Percent of total aerosol	Cumulative percentage
>9.4	6.30	28.6	100
4.5	4.85	22	71.5
2.7	4.84	22	49.5
1.6	2.52	11.5	27.5
<1.6	3.52	16	16

[A]One sample collected with a 5-stage Andersen Impactor.

DISCUSSION

Exposure to egg proteins is an important cause of IgE-mediated occupational asthma.[10] Presently there is an incomplete understanding of the exposure conditions causing respiratory sensitization to egg proteins, especially concerning the magnitude and duration of exposure necessary for this to occur. Sensitization may occur with repeated exposure over time, and workers may chose to continue to work after sensitization occurs as long as it does not progress to severe respiratory distress. Because this was a cross-sectional study and many of the sensitized workers at this facility were long-term employees, it was not possible in this study to definitively determine what conditions of exposure in these workers were necessary to induce sensitization. Therefore, it is unknown whether the current air concentrations documented in this paper are capable of causing IgE-mediated allergy to egg proteins. Because the concentrations of this allergen were

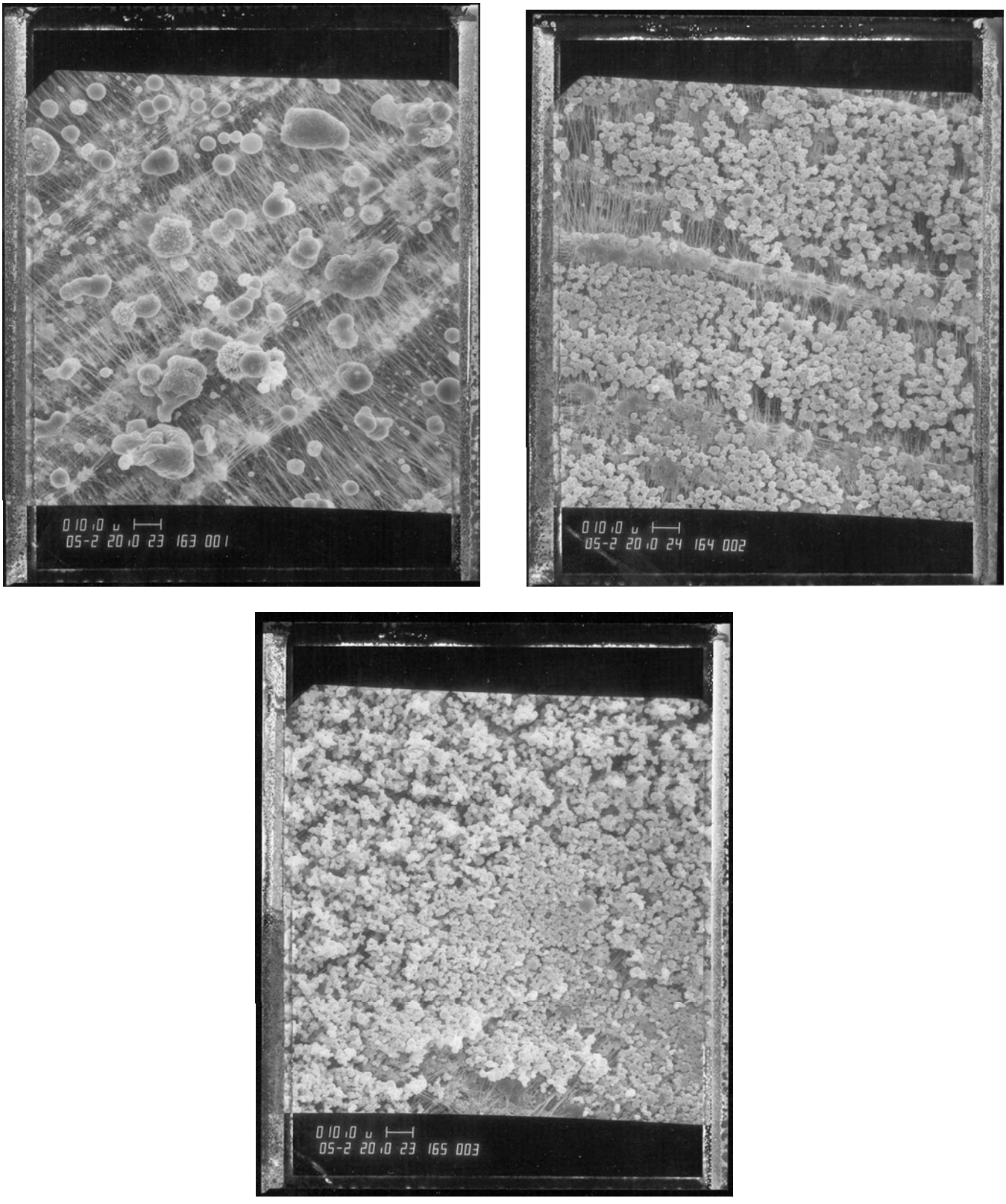

FIGURE 4

Photomicrographs of egg aerosols in the breaking room of a egg processing plant. The size selective 50% aerodynamic cut-offs for the multi-stage cascade impactor were: stage 1, >9.4 μm; stage 2, 4.5 μm; stage 3, 2.7 μm; stage 4, 1.6 μm and stage 5, <1.6 μm. The reference bar ⌴ is equal to 10 μm. *(Continued)*

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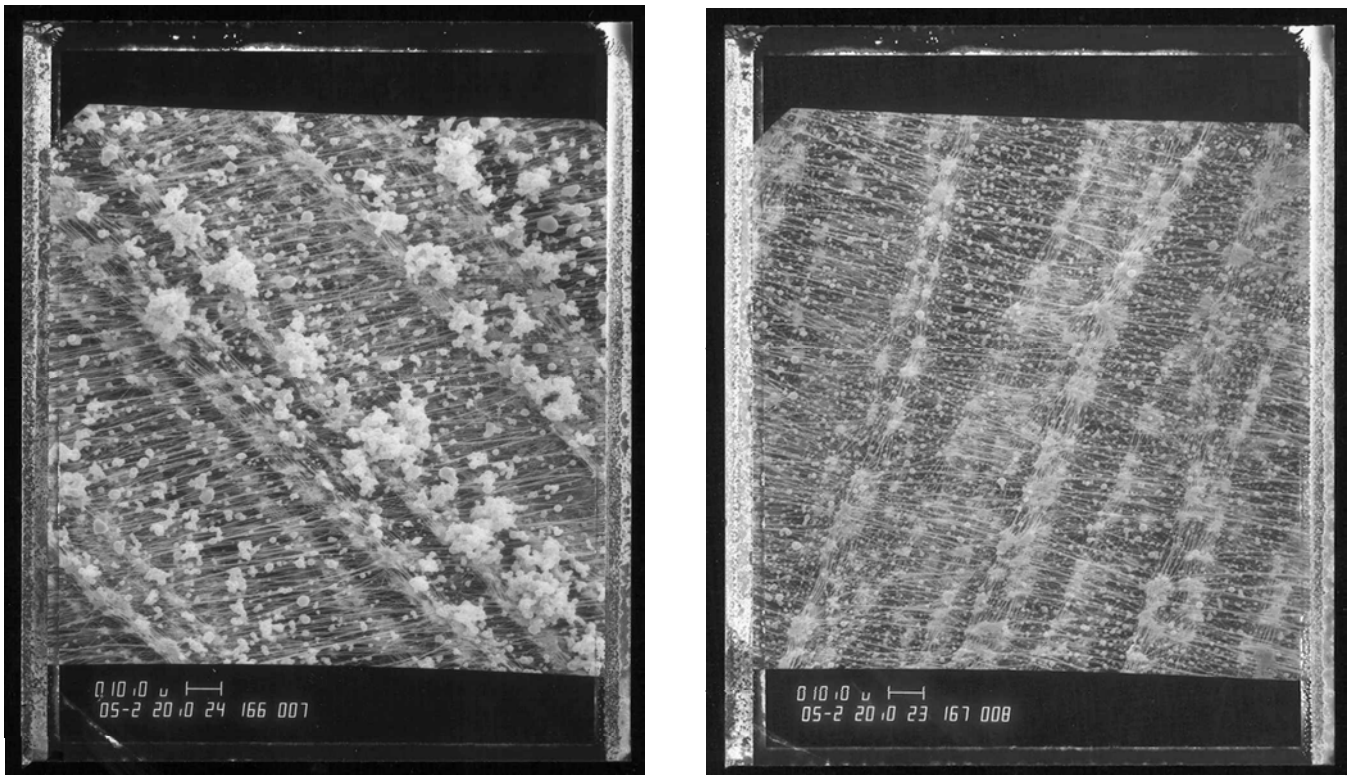

FIGURE 4
(Continued)

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relatively high compared to reported exposures to other known occupational allergens, it is likely that the measured egg protein concentrations are capable of inducing sensitization.

In addition to exposure, susceptibility may be determined by important host differences within individuals. In the current study, there was a higher frequency of atopy among workers with cutaneous sensitization to egg proteins.[23] The airborne concentrations documented in the egg processing facilities included in this report were in each case associated with the existence of IgE-mediated allergy to egg proteins.[24] The concentrations of airborne egg protein are 50–300 times higher than that found for protein aerosols inducing asthma in most other occupational settings.[25–28] In ragweed pollen, the predominant antigenic protein accounts for only 0.2 percent of the total solids and a high daily exposure to this protein may amount to only 40 nanograms.[29] In contrast, liquid whole egg contains about 26 percent solids, of which about 50 percent is protein. The particulate size of the aerosol also predicts where in the respiratory tract the aerosol is most likely to be deposited. Based upon the high protein content and aerosol size characteristics of the egg aerosol in these modern, high-through-put facilities, the cumulative protein exposure which could reach the small airways of egg processing workers may be extraordinarily high.

Comparing the results from the current study with those reported in Table I indicates general agreement between most of the areas, with some exceptions. Individual plant air concentrations will depend on the particular processing equipment used, the speed of operation, and ventilation controls. Air flow patterns between departments may also be important. For instance, the USDA requires egg breaking rooms to be positively pressured relative to adjacent rooms to keep air contaminants from entering. Thus, airborne aerosols in the egg breaking room may enter the transfer room and other adjacent rooms if there is a large difference in supplied air. In this facility, employees at the candler's station definitely received air from the egg breaking room, but also likely received aerosols from the washing machines due to incomplete control of mists generated from within the washers.

As an example of the benefit of exhaust ventilation and different work practices, the container filling operation in the most recently studied site (facility E, Table I) used a closed filling procedure that was under negative pressure. The operator could accurately determine when the box was full while it was still covered by the chute. Personal air exposure concentrations were 185–604 $\mu g/m^3$ total protein. At facility A, filling was not performed under negative pressure and the final weight was accomplished by manually filling the box with a scoop. Visible airborne powder was visible in video taped footage of this operation and personal air exposure concentrations averaged 1615 $\mu g/m^3$ total protein.

In the egg transfer and breaking departments, the personal exposures were generally similar at each facility and were among

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the areas with the highest concentrations. As received from the manufacturers, the design of the egg washing and breaking equipment at the five facilities mentioned in this report provides little or no provision for ventilation control of escaping egg aerosols. The egg processing facilities are left to their own resources to design and install controls. The most likely sources of aerosolized egg protein are where high speed mechanical contact with egg product or air jets are involved. Local exhaust ventilation of points of emission and fresh air showers directed over work stations are some ways of reducing employee exposure. These controls should be designed and an air balance should be performed by a qualified heating, ventilating and air conditioning (HVAC) engineer.

While there was generally a good correlation between total and specific egg protein, and the simpler total protein determination would suffice for most exposure assessment purposes, for clinical research studies it is of particular importance to measure airborne concentrations of specific egg proteins as well. In the egg processing industry, aerosolized egg proteins are the predominant source of all proteins found in the air. However, in occupations with exposures to multiple proteins from various sources, or where the allergenic protein is only a small constituent of the total mass (as with ragweed), studies involving allergic sensitization to specific proteins greatly benefit from quantifying the specific aeroallergen concentrations to which those persons may be exposed.

The "total" aerosol sampling results, compared to published inhalable efficiencies at the aerosol sizes measured in this facility, probably accurately represents the actual inhaled mass of egg aerosol.[30] The respirable-selective versus total aerosol sampling results collected in the wet versus dry products areas suggests that the aerosol size in the wet areas might be smaller than in the packaging room, making the particles more likely to reach the lower regions of the respiratory system. The larger mean spray-dried particle size of 24 μm reported elsewhere would support the suggestion that dried egg protein is less inhalable.[16] It is not known with certainty of what significance predominately respirable particles have in relation to induction of asthma, since asthmatic episodes involve the bronchial region where larger particles (3.5–10 μm) will be more likely to impact. However, it appears from this limited data that practically all the aerosolized egg protein might at least be able to deposit in the upper respiratory regions if inhaled (MMD < 25 μm), at least in the wet processing areas, and thus constitute an aerosol that is potentially capable of eliciting an asthmatic episode.

The physical structure of several egg proteins is expected to be altered by the pasteurization and drying processes and may be allergenically different than the raw egg protein. There is some evidence of this found in the environmental sample results. The relative difference found in the egg white packing area between the concentration of total protein (Figure 2) and in the concentration of egg-specific protein (Figure 3) suggest that protein conformational transformations may have occurred which are not detectable by our epitope-sensitive immunoassay. Thus, the final egg product proteins may be antigenically different than the raw egg proteins, at least for those egg proteins that are heat labile. It may be possible for someone to be sensitized to proteins in the raw egg product that are not the same as in the finished egg, and vice versa. The allergenicity of processed egg protein and the prevalence of sensitization and asthma among food preparation workers exposed to processed egg protein should be studied, as this potentially represents an additional large population of workers who may be at risk.

Chlorinated wash solution products and acids are often used in the egg processing industry to disinfect the eggs and equipment. There is evidence that hypochlorite alone on contact can cause immediate type and delayed type sensitization.[31] There is additional literature suggesting that combined exposure to irritants and allergens may potentiate allergic sensitization.[32–35] Although concentrations of chlorine gas, and possibly chloramine, were sampled but were low at the time we studied the present facility, exposure to higher concentrations may have existed in the past. Potentiation of ovalbumin sensitization by coexposure to irritants in experimental animal studies supports our recommendation to maintain low exposures to irritants in this industry.

The possible significance of a cutaneous route of exposure in regards to sensitization to egg proteins should not be overlooked. For healthy, intact skin, the large molecular size of egg proteins would prevent these proteins from passing through the stratum corneum. However, a damaged skin barrier may facilitate penetration of egg proteins into the epidermis which could readily come in contact with antigen presenting cells and helper T cells leading to activation of B cell production of specific IgE antibodies. Intradermal injection of allergens, including ovalbumin, has been found to be highly effective in producing respiratory sensitization in experimental animals.[36] Local dermal sensitization might well progress to systemic sensitization and respiratory asthma.[37] Such a progression from localized eczema to asthma has been observed in natural rubber latex sensitive persons.[38] Several workers in this facility had severely damaged skin on their hands. Protecting the skin from physical and chemical damage and preventing skin contact with potential allergens might be prudent.

The sampling and analytical methods and recommendations presented here could potentially be applied to protein allergen exposures in other occupations. Our larger study that involved clinical and immune testing of workers in facility E clearly showed an exposure-effect association. These latter findings will be presented separately.

In conclusion, airborne egg protein concentrations have been documented to be quite high in several egg processing facilities. Understanding the magnitude of exposure for each job is important to know regarding both the relative risk of initial sensitization as well as subsequent response in sensitized persons. Knowledge of the magnitude of exposure in each area of a facility, coupled with methods to reduce exposures, can serve to

prevent new cases of sensitization and assist in efforts to place already sensitized workers into safer work environments.

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